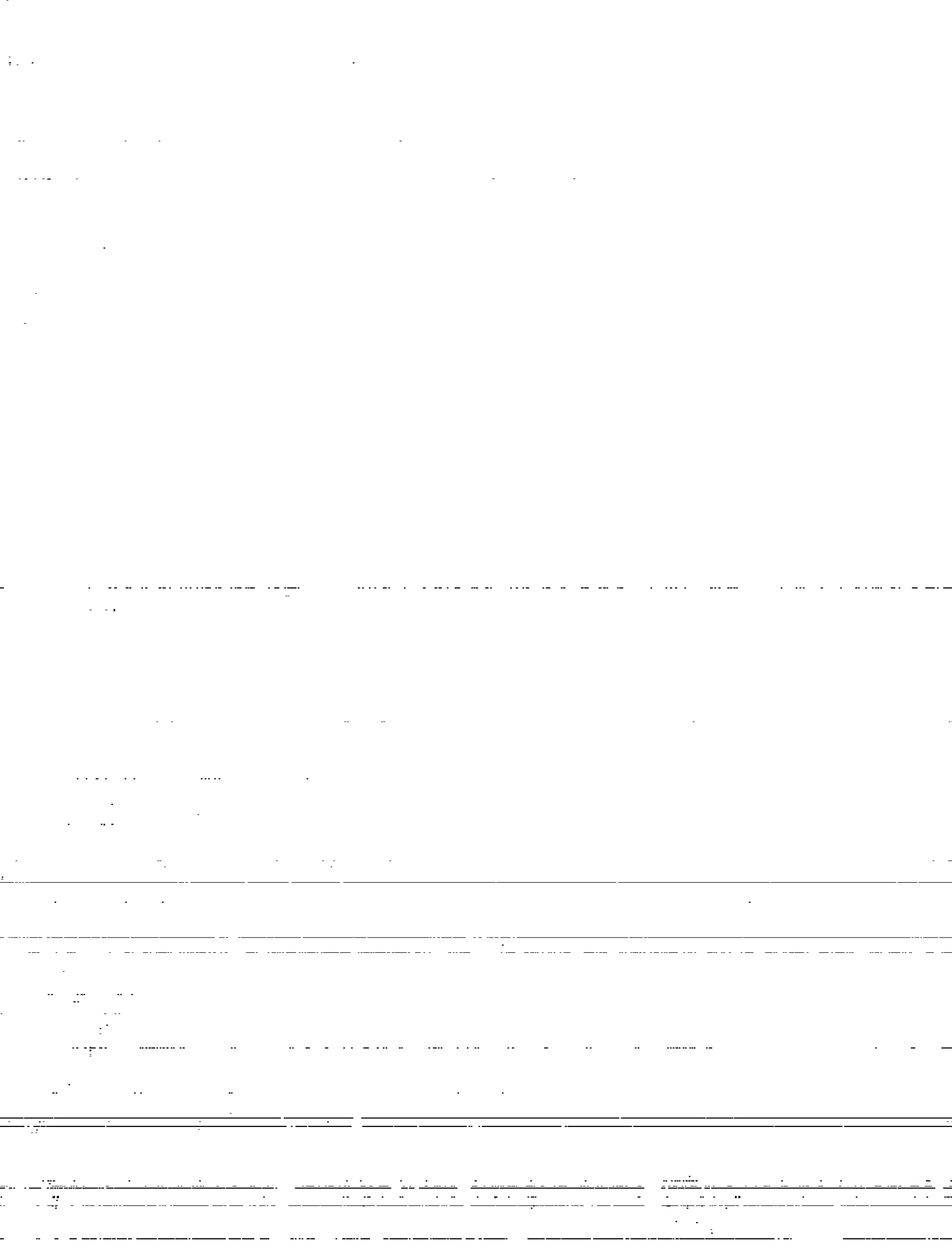


These records are from CDER's historical file of information previously disclosed under the Freedom of Information Act (FOIA) for this drug approval and are being posted as is. They have not been previously posted on Drugs@FDA because of the quality (e.g., readability) of some of the records. The documents were redacted before amendments to FOIA required that the volume of redacted information be identified and/or the FOIA exemption be cited. These are the best available copies.

NDA: 19-085 SPONSOR: BOEHRINGER INGELHEIM

1 OF 3

TRADE: ATROVENT GENERIC: IPRATROPIUM BROMIDE



APRVL

LTR



Washington, D.C. 20205

Dr. [Name],  
[Address]  
[City, State, Zip]

Dear Dr. [Name]:  
[Subject]

Dear Dr. [Name]:

Reference is made to your application for approval of the drug [Name] and to the information submitted in support of the application. The application is approved, effective as of the date of this letter.

We also refer to our letter dated [Date], [City, State, Zip], in which we advised you of the requirements for labeling of this drug.

Additional information is contained in Section 301.33 of the Code of Federal Regulations, Title 21, Chapter 310, Subchapter B, Part 310.33. The labeling must be identical to the labeling of [Name] within 6 months or at the next printing, whichever occurs first.

We have completed the review of this application including the draft labeling submitted in your last amendment and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in that draft labeling. Accordingly, the application is approved, effective as of the date of this letter.

The final approved labeling must be identical to the draft labeling. Marketing the product with a label that is not identical to the draft labeling may render the product misbranded and an unapproved new drug. Please submit twelve copies of the final label to FDA as soon as available. For administrative purposes this submission should be designated "Final Labeling" to the approved NDA [Number]. Approval of this submission by FDA is not required before the labeling is used. Should additional information relating to the safety and effectiveness of this drug product become available prior to our receipt of the final printed labeling, revision of that labeling may be required.

display both the market package of the drug product when it is available,  
and remind you that you must comply with the requirements set forth under  
21 CFR 314.19 and 314.21 for an approved NDA.

Sincerely yours,

*Paula Wickham MD*

Paula Wickham, M.D.  
Acting Deputy Director (Medical Affairs)  
Office of Drug Research and Review  
Center for Drugs and Biologics

F P L

11/27/86  
Appendix 2-#-87

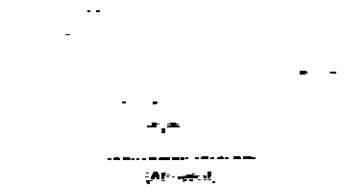
Pharmacist  
Year off Instructions for use and diagnosis with  
containing in container

# Atrovent<sup>®</sup>

(Ipratropium Bromide)  
Inhalation Aerosol

## Prescribing Information

**DESCRIPTION:** The active ingredient in Atrovent<sup>®</sup> Inhalation Aerosol is ipratropium bromide. It is a synthetic quaternary ammonium salt of the naturally occurring alkaloid atropine. The chemical structure of ipratropium bromide is shown below.



Ipratropium bromide is a white crystalline substance freely soluble in water and a very slightly soluble in lipophilic solvents such as ether, chloroform, and fluorocarbon.

Atrovent Inhalation Aerosol is an inhalation aerosol for the relief of bronchospasm. The net weight is 1.4 grams of a ready-to-use 200 actuations. Each actuation of the metered dose is 18 mcg of ipratropium bromide from the mouthpiece. The inert ingredients are propellant dibromofluoromethane, octafluorocyclopentane, and hexafluoroethane as propellant and hexafluoroethane as propellant and octafluorocyclopentane as propellant.

**CLINICAL PHARMACOLOGY:** Atrovent<sup>®</sup> (ipratropium bromide) is an anti-cholinergic (para-sympatholytic) agent which blocks the cholinergic reflexes by antagonizing the action of acetylcholine, the transmitter agent released from the vagus nerve. Anticholinergics prevent the increases in intracellular concentration of cyclic guanosine monophosphate (cyclic GMP) which are caused by stimulation of smooth muscle by the muscarinic receptor on bronchial smooth muscle.

The bronchodilation following inhalation of Atrovent is primarily a local site specific effect, not a systemic one. Much of an inhaled dose is swallowed as shown by fecal excretion studies. Atrovent is not readily absorbed into the systemic circulation either from the surface of the lung or from the gastrointestinal tract as confirmed by blood level and renal excretion studies.

The half-life of elimination is about 7 hours after inhalation or intravenous administration. Autoradiographic studies in rats have shown that Atrovent does not penetrate the blood-brain barrier.

In controlled 30 day studies in patients with bronchospasm associated with chronic obstructive pulmonary disease, asthma, bronchitis and emphysema, ipratropium bromide demonstrated an increase in FEV<sub>1</sub> and FEV<sub>25-75</sub>, increases of 15% or more occurred after 15 minutes, reached a peak in 1-2 hours, and persisted for periods of 3 to 4 hours in the majority of patients.

INDICATIONS AND USAGE  
Atrovent Inhalation Aerosol

Atrovent Inhalation Aerosol is indicated for the relief of bronchospasm associated with chronic obstructive pulmonary disease, asthma, bronchitis and emphysema.

Atrovent Inhalation Aerosol is also indicated for the relief of bronchospasm associated with acute bronchitis and for the relief of bronchospasm associated with upper respiratory tract infection.

Atrovent Inhalation Aerosol is also indicated for the relief of bronchospasm associated with acute bronchitis and for the relief of bronchospasm associated with upper respiratory tract infection.

**INDICATIONS AND USAGE:** Atrovent<sup>®</sup> (ipratropium bromide) is indicated as a bronchodilator for the relief of bronchospasm associated with chronic obstructive pulmonary disease, asthma, bronchitis and emphysema.

**CONTRAINDICATIONS:** Hypertension, glaucoma, and other conditions in which the use of anticholinergics is contraindicated.

**WARNINGS:** Atrovent<sup>®</sup> (ipratropium bromide) is not indicated for the initial treatment of acute episodes of bronchospasm where rapid response is required.

**PRECAUTIONS:** General Atrovent<sup>®</sup> (ipratropium bromide) should be used with caution in patients with narrow angle glaucoma or acute hyper-tension or tachycardia or obstruction.

Information for Patients: Patients should be advised that temporary blurring of vision may result if the aerosol is sprayed into the eyes.

Drug Interactions: Atrovent has been used concurrently with other drugs, including sympathomimetic bronchodilators, methylxanthines, steroids and cromolyn sodium commonly used in the treatment of chronic obstructive pulmonary disease without adverse drug reactions. There are no formal studies fully evaluating the additive effects of Atrovent and these drugs with respect to effectiveness.

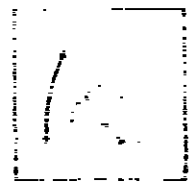
**Carcinogenesis, Mutagenesis, Impairment of Fertility:** Two year oral carcinogenicity studies in rats and mice have revealed no carcinogenic potential at doses up to 1,250 times the maximum recommended human daily dose for Atrovent. Results of various mutagenicity studies were negative.

Fertility of male or female rats at oral doses up to approximately 10,000 times the maximum recommended human daily dose was unaffected by Atrovent administration. At doses above 10,000 times the maximum recommended human daily dose increased resorption and decreased conception rates were observed.

**Pregnancy (PREGNANT EFFECTS):** Pregnancy Category B. Oral reproduction studies performed in mice, rats and rabbits in doses approximately 2,000, 200,000 and 70,000 times the maximum recommended human daily dose, respectively, and embryonal reproduction studies in rats and rabbits at doses approximately 312 and 375 times the maximum recommended human daily dose, respectively, have demonstrated no evidence of teratogenic effects as a result of Atrovent. However, no adequate or well controlled

## Instructions for Use

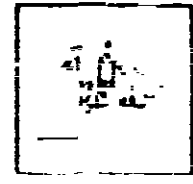
1. Shake the container well before use.  
2. Remove the cap and insert the mouthpiece into the mouth.



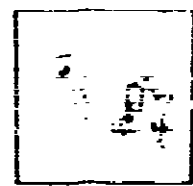
3. Exhale completely and hold the breath for 10 seconds. The label on the container should be held upright. Repeat the steps above for each second inhalation. Hold your breath for 10 seconds after each inhalation.



4. Exhale slowly through the mouth and at the same time firmly press once on the upturned lever at the base. Continue to exhale slowly.



5. Hold your breath for a few seconds and then remove the mouthpiece from the mouth and exhale slowly. Wait approximately 15 seconds and repeat second inhalation as outlined above.



Boehringer Ingelheim, Inc.  
 2800 Quince Orchard Road  
 Gaithersburg, MD 20878  
 (301) 271-4000

...the most common side effect is dizziness...  
 ...the most common side effect is dizziness...  
 ...the most common side effect is dizziness...

...the most common side effect is dizziness...  
 ...the most common side effect is dizziness...  
 ...the most common side effect is dizziness...

| Reaction                      | Percent of Patients |                         |
|-------------------------------|---------------------|-------------------------|
|                               | Symptoms<br>n = 254 | Side Effects<br>n = 244 |
| <b>Cardiovascular</b>         |                     |                         |
| Hypotension                   | 1.2                 | 1.2                     |
| <b>Central Nervous System</b> |                     |                         |
| Dizziness                     | 1.1                 | 6.8                     |
| Headache                      | 0.8                 | 0.8                     |
| Nausea                        | 0.4                 | 0.4                     |
| <b>Dermatological</b>         |                     |                         |
| Rash                          | 1.2                 | 0.8                     |
| <b>Gastrointestinal</b>       |                     |                         |
| Nausea                        | 0.8                 | 1.2                     |
| Constipation                  | 0.4                 | 0.4                     |
| Diarrhea                      | 0.4                 | 0.4                     |
| Vomiting                      | 0.4                 | 0.4                     |
| <b>Musculoskeletal</b>        |                     |                         |
| Pain                          | 0.4                 | 0.4                     |
| <b>Ophthalmological</b>       |                     |                         |
| Blurred vision                | 0.4                 | 0.4                     |
| <b>Oro-pharyngeal</b>         |                     |                         |
| Dry mouth                     | 0.4                 | 0.4                     |
| Inflamed throat               | 0.4                 | 0.4                     |
| <b>Respiratory</b>            |                     |                         |
| Cough                         | 0.4                 | 0.4                     |
| Exacerbation of symptoms      | 0.4                 | 0.4                     |

Additional adverse reactions reported in more than one percent of the patients receiving placebo due to known or unknown causes are listed in the following table.

The large-scale, randomized, double-blind, placebo-controlled study included 500 patients. About 70% of patients involved desired to be in the program despite the adverse events.

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...the most common side effect is dizziness...  
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 ...the most common side effect is dizziness...

**CONTRAINDICATIONS:** Do not use if you are allergic to any of the ingredients. Do not use if you are pregnant or breastfeeding. Do not use if you are taking other medications that may interact with this drug.

**CAUTION:** Federal law prohibits dispensing without prescription.



Distributed by  
 Boehringer Ingelheim  
 Pharmaceuticals, Inc.  
 Gaithersburg, MD 20878

Manufactured by  
 Bayer Laboratories, Inc.  
 2800 Quince Orchard Road  
 Gaithersburg, MD 20878

Patent No. 4,811,500

Developed by  
 Boehringer Ingelheim  
 Pharmaceuticals, Inc.  
 Gaithersburg, MD 20878

Licensed from  
 Boehringer Ingelheim  
 International GmbH

Manufactured by  
 Bayer Laboratories, Inc.  
 2800 Quince Orchard Road  
 Gaithersburg, MD 20878

Patent No. 4,811,500

MED

REV

200A (REVISED) Application for New Drug  
Application Form  
Manufacturer

Application Number: 777001  
Date Recd: 8/22/84

1. **PLATEAU AND STAIRS, 10101 10101 10101**

Draft labeling for Plateau was submitted as a result of a meeting between representatives of the firm and this Agency held on June 19, 1984. The topic of the meeting was a discussion of labeling for this new NDA.

The submitted, revised draft labeling contains the sections titled **PHARMACOLOGY, PHARMACOKINETICS, INDICATIONS, and USAGE, PRECAUTIONS,** and a statement concerning the legend of the Bc legend in the insert.

As a result of the meeting, the submitted draft labeling reflects the comments reached at the above meeting with one exception, concerning the statement made under the section titled **INDICATIONS and USAGE** reading "approximately one-half of asthmatic patients have been shown to respond to AIDIVINE". (See **CLINICAL PHARMACOLOGY**.)

**RECOMMENDATIONS:**

1. The statement, which is the final sentence of the section titled **INDICATIONS and USAGE** including the words, in parentheses regarding use in asthmatic patients should be deleted from that section of the labeling.
2. Providing 1) above is implemented, the submitted draft labeling should be approved.
3. Adverse experience tables for patients treated in the 90-day controlled clinical trials in support of the Package Inserts: For Airovent: This tabulation shows that the total number of patients included in the NDA submission was 228 while the number that completed study was 254 - Table 1. This table is a breakdown by body systems showing the number and percentage of patients reporting adverse experiences under each system and, where available, the numbers and percentages for each sign/symptom for each body system. This was for both the 228 patients in the NDA submission and those under "Completed Study."

There are no remarkable differences between the latter two groups, i.e. "NDA submission" and "completed study". The highest incidences reported were in order of frequency:

- |                |                             |
|----------------|-----------------------------|
| 1. Cough       | 4. Exacerbation of Symptoms |
| 2. Nausea      | 5. Dizziness                |
| 3. Nervousness | 6. G.I. Distress            |

NOV 22 1985

Table 2 - Overall Incidence of Adverse Experiences Among Patients Treated with Alupont for 20 Weeks.

|                        |                       |
|------------------------|-----------------------|
| Number of patients (%) | NDA Submission - 273  |
|                        | Completed Study - 249 |

Incidence in order of frequency was:

1. Nervousness
2. Exacerbation of Symptoms
3. Anxiety Distress
4. Tremor

There was no statistically significant difference between the Alupont and Alupont treatment groups regarding incidence nor severity of adverse experience reporting, except for more cases of insomnia reported in the Alupont-treated patients.

4. "A safety updated section comprising a summary of foreign data from clinical trials and spontaneous post-marketing reports as well as a survey of the literature available since the NDA submission." The review of unpublished and published reports of adverse reactions associated with the use of Atrovent MDI from foreign data sources is consistent with the adverse reactions seen in clinical trials and the Atrovent compassionate use program in the United States. The analysis of these data shows Atrovent MDI is safe.

#### 5. Foreign Marketing Status

There is a list of 25 countries where Atrovent Inhaler is currently marketed as well as the dates introduced in each, 1974-1984. The amount distributed for this period of time represents approximately 3 million single doses.

Atrovent has also been approved in France and Italy where marketing has not begun. There are no pending registrations, nor have any registrations been refused.

6. Patient information and exclusivity information is given in this section. Most of the information presented in this section is in the submitted NDA. Exclusivity - date first ANDA could be approved and length of exclusivity period is given as five (5) years from date of NDA approval.

Applicable patent numbers and expiration date of each are given as

3,681,500 - August 1, 1989

4,385,048 - May 24, 2000

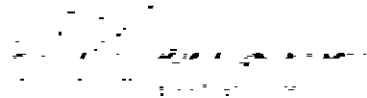
CONCLUSION: Sections 2 through 4 above are in compliance with the agreements reached with the meeting with the representatives of the firm held on June 19, 1985.

Page 12

1. The first condition is that the system must be in a state of equilibrium.

2. The second condition is that the system must be in a state of equilibrium.

3. The third condition is that the system must be in a state of equilibrium.



4. The fourth condition is that the system must be in a state of equilibrium.

5. The fifth condition is that the system must be in a state of equilibrium.

6. The sixth condition is that the system must be in a state of equilibrium.

7. The seventh condition is that the system must be in a state of equilibrium.

8. The eighth condition is that the system must be in a state of equilibrium.

9. The ninth condition is that the system must be in a state of equilibrium.

10. The tenth condition is that the system must be in a state of equilibrium.

11. The eleventh condition is that the system must be in a state of equilibrium.

5

Appendix 1

An explanation of the in patients who discontinued for adverse experience from December 20, 1985 to present (cumulative total - 140 patients). The following was reported.

Patient \_\_\_\_\_ - Burning chest discomfort, worsening of dyspnea; post Atrovent FEV<sub>1</sub> improved but patient discontinued voluntarily.

Patient \_\_\_\_\_ - Blurring of vision, Atrovent stopped, ophthalmological evaluation - early cataracts.

Patient \_\_\_\_\_ - Patient's condition worsened due to myasthenia gravis - investigator noted possible retained secretions.

Patient \_\_\_\_\_ - Patient discontinued due to dry mouth.

Patient \_\_\_\_\_ - Cough aggravated on Atrovent.

Patient \_\_\_\_\_ - Patient developed a mild irritant reaction - discontinued.

Patient \_\_\_\_\_ - Patient experienced recurrent nausea on Atrovent, therefore discontinued.

Patient \_\_\_\_\_ - No significant improvement; irritation and coughing - discontinued.

Patient \_\_\_\_\_ - Chest burning on Atrovent - subsided after discontinuing treatment.

Patient \_\_\_\_\_ - Poor patient compliance.

Patient \_\_\_\_\_ - Patient experienced muscle spasm.

Patient \_\_\_\_\_ - Increased shortness of breath, coughing, chest tightness on Atrovent 3 days.

Patient \_\_\_\_\_ - Bronchospasm, no significant improvements.

Patient \_\_\_\_\_ - Developed cough, stopped Atrovent. Status pending awaiting evaluation of cough.

Appendix 2 is an explanation of fatalities in 38 patients who died on treatment or within two weeks after discontinuation of Atrovent. This covers the period of December 20, 1985 to present, (cumulative total 118). None could be shown to be caused by administration of Atrovent.

Discontinued patients:

This section is in tabular form listing the investigator (study number), total enrolled in NDA both as of April 5, 1984 and total number of patients enrolled as of 10/21/86 (as given in the original NDA). The total enrolled as of November 13, 1986 is given as 2627 patients, and the number in the NDA is given as 1801. The number of dropouts since the NDA of those on Atrovent is tabulated as 23 and reasons given are those hospitalized in some cases, protocol violation, lack of efficacy, lost to follow up, patient unable to be off previous medication, lost interest in study, administrative failure. These apply only to controlled studies - (107).

The number of adverse reactions for this same group, i.e. patients on Atrovent in controlled studies, (107) is given as 16 for adverse reactions in this group since NDA (page 80-101 section 6 of submission).

The number of adverse reactions in the controlled studies - (107) since NDA is given for Atrovent as 8.

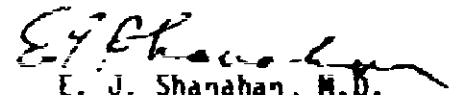
This concludes the review of the submitted data listed in the cover letter. In addition copies of the five preceding annual reports are submitted as requested by HFN 101. The sixth, reviewed report in this MOR is cumulative both qualitatively and quantitatively.

Conclusion

The sixth annual safety report on the emergency use program for Atrovent is complete with no new serious reactions nor significant increases in numbers of reactions relative to the population study numbers.

Recommendation

Continue ongoing reports on safety data for the Atrovent emergency program.

  
E. J. Shanahan, M.D.  
12/10/86

NDA 19 085  
HFN-160  
HFN-340  
HFN-160 Dr. Winkler  
R/D EJShanahan 12/10/86  
R,D Init. by JWWinkler 12/10/86, PGWalters 12/11/86  
FI OLA 2505N 0156N 12/15/86  
Doc. Room 160

CHEM

REV



DIVISION OF SURGICAL DENTAL DRUG PRODUCTS  
FD-302 (Rev. 11-17-64)

1. NDA 19-085

Date Completed: 10/23/65

APPLICANT: Bausch & Lomb, Inc.  
ADDRESS: 70 East Wacker, P.O. Box 519  
Ridgfield, CT 06272

2. PRODUCT NAME (s)

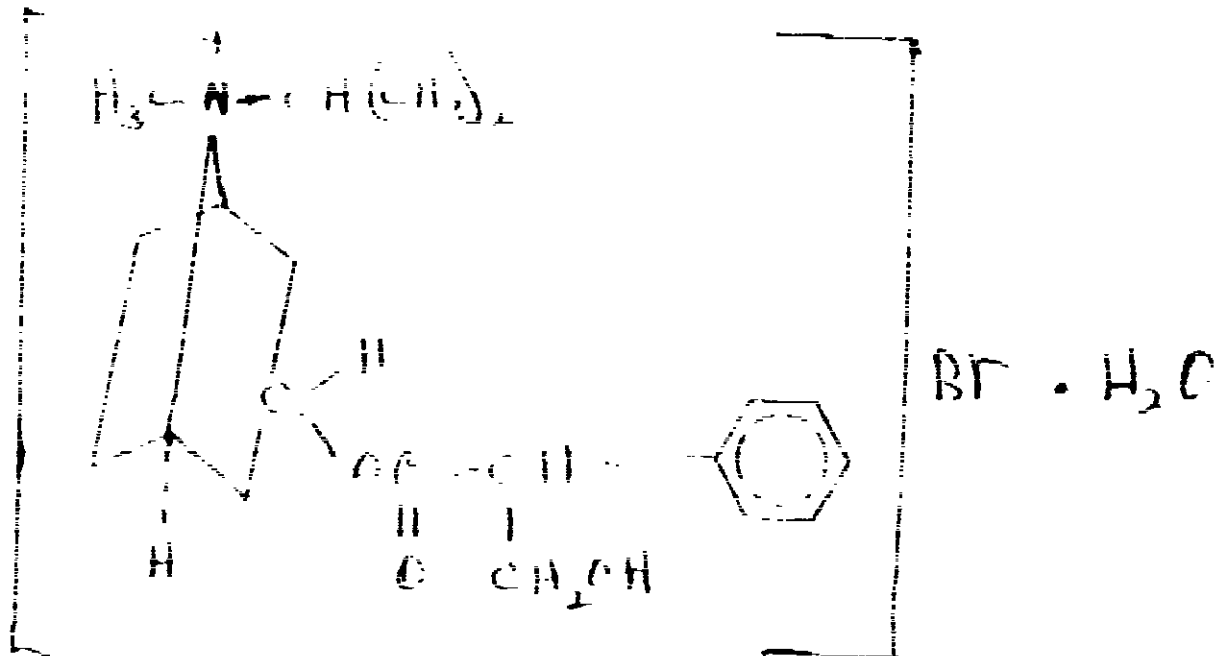
Proprietary: Atrovent Inhaler  
Non proprietary: Ipratropium bromide  
Code Name and/or Number: BBL 1000

3. DRUG FORM AND ROUTE OF ADMINISTRATION: Inhaler

4. PHARMACOLOGICAL CLASSIFICATION AND/OR PRINCIPAL INDICATIONS:

Anticholinergic bronchodilator used to treat bronchospasm.

5. Chemical Name and/or Chemical Name



OCT 26 1965

8. 3. Initial Submission: July 29, 1965

4. **AMENDMENT:** October 11, 1965; Rec'd by Chemist on October 21, 1965.

5. **RELATED DOCUMENTS:**

Telecon between G. H. Riggles and Dr. J. E. Hammarlin (HFN-102) dated September 24, 1965.

6. **REMARKS:**

1. The labeling deficiencies have been resolved. The fluorochloroacrylamides will be identified by their specific names where space permits (PI, container carton)
2. The term "Inhaler" has been permitted in its name. The term "Inhalation Aerosol" appears in the third paragraph of the description section.
3. The storage statement has been revised from "Store at room temperature. Avoid excessive humidity," to "Do not store above 30°C (85°F). Avoid excessive humidity."
4. The consultation reviews from microbiology, pharmacology, and biometrics are still out.

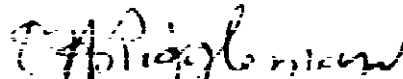
E.

9. CONCLUSIONS AND RECOMMENDATIONS:

From a chemistry standpoint, the NDA can be approved.

The nonclinical studies from toxicology, pharmacology, and microbiology should concur before approval is granted.

Toxicology only has been reviewed. MV is in progress. LIR's are satisfactory, an update has been requested by HFN-322.

  
G. H. Riggleman

NDA 19-085  
HFN-150  
DocRm. 150  
R.D. GRiggleman 10/24/85  
R.D. Init by:CPHoiberg 10/24/85  
shj X214/K 10/25/85

DRAFT OF CHEMIST'S PART, LETTER TO APPLICANT

1. Final chemistry review is awaiting consultation reviews from pharmacology, microbiology, and biometrics.

PHARM

REV

NDA 19-038

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY OF \_\_\_\_\_

Original submission dated 7/29/78

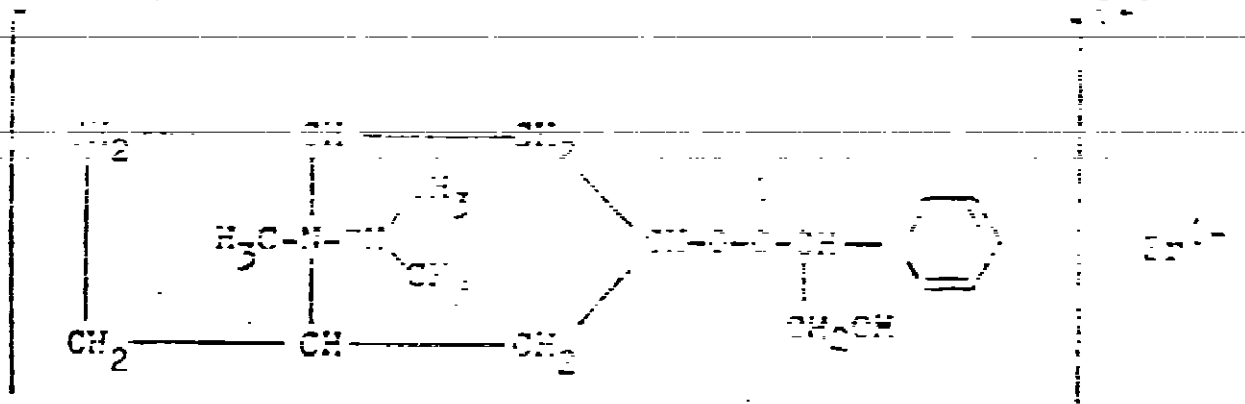
APPLICANT: Boehringer Ingelheim Ltd.  
20 East 71st St.  
P.O. Box 308  
Ridgefield, CT 06377  
(203) 438-0011

NAME OF DRUG: Descriptive: 100 mg/ml solution for injection  
or 100 mg/ml oral solution

USAN: Descriptive: \_\_\_\_\_

Chemical: 1-(2-(4-hydroxy-1-(2-(2-phenyl-1-ethoxyethoxy)ethyl)ethyl)ethyl)ethoxyethane, hydrochloride, monohydrate

1-(2-(4-hydroxy-1-(2-(2-phenyl-1-ethoxyethoxy)ethyl)ethyl)ethyl)ethoxyethane, hydrochloride, monohydrate



Empirical Formula: C<sub>29</sub>H<sub>50</sub>O<sub>5</sub> · H<sub>2</sub>O · HCl

Molecular weight: 430.4

[Faint, mostly illegible text, possibly a list or table of contents]

[Faint text, possibly a section header or title]

[Faint text, possibly a paragraph of a report or letter]

DOSAGE FORM: Suspension for oral use, 100 mg/ml, 100 ml

DATE: 11/11/77

INDICATIONS: For the treatment of the acute and chronic phases of the disease.

COMPARISON: Appropriate comparison.

PHARMACOLOGY: See below.

PHARMACOKINETICS: See below.

PHARMACODYNAMICS: See below.

The following information is based on the results of clinical trials conducted in patients with the disease. The drug is well absorbed and reaches its peak concentration in the blood within 1-2 hours. The half-life is approximately 4-6 hours. The drug is excreted in the urine and feces. The drug is safe and effective in the treatment of the disease. The drug is well tolerated and does not cause any significant side effects. The drug is effective in the treatment of the acute and chronic phases of the disease. The drug is safe and effective in the treatment of the disease. The drug is well tolerated and does not cause any significant side effects. The drug is effective in the treatment of the acute and chronic phases of the disease.

Review of individual data:

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PHARMACOLOGICAL/BIOCHEMISTRY

Antispasmodic Effects

A. Gastrointestinal Tract

(1) Gastric Smooth Muscle Contractility by 100 mg

When administered IV, 100 mg introduced orally, 100 mg administered orally and 100 mg administered intraperitoneally were effective in producing a dose-dependent inhibition of gastric smooth muscle contractility by approximately 50% in the rat.

Administered in 100 mg doses showed 100% as effective as 100 mg administered orally. No significant inhibition was noted at 100 mg. Heart rate was not significantly altered. The only effect produced by 100 mg was only slightly, but not significantly, at 100 mg.

When administered in 100 mg doses, 100 mg was effectively inhibited and 100 mg administered intraperitoneally, 100 mg heart rate or ECG consistently.

In the rat, 100 mg was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat. 100 mg was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat. 100 mg was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat.

(2) Intra-Peritoneal

Volume of 100 mg administered intraperitoneally was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat. 100 mg administered intraperitoneally was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat.

(3) Intra-Venous

When administered IV, 100 mg was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat. 100 mg administered IV was 100% effective in producing a dose-dependent inhibition of gastric smooth muscle contractility in the rat.

(d) Bladder:

When 100 mg was administered IV to anesthetized rats, a dose-dependent effect was as effective as 100 mg administered orally. A dose-dependent response was a maximum in 7 minutes. A dose-dependent response in heart rate was noted. Respiratory rate was not altered. A slight transient decrease in BP occurred. When administered intraperitoneally, 100 mg was 100% effective as 100 mg administered orally and onset of effect was longer.

In the case of the rabbit, the effect of SCH was observed as a decrease in heart rate and a slight increase in blood pressure.

In the case of the dog, the effect of SCH was observed as a decrease in heart rate and a slight increase in blood pressure.

SCH was also twice as effective as atropine in the rabbit. At a dose of 0.3 mg/kg and atropine 0.1 mg/kg, SCH was administered to dogs in a similar manner. Effects occurred at a similar rate after IV administration.

SCH was twice as effective as atropine in the rabbit. At a dose of 0.3 mg/kg and atropine 0.1 mg/kg, SCH was administered to dogs in a similar manner. Effects occurred at a similar rate after IV administration.

In the case of the rabbit, the effect of SCH was observed as a decrease in heart rate and a slight increase in blood pressure.

In the case of the dog, the effect of SCH was observed as a decrease in heart rate and a slight increase in blood pressure.

when given SC to infant dog SCH was as effective as atropine but only 1/5 as effective when given PO. In dogs, 0.03-0.1 mg/kg IV had no effect on the circulatory reflex. 0.3 mg/kg produced a slight and respiratory increase in blood pressure reflex. It was noted that it is indicated for a few minutes.

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2. The second part of the report deals with the results of the work during the year. It is divided into two main sections, the first of which deals with the results of the work during the year and the second with the results of the work during the year.

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9. The ninth part of the report deals with the results of the work during the year. It is divided into two main sections, the first of which deals with the results of the work during the year and the second with the results of the work during the year.

10. The tenth part of the report deals with the results of the work during the year. It is divided into two main sections, the first of which deals with the results of the work during the year and the second with the results of the work during the year.

Results:

Following oral administration of the drug... 3 hours... 22.8% of the drug... and 72% of the drug... drug is given... and 42% of the drug... when given orally...

In man, the metabolism of the drug... The maximum concentration of the drug... 3-4 hours. The... 27%... 37% is excreted... orally, the drug is excreted...

C. Pharmacokinetics in the Dog

man (age 22-34)

# dose level 40-100

dose 169-614 mg. average 400 mg.

administration and duration

Inhalation, 2 daily administrations  
Sch 1000 given as a radioactive...

Results:

The... 10.1% of the drug... 3.1% to 10.1% of the drug...

The maximum plasma levels observed for the drug... per gram of tissue.

D. The Excretion and whole-blood concentrations of Radioactive Sch 1000 after 1.15 administration to monkeys during a chronic inhalation toxicity study of the Non-Radioactive Drug.

Rhesus Monkeys (*M. mulatta*) Sch 1000

#Animals/Dose Levels: 1F and 1M

Dose: Control and 1 mg/kg

Administration and Duration:

Animals used were on a chronic inhalation study. Animals used were controls and the 1600 mcg/da groups. These animals were given radioacti Sch 1000 i.v., a one shot dose.

Results:

An i.v. dose of Sch 1000 at 1 mg/kg given to the monkeys before the initiation of the chronic inhalation study, and at 3 and 6 months during the inhalation study showed no significant differences in the rates of excretion. A total of 66.7% of the dose was found in the urine of the monkeys in the control group and 62.9% in the 1600 mcg/da group at the 3 months evaluations. At the same evaluations, 25.6% of the dose was found in the fecal matter of the control monkeys and 24.9% in the feces of the 1600 mcg/da monkeys. Results for pre-exposure and at 6 months were similar. Similar results were obtained when the whole blood of these monkeys was evaluated for radioactivity. The radioactivity of the control and experimental monkeys were comparable at pre-exposure, 3 and 6 months. Spec suggests that these data show there is no accumulation of drug or inhibition of drug metabolizing enzyme(s) during the administration of Sch 1000. Thin-layer chromatography analysis of the urine show that the drug is excreted unchanged by the monkeys.

E. Species Comparison of Biochemical Findings Sch 100 in Rat, Dog and Man  
(C.H. Boehringer Sohn, Germany) (3/14/77 review)Rat

Rats, given an oral dose of 100 mg/kg Sch 1000 showed a maximum concentration in the blood within 2-4 hours, drug remained in the blood at high levels for several hours (approximately 7). Rats, given a i.v. dose of Sch 1000 (1mg/kg) had a rapid fall in blood drug concentration during the first 4.2 minutes (50% drop). During the next 3 hours, drug was lost at a slower rate. During the first 24 hours, 3.5% of drug was found in the urine and 76.9% was found in feces when Sch 1000 is given to the rat orally. When given by i.v., 57.1% was found in the urine and 39.5 was noted in the feces. A total of 5 components were found in the urine of rats given Sch 1000 orally. The largest amount of these components was unchanged drug.

Dog

Dogs, given Sch 1000 orally at a dose of 5 mg/kg showed a maximum drug concentration in the blood within 2-4 hours. Large concentrations were noted for up to 10 hours in the blood. 23.2% of oral Sch 1000 was found in the urine of the dog within 24 hours and 72.5% was found in the feces. After i.v. administration 39.4% of the dose was found in the urine and 42.6% in the feces.

Man

An oral dose of 30 mg was administered to 10 subjects. The maximum concentration was noted in plasma 1 hour after the dose when this drug was given as was by the following: The study were 500 mg. The mean excretion was 7.2% of the dose in the urine, 85% in feces. Excretion in feces was 7.7% of the dose and 6.3% in feces. By the following 7.2% was excreted in urine.

The metabolic stability of 1 and 7 hour urine samples of a preliminary study after administration of 30 mg of the drug were 100% and 60.4% respectively. The following table shows the percentage of unchanged drug in the urine and feces at 1, 2, 4, 6, and 7 hours after the dose. The following table shows the percentage of unchanged drug in the urine and feces at 1, 2, 4, 6, and 7 hours after the dose.

The only metabolite found in the urine and feces was the hydrolysis product and the proportion of hydrolysis product was increased from 4.0% in 1 hour urine and 40.6% in 7 hour urine, whereas unchanged drug was 27% in 1 hour urine, and the level remained relatively constant between 10.4% and 15% of total radioactivity from 2 hour to 7 hour post dose. This may explain its long duration of effect.

II. ACUTE TOXICITY STUDIES:

1. LD50 by Various Routes: See the following table:



**A. IN-VITRO PROTEIN BINDING OF SCH 1000\* (from 11/9/83 review)**

Lab. Performing the Study: I.D. Boehringer Sohn

Methods: equilibrium dialysis with and without plasma  
Batch No. 1000 (Sch 1000 1711)

Plasma 1 was a mixture of old plasma samples from 10 persons, and was older than 1 week.

Plasma 2 was from 1000 male subjects and used separately.

Red plasma was from several male rats of Chab. 7.1.1983  
see pages 1-3 for details.

**RESULTS:**

The Sch 1000 binding to human plasma was reported as in Table 1: 18% (14-20%), to human albumin as 6% (4-10%), and to rat plasma as 15% (9-22%), and there were no significant differences between old and fresh plasma samples.

**II. ADME AND PHARMACOKINETICS (from the original review)**

**A. Pharmacokinetics**

The pharmacokinetics of Sch 1000 were studied in man and rat. In man, the drug was administered orally as a single dose of 100 mg. The plasma concentration of Sch 1000 was determined at various times up to 24 hours. The elimination half-life of Sch 1000 in man was 1.5 hours. In rat, the drug was administered orally as a single dose of 100 mg. The plasma concentration of Sch 1000 was determined at various times up to 24 hours. The elimination half-life of Sch 1000 in rat was 1.5 hours. The pharmacokinetics of Sch 1000 were similar in man and rat.

CONFIDENTIAL

On 7/14/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 7/20/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 7/26/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 8/1/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 8/7/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 8/13/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

On 8/19/54, Subject was administered 300 mg of DDT via the oral route. The subject was observed for 72 hours post-administration. No significant changes in body weight or clinical observations were noted during this period.

The absorption of 50µ in rat, dog, and man, based on the data obtained following IV and PO use, was 1.4, 1.6, and 1.0, respectively. It would appear that absorption in rat and dog is considerably higher than in man. Only small percent of 50µ was found in total bilirubin administered to man. Apparently, drug administered in total bilirubin administered to the rat and dog is absorbed and excreted. It is assumed that this is true for the rat and dog.

Based on the quantitative distribution of 50µ in urine following IV administration in the rat and dog (see Table 1) and the 24-hour excretion in the dog (see Table 2) and (see Table 3) however, since absolute work-up structural formulae are to be used, and if they were identical, sufficient information was not available to make a definite statement about the rat metabolism case.

**B** Pharmacokinetics of 14C-50µ - See 200 Mastered Record - 2, 200 - 11/28/78

Rats (Radioactive 50µ - 1000)

# animal/dose level 2/2

dose 1 mg/kg

administration and duration

I.V. and oral 15 levels were administered.

Urine levels could not be obtained in the rat. The amount of 14C was fairly small in the urine. The amount of 14C in the urine was approximately 1% of the total amount of 14C administered. The amount of 14C in the urine was given in the following table:

Rats (Radioactive 50µ - 100)

# animals/dose 2

dose level 5 mg/kg

administration and duration

I.V. and oral 15 levels were administered.

C. Subacute Toxicity

Species: *Rattus norvegicus*

Sex: Both

Age: 6 weeks

Group: 10/Group

Duration: 14 days

Dose: 100 mg/kg

Route: Oral

Observations: None

Mortality: 0/10

Signs: None

Pathology: None

Conclusions: None

References: None

Comments: None

Summary: None

References: None

Comments: None

Summary: None

References: None

Comments: None

Summary: None

References: None

Comments: None

Summary: None

References: None

Comments: None

Subacute Toxicity (Abstracts for Industrial and Biological Testing)

Dose (mg/kg): 10, 100, 1000

Duration and Route: Thirty days, oral gavage

Dose: 10, 100, 1000 mg/kg body weight of rats

Mortality: Three of dose 1000 rats were moribund.

Observations: At 1000 mg/kg, rats were moribund and died.

Pathology: At 1000 mg/kg, rats had severe liver damage.

Conclusions: The LD50 of the compound is between 100 and 1000 mg/kg.

References: None

Comments: None

Summary: None

References: None

Comments: None

Summary: None

References: None

Body weight was maintained...  
Organ weight deviations were difficult to...  
loss that occurred. Relative liver and kidney weights  
from overdose were increased. Spleen, heart, and  
spleen, heart, and kidney weights were also increased.  
Tissue in all animals was examined microscopically,  
searched noteworthy:

Adrenals  
Vacuolization  
Information of adrenal cortex

Other Comments:

Generally, behavior was normal. Extreme mydriasis was noted in addition to dry nasal and oral mucosa and irritated and inflamed conjunctiva.

MS 14-001  
Page 11

Heart rate: 140  
No relief of fever  
No blood  
No diarrhea

Weight: 100g  
1/3 of dose

Temp: 40.0

Appetite: 1/2

Stool: 1/2

Urine: 1/2

1/2

1/2

(2) Total

Mortality: None

Body weight (day 1) 45 records for 100g 3 accompanied by 100g 55  
food intake.

RESULTS

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Microbiology

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Monkey (Rhesus) Six - Seven

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Body weight and food consumption were normal. (10 days before death after), heart rate, respiratory rate, body temperatures and neurological and ophthalmological examinations were within normal limits. Except for decreased Hgb, RBC and WBC values, in monkey that died, other treated values were unremarkable. Complete clinical chemistries and qualitative and microscopic urinalysis were also not unusual. Organ weights were within normal range. Histological alterations were not related to drug. Two special studies were performed. One was surface tension which was found to be comparable in all monkeys. Second was determination of urinary activity which was somewhat increased in treated animals as compared to controls but difference was short of significance.

Table 1. Summary of Acute Toxicity Studies with Approved New Active Ingredients

| Host Animal             | Administration  | Species  | Sex   | Body Weight | No. of Animals per Group | Drug Administration  | Day of Death | Ref. Range |
|-------------------------|-----------------|----------|-------|-------------|--------------------------|----------------------|--------------|------------|
| Mice                    | Oral            | C57BL/6J | F     | 20.0g       | 10                       | Acute toxicity study | 1000         | 1000-1500  |
|                         |                 |          | M     | 20.0g       | 10                       | Acute toxicity study | 1000         | 1000-1500  |
|                         | Intraperitoneal | C57BL/6J | F     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
|                         |                 |          | M     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
|                         | Intravenous     | C57BL/6J | F     | 20.0g       | 10                       | Acute toxicity study | 1000         | 1000-1500  |
|                         |                 |          | M     | 20.0g       | 10                       | Acute toxicity study | 1000         | 1000-1500  |
|                         | Subcutaneous    | C57BL/6J | F     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
|                         |                 |          | M     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
|                         | Intramuscular   | C57BL/6J | F     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
|                         |                 |          | M     | 20.0g       | 5                        | Acute toxicity study | 1000         | 1000-1500  |
| Intracerebral           | C57BL/6J        | F        | 20.0g | 5           | Acute toxicity study     | 1000                 | 1000-1500    |            |
|                         |                 | M        | 20.0g | 5           | Acute toxicity study     | 1000                 | 1000-1500    |            |
| Intracerebroventricular | C57BL/6J        | F        | 20.0g | 5           | Acute toxicity study     | 1000                 | 1000-1500    |            |
|                         |                 | M        | 20.0g | 5           | Acute toxicity study     | 1000                 | 1000-1500    |            |

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| no | week | 1 | 2 | 3 | 4 | total |
|----|------|---|---|---|---|-------|
| M  | 13   | 1 | 9 | 2 | 0 | 12    |
| F  | 14   | 0 | 4 | 4 | 1 | 9     |

Table 1 - Circuit Judges

| Source of Administrative Information | Specialist | Structure | Group | Family Weight | No. of Available Per. Group | Group Administration | Group Title | 95% Fiduciary Limit | References |
|--------------------------------------|------------|-----------|-------|---------------|-----------------------------|----------------------|-------------|---------------------|------------|
| Miles                                | Ch. 1      | Ch. 1     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 2      | Ch. 2     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 3      | Ch. 3     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 4      | Ch. 4     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
| Miles                                | Ch. 1      | Ch. 1     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 2      | Ch. 2     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 3      | Ch. 3     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 4      | Ch. 4     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
| Miles                                | Ch. 1      | Ch. 1     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 2      | Ch. 2     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 3      | Ch. 3     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 4      | Ch. 4     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
| Miles                                | Ch. 1      | Ch. 1     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 2      | Ch. 2     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 3      | Ch. 3     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 4      | Ch. 4     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
| Miles                                | Ch. 1      | Ch. 1     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 2      | Ch. 2     | 1     | 11-24 B       | 10                          | Technology           | 11-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 3      | Ch. 3     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |
|                                      | Ch. 4      | Ch. 4     | 2     | 20-24 B       | 10                          | Technology           | 20-24 B     | (11,112,100)        | 10         |



Table 1. (Continued)

| Route of Administration | Species          | Strain               | Sex | Body Weight  | No. of Animals Per Group | Drug Administration                     | LC <sub>50</sub> LD <sub>50</sub> 95% Fiducial Limits | References |
|-------------------------|------------------|----------------------|-----|--------------|--------------------------|-----------------------------------------|-------------------------------------------------------|------------|
|                         | Rat              | CD-1                 | M   | 170-197      | 6                        | Suspension in saline                    | > 11.5 ug/L for 4 hrs                                 | 26         |
|                         | Rat              | SM-49<br>Bibby ranch | M   | 180-200 H    | 5                        | Aerosol with 0.9% and 2.1% Bay solution | > 1200 ug (20 ug/stroke for 100 strokes)              | 27         |
|                         | Rat              | Chick<br>TUM         | M   | (M)=<br>(F)= | 5                        | Aerosol with 0.9% and 2.1% Bay solution | > 1200 ug (20 ug/stroke for 100 strokes)              | 27         |
|                         | Golden<br>Pigeon | Unselected           | M   | 110-140 g    | 6                        | Aerosol with 0.9% and 2.1% Bay solution | > 1200 ug (20 ug/stroke for 100 strokes)              | 27         |
|                         | Poultry          | Unselected           | F   | 120-140 g    | 6                        | Aerosol with 0.9% and 2.1% Bay solution | > 1200 ug (20 ug/stroke for 100 strokes)              | 27         |
|                         | Monkey           | Unselected           | F   | 100-120 kg   | 1                        | Aerosol with 0.9% and 2.1% Bay solution | > 1200 ug (20 ug/stroke for 100 strokes)              | 27         |

Route of Administration  
Administration

LC<sub>50</sub>  
LD<sub>50</sub>  
95% Fiducial  
Limits

References

See pages 7-9, Vol. I, 22 of NDA for the references.

OBSERVATIONS:

1. No significant differences between 74 hour or 14 day LD<sub>50</sub>.
2. Toxic symptoms in rodents were apathy, reduced activity, varying degrees of paralysis of skeletal muscles, slight to moderate tonic convulsions and chronic type of spasms just prior to death, labored breathing, and respiratory failure preceded cardiac arrest.  
Dogs showed a pronounced increase in heart rate and a fall in rectal temperature which lasted up to 6 hours after oral and iv doses with long lasting mydriasis and drying of mucosa (anticholinergic action), sedation, and ataxia, and the death was due to cardiac or circulatory failure.
3. Toxicity was 6 times greater by intraduodenal administration than after sc administration, which that was similar to atropine (Box, 22).

1000

1000

1000

1000

Organ weight:

significant increase in organ weight of heart, lung, spleen, kidney, ovary, thymus, and prostate. The liver of mice was grossly enlarged and heart, thymus and prostate in the gross image of mice. The spleen of mice was grossly enlarged. The organ weight of the female mice was significantly increased.

100

- Group 1 - 100 mg/kg
- M 11 Agnesia testis or epididymis
- Group 2 - 100 mg/kg
- M 12 Hypertrophy of testes and accessory sex glands
- P 13 Focal pneumonia
- P 14 Obstipation
- Group 3 - 100 mg/kg
- M 15 Obstipation
- M 17 Enteritis catarrhalis
- M 19 Enteritis catarrhalis et peritonitis fibrinosa
- M 20 Dilatation of intestine
- M 21 Obstipation, cachexia
- M 22 Enteritis catarrhalis
- M 23 Enteritis catarrhalis
- M 24 Enteritis catarrhalis
- M 25 Cachexia
- M 26 Obstipation
- M 27 Cachexia
- P 28 Cachexia
- P 29 Cachexia
- P 30 Enteritis catarrhalis
- P 31 Enteritis catarrhalis
- P 32 Cachexia
- P 33 Cachexia
- P 34 Cachexia
- P 35 Round heart, enteritis catarrhalis, dilatation of intestine

Autopathology:

The following reports are submitted as drug related changes in the high dose group. In addition to "Drug" or "findings" on pages 15-16.

**INHALATION STUDIES:**

**REFERENCES:** The following reviews are from the previous reviews in our file with reference number if possible.

a) Air-Phase Inhalation of 1,1,1,2,2,2-hexachloroethane (HFC-113) in rats.  
Rat: Sprague-Dawley, Male, 100-150 g (1984/75 review)  
# animals/dose level: 4M + 4F (10, 30, 100 mg/day)  
Dose: 10, 30, 100 mg/day (10, 30, 100 mg/day)  
Administration: Air, 6 hours/day  
Inhalation 4 times daily for 1 hour each / day study

**RESULTS**

Clinical Signs: No abnormalities were seen during exposure or post exposure.

Food Consumption: Food consumption was similar in both groups.

Bodyweight: Body weights were similar for both groups.

Blood Analysis: A blood analysis was done 15 minutes after exposure. The maximum blood level was 78 mg/ml after 30 minutes.

Terminal studies: At autopsy, 75% of the rats had no gross pathology.

b) Watered Aerosol with Sol 1000 to the inhalation toxicity of Sol 1000.

Rat: Sprague-Dawley, Male, 100-150 g (1984/75 review)

# animals/dose level: 2 M + 2 F

dose: 20-300 mg/day

Administration and duration: Inhalation administration at 5, 20, 50, 100, and 300 strokes, each stroke delivered 20 mg of Sol 1000. 14-day observation period.

**Results:**

All animals survived this test. No incompatibility was observed. No morphological examinations were carried out.

c) Effect of Inhaled Aerosol on the Lung  
 Rhesus Monkeys, *M. mulatta*  
 4 animals/dose levels: 1000, 2000, 4000, 8000  
 dose levels: 1000 mcg/day  
 administration: 5 days/week  
 60-1000 mcg/day for 10 days, 2000-8000 mcg/day for 10 days  
 Animals given 1000 mcg/day were sacrificed after 10 days  
 Results:  
 Animals survived, and there were no significant changes in body weight, hematology, or clinical chemistry. Lungs were examined grossly and histologically. No significant changes were observed between the treated and control groups.

d) Effect of Inhaled Aerosol on the Lung  
 Rhesus Monkeys, *M. mulatta* Jan 1970  
 4 animals/dose levels: 1000, 2000, 4000, 8000  
 dose levels: 1000 mcg/day  
 administration: 5 days/week  
 Inhalation - Nasal, oral, + olivary, etc. at 1000, 2000, 4000, 8000  
 mcg/day

Results:  
Survival  
 No adverse effects to the respiratory tract were observed.  
Body Weight Body weights of these animals were maintained.  
Food Consumption Food consumption for all animals was maintained.  
Laboratory Investigations

Hematology Hematology tests carried out one and two months after exposure showed all values within normal limits.

Biochemistry Inorganic phosphorus in the treated animals was high, but levels of  $Ca^{45}$  were low; however, 1 monkey in the control group also had a high level of  $Ca^{45}$ .

Fluorescein Na aerosol had same "particle size distribution and aerodynamic properties" similar to those of ZnO aerosol.

1. In the  $400 \text{ mg/kg}$  dose the animals usually have shown marked changes of liver ( $F_2$  and  $F_3$ ), namely at a big droplet like change of lobules of the liver (M 22, 16, 18, F 22, 24, 25, 26, 27, 28) at a severe big droplet like change of fatty change and in the liver lobules. In the  $200 \text{ mg/kg}$  dose (M 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100) at a severe big droplet like change of fatty change and in the liver lobules.

Mesenchymal reactions are observed in these cases.

2. On second place a diffuse fatty change ( $F_2$ ) of the myocardium was seen more often (M 27, 28, 31, 32, 33, 37, 38, F 26, 33, 34, 39, 48). The fat is dispersed within the muscle fibers in extremely fine vacuoles and degenerative damages of nuclei occur.

3. In the kidneys fatty change could be observed in the cytoplasm of some proximal convoluted tubules in several animals either sex at  $400 \text{ mg/kg}$  dosage only. The fat vacuoles surrounding the nuclei of the epithelial cells ( $F_2$ ).

4. Post mortem changes may prohibit the microscopical proof of slight inflammatory lesions of intestine, as supposed by the diarrhea seen at autopsy in animals of high dosage. 11

**I: FOUR WEEK ORAL TOXICITY STUDY IN RATS: [83-123]**

Lab Performing the Study: Same as above.

Strain: SD-JCL Sprague-Dawley

No. of Animals/Level: 15 M + 15 F except that the high dose group had an additional 10 M + 10 F recovery group with 6 weeks untreated period.  
M = 187 ± 17 g, F = 154 ± 10 g, both 6 weeks old.

Dosage Level: 0, 12, 120, or 1200 mg/kg/day  
Sch 1000 (Batch No. 1/74).

Duration and Route: . or 4 weeks by stomach tube in 1.0 ml/100 g in distilled water.

Mortality: Only 1 M died in the mid dose group, but 9 M + 4 F in the high dose group died during the study.

Clinical Signs: Mid dose group (120 mg/kg) showed a slight weakness. Rats in high dose group were very weak with ruffling up of coat, red incrustated eyes and nose, and ataxia, especially during the first week, and they disappeared during the first week of recovery period.

Growth: Body weight gain of treated groups was generally significantly ( $p < 0.01$  in M in two high dose groups and  $p < 0.05$  in F high dose group) depressed from the first week and also dose-related especially in M.

See pages 21 and 31.

Food Consumption: Both M and F of the treated groups had generally less food consumption than respective controls and significantly ( $p < 0.05$ ) less in the high dose group during the first week. But F of the high dose group had significantly higher consumption during weeks 2-4, due to decreased consumption in the control F, and M in the same group had lesser food than control M only during week 3. See page 32.

toxicity suggested that respiratory distress was not drug related. The total volume in the treated group was 1.17 g (0.02 volume) and a residual of 0.07 g.

Urinalysis

All urine samples were considered normal, except for a trace amount in the treated group. The urine of this monkey, 1079, was 0.01 mg/ml and the other two monkeys, 1078 and 1080, were drug related.

Electrocardiogram

Pre and post drug administration showed various changes with a normal rhythm.

Blood Levels of Free Drug

Relatively high levels of free drug were found in the group of the two animals after the first dosing on the third day. The half-life of the free drug was found to be about 3 minutes. No free drug was detected in any of the samples. Free drug levels were detected to be higher in the nasal catheter group than in the nasal catheter group, 28.3 mcg/ml to 4.85 mcg/ml.

Terminal Studies

No abnormalities were observed in these animals which were considered to be drug-related.

e) The Deposition of Inhaled Fluorescein - Part 2: Deposition in Rat Lungs

ADP

4 animals (2000, 2001, 2002, 2003)

ADP

Administration and Duration

Fluorescein administration for 30 and 45 minutes, group of 20 mg/ml and 15 mg/ml.

**RESULTS:** Fluorescent material was found in lumen of the trachea of the animals. However more material was deposited in the trachea in 45 minutes treated animals than in 30 minute treated ones. Results unexplainable. Minimal deposits of the material was found in the lung parenchyma of animal exposed for 40 minutes. Animals were evaluated using fluorescence microscope. The material was found to be "granular in size distribution and morphology" similar to those of sea salt aerosol.

Water Consumption:

Both M and F of the high dose group had significantly ( $p < 0.01$ ) higher water consumption during weeks 2 to 4 over control group or other 2 groups. See page 33.

COMMENTS: Page numbers for the results of each parameter were missing in the RESULTS section of this study. See pages 14-15.

Hematology:

Some values were significantly different from the control values, but they were reported as "in normal range". See pages 14 and 34-45.

Coagulation Time:

Prothrombin time showed no difference between groups.

Clinical Chemistry:

Although some values were significant, no consistent dose-related or time-related changes were seen. See pages 46-57.

Urinalysis:

No dose-related changes were reported. See pages 58-80.

Organ Weight:

It was stated that "parallel to the decrease of body weight of male and female rats at 1200 mg/kg dose all weights of the main parenchymatous organs, as heart, liver, kidneys and endocrine glands, decrease significantly too. To the contrary, the weight of the adrenals increases in both sexes."

The difference was generally at  $p < 0.01$  in M (see page 81) and  $p < 0.05$  in F (see page 82), and most of them showed a trend of dose-relationship.

RESULTS ACUTE TOXICITY STUDIES

A group of 18 mice of 19 and 21 days of age were exposed to 18 mg/kg of 2,4-D for 10 min. 1, 2, 4, and 6 hours with 18 mg/kg of 2,4-D. All the animals survived and no deaths were observed in the 6 days of observation.

CONCLUSION

The results showed that a 10 min. exposure to 18 mg/kg of 2,4-D was well tolerated by 18 mice of 19 and 21 days of age. The 10 min. exposure was well tolerated for 10 min. but after 2 hours of exposure the animals displayed severe respiratory distress and some died. All the animals survived.

An acute toxicity study of 2,4-D was conducted in mice after 4.5-5 mg/kg. The mice displayed severe respiratory distress and some died. The histopathology revealed the emphysema and hyperemia of the lungs in two dead ones.

COMMENTS ON ACUTE TOXICITY STUDIES.

1. IV LD<sub>50</sub>s in M and F mice were reported as 17 and 18 respectively in 7/23/93's protocol, in Table 1. The LD<sub>50</sub> values are 17.18 and 18.00 mg/kg.

2. There was no submission in 1993 for the acute toxicity study.

Dissection Data:

Group 0 (control)

M 4 necroses of liver  
 M 10 hydronephrosis  
 F 8 hydrometra

Group 1 (100 mg/kg)

N A.D.

Group 2 (100 mg/kg)

F 56 kidney infarct  
 F 59 hydronephrosis

Group 3 (1200 mg/kg)

M 50 cachexia, obstipatio, edema pulmonum  
 M 52 cachexia  
 M 53 edema pulmonum  
 M 54 meteorism  
 M 56 gastric dilatatio, obstipatio of colon,  
 edema pulmonum  
 M 59 obstipatio, pneumonia  
 M 60 cachexia, enteritis catarrhalis, pneumonia  
 M 69 cachexia, enteritis catarrhalis, pneumonia  
 F 53 enteritis catarrhalis, pneumonia  
 F 54 enteritis catarrhalis  
 F 68 cachexia, enteritis catarrhalis

Histopathology:

It was reported that "except the atrophy of male accessory sex glands following loss of body weight at high dosage, no dose-related changes occur."

4) Study of Acute Toxicity of an Acquired Immunodeficiency Virus  
with Spongiform Encephalopathy (S.E.) and A.S. of in 1981

Lab. performing the study: J. W. Sommerer, Bonn, Germany (E. Lind review)  
Date of the study: 6/7/81

Investigator: J. W. Sommerer  
Title: Study of Acute Toxicity of an Acquired Immunodeficiency Virus  
with Spongiform Encephalopathy (S.E.) and A.S. of in 1981

Design: Study of Acute Toxicity of an Acquired Immunodeficiency Virus  
with Spongiform Encephalopathy (S.E.) and A.S. of in 1981  
Animals: 10 mice were used. They were kept in a clean environment  
with a constant temperature of 22°C and a relative humidity of 50%.  
The mice were divided into two groups of 5 mice each. One group  
received 2 sessions with 100 µg of virus at the age of 3 weeks.  
The other group was subjected to a control examination. The mice  
were followed up for 14 days and necropsied. Another 10 mice  
served as control (without virus).

- Results:
1. The 50% of the animals generated in the younger age were reported as 100% of deaths.
  2. The amount of virus reported as 50% of the total amount.
  3. It was estimated that the amount of virus reported during 100% of the total amount.
  4. It was reported that the amount of virus reported during 100% of the total amount.
  5. Necropsy findings: Histological studies reported later by Dept. of Exp. Pathology and Microbiology.

COMMENT: It was only a summary type of report without any review.

**XII. SUBACUTE TOXICITY STUDIES:**

**A. Subacute Toxicity:**

Species: rat

Sex: male

Strain: Wistar

Mortality: 100%

Body weight (g) was decreased to 50% of the control value. Food consumption (g/kg body weight) was severely depressed in these animals. Water consumption (ml/kg body weight) was normal. Hematology (hemoglobin, hematocrit, erythrocytes, leukocytes) were normal. Blood urea nitrogen (BUN) and creatinine were normal. Terminal qualitative and quantitative urinalysis were normal. Organ weights were not remarkable. No gross pathology was observed.

Notes: 15% and 10% of all doses were maintained without treatment for 5 weeks after the study was completed. These animals were sacrificed.

For the detailed description of the study, see pages 18-19 and 22 to the end of the report.

4- FOUR-WEEK INHA: AEROSOL STUDY - 19 085, 10/1/74.

Lab. Performed: the study was done as above.

Strain: SD and albino rats.

no. of animals/group:

12 M + 12 F for all 4 groups except that the high dose group had an additional 6 M + 6 F for "recovery group"

M = 178 ± 17 g, F = 144 ± 11 g, both 6 weeks old.

Dosage Levels:

| Groups         | Dose Group               |                             |                      |
|----------------|--------------------------|-----------------------------|----------------------|
|                | number of aerosol puffs* | period of inhalation (min.) | number of rats       |
| D <sub>0</sub> | 25                       | 10                          | M 1-12<br>F 1-12     |
| D <sub>1</sub> | 10                       | 10                          | M 13-24<br>F 13-24   |
| D <sub>2</sub> | 50                       | 10                          | M 25-36<br>F 25-36   |
| D <sub>3</sub> | 300                      | 10                          | M 37-54**<br>F 37-54 |

\* One aerosol puff contains 0.02 mg Sch 1000

\*\* In the high dose group 6 animals of both sexes were observed untreated for 6 weeks following the end of substance administration (recovery group).

The content of Batch No. 31229 was follows:

Follows:

|                                                                                                                | stated content<br>in g/aerosol | total content<br>aerosol incl.<br>overfill |
|----------------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------------------|
| Sch 1000, micronized                                                                                           | 0.0060                         | 0.00640                                    |
| Saja-lecthine standardized                                                                                     | 0.0105                         | 0.01210                                    |
| Monofluorotrichloromethane                                                                                     | 0.4200                         | 0.48300                                    |
| Propellant as mixture:<br>Hexafluoroethane/<br>Difluorodichloromethane/Tetra-<br>fluorodichloroethane 33:54:23 | 20.3635                        | 23.64800                                   |

Control group received placebo (Batch No. 40224).

**Duration and Route:**

Inhalation for 4 weeks (9/23-10/25/74, and recovery group for additional untreated 6 weeks until 12/6/74. See pages 4 and 22 for drug administration procedure.

**Clinical Signs:**

High dose group had "unrest and slight ataxia" immediately after drug administration but "recovered within half a minute".

**Growth:**

Body weight gain of the high dose group (M + F) was significantly ( $p < 0.05$ ) less only during the first week, but the same group gained more than the respective control group in later weeks, without dose relationship. See pages 23-26.

**Food and Water Consumption:**

Food consumption of treated groups was significantly ( $p < 0.01$ ) higher than the control group except high dose F, but only during the first week, and it was attributed to the "comparatively low intake of the control groups." See page 27.

No differences between groups in water consumption were reported. See page 28.

**Hematology:**

It was reported that "hematological values throughout were in normal range." See pages 29-44.

Mortality: One mid and 1 hi dose

Body weight was decreased in a dose related manner; 23 in hi dose M and F. Food consumption was correspondingly decreased in early weeks of treatment only. Substantial recovery occurred in non-treatment period. Water intake was not consistently altered. Indicators of segmental edema were limited to inflammation and infection at site of injection. This parameter showed subsequent recovery after discontinuation of drug in hi dose animals for 5 additional weeks. Other hematological values were within normal limits as were usual clinical chemistries. Terminal qualitatively and microscopic urinalysis on hi dose animals were normal. Gross pathology not remarkable.

The following histological changes were observed in hi dose animals:

|                                                                              | Incidence |         |
|------------------------------------------------------------------------------|-----------|---------|
|                                                                              | Control   | Hi Dose |
| Inflammation and necrosis at injection site usually accompanied by hair loss | 0         | 3       |
| Depletion of birefringent** compounds in adrenals                            | 0         | 3       |
| Hemorrhagic necrosis of adrenals **                                          | 0         | 3       |

\* In hi dose rats carried 5 additional days

\*\* Said to be due to inflammatory response

Of the two animals that died, one had a necrotic area of stomach and intestine with bloody exudate; the other had a necrotic area of stomach.

Autopsies: Dissection of abdomen in the study hi dose.

NDA: 19-085 SPONSOR: BOEHRINGER INGELHEIM 2 OF 3

TRADE: ATROVENT GENERIC: IPRATROPIUM BROMIDE

COMMENTS: Some of the values fluctuated a lot; for example, reticulocytes of M of all 4 groups decreased to less than 25 at week 4 from 80-140 from week 0 (on page 32) although none of them were reported as significantly different.

Coagulation Time:

There were no significant differences between groups in prothrombin time.

Clinical Chemistry:

Slight but significant ( $p < 0.01$  or  $0.05$ ) decrease in total protein and cholesterol values in all three treated F were attributed to rather high control values at week 4, and there were no-dose related changes. See pages 15 and 45-49. Glucose value in F high dose group and  $Ca^{++}$  in M high dose group were slightly but significantly ( $p < 0.05$ ) higher than respective control values. See page 50. The high glucose was attributed to the "technique of blood sampling", and the high  $Ca^{++}$  value was reported as "of no biological relevance," due to the fact that some of week 0 values were also significant. See pages 14-15 and 45-60.

COMMENTS: 1. Some of control values fluctuated so much that the data submitted may not be reliable, as was the case in Hematology above.

2. Mistake on line 4 from the bottom of page 15: "increased" values of cholesterol should be changed to "decreased".

Urinalysis:

No significant dose-related changes were reported.

Organ Weight:

Absolute weight of pituitary gland of all treated M were significantly ( $p < 0.01$ ) smaller, than that of control M, but not dose-related, and the difference was attributed to rather high control value. See page 50.

**Necropsy:**

Urinary calculi <sup>were</sup> seen in an F(24) in low dose group and perisplentis fibrosa in a M (25) in mid dose group.

**Histopathology:**

No drug induced changes were reported. For the detailed report, see page 17-18 and 92-101.



**Toxicity Subacute (from 1/24/75 review)**

The Toxicity of Rats to Sch 1000 administered by inhalation over a period of 30 days

Rats ( Charles Rivers)

Sch 1000

# animals/dose level:

10F + 10 M

dose level: 21,600 mcg/ds, 14,400 mcg/ds and 7,200 mcg/ds, plus control and vehicle groups. administration and duration

Inhalation - 20 mcg spray every 5 seconds for 15 minutes. Animals were exposed 5 minutes, 5, 4, 2 times a day in the high, medium and low dose groups respectively.

30 day study

**Results:**

**Clinical Signs**

Animal behavior appeared normal after an adjustment period.

Mortalities

Nine animals (all groups) died during this study. Animals deaths were not drug-related.

Food Consumption

Food consumption of the animals was normal following an initial drop in consumed food during the first week.

Body Weight

Animals had an initial weight loss during the first week. Animals (majority) gained weight during the final 3 weeks of the study.

Ophthalmoscopy

Examinations carried out pre-exposure and at termination recorded no abnormalities that could be attributed to treatment with Sch 1000.

Laboratory Investigations

Hematology Values obtained pre-exposure and at termination revealed no significant changes due to Sch 1000.

Biochemistry Biochemical values obtained for these animals were within normal limits.

Urinalyses

All values were within normal limits at both the pre-exposure and at termination.

Macroscopic Pathology

No morphological changes were observed which were considered to be drug induced.

Subacute Toxicity Study in Male Dogs

Dogs, Beagles

Sch 1000

1 animal/dose level 4 M - 6 M in control group.

Dose levels: 1.5, 3.0 and 15.0 mg/kg

administration and duration: Oral administration, 7 days weekly for 13 weeks.

Results:

Clinical Signs

All animals survived the tests. Test animals exhibited dryness of mouth, mydriasis and inhibition of lacrimation. Vomiting and diarrhea was seen in several animals of every group.

Body Weight

Dogs in 15.0 mg/kg group showed a slight weight loss. All other dogs had comparable body weights.

Food Consumption

Food Consumption of the dogs in treatment groups was not significantly different from the control group.

Hematology

The erythrocyte sedimentation rate of 2 dogs in the 3.0 mg/kg groups were slightly accelerated during weeks 0 and 6. All other values for all dogs were within normal range.

Biochemistry

No significant differences were noted between the treated groups and the control group.

Urinalysis

Values for urine specimen were within normal limits.

Ophthalmoscopic

Examination of the eyes of these dogs showed that the animals in the 3.0 and 15.0 mg/kg groups had drug induced keratoconjunctivitis sicca. Daily rinsing with 3% boric acid with applications of aureomycin and Vitamin A ointment healed this condition in 5 of the 8 dogs in the abovementioned groups. Histological investigation showed that keratitis sicca is due to a dose-dependent inhibitory effect of Sch 1000 on lacrimation.

Autopsy Findings

Several dogs in the control group as well as the 3.0 and 15.0 mg/kg had slight hyponatremia, mainly in the right ventricle. The livers of all the dogs in this study had parasitic or bacterial granulomas. In this test, 1 dog in each of the control group and the 1.5 mg/kg group had edema in the lungs. This condition was also noted in 1 dog of the 3.0 mg/kg and 2 dogs in

the 15.0 mg/kg group. In several of these dogs the inflammation was bilateral. In 2 control dogs, 5 dogs of the 1.5 and 15.0 mg/kg groups, and 1 dog in the 15.0 mg/kg a partial atrophy of the glandular epithelium of the prostate was observed.

#### M. Subacute

##### • Inhalation Study in the Rat (Huntingdon Research Center, Huntingdon, England)

In this study, 5 samples of the upper and lower trachea, bronchus and lung parenchyma were taken from each of 3 males and 3 females (rat) for electronic microscopic examination. However, study can not be evaluated because dose levels, administration and duration are not included in the submission. Apparently, these particulars were submitted earlier; however, I can not locate them.

##### • Inhalation Study in Monkeys

##### Electron Microscopy Report (C.H. Boehringer Solm, Germany)

This is another study in which samples were taken from trachea, bronchus bronchiolus and lung parenchyma of the monkey for electron microscopic examination again, important details of the study are missing.

#### N. 6-WEEK INHALATION STUDY ON RATS:

No. of Animals/group: 15 M + 15 F (average wt: M=290 g, F=225 g)

Strain: Albino SPF rats of Chbb:THOM(SPF)

Age at the beginning: M=64 days, F=78 days, + / - days

| Dosage Level: | Group         | No. of whiffs/min | Actual dose (mg/ |
|---------------|---------------|-------------------|------------------|
|               | 0(Handling)   | -                 | -                |
|               | 1(propellant) | 36                | -                |
|               | 2             | 12                | 0.17             |
|               | 3             | 12                | 0.34             |
|               | 4             | 36                | 1.0              |

Duration in chamber: all 120 min.

Duration and Route: 6-weeks by Inhalation (5/10/77-6/27/77)

|                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Growth Effect       | There was no difference. 2 rats from each group except group 2 showed decrease in body weights with unknown reasons.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Food Consumption    | There was no difference.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Mortality:          | One of group 1 and 2 or group 2 died, but the death seemed to be not drug related. No deaths were seen in groups 3 and 4.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Hematology:         | There were no drug induced changes.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Coagulation Test.   | No test was performed as such.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Clinical Chemistry: | GPT of groups 2 and 4 females and GOT of all females were slightly increased, and glucose of females of three treated groups was decreased, but they were not dose-related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Urinalysis:         | No dose related change was seen.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Organ Weights:      | There was no dose related change.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Gross pathology:    | There were some changes such as pleural adhesion or small brownish-red miliary foci of lung in all groups except group 2, but those changes were not dose related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Histopathology:     | <p>The examination was performed by Prof. K. Paulini of the Pathological Institute, Clinics of the University of Mainz. There was no dose related change. Common findings were bronchiectases, foam-cell-rich interstitial pneumonia, slight and various eosinophilia of heart muscle fibers, dysatelectases, PAS-positive cytoplasm of interstitial tissue and alveoli, fatty infiltration of hepatocytes, siderosis of spleen reticulum cells, and calcium-iron incrustations of distal tubules of females.</p> <p>Glandular cysts of the gastric mucosa found in 5 rats of group 1 and dilatation of the bladder found in 6 rats of group 3 were distinct. Other findings were numerous, but they were found in 1-3 rats of each group. The single finding of retinal atrophy in group 4 may be worth mentioning.</p> |

13 WEEK INHALATION TOXICITY STUDY IN DOGS WITH 1000-BR PONDUR  
APPROX (11/9/88 review)

Lab. Performing the Study:

C.H. Boehringer Sohn

(Dr. Kollmer, 12/15/79)

Strain:

Beagle dogs

No. of Animals/Level:

3 M + 3 F

1M = 13.5 kg and 14 months, and  
F = 11.5 kg and 13 months in  
average).

Dosage Level

| Groups | Dose in $\mu\text{g kg}^{-1}$ |                      | No. of dogs |   | Animal No.         |
|--------|-------------------------------|----------------------|-------------|---|--------------------|
|        | nominal                       | actual <sup>a)</sup> | m           | f |                    |
| 0      | placebo                       |                      | 3           | 3 | 001-003<br>051-053 |
| 1      | 10                            | 7                    | 3           | 3 | 101-103<br>151-153 |
| 2      | 50                            | 36                   | 3           | 3 | 201-203<br>251-253 |
| 3      | 250                           | 179                  | 3           | 3 | 301-303<br>351-353 |

a) the nominal dose is the intended dose and the total dose administered; the actual dose was calculated by subtracting from the total amount administered the substance residue demonstrated by quantitative analysis in the inhalation apparatus.

"The experimental animals received Sch 1000-BR on inspiration in the form of a powder aerosol at the same time each day (between 8.00 and 15.00 h). Animals in groups 2 and 3 received Sch 1000-BR powder capsules containing 0.2 mg Sch 1000-BR. The fine adjustment of the dosage in accordance with body weight was carried out using capsules containing 0.04 mg Sch 1000-BR in animals from all the treated groups. Control animals received an aerosol with placebo capsules containing only glucose."

|                                |                                                                                                                                                                                                                                                                    |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Justification of the dose.     | See pages 10-11.                                                                                                                                                                                                                                                   |
| Dose Levels                    | 25000 ug/kg/day (100 mg/kg/day) appeared to be the most effective dose.<br>25000 ug/kg/day (100 mg/kg/day) and 75000 ug/kg/day (300 mg/kg/day) appeared to be the most effective doses.<br>75000 ug/kg/day (300 mg/kg/day) appeared to be the most effective dose. |
| Duration of Treatment          | 3 weeks. Started on 4/27/73 for 3 weeks on 5/19/73. Total duration of treatment was 6-12/73.                                                                                                                                                                       |
| Mortality                      | None                                                                                                                                                                                                                                                               |
| Growth                         | There were no significant differences between groups, although F showed a slight trend of depression.                                                                                                                                                              |
| Food Consumption               | No significant differences between groups were reported.                                                                                                                                                                                                           |
| Clinical Symptoms and Behavior | F and F exhibited dryness of the nose in group 2 (50 ug/kg/day) and dryness of the nose and oral mucosa in group 3 (250 ug/kg/day), and it lasted 1-2 hours in 50 ug/kg/day group and 4-5 hours in 250 ug/kg/day group.                                            |

hematology:

Incidence of diarrhea and  
vomiting were reported on pages  
200 and 201, but there was no  
dose or time relationship.

There were no significant  
differences between groups.

COMMENTS:

Values were presented for  
weeks 4, 5, 6, and 13.

Myelogram (bone marrow):

No dose or time related  
changes were reported for  
bone marrow investigations.  
(See page 111-114.)

Coagulation Times:

No significant differences  
between groups were noted for  
partial thromboplastin time,  
thromboplastin time, or  
thrombin time.

Clinical Chemistry:

Urea of 3 treated groups was  
statistically significantly  
higher than control group,  
but only at week 5, and  
without dose-relationship.  
(The extent of 3 treated  
groups was significantly higher  
than control group at week  
13, but without dose-relationship.)

Urinalysis:

There were no significant  
differences between groups.

Organ Weights:

There were no significant  
differences between groups in  
selected organ weights. (The  
high dose group had larger  
weight of left ovary, but not  
significant.)

COMMENTS:

It was reported that the  
weight of the lung was  
inadvertently omitted.

Summary

Conclusion

The following changes were observed in the lungs of the animals which were given the substance by inhalation: slight interstitial inflammatory changes, slight hyperplasia of the alveolar cells, slight hyperplasia of the bronchial epithelium, slight hyperplasia of the capillary endothelium, slight hyperplasia of the connective tissue cells, slight hyperplasia of the fibroblasts, slight hyperplasia of the macrophages, slight hyperplasia of the lymphocytes, slight hyperplasia of the plasma cells, slight hyperplasia of the eosinophils, slight hyperplasia of the neutrophils, slight hyperplasia of the monocytes, slight hyperplasia of the mast cells, slight hyperplasia of the histiocytes, slight hyperplasia of the dendritic cells, slight hyperplasia of the Langerhans cells, slight hyperplasia of the melanocytes, slight hyperplasia of the keratinocytes, slight hyperplasia of the melanocytes, slight hyperplasia of the keratinocytes, slight hyperplasia of the melanocytes, slight hyperplasia of the keratinocytes.

\*A slight increase in the number of eosinophils in the lungs of the animals which were given the substance by inhalation was observed. This increase may be due to the fact that the substance has a damaging effect. In my opinion all organ changes observed belong to the secondary pathological processes in the lung.

Slight inflammatory processes in the lung, especially those of an interstitial inflammatory nature, were found both in the control animals and in the animals of the test group. They are also known to us from control animals of other experimental groups. Some of the inflammatory changes are, without doubt, of parasitic origin. In other cases in which no parasites are detectable in the section this aetiology is at least probable. Unilateral, or bilateral, non-purulent pyelonephritis was also found in dogs. The fact that, in this experiment, it was only found in animals of the test group, is pure coincidence. In other experiments with dogs of the same age I have encountered it in control animals.

In summary it can be said that the histopathological examination provided no indication that the substance administered by inhalation had any harmful effects.))

QSA

The mean heart rates of both sexes were approximately 200-220 (increased to 240-260) after 10 puffs/day for 13 weeks and after 10 puffs/day for 13 weeks. No significant changes were reported without pathology.

Long Term Maintenance and Acid-Base Balance

No significant changes were reported.

Ophthalmology and Histology of Eyes

No drug induced changes were reported. See pages 39-41 for detailed reports.

12. THREE MONTH INHALATION TOXICITY STUDY IN DOGS WITH AEROSOL FORMS (2/22/94 revised)  
QSA-1251

Lab. performing the Study: Les Oncins Research and Breeding Center  
Saint-Germain sur l'Arbresle.

Species: Beagle dogs, female, 6 months and 12 months.

No. of Animals/Level: 3 M + 3 F

Doseage Level: 10 puffs (1000 µg/day) 5ch 1000 µg or propellant (additional group). Mean dose for M = 17 µg/kg and for F = 34 µg/kg.

Procedure and Route: Dogs were treated twice a day, 10 puffs/day, 5 days/week for 13 weeks via a mask while held by two aides using the following method from metered aerosol containers from Boehringer Ingelheim:

As an example, the results of the investigation are given in Table 1. The results show that the rate of absorption of the drug is very high and that the drug is absorbed almost completely. The results also show that the rate of absorption is not affected by the dose of the drug.

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The percentage of absorbed substance reaching the general circulation is a very high one. The results of the investigation are given in Table 1. The results show that the rate of absorption of the drug is very high and that the drug is absorbed almost completely. The results also show that the rate of absorption is not affected by the dose of the drug.

a.

to inform them of the results of the investigation. The results of the investigation are given in Table 1. The results show that the rate of absorption of the drug is very high and that the drug is absorbed almost completely. The results also show that the rate of absorption is not affected by the dose of the drug.

The body weight of the treated group was more or less the same for the first 9 weeks but slightly less than the control group, while the control group followed the exactly the same pattern as the control group. See pages 12-14.

|                    |                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Food Consumption   | Food consumption was less in the F treated group, only week 13; but all other times, it was similar to or more than the respective control group. See page 157.                                                                                                                                                                                                 |
| Remedial Work      | Some showed slight changes, but they were reported as "within normal limits." See pages 18-19.                                                                                                                                                                                                                                                                  |
| Coagulation Times  | Not done.                                                                                                                                                                                                                                                                                                                                                       |
| Clinical Chemistry | No significant changes were reported.                                                                                                                                                                                                                                                                                                                           |
|                    | COMMENTS: Some of control values fluctuated more than treated group. See pages 25-29.                                                                                                                                                                                                                                                                           |
| Urinalysis         | No drug-induced changes were reported. See pages 36-38.                                                                                                                                                                                                                                                                                                         |
| Organ Weight       | The absolute weight of heart and liver was slightly smaller in F treated group than control F, but gonad in M treated group was slightly larger than control M. See pages 41-42.                                                                                                                                                                                |
| Gross Pathology    | No drug related abnormalities were reported.                                                                                                                                                                                                                                                                                                                    |
| Histopathology     | It was stated by a pathologist that "in most of the dogs there were discrete inflammatory lesions at various sites in the respiratory tract. As they also occurred in some of the controls, it seems that they are of little significance. No significant lesion was found in any of the organs studied." For the detailed report on all dogs, see pages 44-51. |

Arterial blood pressure:

to signs of myocardial damage and hypertrophy. See pages 73-77.

It was reported that lungs of dogs receiving 10 puffs of SCH 1000 in 20 seconds showed "a slight reduction in SP of 10-15 mm Hg" returning to normal within 2 min., but all dogs did not show any changes.

COMMENT: It was stated that "examination of the fundi showed no deterioration," but it was not clear whether the fundi was meant for eye or not. See on page 5 for protocol and on page 8 for results.

## CHRONIC TOXICITY STUDIES:

### A. THE ADMINISTRATION BY INHALATION TO RHESUS MONKEYS OF SCH 1000 FOR 6 MONTHS: (1/30/75 review)

Huntingdon Research Center  
Huntingdon, England

Rhesus Monkeys (M. Mulatta)

SCH 1000

NO. ANIMALS/DOSE LEVEL: 3 F & 3 M

DOSE LEVELS: 200, 800 and 1600 mg per day, control and vehicle control

ADMINISTRATION AND DURATION: Inhalation, 2 X daily, 5 days a week, and 1 X the seventh day.

#### RESULTS:

Mortality: One animal in the control group died.

Clinical signs: Animals physical condition and behavior were within normal limits.

Body Weight: Body weight of animals in all groups was similar. Animals gained weight throughout the study.

Food Consumption: Feeding of animals with Sch 1000 seems to have little or no effect on their food consumption.

Ophthalmoscopy: Eyes of the monkeys were examined at pre-dosing, 12 and 26 weeks. No abnormalities were observed which were due to the administration of this drug. A mydriasis was observed during 2nd or 3rd examination.

Electrocardiography: ECG's taken at pre-dosing, 4, 12, and 26 weeks revealed no biologically significant differences between treated and control groups.

Lung Function Tests: Lung function tests performed at pre-exposure, 8 and 24 weeks showed no significant changes in lung mechanics and ventilation or blood gases.

Estimation of Fluorocarbon-11 In the Blood:

The concentration of fluorocarbon-11 in the blood was found to be greater as the dose of Sch 1000 was increased. There was a wide variation between individual monkeys.

Biochemistry: Tests carried out at pre-exposure, 4, 12, and 26 weeks show values for all animals were within normal limits except for 2 animals in each of the 500 and 1500 mg dose groups who had elevated SAP's at the 26 week evaluations.

Hematology: Hematology values were within normal limits for all monkeys.

Urinalysis: Urine values for all monkeys were comparable and unremarkable.

Terminal studies: Lung abnormalities were seen in monkeys in the control and treated groups. The most severe lesions were seen in the treated monkeys.

No biologically significant changes were seen in the organ weight of these monkeys. No drug-related changes were seen in the respiratory tracts, livers, or G.I. tracts of these animals when the tissues were examined grossly and histologically.

B. RATS: 26 WEEK INHALATION: (Boehringer Sohn) (11/2/78 review)

|                       |                      |
|-----------------------|----------------------|
| No. of Animals/group: | Group 1: 20 M + 20 F |
|                       | Group 2: 30 M + 30 F |
|                       | Group 3: 20 M + 20 F |
|                       | Group 4: 20 M + 20 F |
|                       | Group 5: 30 M + 30 F |

**Strain:**

hysterectomy derived, specific pathogen free albino rats, CD strain (Kent, England)

**Dosage Levels**

**Group:**

(ap-dose)

- 1: Chamber control.
- 2: Vehicle control: 0 x 15 min/day (freons)
- 3: Low dose: 2 x 15 " (128 ug/freons)
- 4: Medium dose: 4 x 15 " (256 ug/freons)
- 5: High dose: 6 x 15 " (512 ug/freons)

\* 11(24.4%), 12(52.9%), and 114(22.5%)

**Exposure regime:** 1 burst of aerosol in the chamber every 5 seconds for 15 min. (64 ug)

Exposures were divided into a morning and afternoon sessions, and morning only on 7th day.

**Duration and Route:  
Growth Effect:**

7 days/week for 26 weeks by inhalation. There was dose-related depression in weekly mean body weight gain in males up to about 16% with highest dose group, but it was considered in the normal range. There was no difference in females.

**Mortality:**

Total of 32 died, 8 each/group except group 1 with 3 and group 4 with 5.

27 of those which died were the result of over compression in the restraining tubes, 4 due to periodic bleeding, and one with broken tail.

**Food Consumption:**

The amount consumed expressed as g/kg/group/week was slightly higher in females than males, but no dose related change was seen.

There were no differences in water consumption.

|                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Hematology:</b>             | No dose-related changes were seen.                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Coagulation Test:</b>       | No test was done.                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Clinical Chemistry:</b>     | There were no differences between groups. Some of values at 6 week were quite low, less than 50% of pre or 12 week values, but no dose-related change was seen.                                                                                                                                                                                                                                                                                               |
| <b>Urinalysis:</b>             | There were no abnormalities.                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Organ Weights:</b>          | Both absolute and relative weights showed no drug related changes. Both males and females showed slightly higher relative weights of adrenal glands without pathology.                                                                                                                                                                                                                                                                                        |
| <b>Macroscopic pathology:</b>  | Severe hydrocephalus was noted in one rat each of groups 1, 2, and 5, and clear fluid filled cysts enveloping in one rat each of groups 1, 3, and 4. Other findings seem to be sporadic, and noted in one rat or two.                                                                                                                                                                                                                                         |
| <b>Microscopic Pathology:</b>  | <p>Infiltration of acute inflammatory cells were conspicuous in occasional mucous glands of the trachea in 2 rats of group 2, and 4 rats of group 5.</p> <p>Dystrophic mineralization was recorded in the kidneys of a rat of group 2 and 2 rats of group 5.</p> <p>Testicular atrophy with reduced spermatogenesis was noted in one rat each of group 1 and group 5.</p> <p>These findings and other sporadic findings were considered not drug induced.</p> |
| <b>Ophthalmoscopy:</b>         | No dose related change was noted.                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Surfactant Production:</b>  | <p>Pattle bubble stability ratio test on random samples of rat lung at autopsy and 1 um epon resin sections of lung showed no drug related changes.</p> <p>Analysis on ciliary beat, lung mechanics, and blood gas levels did not show any drug related changes.</p>                                                                                                                                                                                          |
| <b>Plasma fluorocarbon II:</b> | Freon II was found to be increased with dose administered.                                                                                                                                                                                                                                                                                                                                                                                                    |

C. Subacute Toxicity: (Rabbits)

Strain (NDA, strain TW 49) - 10M, 10F/10M

Dose: 0, L, M, H (100-300 mg/kg) (10 weeks after 13th week)

Duration and Route: 10 weeks, PO (daily)

Mortality: One animal died, accidentally

Body weight, determined weekly, then biweekly, was decreased in mid (11B) and hi dose F (27A) and hi dose M (28A). Food consumption was correspondingly reduced in these animals. Water intake was variable but decreased somewhat in hi dose M. Hematological parameters, determined periodically during the study, were not unusual. Blood glucose and BUN's were essentially normal; however, SAP and SGPT were decreased significantly in treated M and F throughout the study. Term. 11 serum K and cholesterol were not remarkable. Slight decrease in adrenal weight was noted in mid and hi dose F.

The following histological alterations appeared noteworthy:

|                    | Control | Lo    | Mid   | Hi    |
|--------------------|---------|-------|-------|-------|
| <u>Liver</u>       |         |       |       |       |
| fatty degeneration |         |       |       |       |
| cellular           | 14/20   | 11/20 | 13/20 | 6/20  |
| peripheral         | 3/20    | 3/20  | 1/20  | 9/20  |
| periportal         | 4/20    | 7/20  | 7/20  | 12/20 |
| <u>Intestine</u>   |         |       |       |       |
| dilation           | 3/20    | 2/20  | 6/20  | 15/20 |
| <u>Intestine</u>   |         |       |       |       |
| dilation           | 0/20    | 11/20 | 16/20 | 12/20 |
| edema              | 0/20    | 4/20  | 1/20  | 5/20  |
| <u>Bladder</u>     |         |       |       |       |
| dilation           | 4/20    | 3/20  | 10/20 | 16/20 |

Other Comments:

Hypersecretion of Harder's gland in mid and hi dose F after 13th week.  
Hi dose M and F were hyperactive. Retention of urine and feces was noted in treated animals.

D. Chronic Toxicity: (Boehringer)

Species: Dog (Beagle, age 7-11 months)

Duration and Route:

One year, interim sacrifice at 6 months, PO, powder in capsules, 7 days/week for 1st 90 days then tablets were administered in capsules, group 1 received tablets throughout the study.

Schedule:

| Group   | Dose (mg/kg) | Sacrificed<br>6 mo. | Sacrificed<br>1 yr. |
|---------|--------------|---------------------|---------------------|
| Control | 0            | 2M, 2F              | 2M, 2F              |
| 1*      | 1.5          | 3M, 3F              | ---                 |
| 2       | 3.0          | 2M, 2F              | 3M, 3F              |
| 3       | 15.0         | 2M, 2F              | 3M, 3F              |
| 4       | 75.0         | 2M, 2F              | 3M, 3F              |

\* This group started about 6 months after experiment began because lacrimal secretions were decreased in treated dogs.

Mortality:

One group 4 dog sacrificed in moribund condition.

Body weight, determined weekly, was decreased in group 3 and 4 dogs in early part of the study. Terminal values approached initial level in group 3 but not 4. Food intake was reduced in a dose-related manner from group 2 on. Water intake was not consistently altered. Raw data were not included for body weight, food consumption, hematology and clinical chemistries, including urinalysis.

Generally, SGPT and SGOT values were said to be increased in groups 3 and 4 dogs during last half of the study and SAP was increased only in group 4. In the latter part of the study, creatinine was reported to be increased in group 3 and decreased in group 4 from early part on. BUN's were increased in group 3 in last half of the study and decreased in group 4 throughout most of the study. Terminal bilirubin and globulin values were increased in group 4 dogs and cholesterol levels were increased midway in the study only. Blood glucose was essentially normal. Other electrolytes were not appreciably altered. Qualitative and microscopic urinalyses, performed on several occasions during the study, were normal. Organ weights were remarkable.

In the dog sacrificed on day 111 of the study, fatty degeneration of hepatic and renal epithelium, involution of thymus and decreased RBC and WBC in bone marrow was noted. In animals sacrificed at 6 months, fatty liver degeneration was observed in 1 group 4 dog and atrophy of germinal epithelium of testes and epididymus was seen in another dog in this group. In dogs sacrificed at one year, atrophy of epithelium of prostate was seen in one dog each in groups 1, 2 and 4. Localized necrosis of gastric mucosa was observed in 2/6 group 4 dogs.

Other Comments:

Emesis was noted by all treated animals from group 1 on. A dose related decrease in mucosal secretions occurred. Due to decreased salivary secretion at hi dose, food was mixed with water. Decreased conjunctival secretions led to keratoconjunctivitis sicca and animals had to be treated with boric acid, antibiotic and vitamin A and D ointment -- no healing took place. Purulent nasal discharge was also noted occasionally.

E. Chronic Toxicity: (Boenringer)

Rat (SPF, strain FW 49): 35M, 35F/level

Dose: 0, 6, 30 or 150-200 mc/kg (increased during the 14th week).

Duration and Route: Fifty-five weeks, PO (Jiet).

Mortality: Control, 4; Low, 3; Mid, 7; Hi, 24.

Note: All deaths except one occurred after 14th week.

Hi dose M and F gained 29 and 20% respectively of weight gained by controls. Food consumption was correspondingly decreased in these animals. Water intake, measured several times during the study, was decreased in hi dose M and to a lesser extent in F. Hematological parameters were unremarkable. SAP values were decreased in the middle of the study in hi dose F and to a lesser degree in M when compared to corresponding controls. Blood glucose, BUN, SGPT and serum K and cholesterol values were within normal limits. Terminal microscopic and qualitative urinalysis of hi dose animals were normal. 3/14 hi dose M had distinct increase in prostate weight.

The following histological changes seemed drug-related.

|                                        | Control | Incidence |         | iii      |
|----------------------------------------|---------|-----------|---------|----------|
|                                        |         | Lo        | Mid     |          |
| Small Intestine<br>dilation            | 4       | 8         | 19      | 66       |
| Large Intestine<br>dilation<br>colitis | 4<br>0  | 10<br>0   | 17<br>0 | 42<br>18 |
| Bladder<br>dilation                    | 2       | 2         | 15      | 50       |
| Gastric Mucosa<br>dyschylic cysts      | 4       | 22        | 7       | 29       |

Other Comments:

A dose-related hypersecretion of Harder's gland was noted. After 4 months, increased irritability was seen in hi dose animals.

F. Chronic toxicity test in Dogs Using Sch 1000 (1/24/75 review)

Dogs, beagle (7-11 months) Sch 1000

# animals/dose level: 2F + 2 M in 26 week study, 3F+3M in 52 week study

dose level: 1.5, 3.0, 15.0 and 75.0 mg/kg plus controls.

administration and duration:

7 day - oral administration, duration - 26 and 52 weeks.

Results

Clinical Signs

Only 1 death occurred in this study. Animals in the treated groups had a reduction in secretion of conjunctiva, nose and oral mucosa. This was dose-dependent and animals in higher dose groups had difficulty eating dry foods.

### Body Weight

Body weight of dogs was slightly less in the higher dose groups when compared to the weight of the control animals.

### Food Consumption

The food consumption of the animals in the 3.0, 15.0 and 75.0 mg/kg was below that of the animals in the control group.

### Hematology

Erythrocytic hemoglobin and hematocrit values were slightly higher in the treated animals however hematology values for all animals were within normal ranges.

### Biochemistry

Dogs in dose levels groups of 15.0 and 75.0 mg/kg had increased SGPT from week 25 to week 52. Creatinine and Urea N values were slightly confusing in that the 15.0 mg/kg group had increased creatinine and urea-N and the 75 mg/kg group had decreased creatinine and urea-N values.

### Urinalysis

No significant differences was noted between groups.

### Ophthalmoscopic Investigation

A dose dependent keratitis sicca was observed in all dogs receiving dose levels of 3.0 mg/kg or higher. Dogs had difficulty in opening eyelids, minimal lacrimation, cloudiness in corneal epithelium and circumferential fluorescence - positive epithelial lesions. Histological examinations were used to help clarify and diagnose these conditions.

### Autopsy Findings

Autopsy findings at the end of 26 weeks showed 1 dog in the 75.0 mg/kg with fatty degeneration of the liver, as well as a reduction of erythropoiesis and leucopoiesis in the bone marrow. Another dog in the same group showed incomplete spermatogenesis with incipient atrophy of germinal epithelium. These conditions were thought to be drug-induced. Three dogs in the 15.0 and 75.0 mg/kg had vesicles resembling status spongiosus. The 52 week autopsy findings reveal fine-particled fatty degeneration of the myocardial fibers in all dogs. All dogs in this study showed a diffuse fatty degeneration of the liver. In the two highest dose groups, the fatty degeneration was more diffused. Several animals in the 3.0, 15.0 and 75.0 mg/kg groups had bilateral atrophy of the spermatogenic epithelium and cellular interstitial inflammatory infiltrates with incipient fibrosis.

C. Chronic Toxicity Study with Sch 1000 in male dogs for 52 weeks

Dogs, beagle ... Sch 1000

# animals/ dose level: 4 M with 2M for control

dose level: 3, 15 mg/kg Sch 1000, and 0.5 mg/kg atropine sulphate.

administration and duration - oral administration, 7 days a week for 52<sup>1/2</sup> weeks.

Results:

The body weight of the dogs in the 15.0 mg/kg dropped sharply during the first weeks, the dogs did gain weight during the study but never reached their original weights.

Food Consumption

Food consumption of the dogs in the 15.0 mg/kg groups was markedly reduced especially during the first few weeks of the study.

Clinical Observations

Some vomiting and diarrhea was noted in the 3.0 and 15.0 mg/kg dogs. Marked inhibition of secretion was observed in the 15.0 mg/kg dogs throughout the entire study. The oral and nasal membranes were found to be very dry with fissures and dogs had to be fed their food as a wet pulp.

Mortality

One dog in the highest dose group of Sch 1000 died on the 15th day of the study.

Hematology

Dogs in the 15.0 mg/kg group had low total erythrocyte, hemoglobin and hematocrit values when evaluations were made during the 52nd week of this study.

Biochemistry One dog in each of the 3.0 and 15.0 had high SPGT-52nd week.  
Urinalysis

No differences between the groups were observed.

Ophthalmoscopic findings

One dog in the 3.0 mg/kg and all the dogs in the 15.0 mg/kg group had doses-dependent keratitis sicca. No pathological changes were noted in the eyes of the dogs receiving atropine sulphate.

Autopsy Findings

All dogs in this study had parasites or bacterial granulomas of the liver. 3 dogs in the 1.0 mg/kg and 2 dogs in the 15.6 mg/kg group had chronic interstitial epididymitis. Subepithelial lymphohistiocytic infiltration were observed in the tissue of the tongues of several dogs in the 7 groups of dogs dosed with Sch 1000.

4. 18 MONTH ORAL CHRONIC TOXICITY STUDY IN RATS: (10/26/83 review)

Lab. Performing the Study: C. H. Boehringer Sohn in West Germany

Strain: CHbb-THOM (SPF)

No. of Animals/Level: 25 M + 25 F (182.4 g and 142.4 g average weight, and about 43 day old)

Dosage Level: 0, 0.5, 6, 30 mg/kg/day Sch 1000-Br  
Batch No: IV-X and 810783

COMMENTS: See pages 437-440 for actual weekly intake of the drug.

The following table indicates the actual amounts of compound Sch-Br consumed from the food (deviations from theory in percent):

| Week | G <sub>1</sub> |        | G <sub>2</sub> |        | G <sub>3</sub> |        |
|------|----------------|--------|----------------|--------|----------------|--------|
|      | Male           | Female | Male           | Female | Male           | Female |
| 1    | - 0.18         | +56.35 | - 2.12         | -11.02 | - 7.95         | + 8.57 |
| 12   | - 2.83         | + 5.52 | -23.1          | -10.21 | -18.32         | -11.24 |
| 33   | +17.46         | + 2.73 | -17.1          | -15.91 | - 7.18         | - 3.58 |
| 52   | +19.94         | +17.22 | -10.47         | -10.77 | - 9.04         | + 6.70 |
| 65   | -14.06         | + 8.00 | - 4.88         | +12.78 | + 1.40         | - 1.09 |
| 78   | + 7.38         | -19.32 | - 1.58         | - 9.76 | -10.66         | + 9.76 |

**Duration and Route:**

Orally in food for 78 weeks (from 10/10/77 and necropsy on 4/23/79)

**Clinical Standpoint:**

"Small abscesses around the mouth" were observed in many rats of all groups, and they were results of *Pasteurella pneumotropica* infection. No abnormal behavior was observed.

**Growth:**

0.5 mg/kg/day group showed slight but significantly increased body weight gain in both M and F. Six mg/kg/day group also showed a slightly larger gain, but not significant.

**COMMENTS:**

No Table for group means was submitted for body weight gain.

|                      |                                                                                                                                                                                                                                                                                                                                          |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Food Consumption:    | General trend was that weekly food consumption in both M and F showed a great increase in first 3-5 weeks, followed by a gradual decrease up to week 33, then the consumption jumped up to a higher level and stayed to the end. However, M in 30 mg/kg/day showed a significantly smaller food consumption throughout the study period. |
| COMMENTS:            | No Table for groups means was submitted, but figures were submitted on pages 94 for M and 99 for F.                                                                                                                                                                                                                                      |
| Water Consumption:   | Water consumption (every 13 weeks) in M and F of 0.5 mg/kg/day group showed a increase at every point of examination, and it was correlated with the increased body weight gain in this group.                                                                                                                                           |
| Hematology:          | Some values were significantly different from the control, but there were no dose-related or time-dependent biologically significant changes.                                                                                                                                                                                            |
| Thromboplastin Time: | Although the values of 30 mg/kg/day group (M and F) were higher (some significantly) than control values, starting from week 6, they were reported to be within normal ranges for untreated rats. (Compare pages 104-263).                                                                                                               |
| Clinical Chemistry:  | Although some values were significantly different from the control values, only BUN showed a persistent change: BUN in both M and F of 30 mg/kg/day showed significant decrease starting from week 6 to the end, and some of the values in 6.0 mg/kg/day group also showed decrease during the same time.                                |
| Urinalysis:          | No drug-related pathological findings were reported.                                                                                                                                                                                                                                                                                     |
| Necropsy:            | Drug-related finding was "coprostatics" in 1 in 6.0 mg/kg/day group and 4 in 30 mg/kg/day group. See pages 48-61.                                                                                                                                                                                                                        |
| COMMENTS:            | This finding was known to be drug-induced from earlier studies.                                                                                                                                                                                                                                                                          |
| Organ Weight:        | No dose-related significant changes were reported. See pages 352-363.                                                                                                                                                                                                                                                                    |

**Histopathology:**

No drug-induced organ damages were reported. However, there were large number of tumors in this study. 73 M + 12 F in the control group had a total 37 tumors, showing 56%, due to concurrent neoplasias (primary multiplicity of tumors): Among these tumor bearing rats, 3 had 2, 1 had 3, and the other had 5 concurrent neoplasias. See pages 64-66.

10 M + 12 F in the 0.5 mg/kg/day group had a total 34 neoplasias (44%); and of these, 5 had 2, 2 with 3, 1 with 4 concurrent neoplasias. See pages 67-70.

6 M + 9 F in 6.0 mg/kg/day group had a total of 27 neoplasias (30%), and of these, 5 had 2, and 1 with 3 concurrent neoplasias. See pages 70-73.  
6 M + 9 F in 30.0 mg/kg/day group had a total of 23 neoplasias (34%), and of these, 4 had 2 and 1 with 3 concurrent neoplasias. See pages 73-77.

Among them the following carcinomas were noted: hepatocellular carcinoma of the liver and carcinoma in thyroid and pituitary glands in the control group; lymphosarcoma of thymus, hemangiosarcoma of spleen and salivary gland, malignant ependymoma of cerebrum and pituitary, adenocarcinoma of mamma (2 rats) cystadenocarcinoma of mamma in low dose group; hemangiosarcoma of a lymph node, carcinoma of mamma, lymphosarcoma of thymus, subcutaneous fibrosarcoma in mid dose group; lymphosarcoma of thymus (2 rats), adenocarcinoma of thyroid (2 rats), carcinoma of lung, adenoid uterine carcinoma in high dose group. One rat in high dose group had leiomyoma of the cervix. All those tumors were reported to be due to "normal aging pathology" or "the spontaneous pathology" of this rat strain.

**6.0 mg/kg/day group:**

"Animal No. 271; died intercurrently; the cause of death was an extensively metastasizing, solid, anaplastic and partially cirrhotic mammary carcinoma."



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From the 27th week onwards the substance was dosed consistently in ppm. The doses were set on the basis of the food concentrations in the weeks 14 - 26. From the 27th week of treatment until the 95th the dosage scheme was as follows:

|                  | mg/kg | av. concentr.<br>week 14 - 26<br>in ppm | constant dosage from week 27<br>in ppm |
|------------------|-------|-----------------------------------------|----------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 11.19                                   | 12                                     |
| G <sub>1</sub> ♀ | 0.5   | 9.24                                    | 10                                     |
| G <sub>2</sub> ♂ | 2     | 44.44                                   | 45                                     |
| G <sub>2</sub> ♀ | 2     | 36.70                                   | 37                                     |
| G <sub>3</sub> ♂ | 6     | 132.25                                  | 135                                    |
| G <sub>3</sub> ♀ | 6     | 110.77                                  | 110                                    |

From week 78 of the experiment the rats consumed less food so that the effective intake of substance became less. It was therefore necessary to increase the ppm dosages of Sch 1000-Br in the food. From week 86 onwards the dosages were as follows:

|                  | mg/kg | Constant dosage<br>weeks 27 - 85<br>in ppm | Constant dosage<br>from week 86<br>in ppm |
|------------------|-------|--------------------------------------------|-------------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 12                                         | 25                                        |
| G <sub>1</sub> ♀ | 0.5   | 10                                         | 12                                        |
| G <sub>2</sub> ♂ | 2     | 45                                         | 55                                        |
| G <sub>2</sub> ♀ | 2     | 37                                         | 45                                        |
| G <sub>3</sub> ♂ | 6     | 135                                        | 170                                       |
| G <sub>3</sub> ♀ | 6     | 110                                        | 120                                       |

The control group received the same food without 5 $\alpha$ -DHT.

**Average Drug Intake:**

\*The effective amount of substance ingested did not always correspond with the planned dosages because of the varying food consumption (see pages 31 to 32 / Part II).

On average the following amounts were ingested (given in mg/body weight):

| sex | group 1 | group 2 | group 3 |
|-----|---------|---------|---------|
| m   | 0.49    | 1.87    | 5.59    |
| f   | 0.52    | 1.38    | 5.83    |

**Duration and Route:** 114 weeks mixed in the food as 0.1% "trituration with lactose."

Initiated on 9/28-29/77 and autopsied from 11/23/79 to 12/6/79.

**Mortality:** Total 157 out of 500 died intercurrently or sacrificed moribund as shown in the following Table. There were more F than M, but the difference between F and M was significant ( $p < 0.05$ ) only in low and mid dose groups, and there were no significant differences between groups, and none were reported to be drug related. See pages 055 to 122 in Part II for findings on dead animals.

| groups | dose      | animals used |     | died / sacrificed -<br>intercurrently |    |       |
|--------|-----------|--------------|-----|---------------------------------------|----|-------|
|        |           | ♂            | ♀   | ♂                                     | ♀  | total |
| 0      | control   | 100          | 100 | 25                                    | 34 | 59    |
| 1      | 0.5 mg/kg | 50           | 50  | 10                                    | 22 | 32    |
| 2      | 2 mg/kg   | 50           | 50  | 8                                     | 22 | 30    |
| 3      | 6 mg/kg   | 50           | 50  | 16                                    | 20 | 36    |
|        | total     | 250          | 250 | 59                                    | 98 | 157   |

**Clinical Observations:** No drug related findings were reported. See page 33 in Part II.

**Growth:** There were no significant differences between groups. See pages 001-004 in Part II for weekly for 26 weeks then every 4 weeks' growth.

**Food Consumption:** No differences between groups were reported for weekly consumption.

**Water Consumption:** M of mid dose consumed less water, but M in low dose group consumed more water than control M. See page 018 in Part II.

F of treated groups consumed dose-dependently more water than the control F most of the time. See page 020 in Part II.

**COMMENTS:** "Every three months the water consumption was determined for one week by measuring out the water and reweighing the volume not consumed. The consumption of drinking water was measured for the first 12 living male and the first 12 living female animals of each group."

**Hematology:**

Leucocyte counts at the end of the study showed an increase in all groups including the control groups, and the differential counts showed "no deviation from normal findings," although percent of monocyte and banded cells were significantly ( $p < 0.05$ ) increased in the high dose M. Monocytes were significantly ( $p < 0.01$ ) lower in the mid dose group M. See pages 034 and 035 in Part II for group means.

**Other's usual Parameters:** Not done.

**Autopsy Findings:**

No dose-related or drug-induced findings were reported, and the changes reported on pages 123-173 in Part II were regarded as "spontaneous" lesions.

**Histopathology:**

The following report was submitted:

"The neoplasias detected are compiled in tables I to 7. Table 8 gives a survey of the non-neoplastic lesions. The individual histopathological findings are to be found in the protocols (page 178 to 676) in part II of the report. A list of the abbreviations used in the microscopic diagnoses is given on pages 174 to 177 in part II."

In addition, the following results were also submitted:

- Table 2: Frequencies of mammary tumors/100 animals/group
- Table 3: Metastasising tumors classified according to groups
- Table 4: Benign and malignant tumors and metastases of all animals used/groups and sex
- Table 5: Numbers of animals with multiple or solitary primary tumors/group and sex
- Table 6: Neoplasms of the rats dying intercurrently or sacrificed moribund
- Table 7: Time of the first occurrence of the different types of tumor in rats dying intercurrently or sacrificed moribund
- Table 8: Non-neoplastic lesions/group/sex/organ

In the statistical analysis of the types of tumours listed in Table I with the exact test according to Fisher, no significantly increased incidence was calculated either for the male or the female rats of the three dose groups in comparison to the control group. Concerning the aim of the trial, this means that a 114-weeks administration of Sch 1000-ör induced no increase in tumour frequency in the animals of the three dose groups. Due to the one-sided treatment of the problem by the test, a possible decrease of the tumour incidence could not be checked.

A comparison between the sexes within each group revealed the following significant differences ( $P < 0.05$ ):

1. Adenomas of the adrenal cortex are significantly more frequent in the male animals of groups 0, 1 and 2.
2. Pituitary adenomas are, in all groups, significantly more frequent in female animals.
3. Pituitary carcinomas are significantly more frequent in female control rats.
4. Lipomas of the skin/subcutis are significantly more frequent in male control rats.

Distribution and frequency of the different primary tumours, as well as the more frequent-occurrence of pituitary tumours in female animals or of tumours of the adrenals and of the skin/subcutis in male animals, are typical for this rat strain (TILOV et al., 1976, UEBERBERG and Lützen, 1979). There is, therefore, no influence of the substance on the calculated differences between the sexes. 99

The following was the Evaluation on Histopathological Findings submitted by the Sponsor:

"The aim of this 114 week long-term study in the rat was to determine if the substance used, Sch 1000-Br, has a tumorigenic or carcinogenic effect. In order to answer this, the following questions are important from the pathological point of view:

1. Is there an increased occurrence of a certain type of tumour, or of several tumour types, in the treated animals of the three dose groups that also occurred in the control animals?
2. Are types of tumours observed in the treated rats of the three dose groups which do not occur in the control animals?
3. Is the number of malignant tumours increased in the animals treated with Sch 1000-Br in comparison with the control rats?
4. Is the number of multiple tumour carriers or the tumour variety for each individual animal higher in the treated rats of the three dose groups than in control animals?
5. Do certain types of tumour occur earlier in the treated rats than in the control animals of the same age?

When recording the non-neoplastic lesions the following question must also be answered:

6. After this long-term administration of the substance Sch 1000-Br do substance-induced changes occur which had not been observed up to now in previous toxicological experiments in the rat or other species?

Question 1:

In the rats of the three dose groups there was no significant increase in any of the types of tumours detected in comparison with the control animals of the same group.

Within the four experimental groups there were significant sex differences for individual tumour types ( $p < 0.05$ ):

Cortical adenomas of the adrenals were found more frequently in the male animals of groups 0, 1 and 2.

Pituitary adenomas were increased in the female animals of all four experimental groups.

Pituitary carcinomas and lipomas of the skin/subcutis were observed more frequently in the female and male rats of the control group.

Such sex differences are characteristic for the strain of rat used. They are therefore to be evaluated as a spontaneous occurrence independent of the substance.

Question 2:

Some benign and malignant neoplasms at various primary sites occurred only in the rats of the three dose groups (group 1 = 13 tumours, group 2 = 14 tumours, group 3 = 16 tumours). For these tumours no comparable types of tumour were found in the animals of the control group.

The primary sites of these tumours were mainly organs in which the tumor rate is relatively low (e.g. lung).

pancreas, colon, stomach, salivary gland, eyes etc.) On the other hand organs were also concerned, the tumour rate of which was high and which showed a broad spectrum of tumours (e.g. mammary gland, uterus, ovary, skin).

These individually occurring tumours are spontaneous tumours, i.e. not substance-induced.

This statement can be supported by the fact that some other types of tumour at various primary sites were found exclusively in the control animals (Group 0 = 33 tumours). If one examines the distribution of these with regard to their primary sites, it is clear that similar conditions exist in the control as in the treated animals. On the one hand there are again organs with low tumour rates (e.g. aorta, colon, bones, brain etc.), and on the other hand, primary sites with a high tumour rate and a wide tumour spectrum.

Furthermore, the investigations into the spontaneous tumour rate of the strain used (TILOV et al., 1976, UEBERBERG and LÜTZEN, 1979) and surveys on the spontaneous tumor rates of other strains of rats (e.g. MACKENZIE and GARNER, 1973) show that, with regard to primary site, frequency and type of tumour, such a wide variation is to be expected in aging rats.

### Question 3

From the statistical analysis it can be seen that after a 2 year administration of Sch 1000-8r the number of malignant tumours in the treated animals is not increased in comparison with the control animals of the same sex respectively. The same applies to the benign neoplasms.

Question 4:

No effect of the substance, in the sense of an increase in the number of multiple tumour carriers in the dose groups, or with regard to the variation in types of tumour, could be established statistically in comparison with the control animals.

Question 5:

Apart from isolated cases the most common tumours occurred in a certain sequence. Tumours of the mammary glands occurred in the first year of life, adenomas of the interstitial cells of Leydig, thymic lymphomas and adenomas of the pituitary occurred most frequently at the beginning of the second year of life, followed by tumours of the adrenal cortex. If the four experimental groups are compared, it becomes clear that, in spite of this difference in timing, the most common neoplasms were observed simultaneously in the control and the treated animals.

Question 6:

No substance-induced, non-neoplastic lesions could be detected on histopathological examination. The findings recorded were inflammatory and degenerative changes and changes due to age. They are distributed among all four experimental groups with varying frequency

and are to be attributed exclusively to the spontaneous pathology of the strain of rat used. Comparable spontaneous findings are already known from previous long-term studies.

In summary it can be said that after administration of Scl 1000-Ba, in doses of 0.5 mg/kg, 2.0 mg/kg and 6.0 mg/kg, to rats, for 114 weeks, no tumourigenic or carcinogenic effect was observed. Frequency, types of tumour and time of occurrence of the neoplasms were not affected by the administration of the substance.

Likewise no substance-induced, non-neoplastic lesions could be detected histopathologically.

All histopathological findings obtained are, without exception, to be attributed to the spontaneous pathology of the strain of rat used."

**8. ORAL CARCINOGENICITY STUDY OF SCH 1000-BR IN MICE:**

Strain: Cbb: NMR1 (SPF)

No. of Animals/Level: 100 M + 100 F for the control group  
50 M + 50 F for the treated groups  
(M = 31.45 g and 50 + 2 days old  
F = 25.58 g and 49 + 2 days old)

Dosage Level: 0.5, 2, or 6 mg/kg/day in food  
Batch No. = IV/X

From the 27th week onwards the substance was dosed consistently in ppm. The doses were set on the basis of the food concentrations in the weeks 14 - 25. From the 27th week of treatment onwards the dosage scheme was as follows:

|                  | mg/kg | av. concentr.<br>week 14 - 25<br>in ppm | constant dosage<br>from week 27<br>in ppm |
|------------------|-------|-----------------------------------------|-------------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 4.077                                   | 5                                         |
| G <sub>1</sub> ♀ | 0.5   | 2.754                                   | 3                                         |
| G <sub>2</sub> ♂ | 2     | 16.739                                  | 15                                        |
| G <sub>2</sub> ♀ | 2     | 11.032                                  | 12                                        |
| G <sub>3</sub> ♂ | 6     | 51.686                                  | 50                                        |
| G <sub>3</sub> ♀ | 6     | 33.307                                  | 35                                        |

The control group received the same food without Sch 1000-Br.

**Average Drug Intake:**

"The effective amount of substance ingested did not always correspond with the planned dosages, especially towards the end of the experiment, because of the varying food consumption. On average, the following amounts were ingested (given in mg/body weight):

| sex | group 1 | group 2 | group 3 |
|-----|---------|---------|---------|
| m   | 0.56    | 1.76    | 5.59    |
| f   | 0.48    | 1.87    | 5.50    |

(see pages 080 - 090/Part II)."

**Duration and Route:** 107 weeks mixed in food.  
Initiated on 6/23-21/77 and autopsied on 7/2-6/79.

**Mortality:** Total 170 out of 500 mice died intercurrently or were sacrificed moribund as shown in the following Table.

| groups | dose      | animals used |     | died/sacrificed intercurrently |     | total |
|--------|-----------|--------------|-----|--------------------------------|-----|-------|
|        |           | d            | q   | d                              | q   |       |
| 0      | control   | 100          | 100 | 29                             | 43  | 72    |
| 1      | 0.5 mg/kg | 50           | 50  | 1                              | 21  | 34    |
| 2      | 2 mg/kg   | 50           | 50  | 13                             | 21  | 34    |
| 3      | 6 mg/kg   | 50           | 50  | 9                              | 21  | 30    |
| total  |           | 250          | 250 | 64                             | 106 | 170   |

There were more F than M as shown, but the difference between F and M was significant ( $p < 0.05$ ) only in the control and high groups; but there were no significant differences between groups.

For the probable causes of death or illness of these mice, see page 115-161 in Part II.

**Clinical Observation:** No-drug related findings were reported.  
See pages 091-093 in Part II.

**Growth:** No differences between groups were reported. See pages 055-061 in Part II.

**Food Consumption:** No significant differences between groups were reported.

**Water Consumption:** Only mid dose group M had less water consumption during the second year. See pages 078-079 in Part II.

**Hematology:** Leucyte count at the end of the study showed no dose-related changes, and there were no significant differences between groups.

"In all experimental groups individual animals showed in some cases considerably increased total leucocyte values (group 0: 2 m, 4 f; group 1: 2 f; group 2: 1 m, 1 f; group 3: 4 m, 3 f). This includes only those animals whose leucocyte values were over 20,000/mm<sup>3</sup>. The differential blood count of these 17 animals showed in 11 cases (nos. 012, 043, 553, 579, 612, 659, 218, 227, 228, 241, 704) a clearly increased percentage of lymphocytes, in 5 cases (nos. 528, 643, 191, 711, 743) an increase in the granulocytes and lymphocytes and in one case (no. 542) an extreme increase in the number of granulocytes. In a total of 8 (nos. 512, 542, 553, 579, 659, 227, 241, 743) of the 17 mice the increased total leucocyte value correlated with a histopathologically detected lymphatic or plasma cell leukemia. In the remaining 9 cases the increased total leucocyte value is a sign of an acute/chronic inflammation. (see pages 094 to 114/Part II)."

**COMMENTS:** Among the 8 mice with leukemia, 1 M + 3 F belonged to the control group; 1 F, to mid dose group; and 2 M + 1 F, to the high dose group.

**Other usual parameters:** Not done.

**Autopsy Findings:** No significant drug-induced changes were reported, and the changes reported on pages 162 to 206 in Part II were regarded as "spontaneous" lesions.

**Histopathology:** The following reports were submitted.

"In the carcinogenicity study in mice after administration of the substance Sch 1000-Br, the histological investigations were carried out by Prof. Dr. W. Drommer and Prof. Dr. G. Trautwein, Institut für Pathologie of the Tierärztliche Hochschule, Hannover.

The neoplasias observed are summarized in tables 1 - 5. A survey of the non-neoplastic alterations is given in table 6. The individual findings of each experimental animal can be found in the protocols (Pages 211 to 710) in part II of the report. "

"Comparing the dose groups with the control group, the statistical analysis reveals only a few significant findings ( $P < 0.05$ ) for the different types of tumour:

1. Lung adenomas are significantly more frequent in the female animals of group 2, in comparison to the female mice of group 0.
2. This is also true for the reticulosarcomatosis.
3. Hepatocellular adenomas are significantly more frequent in the male mice of group 2 than in the male control animals.
4. Adenocarcinomas of the ovary are significantly more frequent in the female animals of group 1 than in the female control animals.

Since no dose-dependency could be established for the tumor frequency, these slight statistical differences are to be considered as having no biological importance. The statistical test treats the one-sided question of an increase in the tumour rate in the dose groups as compared to the control animals. For this reason, no statement can be given on the remarkably low tumour frequency in group 3.

In comparing the sexes the following significant differences ( $P < 0.05$ ) can be recognized:

1. Lung adenomas are in groups 0, 1 and 3 significantly more frequent in male animals.
2. Reticulosarcomatoses (group 2), lymphatic leucoses (group 0, 3), and thymomas (group 3) are significantly more frequent in the female animals of these groups.

3. Hepatocellular adenomas are in group 2 significantly more frequent in male animals.

The results of Stötzer and Notman (1982) confirm a possible influence of the sex concerning the higher frequency of lung adenomas in male animals of all 3 experimental groups in this long-term study. In their investigation of age-dependent occurrence of tumours in the same mouse strain, they showed that lung tumours are more frequent in 27 months old male animals. The significantly higher incidence of the lymphatic leucosis, the reticulosarcomatosis and the thymomas in the female animals are characteristic of this strain (Weisse et al., 1975, Stötzer and Notman, 1982). The significantly higher rate of hepatic cell adenomas in the male animals of group 2 coincides also with the results of Weiss et al. (1975). In their investigation they detected a higher incidence of hepatocellular adenomas in male mice.

In table 1 the metastasising tumours are compiled according to groups. The primary tumours and their organic localisation are listed in table 1, but not the organic metastases (secondary tumours). A statistical evaluation of the data of this table was not carried out for methodical reasons, since the metastases were found in a varying number of organs in different animals. The number of metastasising tumours as well as the total number of organs with metastases show no influence of substance or dose. Metastases were found most frequently in liver, ovary and lung.

In table 2 the benign and malignant tumours are summarized for each group separately according to sex.

A statistical evaluation of the data of these tables cannot be carried out for methodical reasons, since the numbers include animals with two and more benign or malignant tumours.

Considering the total number of tumours the malignant neoplasias predominate (cf. Weisse et al., 1975). This predominance is mainly due to the fact that the number of malignant tumours is in female mice in the different experimental groups up to four times higher than the number of benign tumours. The basis of this is the high rate of lymphatic leucoses and thymomas in the female mice (cf. tab. 1). In the male mice, on the contrary, the number of benign tumours does not differ greatly from that of malignant tumours in the different experimental groups.

A significant increase due to an influence of the substance either of benign or of malignant tumours is not observed for the 3 dose groups for either sex.

Table 3 lists the tumours of the animals which died intercurrently or were sacrificed moribund.

Tumour spectrum and primary site of the most frequently diagnosed neoplasias coincide largely with the findings at the end of the trial (Tab. 1). The dominating tumours are lymphatic leucoses, reticulosarcomatoses, lung adenomas and carcinomas.

Comparing the number of control and dose animals which died intercurrently or were sacrificed moribund, no significant differences are found for either sex in the groups. However, within groups 0 and 3 significantly ( $P < 0.05$ ) more intercurrent deaths are calculated for the female mice.

Significant differences ( $P < 0.05$ ) result for the different types of tumour when comparing the three dose groups with group 0.

1. Adenocarcinomas of the lung are significantly more frequently the cause of disease and death in the female animals of group 3 compared to the female control animals.
2. The reticulosarcomatosis is significantly more often the reason for sacrifice or the cause of death in the female animals of group 2 in comparison to the female animals of group 0.

Just as for the incidence of tumours in all animals used, no substance influence can be detected for the tumour frequency of the animals which died intercurrently or were sacrificed moribund.

A significant ( $P < 0.05$ ) influence of the sex was found in the animals which died intercurrently only for the adenocarcinomas of the lung, i.e. this tumour is more frequent in the male mice than in the female mice of group 0 (cf. Stötzer and Norman, 1982).

In addition, the following results were also submitted:

Table 4: Lists of the animals with 2 or more primary tumors/group and sex.

Table 5: Time of the first occurrence of the different types of tumors in mice which died intercurrently or sacrificed moribund.

Table 6: Non-neoplastic lesions/group/sex/organ.

The following was the "Evaluation on Histopathological Findings" submitted by the consultants:

"This long-term study was to investigate whether the substance Sch 1000-Br has a tumorigenic or cancerogenic effect in the mouse. In order to find an answer to this, several questions are important from the pathological point of view:

1. In the animals of the three dose groups is there an increased occurrence of a tumour type or several tumour types which were also observed in the control animals?
2. Are types of tumour observed in the mice of the three dose groups which do not occur in the control animals?
3. Is the number of malignant tumours increased in the animals treated with Sch 1000-Br in comparison with the control mice?
4. Is the number of multiple tumour carriers or the tumour variety for each individual animal higher in the mice of the three dose groups than in the control group?
5. Do certain tumours occur earlier in the mice treated with the test substances than in the control animals of the same age?

Finally in the diagnosis of the non-neoplastic lesions the following question should be considered:

6. After this long-term administration of Sch 1000-Br is it possible that substance induced organ changes occur in the mouse which had not been observed up to now in the previous toxicological experiments with other animal species?

Question 1:

In comparison to the control animals of the same sex the following types of tumours occurred significantly more frequently in the dose animals:

pulmonary adenomas and reticulosarcomatosis in the female animals of group 2,

hepatocellular adenomas in the male animals of group 2,

adenocarcinomas of the ovary in the female animals of group 1.

Since, with regard to the overall frequency of tumours and, therefore, also to the incidence of the four above named types of neoplasia, no dose-dependency can be detected, these statistical differences are to be interpreted as coincidental, i.e. not substance induced.

Question 2:

In isolated cases benign or malignant tumours with various primary sites occurred only in animals of the three dose groups (group 1 = 5 tumours, group 2 = 11 tumours, group 3 = 6 tumours). In general, organs were concerned of which the tumour rate is very low (e.g. mammary gland, uterus, urinary bladder, intestine). Although the control animals used in this study did not show comparable neoplasias, in our opinion these were spontaneous occurrences, independent of the substance. This statement can be supported by the fact that, certain other types of tumour with various primary sites and low incidence occurred only in the control animals (group 0 = 11 tumours, e.g.

mammary gland, testes, skin, thoracic and abdominal walls). Furthermore, investigations into the spontaneous tumour rate of the strain of mice used (Gersheff et al., 1976) and the summarized results of other authors (Benirschke et al., 1978, Yarusso et al., 1979, Faith and Ward, 1980), show that, with regard to primary site and tumour frequency, such a wide variation is to be expected in aging mice.

Question 3:

From the statistical analysis it can be seen that after a 2-year administration of Sch 1000 B<sub>2</sub> the number of malignant tumours in the dose animals is not increased in comparison with the non-treated animals.

Question 4:

With regard to the number of multiple tumour carriers and the multiplicity of tumours in each individual animal there are no statistically significant differences between control and dose animals.

Question 5:

Tumours of individual organs or organ systems appear at different times. Thus, broadly speaking, the lymphatic leucoses are followed by the pulmonary tumours and then the hepatic and ovarian neoplasias. However, it can be seen that these most frequent tumours were to be detected within certain periods simultaneously in control and dose animals.

No substance-induced, non-neoplastic changes could be detected histologically. The inflammatory and degenerative changes observed and those caused by age are exclusively spontaneous lesions which are distributed among the four experimental groups with varying frequency. Type and frequency of the lesions are already known from previous long-term studies with the same strain of mouse.

To summarize these results, it can be said that after oral administration of Sch 1000-Br in dosages of 0.5 mg/kg, 2.0 mg/kg and 6.0 mg/kg for 107 weeks no substance induced neoplasias could be detected. The incidence, type and time of appearance of the tumours were not affected by the substance administered.

Likewise no substance induced, non-neoplastic organic lesions could be observed.

All histopathological findings observed are, without exception, to be attributed to the spontaneous pathology of the strain of mouse used.<sup>28</sup>

**ANTIGENICITY and MUTAGENICITY TESTS: (Report No. 0100)**  
Lab performing study: Teljin Institute for Bio-medical  
Research, Japan

1. Antigenicity tests:

Group of 5 M guinea-pigs (Hartley strain, 250-330 g) were sensitized with Sch 1000 (1 mg/ml/kg) with/without Freund's complete adjuvant by I.M. injection every other day for 6 times along with human serum (positive control) and saline control (negative control), both 4 times injections, and they were challenged 2 weeks later from the last day of sensitization by following method:

- a. Active anaphylactic shock reactions: Sch 1000 (1 mg/kg) and human serum (1 mg/kg) were administered to the sensitized animals, and they were observed 3 hours for anaphylactic shock reaction.
- b. Schultz-Dale reaction: Antigenicity was assessed by contraction of isolated ileum from sensitized animals by adding the sensitizing agent in the tissue bath.
- c. Passive cutaneous anaphylaxy test (PCA): Four intradermal injections of 0.1 ml sensitized serum were made on side of the (served) dorsal skin of a normal guinea-pig, and the animals were injected 30 min. later with mixture of 1 ml of Evans blue and sensitizing agents (Sch 1000 0.1 mg or human serum 5 mg) in saline, and the animals were killed 30 min. later, and their inverted skins were examined for the leakage of the dye.
- d. Acute cutaneous anaphylaxy test (ACA): 0.1 ml of 10 ug of Sch 1000 or human serum was injected intradermally on the back (served) of sensitized animals, and the reaction at the injection site was observed 24 hours later.

RESULTS OF ANTIGENICITY TESTS: Sch 1000 with/without adjuvant did not show antigenicity in the tests used while human serum did show antigenicity.

**1. MUTAGENICITY TEST:**

Reverse mutation test (Ames test) using *Salmonella typhimurium* TA 98 and TA 100 and aqueous solution of Sch 1000 (0.1 ml of 0.1-10 mg/ml) was used along with AP-2 as a positive control and distilled water as negative control groups, with/without S-9 mixture.

**RESULT:** It was reported as negative, but close examination of data indicated that the results are not conclusive.

**2. MUTAGENICITY STUDY OF THE COMPOUND SCH1000 BR (Ames Test): (9/17/81 rev)**

Lab. Performing the Test: Boehringer, Schm, Germany

Date of Test: 5/27/80 - 6/27/80 (page 6)

GLP Statements: Not submitted.

- Methods:**
1. Sch 1000 BR (Batch no. 709078/3) was dissolved in DMSO, and the concentrations tested were 25, 250, 2500, 5000, and 10,000 ug/plate (only once for TA 1537) against benzo(a)pyrene(BP) as positive control in most mutagenicity testings; and MNNG, twice; and DENA, once.
  2. Sch 1000 was investigated for a point mutagenic effect in reverse mutation tests in a total of 5 *Salmonella typhimurium* strains (TA 1535, TA 1537, TA 1538, TA 98, TA 100) using microsomal enzyme fractions and an NADPH-generating system. See pages 4-8 for details.

- Results:**
1. Due to extremely high spontaneous mutation rate (mean = 560/plate) of TA 100, the test was repeated without metabolic activation. See Table 2.
  2. Up to 5000 ug/plate of Sch 1000 had no bacteriostatic effect on strains tested, but 10,000 ug/plate produced a toxic effect on TA 1537, and this concentration was used only once for repeated test in Table 6.

3. There were no differences in number of colonies (mean) between control and Sch for all for strains, TA 1535, TA 1538, TA 98, and TA 100 with/without addition of various activation systems.

But, Sch produced dose-dependent increase of revertant colonies in TA 1537, only with addition of Aroclor-induced microsomal fractions. Number of colonies with 5000 ug/plate was 70 while control was only 23, indicating positive mutation, as BP. See Table 5 for details.

4. The repeated test with the same system did not show any difference between Sch and control plate with addition of the same Aroclor induced fraction. However, this time the results after addition of phenobarbital induced fraction increased the revertant mutants slightly (average mean 20) from 250 up over the control mean of 14, while the results of studies without any fraction were only 4 -2/plate in all concentrations including control and BP.

### 3. MUTAGENICITY STUDIES: DOMINANT LETHAL STUDY IN MICE:(11/9/83 review)

#### A. DOMINANT LETHAL STUDY IN MICE:

IRI Project No. 703287:

Lab. Performing the Study: Inveresk Research International  
Musselburgh, EH21 7UB, Scotland

Methods: See the pages 8-15. Batch No. = 709078.

#### RESULTS:

##### 1). Dose-Finding Study:

Two M/group of CD-1 mice (11-13 week olds) were dosed by gavage with 100, 250, 500, 1000, or 2000 mg/kg/day, as suspension in distilled water (10-20 ml/kg) for 5 days (7/7/-11/80).

Two rats each in 1000 mg/kg/day group and 2000 mg/kg/day groups died on days 6 and 7, and they had signs of Piloerection, hypokinesia, and ataxia as shown in the following Table:

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                    | Duration of Clinical Signs |
|-----------|------------------|------------|--------|------------|----------------------------------|-----------------------------------|----------------------------|
|           |                  |            | No.    | Day        |                                  |                                   |                            |
| 1         | 100              | 1<br>2     | 0/2    | K          | 5(2)                             | None                              | -                          |
| 2         | 150              | 3<br>4     | 0/2    | K          | 5(2)                             | None                              | -                          |
| 3         | 300              | 5<br>6     | 0/2    | K          | 5(2)                             | None                              | -                          |
| 4         | 1000             | 7<br>8     | 2/2    | 1(2)       | 5(3)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |
| 5         | 3000             | 9<br>10    | 2/2    | 6(1), 7(1) | 5(2)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |

K = Killed at end of 15 day observation period.

See pages 16-17 for other findings.

2). Main Toxicity Study:

Six M/group of mice were dosed by gavage with 350 to 1772 mg/kg/day for 5 days (7/15-19/80). The following Table shows the results of this study.

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                                    | Duration of Clinical Signs           |
|-----------|------------------|------------|--------|------------|----------------------------------|---------------------------------------------------|--------------------------------------|
|           |                  |            | No.    | Day        |                                  |                                                   |                                      |
| 1         | 350              | 11-16      | 0/6    | K          | 5(6)                             | None                                              | -                                    |
| 2         | 525              | 17-22      | 0/6    | K          | 5(6)                             | Piloerection                                      | day 2-6                              |
| 3         | 780              | 23-28      | 2/6    | 6(2), K(4) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia<br>Sedation | day 3-5<br>day 3-7<br>day 5<br>day 5 |
| 4         | 1181             | 29-34      | 6/6    | 6(6)       | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 4-5<br>day 2-5<br>day 4-5        |
| 5         | 1772             | 35-40      | 6/6    | 8(1), 6(5) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 3-5<br>day 3-5<br>day 3-5        |

K = Killed at end of 15 day observation period.

"From the results of this main toxicity test the LD<sub>50</sub> (5-day) value was calculated as 842 mg/kg with 95% confidence limits of 710-1000 mg/kg. The dose levels used in the main dominant lethal test were as follows:

| <u>Group</u>                     | <u>Dose Level</u> |
|----------------------------------|-------------------|
| Low - 0.05 x LD <sub>50</sub>    | 42 mg/kg          |
| Middle - 0.16 x LD <sub>50</sub> | 135 mg/kg         |
| High - 0.5 x LD <sub>50</sub>    | 421 mg/kg         |

### 3). Dominant Lethal Study:

After a preliminary pregnancy study with six M/group and F, 15 M/group of mice were selected (see page 21) and dosed by gavage for 5 days with 42, 135, 421 mg/kg/day (10 ml/kg) Sch 1000-BR along with a vehicle control group receiving 10 ml/kg distilled water and a positive control group receiving 100 mg/kg/day ethyl methane sulphonate in 10 ml/kg. Then, 1 M was mated with 2 F virgin mice (8-10 weeks old) in each cage (Day 0) for 5 days (total 8 sets of matings during 8 weeks), and F were separated from the M, killed, and examined.

There were no deaths in any group, and no toxic signs were noted in vehicle control, low, or mid dose groups. Two mice (Nos. 57 and 62) in the high dose group had piloerection and lethargy during dosing, and one in the positive control group (no. 153) had piloerection on day 4.

The following Table showed pregnancy rate for F:

| Week    | Distilled Water<br>10 ml/kg, n 5 |     | Sch 1000 BR<br>42 mg/kg, n 5 |     | Sch 1000 BR<br>125 mg/kg, n 5 |     | Sch 1000 BR<br>421 mg/kg, n 5 |     | CNS<br>100 mg/kg, n 5 |     |
|---------|----------------------------------|-----|------------------------------|-----|-------------------------------|-----|-------------------------------|-----|-----------------------|-----|
| 1       | 7/10                             | 87% | 22/30                        | 73% | 28/30                         | 93% | 26/30                         | 87% | 24/30                 | 80% |
| 2       | 29/30                            | 97% | 10/30                        | 33% | 29/30                         | 97% | 24/30                         | 80% | 22/30                 | 73% |
| 3       | 24/30                            | 80% | 25/30                        | 83% | 27/30                         | 90% | 24/30                         | 80% | 22/30                 | 73% |
| 4       | 27/30                            | 90% | 25/30                        | 83% | 26/30                         | 87% | 23/30                         | 77% | 24/30                 | 80% |
| 5       | 24/30                            | 80% | 26/30                        | 87% | 24/29                         | 83% | 21/29                         | 72% | 24/30                 | 80% |
| 6       | 28/30                            | 93% | 26/29                        | 90% | 24/30                         | 80% | 26/30                         | 87% | 22/30                 | 73% |
| 7       | 26/30                            | 87% | 25/29                        | 86% | 27/29                         | 93% | 27/30                         | 90% | 24/30                 | 80% |
| 8       | 27/28                            | 96% | 24/28                        | 86% | 24/28                         | 86% | 24/30                         | 80% | 24/30                 | 80% |
| Overall | 211/230                          | 92% | 193/230                      | 84% | 207/230                       | 90% | 197/230                       | 86% | 186/230               | 81% |

\* - Actual pregnancies/possible pregnancies

There were no significant differences between 4 groups in mean numbers of implantations per pregnant F, but during mating weeks 1 and 2, the positive control group had a slightly less implantation. See Table 7 on page 34.

"Early deaths" were assessed by 4 methods as the following 4 Tables, but only method 4 was statistically analyzed.

Method 1:

Pregnancies with 3 or More Early Deaths  
Sediment Inchi Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, n 5 |       | Sch 1000 BR<br>42 mg/kg, n 5 |       | Sch 1000 BR<br>125 mg/kg, n 5 |       | Sch 1000 BR<br>421 mg/kg, n 5 |       | CNS<br>100 mg/kg, n 5 |       |
|------|----------------------------------|-------|------------------------------|-------|-------------------------------|-------|-------------------------------|-------|-----------------------|-------|
| 1    | 3/26                             | 11.5% | 5/23                         | 21.7% | 3/26                          | 11.5% | 1/20                          | 5.0%  | 14/24                 | 58.3% |
| 2    | 1/29                             | 3.4%  | 3/30                         | 10.0% | 3/29                          | 10.3% | 2/26                          | 7.7%  | 11/22                 | 50.0% |
| 3    | 0/24                             | 0.0%  | 1/25                         | 4.0%  | 5/27                          | 18.5% | 1/24                          | 4.2%  | 3/22                  | 13.6% |
| 4    | 7/27                             | 25.9% | 2/25                         | 8.0%  | 4/26                          | 15.4% | 3/23                          | 13.0% | 1/24                  | 4.2%  |
| 5    | 3/24                             | 12.5% | 3/26                         | 11.5% | 2/24                          | 8.3%  | 1/21                          | 4.8%  | 5/24                  | 20.8% |
| 6    | 5/28                             | 17.9% | 2/26                         | 7.7%  | 1/24                          | 4.2%  | 1/26                          | 3.8%  | 5/22                  | 22.7% |
| 7    | 4/26                             | 15.4% | 1/25                         | 4.0%  | 4/27                          | 14.8% | 1/27                          | 3.7%  | 3/24                  | 12.5% |
| 8    | 4/27                             | 14.8% | 1/24                         | 4.2%  | 3/24                          | 12.5% | 1/24                          | 4.2%  | 1/24                  | 4.2%  |

Method 2

Estimated Percentage of Implants Reported as Early Deaths in  
Dominant Lethal Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, x 3 | Sch 1000 BR<br>42 mg/kg, x 3 | Sch 1000 BR<br>135 mg/kg, x 3 | Sch 1000 BR<br>421 mg/kg, x 3 | DBP<br>100 mg/kg, x 3 |
|------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1    | 1.0                              | 6.8                          | 3.6                           | 1.5                           | 20.4                  |
| 2    | 1                                | 4.6                          | 1.5                           | 7.3                           | 18.2                  |
| 3    | 1.8                              | 2.3                          | 4.9                           | 7.0                           | 3.5                   |
| 4    | 7.0                              | 3.0                          | 4.0                           | 5.3                           | 2.1                   |
| 5    | 7.7                              | 6.1                          | 4.1                           | 3.4                           | 5.4                   |
| 6    | 6.0                              | 3.8                          | 2.0                           | 2.1                           | 5.0                   |
| 7    | 6.0                              | 1.9                          | 8.2                           | 7.8                           | 6.8                   |
| 8    | 3.0                              | 7.9                          | 4.0                           | 3.1                           | 2.3                   |

Method 3:

Mean Number of Live Implants and Late Deaths per Pregnancy  
Dominant Lethal Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, x 3 | Sch 1000 BR<br>42 mg/kg, x 3 | Sch 1000 BR<br>135 mg/kg, x 3 | Sch 1000 BR<br>421 mg/kg, x 3 | DBP<br>100 mg/kg, x 3 |
|------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1    | 12.38                            | 11.27                        | 11.38                         | 12.77                         | 8.46                  |
| 2    | 11.66                            | 11.48                        | 11.45                         | 11.92                         | 8.39                  |
| 3    | 12.29                            | 11.88                        | 12.78                         | 12.38                         | 11.68                 |
| 4    | 11.15                            | 11.68                        | 12.31                         | 12.80                         | 11.87                 |
| 5    | 11.96                            | 11.77                        | 12.78                         | 12.18                         | 11.67                 |
| 6    | 11.54                            | 11.77                        | 12.83                         | 11.77                         | 11.68                 |
| 7    | 11.63                            | 12.28                        | 11.91                         | 11.81                         | 11.21                 |
| 8    | 11.85                            | 11.64                        | 10.96                         | 10.96                         | 10.92                 |

METHOD 2:

Estimates of  $\theta$ , the Expected  
Proportion of Early Deaths  
Extra Binomial Variation Assumed

| Week | Treatment Group | $\hat{\theta} \pm S.E. \hat{\theta}$<br>$\hat{\theta} = \log \theta / (1 - \theta)^{-1}$ |
|------|-----------------|------------------------------------------------------------------------------------------|
| 1    | 1               | -3.480 ± 0.4025                                                                          |
|      | 2               | -2.857 ± 0.3075                                                                          |
|      | 3               | -3.394 ± 0.3500                                                                          |
|      | 4               | -4.192 ± 0.3413                                                                          |
|      | 5               | -1.291 ± 0.1798*                                                                         |
| 2    | 1               | -3.714 ± 0.4158                                                                          |
|      | 2               | -3.364 ± 0.3000                                                                          |
|      | 3               | -3.307 ± 0.3445                                                                          |
|      | 4               | -3.802 ± 0.4945                                                                          |
|      | 5               | -1.503 ± 0.1972*                                                                         |
| 3    | 1               | -4.289 ± 0.5395                                                                          |
|      | 2               | -3.731 ± 0.4103                                                                          |
|      | 3               | -2.560 ± 0.2660                                                                          |
|      | 4               | -3.493 ± 0.3487                                                                          |
|      | 5               | -3.228 ± 0.3460                                                                          |
| 4    | 1               | -2.594 ± 0.3050                                                                          |
|      | 2               | -3.466 ± 0.4824                                                                          |
|      | 3               | -2.173 ± 0.3958                                                                          |
|      | 4               | -2.892 ± 0.3725                                                                          |
|      | 5               | -3.504 ± 0.5050                                                                          |
| 5    | 1               | -3.597 ± 0.3940                                                                          |
|      | 2               | -3.183 ± 0.3103                                                                          |
|      | 3               | -3.153 ± 0.3106                                                                          |
|      | 4               | -3.350 ± 0.3738                                                                          |
|      | 5               | -2.855 ± 0.2801                                                                          |
| 6    | 1               | -2.614 ± 0.2992                                                                          |
|      | 2               | -2.784 ± 0.4181                                                                          |
|      | 3               | -3.890 ± 0.5772                                                                          |
|      | 4               | -3.961 ± 0.4769                                                                          |
|      | 5               | -2.945 ± 0.3888                                                                          |
| 7    | 1               | -2.761 ± 0.2891                                                                          |
|      | 2               | -3.943 ± 0.5071                                                                          |
|      | 3               | -2.718 ± 0.2759                                                                          |
|      | 4               | -2.498 ± 0.2505                                                                          |
|      | 5               | -2.415 ± 0.2852                                                                          |
| 8    | 1               | -3.251 ± 0.3222                                                                          |
|      | 2               | -3.500 ± 0.3892                                                                          |
|      | 3               | -3.171 ± 0.3337                                                                          |
|      | 4               | -3.389 ± 0.3709                                                                          |
|      | 5               | -3.772 ± 0.4462                                                                          |

1 = Distilled water, 10 ml/kg    2 = Sch 1000 SR, 42 mg/kg  
3 = Sch 1000 SR, 133 mg/kg    4 = Sch 1000 SR, 421 mg/kg  
5 = EMS, 100 mg/kg

\* = Significantly increased from the control level,  $P < 0.5$

See pages 23-24 for discussion on these methods.

**B** CYTOGENETIC STUDY IN CHINESE HAMSTERS OF SCH 1000-BR.

IRI Project No. 703308

Lab. Performing the Study: Inverack Research International.

Methods. See pages 8-11. Batch No. = 709078

RESULTS:

1) Dose-Finding Study:

Two M/group of Chinese hamsters (8-10 weeks old) were dosed orally by gavage with 100 to 2000 mg/kg/day Sch-1000 Br suspended in distilled water (10 ml/kg) for 5 days (8/13-17/80). All treated hamsters had piloerection from day 1 until the death or termination on day 14. One M died on day 7 and one on day 8 after 1000 mg/kg/day, and both M in 2000 mg/kg/day died on day 6, and they also had hypokinesia on day 3 and sedation on day 5, as shown in the following Table. Autopsy showed red lung and red/orange fluid in the gut of dead animals.

| Group no. | Dose Level mg/kg | Animal no. | Deaths |      | No. of Doses Given (No. of Hamsters) | Clinical Signs                                         | Duration Clinical Signs |
|-----------|------------------|------------|--------|------|--------------------------------------|--------------------------------------------------------|-------------------------|
|           |                  |            | No.    | Day  |                                      |                                                        |                         |
| 1         | 100              | 1          | 0/2    | X    | 5(2)                                 | Piloerection                                           | day 1-14                |
|           |                  | 2          |        |      |                                      |                                                        |                         |
| 2         | 250              | 3          | 0/2    | X    | 5(2)                                 | Piloerection                                           | day 1-14                |
|           |                  | 4          |        |      |                                      |                                                        |                         |
| 3         | 500              | 5          | 0/2    | X    | 5(2)                                 | hypokinesia, piloerection, sedation                    | day 1-14                |
|           |                  | 6          |        |      |                                      |                                                        |                         |
| 4         | 1000             | 7          | 2/2    | 7(1) | 5(2)                                 | hypokinesia, piloerection, sedation, coma, prostration | day 1-7                 |
|           |                  | 8          |        | 8(1) |                                      |                                                        |                         |
| 5         | 2000             | 9          | 2/2    | 4(2) | 5(2)                                 | Hypokinesia, piloerection, sedation                    | day 1-5                 |
|           |                  | 10         |        |      |                                      |                                                        |                         |

X = killed at end of 14 day observation period.

See page 19 for body weight.

2) Main Toxicity Study:

Six M/group of Chinese hamsters were dosed with 400 to 1142 mg/kg/day Sch 1000-Br for 5 days. The following Table was the results of this study. One M in 520 mg/kg/day group, 3 in 676 mg/kg/day group, 4 in 879 mg/kg/day group, and 5 in 1142 mg/kg/day died between days, 7 and 10. Autopsy showed no significant pathology in 400 and 1000 mg/kg/day groups, but other groups had red lungs, red/orange gut fluids, pale kidneys, patchy lungs, and they were dose-related.

| Group No. | Animal No. | Dose Level mg/kg | Deaths |                               | No. of Doses Given (No. of Hamsters) | Clinical Signs                                    | Duration of Clinical Signs                            |
|-----------|------------|------------------|--------|-------------------------------|--------------------------------------|---------------------------------------------------|-------------------------------------------------------|
|           |            |                  | No.    | Day                           |                                      |                                                   |                                                       |
| 1         | 11-16      | 400              | 0/6    | K                             | 5(6)                                 | Hypokinesia<br>Piloerection<br>Soiled snout       | on day 3<br>day 2-10<br>day 6-10                      |
| 2         | 17-22      | 520              | 1/6    | 9(1), K(5)                    | 5(6)                                 | Hypokinesia<br>Piloerection<br>Ataxia             | day 3-6<br>day 3-14<br>day 5-7                        |
| 3         | 23-28      | 676              | 3/6    | 7(1), 9(1),<br>10(1),<br>K(3) | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | days 3-5 and<br>7-8<br>day 4-5<br>day 3-14<br>day 5-8 |
| 4         | 28-34      | 879              | 4/6    | 7(2), 9(1),<br>10(1),<br>K(2) | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | day 3-4<br>day 5-8<br>day 2-14<br>day 4-10            |
| 5         | 35-40      | 1142             | 5/6    | 7(3), 8(2),<br>K(1)           | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | day 3-7<br>day 5-8<br>day 1-14<br>day 3-9             |

K = Killed at end of 14 day observation period.

See page 21 for body weight.

From the results of this study, the 100% (5 day) value was calculated as 722 mg/kg/day (500-950 mg/kg for 95% conf. limit), and the dose levels were chosen as 72 and 361 mg/kg/day for cytogenetic study.

### 3) Cytogenetic Study:

Ten M/group of Chinese hamster (20-27 g, 8-10 weeks old) were dosed by gavage with 72 or 361 mg/kg/day Sch 1000-BB in 10 ml/kg, along with a vehicle control group receiving 10 ml/kg of distilled water and a positive control group receiving 200 mg/kg/day ethylmethane sulphamate for 5 days (9/12-16/80).

Four hours after the last dose, each hamster was injected with 6 mg/kg ip colchicine dissolved in Hank's Balanced Salt Solution, and 2 hours later hamsters were killed by neck dislocation, then bone marrow smears were made from "femora" for metaphase examination of chromosomes. See pages 8-11 for detailed procedure for metaphase cell preparation.

Body weight of positive control group was slightly less than other groups (see page 22).

On M (no. 67) in 72 mg/kg/day group had diarrhea on day 3, and one M (No. 78) in 361 mg/kg/day group was "subdued" on day 3 with closed eyes, and No. 79 was found moribund on the morning of day 5, and it was killed in extremis, but autopsy did not show any specific pathology.

The following Table was the summary of metaphase chromosome analysis:

Cytogenetic Analysis of Chinese Hamster Bone Marrow Cells

Sch 1000 BR

Chromatid/Chromosomal Aberrations Scored

Table 1

Sampling Time: 6

| Group                                    | Number of Spreads Observed | Observed Aberrations |       |         |            |       |         | Miscellaneous                                         |
|------------------------------------------|----------------------------|----------------------|-------|---------|------------|-------|---------|-------------------------------------------------------|
|                                          |                            | Chromatid            |       |         | Chromosome |       |         |                                                       |
|                                          |                            | Gap                  | B w F | B w/o F | Gap        | B w F | B w/o F |                                                       |
| Distilled water<br>10 ml/kg x 3          | 300                        | 4                    | 1     | 7       | 1          | 0     | 0       | -                                                     |
| Sch 1000 BR<br>12 mg/kg x 3              | 500                        | 9                    | 1     | 1       | 3          | 1     | 0       | 1 Chromatid fragment                                  |
| Sch 1000 BR<br>161 mg/kg x 3             | 450                        | 14                   | 2     | 1       | 3          | 1     | 0       | -                                                     |
| Ethyl methanesulphonate<br>200 mg/kg x 3 | 570                        | 29                   | 25    | 0       | 8          | 1     | 0       | 2 Multi Aberrations<br>5 Exchanges<br>1 Translocation |

\* One animal died

B w F = Break with Fragment

B w/o F = Break without Fragment

The following summary was also submitted:

"The quality of the metaphase chromosomes observed was variable. It was possible, however, to read 50 metaphase spreads for each of the 40 animals used in the cytogenetic test (apart from one hamster which died). Details are given in Appendix Table 3.

Chromosomal damage was seen in all 4 groups. Both gaps and breaks were observed. Most damage was seen in the positive control group, 200 mg EMS/kg, in which was observed not only gaps and breaks, but exchanges, multi-aberrations and a translocation. There was little difference in the numbers or frequencies of aberrations between the low and high dose groups of Sch 1000 BR. In

the vehicle control group fewer gaps and breaks were observed than the other groups. There is statistical evidence that Sch 1000 BR does increase the incidence of cells with any type of aberration in both the low and high dose groups. The significance levels were 0.05 and 0.07 respectively. If cells containing gaps only are rejected from analysis, then there were no significant differences between the vehicle control group and the groups dosed with Sch 100 BR. 2)

C. POINT MUTAGENIC ACTIVITY OF SCH 1000-BR IN SALMONELLA TYPHIMURIUM:

Lab. Performing the Study: Boehringer Ingelheim KG in Rhein

NOTE: This study was repetition of earlier report dated 7/10/80.

Methods: Sch 1000-BR was tested from 10 to 10000 ug-per plate on TA 1535, TA 1537, TA 1538, TA 100, and TA 98 with/without S-9 metabolic activation, along with a vehicle control and respective positive control groups. See pages 1-9 for detailed procedure.

RESULTS: See the following two Tables.

Table 1: Induction of His<sup>r</sup> mutations in *S. typhimurium* by the substance Sch 1000-Br without metabolic activation with S-9 mix.  
(Experimental part No. NCI 0002701)

| substance <sup>a</sup> | concentration<br>(µg/plate) | number of h <sub>i</sub> <sup>b</sup> |     | revertants per plate <sup>b, c</sup> |    |        |     |       |     |      |    |
|------------------------|-----------------------------|---------------------------------------|-----|--------------------------------------|----|--------|-----|-------|-----|------|----|
|                        |                             | TA1535                                |     | TA1537                               |    | TA1538 |     | TA100 |     | TA98 |    |
|                        |                             | n                                     | ̄   | n                                    | ̄  | n      | ̄   | n     | ̄   | n    | ̄  |
| control                |                             | 29                                    |     | 17                                   |    | 10     |     | 191   |     | 17   |    |
|                        |                             | 42                                    |     | 12                                   |    | 15     |     | 180   |     | 16   |    |
|                        |                             | 36                                    | 36  | 20                                   | 16 | 16     | 16  | 184   | 185 | 13   | 15 |
| Sch 1000-Br            | 10                          | 37                                    |     | 16                                   |    | 13     |     | 178   |     | 12   |    |
|                        |                             | 39                                    |     | 7                                    |    | 13     |     | 205   |     | 22   |    |
|                        |                             | 30                                    | 35  | 12                                   | 12 | 15     | 14  | 207   | 197 | 17   | 12 |
|                        | 100                         | 36                                    |     | 17                                   |    | 27     |     | 184   |     | 15   |    |
|                        |                             | 32                                    |     | 26                                   |    | 16     |     | 161   |     | 18   |    |
|                        |                             | 33                                    | 34  | 15                                   | 19 | 12     | 18  | 176   | 174 | 24   | 19 |
|                        | 1000                        | 37                                    |     | 10                                   |    | 14     |     | 174   |     | 14   |    |
|                        |                             | 30                                    |     | 13                                   |    | 11     |     | 171   |     | 17   |    |
|                        |                             | 40                                    | 36  | 11                                   | 11 | 18     | 14  | 189   | 178 | 23   | 18 |
|                        | 5000                        | 38                                    |     | 16                                   |    | 20     |     | 160   |     | 20   |    |
|                        |                             | 38                                    |     | 21                                   |    | 11     |     | 218   |     | 16   |    |
|                        |                             | 38                                    | 38  | 19                                   | 19 | 15     | 15  | 181   | 186 | 20   | 19 |
|                        | 10000                       | 49                                    |     | 17                                   |    | 16     |     | 188   |     | 17   |    |
|                        |                             | 45                                    |     | 13                                   |    | 13     |     | 178   |     | 16   |    |
|                        |                             | 42                                    | 45  | 12                                   | 13 | 16     | 15  | 176   | 174 | 14   | 14 |
| 4-NQO                  | 1                           | 171                                   |     | 47                                   |    | 258    |     | >3000 |     | 548  |    |
|                        |                             | 179                                   |     | 54                                   |    | 221    |     | >3000 |     | 531  |    |
|                        |                             | 150                                   | 167 | 39                                   | 45 | 213    | 231 | >3000 |     | 17   | 54 |

<sup>a</sup> control, 50 µl DMSO/plate; 4-NQO, 4-nitroquinoline-1-oxide  
<sup>b</sup> titer of the bacterial suspensions (cells/ml): TA1535,  $2.9 \times 10^9$  (290, 292, 281); TA1537,  $2.0 \times 10^9$  (206, 190, 201); TA1538,  $2.2 \times 10^9$  (222, 208, 237); TA100,  $0.5 \times 10^9$  (58, 49, 51); TA98,  $1.5 \times 10^9$  (156, 160, 171); the data in parentheses give the number of colonies counted per plate.  
<sup>c</sup> n, absolute number of revertants per plate; ̄, mean value of the parallel plates; IF, infection.

Table 2: Induction of his<sup>r</sup> mutations in *E. typhimurium* by the substance Sch 1000-Br with metabolic activation with S-9 mix.  
 (Experimental part No. MUT 0007/02)

| substance <sup>a</sup> | concentration<br>(µg/plate) | number of his <sup>r</sup> |           | revertants per plate <sup>b, c</sup> |           |        |           |       |           |      |           |
|------------------------|-----------------------------|----------------------------|-----------|--------------------------------------|-----------|--------|-----------|-------|-----------|------|-----------|
|                        |                             | TA1535                     |           | TA1537                               |           | TA1538 |           | TA100 |           | TA98 |           |
|                        |                             | n                          | $\bar{x}$ | n                                    | $\bar{x}$ | n      | $\bar{x}$ | n     | $\bar{x}$ | n    | $\bar{x}$ |
| control                |                             | 24                         |           | 23                                   |           | 17     |           | 133   |           | 28   |           |
|                        |                             | 22                         |           | 20                                   |           | 26     |           | 153   |           | 29   |           |
|                        |                             | 11                         | 23        | 18                                   | 20        | 70     | 21        | 127   | 138       | 40   | 32        |
| Sch 1000-Br            | 10                          | 26                         |           | 12                                   |           | 17     |           | 143   |           | 30   |           |
|                        |                             | 13                         |           | 18                                   |           | 31     |           | 139   |           | 30   |           |
|                        |                             | 27                         | 22        | 15                                   | 15        | 19     | 22        | 127   | 134       | 34   | 31        |
|                        | 100                         | 18                         |           | 14                                   |           | 27     |           | 165   |           | 25   |           |
|                        |                             | 22                         |           | 20                                   |           | 17     |           | 161   |           | 24   |           |
|                        |                             | 18                         | 20        | 18                                   | 21        | 24     | 23        | 150   | 159       | 28   | 26        |
|                        | 1000                        | 23                         |           | 17                                   |           | 22     |           | 191   |           | 27   |           |
|                        |                             | 22                         |           | 21                                   |           | 33     |           | 140   |           | 27   |           |
|                        |                             | 29                         | 22        | 19                                   | 19        | 29     | 28        | 131   | 141       | 27   | 27        |
|                        | 5000                        | 14                         |           | 21                                   |           | 11     |           | 158   |           | 22   |           |
|                        |                             | 27                         |           | 25                                   |           | 34     |           | 187   |           | 45   |           |
|                        |                             | 25                         | 22        | 26                                   | 24        | 21     | 22        | 133   | 159       | 24   | 30        |
|                        | 10000                       | 23                         |           | 16                                   |           | 18     |           | 171   |           | 25   |           |
|                        |                             | 21                         |           | 26                                   |           | 24     |           | 153   |           | 24   |           |
|                        |                             | 18                         | 21        | 18                                   | 20        | 27     | 23        | 150   | 158       | 29   | 28        |
| B(a)P <sup>a</sup>     | 5                           | 18                         |           | 22                                   |           | 65     |           | 640   |           | 193  |           |
|                        |                             | 20                         |           | 24                                   |           | 56     |           | 558   |           | 186  |           |
|                        |                             | 24                         | 21        | 24                                   | 24        | 51     | 54        | 572   | 590       | 232  | 208       |

<sup>a</sup> control, 50 µl DMSO/plate; B(a)P, benzo(a)pyrene

<sup>b</sup> titer of the bacterial suspensions (cells/ml): TA1535,  $3.1 \times 10^9$  (335, 292, 308); TA1537,  $1.5 \times 10^9$  (112, 160, 191); TA1538,  $2.6 \times 10^9$  (282, 254, 252); TA100,  $0.5 \times 10^9$  (44, 50, 60); TA98,  $2.4 \times 10^9$  (243, 256, 229) the data in parentheses give the number of colonies counted per plate.

<sup>c</sup> n, absolute number of revertants per plate;  $\bar{x}$ , mean value of the parallel plates; IF, infection; sterility control(s); S-9 mix was germ-free.

D. MICRONUCLEUS TEST OF SCH 1000 BR:

Project NO 703292 and Extension 1

Lab. Performing the Study: Inveresk Research International

Methods: See pages 8-15.

RESULTS:

1). Two Day LD<sub>50</sub> Study:

Two M/group of CD-1 mice (8-10 weeks, 35-40 g) were dosed by gavage in the dose-finding study, and 5 M/group were dose for 2 days in the main toxicity study to determine the oral LD<sub>50</sub>. The LD<sub>50</sub> was estimated as 1044 mg/kg from the following Tables. Toxic signs were the same as before and as in the hamster studies.

Two Day LD<sub>50</sub> Study  
Dose Finding Test  
SCH 1000 BR

| Group No. | Dose Level mg/kg | Deaths |                   | No. of Doses Given (No. of Mice) | Clinical Signs                                    | PM-Observations (No. of mice)                        | Duration of Clinical Signs |
|-----------|------------------|--------|-------------------|----------------------------------|---------------------------------------------------|------------------------------------------------------|----------------------------|
|           |                  | No.    | Day (No. of Mice) |                                  |                                                   |                                                      |                            |
| 1         | 100              | 0/2    | K                 | 2(2)                             | NAD                                               | NAD                                                  | None                       |
| 2         | 250              | 0/2    | K                 | 2(2)                             | NAD                                               | NAD                                                  | None                       |
| 3         | 500              | 0/2    | K                 | 2(2)                             | Piloerection                                      | NAD                                                  | 2-5 days                   |
| 4         | 1000             | 1/2    | 3(1)<br>K(1)      | 2(2)                             | Hypokinesia<br>Piloerection<br>Ataxia             | Lungs red, kidneys pale.<br>1/2                      | 2-8 days                   |
| 5         | 2000             | 2/2    | 5(1)<br>7(1)      | 2(2)                             | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | Lungs pink, kidneys pale, gut contents fluid.<br>2/2 | 2-6 days                   |

NAD = No abnormalities detected.

K = Animals killed at end of 14 day observation period.

Two Day LD<sub>50</sub> Study  
Main Toxicity Test  
JUN 1968 SF

| Group No. | Dose Level mg/kg | Deaths |                      | No. of Doses Given (No. of Mice) | Clinical Signs                        | PM Observations (No. of Mice)                                                  | Duration of Clinical Signs |
|-----------|------------------|--------|----------------------|----------------------------------|---------------------------------------|--------------------------------------------------------------------------------|----------------------------|
|           |                  | No.    | Day (No. of Mice)    |                                  |                                       |                                                                                |                            |
| 1         | 350              | 0/6    | 0                    | 2(6)                             | Piloerection                          | NAD                                                                            | 2-3 days                   |
| 2         | 525              | 1/6    | K(5)<br>5(1)         | 2(6)                             | Hypokinesia<br>Piloerection           | Autolysis<br>1/6                                                               | 2-3 days                   |
| 3         | 788              | 1/6    | K(5)<br>5(1)         | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Lungs pink,<br>kidneys pale,<br>liver mottled.<br>1/6                          | 2-3 days                   |
| 4         | 1181             | 4/6    | K(2)<br>4(2)<br>5(2) | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Lungs pink,<br>kidneys pale,<br>liver mottled.<br>2/6                          | 2-3 days                   |
| 5         | 1772             | 6/6    | K(2)<br>5(5)         | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Gut contents -<br>fluid, kidneys<br>pale, liver<br>mottled, auto-<br>lysis 5/6 | 1-4 days                   |

NAD - No abnormalities detected.

K - Animals killed at end of 14 day observation period.

2) Micronucleus Tests:

A total of 4 series of tests were done, and the first 3 tests used a standard micronucleus protocol (project 703292), but the 4th test, (Project 703292, Extension-1) used a modified version of the same protocol, due to positive results in the first 3 tests.

The first study was done in two part, as shown below.

Phase 1:

"A small but significant increase in the frequency of micronuclei was detected when 15 mice were dosed on 2 consecutive days (0 h and 24 h) with 803.5 mg/kg (0.8 x LD<sub>50</sub>) SCH 1000 BR. Since only the 72 h sample from time 0 h was positive, as judged by the number of micronuclei within any one group, Phase 2 was initiated with a probable positive response for this particular time sample in mind. In Phase 1 the number of micronucleated normochromatic erythrocytes was not significantly increased.

The vehicle control group did not show an increased number of micronuclei."

Phase 2:

"Again, small increases in the numbers of micronuclei were observed when 3 groups of 5 mice were dosed at 0 h and 24 h with: 0.4 x LD<sub>50</sub>, 401.8 mg/kg/day SCH 1000 BR; 0.6 x LD<sub>50</sub>, 602.6 mg/kg/day SCH 1000 BR; and 0.8 x LD<sub>50</sub>, 803.5 mg/kg/day SCH 1000 BR.

Sampling was done at 72 h only from time 0 h.

All 3 groups of mice dosed with the test compound had increased numbers of micronuclei when compared with the vehicle control group of mice. In one dose group (0.4 x LD<sub>50</sub>) there was more than a 2-fold increase: 20 micronuclei per 5000 polychromatic erythrocytes (PCE) from 5 mice (frequency 0.40%), compared to 8 micronuclei per 5000 PCE from 5 mice (frequency 0.16%) for the vehicle control group. The frequencies of micronuclei in the 0.6 x LD<sub>50</sub> and 0.8 x LD<sub>50</sub> dose group were 0.26% and 0.24% respectively. As in Phase 1, the number of micronucleated normochromatic erythrocytes was marginally, but not significantly, increased.

However, the obtained results failed to indicate any correlation between increasing dose levels and severity of response in terms of induced micronuclei in either polychromatic or normochromatic erythrocytes. Consequently, it was decided to repeat the Phase 2 experiment in its entirety in an attempt to resolve the remaining ambiguities."

Phase 2-Repeat Study:

"Again, 3 groups of 5 mice were dosed at 0 h and 24 h with: 0.4 x LD<sub>50</sub>, 401.8 mg/kg/day SCH 1000 BR; 0.6 x LD<sub>50</sub>, 602.6 mg/kg/day SCH 1000 BR; and 0.8 x LD<sub>50</sub>, 803.5 mg/kg/day SCH 1000 BR.

Sampling was carried out, as before, at 72 h from time 0 h.

The 3 groups of mice dosed with SCH 1000 BR showed, as before, slight increases in the frequencies of micronuclei when compared with the vehicle control group. Once more, the 0.4 x LD<sub>50</sub> dose group showed the highest number of micronuclei, i.e. 16/5000 polychromatic erythrocytes (PCE) from 5 mice (frequency 0.32%), compared to 6 micronuclei/5000 PCE from 5 mice for the vehicle control group (frequency 0.12%). The increase in the 0.4 x LD<sub>50</sub> group, however, was largely due to one mouse having an unusually high proportion of micronucleated PCE (10 micronuclei, frequency 1%). None of the other mice in this dose group showed any significant response. When this one mouse was excluded from analysis, the mean frequency for the group was 0.15%. Normal levels of micronuclei were in all dose groups observed when normochromatic erythrocytes were analysed for presence of chromosome fragments.

All animals survived dosing in Phase 1, Phase 2 and the repeat study of Phase 2. 2)

The following Table was the summary of all 3 above studies:

**Microenclosure Test**  
**Number and Mean ± Microenclosed Polychromatized Erythrocytes**  
**(CA 1000 or)**

|                                                      | Number of Mice |      |      | Number of Microenclosed Polychromatized Erythrocytes |                         |            | Number of Microenclosed Mitochondrial Erythrocytes |      |      |
|------------------------------------------------------|----------------|------|------|------------------------------------------------------|-------------------------|------------|----------------------------------------------------|------|------|
|                                                      | 00 h           | 12 h | 96 h | 00 h                                                 | 12 h                    | 96 h       | 00 h                                               | 12 h | 96 h |
| Phase 1                                              |                |      |      |                                                      |                         |            |                                                    |      |      |
| Vehicle Control<br>Distilled Water                   | 5              | 5    | 5    | 3<br>0.10                                            | 11<br>0.22              | 11<br>0.22 | 0                                                  | 0    | 0    |
| 0.1 x 10 <sup>10</sup><br>50% 5 mg/kg<br>SCN 1000 or | 5              | 5    | 5    | 10<br>0.20                                           | 20 <sup>a</sup><br>0.40 | 10<br>0.20 | 0                                                  | 0    | 0    |
| Phase 2                                              |                |      |      |                                                      |                         |            |                                                    |      |      |
| Vehicle Control<br>Distilled Water                   | 5              | 5    | 5    | 3<br>0.10                                            | 11<br>0.22              | 11<br>0.22 | 0                                                  | 0    | 0    |
| 0.1 x 10 <sup>10</sup><br>50% 5 mg/kg<br>SCN 1000 or | 5              | 5    | 5    | 10<br>0.20                                           | 20 <sup>a</sup><br>0.40 | 10<br>0.20 | 0                                                  | 0    | 0    |
| Phase 2 (B)                                          |                |      |      |                                                      |                         |            |                                                    |      |      |
| Vehicle Control<br>Distilled Water                   | 5              | 5    | 5    | 3<br>0.10                                            | 11<br>0.22              | 11<br>0.22 | 0                                                  | 0    | 0    |
| 0.1 x 10 <sup>10</sup><br>50% 5 mg/kg<br>SCN 1000 or | 5              | 5    | 5    | 10<br>0.20                                           | 20 <sup>a</sup><br>0.40 | 10<br>0.20 | 0                                                  | 0    | 0    |

<sup>a</sup> Significant response for microenclosed group at 12 h of 1000 or

"On the basis of these test findings, SCH 1000 HF might appear to induce micronuclei at very low and occasionally detectable rates when administered at levels approximately half that of the  $LD_{50}$  value. To eliminate any spurious result or false conclusions arising from the small samples of animals, a different protocol was designed which, it was hoped, would provide additional and conclusive information about the possible clastogenic potential of the test compound. The findings from this modified micronucleus test are given below."

Phase 2 - Extension Study (Project 703292, Extension No. 1)

The results of this study were in the following Table and Appendix Table 4.

MICRONUCLEUS TEST  
Number and Mean of Micronucleated Polychromatic Erythrocytes  
SCH 1000 HF

| Phase 2 - Extension Study                        | Number of NCE | Number of Micronuclei Observed | Number of Micronucleated Normochromatic Erythrocytes |
|--------------------------------------------------|---------------|--------------------------------|------------------------------------------------------|
|                                                  | 72 h          | 72 h                           |                                                      |
| Vehicle Control<br>15 ml/kg<br>distilled water   | 15            | 19<br>0.13                     | 10                                                   |
| 0.5 x $LD_{50}$<br>100 mg/kg<br>SCH 1000 HF      | 15            | 23<br>0.15                     | 6                                                    |
| Positive Control<br>50 mg/kg<br>Cyclophosphamide | 15            | 170<br>1.10                    | 130                                                  |

NCE = Polychromatic erythrocytes

"No increased frequencies of micronuclei were observed when 15 mice were dosed at 0 h and 24 h with  $0.5 \times LD_{50}$ , 502.2 mg/kg/ day SCH 1000 BR. The number of micronucleated normochromatic erythrocytes also was not increased. Sampling was done at 72 h only.

The positive control animals, on the other hand, showed huge increases in the numbers of micronuclei both in polychromatic and normochromatic erythrocytes. The frequency values in the 2 types of erythrocytes were 1.19% and 0.93% respectively.

There was nothing to suggest that SCH 1000 BR induced micronuclei in CD-1 mice when tested at a dose level of  $0.5 \times LD_{50}$ . Accordingly the weak positive findings from the 3 previous micronucleus tests were in no way confirmed or sustained by evidence from this extension study.

As before, no animal deaths were recorded during or after dosing with the test substance. "

The following were the conclusions submitted on these series tests:

There was no strong and reproducible evidence to show that SCH 1000 BR, in these tests, induced micronuclei at rates which were clearly above the levels at which micronuclei can spontaneously occur. The number of micronuclei observed in the first 3 tests with SCH 1000 BR indicated a slight, albeit ambiguous, effect by the test substance particularly at dose levels approximately half the LD<sub>50</sub> value. These findings were, however, not substantiated when a final, more comprehensive micronucleus test using the 0.5 x LD<sub>50</sub> dose level was run.

The pattern of responses in the series of tests was as follows:

| No. of Animals | Dose Level             | Test 1 | Test 2 | Test 3 | Test 4 |
|----------------|------------------------|--------|--------|--------|--------|
| 5              | 0.8 x LD <sub>50</sub> | +      | -      | -      | 0      |
| 5              | 0.6 x LD <sub>50</sub> | 0      | -      | -      | 0      |
| 15             | 0.5 x LD <sub>50</sub> | 0      | 0      | 0      | -      |
| 5              | 0.4 x LD <sub>50</sub> | 0      | +      | +      | 0      |

- + increase in micronuclei
- no increase in micronuclei
- 0 not tested

The + result in Test 3 mice given 0.4 x LD<sub>50</sub> was due entirely to one mouse out of the 5 treated. The minimal increases in micronucleus frequency observed and the lack of any dose related response led to the conclusion that SCH 1000 BR probably was not a clastogen.

### A. Toxicology

- (1) Mice (Strain, ~~Swiss~~) were given ~~PO~~ by intubation ~~at~~ doses of 0, 2 or 10 mg/kg from day 6-15 of pregnancy. Dams were sacrificed on day 18. Fetuses were examined externally; 2/3 were cleared and stained for skeletal abnormalities and 1/3 were examined for visceral defects.

Weight gain of dams was normal. None of the dams died. Number of live fetuses and total number of implantations were decreased and total number of resorptions was increased but these were within physiologic limits. None of fetuses died. Weight of fetuses was comparable in all groups. No malformations, either skeletal or visceral, were reported to occur.

- (2) Pregnant rats were dosed in the same manner as outlined above.

No behavioral effects were seen. None of the dams died. Weight gain of dams was normal. Number of implantation sites, live fetuses, dead fetuses, resorption sites, and mean fetal weight, were comparable in all groups. In hi dose group one fetus had anophthalmia and hydrocephalus interna.

- (3) Due to the unusual abnormality (anophthalmia and hydrocephalus interna) found in the rat study above, study was repeated by Boehringer.

Same schedule followed as outlined above except doses were 0, 10 or 20 mg/kg.

One hi dose dam died. All parameters measured above in the previous study were comparable in treated and control groups.

- (4) Pregnant rabbits (Russian) were given 0, 2 or 10 mg/kg PO by intubation from day 6-18 of pregnancy. Dams were sacrificed on day 9. One-half fetuses were sacrificed and remainder were placed in an incubator for 24 hours. The latter were examined for visceral and skeletal anomalies.

No unusual behavioral effects were noted. No dams died. Weight gain of dams was reduced in treated groups (125 gm., controls, 25 gm., low and 9 gm., Hi). Number of live and dead fetuses and number of resorptions was comparable in all groups but number of implantation sites was lower in hi dose group than controls. All the pups survived the 24 hour incubation period. Average fetal weight was comparable in all groups. No skeletal or visceral anomalies were reported; three fetuses in hi dose groups showed slight alteration in gall bladder which was considered spontaneous.

Termination of Pregnancy in the First Trimester of Pregnancy

Rabbits were divided into two groups

and administered the following

dose levels: 250, 1000 mg/kg body weight

administration and duration

Administered by stomach tube, day 6-10 day of pregnancy dose was 250 mg/kg body weight, day 11-14 day of pregnancy

Results

Clinical Symptoms

Rabbits behaved normally during study

Body Weight

Animals Pregnant in Sch 1000 dose groups had slight weight loss.

Spontaneous Births and Abortions

All mothers carried their fetuses until surgery.

Deaths

No deaths occurred during this study.

Conceptions

Conception rates were normal and similar

Implantations

There was a reduced number of implantations in the 10.0 mg/kg group. Difference was not significant.

Living Fetuses

No significant difference was noted in the number of living fetuses between the groups.

Reabsorptions

The number of reabsorptions was similar and unremarkable.

Survival Rate

Survival rate was 100% in all groups.

Litter Weight

Litter weight was similar in all groups.

Malformations

No major malformations were noted in the fetuses. Bone staining using Alizarin red S was carried out as part of the examination.

**C. TESTING OF SCH 1000 FOR FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE IN RATS**  
(SECRET) (1/30/75 review)

**Rats**

Sch 1000

**#Animals/Dose Levels:** 10 M & 20 F

**Dose Levels:** 10, 30, and 90 mg/kg

**Administration:** Added to food, 75 da prior to mating for males and 14 days for females. Treated rats mated with untreated rats in both cases.

**Results:**

**Clinical Signs:** Behavior of animals was normal, no mortalities.

**Body Weights:** Body weight of both the males and females was similar.

**Food Consumption:** Food consumption was similar in all groups. The rats in the 10.0 mg/kg seems to consume slightly more food than rats in other groups.

**Mating Period:** There were no significant differences in the conception and implantation rates between the groups.

**Weight of Testicles:** Macroscopic and histological examinations of the testes gave no indication of damage due to Sch 1000.

Litter data of Pregnant Females:

The percentage of implantations, living fetuses and resorptions was similar in all groups. There were no dead fetuses in any group. The mean fetus weight was slightly higher in the low and high dose groups. Examination with a Gillian Red 5 staining method revealed no major abnormalities in the skeletons of the fetuses.

Results of the Treated Females Clinical Observations:

Normal behavior, no mortalities.

Body Weight: Body weight in these females was normal with a constant weight increase.

Food Intake: Food intake was comparable in all groups.

Data from 14 Day Gestation:

There were no differences in the average number of implantations between the control and the treated animals. The number of resorptions in the 10 and 90 mg/kg groups was higher than resorptions in the control groups.

21 Day Data-Duration of Gestation:

The duration of gestation in these pregnant rats was normal. The mean litter weight of the pups in the 10 mg/kg group was slightly less than the weight of the pups in the other 3 groups. The differences in weight between the groups was not significant.

Mortality: at birth

Only one pup in the 10 mg/kg group was found dead at birth.

Malformations: No major malformations were observed when pups were examined at autopsy.

Data from F<sub>1</sub> Generations:

Mating of Mothers-

Fourteen days after weaning of the pups, the dams were mated for the 2nd time. The conception rate in the control, 30 and 90 mg/kg groups was 100% and 70% in the 10 mg/kg group.

Total Implantations: Total implantations were similar in all groups.

Viable Fetuses: The mean value for viable pups was similar in all groups.

Percent Implantations: The percentage of implantations in the 10 mg/kg group was lower than in the 3 other groups.

Terminal Studies: No serious abnormalities were observed when these pups were examined at autopsy.

D. Testing of the substance - Sch 1000 for its Effects on Rats in the Perinatal and Postnatal Phase (Segment III)

Rats Sch 1000

#Animals/Dose Level: 20F.

Doses: 10, 30, and 90 mg/kg plus control.

Administration and Duration: Given by stomach tube from day 14 of gestation until the weaning of pups on the 21st day after parturition.

Results:

Clinical Signs: Behavior of Pregnant rats was normal.

Bodyweights: Body weight of the dams was comparable.

Conception Rates: All conception rates were between 90 and 100%.

Duration of Gestation: All the dams delivered their young on the 21st and 22nd days of pregnancy.

Autopsy of Dams:

No abnormalities were observed in the dams when they were examined by gross methods at autopsy.

Data concerning Pups:

Litter Size: The mean number of pups in each litter was within normal limits.

Body Weight of Pups at Birth: The mean body weight of the pups between groups was comparable.

Mortality 0-21 days: The mortality of the pups in the 90 mg/kg groups was higher (16.9%) than in the control (11.9%) the 10 mg/kg (11.0%) and the 30 mg/kg group (8.8%). Difference was not significant.

Finding in 21 days old pups: The mean litter weight of the pups in the control and 90 mg/kg was slightly lower than the weight of the pups in the other 2 groups.

Autopsy Findings - Pups: Examination of pups at autopsy did not reveal any remarkable findings.

Mating of Pups F1 Generation:

Several pups from the control and 90 mg/kg groups were cross mated, i.e. female pups from control with male pups from 90 mg/kg group. The conception rates were found to be 65 to 92%.

**E. SEGMENT I REPRODUCTION STUDY IN RAT BY ORAL (12/17/76 review)**

Lab performed study: C. H. Schriber Ingelheim in JAPAN. The study is done according to the notification No. 429 of 1/31/74 of Japanese National Council of Pharmacy. Methods for reproduction studies of new drug.

No. of Animals/Level: 20 M (7 weeks old, 230±27 g) + 20 F (10 weeks old, 193±7 g)

Strain: Wistar Sprague-Dawley

| Dosage Levels | GROUP                 | Dose (mg/kg/day) |
|---------------|-----------------------|------------------|
|               | D <sub>0</sub>        | 0                |
|               | D <sub>1</sub> (Low)  | 4                |
|               | D <sub>2</sub> (Mid)  | 50               |
|               | D <sub>3</sub> (High) | 500              |

Duration and Route: Males were treated for 60 days before the mating through copulation (17 to 20 days) and killed after the mating by gastric intubation.

Females were treated from 14 days before mating through day 7 of gestation by gastric intubation. Caesarean section was performed on day 21 of gestation under ether (5/24/76 to 8/4/76). Drug was dissolved in distilled water and administered in 0.5 ml/100 g.

Mortality: None

General Signs: It was reported that except one M (No. 017) of control group all M of control and low dose groups behaved "unobtrusively". No. 017 of control, No. 211(M) and 217(M) of mid dose group, and No. 307(M) of high dose groups had bronchial breathing sounds which started one or two weeks after beginning of treatment through mating, except the M of control group which started toward the end. 3 M of mid dose groups (No. 210, 216, and 217) and all M of high dose groups showed red "incrusted eyes" or nose starting one or two weeks after beginning of treatment through mating.

All F of high dose group had red increased eyes from about 3 or 4 days of treatment through several days and 2 F of mid dose groups had this sign during a few days after the start of treatment.

COMMENT:

All the results were given separately, for males in one section and for females in another section, which required more time for review.

Growth Effect:

M of high dose group showed depression in mean body weight gain starting around day 15 which became significantly different from controls after about 30 days through 40 days. Mean body weight of F of high dose group showed a decrease (loss) on the third day, but later it became only slightly depressed, and the total group weight gain during pregnancy was very slightly smaller in high dose, and very slightly larger in two low dose groups when compared to control group.

COMMENT:

500 mg/kg group in Figure 2 (p. 77) is not correct.

Food and Water Consumption:

Before mating the intake of food in F was significantly depressed in two high dose groups, and it remained slightly lower in high dose during gestation. No dose related differences in water consumption in F were observed, but some of values in two high dose groups were significantly lower than that of control F.

COMMENT:

No values for M on food consumption or water consumption was reported.

| Group Fertility Data:             |                         | Sub 1000 | Ref     | p.c.     | Sub 1     |
|-----------------------------------|-------------------------|----------|---------|----------|-----------|
| Males                             |                         | Control  | 5 mg/kg | 10 mg/kg | 100 mg/kg |
| Number mated                      |                         | 20       | 20      | 20       | 20        |
| Mortality                         |                         | 0        | 0       | 0        | 0         |
| Number mated                      |                         | 20       | 20      | 20       | 20        |
| Inseminated with female partner   |                         | 20       | 20      | 20       | 19        |
| - at first mating                 |                         | 20       | 20      | 20       | 19        |
| - at 2nd or period                |                         | 0        | -       | -        | 7         |
| Inseminated with female partner   |                         | 0        | 0       | 0        | 1         |
| - inseminated with substitute     |                         | -        | -       | -        | 0         |
| - not inseminated with substitute |                         | -        | -       | -        | 1         |
| with lesions in sex organs        |                         | 7        | 12      | 9        | 7         |
| Females                           |                         |          |         |          |           |
| Number mated                      |                         | 20       | 20      | 20       | 20        |
| Mortality before mating           |                         | 0        | 0       | 0        | 0         |
| Estrous cycles before mating      | normal                  | 20       | 20      | 20       | 15        |
|                                   | with spurious pregnancy | 0        | 0       | 0        | 2         |
|                                   | no cycles               | 0        | 0       | 0        | 0         |
| Number mated                      |                         | 20       | 20      | 20       | 20        |
| Estrous cycles during mating      | normal                  | 20       | 20      | 20       | 16        |
|                                   | with spurious pregnancy | 0        | 0       | 0        | 4         |
|                                   | no cycles               | 0        | -       | 0        | 0         |
| Inseminated from male partner     |                         | 20       | 20      | 20       | 19        |
| - pregnant                        |                         | 10       | 11      | 10       | 10        |
| - non-pregnant                    |                         | 0        | 0       | 0        | 7         |
| Not inseminated from male partner |                         | 0        | 0       | 0        | 1         |
| - inseminated from substitute     |                         | -        | -       | -        | 1         |
| - pregnant                        |                         | -        | -       | -        | 1         |
| - non-pregnant                    |                         | -        | -       | -        | 0         |
| - not inseminated from substitute |                         | -        | -       | -        | 0         |
| with lesions in sex organs        |                         | 0        | 0       | 0        | 0         |

Only one M (No. 311) in high dose group failed to inseminate two untreated substitute females, in addition to original F partner (No. 361), that was later inseminated from an untreated substitute M. 7 M of high dose group showed spurious pregnancy before the mating. Two F (one in control and another in high dose group) had no sperm, but their estrus cycles disappeared, and later they were found pregnant. Therefore, the copulation rate for M is 100% for all groups except high dose group with 95% (19/20). The pregnancy rate for F is reported as 100% for control and low dose group, 95% for mid dose (19/20), and 75% for high dose (15/20) including the one F that was pregnant by untreated M partner.

**COMMENT:**

- 1) Only 18/20 in mid dose and 13/20 in high dose group F seemed to be pregnant (Table 1, p. 34), that make pregnancy rate of 90% for mid and 65% for high dose group instead of 95% and 75% reported in p. 14.
- 2) It was reported that ovulation and nidation seemed to be slightly affected in F of high dose group.

**Spontaneous Parturition:** No. 358 of high dose and No. 260 of mid dose F on day 21.

**Necropsy:**

At the end of 12 day mating period or after insemination was demonstrated, all M were killed and necropsied. It was found that No. 208 had edematously swollen testes, No. 214 had edematously swollen prostate, and No. 218, hydronephrosis.

Among dams examined after caesarean section No. 064 of control and 250 of mid dose had hydronephrosis, and No. 358 of high dose had petechiae in gastric and intestinal mucosa.

**Weight of Male Sex Organs:**

Mean absolute weight of testes showed a dose-related depression, and it was

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Mean Litter Data:

|                                              | Cont. 1               | Cont. 2               | Cont. 3               | Cont. 4               |
|----------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| No. of dams pregnant                         | 20                    | 20                    | 20                    | 20                    |
| No. of dams died during pregnancy            | 0                     | 0                     | 0                     | 0                     |
| No. of dams with all fetuses dead            | 1                     | 0                     | 0                     | 0                     |
| No. of dams with live fetuses                | 19                    | 20                    | 19                    | 20                    |
| Total no. of corpora lutea                   | 287                   | 310                   | 287                   | 302                   |
| Ave. no. of corpora lutea per dam            | 15.11 <sup>a)</sup>   | 15.511.0              | 15.1 <sup>a)2.2</sup> | 15.1 <sup>a)2.1</sup> |
| Total no. of implantations                   | 265                   | 281                   | 269                   | 260                   |
| Ave. no. of implantations per dam            | 13.951.8              | 14.051.7              | 14.171.7              | 13.0 <sup>a)2.8</sup> |
| % impl. ntations/corpora lutea <sup>a)</sup> | 92.6                  | 91.8                  | 94.4                  | 83.6                  |
| Total no. of fetal deaths:                   |                       |                       |                       |                       |
| early resorptions                            | 22                    | 27                    | 18                    | 14                    |
| late resorptions                             | 15                    | 11                    | 15                    | 13                    |
| dead fetuses                                 | 7                     | 5                     | 3                     | 1                     |
| total                                        | 0                     | 5                     | 0                     | 0                     |
| Ave. no. of fetal deaths per dam             | 1.211.5               | 1.351.9               | 0.910.9               | 1.011.3               |
| % fetal deaths/implantations <sup>a)</sup>   | 8.2                   | 10.1                  | 10.3                  | 8.7                   |
| Total no. of live fetuses                    | 243                   | 256                   | 251                   | 155                   |
| Ave. no. of live fetuses per dam             | 12.8 <sup>a)2.1</sup> | 12.8 <sup>a)2.9</sup> | 13.2 <sup>a)1.6</sup> | 11.2 <sup>a)2.9</sup> |
| % live fetuses/implantations <sup>a)</sup>   | 91.8                  | 89.9                  | 89.2                  | 91.8                  |
| Sex ratio (male/female) <sup>a)</sup>        | 0.9                   | 1.0                   | 1.2                   | 1.2                   |
| Ave. fetal weight <sup>a)</sup>              | 5.4 <sup>a)0.3</sup>  | 5.5 <sup>a)0.2</sup>  | 5.4 <sup>a)0.3</sup>  | 5.4 <sup>a)0.4</sup>  |

a) calculated using litter value as a basic unit

**Malformation:** There were no external malformations of fetuses. In the skeletal examination, 100% of control, 170/256 of low, 167/251 of mid, and 104/155 of high dose fetuses, it was found that skeletal variations noted was not dose related or smaller in high dose group.

About half of fetuses were examined for the visceral malformation, and only one of control seemed to have veristic

**Comments:** It was reported by the sponsor that fetus No. 370-x of high dose had discurved (waved) ribs and another fetus (No. 053) of control had internal hydrocephalus as malformation.

**F. EFFECT OF METERED AEROSOL ON PREGNANCY OF THE RATS: E83-130: (2/22/84)**

**Lab. Performing the Study:** Huntingdon Research Center  
Huntingdon, Cambridshire, England

**Strain:** Not given.

**Methods:** 20 F/group of pregnant rats were treated (head only) for 15 min with metered aerosol from day 6 to 15 of gestation, daily dose of 0.512, 1.024, or 1.536 mg/kg/day Sch 1000 (Lot No. 30336), along with a sham dosed control group and vehicle dosed control group (Lot. No. 30335). On day 20, the rats were killed by CO<sub>2</sub> euthanasia and examined. Sch 1000 was a suspension in aerosol formulation, and it delivered 0.02 mg Sch 1000 per valve depression, and vehicle contained 24.4% Fluorocarbon 11, 52.9% Fluorocarbon 12, and 22.5% Fluorocarbon 114 propellants. For detailed methods and procedure, see pages 4-5, 8, and 14-16. Particle size was reported to be less than 0.01 µm (see page 14).

**COMMENTS:** (1) Pages 2 and 3 are missing from the submission.

(2) The unit of the particle size as 0.01 mg must be wrong.

Table 1

Table 1

Table 1

Table 1

Table 1

Table 1

Table 1

Table 1

No effect was produced by drug treatment. See Table 1 and Litter data below.

7. LITTER DATA:

TABLE 2

Gestational litter data

Group: 1 2 3 4 5  
 Drug: SHAM DOSED CONTROL VEHICLE DOSED CONTROL SCH 1000  
 Dose (mg/kg/day): - 0.512 1.024 1.536

| GROUP | NO. OF ANIMALS | PREGNANCY RATE % |     | VIALE YOUNG |            | DESCRIPTIONS |     | IMPLANT ATIONS | CORPORA LUTEA | LOSS % | FOETAL LOSS % | LITTER WT (g) | MEAN FOETAL WEIGHT (g) |      |
|-------|----------------|------------------|-----|-------------|------------|--------------|-----|----------------|---------------|--------|---------------|---------------|------------------------|------|
|       |                | 3 d              | 5 d | Total       | Early Late | Total        |     |                |               |        |               |               |                        |      |
| 1     | n=17           | 85.3             | 6.5 | 5.2         | 11.7       | 0.6          | 0.1 | 0.7            | 12.4          | 14.0   | 14.0          | 5.6           | 4.2                    | 3.01 |
| 2     | n=20           | 100.0            | 5.6 | 4.4         | 11.0       | 0.9          | -   | 0.9            | 11.0          | 14.0   | 17.5          | 7.1           | 4.2                    | 3.81 |
| 3     | n=18           | 90.0             | 5.7 | 5.0         | 10.7       | 1.1          | 0.1 | 1.2            | 11.8          | 14.0   | 15.5          | 9.1           | 4.2                    | 3.32 |
| 4     | n=18           | 94.7             | 5.7 | 5.6         | 11.2       | 0.4          | -   | 0.4            | 11.7          | 11.7   | 16.0          | 5.0           | 4.8                    | 3.94 |
| 5     | n=18           | 100.0            | 5.3 | 4.1         | 11.4       | 0.7          | 0.1 | 0.7            | 12.3          | 15.3   | 18.1          | 6.1           | 4.3                    | 3.40 |

Difference from control groups 1 and 2 statistically significant or  
 Wilcoxon test  
 p < 0.01

includes all surviving animals that provided evidence of implantation,  
 including those showing resorption

includes all animals bearing viable young at termination

b. Major and Minor Abnormalities

The following table shows the

total number of young showing

abnormalities in each group. The total number of young examined in each group is also shown.

The following table shows the

total number of young showing major abnormalities in each group. The total number of young examined in each group is also shown.

The following table shows the total number of young showing major abnormalities in each group.

The incidence of major, visceral and skeletal abnormalities was not generally affected by treatment.

c. Group Mean Incidence of Abnormalities:

| GROUP | NUMBER OF YOUNG     |           |        |                             |           |        |          |           |        |
|-------|---------------------|-----------|--------|-----------------------------|-----------|--------|----------|-----------|--------|
|       | MAJOR MALFORMATIONS |           |        | MINOR ANOMALIES             |           |        |          |           |        |
|       | Examined            | Total No. | Mean % | Visceral (Wilson Technique) |           |        | Skeletal |           |        |
|       |                     |           |        | Examined                    | Total No. | Mean % | Examined | Total No. | Mean % |
| 1     | 199                 | 0         | 0      | 39                          | 0         | 0      | 130      | 6         | 5.0    |
| 2     | 219                 | 0         | 0      | 43                          | 3         | 5.2    | 136      | 7         | 4.2    |
| 3     | 192                 | 1         | 0.4    | 59                          | 2         | 2.8    | 132      | 3         | 2.2    |
| 4     | 232                 | 1         | 0.4    | 45                          | 1         | 1.4    | 136      | 2         | 1.2    |
| 5     | 208                 | 0         | 0      | 67                          | 0         | 0      | 141      | 7         | 4.9    |

/ Young showing major malformations excluded

| Group     | No. of pups | Mean body weight (g) |            |
|-----------|-------------|----------------------|------------|
|           |             | At birth             | At 14 days |
| Control 1 | 10          | 4.8                  | 11.0       |
| Control 2 | 10          | 4.8                  | 11.0       |
| Test 1    | 10          | 4.2*                 | 10.0       |
| Test 2    | 10          | 4.2*                 | 10.0       |
| Test 3    | 10          | 4.2*                 | 10.0       |
| Test 4    | 10          | 4.2*                 | 10.0       |
| Test 5    | 10          | 4.2*                 | 10.0       |
| Test 6    | 10          | 4.2*                 | 10.0       |
| Test 7    | 10          | 4.2*                 | 10.0       |
| Test 8    | 10          | 4.2*                 | 10.0       |
| Test 9    | 10          | 4.2*                 | 10.0       |
| Test 10   | 10          | 4.2*                 | 10.0       |

\*Pups showing major malformations excluded.

\*Difference from control groups 1 and 2 statistically significant at Wilcoxon test (p < 0.05).

**6. ORAL TERATOLOGICAL TESTING IN RATS: E83-125:**

Lab. Performing the Study: **Boehringer Ingelheim GmbH  
Pharma-Research Japan.**

Strain: **SD-JCL Sprague-Dawley female albino  
(300 g average body weight)**

Measures of mortality of pregnant rats and their pups were similar to those of non-pregnant rats and pups. In the 1000 mg/kg/day group, 100% of the dams and 100% of the pups died during the dosing period. In the 100 mg/kg/day group, 100% of the dams and 100% of the pups died during the dosing period. In the 100 mg/kg/day group, 100% of the dams and 100% of the pups died during the dosing period. In the 100 mg/kg/day group, 100% of the dams and 100% of the pups died during the dosing period.

Ret No. 10 had red, increased eyes and nose from day 10 and Ret No. 11 from day 10 in 100 mg/kg/day group.

Ret No. 10 had red, increased eyes and nose from day 10 and Ret No. 11 from day 10 in 100 mg/kg/day group.

Ret No. 11 had red, increased eyes and nose from day 10 and day 14 respectively in 1000 mg/kg/day group.

b. Bodyweight Changes:

Only 1000 mg/kg/day group had significant loss of weight during the dosing period, but it started to gain afterward. See Fig. 1 on pages 15 and 20-25.

Mortality:

10 F died in 1000 mg/kg/day group as the following table shows:

| Dams/No.             | Day of death     | Administration of substance |
|----------------------|------------------|-----------------------------|
| 17d, 20d             | 10 <sup>th</sup> | 4 days                      |
| 1d, 3d, 7d, 14d, 15d | 11 <sup>th</sup> | 5 days                      |
| 16d, 19d             | 12 <sup>th</sup> | 6 days                      |
| 4d                   | 16 <sup>th</sup> | 10 days                     |

Therefore, the mortality rate in this group was 50%.

d. Autopsy Findings.

Red No. 50 in 10 mg/kg/day group (that was a 10 mg/kg/day group) had urinary calcium which led to hydroxyproline excretion and uric acid levels, as shown on page 14.

e. Frequency Rates:

10 and 1000 mg/kg/day groups had less pregnancies than other groups as shown below:

| Group          | Pregnant/total rat |
|----------------|--------------------|
| D <sub>0</sub> | 19/20 (95 %)       |
| D <sub>1</sub> | 20/20 (100 %)      |
| D <sub>2</sub> | 18/20 (90 %)       |
| D <sub>3</sub> | 17/20 (85 %)       |
| D <sub>4</sub> | 18/20 (90 %)       |

f. Other Data:

No significant differences between groups were reported for implantation sites/dam, total viable fetus/dam, resorption site/dam. See Tables below for details.

g. GROUP MEAN LITTER DATA:

a.

"A total number of 1108 fetuses was produced and examined for the presence of malformations.

| Group                  | liv. fet.   | ♂          | ♀          | skelatal exam. | visceral exam. |
|------------------------|-------------|------------|------------|----------------|----------------|
| D <sub>1</sub> control | 242         | 110        | 132        | 161            | 81             |
| D <sub>2</sub> 1.0     | 247         | 124        | 123        | 165            | 82             |
| D <sub>3</sub> 10.0    | 237         | 118        | 119        | 156            | 81             |
| D <sub>4</sub> 100.0   | 266         | 128        | 138        | 177            | 88             |
| D <sub>5</sub> 1000.0  | 116         | 47         | 69         | 77             | 39             |
| <b>Total:</b>          | <b>1108</b> | <b>527</b> | <b>581</b> | <b>737</b>     | <b>371</b>     |

|                                             | 1957        | 1958        | 1959        | 1960        | 1961       |
|---------------------------------------------|-------------|-------------|-------------|-------------|------------|
| Total number of implantation sites<br>总植入位点 | 25          | 27          | 20          | 20          | 26         |
| Total number of implantation sites<br>总植入位点 | 0           | 0           | 0           | 0           | 0          |
| Total number of implantation sites<br>总植入位点 | 0           | 0           | 0           | 0           | 0          |
| Total number of implantation sites<br>总植入位点 | 0           | 0           | 0           | 0           | 0          |
| Total number of implantation sites<br>总植入位点 | 15          | 15          | 18          | 19          | 19         |
| Total number of implantation sites<br>总植入位点 | 10          | 12          | 12          | 17          | 17         |
| Per dam<br>每母体总植入位点                         | 1.0 ± 0.2   | 1.1 ± 0.2   | 1.2 ± 0.2   | 1.4 ± 0.2   | 1.4 ± 0.2  |
| Total viable fetuses<br>总存活胎仔数              | 24          | 27          | 27          | 26          | 17         |
| Male %<br>雄仔率 (%)                           | 50.0        | 49.8        | 49.8        | 48.1        | 40.5       |
| Female %<br>雌仔率 (%)                         | 50.0        | 50.2        | 50.2        | 51.9        | 59.5       |
| % of implantation sites<br>胎仔存活率 (%)        | 96.8        | 95.7        | 96.0        | 95.7        | 97.5       |
| Per dam<br>每母体总存活胎仔数                        | 12.7 ± 2.8  | 12.4 ± 3.5  | 13.2 ± 3.9  | 14.0 ± 2.1  | 14.5 ± 1.7 |
| Total resorption sites<br>总吸收位点             | 7           | 11          | 9           | 12          | 3          |
| Embryos<br>胚胎吸收数                            | 7           | 10          | 9           | 12          | 2          |
| Fetuses<br>胎仔吸收数                            | 0           | 1           | 0           | 0           | 1          |
| % of implantation sites<br>胎仔吸收率 (%)        | 2.8         | 3.9         | 3.6         | 4.7         | 3.5        |
| Per dam<br>每母体总吸收胎仔数                        | 0.4 ± 0.8   | 1.6 ± 0.6   | 0.5 ± 0.8   | 0.6 ± 0.8   | 0.4 ± 0.0  |
| Total dead fetuses<br>总死亡胎仔数                | 1           | 0           | 1           | 0           | 0          |
| % of implantation sites<br>胎仔死亡率 (%)        | 0.40        | 0           | 0.40        | 0           | 0          |
| Per dam<br>每母体总死亡胎仔数                        | 0.05        | 0           | 0.06        | 0           | 0          |
| Mean litter weight (g)<br>平均胎仔体重 (g)        | 70.2 ± 14.4 | 67.6 ± 18.8 | 72.0 ± 20.4 | 76.8 ± 12.2 | 73.9 ± 8.0 |
| Mean weight total litter (g)<br>平均胎仔总重量 (g) | 1685.0      | 1720.0      | 1824.0      | 1881.6      | 1411.0     |

|                                                | Group<br># | 100 = 100 | 100 = 100 | 100 = 100 | 100 = 100 |
|------------------------------------------------|------------|-----------|-----------|-----------|-----------|
| Group total<br>Z. G. B.                        | 277        | 310       | 279       | 202       | 127       |
| Per incident (no. of<br>individuals) (Z)       | 9.0        | 15.8      | 9.0       | 7.9       | 9.3       |
| Per experiment (no. of<br>individuals) (Z)     | 3.2        | 7.3       | 3.0       | 4.7       | 3.5       |
| Total malformations<br>畸形总数                    | 34         | 61        | 52        | 11        | 0         |
| % of viable fetuses<br>畸形占活胎 (%)               | 14.0       | 24.7      | 21.6      | 15.0      | 0.0       |
| Per dam<br>每胎母体畸形数                             | 1.8        | 3.1       | 2.4       | 2.1       | 0.4       |
| Total malformations<br>畸形总数                    | 0          | 0         | 0         | 0         | 0         |
| % of viable fetuses<br>畸形占活胎 (%)               | 0          | 0         | 0         | 0         | 0.0       |
| Per dam<br>每胎母体畸形数                             | 0          | 0         | 0         | 0         | 0.13      |
| Spontaneously delivered groups<br>自产胎组         |            |           |           |           |           |
| Number of animals<br>胎数                        | 3          | 3         | 7         | 3         | 3         |
| Number of pregnant animals<br>妊娠母体数            | 1          | 2         | 2         | 3         | 2         |
| Total number of young<br>胎仔数                   |            |           |           |           |           |
| At birth<br>分娩时                                |            |           |           |           |           |
| Viable young per dam<br>1母体活胎仔数                |            |           |           |           |           |
| Dead young per dam<br>1母体死胎仔数                  |            |           |           |           |           |
| Mean litter weight (g)<br>平均胎仔体重 (g)           |            |           |           |           |           |
| Mean weight of viable young (g)<br>平均活胎仔体重 (g) |            |           |           |           |           |
| 11 <sup>th</sup> day<br>11日                    |            |           |           |           |           |
| Viable young<br>活胎仔数                           | 10         | 20        | 23        | 38        | 5         |
| Viable young per dam<br>1母体活胎仔数                | 10.0       | 10.0      | 11.5      | 12.7      | 2.5       |
| Mean litter weight (g)<br>平均胎仔体重 (g)           |            |           |           |           |           |
| Mean weight of young (g)<br>平均活胎仔体重 (g)        |            |           |           |           |           |
| Mortality of young<br>活胎仔死亡率                   |            |           |           |           |           |
| Variations<br>变异胎仔数                            | 0          | 13        | 7         | 15        | 1         |
| Malformations<br>畸形胎仔数                         | 0          | 0         | 0         | 0         | 0         |

Observations:

1. Mean body weight gain of pups in 1000 mg/kg/day group was significantly lower than that of control group. The difference was significant (p < 0.05) at the end of the study. The mean body weight gain of pups in 1000 mg/kg/day group was 10.5% of the initial weight, while that of control group was 15.5%.

The following skeletal variations were observed:

| Group          | No. | Skeletal variations |          | Skeletal deformity  |                            |
|----------------|-----|---------------------|----------|---------------------|----------------------------|
|                |     | Number of pair      | %        | % missing of center | % splintering of sternbrae |
| D <sub>0</sub> | 161 | 34                  | (21.1 %) | 0                   | 0                          |
| D <sub>1</sub> | 165 | 60                  | (36.4 %) | 0                   | 1                          |
| D <sub>2</sub> | 155 | 32                  | (33.3 %) | 0                   | 0                          |
| D <sub>3</sub> | 175 | 38                  | (21.3 %) | 1                   | 0                          |
| D <sub>4</sub> | 77  | 1                   | ( 1.3 %) | 0                   | 2                          |

iii. Major Malformations:

One fetus (No. 10d-7 in 1000 mg/kg/day group had no tail and an atresia ani.

See Figs. on page 14.

3. Spontaneously Delivered Groups.

a. Conception Rate:

Only 1 out of 3 in control group was pregnant while 2-3 out of 3 were pregnant in the treated groups.

COMMENTS: Group "D<sub>4</sub>" should be added on line 2 on page 11, since it has only 2 pregnancies out of 3 rats as other groups.

b. Bodyweight gain

It was reported that "the body weight gain of pregnant dams were similar to the values of the main groups."

c. Mortality

One of the 4 pregnant dams in 1000 mg/kg/day group (No. 16) died on day 4 of gestation with 8 fetuses in her uterus.

d. Fetuses

One F in 1000 mg/kg/day group delivered 5 live youngs but killed and ate them herself 3 days after parturition. Five pups survived - the 3rd dams in 1000 mg/kg/day group. All other youngs grew up normally.

A total of 96 fetuses was living after 3 weeks and examined for the presence of variations and malformations. Not only several additional 14th costal ribs were found. See detailed data in table above.

COMMENTS:

1. The bottom of the whole report is missing (cut off); therefore, some pages are not complete, especially page 1 of summary.
2. The bottom of the page 10 was cut off; therefore, the exact figures for mean viable fetal weight were missing in the above table (5.5 was the figure for all by calculation based on the submitted data.)
3. The result section reported that 18 out of 20 F were pregnant in 1000 mg/kg/day group on page 9 but the Table for Group Mean Litter Data above shows only 8 were pregnant in this group, and total implantation sites and total corpora lutea etc. were presented for only these 8 rats (other rats died or not pregnant).

H. ORAL TERATOLOGY STUDY IN RATS: E83-127:

Lab. Performing the Study: The same as above.

Strain: SD-JCL Sprague-Dawley albino F, about 120 days old and 270 g.

Method: The experiment consisted of two phases. In the first phase, the animals were divided into four groups: control, 100 mg/kg/day, 500 mg/kg/day, and 1000 mg/kg/day. The animals were housed in cages and fed a standard diet. The animals were observed for signs of toxicity. In the second phase, the animals were sacrificed and the organs were examined for gross pathology. The results of the experiment are shown in Table 1.

RESULTS

1. Clinical Observations:

a. General Appearance:

A few rats had red eyes and nose in 500 mg/kg/day group.

b. Body weight:

500 mg/kg/day group did not gain any weight between day 8 and day 11, then started to gain (see page 20).

c. Food and Water Consumption:

Food consumption of treated groups was slightly depressed with trend of dose-relation during the dosing period, but the water consumption showed no dose-related differences between groups. See page 22.

d. Spontaneous Parturition:

Several fetuses were spontaneously delivered short before Caesarean section as in the following table:

| Group     | Dam No. | Number of Fetuses |
|-----------|---------|-------------------|
| Control   | 4       | 12                |
|           | 10      | 2                 |
|           | 12      | 4                 |
| 5 mg/kg   | 11a     | 14                |
|           | 9b      | 3                 |
|           | 7b      | 11                |
|           | 8b      | 14                |
|           | 9a      | 2                 |
| 500 mg/kg | 10b     | 11                |
|           | 17b     | 1                 |
|           | 3a      | 3                 |
|           | 15a     | 9                 |

These fetuses were randomly assigned to the implantation sites and remaining fetuses were produced by Caesarean section.

e. Mortality:

One control rat died on day 21 spontaneously.

f. Autopsy Findings:

The following findings were reported from the examination of all dams from C section group: Uterine bleeding in one control rat (15), swelling and focal necrosis of liver and splenomegaly in one control rat(20), and perisplenitis adhesiva in one rat in 500 mg/kg/day group...

NDA: 19-085 SPONSOR: BOEHRINGER INGELHEIM

3 OF 3

TRADE: ATROVENT GENERIC: IPRATROPIUM BROMIDE

g. Pregnancy Rate:

20/20 in control group vs. 19/20 (= 95%) in all 3 treated groups.

h. Implantation and Corpora Lutea:

No significant differences between groups in number of implantations/dam or corpora lutea.

2. GROUP MEAN LITTER DATA:

a. Viable Fetuses:

|                | group<br>(mg/kg) | living<br>fet. | ♂   | ♀   | skeletal<br>exam. | visceral<br>exam. |
|----------------|------------------|----------------|-----|-----|-------------------|-------------------|
| D <sub>0</sub> | control          | 233            | 123 | 110 | 157               | 76                |
| D <sub>1</sub> | 5                | 219            | 112 | 103 | 143               | 72                |
| D <sub>2</sub> | 50               | 255            | 128 | 127 | 169               | 86                |
| D <sub>3</sub> | 500              | 223            | 108 | 113 | 148               | 79                |
| Total:         |                  | 926            | 471 | 455 | 617               | 309               |

C. Table 1

Group Mean Litter Data  
グループ別胎仔記録

Species: Rat      P.O.:      Substrate: Sch 1000 Br  
種別:      産卵期:      飼料:

|                                              | Control<br>対照 | 50 mg/kg    | 50 mg/kg    | 500 mg/kg   |
|----------------------------------------------|---------------|-------------|-------------|-------------|
| Number of animals<br>動物数                     | 20            | 20          | 20          | 20          |
| Maternal mortality<br>母体死亡率                  | 1             | 0           | 0           | 0           |
| Abortions<br>墮胎                              | 0             | 0           | 0           | 0           |
| Natural Abortions<br>自然墮胎                    | 0             | 0           | 0           | 0           |
| Total number of pregnant animals<br>妊娠動物数    | 19            | 19          | 19          | 19          |
| Total implantation sites<br>着床数              | 245           | 223         | 263         | 239         |
| Per dam<br>1胎仔あたり                            | 12.9 ± 3.7    | 11.7 ± 3.2  | 13.8 ± 1.5  | 12.6 ± 2.9  |
| Total viable fetuses<br>生存胎仔数                | 233           | 215         | 255         | 223         |
| Males as %<br>雄胎仔 (%)                        | 53            | 52          | 50          | 48          |
| Females as %<br>雌胎仔 (%)                      | 47            | 48          | 50          | 52          |
| In % of implantation sites<br>着床数に対する (%)    | 95.1          | 96.4        | 97.0        | 93.3        |
| Per dam<br>1胎仔あたり                            | 12.3 ± 4.0    | 11.3 ± 3.6  | 13.4 ± 1.4  | 11.7 ± 3.3  |
| Total resorption sites<br>吸収数                | 12            | 8           | 8           | 16          |
| Early<br>早期                                  | 10            | 6           | 8           | 13          |
| Late<br>晚期                                   | 2             | 2           | 0           | 1           |
| In % of implantation sites<br>着床数に対する (%)    | 4.9           | 3.6         | 3.0         | 6.7         |
| Per dam<br>1胎仔あたり                            | 0.6 ± 1.1     | 0.4 ± 0.8   | 0.4 ± 0.8   | 0.8 ± 1.8   |
| Total dead fetuses<br>死胎数                    | 0             | 0           | 0           | 0           |
| In % of implantation sites<br>着床数に対する (%)    | 0             | 0           | 0           | 0           |
| Per dam<br>1胎仔あたり                            | 0             | 0           | 0           | 0           |
| Mean litter weight (g)<br>胎仔平均重 (g)          | 71.5 ± 23.0   | 66.8 ± 20.2 | 76.4 ± 7.9  | 66.8 ± 15.7 |
| Mean viable litter weight (g)<br>生存胎仔平均重 (g) | 9.84 ± 0.39   | 9.90 ± 0.43 | 9.70 ± 0.41 | 9.74 ± 0.49 |

|                                               | Control<br>对照组 | 5 mg/kg    | 50 mg/kg    | 500 mg/kg   |
|-----------------------------------------------|----------------|------------|-------------|-------------|
| Cows per lot<br>每批头数                          | 263            | 261        | 266         | 282         |
| Pre-implantation loss %<br>植入前丢失率 (%)         | 6.8            | 14.6       | 1.1         | 15.2        |
| Post-implantation loss %<br>植入后丢失率 (%)        | 4.3            | 3.7        | 3.0         | 6.7         |
| Total variations<br>总变异数                      | 66             | 67         | 34          | 24          |
| In % of viable fetuses<br>占可育胎儿 (%)           | 42.0           | 46.9       | 32.0        | 16.2        |
| Per dam<br>每头母牛                               | 1.5            | 3.5        | 2.8         | 1.3         |
| Total malformations<br>总畸形数                   | 3              | 4          | 0           | 3           |
| In % of viable fetuses<br>占可育胎儿 (%)           | 1.3            | 0.5        | 0           | 1.3         |
| Per dam<br>每头母牛                               | 0.16           | 0.05       | 0           | 0.16        |
| Spontaneously delivered groups<br>自然分娩组       |                |            |             |             |
| Number of animals<br>动物数                      | 10             | 10         | 10          | 10          |
| Number of pregnant animals<br>怀孕动物数           | 7              | 9          | 10          | 9           |
| Total number of young<br>总仔数                  | 72             | 107        | 120         | 104         |
| All born<br>全部出生                              |                |            |             |             |
| Viable young per dam<br>每头母牛可育仔数              | 10.1 ± 2.3     | 11.8 ± 1.6 | 11.9 ± 2.0  | 11.0 ± 3.3  |
| Dead young per dam<br>每头母牛死仔数                 | 0.1 ± 0.4      | 0.1 ± 0.1  | 0.1 ± 0.1   | 0.6 ± 0.7   |
| Mean litter weight (g)<br>平均胎重 (g)            | 62.5 ± 20.1    | 73.7 ± 8.8 | 73.6 ± 15.4 | 61.6 ± 20.3 |
| Mean weight of viable young (g)<br>可育仔平均重 (g) | 6.2 ± 0.7      | 6.3 ± 0.6  | 6.2 ± 0.8   | 5.5 ± 0.7   |
| All dead<br>全部死亡                              |                |            |             |             |
| Viable young<br>可育仔数                          | 63             | 98         | 114         | 85          |
| Viable young per dam<br>每头母牛可育仔数              | 9.9 ± 4.2      | 10.9 ± 2.5 | 11.4 ± 2.5  | 9.8 ± 4.8   |
| Mean litter weight (g)<br>平均胎重 (g)            | 371 ± 161      | 455 ± 45   | 447 ± 78    | 369 ± 166   |
| Mean weight of young (g)<br>仔平均重 (g)          | 41.2 ± 9.0     | 41.7 ± 7.5 | 39.2 ± 4.2  | 37.7 ± 5.3  |
| Number of young<br>仔数                         | 7              | 8          | 3           | 11          |
| Abortions<br>流产数                              | 2              | 10         | 8           | 0           |
| Stillborns<br>死胎数                             | 0              | 0          | 0           | 2           |

e. Observations.

- i. Mean viable fetal weight of 500 mg/kg/day group was slightly but significantly ( $p < 0.01$ ) smaller than that of control group, but it was reported to be within the background for normal data (see page 70,  $5.55 \pm 0.51$  g).

COMMENTS: Mean body weight of viable young in 50 and 500 mg/kg/day groups on day 21 after birth was also highly significantly ( $p < 0.01$ ) smaller than that of control group, showing retarded weight gains in these two groups in spontaneously delivered group. See below.

There were no stillbirths or "runts" in the fetuses.

ii. Skeletal Variations:

|                | n   | lumbar ribs (%) | lumbri- zation | splintering or missing of sternal centers | misshaped vertebral body | others | Total*   |
|----------------|-----|-----------------|----------------|-------------------------------------------|--------------------------|--------|----------|
| D <sub>0</sub> | 197 | 64(40.8)        | 2(1.7)         | 0                                         | 2 (1.3)                  | 0      | 66(42.0) |
| D <sub>1</sub> | 143 | 64(44.8)        | 0              | 1(0.7)                                    | 1 (0.7)                  | 1(0.7) | 67(46.0) |
| D <sub>2</sub> | 169 | 49(29.0)        | 4(2.4)         | 1(0.6)                                    | 2 (1.2)                  | 1(0.6) | 54(32.0) |
| D <sub>3</sub> | 148 | 19(10.1)**      | 2(1.4)         | 0                                         | 4 (2.7)                  | 3(2.0) | 24(16.2) |

\* Several fetuses have shown two or more variations.

As shown above, the rate of fetuses with lumbar ribs in 500 mg/kg group was significantly smaller than control, and the decrease was dose-related.

iii. Malformations: skeletal:

The following report was submitted:

Control

4-x Vertebrae schisis

5-x Vertebrae schisis

10-x Vertebrae schisis

D<sub>1</sub> (50 mg/kg)

18a-x Discurvatio costae

D<sub>2</sub> (50 mg/kg)

N.A.D.

D<sub>3</sub> (500 mg/kg)

1c-x Vertebrae schisis

3c-x Vertebrae schisis

13c-x Discurvatio costae

3. Spontaneously Delivered group.

Dams: Clinical symptoms and body weight gain of pregnant dams paralleled about to that of E section group above.

500 mg/kg/day group actually lost some weight during day 8 and day 11 before starting to gain again. See Fig. on page 21 and Table on page 19.

At autopsy no abnormalities were noted.

Pups:

a. At Birth:

Pups (of viable young) in 500 mg/kg/day group had slight but significantly ( $p < 0.01$ ) smaller birth weight, and the increased number of dead youngs per dam was produced in this group (0.1 in others vs. 0.6.).

b. During Weaning Period:

i. Pups from 50 and 500 mg/kg/day group were slightly smaller than those of control but not significant. See Graph on page 23 for the growth curves, and group mean body weight in Table below.

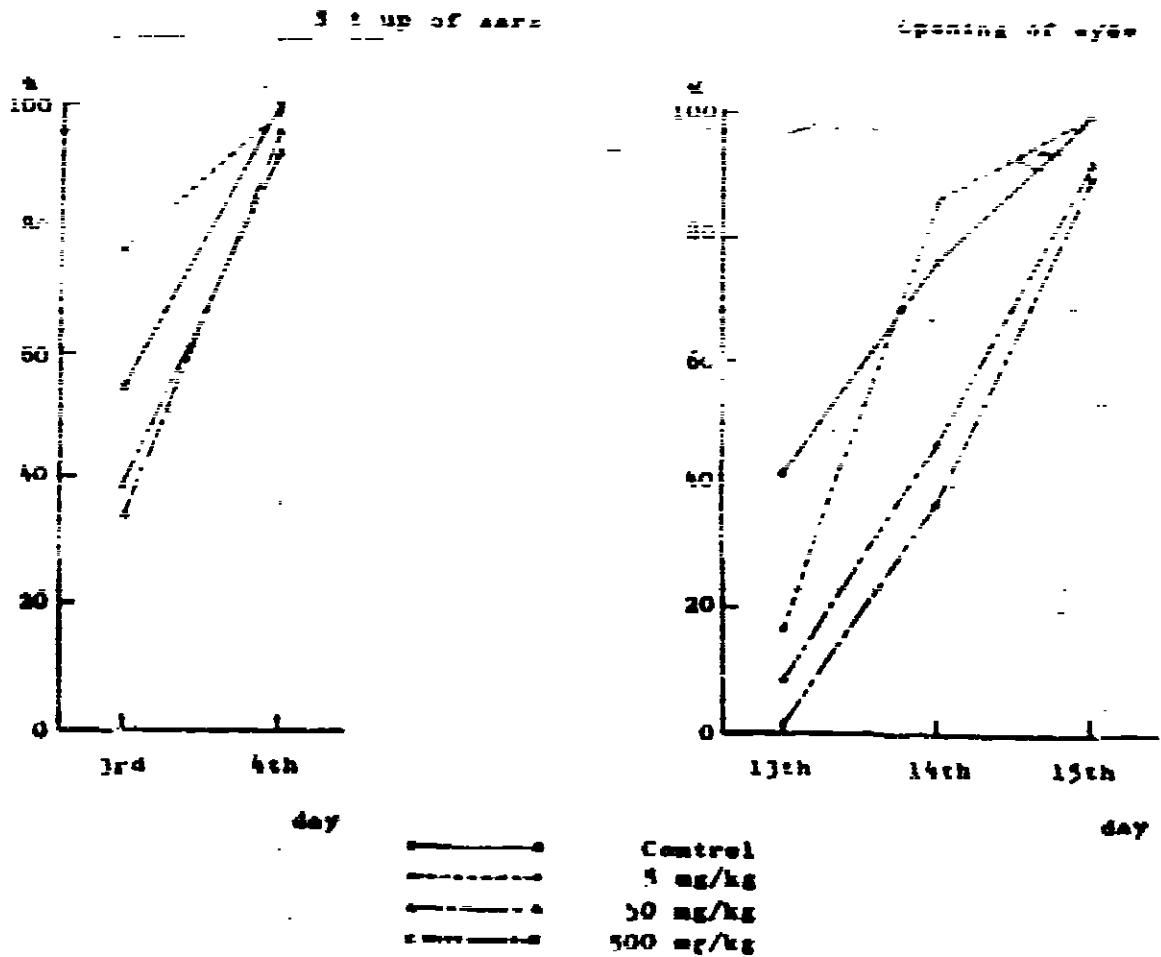
| Group   | Day after birth |         |          |          |          |
|---------|-----------------|---------|----------|----------|----------|
|         | 1               | 4       | 7        | 14       | 21       |
| Control | 6.2±0.7         | 9.2±1.8 | 13.1±1.6 | 23.6±3.5 | 41.2±5.0 |
| 5       | 6.3±0.6         | 9.6±1.0 | 14.1±1.4 | 27.5±4.7 | 41.7±7.5 |
| 50      | 6.2±0.8         | 9.3±1.2 | 12.9±1.5 | 24.9±3.5 | 39.2±4.2 |
| 500     | 5.6±0.7         | 8.5±1.1 | 12.2±1.5 | 24.0±2.7 | 37.7±5.3 |

ii. Ear and Eye Openings:

It was reported that "the setting up of ears and the opening of eyes seemed to be dose-dependently retarded for about one day; however, no background data are existing in case of this new investigation method." See Graph 5 and Table 12 below:

Graph 3 Development of Ears and Eyes of the Pupae

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| Day         | No. of pups, the ears of which were setting up |                   | No. of pups, the eyes of which opened |                  |                   |
|-------------|------------------------------------------------|-------------------|---------------------------------------|------------------|-------------------|
|             | 3th                                            | 4th               | 13th                                  | 14th             | 15th              |
| Control (%) | 36/56<br>(64.3)                                | 64/64<br>(100.0)  | 20/63<br>(31.7)                       | 48/51<br>(94.1)  | 63/63<br>(100.0)  |
| 5 mg/kg     | 75/98<br>(76.5)                                | 97/98<br>(99.0)   | 16/98<br>(16.3)                       | 84/98<br>(85.7)  | 98/98<br>(100.0)  |
| 50 mg/kg    | 39/114<br>(34.2)                               | 109/114<br>(95.6) | 10/114<br>(8.8)                       | 52/114<br>(45.6) | 105/114<br>(92.1) |
| 500 mg/kg   | 35/90<br>(38.9)                                | 81/88<br>(92.1)   | 1/88<br>(1.1)                         | 34/88<br>(38.6)  | 79/88<br>(89.8)   |

111. Locomotion and Behavior:

No significant difference between groups was reported for open-field test as shown in Table below:

Results of Open-field Test\*

Sch 1000 Br p.o.

Rats

| Group             | loco-<br>motion | rearing<br>up | preening |
|-------------------|-----------------|---------------|----------|
| Control<br>N = 11 | 32.5±12.4       | 1.8±1.8       | 0.5±0.6  |
| 5<br>mg/kg        | 28.8±10.6       | 2.0±1.9       | 0.6±0.7  |
| 50<br>mg/kg       | 29.8±12.0       | 1.8±1.8       | 0.7±0.6  |
| 500<br>mg/kg      | 33.1±17.2       | 1.6±1.7       | 0.5±0.5  |

\* Makroton (R) -cages, Type III (floor space approximately 700 cm<sup>2</sup>), were used, the floor of which was divided into 17 equal squares. The individual pups were individually placed in the center and the movements were registered during the period of one minute.

locomotion: Number of squares, crossed per minute

rearing up: Number per minute

preening: Number of wiping the face by forelimbs

c. 21st Day:

- i. Many pups died during weaning: 7 in control, 8, 5, and 11 in 5, 50, and 500 mg/kg/day group respectively, but others grew up normally.
- ii. A total of 363 pups alive after 3 weeks, and among them the following variations were found with two malformations in 500 mg/kg/day group.

|                | n   | additional sternbrae (%) | additional lumbar vertebrae (%) |
|----------------|-----|--------------------------|---------------------------------|
| D <sub>0</sub> | 63  | 2 (3.2)                  | 0                               |
| D <sub>1</sub> | 98  | 9 (9.2)                  | 1 (1.0)                         |
| D <sub>2</sub> | 114 | 8 (7.0)                  | 0                               |
| D <sub>3</sub> | 88  | 1 (1.1)                  | 0                               |

Two malformations in 500 mg/kg/day group were a case of nanism with absence of one thoracic vertebra and three ribs, vertebraschisis, deformity of lumbar vertebrae and discurvatio of spinal column seen in a pup(21c-x), and discurvatio costae seen in the second pup(25c-x).

COMMENTS: 1) A total of 7 malformations was reported for C section group above; therefore, a total of 9 malformations was found in this rat study.

2) Line 9 from the bottom of page 17: 7 should be changed to 9, due to 2 more in 500 mg/kg/day group: See top of the page 18.

iii. Significantly ( $p < 0.01$ ) smaller mean body weight was seen in 50 and 500 mg/kg/day group. See Table above.

I. ORAL TERATOLOGY STUDY IN RABBITS: -E83-128:

Lab. Performing the Study: See above, but according to recommendations of the National Council of Pharmacy, Japan.

Strain: Himalayan (small Russian breed), about 2 kg and 4 month old.

Methods: 15 pregnant F/group of rabbits were treated with 5, 25, or 125 mg/kg/day Sch 1000-Br (Batch No. 1/74) from day 6 to day 18 of gestation via stomach tube along with a control group receiving distilled water. They were killed by air embolism on day 29, about 24 hours before parturition, and examined. See pages 1-6 for detailed information.

RESULTS:

I. PARENT:

a. Clinical Symptoms:

Several dams in 125 mg/kg/day group had misshaped feces, and the amount was markedly diminished.

b. Body Weight:

All 3 treated groups did not gain any weight during the first 6 days treatment, then started to gain slowly during the next 6 days treatment period. 5 and 125 mg/kg/day groups gained much less than the other groups during day 24 and day 29. See graph i on page 14 and Tables 2 and 3.

c. Food and Water Consumption:

Food consumption of 25 and 125 mg/kg/day groups was distinctly (but not significantly) decreased during the dosing period. Water consumption did not show dose related changes. See page 15 and Table 4 on page 24.

d. Spontaneous Parturition:

One dam in the control group and one of 125 mg/kg/day group aborted during the last week of gestation as shown below:

e. Pregnancy Rate:

100% in control group (15/15), 80% in 5 mg/kg/day group (12/15) with 86% (13/15) and 73% in high dose group (11/15) including aborted (see). See Table 1 below.

COMMENTS: On page 8, it was reported that the high dose had 80% (12/15), but it must be a mistake, since all other figures are based only on pregnant dams in Table 1 below.

f. Mortality:

One in 5 mg/kg/day group died "a couple of days" before the drug treatment, and at autopsy "deposition of left kidney and bleeding into the gastric wall have been found" in this dam (6a).

g. Autopsy Finding After Caesarean Section on All Dams:

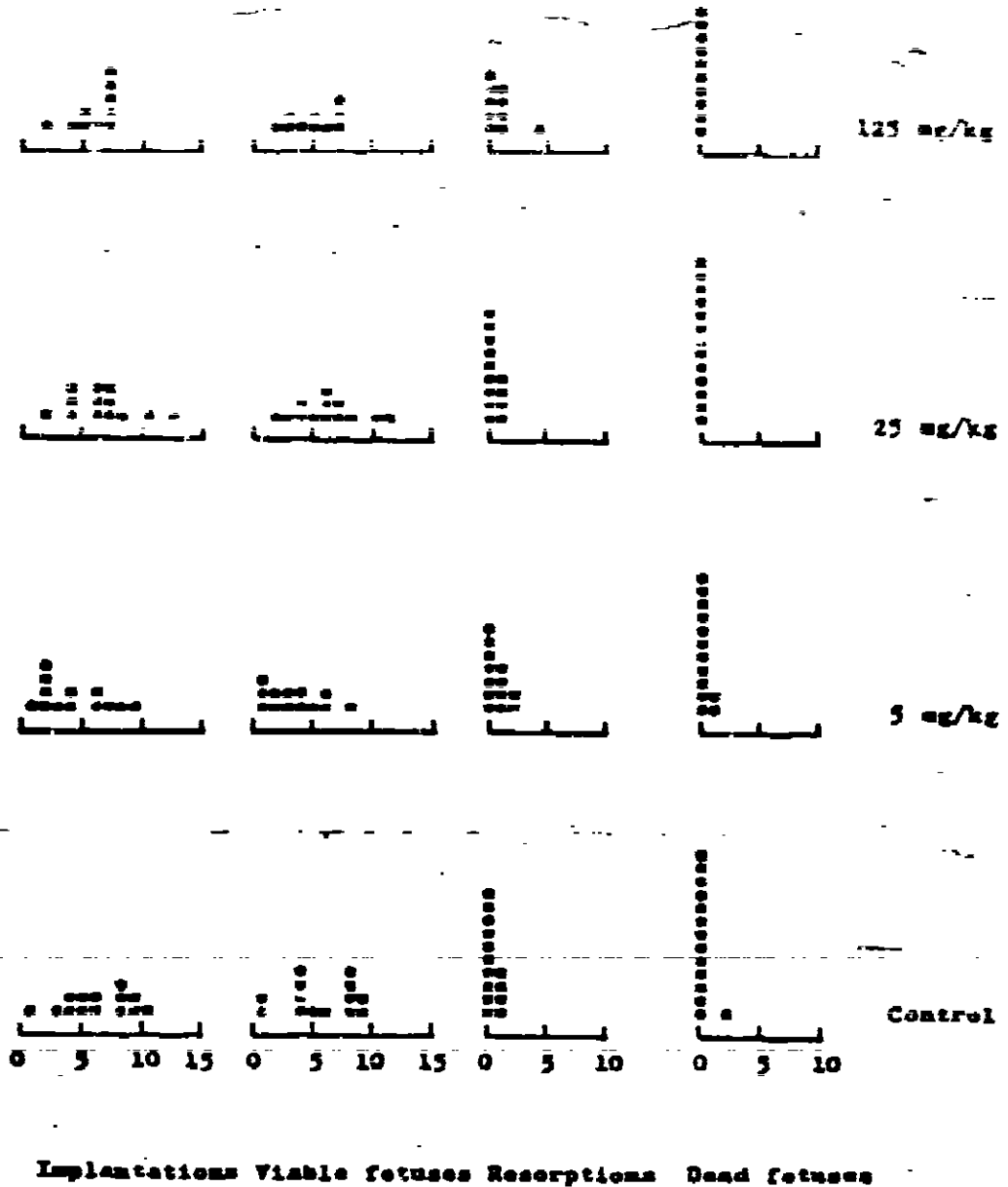
Only one dam (11b) in 25 mg/kg/day group had "hydrothorax, ascites, and adipositas hepatica."

h. Implantation:

Number of implantation sites/dam was lowest in 5 mg/kg/day group, followed by the 125 mg/kg/day group, that was slightly lower than control group. See Table 1 and Graph 3 below for details.

COMMENTS: Mistake: Table 4 under Implantations on page 8 is wrong, since it contains food and water consumption only.

Graph 3 Litter Distribution of Each Parameter



COMMENTS: Mistakes: Graph 2 on lines 13, 7, and 2 from the bottom of page 8 should be changed to Graph 3.

1. Resorption:

Number of resorptions was significantly ( $p < 0.05$ ) larger in 5 and 125 mg/kg/day groups than control group.

2. Corpora lutea:

Number of corpora lutea was greater than the total number of implantations.

Pre-implantation loss was greater in 5 mg/kg/day group than other groups, and post-implantation loss was significantly ( $p < 0.05$ ) greatest in this 5 mg/kg/day group followed by 125 mg/kg/day group, then the control group. See Table 1 below.

3. LITTER DATA:

a. Viable Fetuses:

Number of live fetuses per implantation sites and per dam was significantly ( $p < 0.05$ ) lower in 5 mg/kg/day than control group, followed by 125 mg/kg/day group, then control group.

b. Dead Fetuses:

A total of 4 still born were observed; 2 in control and 2 in 5 mg/kg/day group, as shown in the above Graph 3 but these fetuses had no external and skeletal malformations.

c. GROUP MEAN LITTER DATA: Table 1.

|                                                 | Control<br>對 照 | 5 mg/kg    | 25 mg/kg   | 125 mg/kg  |
|-------------------------------------------------|----------------|------------|------------|------------|
| Number of litters<br>胎 鼠 數                      | 15             | 15         | 15         | 15         |
| Aborted in utero<br>胎 鼠 死 亡 數                   | 0              | 1          | 0          | 0          |
| Abortions<br>墮 胎 數                              | 1              | 0          | 0          | 1          |
| Pre-implantation loss<br>着 床 前 胎 鼠 數            | 0              | 0          | 0          | 0          |
| Total number of progeny animals<br>胎 鼠 總 數      | 14             | 13         | 15         | 10         |
| Total implantation sites<br>着 床 點 數             | 86             | 56         | 83         | 57         |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 6.1 ± 2.6      | 3.7 ± 2.6  | 6.4 ± 2.7  | 5.7 ± 1.7  |
| Total viable fetuses<br>實 存 胎 鼠 數               | 80             | 46         | 79         | 49         |
| Males in %<br>公 胎 鼠 (%)                         | 46.3           | 43.5       | 46.8       | 46.9       |
| Females in %<br>母 胎 鼠 (%)                       | 53.7           | 56.5       | 53.1       | 53.1       |
| In % of implantation sites<br>胎 鼠 存 活 率 (%)     | 93.0           | 82.1*      | 95.1       | 86.0       |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 5.7 ± 2.6      | 3.3 ± 2.2* | 6.1 ± 2.6  | 4.9 ± 1.9  |
| Total resorptions seen<br>吸 收 胎 鼠 數             | 4              | 8          | 4          | 8          |
| Early<br>早 期 吸 收                                | 4              | 8          | 4          | 7          |
| Late<br>晚 期 吸 收                                 | 0              | 0          | 0          | 1          |
| In % of implantation sites<br>胎 鼠 吸 收 率 (%)     | 4.7            | 14.3*      | 4.8        | 14.0*      |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 0.3 ± 0.5      | 0.6 ± 1.8  | 0.3 ± 0.5  | 0.8 ± 1.2  |
| Total dead fetuses<br>死 亡 胎 鼠 數                 | 2              | 2          | 0          | 0          |
| In % of implantation sites<br>胎 鼠 死 亡 率 (%)     | 2.3            | 3.6        | 0          | 0          |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 0.1 ± 0.5      | 0.2 ± 0.4  | 0          | 0          |
| Mean litter weight (g)<br>胎 鼠 總 體 重 (g)         | 210 ± 79       | 153 ± 89   | 209 ± 63   | 177 ± 55   |
| Mean viable foal weight (g)<br>實 存 胎 鼠 體 重 (g)  | 36.7 ± 5.7     | 39.7 ± 4.5 | 34.4 ± 6.7 | 36.1 ± 6.8 |
| Contrace failure<br>避 孕 失 效 數                   | 126            | 99         | 101        | 68         |
| Pre-implantation loss %<br>着 床 前 胎 鼠 失 效 率 (%)  | 31.7           | 43.4       | 21.7       | 16.2       |
| Post-implantation loss %<br>着 床 後 胎 鼠 失 效 率 (%) | 7.0            | 17.9*      | 4.8        | 14.0       |
| Total variations<br>變 異 胎 鼠 數                   | 0              | 0          | 0          | 1          |
| In % of viable fetuses<br>變 異 胎 鼠 率 (%)         | 0              | 0          | 0          | 2.0        |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 0              | 0          | 0          | 0.1        |
| Total malformations<br>畸 形 胎 鼠 數                | 0              | 0          | 1          | 0          |
| In % of viable fetuses<br>畸 形 胎 鼠 率 (%)         | 0              | 0          | 1.3        | 0          |
| Per dam<br>1 胎 鼠 母 體 均 值                        | 0              | 0          | 0.1        | 0          |

d. Weight of Fetuses:

No differences between groups in mean viable fetal birth weight were shown, but there were several fetuses with the weight below 65% of the normal fetal weight ("runt") in 25 and 125 mg/kg/day groups as shown in the following, but they had no other abnormalities.

|           |      |        |   |                     |
|-----------|------|--------|---|---------------------|
| Fetus No. | 2b-6 | 16.0 g | ♀ | 25 mg/kg/day group  |
|           | 3b-3 | 21.6   | ♂ |                     |
|           | 3b-5 | 33.7   | ♂ |                     |
|           | 3b-6 | 22.4   | ♀ |                     |
|           | 3b-7 | 22.5   | ♂ |                     |
|           | 5b-6 | 23.2   | ♂ |                     |
|           | 5c-5 | 22.8   | ♀ | 125 mg/kg/day group |

e. Variations:

One fetus in 125 mg/kg/day group had asymmetrical sternbrae.

f. Malformations:

Among 254 fetuses examined, one malformation occurred in 25 mg/kg/day group (11b-6 ♀, 20.7 g) with pseudotruncus aortae (dextropositio et dilatatio aortae, atresia pulmonalis, agenesia septi ventriculorum). See Fig. 1 and 2 on page 13.

COMMENTS: It was stated that the deformed heart found in 25 mg/kg/day group was seen before in the strain of animals used, and the 1.3% malformation among viable fetuses are within normal range. See background control data on Himalayan rabbits on page 41.



- a. No. of Animals/Level: 15 pregnant ♀
- b. Dosage Level:

| Group | Treatment            | Dosing schedule<br>(No. of metered doses/<br>rabbit/session) | No. of<br>sessions/day | No. of rabbits |
|-------|----------------------|--------------------------------------------------------------|------------------------|----------------|
| 1     | Control (sham)       | —                                                            | 4                      | 12             |
| 2     | Control (unintended) | 40                                                           | 4                      | 12             |
| 3     | Sen 1000, 0.3 mg/kg  | 4                                                            | 4                      | 12             |
| 4     | Sen 1000, 0.9 mg/kg  | 15                                                           | 4                      | 12             |
| 5     | Sen 1000, 1.8 mg/kg  | 30                                                           | 4                      | 12             |

- c. Blood samples collected from 5 rabbits of Groups 2 and 5, 30 seconds after administration of the first dosing on days 6 and 18.

See detailed procedures on pages 4-6.

## RESULTS:

### i. Preliminary Study:

- a. Signs: Transient tachypnea was observed in the majority of rabbits in both groups immediately after dosing.

Freon 12 was detected in all animals immediately after dosing, and the level declined rapidly during the subsequent 12-15 min, although there were individual variations. See page 20 for blood levels.

COMMENTS: Time of blood collection was not same as indicated in Methods for 3 rabbits.

### b. Body Weight:

No difference between two groups in weight gain. See Fig. 1 on page 10.

c. Litter Data:

- i. All 6 control rabbits and 5 treated rabbits were pregnant (100% vs. 83%) and had viable youngs, and only one in treated group was not pregnant.
- ii. Treatment did not produce adverse effect on litter size, mean fetal weight, implantation, and no major malformation was produced. Slightly higher fetal loss was noted in the treated group (6.2% vs. 8.5%). See Table 2 on page 13. The incidence of minor skeletal abnormalities were reported to be normal limits. See Table 3 on page 14 and Table 4 on page 15.

2. MAIN TERATOLOGY STUDY:a. Signs and Mortalities:

- i. "Several" rabbits in 1.8 mg/kg/day group showed transient anorexia during dosing period.
- ii. One rabbit (No. 47) was sacrificed on day 26 with apparent middle ear disease with tilting of the head to one side, muscular incoordination, and marked nystagmus of right eye. Liver and renal cortex were congested at autopsy, with 8 implantation.

iii. Freon Analysis:

Apparently much higher levels were presented in Sch-1000 treated group than vehicle control group on both days as shown in the following Table:

**GROUP 2 : Proflant :**

| PARENTE<br>No. MICE | Blood levels of Proflant (µg/ml) 30 seconds<br>after administration of the first dosing water on day |     |
|---------------------|------------------------------------------------------------------------------------------------------|-----|
|                     | 6                                                                                                    | 18  |
| 4                   | 13.0                                                                                                 | 3.5 |
| 3                   | 3.0                                                                                                  | 2.5 |
| 24                  | 1.0                                                                                                  | 1.0 |
| 25                  | 2.0                                                                                                  | 1.0 |
| 26                  | 11.0                                                                                                 | 2.0 |

**GROUP 5 : 1.8 mg/kg/day :**

|    |       |      |
|----|-------|------|
| 51 | 14.0  | 11.0 |
| 52 | 9.0   | 3.0  |
| 53 | 23.0  | 29.0 |
| 54 | 200.0 | 26.0 |
| 55 | 33.0  | 23.0 |

**b. Body Weight Change of Dams with Viable Youngs:**

Retardation of weight gain occurred in 1.8 mg/kg/day group during dosing period (See Fig. 2 on page 11), but the weight of this group was comparable to the control group by day 28. 0.3 mg/kg/day group also had some retardation.

**COMMENTS:** It was stated that lower weight gain on the high dose group could not be conclusively related to treatment, since the same dose in the preliminary study showed comparable weight gain as the control group.

**c. Pregnancy Rate:**

First 4 groups had 92%, and the 1.8 mg/kg/day group had 85%, but the difference between groups was not significant.

d. Pre-implantation Loss:

Preimplantation loss of all treated groups was higher than control or vehicle-control group, but it was stated as "within the Lab. Standard Range" and not significant.

2. LITTER DATA:

Two rabbits in 1.8 mg/kg/day group and one in 0.9 mg/kg/day group aborted. It was stated that 2 in 1.8 mg/kg/day group had previously been bled for Freon analysis, and this influence and "since the frequency of total litter losses was in a range previously encountered in control groups, the following comparison were restricted to mean B values (i.e. only litters with viable young)". See Table below.

a. Litter Size and Fetal Loss:

Litter size and fetal loss were not significantly different between group (B values only). See Table below and Appendix 9.

COMMENTS: Fetal loss of two treated groups was much higher than sham control or vehicle control group and dose related (see A values: 15.3% for sham control, 14.9% for vehicle control, but 13.6%, 21.3%, and 29.1% for low, med, and high dose groups for A values).

b. Litter and Mean Pup Weight:

Slightly lower mean pup weight in treated groups was not significant.

Group mean litter data - teratology study

|              |                  |   |     |     |     |
|--------------|------------------|---|-----|-----|-----|
| Group        | 1                | 2 | 3   | 4   | 5   |
| Drug         | CONTROL          |   |     |     |     |
|              | Schem Propellant |   |     |     |     |
|              | SCH 1000         |   |     |     |     |
| Dose (mg/kg) | -                | - | 0.3 | 0.9 | 1.8 |

| GROUP | NO. OF ANIMALS | PREGNANCY RATE % | VIABLE YOUNG |       | RESORPTIONS |      | ABORTIONS | IMPLANT-ATIONS | CORPORA LUTEA | PRE-IMPLANTATION LOSS % | FOETAL LOSS % | LITTER WT. (g) | MEAN FOETAL WEIGHT (g) |       |      |
|-------|----------------|------------------|--------------|-------|-------------|------|-----------|----------------|---------------|-------------------------|---------------|----------------|------------------------|-------|------|
|       |                |                  | d            | Total | Early       | Late |           |                |               |                         |               |                |                        | Total |      |
| 1     | A=0-12         | 92               | 4.0          | 1.3   | 7.3         | 0.6  | 0.8       | 1.3            | -             | 0.7                     | 0.9           | 1.0            | 15.3                   | 519.7 | 44.8 |
| 2     | A=0-12         | 92               | 3.8          | 1.6   | 7.4         | 1.1  | 0.3       | 1.4            | -             | 0.8                     | 0.6           | 7.4            | 14.9                   | 119.5 | 16.1 |
| 3     | A=0-11         | 92               | 3.4          | 1.6   | 7.0         | 0.8  | 0.5       | 1.3            | -             | 0.3                     | 0.5           | 12.6           | 13.6                   | 111.0 | 14.8 |
| 4     | A=11           | 92               | -            | -     | 6.8         | 0.5  | 0.6       | 1.2            | 0.5           | 0.5                     | 0.9           | 16.6           | 21.9                   | -     | -    |
|       | B=10           |                  | 4.2          | 1.3   | 7.5         | 0.6  | 0.7       | 1.3            | -             | 0.8                     | 10.2          | 16.0           | 13.4                   | 196.4 | 40.3 |
| 5     | A=11           | 85               | -            | -     | 6.3         | 0.4  | 0.5       | 0.9            | 0.8           | 0.0                     | 9.1           | 13.0           | 29.1                   | -     | -    |
|       | B=9            |                  | 4.4          | 1.2   | 7.7         | 0.4  | 0.7       | 1.1            | -             | 0.8                     | 9.8           | 9.7            | 121.7                  | 41.9  |      |

Group mean values were then calculated from individual observations in the way:

Mean A: includes all surviving animals that provided evidence of nidation including those aborting or exhibiting total resorption

Mean B: includes all animals bearing viable young at termination

Mean B has more meaning when there is a small number of animals per group. In which case the mean values would be unduly influenced by the presence of a single animal aborting or exhibiting total resorption. Mean A is a more accurate index when there is a large number of animals per group or when several animals abort or exhibit total resorption.

c. Major and Minor Malformations:

Four major malformations occurred, 2 in 0.3 mg/kg/day group and 2 in control group. But similar abnormalities have been occurred spontaneously among control young; therefore, not drug related. See pages 40-52 for detailed descriptions of malformations and minor variations in Appendix 10. No significant differences between groups were reported for incidence of minor visceral and skeletal anomalies.

d. Skeletal Variations:

No significant differences between groups were shown, although there were higher incidence of both 12 and 13-ribbed pups with variant sternbrae in treated pups as shown in the following Table:

Group mean incidence of skeletal variants - ceratology study

| Group        | 1                          | 2                    | 3          | 4   | 5 |
|--------------|----------------------------|----------------------|------------|-----|---|
| Drug         | CONTROL                    |                      | SCH 1000   |     |   |
|              | Sham                       | Propellant           |            |     |   |
| Dose (mg/kg) | -                          | -                    | 0.3        | 0.3 | 1 |
| GROUP        | PERCENTAGE OF YOUNG * WITH |                      |            |     |   |
|              | EXTRA RIB(S)               | VARIANT STERNEBRA(E) |            |     |   |
|              |                            | Normal ribs          | Extra ribs |     |   |
| 1            | 40.7                       | 7.6                  | 0.0        |     |   |
| 2            | 44.0                       | 14.2                 | 12.9       |     |   |
| 3            | 35.8                       | 18.3                 | 2.8        |     |   |
| 4            | 22.6                       | 21.3                 | 10.7       |     |   |
| 5            | 48.1                       | 20.8                 | 12.5       |     |   |

\*Young showing major malformations excluded.

COMMENTS: A mistake on line 2 on page 9: The propellant control group should be changed to sham control group since two major malformations were seen in group 1 instead of group 2 on pages 40 and 41.

R. ORAL PERI-AND POST NATAL REPRODUCTION STUDY IN RATS: E83-13?  
(SEGMENT III):

Lab. Performing the Study: Boehringer Ingelheim GmbH  
Pharma-Research, Japan: (according to  
Jap. Coun: of Pharmacy methods).  
Strain: SD-JCL Sprague-Dawley, about 11 weeks  
old and 229 ± 22 g.

Methods: 20 pregnant F/group of rats were treated by gastric intubation with 5, 50, or 500 mg/kg/day sch TOCJ-Br (Batch No. VIII) from day 17 of gestation through lactation until day 21 after parturition along with a vehicle control group receiving distilled water (0.5 ml/100 g). 8 pups out of each litter were nursed until day 21, and one M and one F per litter were mated at the age of 12 weeks for the evaluation of their reproductive capability (Segment III). See pages 1-9 for detailed procedure.

RESULTS:

1. PARENTS:

a. General Observations:

One (No. 256) in 50 mg/kg/day group became weak after dosing during pregnancy. In 500 mg/kg/day group, one (No. 354) had weakness, and 3 (No.s 351, 360, 367) had red incrusted eyes during pregnancy. After delivers, other 3 (No.s 355, 359, 365) had red incrusted eyes and No. 354 had prostration.

b. Body Weight Gain:

Total group mean weight gain during gestation period was depressed in 50 mg/kg/day group ( $P < 0.05$ ) and 500 mg/kg/day group ( $p < 0.01$ , see Fig 1 on page 24 and Table 2-1 on page 32), but after parturition, the group mean gain was smallest in 50 mg/kg/day group especially first 5 days. See page 24 and Table 2-2 on page 33.

COMMENTS: The second paragraph on body weight under results section on page 10 was not accurate strictly speaking.

c. Food and Water Consumption

No significant differences between groups showed in both parameters. See Fig. 2 on page 25 and Table 4 on page 42.

d. Mortality and Necropsy:

Two rats (No.s 359 and 356) died in 500 mg/kg/day group on the first and day 18 from accidental drug delivery.

No. 256 in 50 mg/kg/day group became progressively weak after dosing on day 21 and died on the following morning before delivery, with 14 ripe fetuses in the uterine horns. Shrinking of spleen and hypertrophy of adrenals were seen in this rat at autopsy.

No. 357 in 500 mg/kg/day group died after delivery of only one fetus without remarkable signs. Congestion of lungs was seen in this rat at autopsy.

Two (No.s 354 and 356) in 500 mg/kg/day group suffered from fibrinous pneumonia and loss of body weight and died on day 6 after parturition.

e. Conception Rate:

All three treated groups had 100% (20/20) pregnancy, but the control had 95% (19/20--No. 058 in this group did not deliver and had no implantation sites in her uteri).

f. Gestation Length:

Except 4 control and 2 in 500 mg/kg/day group, which delivered on day 23, all others delivered on day 22 of gestation (exactly between 12 noon on day 21 and 12 noon on day 22). See Table 7.

2. LITTER DATA:

a. At Birth:

1. The number of viable pups per dam was slightly decreased with increased number of dead pups in 500 mg/kg/day group.

ii. Group mean fetal body weight was slightly but significantly ( $p < 0.01-0.05$ ) smaller in 50 mg/kg/day group and 500 mg/kg/day group. See Table below.

a. During the Nursing Period:

i. Mortality:

After reduction of litter size to maximum 8 pups per litter, the mortality was still significantly higher in 500 mg/kg/day group where a total of 23 pups died during the first week. All pups died in 6 litters (No. 167 in 5 mg/kg/day group, No. 254 in 50 mg/kg/day group, No.s 354, 359, 364, 365 in 500 mg/kg/day group), and they were eaten by their mothers (missed the next morning).

Table 5

Group Mean Number of Pups  
Sch 1000-BR Rat p.o. Seg. III

|           |       | day 0     |           | viable pups after selection |           |            |           | viability at       |
|-----------|-------|-----------|-----------|-----------------------------|-----------|------------|-----------|--------------------|
|           |       | viables   | deads     | day 0                       | day 7     | day 14     | day 21    | day 21 (%)         |
| Control   | total | 110       |           | 73                          | 72        | 71         | 70        |                    |
|           | mean  | 5.8 ± 2.4 | 17        | 3.8 ± 0.8                   | 3.8 ± 0.9 | 3.7 ± 0.9  | 3.7 ± 0.9 | 95.9               |
|           | total | 111       | 0.7 ± 2.3 | 78                          | 78        | 78         | 78        |                    |
|           | mean  | 5.8 ± 2.1 |           | 4.1 ± 0.9                   | 4.1 ± 0.9 | 4.1 ± 0.9  | 4.1 ± 0.9 | 100.0              |
| 5 mg/kg   | total | 124       |           | 78                          | 74        | 72         | 71        |                    |
|           | mean  | 6.2 ± 1.6 | 1         | 1.9 ± 0.4                   | 3.7 ± 1.1 | 3.6 ± 1.0  | 3.6 ± 1.1 | 91.0               |
|           | total | 119       | 0.1 ± 0.2 | 82                          | 76        | 76         | 76        |                    |
|           | mean  | 6.0 ± 1.3 |           | 4.1 ± 0.4                   | 3.8 ± 1.1 | 3.8 ± 1.1  | 3.8 ± 1.1 | 92.7 <sup>oo</sup> |
| 50 mg/kg  | total | 113       |           | 77                          | 73        | 71         | 71        |                    |
|           | mean  | 5.9 ± 2.4 | 10        | 4.1 ± 1.0                   | 3.8 ± 1.3 | 3.7 ± 1.4  | 3.7 ± 1.4 | 92.2               |
|           | total | 94        | 0.5 ± 0.9 | 74                          | 68        | 67         | 67        |                    |
|           | mean  | 4.9 ± 2.1 |           | 3.9 ± 1.0                   | 3.6 ± 1.2 | 3.5 ± 1.3  | 3.5 ± 1.3 | 90.5 <sup>oo</sup> |
| 100 mg/kg | total | 94        |           | 73                          | 57        | 57         | 57        |                    |
|           | mean  | 4.9 ± 2.0 | 29        | 3.8 ± 1.0                   | 3.0 ± 1.8 | 3.0 ± 1.85 | 3.0 ± 1.8 | 78.1 <sup>oo</sup> |
|           | total | 86        | 1.5 ± 2.9 | 69                          | 58        | 58         | 57        |                    |
|           | mean  | 4.9 ± 2.0 |           | 3.6 ± 1.2                   | 3.1 ± 1.9 | 3.1 ± 1.9  | 3.0 ± 1.9 | 82.6 <sup>oo</sup> |

Table 6

Group Mean Body Weight of Pups  
Sch 1000-BR Rat p.o. Seg. III

| dose      | sex | after selection         |                         |                          |                          |                          |
|-----------|-----|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
|           |     | day 0                   | day 0                   | day 7                    | day 14                   | day 21                   |
| Control   | ♂   | 6.2 ± 0.9               | 6.3 ± 1.0               | 16.3 ± 3.4               | 31.6 ± 4.7               | 51.2 ± 6.6               |
|           | ♀   | 5.9 ± 0.7               | 6.0 ± 0.7               | 15.9 ± 2.2               | 30.7 ± 4.1               | 49.5 ± 6.2               |
| 5 mg/kg   | ♂   | 6.2 ± 0.5               | 6.3 ± 0.5               | 16.7 ± 2.1               | 33.1 ± 3.0               | 53.4 ± 5.5               |
|           | ♀   | 6.0 ± 0.6               | 6.0 ± 0.6               | 16.0 ± 2.2               | 30.3 ± 7.9               | 52.0 ± 5.3               |
| 50 mg/kg  | ♂   | 5.9 ± 0.7 <sup>oo</sup> | 5.9 ± 0.7 <sup>oo</sup> | 15.2 ± 2.6 <sup>oo</sup> | 30.4 ± 2.8               | 48.9 ± 4.6 <sup>oo</sup> |
|           | ♀   | 5.6 ± 0.7 <sup>oo</sup> | 5.5 ± 0.7 <sup>oo</sup> | 13.9 ± 2.1 <sup>oo</sup> | 30.1 ± 2.9               | 46.0 ± 4.2 <sup>oo</sup> |
| 500 mg/kg | ♂   | 5.8 ± 0.9 <sup>oo</sup> | 5.8 ± 0.9 <sup>oo</sup> | 15.2 ± 1.9 <sup>oo</sup> | 27.3 ± 3.1 <sup>oo</sup> | 45.1 ± 5.2 <sup>oo</sup> |
|           | ♀   | 5.6 ± 0.8 <sup>oo</sup> | 5.6 ± 0.8 <sup>oo</sup> | 13.9 ± 1.9 <sup>oo</sup> | 27.3 ± 3.4 <sup>oo</sup> | 43.2 ± 5.1 <sup>oo</sup> |

ii. General Observations:

- a) One control fetus and one fetus in 50 mg/kg/day group had red incrusted eyes, without any other abnormal signs.
- b) 15 M + 16 F fetuses in 500 mg/kg/day group had red incrusted eyes, either unilateral or bilateral, that was observed after opening of eyelids and gradually disappeared until weaning. Two among these 31 pups suffered from a mild purulent inflammation (histologically confirmed) of lacrimal glands (see photo 1).

iii. Body Weight:

Body weight gain was slightly but significantly ( $p < 0.01 - 0.05$ ) retarded in both sexes in 50 and 500 mg/kg/day groups, with a trend of dose-relationship. See Fig. 3 on page 26.

iv. Setting up of Pinnae and Opening of Eyes:

Setting up of pinnae of 3 treated groups was slightly retarded, but without dose-relationship, but opening of eyes of 3 treated groups was slightly but significantly ( $p < 0.01-0.05$ ) retarded with a dose-relationship, as shown in Fig. 4 below and Table 9 on pages 61-62.

v. Swimming Test:

No significant differences between groups were shown in development of swimming ability. See Fig. 5a on page 28 and Table 10.

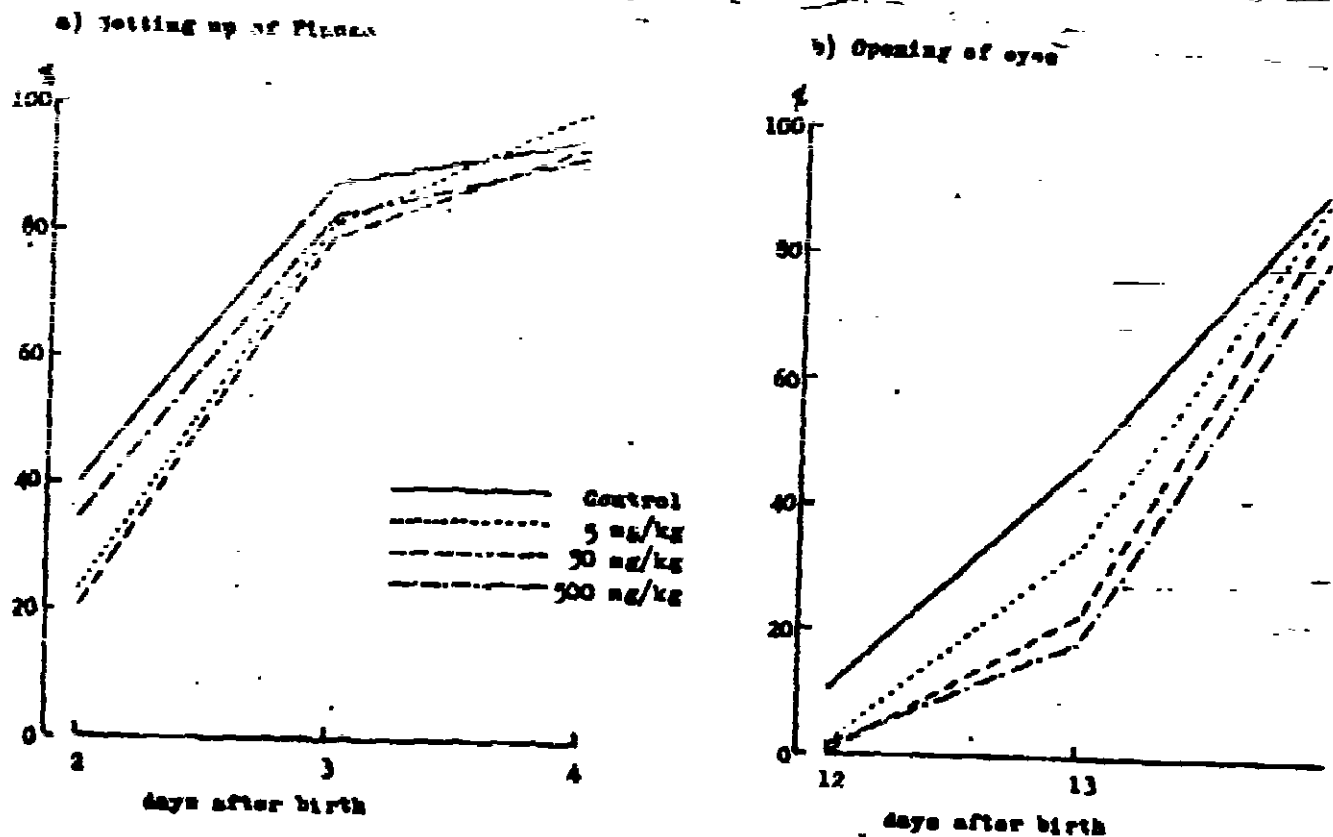
vi. Open-field Test:

Behavior studies in "open-field" as locomotion, rearing, or preening showed no significant differences between groups.

Figure 6

Development of Fins and Eyes of the Pupae

Sub 1000-22 mg P.O. Sub. III



vii. Variations

The following skeletal variations were observed on day 21:

| Group          | n   | Sternebrae<br>additives (%) | Coxae<br>additives | Lumbarisatio<br>vertebrae<br>sacralis | Total (%) |
|----------------|-----|-----------------------------|--------------------|---------------------------------------|-----------|
| D <sub>0</sub> | 148 | 8 (5.4)                     | 4 (2.7)            | 3 (2.0)                               | 15 (10.1) |
| D <sub>1</sub> | 147 | 15 (10.2)                   | 1 (0.7)            | 3 (2.0)                               | 19 (12.9) |
| D <sub>2</sub> | 138 | 2 (1.4)                     | 0                  | 3 (2.2)                               | 5 (3.6)   |
| D <sub>3</sub> | 114 | 0                           | 1 (0.9)            | 7 (6.1)                               | 8 (7.0)   |

viii. Malformations

The following abnormalities were reported:

|           |                          |                                          |
|-----------|--------------------------|------------------------------------------|
| Control   | No. 057-X <sub>1</sub> ♀ | Hydrocephalus internus                   |
|           | 057-X <sub>2</sub> ♀     | Aplasia costae dextrae XIII              |
|           | 063-X <sub>1</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>2</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>1</sub> ♀     | Hydrocephalus internus                   |
|           | 066-X <sub>1</sub> ♂     | Hypoplasia testis dextri                 |
| 3 mg/kg   | N.A.D.                   |                                          |
| 50 mg/kg  | N.A.D.                   |                                          |
| 500 mg/kg | No. 353-X <sub>1</sub> ♀ | pes varus extremitatis anterior sinister |
|           | 350-X <sub>1</sub> ♂     |                                          |
|           | 370-X <sub>1</sub> ♀     | Hydrocephalus internus                   |

By that, the malformation rate at day 21 was as follows:

|                |           |         |
|----------------|-----------|---------|
| D <sub>0</sub> | control   | : 4.7 % |
| D <sub>1</sub> | 3 mg/kg   | : 0.0 % |
| D <sub>2</sub> | 50 mg/kg  | : 1.4 % |
| D <sub>3</sub> | 500 mg/kg | : 2.6 % |

### 3. REPRODUCTION CAPACITY OF F<sub>1</sub> OFFSPRINGS:

#### a. Clinical Signs and Mortality:

No abnormal signs or mortality were observed.

#### b. Body Weight:

Body weight gain was slightly but significantly ( $p < 0.01-0.05$ ) retarded in F of 50 mg/kg/day group and M + F of 500 mg/kg/day group from week 3 through week 11 after weaning as shown in Table 12 below: See also Table 6 above.

Table 12 Mean Body Weight Development of the Offspring (F1) after Weaning

Sch 1000-BB Int r a. Sec. III

mean ± S.D.

| Dose      | Sex | n  | Age of week                |                            |                              |                              |                              |                              |                              |                             |                             |
|-----------|-----|----|----------------------------|----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-----------------------------|-----------------------------|
|           |     |    | 3                          | 4                          | 5                            | 6                            | 7                            | 8                            | 9                            | 10                          | 11                          |
| Control   | ♂   | 19 | 50.7<br>±5.2               | 91.0<br>±11.4              | 145.1<br>±15.1               | 205.3<br>±19.0               | 262.3<br>±26.2               | 317.6<br>±30.7               | 359.5<br>±33.9               | 396.3<br>±36.9              | 424.8<br>±40.8              |
|           | ♀   | 19 | 51.0<br>±5.9               | 87.9<br>±8.3               | 130.8<br>±10.1               | 165.1<br>±11.8               | 187.4<br>±11.8               | 207.7<br>±12.0               | 225.6<br>±12.4               | 238.9<br>±12.8              | 252.0<br>±13.6              |
| 5 mg/kg   | ♂   | 19 | 54.1<br>±7.7               | 94.5<br>±7.2               | 149.1<br>±11.4               | 211.5<br>±17.8               | 269.7<br>±23.5               | 324.9<br>±25.5               | 367.1<br>±29.6               | 405.2<br>±28.5              | 435.1<br>±34.3              |
|           | ♀   | 19 | 52.2<br>±6.3               | 89.0<br>±5.7               | 131.5<br>±8.1                | 168.9<br>±10.7               | 193.6<br>±13.2               | 217.4<br>±15.9               | 237.0<br>±18.8               | 252.2<br>±19.1              | 266.3<br>±21.4              |
| 50 mg/kg  | ♂   | 18 | 49.7<br>±4.4               | 89.3<br>±6.6               | 143.9<br>±11.7               | 211.6<br>±20.7               | 265.6<br>±21.1               | 319.2<br>±26.2               | 362.1<br>±30.5               | 396.2<br>±34.2              | 425.9<br>±37.2              |
|           | ♀   | 18 | 45.4<br>±5.1               | 78.7 <sup>**</sup><br>±7.5 | 120.1 <sup>**</sup><br>±8.7  | 152.1 <sup>**</sup><br>±9.5  | 175.5 <sup>**</sup><br>±11.7 | 195.2 <sup>**</sup><br>±12.0 | 213.9 <sup>°</sup><br>±14.7  | 227.0 <sup>°</sup><br>±17.7 | 239.3 <sup>°</sup><br>±18.4 |
| 100 mg/kg | ♂   | 14 | 47.5<br>±5.6               | 83.2 <sup>°</sup><br>±7.6  | 135.3<br>±20.7               | 185.9 <sup>°</sup><br>±21.4  | 235.4 <sup>**</sup><br>±26.8 | 282.3 <sup>**</sup><br>±33.4 | 332.1 <sup>°</sup><br>±33.6  | 369.7<br>±38.3              | 397.4 <sup>°</sup><br>±37.9 |
|           | ♀   | 14 | 44.2 <sup>**</sup><br>±5.2 | 76.2 <sup>**</sup><br>±7.8 | 112.1 <sup>**</sup><br>±12.7 | 145.7 <sup>**</sup><br>±13.7 | 170.2 <sup>**</sup><br>±15.7 | 192.1 <sup>**</sup><br>±16.7 | 207.9 <sup>**</sup><br>±18.4 | 224.2 <sup>°</sup><br>±18.4 | 237.4 <sup>°</sup><br>±19.5 |

c. Copulation Rate and Pregnancy Rate:

Copulation rate of the control group was 100%, but 1 treated groups had 94-95%. However, 2 females in control group and one in 5 mg/kg/day group were not pregnant as shown on the Table 16 below:

Table 16 Group Fertility Data  
Sch 1000-BR 3sg. III - F<sub>1</sub>

|                                           | Control   | 5 mg/kg   | 50 mg/kg  | 500 mg/kg |
|-------------------------------------------|-----------|-----------|-----------|-----------|
| Couples for mating                        | 19        | 19        | 18        | 17        |
| Females not inseminated                   | 0         | 1         | 1         | 1         |
| Couples copulated<br>(copulation rate)    | 19 (100%) | 18 (95 %) | 17 (94 %) | 16 (94 %) |
| at 1 <sup>st</sup> estrus<br>in 5 days    | 14        | 16        | 16        | 13        |
| at 1 <sup>st</sup> estrus<br>after 5 days | 2         | 0         | 0         | 0         |
| at 2 <sup>nd</sup> estrus                 | 1         | 0         | 0         | 0         |
| at estrus after<br>pseud-pregnancy        | 1         | 2         | 0         | 2         |
| at diestrus                               | 1         | 0         | 0         | 0         |
| SPERM-negative<br>but pregnant            | 0         | 0         | 1         | 1         |
| non-pregnant                              | 2         | 1         | 0         | 0         |
| pregnant<br>(conception rate)             | 17 (89 %) | 17 (94 %) | 16 (94 %) | 15 (94 %) |

At autopsy, three non-pregnant females did not show any abnormal findings.

d. Final Tests on F<sub>1</sub> Offsprings (Autopsy):

Males were killed and necropsied after copulation, and females, at Caesarean section. One control M(No. 059) had atrophy of seminiferal tubule in one testis, and one F in 500 mg/kg/day group (No. 351), bilateral hydronephrosis; and one in 50 mg/kg/day group, multiple anemic splenic infarcts.

e. Corpora Lutea:

The number of corpora lutea per dam was decreased significantly ( $p < 0.01$ ) in 500 mg/kg/day group. See Table for group mean litter data below.

f. Implantation:

The number of implantation sites per dam was also significantly ( $p < 0.01$ ) decreased in 500 mg/kg/day group.

g. LITTER DATA:

A total of 784 fetuses (F<sub>2</sub>) was produced and examined as in the following Table:

|                | Group<br>(mg/kg) | living<br>fetuses | df  | qq  | skeletal<br>exam. | visceral<br>exam. |
|----------------|------------------|-------------------|-----|-----|-------------------|-------------------|
| D <sub>0</sub> | Control          | 230               | 100 | 110 | 154               | 79                |
| D <sub>1</sub> | 5                | 203               | 98  | 111 | 133               | 68                |
| D <sub>2</sub> | 50               | 197               | 102 | 94  | 132               | 63                |
| D <sub>3</sub> | 500              | 154               | 88  | 66  | 102               | 52                |
| total:         |                  | 784               | 388 | 401 | 520               | 264               |

h. Group Mean Litter Data of F<sub>2</sub> generation:

Table 17 Group Mean Litter Data  
Sch 1000-BR Rat p.o. Seg. III - F<sub>2</sub>

|                                        | Control   | 5 mg/kg            | 50 mg/kg           | 500 mg/kg |
|----------------------------------------|-----------|--------------------|--------------------|-----------|
| No. of litters                         | 17        | 17                 | 16                 | 13        |
| Total no. Corpora lutea                | 270       | 262                | 242                | 174       |
| Avg. no. Corpora lutea                 | 15.9±2.1  | 15.4±2.2           | 15.1±2.0           | 13.4±2.5  |
| Range no. Corpora lutea                | 13-21     | 12-20              | 12-19              | 8-19      |
| Total no. implantation sites           | 246       | 249                | 224                | 164       |
| Avg. no. implantation sites            | 14.6±1.4  | 14.6±2.3           | 14.0±2.2           | 12.6±1.5  |
| Range no. implantation sites           | 12-17     | 10-20              | 10-18              | 8-16      |
| Ratio (implantations/Corpora lutea) %  | 91.9      | 95.0               | 92.6               | 94.3      |
| Total no. resorptions                  | 15        | 38                 | 26                 | 9         |
| Early                                  | 14        | 36                 | 17                 | 9         |
| Late                                   | 1         | 2                  | 9                  | 0         |
| Avg. no. resorptions                   | 1.1±1.6   | 2.2±2.4            | 1.6±1.7            | 0.7±0.8   |
| Range no. resorptions                  | 0- 6      | 0- 7               | 0- 5               | 0- 2      |
| Ratio (resorptions/implantations) %    | 7.3       | 13.7 <sup>a</sup>  | 11.6 <sup>a</sup>  | 5.5       |
| Total no. dead fetuses                 | 0         | 0                  | 1                  | 1         |
| Avg. no. dead fetuses                  | 0         | 0                  | 0.1±0.3            | 0.1±0.3   |
| Range no. dead fetuses                 | 0- 0      | 0- 0               | 0- 1               | 0- 1      |
| Total no. viable fetuses               | 270       | 203                | 197                | 154       |
| Avg. no. viable fetuses                | 13.5±2.3  | 11.9±3.9           | 12.3±3.1           | 11.8±2.2  |
| Range no. viable fetuses               | 8-18      | 4-20               | 6-17               | 6-14      |
| Sex ratio (male:female)                | 100:130   | 92:111             | 103:94             | 88:66     |
| Ratio (viable fetuses/implantations) % | 92.7      | 81.5 <sup>ab</sup> | 87.9 <sup>ab</sup> | 93.9      |
| Avg. weight of viable fetuses (gms)    | 5.39±0.50 | 5.42±0.47          | 5.33±0.55          | 5.48±0.45 |
| Range weight of viable fetuses (gms)   | 3.7-8.2   | 3.9-6.3            | 3.7-6.8            | 4.2-6.5   |
| No. variations                         | 38        | 36                 | 37                 | 40        |
| Variation rate (%)                     | 38.4      | 41.5               | 28.0               | 39.2      |
| No. malformations (skeletal)           | 3         | 3                  | 9                  | 2         |
| Malformation rate (%)                  | 1.3       | 1.5                | 4.6                | 1.3       |

1. Dead Fetuses:

Two dead fetuses were reported;  
One in 50 mg/kg/day group and one in 500  
mg/kg/day group.

j. Viabile Fetuses:

The number of viable fetuses per dam was slightly but significantly smaller in 500 mg/kg/day group. The percentage of implantation yielding viable fetuses was significantly smaller in two low dose groups. See Table above.

k. Weight of Fetuses:

The average weight of viable fetuses showed no differences between groups.

l. Skeletal Variations:

The following report was submitted, but it was stated that "the variation rate was within normal range".

Skeletal Variations in F<sub>2</sub> - offspring

| N   | Costae<br>additiae<br>(%) | Costae<br>cervicales | Deformatis<br>vertebrae | Lumbosacralis<br>vertebrae<br>sacralis | Sternebra-<br>schinia | Total No. of<br>fetuses with<br>variations |
|-----|---------------------------|----------------------|-------------------------|----------------------------------------|-----------------------|--------------------------------------------|
| 131 | 35<br>(26.4)              | 0                    | 3<br>(2.0)              | 2<br>(1.3)                             | 0                     | 38<br>(28.4)                               |
| 135 | 53<br>(39.3)              | 1<br>(0.7)           | 2<br>(1.5)              | 3<br>(2.7)                             | 0                     | 56<br>(41.5)                               |
| 132 | 33 <sup>a</sup><br>(25.0) | 0                    | 4<br>(3.0)              | 1<br>(0.8)                             | 1<br>(0.8)            | 37<br>(28.0)                               |
| 102 | 38<br>(37.3)              | 0                    | 3<br>(2.9)              | 6<br>(5.9)                             | 1<br>(1.0)            | 40<br>(39.2)                               |

Mean Number of ossification centers in F<sub>2</sub> - offspring

| Pars phalax |      | Mids phalax |      | Caudal<br>vertebrae |
|-------------|------|-------------|------|---------------------|
| Right       | Left | Right       | Left |                     |
| 11.0        | 10.8 | 10.5        | 10.3 | 7.3                 |
| ±2.0        | ±2.0 | ±2.0        | ±2.0 | ±1.4                |
| 11.0        | 10.6 | 10.4        | 10.3 | 7.1                 |
| ±2.1        | ±2.2 | ±2.3        | ±2.3 | ±1.3                |
| 11.7        | 11.6 | 11.1        | 11.1 | 7.3                 |
| ±1.9        | ±1.7 | ±2.4        | ±2.4 | ±1.6                |
| 11.2        | 10.8 | 10.6        | 10.7 | 7.0                 |
| ±1.9        | ±2.1 | ±2.5        | ±2.5 | ±1.1                |

K. Malformations:

The following abnormalities were reported:

Control

051 - X<sub>1</sub>      Synostosis sternebrae III-V  
051 - X<sub>2</sub>      Synostoses sternebrae I-V et deformatio  
                  costae  
051 - X<sub>3</sub>      Synostoses arcuum vertebrarum  
                  thoracarum dextrarum III-VII et  
                  sinistrarum II-VII, vertebraschisis  
                  thoracii II et XIII, hemivertebra  
                  thoracii XII et deformatio costae

2 mg/kg

154 - X<sub>1</sub>      Vertebraschisis thoracii XII  
161 - X<sub>1</sub>      Synostosis sternebrae III-V  
161 - X<sub>2</sub>      Synostosis sternebrae III-V

20 mg/kg

266 - X<sub>1</sub>      Synostosis sternebrae III-V et  
                  deformatio costae  
266 - X<sub>2</sub>      Synostoses sternebrae II, III et V et  
                  deformatio costae  
266 - X<sub>3</sub>      Deformatio costae  
266 - X<sub>4</sub>      Deformatio costae  
267 - X<sub>1</sub>      Vertebraschisis thoracii XII  
267 - X<sub>2</sub>      Synostoses sternebrae III-V  
267 - X<sub>3</sub>      Vertebraschisis thoracii XIII  
267 - X<sub>4</sub>      Vertebraschisis thoracii XIII et  
                  synostosis sternebrae II-V  
267 - X<sub>5</sub>      Vertebraschisis thoracii XIII

300 mg/kg

356 - 9        Hernia umbilicalis  
362 - X<sub>1</sub>      Synostosis sternebrae IV et V et  
                  deformatio costae

In addition to above abnormalities, a few edematous fetuses were noted at Caeserean section, although it was stated that "it was not ascertained whether the skeletal malformations occurred in edematous fetuses or others." See below:

Control

051 - 3 Anasarca  
051 - 7 Anasarca

2 mg/kg

168 - 6 Edema

20 mg/kg

266 - 4 Edema, pes varus extremitatis anterioris  
269 - 5 Edema et pes varus extremitatis anterioris  
266 - 6 Edema et pes varus extremitatis inferioris sinistri  
267 - 7 Edema et pes varus extremitatis anterioris  
266 -12 Edema et pes varus extremitatis anterioris  
266 -14 Edema et pes varus extremitatis anterioris

200 mg/kg

N. A. D.

DRUG INTERACTION OF THE 1165a( $\alpha$ -adrenergic agent) and SCH 1000-BR:  
32 DAY INTRAVENOUS TOXICITY STUDY IN BEAGLE DOGS: EE3-133:

Lab. Performing the Study: C.N. Boehringer Sohn Ingelheim a. Rhein.

Strain: Pure-bred Beagles, (M about 12 kg,  
F = 12 kg, 10 month old)Test Substances: Sch 1000-BR, Batch VIII.  
Th 1165a(1-(3,5-dihydroxyphenyl)-  
2-[[1-(4-hydroxybenzyl)-ethyl]-  
amino]-ethanol hydrobromide. Batch  
60901

## Dosage Level:

| Group | Dose $\mu\text{g}/\text{kg}$ |          | total | Volume administered<br>ml/kg | Number of animals      |         |
|-------|------------------------------|----------|-------|------------------------------|------------------------|---------|
|       | Th 1165a                     | Sch 1000 |       |                              | serial no.             | sex     |
| 0     | 0.9% NaCl solution           |          |       | 0.25                         | 001 - 003<br>051 - 053 | m-<br>f |
| 1     | 3.32                         | 1.32     | 4.64  | 0.25                         | 101 - 103<br>151 - 153 | m<br>f  |
| 2     | 20                           | 8        | 28    | 0.25                         | 201 - 203<br>251 - 253 | m<br>f  |
| 3     | 60                           | 24       | 84    | 0.25                         | 301 - 303<br>351 - 353 | m<br>f  |
| 4     | 60                           | -        | 60    | 0.25                         | 401 - 403<br>451 - 453 | m<br>f  |
| 5     | -                            | 24       | 24    | 0.25                         | 501 - 503<br>551 - 553 | m<br>f  |

## Duration and Route:

Twice daily, 7 days a week for 4 weeks  
(11/11/76-12/13/76), intravenously into  
the antebrachial cephalic vein, while  
animal fasting.

## COMMENTS:

No rate of infusion was given.

**Reason for the Dosage:** The ratio of the two substances in the combination tested was 1 part Sch 1000-Br to 2.5 parts Th 1165a, and that is also the ratio intended for man. See page 7 for detailed reasons.

**Mortality:** None.

**Clinical Signs:** After the injection, all in group 3 (highest dose combination) and group 5 (Sch 1000-Br) showed dryness of mucosa in the nose and mouth that lasted for 45-75 min. Two (Nos. 252 and 253) in group 2 (mid dose combination) also had the same symptoms as above.

Dog no. 453 in group 4 (Th 1165a) showed increased lacrimation from the 3rd day, and both eyes had a purulent conjunctivitis on 5th day that subsided in the 4th week. But the increased lacrimation persisted until the end of the study, but no treatment was given.

See Tables 013-015 for other symptoms.

**Body Weight:** There were no significant differences between groups in body weight gains. See Fig. 1 and 2.

**COMMENTS:** No page numbers for the report except the first 3 pages of the summary of the study.

**Food Intake:** There were no differences between groups except M in group 5 had slightly smaller consumption.

**Hematology:** No dose-related or time related significant changes were reported.

**Coagulation Time:** No significant differences between groups were reported for thrombin time and partial thrombin time.

**Clinical Chemistry:** LDH in groups 3 and 4 was highest at weeks 2 and 4, then followed by group 5 that was also higher than respective control values.

HCDB( $\alpha$ -hydroxybutyrate dehydrogenase) in weeks 2 and 4 was dose-relatedly increased in groups 1, 2, and 3, and that in group 4 was also higher than respective control group at week 4 (control value was smallest).

COMMENTS: It was stated that "no important shift in the  $\alpha$ -HBDH/LDH ratio was found."

CK in groups 1, 2, and 3 showed dose-related increase in week 2 and 4, and that in group 4 was highest that was followed by group 5 on both weeks 2 and 4.

CK Isoenzymes: It was reported that "slight increase in this enzyme consisted mainly of a rise in the muscle-specific CK MM band, not the cardiospecific isoenzyme CK-MB".

Cholesterol in groups 3 and 4 was slightly higher than control group in weeks 2 and 4.

AP in groups 1, 2, and 3 was higher than respective control values in weeks 2 and 4, but without dose-relationship, and that in group 4 was highest in both times.

$K^+$  in groups 2, 3, and 4 was slightly higher than control values in weeks 2 and 4.

COMMENTS: There were some animals with unusually high values in the report submitted.

Urinalysis: No significant differences between groups were reported.

Organ Weight: There were no dose-related changes in absolute weight of selected organs, although kidneys and thyroid of all treated groups were generally slightly smaller than respective control values.

Weight of testis in group 5 was smaller than control group.

COMMENTS: Many of figures in Table were not legible due to poor copies.

Necropsy Findings: The following report had been submitted:

Group 0 (controls):

Animal No. 001 m Spleen: peripheral haematoma, 0.2 cm in diameter  
Animal No. 002 m Kidneys, bilaterally: slight loss of substance when  
the capsule removed.  
Animal No. 003 m No pathological findings macroscopically.  
Animal No. 051 f Lung: isolated parasitic granulomas in all lobes.  
Animal No. 052 f Injection site: slight haemorrhage.  
Animal No. 053 f Lung: isolated parasitic granulomas in all the lobes.

Group 1:

Animal No. 101 m Liver: a grey-brown elevated calcified focus, 0.5 cm  
in diameter, in the left lateral lobe.  
Animal No. 102 m No pathological findings macroscopically.  
Animal No. 103 m Tonsils: tonsillitis.  
Animal No. 151 f No pathological findings macroscopically.  
Animal No. 152 f Thymus: multiple petachial haemorrhages in the  
parenchyma (probably due to the killing).  
Animal No. 153 f Lung: isolated parasitic granulomas in all the lobes.

Group 2:

Animal No. 201 m Injection site: slightly bloodstained  
Animal No. 202 m Injection site: slightly bloodstained  
Animal No. 203 m Spleen: two small peripheral haematomas.  
Animal No. 251 f No pathological findings macroscopically.  
Animal No. 252 f No pathological findings macroscopically.  
Animal No. 253 f Lung: a few parasitic granulomas in all lobes.  
Spleen: a small peripheral haematoma.  
Liver: clearly outlined lobules.

Group 3:

Animal No. 301 m No pathological findings on macroscopic examination.  
Animal No. 302 m Lung: isolated parasitic granulomas with peripheral  
emphysema in all the lobes; in the right  
diaphragmatic lobe there was a focus of  
pneumonia, 0.5 cm in diameter.  
Animal No. 351 m No pathological findings on macroscopic examination.  
Animal No. 352 f No pathological findings on macroscopic examination.  
Animal No. 353 f Lung: isolated parasitic granulomas in all the lobes.

Group 4 (Th 1152a)

- Animal No. 401 m No pathological findings on macroscopic examination.
- Animal No. 402 m Liver: very marked grey-yellow outlining of lobules,  
fatty liver suspected.  
Spleen: two small peripheral haematomas, and one raised  
focus, 0.3 cm in diameter, in the natural  
colour of the spleen.
- Animal No. 403 m Lung: isolated parasitic granulomas in all lobes.
- Animal No. 451 f No pathological findings on macroscopic examination.
- Animal No. 452 f Lung: isolated parasitic granulomas in all lobes,  
mild peripheral emphysema, probably due to the  
killing.
- Animal No. 453 f No pathological findings on macroscopic examination.

Group 5 (Sch 1000-BR)

- Animal No. 501 m Lung: isolated parasitic granulomas in all lobes.
- Animal No. 502 m No pathological findings on macroscopic examination.
- Animal No. 503 m Thyroid, right: aplasia.  
Muscles in nape of neck: grey-white discolouration in  
places.
- Animal No. 551 f Thyroid, right: bloodstained, due to killing.
- Animal No. 552 f No pathological findings on macroscopic examination.
- Animal No. 553 f Lung: isolated parasitic granulomas in all lobes.

Histopathology:

Histopathological examinations of beagles  
(excluding eyes) were carried out by Herr Prof.  
Dr. med. vet. J. von Sandersleben at Institute  
of Animal Pathology of the University of Munich,  
and the following summary of his findings was  
submitted, in addition to detailed report on  
each dog.

"When the findings from the controls are first compared with the findings from the animals in the groups given Th 1165a or Sch 1000 alone, the following are the results:

- 1) With Sch 1000 alone (Group 5) there were no indications of substance-related changes.
- 2) With Th 1165a alone (Group 4) one animal (402) showed a marked centrolobular or intermediate fatty change in the liver cells; in another (403) only isolated groups of cells showed fatty change. It is at least doubtful whether this finding was due to the substance.

In the animals in the highest group of the combination (Group 3), which received the same amount of Th 1165, the fatty change in the liver was no more severe. I have seen slight degrees of fatty change of individual liver cells or small groups of liver cells (as in one case each from Groups 1 - 4) in controls in other study series. It is also questionable whether the vacuolar/hydropic degeneration of the convoluted renal tubules in one case in Group 4 (403) was connected with the administration of the substance. Here again, this finding does not appear at the highest dosage in the combination. Out of 12 animals which received the highest dosage of Th 1165a alone or combination the renal abnormality appeared in only one. A connection with the substance therefore seems highly improbable.

When the findings in the animals which were given the substances in combination are compared with those of the controls or of the animals given Th 1165 or Sch 1000 alone, the following statement can be made:

- 1) In the highest group of the combination (Group 3) there were some very conspicuous scars in the left papillary muscle in one dog (302). This finding should be interpreted as being due to the substance. However, it should be emphasised that it appeared in only one out of six animals. The inconspicuous small flecks of fatty change in a few myocardial fibres, seen in two animals in Group 1 and in one in Group 2, was certainly due to chance. The glycogen content in the livers of the animals in the combination group 3 seems to be somewhat lower on average than in all the other groups. This was probably due to the experiment.

- 2) The only other findings to be discussed are those in the testes of some males. I have seen relatively sharply outlined areas of atrophy of the germinal epithelium in the testes, as in one animal in Group 4 and 5, in controls in other study series. The confirmation that this change is a spontaneous pathological process comes from the fact that it did not occur in the highest group of the combination (Group 3). The other findings in the testes of the animals in Groups 1 and 2 should also be regarded as chance findings, as they show no dose-response relationship.

In none of the groups were there any changes at the injection sites which were not also present in the controls. Quantitatively also there were no differences. All the other findings in the dogs which are not discussed in detail here are caused, in my opinion, by spontaneous pathology.

To summarise, it can be said that the myocardial scars found in one dog in highest combination group were due to the substance. The slight glycogen depletion of the liver also points in this direction. It seems at least improbable that the severe fatty change in the liver in one case from Group 4 (Th 1165 alone) and one case of vacuolar-hydropic degeneration of the tubules of the renal cortex in the same group were due to the substance. \*

Long Term Pharmacology: Electrocardiogram:

Heart Rate:

a. First Day of the Study:

There were no differences between groups before the dosing. One min after the morning dosing, the average heart rate of group 1 was markedly increased, and that in groups 2 and 3 was highly significantly ( $p < 0.01$ ) increased over the control value. One hour later, group 1 returned to control value, but groups 2 and 3 still had highly significantly increased heart rates.

Before the second dosing in the afternoon and 6 hours after the morning dose (above), there were no differences between groups in the average heart rates. But one min after the second dosing, all 3 groups (1,2,3) showed highly significantly ( $p < 0.01$ ) increased heart rates over the control value. One hour later, the heart rate of group 1 returned to control value, but that in groups 2 and 3 was still very significantly higher than control value. 18 hours later, the heart rate of all 3 groups was very similar to control group.

b. 2nd Week of the Study:

Before the dosing, all groups had similar heart rates. But one min after the 1st dosing in the morning, the heart rate of groups 1, 2, and 3 was very significantly ( $p < 0.01$ ) higher than that of control group. One hour later, the heart rate of group 1 was still slightly elevated; that of group 2, significantly so ( $p < 0.05$ ); and that of group 3 highly significantly ( $p < 0.01$ ) so.

Before the dosing, all groups had similar heart rates. But one min after the 1st dosing in the morning, the heart rate of groups 1, 2, and 3 was very significantly ( $p < 0.01$ ) higher than that of control group. One hour later, the heart rate of group 1 was still slightly elevated; that of group 2, significantly so ( $p < 0.05$ ); and that of group 3 highly significantly ( $p < 0.01$ ) so.

Before the afternoon dosing, all groups had similar heart rates to control group. The results of one min after the second dosing were the same as the first dosing, as reported above, so were the results of one hour and 18 hour after the dosing.

**COMMENTS:** Nothing was reported for groups 4 and 5.

**4th Week of the Study:**

The results of 1st and the 2nd dosing were exactly the same as the 2nd week's results described above.

**PQ, QRS, AND QT INTERVALS:**

It was reported that the group means of EKG variables were within normal limits for all groups.

**ECG Tracings:**

It was reported that drug treatment did not produce any pathological ECG tracings.

**Ophthalmological Findings:**

A complete reports on ophthalmoscopic examination and histological examination of the eyes of all dogs have been submitted, but no drug related lesions were found.

EVALUATION

Pharmacology:

Atrovent is a synthetic quaternary ammonium compound derived by quaternization of the atropine molecule. Atrovent has anticholinergic activity quite similar to atropine but the precise subcellular mechanism of action as a bronchospasmolytic agent has not been completely elucidated. The compound acts in part by vagal inhibition, preventing reflex bronchoconstriction, and in part by a local effect on the bronchus.

The anticholinergic and spasmolytic activities of atrovent have been demonstrated in numerous animal screening models and comparisons made with other anticholinergic and spasmolytic agents. The drug has greater antispasmodic efficacy than atropine in isolated organ bath preparations. Parenterally, it is more effective than atropine in its ability to inhibit gastric secretions but less effective on salivary secretion. Following enteral administration, the drug was less effective than atropine as antisecretory and antispasmodic agents. When used as an aerosol, the compound appeared to adhere to the oral and pharyngeal mucosa and was then swallowed. When used as a broncholytic agent by aerosol administration, it was more effective than atropine without producing the atropine-like side effects such as the antisialogogue effect. The ED<sub>50</sub> was approximately 25 micrograms per animal for the bronchospasmolytic effect whereas the ED<sub>50</sub> for the inhibition of salivary secretion was about 100 times higher (2 to 3 mg per animal).

In studies with anesthetized beagles using inhalations of 100 to 200 puffs of 40-sec, there were no significant changes in heart rate or any other pharmacological changes in the EKG.

The drug is relatively free of significant central nervous system activity.

Pharmacokinetics:

The biochemical and pharmacokinetic parameters with Atrovent have been investigated and include the determination of absorption, distribution, excretion, and metabolism in the rat, dog, monkey, and man. The drug pharmacokinetically is a quaternary ammonium compound. The pharmacokinetics of the drug are biphasic.

This evaluation has utilized extensively the wording as found in the summary section for preclinical studies. Appropriate modifications have been made where the reviewer differed regarding interpretation of the data.

After parenteral administration in the rat, the compound is rapidly absorbed and has a disposition half-life of about 5 minutes. The second phase has a half-life of 4 hours. Approximately 60% of the drug is excreted in the urine within the first 24 hours and an additional 20% in the feces. That part of the drug excreted in the feces is believed to be the result of the drug reaching the intestinal lumen through direct secretion through the intestinal wall. Tissue distribution studies verify a high level of the drug in the small and large intestines during the first 6-12 hours after administration. After intravenous administration, approximately 40% of the unchanged active drug is recovered in the urine. After oral administration, a major part of the administered dose (70%) is accounted for in fecal excretion. The drug did not penetrate the blood/brain barrier, either following intravenous or oral administration. By the oral route, approximately 26% of the unchanged drug was recovered in the urine the first 8 hours after drug administration.

In man, the comparative pharmacokinetic studies after the inhalation and oral routes indicated that the curves were comparable except for the low peak plasma levels following inhalation. However, the half-life for elimination of the drug from plasma after oral administration was within the same range as the half-life after inhalation. The drug was found to be present in plasma within 2 minutes following inhalation. The peak values for renal excretion of 2-3 hours were comparable for the oral and inhalation routes. The similarity between the absorption-distribution patterns following oral administration and inhalation suggests the inhaled drug is being swallowed. Low blood level and the pharmacodynamic effects do not correlate because bronchodilation occurs despite the much lower blood levels after inhalation. The bronchodilating effect is probably due to a local effect in the lung as well as particulate distribution over the oral cavity resulting in buccal absorption.

Fecal excretion, both by oral administration and inhalation, account for the majority of the drug.

Parenterally, an important excretion route was bile.

Comparative determinations of excretory patterns following initial dosage and that obtained after 13 or 26 weeks of subchronic or chronic administration in rats and monkeys, indicated that there was little difference between the urinary excretion and plasma level when compared to the values obtained pre-exposure.

The drug shows no evidence of accumulating in the body. There do not appear to be major species differences in metabolism, since the principal excretory product is unchanged drug.

### Toxicology and Pathology:

The safety evaluation studies conducted with Atrovent<sup>®</sup> in a variety of animal species have shown that the drug has a relatively wide margin of safety and compares favorably with other anticholinergic or bronchospasmolytic agents.

In acute toxicity studies, the ranges of the LD<sub>50</sub>'s after intravenous or oral administration were 12 to 20 mg/kg and 400 to greater than 4000 mg/kg, respectively. In the case of the inhalation route, monkeys tolerated dosages of 500-1000 micrograms per day for a 7-day period. These dosages, on a microgram/kilogram basis, are several hundredfold the maximum proposed daily administration in man (4.8 mcg/kg). The major signs and symptoms of toxicity were related to the anticholinergic action of the drug. The therapeutic index of Atrovent compares favorably with atropine and is even more favorable by the aerosol inhalation route of administration.

Subchronic toxicity studies ranging from four weeks to three months have been conducted subcutaneously, orally, and by the inhalation route in the rat, dog, rabbit, and monkey. In four-week or one-month oral studies in the rat, rabbit, and dog, dosages in excess of 300 mg/kg produced death. At lower dosages (30-150 mg/kg) anticholinergic effects as evidenced by dryness around the nose, eyes, and oral mucosa, were observed. Dosages in the range of 3-40 mg/kg did not produce any significant signs of anticholinergic effects. Even at lethal dosages of 300-1300 mg/kg, there were no significant pathological findings or clinical laboratory findings that could not be explained by the anticholinergic action of the drug.

Aerosol exposure of rats for 4-6 weeks at dosages up to 750 . as the human daily dose produced no toxic effects. In the monkey, exposures up to 6 weeks and dosages up to 800 mcg/day showed no evidence of anticholinergic effects and no effects on electrocardiogram, neurological parameters, or any of the biochemical measurements. There was no evidence of significant changes demonstrated upon macroscopic or histopathological examination. There were also no effects on the ciliary beat rate; however, there was some evidence of improved mucociliary transport.

In a 13-week dog oral study, at dosages of 1.5, 3 and 15 mg/kg, the anticholinergic action of the drug was manifested by inhibition of lacrimation. In the case of the two highest levels, keratoconjunctivitis sicca was present. However, the ophthalmological and histopathological examinations did not show any toxic damage to the eye.

Two, three-month dog aerosol head only exposure studies were conducted. In one study, the dogs were exposed to approximately 400 mcg/day (about 8-9 times the proposed maximum human dose on mcg/kg basis) without any adverse effects being observed. In the second study, the drug was administered as a powdered aerosol, twice daily, at dosages which were equivalent to 7, 36 and 179 mcg/kg. The highest dose was equivalent to about 40 times the maximal human dose.

No significant effects on pulmonary function or neurological examinations were observed. The electrocardiographic results showed a tachycardia at the highest dose level which was present at the time of the 6 and 13 week evaluations. There was also a dryness of the nose and oral mucosa present, which lasted for 4-5 hours in the high dose level animals but only for 1-2 hours in the intermediate dose level. There were no hematological, ophthalmological or biochemical changes observed. The pathological findings, even at the high dose level, were negative.

Chronic toxicity studies by the oral route of 26 and 52 weeks duration were conducted in purebred beagle dogs. Dosage levels were 1.5, 3.0, 15.0 and 75 mg/kg for the 6-month study. For the one-year study, the levels were identical except that the 1.5 mg/kg level was eliminated. There was a slight decrease in body weight gain in both the 3.0 and 15.0 mg/kg dose levels, with a sharp decrease being present at the highest dose level of 75 mg/kg. The weight reduction was also accompanied by a decrease in food consumption. All of the levels showed an increased frequency of emesis except the 1.5 mg/kg dose. At all the levels but the 1.5 mg/kg dose level, there was a dose dependent reduction in secretion of the conjunctiva, nasal and oral mucosa. Keratoconjunctivitis sicca was histologically evident at the three highest dose levels; however, there was no damage to the retina. There were no toxic effects on the hematopoietic system, whereas the clinical chemical parameters showed that the high dose animals had a shift in the albumin/globulin ratio towards higher globulin levels. The most prominent finding during the course of both the six-month and one-year studies, was the antisecretory effects related to anticholinergic action of the drug. There was a sporadic atrophy of the spermatogenic epithelium found in the treated animals which was not dose related. It was considered that definite evidence of toxicity was observed at the two highest dosage levels of 15 and 75 mg/kg, particularly in the one-year study.

Three chronic toxicity studies, ranging in duration from 26- to 78-weeks, by dietary admixture, were conducted in rats. The dosages employed were 6, 30 and 150 mg/kg for the 26- and 55-week studies. For the 78-week study, the high dose was 30 mg/kg. The lowest dose level was 0.5 mg/kg, a level which is approximately 100 times the maximum human therapeutic dose (12 whiffs at 0.02 mg/whiff or 4.8 mcg/kg daily). There was a marked reduction in the rate of body weight gain in males at the highest dosage level of 150 mg/kg which was accompanied by a reduction in food and water consumptions. There was a hypersecretion of the Harder's glands beginning at the 6 mg/kg dose level and increasing in severity through the 150 mg/kg dose level. Constipation was evident in both the male and female animals at the 30 and 150 mg/kg dose levels.

The hematological values were within the normal range, whereas there was a reduction in blood urea nitrogen in the 30 and 150 mg/kg groups. Coprostasis was observed at all of the dosage levels except 0.5 mg/kg. This effect, as well as the secondary inflammatory changes observed in the intestine are related to the anticholinergic action of the drug. The extensive histopathological examinations did not show any drug related organ damage at dosages up to 30 mg/kg. Therefore, Atrovent, when administered by dietary admixture to rats for 78 weeks at a dosage of 6 mg/kg of body weight, did not cause any toxicologically significant effects.

Six-month rat and monkey head-only aerosol exposure studies revealed no effects on any of the parameters studied related to the exposure of Atrovent. These included observations for effects on body weight, food consumption, normal surfactant pattern, ophthalmological effects, electrocardiographic effects, hematological, clinical chemistry and urinary parameters, ciliary beat, lung mechanics and blood gases, tracheal mucociliary transport, gross pharmacological signs, and gross and microscopic pathology, including organ weights. In the case of the rat study, the dosages employed were equivalent to 128, 256 and 384 mcg/rat per day. For the monkeys, the dosage levels were equivalent to 200, 800 and 1600 mcg/day. Therefore, Atrovent did not produce any adverse effects at dosages several hundredfold those employed clinically in the six-month aerosol inhalation studies.

Two-year carcinogenicity studies were conducted in rats and mice at dosage levels of 0.5, 2 and 6 mg/kg by dietary admixture. This route of administration was justified by the demonstrated absorption orally of the drug in the rodent within 2-3 hours after oral administration, as well as the greatly exaggerated dose administration in relationship to the bronchospasmolytic dose in man. There were no adverse effects observed on the hematological parameters or in the extensive gross and microscopic examination of the tissues. There were no drug induced non-neoplastic or neoplastic lesions that were different from the spontaneous changes normally found in the strain of rat or mouse utilized. There were also no differences in latency period between the controls and Atrovent treated groups for onset of tumor formation. Therefore, there was no evidence of carcinogenicity found following 2-year administration of Atrovent to rats and mice.

The effect of the drug on fertility and reproductive capacity (Segment I) was investigated following oral administration in rats. By dietary admixture, dosages as high as 90 mg/kg did not adversely affect fertility. Higher dosages of 500 mg/kg produced manifestation of the anticholinergic action of the drug.

Teratological studies of the Segment II type were performed in mice, rats and rabbits at oral dosages as high as 1000 mg/kg. This dose level was found to be toxic to the dams and fetuses. At a dosage of 500 mg/kg, a slight evidence of the anticholinergic activity of the drug, manifested by red, encrusted eyes and nose was observed. The same dosage produced a slight reduction in viable fetal weight. The weight of the pups at birth and weaning was also slightly reduced at the 50 and 500 mg/kg dose levels. However, there were no effects on any of the usual parameters or visceral or skeletal malformations observed. In the case of the rabbits, dosages as high as 125 mg/kg did not produce any embryotoxicity or teratogenic effects. There was evidence of the anticholinergic action of the drug at the dosage level of 125 mg/kg, as evidenced by misshaped feces, which were also sharply reduced in quantity. Therefore, Atrovent is not teratogenic at dosages several thousand times those recommended for clinical use. In addition, rat and rabbit aerosol-exposures at dosages as high as 1.5 mg/kg per day and 1.8 mg/kg per day (nose only), respectively, did not produce any effects on litter size, pup weight, pregnancy, embryonic or fetal development. Furthermore, there were no embryotoxic effects observed.

Pre- and postnatal rat studies (Segment III) were performed at dosages of 5, 10, 30, 50, 90 and 500 mg/kg orally. Dosages of 50 and 500 mg/kg produced a lower body weight gain and lower fetal weights. At 90 mg/kg there was a higher pup loss which was not statistically significant. Both the 50 and 500 mg/kg dosage levels produced a retardation of the growth of the pups up to weeks 11 while at the 500 mg/kg level, the anticholinergic activity and an increase in mortality were observed. The parameters measured in the F<sub>1</sub> offspring were comparable to the control values.

The mutagenic potential of the drug was studied in the Ames test at concentrations of 10-10,000 micrograms per plate, with and without S-9 liver cell activation against *S.typhimurium* and also with the NaDPH generating system and was found to be negative.

In the mouse micronucleus test there was no dose response nor reproducible evidence to demonstrate that the drug was clastogenic, based upon the induction of micronuclei in either polychromatic or normochromatic erythrocytes at dose levels of at least 0.5 x the LD<sub>50</sub>.

There was no evidence that Atrovent given at dosages of 42-421 mg/kg per day produced a mutagenic action in the male mouse dominant lethal assay. The cytogenetic assay in hamsters did not generate any clearcut evidence that there were significant differences between the vehicle control groups and groups dosed with Atrovent.

The various techniques that were used to study the antigenic activity of the drug which included both active and passive anaphylactic assays, did not show any evidence of sensitization or allergenicity. The drug was not well tolerated when injected repeatedly subcutaneously over a five-day period.

**CONCLUSIONS:**

It is concluded that Atrovent is reasonably safe for human use by aerosol inhalation as proposed based on preclinical data.

**RECOMMENDATION:**

This NDA is considered approvable based on preclinical data.

Young S. Choi, Ph.D.  
Pharmacologist

cc:  
NDA 19-085  
HFN-160, HFN-220  
HFN-160/YSchoi:  
R/D init. by JKinscoe:  
doc:rm 16U  
f/t jb

STATAT

REV

(15)

Statistical Review and Evaluation

Date:

NDA #: 19-085/Drug Class: B

Applicant: Boehringer Ingelheim

Name of Drug: Atrovent

Documents Reviewed: Two unnumbered volumes (1/25/85)

The medical reviewer for this NDA is Dr. Shanahan (M.D.-M) with whom this review has been discussed.

I. Background

The NDA for Atrovent was previously reviewed for the indications of chronic obstructive pulmonary disease (COPD) and bronchial asthma. In an FDA letter to the sponsor dated May 1, 1984, several statistical deficiencies were cited: (a) In Study II<sub>2b</sub>, patients were divided into day 1 responders and non-responders (to the Atrovent treatment) and efficacy conclusions were based on a statistical analysis conditioned on the patient's response to the drug; (b) the sponsor failed to address Atrovent's potential for use in the maintenance and prevention of COPD as implied in the proposed labeling; (c) the sponsor failed to fully address the use of concomitant medication and drug tolerance in the statistical analysis; (d) The sponsor did not fully explain why Day 1 base line was used to adjust the analysis of pulmonary function parameters on days 45 and 90; (e) the sponsor failed to address some extreme, abnormal laboratory values.

The sponsor resubmitted the NDA on September 14, 1984, but this reviewer found the sponsor's responses to the statistical deficiencies to be inadequate as conveyed to the sponsor in a telephone conversation (with Ms. Wasserman, Dec. 6, 1984). On January 25, 1985, an additional response was sent to the agency.

This review will evaluate the sponsor's response to the above deficiencies, item by item, and include essential portions of my previous review (April 3, 1984) so that a final conclusion can be drawn.

II. Reviewer's Evaluation of The Sponsor's Response

- (a) On the issue of drawing efficacy conclusions from a statistical analysis conditioned on the patient's response to the drug, the sponsor agreed "that statistically it is inappropriate to draw the primary efficacy conclusions ..." from this type of analysis. The sponsor went on to explain that "the only conclusions drawn (based on this restricted subgroup of patients) are: that some asthmatics do respond to the drug; and that in these patients the time profiles of response are similar to those seen in the bronchitic studies where valid comparison can be made."

For Study III<sub>9</sub>, the analysis of data subgrouped by the Day 1 responders was one of two analyses presented in the original submission. In my April 3, 1984 review, the more appropriate analysis (i.e. using all patients) was used to assess the effects of Atrovent on bronchial asthma.

(b) In response to the labeling issue of prophylactic treatment the sponsor agreed to delete the term "prevention" from the label because the studies were not designed to evaluate prevention. Therefore, the attention of this review will be focused on the acute effectiveness of Atrovent in the treatment of COPD and asthma.

(c) The sponsor tabulated for each treatment group the number of patients who used concomitant medications. The percentages of patients using concomitant medications are not entirely comparable between groups for each type of concomitant drug (e.g. theophyllines: 84% on Atrovent vs. 89% on Alupent, p=0.17 in the COPD studies; and 99% vs. 99%, p=0.037 in the asthma studies). On the issue of drug tolerance, the sponsor addressed tolerance development using the change in area under the curve (AUC) of pulmonary functions from baseline to a [AUC on day 45]-[AUC on day 1].

The following is a summary of results:

| Day | Study III <sub>9</sub> |                   |           | Study III <sub>10</sub> |                   |           |
|-----|------------------------|-------------------|-----------|-------------------------|-------------------|-----------|
|     | Atrovent<br>(n=43)     | Alupent<br>(n=37) | Diff/Prob | Atrovent<br>(n=63)      | Alupent<br>(n=50) | Diff/Prob |
| 45  | - .01                  | - .05             | 0.03/.001 | - 0.03                  | - 0.03            | 0.05/.154 |
| 90  | - .06                  | - .17             | 0.10/.001 | - 0.04                  | - 0.04            | 0.01/.800 |

It should be noted that: the mean change from baseline AUC is in units of liters per hour (AUC - 6 hrs); mean differences are differences in least-square means from a model which accounts for clinics, day 1 baseline, and day 1 baseline by treatment interaction; and probability values result from comparisons of the two (treatment) least-square means. These data showed that Atrovent patients experienced a significantly smaller average reduction in AUC after 45 and 90 days of treatment than their Alupent counterparts in Study III<sub>9</sub>, but not in Study III<sub>10</sub>. While these data do not suggest any dramatic change in the significance of the acute effect of Atrovent after continuous use, the results should also be interpreted on medical grounds.

Note that it is difficult to address the issue of drug tolerance without taking into consideration the possible changes in the doses and timing of concomitant drugs. Unfortunately, the sponsor failed to do so.

(d) The sponsor reanalyzed the AUC data for pulmonary function tests using the pre-dosing baseline value on the test day (instead of Day 1) as a covariate. The results are summarized below.

Table 1. Treatment comparisons in the GHS study

| Pulmonary Function    | Study 1115      |                |                 | Study 1119      |                |                 |
|-----------------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|
|                       | <u>Atrivent</u> | <u>Atipent</u> | <u>P-values</u> | <u>Atrivent</u> | <u>Atipent</u> | <u>P-values</u> |
| <b>Day 45:</b>        |                 |                |                 |                 |                |                 |
| Sample Size           | 43              | 41             |                 | 74              | 58             |                 |
| FVC (L)               | 7.85/7.14       | 6.09/6.84      | .071/.003       | 7.70/7.56       | 7.64/7.49      | .023/.00        |
| FEV <sub>1</sub> (L)  | 19.71/17.57     | 15.70/16.68    | .024/.001       | 16.64/16.72     | 15.67/15.89    | .012/.00        |
| FEV <sub>1</sub> /FVC | 2.79/2.48       | 2.57/2.51      | .745/.764       | 3.56/3.68       | 3.61/3.45      | .147/.05        |
| <b>Day 81:</b>        |                 |                |                 |                 |                |                 |
| Sample Size           | 43              | 37             |                 | 51              | 53             |                 |
| FVC (L)               | 7.52/6.98       | 5.93/6.61      | .091/.002       | 7.15/7.68       | 7.25/7.18      | .002/.07        |
| FEV <sub>1</sub> (L)  | 17.88/16.96     | 15.17/15.66    | .523/.639       | 16.19/16.38     | 15.71/15.39    | .011/.01        |
| FEV <sub>1</sub> /FVC | 2.67/2.60       | 2.54/2.79      | .025/.002       | 3.19/3.72       | 3.29/3.35      | .004/.02        |

Note: (1) Mean values are "unadjusted"/"adjusted for the given day baseline."  
 (2) p-values are "adjusted for given day baseline"/"adjusted for day 1 baseline."

The pulmonary function data for days 45 and 90 were previously analyzed by adjusting for Day 1 baseline. The above table presents both p-values resulting from the comparison of average AUC values (i.e., adjusting for the Day 1 baseline and adjusting for the pre-dose baseline measured on the given test day). As shown in the table, the difference in AUC for FEV<sub>1</sub> in Study IIb5 shifted from a highly significantly difference favoring Atrovent to a non-significant result at the 5% level (p=.003 adjusting for day 1 baseline to p=.074 using the pre-dose baseline on day 45; and p=.002 to .091 on day 90). However, in Study IIIa10, p values are shifted in both directions.

The sponsor argued that "measuring the effect of treatment relative to the Day 45 or Day 90 baseline (e.g by analysis of covariance or change from baseline) could be misleading if the preceding continuous therapy had influenced the baseline response." The sponsor continued "as an extreme case, a curative therapy would show no effect relative to the Day 45 baseline because the patients would arrive at testing fully bronchodilated." The sponsor's argument would be correct if the disease or symptom was dramatically improved or completely cured, thus leaving no further room for improvement, as the sponsor tends to imply. However, the data from Study IIb5 showed just the opposite. The mean pre-dosing baseline values showed a consistently deteriorating trend from 1.091 liters on Day 1, 1.054 on Day 45, down to 1.018 liters on Day 90, while the Alupent group exhibited an increasing trend (0.894, 0.977 and 0.941 liters on Days 1, 45, and 90 respectively). Under these conditions (lower daily pre-dosing baselines), Atrovent patients should have even more opportunity to improve. Unfortunately, the AUC for FEV<sub>1</sub> deteriorated from 7.14 on Day 45 to 6.98 on Day 90.

- (e) The sponsor admitted mishandling of some laboratory values as pointed out in my previous review. For examples, in Goldring's study the creatinine 100 mg/DL of patient #1211 should have been reported as the tie value for glucose; and the lymphocyte 2.0% of patient #1213 should have been 37.0%. The sponsor submitted a corrected tabulation of laboratory values, but failed to perform statistical comparisons between treatment groups.

### III Overall results reported by the sponsor

The overall efficacy results are summarized below.

Table 2: Summary of COPD and bronchial asthma studies

| Pulmonary Function    | COPD STUDIES                              |                 |                 |                         |                 |                 |
|-----------------------|-------------------------------------------|-----------------|-----------------|-------------------------|-----------------|-----------------|
|                       | Study II <sub>b5</sub>                    |                 |                 | Study III <sub>a5</sub> |                 |                 |
|                       | Day 1                                     | Day 45          | Day 90          | Day 1                   | Day 45          | Day 90          |
| Sample Size           | 49/48                                     | 43/41           | 43/37           | 82/80                   | 74/58           | 64/53           |
| Baseline              |                                           |                 |                 |                         |                 |                 |
| FEV <sub>1</sub> (L)  | 1.08/.89/.04                              | 1.06/0.98/.06   | 1.12/0.94/.06   | 1.03/1.17/.05           | 1.21/1.13/.05   | 1.12/1.13/.87   |
| FVC (L)               | 2.56/2.24/.08                             | 2.60/2.39/.04   | 2.66/2.31/.03   | 2.21/2.34/.07           | 2.49/2.33/.03   | 2.16/2.31/.58   |
| FEF <sub>25-75%</sub> | 0.38/0.33/.03                             | 0.39/0.36/.13   | 0.35/0.34/.08   | 0.47/0.55/.10           | 0.58/0.52/.04   | 0.53/0.55/.50   |
| AUC                   |                                           |                 |                 |                         |                 |                 |
| FEV <sub>1</sub> (L)  | 7.46/7.08/.03                             | 7.14/6.84/.07   | 6.93/6.67/.09   | 8.07/7.55/.001          | 7.88/7.49/.02   | 7.69/7.18/.01   |
| FVC (L)               | 17.72/17.12/.10                           | 17.57/15.68/.02 | 16.96/16.66/.02 | 17.33/15.60/.001        | 16.73/15.89/.01 | 16.33/15.39/.01 |
| FEF <sub>25-75%</sub> | 2.79/2.47/.03                             | 2.48/2.51/.74   | 2.50/2.39/.04   | 3.72/3.38/.001          | 3.67/3.45/.17   | 3.72/3.35/.01   |
|                       | Bronchial Asthma (Study I <sub>a5</sub> ) |                 |                 |                         |                 |                 |
| Pulmonary Function    | Day 1                                     | Day 45          | Day 90          |                         |                 |                 |
| Sample Size           | 78/77                                     | 70/73           | 69/65           |                         |                 |                 |
| Baseline              |                                           |                 |                 |                         |                 |                 |
| FEV <sub>1</sub> (L)  | 2.44/2.32/.28                             | 2.47/2.22/.13   | 2.47/2.22/.17   |                         |                 |                 |
| FVC (L)               | 3.58/3.39/.19                             | 3.55/3.30/.12   | 3.45/3.25/.09   |                         |                 |                 |
| FEF <sub>25-75%</sub> | 1.93/1.83/.51                             | 1.85/1.64/.15   | 1.93/1.71/.05   |                         |                 |                 |
| AUC                   |                                           |                 |                 |                         |                 |                 |
| FEV <sub>1</sub> (L)  | 14.57/15.12/.15                           | 14.27/14.32/.92 | 14.45/14.11/.38 |                         |                 |                 |
| FVC (L)               | 19.83/20.82/.004                          | 19.58/19.93/.44 | 19.40/19.73/.56 |                         |                 |                 |
| FEF <sub>25-75%</sub> | 12.47/13.24/.13                           | 12.06/11.82/.69 | 12.30/12.11/.08 |                         |                 |                 |

Note: (1) The pair of numbers are sample sizes of "Atravent"/"Alupent"  
 (2) The triple sets of numbers indicate adjusted means of "Atravent"/"Alupent"/"p-value".  
 (In Study II<sub>b5</sub>, means are adjusted for Day 1 baseline.)

Since the sponsor has withdrawn the labelling claim of maintenance and prevention of COPD, the statistical analysis and interpretation will be focused on the acute effects of Atrovent on COPD and bronchial asthma which are primarily measured by the AHC (of pulmonary function) after adjusting for the daily pre-dosing baseline.

#### (c) COPD Studies

As shown in Table 2, the Atrovent patients of Study III<sub>10</sub> obtained significantly greater improvement from the pre-dosing baseline in FEV<sub>1</sub>, FVC, and FEF<sub>25-75</sub> than their Alupent counterparts for each of 3 visits except FEF<sub>25-75</sub> on day 91. In Study III<sub>11</sub>, Atrovent patients showed consistently higher average AHC values than Alupent patients, with significant differences in favor of Atrovent observed in FEV<sub>1</sub> on day 1, day 42 and FEF<sub>25-75</sub> on day 91.

#### (d) Bronchial Asthma Study

In bronchial asthmatic patients (Study III<sub>12</sub>), no significant difference (except 1/3 in favor of Alupent on day 1) was seen between treatment groups.

#### (e) Study 12<sub>10</sub>

This study was designed to evaluate the effects of Atrovent and theophylline given together as compared with theophylline alone. Two groups of bronchial asthmatic patients (11 each) with a relatively stable airway obstruction completed this 90-day randomized double-blind study. The first group of patients received Atrovent (40 mcg QID) on day 1, placebo on day 2, Atrovent for the next 90 days, and then placebo on the last day. Group 2 received the opposite order of treatments. All patients received theophylline during the course of the trial. FEV<sub>1</sub>, the primary efficacy variable, was taken at pre-dosing, and every hour for 6 hours after dosing on days 1, 2, 90 and 91.

The data showed that the addition of Atrovent to theophylline maintenance treatment failed to provide a significant increase in FEV<sub>1</sub> (using the change from pre-dosing baseline on day 90), or mean peak flow rate (43.3 on Atrovent vs. 44.8 on placebo in the morning, and 37.7 vs. 23.4 in the evening). Furthermore, patients receiving Atrovent in addition to theophylline treatment reported a higher rate of acute attacks per day (0.187 with Atrovent vs. 0.120 without Atrovent) and a higher mean daily use of theophylline (4.57 whiffs with Atrovent vs. 4.11 without Atrovent); these differences failed to reach statistical significance at the 5% level.

#### (f) Safety

For safety, several adverse reactions were reported in each of the COPD and Asthmatic studies. However, no statistically significant difference was found between Atrovent and Alupent in each event rate.

The most frequently reported adverse events included cardiovascular palpitations, nervousness, dizziness, all of which, musculoskeletal tremor, dry mouth, and cough in the COPD studies; and nervousness, dizziness, cardiovascular palpitations, headache, nausea, irritation from aerosol, respiratory exacerbation of symptoms, and cough in the bronchial asthma study.

In the reevaluation of laboratory values, the sponsor corrected data handling errors and reanalyzed those patients who experienced abnormal changes. Since the number of patients who experienced an abnormal change in laboratory values was extremely small, statistical procedures could not detect a significant mean difference between treatment groups.

#### iv. Reviewer's Comments:

(1) The original submission of this NDA did not clearly indicate whether this NDA was intended to support a therapeutic or a prophylactic claim for the efficacy of Atrovent in COPD patients. There is a distinction in types of endpoints and statistical analysis used to address these 2 claims.

To establish a therapeutic claim, the sponsor should focus on the acute effects of the drug by evaluating differences between pre- and post-dosing pulmonary function values. Comparing treatment with respect to the change from pre-dosing ABC in pulmonary function variables is a reasonable approach to this question. In terms of maintenance and prevention (or prophylactic treatment), attention should be given to the trend over time in the pre-dosing pulmonary function tests and the frequency of bronchospastic symptom occurrence (such as asthma or wheezing attacks). This reviewer raised this issue in the previous review, as communicated to the sponsor. In response, the sponsor had deleted claims implying maintenance and prevention effects from the proposed labeling for Atrovent. Hence, this review is primarily focused on the assessment of acute effects of the drug in improving pulmonary performance and alleviating symptoms.

(2) The sponsor analyzed the pulmonary function data on days 45 and 90 after adjusting for the baseline measured on day 1. They argued that "measuring the effect of treatment relative to the Day 45 or Day 90 baseline (e.g. by analysis of covariance or change from baseline) could be misleading if the preceding continuous therapy had influenced the baseline response." They went on to say "as an extreme case, a curative therapy would show no effect relative to the Day 45 or Day 90 baseline because the patient would arrive at testing fully bronchodilated."

Indeed, if the disease is cured or the symptoms are completely eliminated as a result of chronic treatment, this leaves no room for improvement over the pre-dosing baseline measured on day 45 or day 90. However, such was not the case for this NDA. Pre-dosing pulmonary function values got worse in study IIIa5. For example, pre-dosing FEV<sub>1</sub> was 1.081 on Day 1, 1.056 on Day 45 and down to 1.018 liters on Day 90 in study IIIa5. Pulmonary function in study IIIa10 showed a slight improvement (8.3% in FEV<sub>1</sub>, 6.5% in FVC) on Day 90

from Day 1, but patients were certainly not fully bronchodilated. In addition, the pre-treatment baseline of Alupent patients showed an increase in FEV<sub>1</sub> from Day 1, which would further increase the (p-value of mean FEV<sub>1</sub>) provide an even greater chance to demonstrate differences in favor of Atrovent.

Therefore, adjustment for the baseline on each test day (rather than Day 1 baseline) is reasonable and fully justified.

(3) Concomitant medications were heavily used in both studies. Theophylline alone was used by 95% of patients in the COPD Study and 100% on Atrovent vs. 92% on Alupent, p=1/20) and 95% of patients in the bronchial asthma study (99% on Atrovent vs. 92% on Alupent, p=0.037). Without a group of patients treated with theophylline alone serving as a control, it is difficult to determine the extent to which treatment effects on pulmonary function might be explained by use of concomitant medications. The sponsor was requested to address this issue in its previous review. However, the sponsor failed to specifically address the impact of concomitant medication use on treatment effects. A simple approach to address this issue is to compare the mean daily use of theophylline between treatments.

The sponsor was also asked to address the issue of drug tolerance. In response, the sponsor compared the changes in all from baseline between treatment groups. This reviewer believes that the comparative use of concomitant medication should be taken into consideration in the analysis of drug tolerance.

(4) In future studies, the sponsor should consider including a group of patients taking only concomitant medications to serve as a control so that the study drug effect can be separated from the effect of background medications. Unfortunately, IrV's study failed to demonstrate that the combination of Atrovent and theophylline provides a significant increase in FEV<sub>1</sub> or mean peak flow rate over levels achieved with theophylline alone.

## V. Summary

This NDA was intended to establish the efficacy of Atrovent for two clinical indications -- chronic obstructive pulmonary disease (COPD) and bronchial asthma. Two multicenter controlled studies (Studies IIB5 and IIA5) involving 98 and 177 patients respectively) were conducted in COPD patients, while only one multicenter study (Study IIA5 with 108 patients) was done in bronchial asthmatics.

In COPD studies, Atrovent was shown to be statistically superior to Alupent in AUC of FEV<sub>1</sub> (8.07 on Atrovent vs. 7.55 on Alupent on Day 1, 7.86 vs. 7.49 on Day 49 and 7.68 vs. 7.18 on Day 90), FVC (17.05 vs. 15.60, 16.72 vs 15.89, and 16.34 vs. 15.39), and FEV<sub>0.75</sub> (3.70 vs. 3.39, 3.68 vs. 3.45, and 3.72 vs. 3.35) in Study IIA10; in Study IIB5, Atrovent patients obtained a consistently greater AUC than their Alupent counterparts in FEV<sub>1</sub> and FVC.

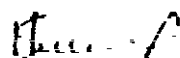
in terms of the onset time and duration of at least a 15% increase in FEV<sub>1</sub> (which occurred in the majority of patients), no statistical difference between groups was found in Study 11b5 (the average time to response on the 3 test days ranged from approximately 15-30 minutes, with a median duration of 1.5 hours). However, in Study 111a10, Atrovent had a similar onset time, but a 2-fold longer median duration of effect than Alupent.

In the bronchial asthmatic study, Alupent patients obtained a consistently greater AUC in FEV<sub>1</sub> and FVC than Atrovent counterparts, even though Alupent patients started out with consistently lower pre-dosing baseline values. However, no significant difference was detected at the 5% level.

For safety, patients in COPD and bronchial asthma studies reported similar adverse reactions. These included cardiovascular palpitations, nervousness, dizziness, GI distress, nausea, headache, and cough. Abnormal changes in laboratory values included white blood cell counts, uric acid, Alkaline phosphatase, LDH, total protein, globulin, Gamma gi, and phosphorous. Goldring's study reported extremely high percentages of abnormalities in laboratory values, e.g. hemoglobin (94% on Atrovent vs. 92% on Alupent), Lymphocyte (56% vs. 33%), eosinophil (71% vs. 100%), Alkaline phosphatase (31% vs. 46%), LDH (75% vs. 100%) and WBC (12% vs. 16%). These abnormalities should be interpreted on medical grounds.

VI. Conclusion (which may be conveyed to the sponsor)

Studies in this RDA provide statistical evidence that Atrovent is at least as effective as Alupent for the acute improvement of pulmonary function and relief of symptoms in COPD patients. However, evidence supporting the efficacy of Atrovent in bronchial asthma was weak for the following reasons: (a) A significantly higher proportion of patients was receiving concomitant theophylline on Atrovent than on Alupent (99% on Atrovent vs. 90% on Alupent, p=0.077), (b) Atrovent patients showed smaller average AUC values for pulmonary function parameters than Alupent patients, even though Alupent patients started out with consistently lower pre-dosing baselines, (c) Kirk's study failed to establish the benefit of adding Atrovent to a theophylline maintenance regimen, and (d) no other independently conducted, well controlled trial was available to support the 11a5 study in evaluating Atrovent for the treatment of bronchial asthma.

  
K.S. Huang

Mathematical Statistician

000 On 1/1/1988

HN 100

HN 160/Dr. Shanahan

HN 344/Dr. L. Scott

~~HN 112/Dr. Tubey~~

HN 713/Dr. Huang

Chron.

File: HNU 1.3.2 NW

Manufacture of plates and film control

Contact: *MT 5/1/88*

STATISTICAL REVIEW AND EVALUATION

Date: APR 3 1984

NDA # 19-085

Drug Class: IC

Applicant: Hoehringer Ingelheim Ltd.

Name of Drug: Atrovent Inhaler (ipratropium bromide)

Documents Reviewed: Volumes 1.1 and 1.10-1.18 and 1.20 dated 07-29 83

The medical officer for this submission is F.J. Shanahan, M.D. (HFN-160), with whom this review has been discussed.

Until the additional information requested in this review is provided by the sponsor, it is not possible to draw firm conclusions regarding the efficacy and safety of Atrovent. In particular, the sponsor has not fully substantiated the claim that Atrovent is effective for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease.

1. SUMMARY

This submission contains studies evaluating the efficacy and safety of Atrovent Inhaler for the treatment of patients with chronic obstructive pulmonary disease (COPD) or with bronchial asthma. There were two multicenter controlled studies and one single-center controlled study in COPD patients, while there was only one multicenter controlled study in bronchial asthma patients. Two hundred and five patients (110 on Atrovent and 95 on Alupent) completed the 90-day COPD study, while 144 patients (71 on Atrovent and 73 on Alupent) completed the 90 day bronchial asthma study.

For efficacy, the COPD studies revealed a significant difference in terms of FEV1.0 in favor of Atrovent. But the bronchial asthma studies failed to demonstrate the superiority of Atrovent over the active control drug. Both Atrovent and Alupent patients exhibited a slight decline in AUC of pulmonary function tests over the study periods (which could be due to deterioration in pulmonary function or increasing tolerance to treatment). This reviewer feels that the statistical analysis should specifically address the sponsor's claim of maintenance treatment and prevention of bronchospastic symptoms associated with COPD or bronchial asthma by clearly defining mathematical models and hypothesis tests. Also, the sponsor should address the extremely high level of concomitant drug use during these trials, including Theophylline (85% of COPD patients and 96% of bronchial asthma patients), steroids (24.1% of COPD patients and 37.2% of bronchial asthma patients), beta-adrenergic agents and other medications that could affect pulmonary functions and complicate the interpretation of the trial results.

For safety, numerous adverse reactions were observed during Atrovent therapy such as cough (6.1% of 228 Atrovent patients), nausea (3.1%), nervousness (3.1%), dizziness (2.6%), headache (2.6%), GI distress (2.6%), and respiratory exacerbation of symptoms (2.6%). Several patients whose initial laboratory

values were within normal ranges before treatment. Blood levels that were elevated or decreased abnormally at the end of the study period. These laboratory parameters, which included BUN, uric acid, phosphorus, LDH and white blood cell count, should be examined on medical grounds.

The pulse rate was reported to drop an average of 7.5 beats/minute after dosing in each treatment group of the COPD and bronchial asthma studies. Blood pressures were also reported to show a declining trend.

## II. Patient Enrollment

### A. COPD Studies

Studies in COPD included multicenter studies IIB5 (Vol. 1.10-1.11) and IIIA10 (Vol. 1.12-1.13), and one single-clinic study (L80-606, Vol. 1. 4) by R. Goldring, M.D. The IIB5 studies involved four investigators, while IIIA10 studies consisted of two investigators. Goldring's study was initiated as one of the IIB5 multicenter studies. However, due to this investigator's elimination of 2-week wash-out periods for beta-adrenergic bronchodilators, the data were analyzed separately.

Of 98 patients enrolled in the IIB5 study, 83 patients (84.7%) completed the study, while 122 of 177 patients (68.9%) completed the IIIA10 study. Early withdrawals were due to: side effects, three on Atrovent (2.3%) and six on Alupent (4.0%); lack of efficacy, one on Atrovent (0.8%) and eight on Alupent (6.2%); and other reasons such as violation of protocol and lost to follow-up, 10 on Atrovent (7.6%) and 15 on Alupent (10.8%). Details are given below.

DISTRIBUTION OF PATIENT'S ENROLLMENT  
AND REASONS FOR EARLY WITHDRAWAL

| Studies                     | Completed/<br>Enrolled |                     | Reasons for Early Withdrawal |     |                  |     |        |      |
|-----------------------------|------------------------|---------------------|------------------------------|-----|------------------|-----|--------|------|
|                             | Atrovent               | Alupent             | Side Effects                 |     | Lack of Efficacy |     | Other  |      |
|                             |                        |                     | Atr                          | Alu | Atr              | Alu | Atr    | Alu  |
| <b>Multicenter IIb5</b>     |                        |                     |                              |     |                  |     |        |      |
| Eugell/M. Gross             | 17/21                  | 16/20               | 2                            | 2   | 1                | 1   | 1      | 1    |
| Emrigil                     | 6/6                    | 5/6                 | 0                            | 0   | 0                | 0   | 0      | 1    |
| Newdehner                   | 13/14                  | 12/14               | 0                            | 0   | 0                | 1   | 1      | 0    |
| Plummer                     | 7/8                    | 7/8                 | 0                            | 0   | 0                | 0   | 1      | 1    |
| Sub-Total                   | 43/49                  | 40/48*              | 2                            | 2   | 1                | 2   | 3      | 3    |
| <b>Multicenter IIa10</b>    |                        |                     |                              |     |                  |     |        |      |
| Ashutosh                    | 10/10                  | 9/10                | 0                            | 0   | 0                | 0   | 0      | 0    |
| B. Becker/B. Pitt           | 7/7                    | 5/8                 | 0                            | 0   | 0                | 0   | 0      | 3    |
| Comiskey                    | 8/9                    | 8/8                 | 0                            | 0   | 0                | 0   | 1      | 0    |
| G. Gross                    | 6/8                    | 4/6                 | 0                            | 0   | 0                | 1   | 2      | 1    |
| N. Gross                    | 8/16                   | 7/16                | 0                            | 1   | 0                | 3   | 1      | 3    |
| Lakshminarayan              | 9/10                   | 8/12                | 0                            | 0   | 0                | 1   | 1      | 2    |
| Miller/Kotch                | 4/6                    | 5/6                 | 1                            | 1   | 0                | 0   | 1      | 0    |
| Renzetti                    | 3/4                    | 2/3                 | 0                            | 0   | 0                | 0   | 1      | 1    |
| Sackner/Wanner              | 4/4                    | 3/5                 | 0                            | 1   | 0                | 0   | 0      | 1    |
| Tashkin                     | 8/9                    | 4/7                 | 0                            | 1   | 0                | 1   | 0      | 0    |
| Sub-Total                   | 67/83 <sup>+</sup>     | 55/81 <sup>++</sup> | 1                            | 4   | 0                | 6   | 7      | 11   |
| Overall total               | 110/132                | 95/129              | 3                            | 6   | 1                | 8   | 10     | 14   |
| % of Overall total          | 83.3                   | 73.6                | 2.3                          | 4.6 | 0.8              | 6.2 | 7.6    | 10.8 |
| Prob. (Fisher's Exact Test) |                        |                     | P=.476                       |     | P=.038           |     | P=.483 |      |

- \* one patient in progress
- + eight patients in progress
- ++ five patients in progress

The Fisher's Exact Test was performed by the reviewer to see if the rate of early withdrawal differed between treatment groups.

In Goldring's study, 29 patients were enrolled (21 males, 8 females), but 27 patients completed the 90-day study. Patients #1209 (Atrovent) and #1220 (Alupent) dropped out after day 1. Patient #1209 had abnormal liver enzymes (SGOT, SGPT and LDH), but patient #1220 was lost due to patient's distant travel to clinic.

B. Bronchial Asthma Studies

Four investigators enrolled 168 patients in this multicenter study IIa5 (Vol. 1.15-1.17). Of these, 144 patients completed the study (71 patients treated with Atrovent (87.6%), 73 on Alupent (88.0%)). Early withdrawals were due to: side effects, 0 on Atrovent and 3 on Alupent; lack of efficacy, 5 on Atrovent and 1 on Alupent; and other reasons such as violation of protocol and

Loss to follow-up, 4 on Atrovent and 3 on Alupent. Details are given below:

DISTRIBUTION OF PATIENT'S ENROLLMENT  
AND REASONS FOR EARLY WITHDRAWAL

| Study:                      | Completed/<br>Enrolled |         | Reasons for Early Withdrawal |        |                  |        |       |       |
|-----------------------------|------------------------|---------|------------------------------|--------|------------------|--------|-------|-------|
|                             |                        |         | Side Effects                 |        | Lack of Efficacy |        | Other |       |
|                             | Atrovent               | Alupent | Atv                          | Alu    | Atv              | Alu    | Atv   | Alu   |
| Multicenter IIA             |                        |         |                              |        |                  |        |       |       |
| Busse/Bush                  | 15/19                  | 15/17   | 0                            | 1      | 1                | 0      | 3     | 1     |
| Falliers                    | 15/15                  | 15/15   | 0                            | 0      | 0                | 0      | 0     | 0     |
| O'Hollaren                  | 11/16                  | 13/17   | 0                            | 0      | 4                | 0      | 0     | 1     |
| Storms                      | 15/16                  | 15/17   | 0                            | 1      | 0                | 0      | 1     | 1     |
| Weg                         | 15/15                  | 15/17   | 0                            | 1      | 0                | 1      | 0     | 0     |
| Total                       | 71/81*                 | 73/83** | 0                            | 3      | 5                | 1      | 4     | 3     |
| Percent of Total            | 87.6                   | 88.0    | 0.0                          | 3.6    | 6.2              | 1.2    | 4.9   | 3.6   |
| Prob. (Fisher's Exact Test) |                        |         |                              | P=.257 |                  | P=.201 |       | P=.97 |

\* one patient in progress

\*\* three patients in progress

The Fisher's Exact Test was performed by the reviewer to see if the rate of early withdrawal differed between treatment groups.

III. STUDY DESIGN AND ANALYSIS

Both COPD and bronchial asthma studies had a 2-week washout period followed by a 90-day randomized double-blind parallel group treatment period. During treatment, patients returned for follow-up on days 1, 45 and 90. On each study day, pulse, blood pressures, pulmonary functions, and body plethysmography were measured at pre-dosing, 0.25, 0.50, 1, 2, 3, 4, 5, and 6 hours after dosing. Pulmonary function tests included FEV<sub>1,0</sub>, FVC and FE<sub>25-75%</sub> (measured in liters). Body plethysmography consisted of airway resistance (Raw), and thoracic gas volume (VTS) and hence specific conductance (SGaw) was derived. The areas under the curves (AUC) of pulmonary functions were computed using Trapezoid-rule approximations.

At bimonthly visits on days 1, 15, 30, 45, 60, 75, and 90, the physician's assessment of overall disease condition was recorded on a 100 mm line ranging from "poor" (0 mm) to "excellent" (100 mm). A similar measurement scale was used by the patient in response to the question "How did you feel during the last two weeks compared to the previous two week period?"

For the safety data, physical examination and laboratory data (such as hematology, SMA-12, and urinalysis) were obtained prior to admission to the study and at the end of the study.

Treatment (either Atrovent or Alupent) was given by oral inhalation. Patients were required to take two whiffs of either Atrovent (20 mcg) or Alupent (750 mcg) on the morning of each test day. During the 90-day treatment

period, patients also took two whiffs of either 20 mcg (q.i.d) of Atrovent or 750 mcg (q.i.d) of Alupent. If necessary, the patient might also take an additional two whiffs, either at bedtime or during the night, but the number of whiffs was not to exceed 12 whiffs/24 hours.

The sponsor analyzed the AUC for each pulmonary function parameter (FEV1.0, FVC, FEF<sub>25-75%</sub> and SGaw) at each visit using analysis of covariance with day 1 predose levels as covariates. The reported means in the subsequent tables are the least-squares adjusted means. The onset time and durations of at least 15% increase in FEV1.0 were reported. The frequencies of adverse reactions were tabulated and percentages were reported. The means of laboratory values were calculated and raw data were displayed for each group.

## RESULTS REPORTED BY THE SPONSOR

### A. Efficacy Data

#### 1. COPD Study

In the IIb5 study, the pre-treatment (day 1) baseline mean of FEV1.0 was significantly lower on Alupent (0.894L) than on Atrovent (1.081L). Hence, the subsequent statistical comparison of treatment means was made after adjustment for the day 1 baseline. Mean FEV1.0 at baseline (pre-dose) in the Atrovent group showed a slight decrease from 1.021 on day 1 to 1.056 (-2.31%) on day 45 and to 1.018 (-5.83%) on day 90, while the corresponding means for the Alupent group increased from 0.894(L) on day 1 to 0.977 (+9.28%) on day 45, and to 0.941 (+5.26%) on day 90, as shown in Table 1. That is, longitudinally, the baseline FEV1.0 profile of Atrovent patients showed a declining trend (deterioration in FEV1.0) while the Alupent group showed an increasing tendency. Cross-sectionally, comparisons of baseline FEV1.0 between Atrovent and Alupent revealed a significant difference on day 1 (P=0.040), but this difference narrowed to borderline significance (P=0.056) on both days 45 and 90. Table 2 shows that there was a statistically significant difference in baseline FVC (pre-dose) between Atrovent and Alupent on days 45 and 90, but these differences may not reflect prophylactic effects of Atrovent since the treatment groups showed a nearly significant difference in pre-dose FVC on day 1 (p=.079). Further, the Atrovent FVC mean on day 1 was exactly the same as that observed on day 90, as shown in Table 1.

In terms of FEV1.0 area under the curve (AUC), the Atrovent group showed a significantly higher mean AUC than the Alupent group on each of the three study days. Longitudinally, both Atrovent and Alupent showed a declining trend in AUC of FEV1.0 (7.455, 7.227 and 7.077 for Atrovent vs. 7.084, 6.524, and 6.254 for Alupent on days 1, 45 and 90 respectively).

Significant differences were seen in AUC for FVC on days 45 and 90, since the Alupent group showed a decline (-7.50%) while Atrovent patients remained fairly stable (-1.86%). No significant difference between groups was observed for SGaw.

The onset of at least a 15% increase in FEV1.0 in the majority of patients was reported as exactly equal for Atrovent and Alupent groups (15, 30 and 15 minutes on days 1, 45 and 90 respectively). The median durations of at least a 15% increase in FEV1.0 on days 1, 45 and 90 were (4,3,3) and (4,1,2) hours for Atrovent and Alupent groups respectively. The median durations were not

| Efficacy Measurements | Atrovent |        | Alupent |       | P-values of Comparisons |        |       |        |        |
|-----------------------|----------|--------|---------|-------|-------------------------|--------|-------|--------|--------|
|                       | Day 1    | Day 45 | Day 90  | Day 1 | Day 45                  | Day 90 | Day 1 | Day 45 | Day 90 |
| Sample Size           | 16       | 15     | 15      | 13    | 12                      | 12     | 29    | 27     | 27     |
| <b>Baseline</b>       |          |        |         |       |                         |        |       |        |        |
| FEV <sub>1.0</sub>    | 1.001    | 0.995  | 0.985   | 0.933 | 0.990                   | 0.925  | 0.623 | 0.974  | 0.682  |
| FVC                   | 2.082    | 2.047  | 2.033   | 1.955 | 1.900                   | 1.898  | 0.593 | 0.524  | 0.555  |
| FEF <sub>25-75%</sub> | 0.409    | 0.437  | 0.381   | 0.391 | 0.435                   | 0.365  | 0.820 | 0.989  | 0.814  |
| SGaw                  | 0.032    | 0.037  | 0.031   | 0.053 | 0.045                   | 0.042  | 0.043 | 0.468  | 0.185  |
| <b>AUC</b>            |          |        |         |       |                         |        |       |        |        |
| FEV <sub>1.0</sub>    | 0.917    | 1.149  | 1.143   | 0.842 | 0.760                   | 0.873  | 0.809 | 0.207  | 0.437  |
| FVC                   | 2.31     | 2.255  | 2.440   | 1.217 | 1.882                   | 1.197  | 0.082 | 0.791  | 0.061  |
| FEF <sub>25-75%</sub> | 0.008    | 0.423  | 0.476   | 0.214 | 0.467                   | 0.481  | 0.903 | 0.066  | 0.983  |
| SGaw                  | 0.098    | 0.098  | 0.137   | 0.043 | 0.057                   | 0.057  | 0.110 | 0.260  | 0.063  |

As shown in the table above, both Atrovent and Alupent groups presented a longitudinal declining trend in pre-dose baseline pulmonary tests, but the groups exhibited an increasing tendency in AUC. However, there were few statistically significant differences between groups observed in either baseline or AUC.

## 2. Bronchial Asthma Study

In the IIa5 study, no statistically significant differences between groups in baseline means for FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> were observed on days 1, 45 or 90. In terms of AUC for FEV<sub>1.0</sub> and FEF<sub>25-75%</sub>, again no statistically significant treatment difference was seen on each study day. However, the AUC for FVC in the Atrovent group (19.83) was significantly smaller (P=0.004) than that of the Alupent group (20.82) on day 1. The difference was no longer significant on days 45 and 90. The SGaw results were not reported by the sponsor. Longitudinally, both Atrovent and Alupent exhibited a declining trend in every pulmonary function in both pre-dose baseline and areas under the curves.

In the majority of patients, the Alupent group had a much shorter time to onset of response than the Atrovent group (5, 5, and 15 minutes on Alupent versus 120, 120, and 60 minutes on Atrovent on days 1, 45 and 90, respectively). Similarly, Alupent had a longer duration than Atrovent (3, 3, and 1 hours on Alupent versus 1/2, 1/2, and 1 on Atrovent).

In terms of physician's global evaluation of the treatment, only group mean scores and raw data were presented. No statistical comparison was made. No data were reported on patient's evaluation of the treatment.

TABLE 3: ADJUSTED MEANS OF EFFICACY DATA

| Efficacy Measurements | IIa5 Study*                 |        |        |         |        |        |
|-----------------------|-----------------------------|--------|--------|---------|--------|--------|
|                       | Atrovent                    |        |        | Alupent |        |        |
|                       | Day 1                       | Day 45 | Day 90 | Day 1   | Day 45 | Day 90 |
| Sample Size           | 78                          | 70     | 69     | 77      | 73     | 68     |
| Baseline              |                             |        |        |         |        |        |
| FEV <sub>1.0</sub>    | 2.44                        | 2.41   | 2.41   | 2.32    | 2.22   | 2.22   |
| FVC                   | 3.58                        | 3.55   | 3.45   | 3.39    | 3.30   | 3.28   |
| FEF <sub>25-75%</sub> | 1.93                        | 1.85   | 1.93   | 1.83    | 1.64   | 1.75   |
| SGaw                  | NOT REPORTED BY THE SPONSOR |        |        |         |        |        |
| AUC                   |                             |        |        |         |        |        |
| FEV <sub>1.0</sub>    | 14.57                       | 14.27  | 14.46  | 15.12   | 14.22  | 14.00  |
| FVC                   | 19.83                       | 19.58  | 19.45  | 20.82   | 19.93  | 19.77  |
| FEF <sub>25-75%</sub> | 12.47                       | 12.06  | 12.38  | 13.24   | 11.82  | 11.71  |
| SGaw                  | NOT REPORTED BY THE SPONSOR |        |        |         |        |        |

NOTE:

+ The entries were extracted from Tables 4.1.1-4.4.3 (p. 45-57, Vol. 1.15) reported by the sponsor.

statistically compared by the sponsor.

TABLE 1: ADJUSTED MEANS OF EFFICACY DATA\*

| Efficacy Measurements | IIBS Study <sup>†</sup>     |        |                       |         |                       |        |
|-----------------------|-----------------------------|--------|-----------------------|---------|-----------------------|--------|
|                       | Atrivent                    |        |                       | Alupent |                       |        |
|                       | Day 1                       | Day 45 | Day 90                | Day 1   | Day 45                | Day 90 |
| Sample Size           | 49                          | 43     | 43                    | 48      | 41                    | 37     |
| Baseline (Pre-dose)   |                             |        |                       |         |                       |        |
| FEV <sub>1.0</sub>    | 1.081                       | 1.056  | 1.018                 | 0.894   | 0.977                 | 0.941  |
| FVC                   | 2.561                       | 2.598  | 2.561                 | 2.242   | 2.390                 | 2.310  |
| FEF <sub>25-75%</sub> | 0.377                       | 0.386  | 0.352                 | 0.427   | 0.356                 | 0.339  |
| SGaw                  | 0.053                       | 0.052  | 0.053                 | 0.046   | 0.049 <sup>(-1)</sup> | 0.047  |
| AUC                   |                             |        |                       |         |                       |        |
| FEV <sub>1.0</sub>    | 7.445                       | 7.127  | 7.077                 | 7.084   | 6.524                 | 6.254  |
| FVC                   | 17.773                      | 17.986 | 17.393                | 17.119  | 15.964                | 15.835 |
| FEF <sub>25-75%</sub> | 2.786                       | 2.550  | 2.518                 | 2.470   | 2.420                 | 2.240  |
| SGaw                  | 0.375                       | 0.393  | 0.398                 | 0.348   | 0.353 <sup>(-1)</sup> | 0.347  |
| Efficacy Measurements | IIBS10 Study <sup>††</sup>  |        |                       |         |                       |        |
|                       | Atrivent                    |        |                       | Alupent |                       |        |
|                       | Day 1                       | Day 45 | Day 90                | Day 1   | Day 45                | Day 90 |
| Sample Size           | 57                          | 74     | 64                    | 80      | 58                    | 53     |
| Baseline              |                             |        |                       |         |                       |        |
| FEV <sub>1.0</sub>    | 1.034                       | 1.206  | 1.170                 | 1.166   | 1.134                 | 1.127  |
| FVC                   | 2.214                       | 2.494  | 2.358                 | 2.335   | 2.334                 | 2.311  |
| FEF <sub>25-75%</sub> | 0.474                       | 0.581  | 0.528 <sup>(+2)</sup> | 0.551   | 0.518                 | 0.552  |
| SGaw                  | NOT REPORTED BY THE SPONSOR |        |                       |         |                       |        |
| AUC                   |                             |        |                       |         |                       |        |
| FEV <sub>1.0</sub>    | 8.067                       | 8.304  | 7.728                 | 7.549   | 7.428                 | 7.238  |
| FVC                   | 17.048                      | 17.158 | 16.485                | 15.957  | 15.471                | 15.178 |
| FEF <sub>25-75%</sub> | 3.700                       | 3.973  | 3.675 <sup>(+2)</sup> | 3.375   | 3.324                 | 3.417  |
| SGaw                  | NOT REPORTED BY THE SPONSOR |        |                       |         |                       |        |

NOTE: † The entries were extracted from Tables 4.1.1-4.4.3 (p. 50-63, Vol. 1.10) reported by the sponsor.

†† The entries were extracted from Tables 4.1.1-4.3.4 (p. 47-57, Vol. 1.12) reported by the sponsor.

(-1) The sample size for the mean was 40.

(+2) The sample size for the mean was 66.

\*Baseline at Day 1 is adjusted for clinics only; other means are adjusted for clinic and Day 1 baseline.

TABLE 2. P-VALUES OF STATISTICAL COMPARISONS OF EFFICACY DATA BETWEEN ATROVENT AND ALUPENT TREATMENTS

| Efficacy Measurements | IIb5 Study <sup>+</sup> |        |        | IIIa10 Study <sup>++</sup> |        |        |
|-----------------------|-------------------------|--------|--------|----------------------------|--------|--------|
|                       | Day 1                   | Day 45 | Day 90 | Day 1                      | Day 45 | Day 90 |
| Baseline (Pre-dose)   |                         |        |        |                            |        |        |
| FEV <sub>1.0</sub>    | 0.040                   | 0.056  | 0.056  | 0.0504                     | 0.0504 | 0.869  |
| FVC                   | 0.079                   | 0.036  | 0.028  | 0.268                      | 0.028  | 0.584  |
| FEF <sub>25-75%</sub> | 0.296                   | 0.132  | 0.578  | 0.104                      | 0.040  | 0.496  |
| SGaw                  | 0.108                   | 0.576  | 0.189  | NOT REPORTED BY SPONSOR    |        |        |
| AUC                   |                         |        |        |                            |        |        |
| FEV <sub>1.0</sub>    | 0.028                   | 0.003  | 0.002  | 0.001                      | 0.001  | 0.076  |
| FVC                   | 0.095                   | 0.001  | 0.009  | 0.001                      | 0.001  | 0.019  |
| FEF <sub>25-75%</sub> | 0.027                   | 0.264  | 0.088  | 0.015                      | 0.001  | 0.283  |
| SGaw                  | 0.176                   | 0.128  | 0.057  | NOT REPORTED BY SPONSOR    |        |        |

NOTE:

- + The entries were extracted from Tables 4.1.1-4.4.3 (p. 50-63, Vol. 1.10) reported by the sponsor.
- ++ The entries were extracted from Tables 4.1.1-4.3.4 (p. 47-57, Vol. 1.12) reported by the sponsor.

In the IIIa10 study, the baseline means for FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> displayed a slight increase in the Atrovent group, while the Alupent group showed a declining tendency. Cross-sectionally, Atrovent baseline means of FEV<sub>1.0</sub> were significantly higher than those for Alupent on days 1 and 45, but the difference was not significant on day 90. Also, on day 45, baseline FVC and FEF<sub>25-75%</sub> were significantly higher in the Atrovent group than in the Alupent group.

In terms of average AUC in FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> Atrovent patients exhibited an increase on day 45 but declined below the day 1 level on day 90, while Alupent patients displayed a longitudinally declining trend. Cross-sectionally, the Atrovent group showed a significantly higher mean ( $p < 0.015$ ) than Alupent in FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> on days 1 and 45. No SGaw significance figures were reported by the sponsor.

In terms of onset of response (at least a 15% increase in FEV<sub>1.0</sub>) in a majority of patients, it was reported as exactly the same in the two treatment groups (onset time of less than 15 minutes on days 1 and 45); on day 90, the Atrovent group had a shorter onset time than Alupent (15 vs 30 minutes). The median duration of response on days 1, 45 and 90 were (5,3,4) and (3,1,0.25) hours for Atrovent and Alupent groups respectively. The median durations were not statistically compared by the sponsor.

In terms of physician's global evaluations of the treatment in COPD patients, only group means scores and raw data were tabulated. No statistical comparison was made. No data were reported on patient's assessment of the treatment.

In the E80-666 study by R. Goldring, M.D., the adjusted means and p-values of statistical comparisons of efficacy data are given below:

**Table 3. Plasma levels of  $^{14}C$  - 5000 in Man**

Comparative presentation of 3 different forms of administration (dosage forms). In the upper section of the Table the experimentally determined plasma concentrations are recorded and in the lower part of those calculated for therapeutic doses. The values are taken from Table 1 and 9 Section 3 report 1 and Table 1 Section 3 report 4. For graphic presentation see Figure 1.

| Administration Form        | Quality of $^{14}C$ (aqueous solution) | Plasma level (1000 ml plasma) | Plasma level (1000 ml plasma) |
|----------------------------|----------------------------------------|-------------------------------|-------------------------------|
| Experiment No.             |                                        |                               |                               |
| No. of subjects            |                                        |                               |                               |
| Activity                   |                                        |                               |                               |
| Specific activity          |                                        |                               |                               |
| 1 ml sample                |                                        |                               |                               |
| Plasma water               |                                        |                               |                               |
| Plasma levels              |                                        |                               |                               |
| are values                 |                                        |                               |                               |
| in $\mu g/g$ plasma        |                                        |                               |                               |
| of administered dose       |                                        |                               |                               |
| in the total               |                                        |                               |                               |
| dose, lower limit          |                                        |                               |                               |
| of detection.              |                                        |                               |                               |
| Plasma levels              |                                        |                               |                               |
| converted to               |                                        |                               |                               |
| therapeutic dose           |                                        |                               |                               |
| therapeutic dose           |                                        |                               |                               |
| are values                 |                                        |                               |                               |
| theoretical plasma         |                                        |                               |                               |
| levels in $\mu g/g$ plasma |                                        |                               |                               |

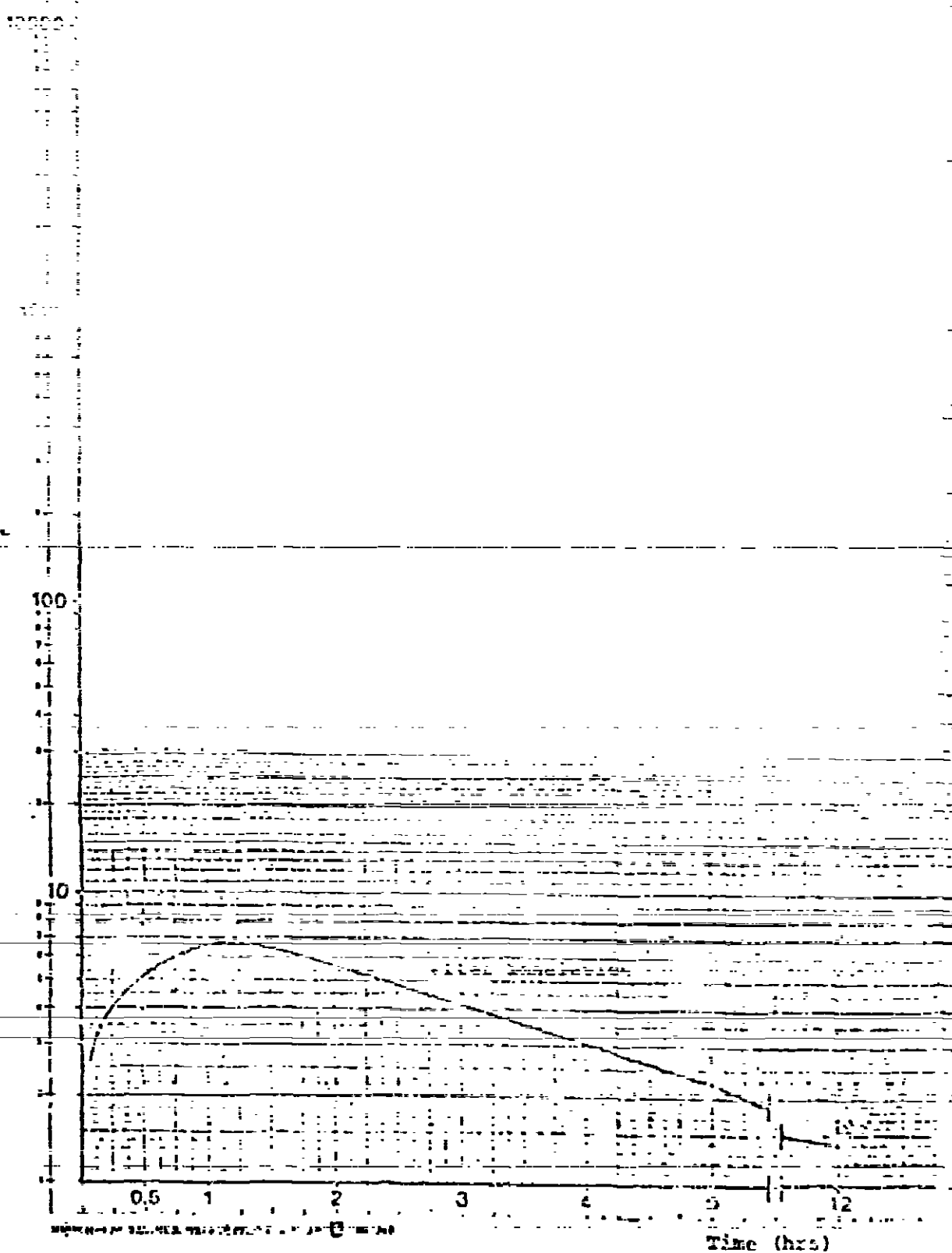


Figure 1: Plasma levels of  $^{14}\text{C}$ -sch 1000 in man. The experimentally determined plasma concentrations following administration of 1000  $\mu\text{g}$  orally, 1  $\mu\text{g}$  i.v., and 555  $\mu\text{g}$  by inhalation were converted to the therapeutic levels 150  $\mu\text{g}$  orally, 150  $\mu\text{g}$  i.v., and 40  $\mu\text{g}$  by inhalation and presented as a graph. The depicted curves are the concentration courses of the total radioactivities of **123** subject 1 (inhal) and subject 11 (i.v.) and subject 14 (i.v.).

TABLE 4. P-VALUES OF STATISTICAL COMPARISON OF EFFICACY DATA BETWEEN ATROVENT AND ALUPENT TREATMENTS

| Baseline                                                                                                    | IIB5 Study*                 |        |        |
|-------------------------------------------------------------------------------------------------------------|-----------------------------|--------|--------|
|                                                                                                             | Day 1                       | Day 45 | Day 90 |
| FEV <sub>1.0</sub>                                                                                          | 0.278                       | 0.127  | 0.174  |
| FVC                                                                                                         | 0.192                       | 0.117  | 0.291  |
| FEF <sub>25-75%</sub>                                                                                       | 0.121                       | 0.150  | 0.288  |
| SGaw                                                                                                        | NOT REPORTED BY THE SPONSOR |        |        |
| <b>All</b>                                                                                                  |                             |        |        |
| FEV <sub>1.0</sub>                                                                                          | 0.148                       | 0.918  | 0.175  |
| FVC                                                                                                         | 0.004                       | 0.438  | 0.535  |
| FEF <sub>25-75%</sub>                                                                                       | 0.170                       | 0.504  | 0.330  |
| SGaw                                                                                                        | NOT REPORTED BY THE SPONSOR |        |        |
| <b>Onset of <math>\geq 10\%</math> FEV<sub>1</sub> Increase<sup>(1)</sup> in Majority of Patients (n/n)</b> |                             |        |        |
| Atrovent                                                                                                    | 1/30                        | 1/30   | 0/30   |
| Alupent                                                                                                     | 2                           | 5      | 15     |
| <b>Median Duration of <math>\geq 10\%</math> FEV<sub>1</sub> Increase (hrs)</b>                             |                             |        |        |
| Atrovent                                                                                                    | 0.5                         | 0.5    | 1      |
| Alupent                                                                                                     | 3                           | 3      | 1      |

**NOTE:**

\* The entries were extracted from Tables 4.1.1-4.4.3 (p. 45-57, Vol. 1.1b) reported by the sponsor.

(1) Taken from Table 4.1.4, p. 48, Volume 1.1b.

**B. Safety Data**

**1. COPD SYMPTOMS**

In IIB5 studies, the most frequently reported possibly drug related adverse reactions were cardiovascular palpitations (2 on Atrovent, 1 on Alupent), nervousness (1 on Atrovent, 2 on Alupent), dizziness (1 on Atrovent, 3 on Alupent), gastrointestinal distress (2 patients in each group), musculoskeletal tremor (3, all treated with Alupent), dry mouth (2 on Atrovent, 1 on Alupent), and cough (3 on Atrovent, 2 on Alupent). Other possibly drug related reactions such as headache, drowsiness, coordination difficulties, tinnitus, rash, nausea, diarrhea, vomiting, urinary difficulty, congestion, irritation from aerosol, decreased visual acuity and ophthalmological difficulties of accommodation were also reported by one or two of the patients.

Numbers of patients who experienced clinically abnormal lab value changes from initial baselines were as follows: 2 elevations in BUN (1 in each group); 3 elevations in uric acid (2 on Atrovent, 1 on Alupent); 1 elevation in total bilirubin (Atrovent group); 1 decrease in hematocrit and hemoglobin (Atrovent group); and 5 increases in blood glucose (2 on Atrovent and 3 on Alupent). On average, the pulse rate tended to drop 5-8 beats/minute below baseline during

Application for  
Initial Review (NDA Form 201)  
Date: 1/27/85

Product Name: [REDACTED]  
Sponsor: [REDACTED]

Due to the fact that the data submitted in support of the application for initial review is inadequate, a waiver of the requirement for submission of additional data is granted.

However, the data submitted in support of the application for initial review may be adequate as evidence of bioequivalence and will be separately reviewed.

Recommendation:

Waiver may not be granted (See Deficiency).

  
J. J. MALINOWSKI, PhD.  
Principal Scientist, Research

NDA INITIALED BY CIVISwanatha

ET INITIALED BY CIVISwanatha



12/2/85 - L. Lopham ([REDACTED])

cc: NDA 19-085 original; HFN-160, HFN-525 (Malinowski), Drug, Chron, Review and Division Files.

JAN 31 1985

the first 3 hours after dosing of Atrovent, while the Alupent group reportedly dropped fewer than 4 beats/minute. This difference was reported to be statistically significant at the 0.05 level. Average blood pressures also showed a declining tendency in each drug group during the treatment period.

In the 111310 study, the most frequently reported possibly drug-related adverse reactions were headache (1 on Atrovent, 2 on Alupent), dizziness (2 on Atrovent, 1 on Alupent), nausea (1 on Atrovent, 2 on Alupent), gastrointestinal distress (3 on Atrovent, 4 on Alupent), respiratory exacerbation of symptoms (4 on Alupent) and cough (3 on Atrovent, 1 on Alupent). Other possibly drug-related reactions such as cardiovascular palpitations, nervousness, coordination difficulties, fatigue, insomnia, weakness, rash, vomiting, change in appetite, constipation, urinary difficulty, musculoskeletal tremor, muscle pain, decreased visual acuity, epistaxis, dry mouth, irritation from aerosol, rhinorrhea, pharyngitis and congestion were each also reported by 1 or 2 patients.

Numbers of patients who experienced a clinically abnormal change in lab values from initial baseline were as follows: 3 elevations in white blood cells (1 on Atrovent, 2 on Alupent), 1 elevation in uric acid (Alupent group), 4 elevations in alkaline phosphatase (2 in each group), 1 elevation in LDH (Atrovent group), 1 decrease in total protein and globulin (Atrovent group), 2 increases in gamma GI (1 in each group), 1 decrease in hematocrit and hemoglobin (Atrovent group), 1 decrease in phosphorus, 2 decreases in sodium and 1 increase in potassium and chloride (all in the Atrovent group), and 8 increases in glucose (3 on Atrovent, 5 on Alupent). The pulse rate tended to drop 1-5 beats below baseline during the first hour after test dosing. Consequently, blood pressures were also reported to show a declining tendency.

In Goldring's study, no adverse reactions were reported by the sponsor. However, numerous observed laboratory abnormalities included hemotocrit (50% on Atrovent, 23% on Alupent), hemoglobin (94% on Atrovent, 92% on Alupent), RBC (31% on Atrovent, 23% on Alupent), WBC (12.5% on Atrovent, 15% on Alupent), lymphocytes (56% on Atrovent, 33% on Alupent), eosinophil (71% on Atrovent, 100% on Alupent), alkaline phosphatase (31% on Atrovent, 40% on Alupent), and LDH (75% on Atrovent, 100% on Alupent).

## 2. Bronchial Asthma Study

In the 1105 study, the most frequently reported possibly drug-related adverse reactions were nervousness (5 on Atrovent, 14 on Alupent; nearly significant difference with  $p=0.056$ ), dizziness (3 in each group), cardiovascular palpitations (3 on Alupent), headache (4 on Atrovent, 2 on Alupent), nausea (2 on Atrovent, 1 on Alupent), irritation from aerosol (2 in each group), respiratory exacerbation of symptoms (6 on Atrovent, 1 on Alupent) and cough (3 on Atrovent and 2 on Alupent). Other possibly drug-related reactions such as tachycardia, insomnia, weakness, drowsiness, rash, hives, GI distress, musculoskeletal tremor, decreased visual acuity and dry mouth were each also reported by 1 or 2 patients. The pulse rate tended to drop 1-5 beats/minute below baseline between hours 1 and 3 after dosing with Atrovent or Alupent. The blood pressures also showed a declining tendency.

MICRO

REV

NDA 19 348

Division of Biologics Control

Washington, D.C. 20205

Application No. 19 348

Applicant: WALLENBERG PHARMACEUTICALS, INC.  
10000 Wilshire Blvd., Suite 1000  
Beverly Hills, California 90210

Product Name

Proprietary Name: Atropine Inhaler

Manufacturer: Wallenberg Pharmaceuticals, Inc.

Package Form and Quantity: Atropine Inhaler

Amount: 100 mg

4. Pharmaceutical Category and Principal Indication.

Anticholinergic bronchodilator used to treat bronchospasm.

a. 1. Initial Submission: July 24, 1983

2. Amendments:

(a) September 12, 1985 - Review No. 1

(b) December 6, 1985 - Subject of Review No. 2

c. Remarks:

1. The amendment addresses deficiencies noted in the applicant's initial review and discussed with Ms. Ruth Wasserman in telephone conversations held on December 2 and 6, 1985.

2. The proposed procedures and specifications for microbiological control of product for lot release has been provided. The applicant has agreed to perform these microbial assays on all batches of the subject drug at the time of release.

JAN - 4 1986

REVIEWER'S COMMENTS

A. Statistical Methodology

1. These trials were longitudinal studies (90 days) and were statistically analyzed in a cross-sectional manner. No consideration was given for the repeated measures aspect of the designs. The sponsor should explain how their analysis addresses the possibility that drug tolerance develops over time.

2. There is no indication as to whether reported p-values were one-sided or two-tailed.

3. The statistical methodology section was too brief. The statistical model should be specifically defined. For example, to analyze a study of two drug groups (Dj) conducted at four clinics (Ci) using baseline (Xijk) as covariate at a specific day, a statistical model can be given in mathematical form as follows:

$$Y_{ijk} = \mu + \alpha C_{ij} + \beta_j + \gamma C_{ij} + \epsilon_{ijk}$$

with terms appropriately defined in the model.

The sponsor should explain why Day 1 baseline was used to adjust analyses for pulmonary function parameters on days 45 and 90, rather than the pre-dose baseline for each test day.

4. Demographic data such as age, sex, race, etc., and disease characteristics such as age of onset and baseline severity were tabulated only, but not statistically compared between treatment groups (e.g., see p. 48, Vol. 1:10).

5. The sponsor should statistically compare Atrovent and Alupent in terms of time to onset of response (at least 15% increase over baseline in FEV<sub>1</sub>) and duration of response. Also, physician's and patient's evaluation of the treatment effects should be analyzed statistically.

6. The results of preliminary statistical tests such as tests of clinic by drug interaction terms should also be presented and examined before proceeding to a new statistical model or pooling insignificant terms into residuals.

7. Appendix G provided the SAS output of ANOVA tables resulting from the analysis of efficacy data. Two types of sum of squares (I and IV) were presented. No indication was given as to which type sum of squares was used for hypothesis-testing.

8. Laboratory values were tabulated but were not statistically compared between treatment groups. The proportions of patients in each treatment group with laboratory parameters which were within the normal range before treatment but outside the normal range after treatment should be statistically compared.

9. In SAS studies, the efficacy data were divided into day 1 responders or non-responder and statistically analyzed separately. It is inappropriate to draw efficacy conclusions from a statistical analysis conditioned on the patient's response to the drug.

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10. During these trials, 85% of COPD patients and 96% of bronchial asthma patients used the concomitant medication theophylline, a potent bronchodilator. Other concomitant medications such as steroids and beta-adrenergic bronchodilators were frequently used during the study period. No consideration for the use of concomitant medication was made in the statistical analysis. The sponsor should address this issue. Details are given in Tables 5 and 6 below.

**TABLE 5: DISTRIBUTION OF PATIENTS TAKING CONCOMITANT MEDICATION IN COPD STUDIES**

| Studies/No. of Patients*       | Theophylline |             | Steroids**  |             |
|--------------------------------|--------------|-------------|-------------|-------------|
|                                | Atr.         | Alu.        | Atr.        | Alu.        |
| <b>Multicenter IIIb</b>        |              |             |             |             |
| Cugell/N. Gross (21/20)        | 17           | 19          | 4           | 6           |
| Emrigil (8/6)                  | 4            | 4           | 2           | 1           |
| Niewoerner (14/14)             | 13           | 11          | 1           | 0           |
| Plummer (7/8)                  | 6            | 7           | 0           | 0           |
| Sub-Total                      | 40           | 41          | 7           | 7           |
| <b>Multicenter IIIa10</b>      |              |             |             |             |
| Achutosh (10/10)               | 10           | 9           | 3           | 3           |
| Bleecker/Britt (7/8)           | 6            | 8           | 3           | 2           |
| Cumiskey (9/8)                 | 7            | 7           | 1           | 1           |
| G. Gross (8/6)                 | 6            | 4           | 5           | 3           |
| N. Gross (16/16)               | 14           | 16          | 6           | 2           |
| Lakshminarayan (10/12)         | 7            | 11          | 2           | 4           |
| Miller/Kotch (6/6)             | 4            | 5           | 4           | 2           |
| Renzetti (4/3)                 | 2            | 2           | 0           | 1           |
| Sackner/Wanner (4/5)           | 3            | 3           | 1           | 1           |
| Tashkin (9/7)                  | 9            | 7           | 2           | 1           |
| Sub-Total                      | 68           | 72          | 27          | 20          |
| <b>Overall Total (132/129)</b> | <b>108</b>   | <b>113</b>  | <b>34</b>   | <b>27</b>   |
| <b>Percent</b>                 | <b>81.8</b>  | <b>87.6</b> | <b>25.8</b> | <b>22.5</b> |

**NOTE:**

\* Sample sizes on Atrovent and Alupent respectively.

\*\* Included both oral and inhaled steroids.

TABLE 6 - DISTRIBUTION OF PATIENTS TAKING (CONCURRENT) MEDICATION  
IN THE BRONCHIAL ASTHMA STUDY

| Studies/No. of Patients* | Theophylline |             | Steroids**  |             |
|--------------------------|--------------|-------------|-------------|-------------|
|                          | Atr.         | Alu.        | Atr.        | Alu.        |
| <b>Multicenter 11a5</b>  |              |             |             |             |
| Busse/Bush (19/17)       | 19           | 17          | 5           | 10          |
| Fallgren (15/15)         | 15           | 15          | 0           | 0           |
| O'Hallaren (16/17)       | 15           | 16          | 17          | 17          |
| Storm (16/17)            | 16           | 17          |             | 7           |
| Weg (15/17)              | 15           | 13          | 4           | 3           |
| <b>Total (81/88)</b>     | <b>80</b>    | <b>78</b>   | <b>31</b>   | <b>30</b>   |
| <b>Percent</b>           | <b>98.8</b>  | <b>94.0</b> | <b>38.3</b> | <b>36.1</b> |

NOTE:

\* Sample sizes on Atrovent and Alupent respectively.

\*\* Included both oral and inhaled steroids.

11. One of the sponsor's claims in the submission was that Atrovent had the potential for maintenance and prevention of the bronchospastic symptoms associated with cold (statement 12, p. 50, Vol. 1.20). The statistical analysis did not specifically address this question. It is suggested that the sponsor re-analyze the efficacy data of pulmonary functions with emphasis on acute effects as well as prophylactic effects of Atrovent, which the wording of the label tends to imply.

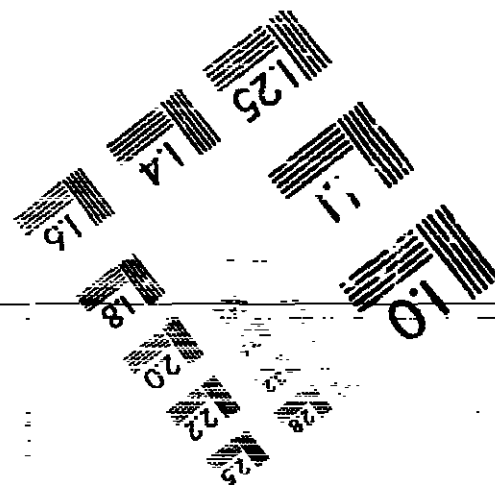
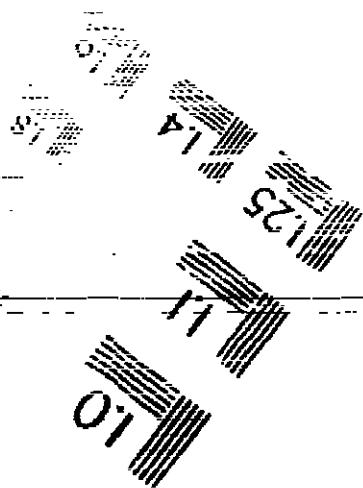
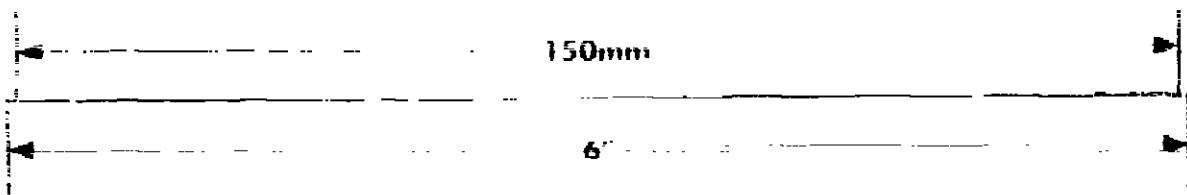
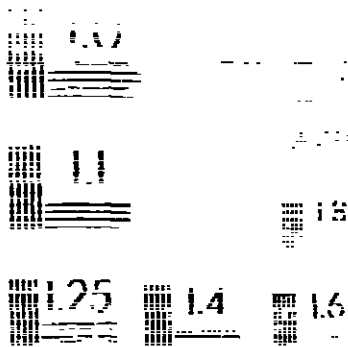
12. For safety data, the statistical analysis of laboratory data can be more meaningfully addressed by grouping lab values in terms of hepatic function (e.g. liver enzymes such as total bilirubin, SGOT, SGPT, and LDH) cardiac, renal and other functions which have a clear medical interpretation.

b. Missing Tables and Inconsistencies

1. COPD Study

a. In the 11bb study, the numbers of patients who had clinically abnormal changes in phosphorus levels from initial values were reported as zero in both Atrovent and Alupent groups, as shown in Table 7.1 (p. 71, Vol. 1.10). However, Appendix F26 (Vol. 1.10) showed that there were nine patients (four on Atrovent and five on Alupent) who started with initial phosphorus within normal range, but were elevated outside the normal range at the end of the trial. These abnormal elevations of phosphorus occurred primarily in Plummer's study (50% and 67% of Atrovent and Alupent groups, respectively). Similar situations also occurred in other laboratory values such as white blood cell counts (p. 138, Vol. 1.10), BUN (p. 173, p. 181, Vol. 1.10), LDH (p. 227, p. 231, Vol. 1.10), and alkaline phosphatase (p. 357, Vol. 1.12). Some explanation should be provided.

b. In the 111a10 study, the tables of adjusted means for SGaw corresponding to Tables 4.1.1-4.1.3 were missing and should be provided (p. 47-49, Vol. 1.12).



**END**

In Appendix I of the IIIa10 study, the numbers of patients taking concomitant medications were not consistent with patient listings (e.g., pp. 189, 193, 196 and 210 in Vol. 13).

d. Appendix B of Goldring's study showed that patient #1209 dropped out for unknown reasons, but Appendices F14, F15, and F16 showed that he had abnormally high levels of liver enzymes (SGOT, SGPT, and LDH respectively). Was this patient in fact dropped due to abnormal liver enzymes? The creatinine of patient #1211 in Goldring's study was extremely high (100 mg/DL) at the end of the study. Some explanation should be provided for the high frequency of abnormal lab values in Goldring's study, when no adverse reactions were reported.

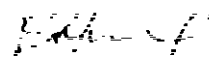
## 2. Bronchial Asthma Study

a. In the Ila5 study, the reported number of treated patients were not consistent. There were 73 patients on Atrovent who completed the 90 days of study as shown in the Abstract Section on p. 22 of Volume 1.15. A total of nine patients on Atrovent dropped out, as shown in Table 1 of page 10. Hence, there should have been 82 patients on Atrovent, but only 81 patients were treated with Atrovent as stated in the same Abstract Section on p. 22. This discrepancy also calls into question the number of patients treated with Alupent.

b. In the Ila5 study, Appendix A (p. 87, Vol. 1.15) showed that no patient treated with Atrovent dropped out due to lack of efficacy in the Busse/Bush study, but Appendix B (p. 89, Vol. 1.15) indicated that patient 902 dropped out on day 16 due to efficacy failure.

## VI. Comments to be conveyed to the firm

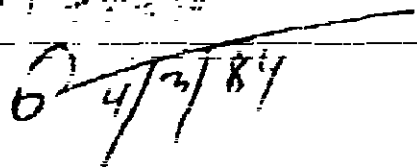
All items in reviewer's comments should be conveyed to the firm except tables 5 and 6.

  
Kao Shing Huang, Ph.D.  
Mathematical Statistician

cc: NDA 19-085  
HFN-160  
HFN-160/Dr. Shanahan  
HFN-224/Dr. Lisook  
HFN-713/Dr. Dubey  
HFN-713/Dr. Huang  
Chron  
File: DRU 1.3.2 NDA  
Khuang/cg/plt/ehd/njs/03/28/84/34594/#1955c

Concur: Dr. Johnson

Dr. Dubey



BIO/DIS

REV

Department of  
Pharmacology  
New York

Department of  
Medicine  
Columbia University  
July 29, 1953

Received: Murray J. Malinowski  
New York

### Review of a Pharmacokinetic Study

#### Background

The study had requested a review of the validity of the data for the study. The work was not critical, however, the data was acceptable using  $^{14}C$  labelled drug and these studies will now be reviewed.

The study was in a systematic comparison of the routes of administration.

1. Comparative pharmacokinetic studies were carried out following oral, intravenous, and inhaled administration. The oral route was compared to the other two routes. The oral dose contained 0.555 mg of drug, containing 0.555 mg of radioactivity, whereas the intravenous administration was 0.555 mg of drug containing 0.555 mg of radioactivity. In the case of inhalation, a metered dose containing 0.555 micrograms with 0.555 micrograms of radioactivity was utilized.

The plasma levels were the same order of magnitude for the oral and intravenous routes, however, when corrected for dose, the levels were much lower after oral administration indicating incomplete oral absorption. The levels following inhalation of drug were extremely low, (a thousandfold less), than that obtained by the other two routes of administration. As an illustration, 1 hour after the inhalation (0.555 mg dose) a plasma level of only 0.6 pg/ml as compared with 0.6 pg/ml after intravenous (0.555 mg dose) and 0.6 pg/ml after oral (0.555 mg dose) administration was found. The mean time of peak plasma level were the same (3 hours after administration) following doses by the inhalation route or oral administration. (See Table 1 & 2).

The half-life for elimination from plasma following oral administration ( $T_{1/2}$ : 3.4 hours) was in the same range as the half-life after inhalation ( $T_{1/2}$ : 1.4-4.1 hours). The active ingredient was shown to be present in the first plasma specimen immediately after inhalation (0-2 minutes). This was in contrast to oral administration. The concentration (22 pg/ml plasma) as a result of rapid distribution declined to a minimum (12 pg/ml) after about 5-10 minutes and then followed the same pattern as after oral administration.

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The State is requesting this facility in accordance with the  
Regulatory Requirements for this application.

*Henry J. Schmidt*  
Henry J. Schmidt, III,  
State of Michigan

For information only, received from M. YAU 12/21/83  
RE: INQUIRY BY CT. UNIVERSITY, P.O. Ct. University  
1/15/84

For information only, received from M. YAU (Michigan). P.O. State,  
Michigan

Table 11. Characteristics of 100-100-1000 in rats, dogs and mice

| Dose                        | Mice |        | Dogs |        | Rats |        |
|-----------------------------|------|--------|------|--------|------|--------|
|                             | Oral | I.V.   | Oral | I.V.   | Oral | I.V.   |
| Speed of onset              | 1 hr | 10 min | 1 hr | 10 min | 1 hr | 10 min |
| Speed of extinction         | 1 hr | 10 min | 1 hr | 10 min | 1 hr | 10 min |
| Total in 24 hours           | 100  | 100    | 100  | 100    | 100  | 100    |
| At end of study (0-15 - 24) |      |        |      |        |      |        |
| Eye Extinction              |      |        |      |        |      |        |
| 2 Total in 24 hours         |      |        |      |        |      |        |
| At end of study (0-15 - 24) |      |        |      |        |      |        |
| Eye Extinction              |      |        |      |        |      |        |
| 2 Total in 24 hours         |      |        |      |        |      |        |
| At end of study (0-15 - 24) |      |        |      |        |      |        |
| Eye Extinction              |      |        |      |        |      |        |
| 2 Total in 24 hours         |      |        |      |        |      |        |
| At end of study (0-15 - 24) |      |        |      |        |      |        |
| Eye Extinction              |      |        |      |        |      |        |
| 2 Total in 24 hours         |      |        |      |        |      |        |
| At end of study (0-15 - 24) |      |        |      |        |      |        |
| Eye Extinction              |      |        |      |        |      |        |
| 2 Total in 24 hours         |      |        |      |        |      |        |
| At end of study (0-15 - 24) |      |        |      |        |      |        |

Number of Mice (M) = 100

Unchanged Subject

ca. = value in table above

ca. = not determined

ref: (8) - White, P. ...

ATROV 1000

- 44 -

TABLE 2

Sch 1000

Pharmacokinetic Findings in Man After 3 Routes of Administration

| Mode of Administration  | I.V. | Oral | Inhalation  |
|-------------------------|------|------|-------------|
| N                       |      |      | 5           |
| Sex                     |      |      | 4 M<br>1 F  |
| Dose                    |      |      | 550 ug      |
| T max plasma            |      |      | 1-3 hrs     |
| T <sub>1/2</sub> plasma |      |      | 2-4 hrs     |
| Renal excretion (6d)    |      |      | 3.2%        |
| T max urine             |      |      | 2-3 hrs     |
| Fecal excretion (6d)    |      |      | 69.4%       |
| Total excretion (6d)    |      |      | 72.4%       |
|                         |      |      | (max 91.0%) |

Ref: (8) - Wahl, D., Pharmacokinetics of 14 C - Sch 1000  
 Metered Dose Inhaler, C.H. Boehringer Sohn,  
 Biochemistry Dept. November & December 1972



N19-085

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Statistical Review and Evaluation

Date:

NDA #: 19-085/Drug Class: 1C

Applicant: Boehringer Ingelheim

Name of Drug: Atrovent

Documents Reviewed: Two unnumbered volumes (1/25/85)

The medical reviewer for this NDA is Dr. Shanahan (HFN-160) with whom this review has been discussed.

I. Background

The NDA for Atrovent was previously reviewed for the indications of chronic obstructive pulmonary disease (COPD) and bronchial asthma. In an FDA letter to the sponsor dated May 1, 1984, several statistical deficiencies were cited: (a) In Study II<sub>5</sub>, patients were divided into day 1 responders and non-responders (to the Atrovent treatment) and efficacy conclusions were based on a statistical analysis conditioned on the patient's response to the drug; (b) The sponsor failed to address Atrovent's potential for use in the maintenance and prevention of COPD as implied in the proposed labeling; (c) The sponsor failed to fully address the use of concomitant medication and drug tolerance in the statistical analysis; (d) The sponsor did not fully explain why Day 1 baseline was used to adjust the analysis of pulmonary function parameters on days 45 and 90; (e) The sponsor failed to address some extreme, abnormal laboratory values.

The sponsor resubmitted the NDA on September 14, 1984, but this reviewer found the sponsor's responses to the statistical deficiencies to be inadequate as conveyed to the sponsor in a telephone conversation (with Ms. Wasserman, Dec. 6, 1984). On January 25, 1985, an additional response was sent to the agency.

This review will evaluate the sponsor's response to the above deficiencies, item by item, and include essential portions of my previous review (April 3, 1984) so that a final conclusion can be drawn.

II. Reviewer's Evaluation of The Sponsor's Response

- (a) On the issue of drawing efficacy conclusions from a statistical analysis conditioned on the patient's response to the drug, the sponsor agreed "that statistically it is inappropriate to draw the primary efficacy conclusions ..." from this type of analysis. The sponsor went on to explain that "the only conclusions drawn (based on this restricted subgroup of patients) are: that some asthmatics do respond to the drug; and that in these patients the time profiles of response are similar to those seen in the bronchitic studies where valid comparison can be made."

For Study II<sub>a</sub>5, the analysis of data subgrouped by the Day 1 responders was one of two analyses presented in the original submission. In my April 3, 1984 review, the more appropriate analysis (including all patients) was used to assess the effects of Atrovent on bronchial asthma.

- (b) In response to the labelling issue of prophylactic treatment the sponsor agreed to delete the term "prevention" from the label because the studies were not designed to evaluate prevention. Therefore, the attention of this review will be focused on the acute effectiveness of Atrovent in the treatment of COPD and asthma.
- (c) The sponsor tabulated for each treatment group the number of patients who used concomitant medications. The percentages of patients using concomitant medications are not entirely comparable between groups for each type of concomitant drug (e.g. theophylline: 83% on Atrovent vs. 89% on Alupent, p=0.17 in the COPD studies; and 99% vs. 90%, p=.037 in the asthma studies). On the issue of drug tolerance, the sponsor addressed tolerance development using the change in area under the curve (AUC) of pulmonary functions from baseline (e.g [AUC of day 45]-[AUC of day 1]).

The following is a summary of results:

| Day | Study II <sub>b</sub> 5 |                   |           | Study III <sub>a</sub> 10 |                   |           |
|-----|-------------------------|-------------------|-----------|---------------------------|-------------------|-----------|
|     | Atrovent<br>(n=43)      | Alupent<br>(n=37) | Diff/Prob | Atrovent<br>(n=63)        | Alupent<br>(n=50) | Diff/Prob |
| 45  | - .01                   | - .06             | 0.08/.001 | 0.03                      | - 0.03            | 0.05/.154 |
| 90  | - .06                   | - .12             | 0.10/.001 | -0.04                     | - 0.04            | 0.01/.800 |

It should be noted that: the mean change from baseline AUC is in units of liters per hour (AUC - 6 hrs); mean differences are differences in least-square means from a model which accounts for clinics, day 1 baseline, and day 1 baseline by treatment interaction; and probability values result from comparisons of the two (treatment) least-square means. These data showed that Atrovent patients experienced a significantly smaller average reduction in AUC after 45 and 90 days of treatment than their Alupent counterparts in Study II<sub>b</sub>5, but not in Study III<sub>a</sub>10. While these data do not suggest any dramatic change in the significance of the acute effect of Atrovent after continuous use, the results should also be interpreted on medical grounds.

Note that it is difficult to address the issue of drug tolerance without taking into consideration the possible changes in the doses and timing of concomitant drugs. Unfortunately, the sponsor failed to do so.

- (d) The sponsor reanalyzed the AUC data for pulmonary function tests using the pre-dosing baseline value on the test day (instead of Day 1) as a covariate. The results are summarized below.

Table 1 Treatment comparisons in the COPD study

| <u>Pulmonary Function</u> | <u>Study II<sub>b</sub>5</u> |                |                 | <u>Study III<sub>a</sub>10</u> |                |                 |
|---------------------------|------------------------------|----------------|-----------------|--------------------------------|----------------|-----------------|
|                           | <u>Atrovent</u>              | <u>Alupent</u> | <u>P-values</u> | <u>Atrovent</u>                | <u>Alupent</u> | <u>P-values</u> |
| Day 45:                   |                              |                |                 |                                |                |                 |
| Sample Size               | 43                           | 41             |                 | 74                             | 58             |                 |
| FEV <sub>1</sub> (L)      | 7.85/7.14                    | 6.09/6.84      | .074/.003       | 7.70/7.86                      | 7.64/7.49      | .023/.00        |
| FVC (L)                   | 18.71/17.57                  | 15.20/16.68    | .024/.001       | 16.64/16.72                    | 15.67/15.89    | .013/.00        |
| FEF <sub>25-75%</sub>     | 2.79/2.48                    | 2.23/2.51      | .745/.264       | 3.56/3.68                      | 3.61/3.45      | .174/.00        |
| Day 90:                   |                              |                |                 |                                |                |                 |
| Sample Size               | 43                           | 37             |                 | 64                             | 53             |                 |
| FEV <sub>1</sub> (L)      | 7.52/6.98                    | 5.93/6.61      | .091/.002       | 7.15/7.68                      | 7.86/7.18      | .002/.07        |
| FVC (L)                   | 17.85/16.96                  | 15.12/16.66    | .523/.009       | 16.19/16.38                    | 15.71/15.39    | .011/.01        |
| FEF <sub>25-75%</sub>     | 2.67/2.50                    | 2.13/2.29      | .038/.088       | 3.18/3.72                      | 3.89/3.35      | .004/.28        |

Note: (1) Mean values are "unadjusted"/"adjusted for the given day baseline."  
 (2) p-values are "adjusted for given day baseline"/"adjusted for day 1 baseline."

The pulmonary function data for days 45 and 90 were previously analyzed by adjusting for Day 1 baseline. The above table presents both p-values resulting from the comparison of average AUC values (i.e., adjusting for the Day 1 baseline and adjusting for the pre-dose baseline measured on the given test day). As shown in the table, the difference in AUC for FEV<sub>1</sub> in Study II<sub>b</sub>5 shifted from a highly significant difference favoring Atrovent to a non-significant result at the 5% level (p=.003 adjusting for day 1 baseline to p=.074 using the pre-dose baseline on day 45; and p=.002 to .091 on day 90). However, in Study III<sub>a</sub>10, p-values are shifted in both directions.

The sponsor argued that "measuring the effect of treatment relative to the Day 45 or Day 90 baseline (e.g by analysis of covariance or change from baseline) could be misleading if the preceding continuous therapy had influenced the baseline response." The sponsor continued "as an extreme case, a curative therapy would show no effect relative to the Day 45 baseline because the patients would arrive at testing fully bronchodilated." The sponsor's argument would be correct if the disease or symptom was dramatically improved or completely cured, thus leaving no further room for improvement, as the sponsor tends to imply. However, the data from Study II<sub>b</sub>5 showed just the opposite. The mean pre-dosing baseline values showed a consistently deteriorating trend from 1.081 liters on Day 1, 1.056 on Day 45, down to 1.018 liters on Day 90, while the Alupent group exhibited an increasing trend (0.894, 0.977 and 0.941 liters on Days 1, 45, and 90 respectively). Under these conditions (lower daily pre-dosing baselines), Atrovent patients should have even more opportunity to improve. Unfortunately, the AUC for FEV<sub>1</sub> deteriorated from 7.14 on Day 45 to 6.98 on Day 90.

- (e) The sponsor admitted mishandling of some laboratory values as pointed out in my previous review. For examples, in Goldring's study the creatinine 100 mg/DL of patient #1211 should have been reported as the the value for glucose; and the lymphocyte 2.0% of patient #1213 should have been 37.0%. The sponsor submitted a corrected tabulation of laboratory values, but failed to perform statistical comparisons between treatment groups.

### III Overall results reported by the sponsor

The overall efficacy results are summarized below.

Table 2: Summary of COPD and bronchial asthma studies

|                       |                  | COPD STUDIES                               |                 |                  |                           |                 |  |
|-----------------------|------------------|--------------------------------------------|-----------------|------------------|---------------------------|-----------------|--|
|                       |                  | Study II <sub>b</sub> 5                    |                 |                  | Study III <sub>a</sub> 10 |                 |  |
| Pulmonary Function    | Day 1            | Day 45                                     | Day 90          | Day 1            | Day 45                    | Day 90          |  |
| Sample Size           | 49/48            | 43/41                                      | 43/37           | 82/80            | 74/58                     | 64/53           |  |
| Baseline              |                  |                                            |                 |                  |                           |                 |  |
| FEV <sub>1</sub> (L)  | 1.08/.89/.04     | 1.06/0.98/.06                              | 1.02/0.94/.06   | 1.03/1.17/.05    | 1.21/1.13/.05             | 1.12/1.13/.87   |  |
| FVC (L)               | 2.56/2.24/.08    | 2.60/2.39/.04                              | 2.56/2.31/.03   | 2.21/2.34/.27    | 2.49/2.33/.03             | 2.36/2.31/.58   |  |
| FEF <sub>25-75%</sub> | 0.38/0.33/.30    | 0.39/0.36/.13                              | 0.35/0.34/.58   | 0.47/0.55/.10    | 0.58/0.52/.04             | 0.53/0.55/.50   |  |
| AUC                   |                  |                                            |                 |                  |                           |                 |  |
| FEV <sub>1</sub> (L)  | 7.46/7.08/.03    | 7.14/6.84/.07                              | 6.98/6.61/.09   | 8.07/7.55/.001   | 7.86/7.49/.02             | 7.68/7.18/.002  |  |
| FVC (L)               | 17.72/17.12/.10  | 17.57/16.68/.02                            | 16.96/16.66/.52 | 17.05/15.60/.001 | 16.72/15.89/.01           | 16.38/15.39/.01 |  |
| FEF <sub>25-75%</sub> | 2.79/2.47/.03    | 2.48/2.51/.74                              | 2.50/2.29/.04   | 3.70/3.38/.015   | 3.68/3.45/.17             | 3.72/3.35/.004  |  |
|                       |                  | Bronchial Asthma (Study II <sub>a</sub> 5) |                 |                  |                           |                 |  |
| Pulmonary Function    | Day 1            | Day 45                                     | Day 90          |                  |                           |                 |  |
| Sample Size           | 78/77            | 70/73                                      | 69/68           |                  |                           |                 |  |
| Baseline              |                  |                                            |                 |                  |                           |                 |  |
| FEV <sub>1</sub> (L)  | 2.44/2.32/.28    | 2.41/2.22/.13                              | 2.41/2.22/.17   |                  |                           |                 |  |
| FVC (L)               | 3.58/3.39/.19    | 3.55/3.30/.12                              | 3.45/3.28/.29   |                  |                           |                 |  |
| FEF <sub>25-75%</sub> | 1.93/1.83/.52    | 1.85/1.64/.15                              | 1.93/1.75/.29   |                  |                           |                 |  |
| AUC                   |                  |                                            |                 |                  |                           |                 |  |
| FEV <sub>1</sub> (L)  | 14.57/15.12/.15  | 14.27/14.32/.92                            | 14.46/14.00/.38 |                  |                           |                 |  |
| FVC (L)               | 19.83/20.82/.004 | 19.58/19.93/.44                            | 19.46/19.77/.54 |                  |                           |                 |  |
| FEF <sub>25-75%</sub> | 12.47/13.24/.13  | 12.06/11.82/.69                            | 12.38/11.71/.33 |                  |                           |                 |  |

Note: (1) The pair of numbers are sample sizes of "Atrovent"/"Alupent"  
 (2) The triple sets of numbers indicate adjusted means of "Atrovent"/"Alupent"/"p-value".  
 (In Study II<sub>b</sub>5, means are adjusted for Day 1 baseline.)

Since the sponsor has withdrawn the labelling claim of maintenance and prevention of COPD, the statistical analysis and interpretation will be focused on the acute effects of Atrovent on COPD and bronchial asthma which are primarily measured by the AUC (of pulmonary function) after adjusting for the daily pre-dosing baseline.

(a) COPD Studies

As shown in Table 2, the Atrovent patients of Study III<sub>a</sub>10, obtained significantly greater improvement from the pre-dosing baseline in FEV<sub>1</sub>, FVC, and FEF<sub>25-75%</sub> than their Alupent counterparts for each of 3 visits except FEF<sub>25-75%</sub> on day 45. In Study II<sub>b</sub>5, Atrovent patients showed consistently higher average AUC values than Alupent patients, with significant differences in favor of Atrovent observed in FEV<sub>1</sub> on day 1, FVC on day 45 and FEF<sub>25-75%</sub> on days 1 and 90.

(b) Bronchial Asthma Study

In bronchial asthmatic patients (Study II<sub>a</sub>5), no significant difference (except FVC in favor of Alupent on day 1) was seen between treatment groups.

(c) Kirk's Study

This study was designed to evaluate the effects of Atrovent and theophylline given together in comparison with theophylline alone. Two groups of bronchial asthmatic patients (11 each) with a relatively stable airway obstruction completed this 90-day randomized double-blind study. The first group of patients received Atrovent (40 mcg QID) on day 1, placebo on day 2, Atrovent for the next 90 days, and then placebo on the last day. Group 2 received the opposite order of treatments. All patients received theophylline during the course of the trial. FEV<sub>1</sub>, the primary efficacy variable, was taken at pre-dosing, and every hour for 6 hours after dosing on days 1, 2, 90 and 91.

The data showed that the addition of Atrovent to theophylline maintenance treatment failed to provide a significant increase in FEV<sub>1</sub> (using the change from pre-dosing baseline on day 90), or mean peak flow rate (43.3 on Atrovent vs. 44.8 on placebo in the morning, and 37.7 vs. 23.4 in the evening). Furthermore, patients receiving Atrovent in addition to theophylline treatment reported a higher rate of acute attacks per day (0.187 with Atrovent vs. 0.120 without Atrovent) and a higher mean daily use of theophylline (4.82 whiffs with Atrovent vs. 4.11 without Atrovent); these differences failed to reach statistical significance at the 5% level.

(d) Safety

For safety, several adverse reactions were reported in each of the COPD and Asthmatic studies. However, no statistically significant difference was found between Atrovent and Alupent in each event rate.

The most frequently reported adverse reactions included cardiovascular palpitations, nervousness, dizziness, GI distress, musculoskeletal tremor, dry mouth, and cough in the COPD studies; and nervousness, dizziness, cardiovascular palpitations, headache, nausea, irritation from aerosol, respiratory exacerbation of symptoms, and cough in the bronchial asthma study.

In the resubmission of laboratory values, the sponsor corrected data handling errors and retabulated those patients who experienced abnormal changes. Since the number of patients who experienced an abnormal change in laboratory values was extremely small, statistical procedures could not detect a significant mean difference between treatment groups.

#### IV. Reviewer's Comments

(1) The original submission of this NDA did not clearly indicate whether this NDA was intended to support a therapeutic or a prophylactic claim for the efficacy of Atrovent in COPD patients. There is a distinction in types of end points and statistical analysis used to address those 2 claims.

To establish a therapeutic claim, the sponsor should focus on the acute effects of the drug by evaluating differences between pre- and post-dosing pulmonary function values. Comparing treatments with respect to the change from pre-dosing AUC in pulmonary function variables is a reasonable approach to this question. In terms of maintenance and prevention (or prophylactic treatment), attention should be given to the trend over time in the pre-dosing pulmonary function tests and the frequency of bronchospastic symptom occurrence (such as asthma or wheezing attacks). This reviewer raised this issue in the previous review, as communicated to the sponsor. In response, the sponsor had deleted claims implying maintenance and prevention effects from the proposed labeling for Atrovent. Hence, this review is primarily focused on the assessment of acute effects of the drug in improving pulmonary performance and alleviating symptoms.

(2) The sponsor analyzed the pulmonary function data on days 45 and 90 after adjusting for the baseline measured on day 1. They argued that "measuring the effect of treatment relative to the Day 45 or Day 90 baseline (e.g. by analysis of covariance or change from baseline) could be misleading if the preceding continuous therapy had influenced the baseline response." They went on to say "as an extreme case, a curative therapy would show no effect relative to the Day 45 or Day 90 baseline because the patient would arrive at testing fully bronchodilated."

Indeed, if the disease is cured or the symptoms are completely eliminated as a result of chronic treatment, this leaves no room for improvement over the pre-dosing baseline measured on day 45 or day 90. However, such was not the case for this NDA. Pre-dosing pulmonary function values got worse in study IIb5. For example, pre-dosing FEV<sub>1</sub> was 1.081 on Day 1, 1.056 on Day 45 and down to 1.018 liters on Day 90 in study IIb5. Pulmonary function in study IIIa10 showed a slight improvement (8.3% in FEV<sub>1</sub>, 6.5% in FVC) on Day 90

from Day 1, but patients were certainly not fully bronchodilated. In addition, the pre-dosing baselines of Alupent patients showed an increasing trend, which would (using the sponsor's line of reasoning) provide an even greater chance to demonstrate differences in favor of Atrovent.

Therefore, adjustment for the baseline on each test day (rather than Day 1 baseline) is reasonable and fully justified.

(3) Concomitant medications were heavily used in these studies. Theophylline alone was used by 86% of patients in the COPD studies (83% on Atrovent vs. 89% on Alupent,  $p=.178$ ) and 95% of patients in the bronchial asthma study (99% on Atrovent vs. 90% on Alupent,  $p=.037$ ). Without a group of patients treated with theophylline alone serving as a control, it is difficult to determine the extent to which treatment effects on pulmonary function might be explained by use of concomitant medications. The sponsor was requested to address this issue in my previous review. However, the sponsor failed to statistically assess the impact of concomitant medication use on treatment effects. A simple approach to address this issue is to compare the mean daily use of theophylline between treatments.

The sponsor was also asked to address the issue of drug tolerance. In response, the sponsor compared the changes in AUC from baseline between treatment groups. This reviewer believes that the compensatory use of concomitant medications should be taken into consideration in the evaluation of drug tolerance.

(4) In future studies, the sponsor should consider including a group of patients taking only concomitant medications to serve as a control so that the study drug effect can be separated from the effect of background medications. Unfortunately, Kirk's study failed to demonstrate that the combination of Atrovent and theophylline provides a significant increase in FEV<sub>1</sub> or mean peak flow rates over levels achieved with theophylline alone.

#### V. Summary

This NDA was intended to establish the efficacy of Atrovent for two clinical indications --- chronic obstructive pulmonary disease (COPD) and bronchial asthma. Two multicenter controlled studies (Studies IIB5 and IIIA10 involving 98 and 177 patients respectively) were conducted in COPD patients, while only one multicenter study (Study IIA5 with 168 patients) was done in bronchial asthmatics.

In COPD studies, Atrovent was shown to be statistically superior to Alupent in AUC of FEV<sub>1</sub> (8.07 on Atrovent vs. 7.55 on Alupent on Day 1, 7.86 vs. 7.49 on day 45 and 7.68 vs. 7.18 on Day 90), FVC (17.05 vs. 15.60, 16.72 vs 15.89, and 16.34 vs. 15.39), and FEV<sub>25-75</sub> (3.70 vs. 3.38, 3.68 vs. 3.45, and 3.72 vs. 3.35) in Study IIIA10; in Study IIB5, Atrovent patients obtained a consistently greater AUC than their Alupent counterparts in FEV<sub>1</sub> and FVC.

In terms of the onset time and duration of at least a 15% increase in FEV<sub>1</sub> (which occurred in the majority of patients), no statistical difference between groups was found in Study IIb5 (the average time to response on the 3 test days ranged from approximately 15-30 minutes, with a median duration of 1-4 hours). However, in Study IIIa10, Atrovent had a similar onset time, but a 2-fold longer median duration of effect than Alupent.

In the bronchial asthmatic study, Alupent patients obtained a consistently greater AUC in FEV<sub>1</sub> and FVC than Atrovent counterparts, even though Alupent patients started out with consistently lower pre-dosing baseline values. However, no significant difference was detected at the 5% level.

For safety, patients in COPD and bronchial asthma studies reported similar adverse reactions. These included cardiovascular palpitations, nervousness, dizziness, GI distress, nausea, headache, and cough. Abnormal changes in laboratory values included white blood cell counts, uric acid, Alkaline phosphatase, LDH, total protein, globulin, Gamma gT, and phosphorous. Goldring's study reported extremely high percentages of abnormalities in laboratory values, e.g. hemoglobin (94% on Atrovent vs. 92% on Alupent), lymphocyte (56% vs. 33%), eosinophil (71% vs. 100%), Alkaline phosphatase (31% vs. 46%), LDH (75% vs. 100%) and WBC (12% vs. 15%). These abnormalities should be interpreted on medical grounds.

VI. Conclusion (which may be conveyed to the sponsor)

Studies in this NDA provide statistical evidence that Atrovent is at least as effective as Alupent for the acute improvement of pulmonary function and relief of symptoms in COPD patients. However, evidence supporting the efficacy of Atrovent in bronchial asthma was weak for the following reasons: (a) A significantly higher proportion of patients was receiving concomitant theophylline on Atrovent than on Alupent (99% on Atrovent vs. 90% on Alupent,  $p=.037$ ), (b) Atrovent patients showed smaller average AUC values for pulmonary function parameters than Alupent patients, even though Alupent patients started out with consistently lower pre-dosing baselines, (c) Kirk's study failed to establish the benefit of adding Atrovent to a theophylline maintenance regimen, and (d) No other independently conducted, well controlled trial was available to support the IIa5 study in evaluating Atrovent for the treatment of bronchial asthma.



K.S. Huang  
Mathematical Statistician

(15)

STATISTICAL REVIEW AND EVALUATION

Date:      APR    3 1984

NDA #: 19-085

Drug Class: 1C

Applicant: Boehringer Ingelheim Ltd.

Name of Drug: Atrovent Inhaler (ipratropium bromide)

Documents Reviewed:      Volumes 1.1 and 1.10-1.18 and 1.20 dated 07-29-83

The medical officer for this submission is E.J. Shanahan, M.D. (HFN-160), with whom this review has been discussed.

Until the additional information requested in this review is provided by the sponsor, it is not possible to draw firm conclusions regarding the efficacy and safety of Atrovent. In particular, the sponsor has not fully substantiated the claim that Atrovent is effective for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease.

I.    SUMMARY

This submission contains studies evaluating the efficacy and safety of Atrovent Inhaler for the treatment of patients with chronic obstructive pulmonary disease (COPD) or with bronchial asthma. There were two multicenter controlled studies and one single-center controlled study in COPD patients, while there was only one multicenter controlled study in bronchial asthma patients. Two hundred and five patients (110 on Atrovent and 95 on Alupent) completed the 90-day COPD study, while 144 patients (71 on Atrovent and 73 on Alupent) completed the 90-day bronchial asthma study.

For efficacy, the COPD studies revealed a significant difference in terms of FEV1.0 in favor of Atrovent. But the bronchial asthma studies failed to demonstrate the superiority of Atrovent over the active control drug. Both Atrovent and Alupent patients exhibited a slight decline in AUC of pulmonary function tests over the study periods (which could be due to deterioration in pulmonary function or increasing tolerance to treatment). This reviewer feels that the statistical analysis should specifically address the sponsor's claim of maintenance treatment and prevention of bronchospastic symptoms associated with COPD or bronchial asthma by clearly defining mathematical models and hypothesis tests. Also, the sponsor should address the extremely high level of concomitant drug use during these trials, including Theophylline (85% of COPD patients and 96% of bronchial asthma patients), steroids (24.1% of COPD patients and 37.2% of bronchial asthma patients), beta-adrenergic agents and other medications that could affect pulmonary functions and complicate the interpretation of the trial results.

For safety, numerous adverse reactions were observed during Atrovent therapy such as cough (6.1% of 228 Atrovent patients), nausea (3.1%), nervousness (3.1%), dizziness (2.6%), headache (2.6%), GI distress (2.6%), and respiratory exacerbation of symptoms (2.6%). Several patients whose initial laboratory

values were within normal ranges before treatment showed levels that were elevated or decreased abnormally at the end of the study period. These laboratory parameters, which included BUN, uric acid, phosphorus, LDH and white blood cell count, should be examined on medical grounds.

The pulse rate was reported to drop an average of 1-5 beats/minute after dosing in each treatment group of the COPD and bronchial asthma studies. Blood pressures were also reported to show a declining trend.

## II. Patient Enrollment

### A. COPD Studies

Studies in COPD included multicenter studies IIB5 (Vol. 1.10-1.11) and IIIA10 (Vol. 1.12-1.13), and one single-clinic study (E80-666, Vol. 1.14) by R. Goldring, M.D. The IIB5 studies involved four investigators, while IIIA10 studies consisted of ten investigators. Goldring's study was initiated as one of the IIB5 multicenter studies. However, due to this investigator's elimination of 2-week wash-out periods for beta-adrenergic bronchodilators, the data were analyzed separately.

Of 98 patients enrolled in the IIB5 study, 83 patients (84.7%) completed the study, while 122 of 177 patients (68.9%) completed the IIIA10 study. Early withdrawals were due to: side effects, three on Atrovent (2.3%) and six on Alupent (4.6%); lack of efficacy, one on Atrovent (0.8%) and eight on Alupent (6.2%); and other reasons such as violation of protocol and lost to follow-up, 10 on Atrovent (7.6%) and 15 on Alupent (10.8%). Details are given below.

DISTRIBUTION OF PATIENT'S ENROLLMENT  
AND REASONS FOR EARLY WITHDRAWAL

| Studies                     | Completed/<br>Enrolled |                     | Reasons for Early Withdrawal |     |                  |     |        |      |
|-----------------------------|------------------------|---------------------|------------------------------|-----|------------------|-----|--------|------|
|                             | Atrovent               | Alupent             | Side Effects                 |     | Lack of Efficacy |     | Other  |      |
|                             |                        |                     | Atr                          | Alu | Atr              | Alu | Atr    | Alu  |
| <b>Multicenter IIb5</b>     |                        |                     |                              |     |                  |     |        |      |
| Cugell/N. Gross             | 17/21                  | 16/20               | 2                            | 2   | 1                | 1   | 1      | 1    |
| Emirgil                     | 6/6                    | 5/6                 | 0                            | 0   | 0                | 0   | 0      | 1    |
| Niewoehner                  | 13/14                  | 12/14               | 0                            | 0   | 0                | 1   | 1      | 0    |
| Plummer                     | 7/8                    | 7/8                 | 0                            | 0   | 0                | 0   | 1      | 1    |
| Sub-Total                   | 43/49                  | 40/48*              | 2                            | 2   | 1                | 2   | 3      | 3    |
| <b>Multicenter IIIa10</b>   |                        |                     |                              |     |                  |     |        |      |
| Ashutosh                    | 10/10                  | 9/10                | 0                            | 0   | 0                | 0   | 0      | 0    |
| Bleecker/Britt              | 7/7                    | 5/8                 | 0                            | 0   | 0                | 0   | 0      | 3    |
| Cummiskey                   | 8/9                    | 8/8                 | 0                            | 0   | 0                | 0   | 1      | 0    |
| G. Gross                    | 6/8                    | 4/6                 | 0                            | 0   | 0                | 1   | 2      | 1    |
| N. Gross                    | 8/16                   | 7/16                | 0                            | 1   | 0                | 3   | 1      | 3    |
| Lakshminarayan              | 9/10                   | 8/12                | 0                            | 0   | 0                | 1   | 1      | 2    |
| Miller/Kotch                | 4/6                    | 5/6                 | 1                            | 1   | 0                | 0   | 1      | 0    |
| Renzetti                    | 3/4                    | 2/3                 | 0                            | 0   | 0                | 0   | 1      | 1    |
| Sackner/Wanner              | 4/4                    | 3/5                 | 0                            | 1   | 0                | 0   | 0      | 1    |
| Tashkin                     | 8/9                    | 4/7                 | 0                            | 1   | 0                | 1   | 0      | 0    |
| Sub-Total                   | 67/83 <sup>+</sup>     | 55/81 <sup>++</sup> | 1                            | 4   | 0                | 6   | 7      | 11   |
| Overall Total               | 110/132                | 95/129              | 3                            | 6   | 1                | 8   | 10     | 14   |
| % of Overall Total          | 83.3                   | 73.6                | 2.3                          | 4.6 | 0.8              | 6.2 | 7.6    | 10.8 |
| Prob. (Fisher's Exact Test) |                        |                     | P=.476                       |     | P=.038           |     | P=.483 |      |

- \* one patient in progress
- + eight patients in progress
- ++ five patients in progress

The Fisher's Exact Test was performed by the reviewer to see if the rate of early withdrawal differed between treatment groups.

In Goldring's study, 29 patients were enrolled (21 males, 8 females), but 27 patients completed the 90-day study. Patients #1209 (Atrovent) and #1220 (Alupent) dropped out after day 1. Patient #1209 had abnormal liver enzymes (SGOT, SGPT and LDH), but patient #1220 was lost due to patient's distant travel to clinic.

B. Bronchial Asthma Studies

Four investigators enrolled 168 patients in this multicenter study IIa5 (Vol. 1.15-1.17). Of these, 144 patients completed the study (71 patients treated with Atrovent (87.6%), 73 on Alupent (88.0%)). Early withdrawals were due to: side effects, 0 on Atrovent and 3 on Alupent; lack of efficacy, 5 on Atrovent and 1 on Alupent; and other reasons such as violation of protocol and

loss to follow-up, 4 on Atrovent and 3 on Alupent. Details are given below:

**DISTRIBUTION OF PATIENT'S ENROLLMENT  
AND REASONS FOR EARLY WITHDRAWAL**

| Studies                     | Completed/<br>Enrolled |                     | Reasons for Early Withdrawal |     |                  |     |        |     |  |
|-----------------------------|------------------------|---------------------|------------------------------|-----|------------------|-----|--------|-----|--|
|                             |                        |                     | Side Effects                 |     | Lack of Efficacy |     | Other  |     |  |
|                             | Atrovent               | Alupent             | Atr                          | Alu | Atr              | Alu | Atr    | Alu |  |
| Multicenter IIa5            |                        |                     |                              |     |                  |     |        |     |  |
| Busse/Bush                  | 15/19                  | 15/17               | 0                            | 1   | 1                | 0   | 3      | 1   |  |
| Falliers                    | 15/15                  | 15/15               | 0                            | 0   | 0                | 0   | 0      | 0   |  |
| O'Hollaren                  | 11/16                  | 13/17               | 0                            | 0   | 4                | 0   | 0      | 1   |  |
| Storms                      | 15/16                  | 15/17               | 0                            | 1   | 0                | 0   | 1      | 1   |  |
| Weg                         | 15/15                  | 15/17               | 0                            | 1   | 0                | 1   | 0      | 0   |  |
| Total                       | 71/81 <sup>+</sup>     | 73/83 <sup>++</sup> | 0                            | 3   | 5                | 1   | 4      | 3   |  |
| Percent of Total            | 87.6                   | 88.0                | 0.0                          | 3.6 | 6.2              | 1.2 | 4.9    | 3.6 |  |
| Prob. (Fisher's Exact Test) |                        |                     | P=.252                       |     | P=.201           |     | P=.975 |     |  |

+ one patient in progress

++ three patients in progress

The Fisher's Exact Test was performed by the reviewer to see if the rate of early withdrawal differed between treatment groups.

**III. STUDY DESIGN AND ANALYSIS**

Both COPD and bronchial asthma studies had a 2-week washout period followed by a 90-day randomized double-blind parallel group treatment period. During treatment, patients returned for follow-up on days 1, 45 and 90. On each study day, pulse, blood pressures, pulmonary functions, and body plethysmography were measured at pre-dosing, 0.25, 0.50, 1, 2, 3, 4, 5, and 6 hours after dosing. Pulmonary function tests included FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> (measured in liters). Body plethysmography consisted of airway resistance (Raw), and thoracic gas volume (VTG) and hence specific conductance (SGaw) was derived. The areas under the curves (AUC) of pulmonary functions were computed using trapezoid-rule approximations.

At bimonthly visits on days 1, 15, 30, 45, 60, 75, and 90, the physician's assessment of overall disease condition was recorded on a 100 mm line ranging from "poor" (0 mm) to "excellent" (100 mm). A similar measurement scale was used by the patient in response to the question "How did you feel during the last two weeks compared to the previous two week period?"

For the safety data, physical examination and laboratory data (such as hematology, SMA-12, and urinalysis) were obtained prior to admission to the study and at the end of the study.

Treatment (either Atrovent or Alupent) was given by oral inhalation. Patients were required to take two whiffs of either Atrovent (20 mcg) or Alupent (750 mcg) on the morning of each test day. During the 90-day treatment

period, patients also took two whiffs of either 20 mcg (q.i.d) of Atrovent or 750 mcg (q.i.d) of Alupent. If necessary, the patient might also take an additional two whiffs, either at bedtime or during the night, but the number of whiffs was not to exceed 12 whiffs/24 hours.

The sponsor analyzed the AUC for each pulmonary function parameter (FEV1.0, FVC, FEF<sub>25-75</sub> and SGaw) at each visit using analysis of covariance with day 1 predose levels as covariates. The reported means in the subsequent tables are the least-squares adjusted means. The onset time and durations of at least 15% increase in FEV1.0 were reported. The frequencies of adverse reactions were tabulated and percentages were reported. The means of laboratory values were calculated and raw data were displayed for each group.

#### IV. RESULTS REPORTED BY THE SPONSOR

##### A. Efficacy Data

##### 1. COPD Study

In the IIB5 study, the pre-treatment (day 1) baseline mean of FEV1.0 was significantly lower on Alupent (0.894L) than on Atrovent (1.081L). Hence, the subsequent statistical comparison of treatment means was made after adjustment for the day 1 baseline. Mean FEV1.0 at baseline (pre-dose) in the Atrovent group showed a slight decrease from 1.081 on day 1 to 1.056 (-2.31%) on day 45 and to 1.018 (-5.83%) on day 90, while the corresponding means for the Alupent group increased from 0.894(L) on day 1 to 0.977 (+9.28%) on day 45, and to 0.941 (+5.26%) on day 90, as shown in Table 1. That is, longitudinally, the baseline FEV1.0 profile of Atrovent patients showed a declining trend (deterioration in FEV1.0) while the Alupent group showed an increasing tendency. Cross-sectionally, comparisons of baseline FEV1.0 between Atrovent and Alupent revealed a significant difference on day 1 ( $P=0.040$ ), but this difference narrowed to borderline significance ( $P=0.056$ ) on both days 45 and 90. Table 2 shows that there was a statistically significant difference in baseline FVC (pre-dose) between Atrovent and Alupent on days 45 and 90, but these differences may not reflect prophylactic effects of Atrovent since the treatment groups showed a nearly significant difference in pre-dose FVC on day 1 ( $p=.079$ ). Further, the Atrovent FVC mean on day 1 was exactly the same as that observed on day 90, as shown in Table 1.

In terms of FEV1.0 area under the curve (AUC), the Atrovent group showed a significantly higher mean AUC than the Alupent group on each of the three study days. Longitudinally, both Atrovent and Alupent showed a declining trend in AUC of FEV1.0 (7.455, 7.227 and 7.077 for Atrovent vs. 7.084, 6.524, and 6.254 for Alupent on days 1, 45 and 90 respectively).

Significant differences were seen in AUC for FVC on days 45 and 90, since the Alupent group showed a decline (-7.50%) while Atrovent patients remained fairly stable (-1.86%). No significant difference between groups was observed for SGaw.

The onset of at least a 15% increase in FEV1.0 in the majority of patients was reported as exactly equal for Atrovent and Alupent groups (15, 30 and 15 minutes on days 1, 45 and 90 respectively). The median durations of at least a 15% increase in FEV1.0 on days 1, 45 and 90 were (4,3,3) and (4,1,2) hours for Atrovent and Alupent groups respectively. The median durations were not

statistically compared by the sponsor.

TABLE 1: ADJUSTED MEANS OF EFFICACY DATA\*

| Efficacy<br>Measurements | <u>IIb5 Study<sup>+</sup></u> |               |               |                |                       |               |
|--------------------------|-------------------------------|---------------|---------------|----------------|-----------------------|---------------|
|                          | <u>Atrovent</u>               |               |               | <u>Alupent</u> |                       |               |
|                          | <u>Day 1</u>                  | <u>Day 45</u> | <u>Day 90</u> | <u>Day 1</u>   | <u>Day 45</u>         | <u>Day 90</u> |
| Sample Size              | 49                            | 43            | 43            | 48             | 41                    | 37            |
| Baseline (Pre-dose)      |                               |               |               |                |                       |               |
| FEV <sub>1.0</sub>       | 1.081                         | 1.056         | 1.018         | 0.894          | 0.977                 | 0.941         |
| FVC                      | 2.561                         | 2.598         | 2.561         | 2.242          | 2.390                 | 2.310         |
| FEF <sub>25-75%</sub>    | 0.377                         | 0.386         | 0.352         | 0.327          | 0.356                 | 0.339         |
| SGaw                     | 0.053                         | 0.052         | 0.053         | 0.046          | 0.049 <sup>(-1)</sup> | 0.047         |
| AUC                      |                               |               |               |                |                       |               |
| FEV <sub>1.0</sub>       | 7.455                         | 7.227         | 7.077         | 7.084          | 6.524                 | 6.254         |
| FVC                      | 17.723                        | 17.986        | 17.393        | 17.119         | 15.984                | 15.835        |
| FEF <sub>25-75%</sub>    | 2.786                         | 2.559         | 2.518         | 2.470          | 2.420                 | 2.240         |
| SGaw                     | 0.375                         | 0.393         | 0.398         | 0.348          | 0.353 <sup>(-1)</sup> | 0.347         |

| Efficacy<br>Measurements | <u>IIIa10 Study<sup>++</sup></u> |               |                       |                |               |               |
|--------------------------|----------------------------------|---------------|-----------------------|----------------|---------------|---------------|
|                          | <u>Atrovent</u>                  |               |                       | <u>Alupent</u> |               |               |
|                          | <u>Day 1</u>                     | <u>Day 45</u> | <u>Day 90</u>         | <u>Day 1</u>   | <u>Day 45</u> | <u>Day 90</u> |
| Sample Size              | 82                               | 74            | 64                    | 80             | 58            | 53            |
| Baseline                 |                                  |               |                       |                |               |               |
| FEV <sub>1.0</sub>       | 1.034                            | 1.206         | 1.120                 | 1.166          | 1.134         | 1.127         |
| FVC                      | 2.214                            | 2.494         | 2.358                 | 2.336          | 2.334         | 2.311         |
| FEF <sub>25-75%</sub>    | 0.474                            | 0.581         | 0.528 <sup>(+2)</sup> | 0.551          | 0.518         | 0.552         |
| SGaw                     | NOT REPORTED BY THE SPONSOR      |               |                       |                |               |               |
| AUC                      |                                  |               |                       |                |               |               |
| FEV <sub>1.0</sub>       | 8.067                            | 8.304         | 7.728                 | 7.549          | 7.428         | 7.238         |
| FVC                      | 17.048                           | 17.158        | 16.485                | 15.957         | 15.471        | 15.178        |
| FEF <sub>25-75%</sub>    | 3.700                            | 3.973         | 3.675 <sup>(+2)</sup> | 3.375          | 3.324         | 3.417         |
| SGaw                     | NOT REPORTED BY THE SPONSOR      |               |                       |                |               |               |

NOTE: + The entries were extracted from Tables 4.1.1-4.4.3 (p. 50-63, Vol. 1.10) reported by the sponsor.

++ The entries were extracted from Tables 4.1.1-4.3.4 (p. 47-57, Vol. 1.12) reported by the sponsor.

(-1) The sample size for the mean was 40.

(+2) The sample size for the mean was 66.

\*Baseline at Day 1 is adjusted for clinics only; other means are adjusted for clinic and Day 1 baseline.

TABLE 2: P-VALUES OF STATISTICAL COMPARISONS OF EFFICACY DATA BETWEEN ATROVENT AND ALUPENT TREATMENTS

| Efficacy Measurements | IIb5 Study <sup>+</sup> |        |        | IIIa10 Study <sup>++</sup> |        |        |
|-----------------------|-------------------------|--------|--------|----------------------------|--------|--------|
|                       | Day 1                   | Day 45 | Day 90 | Day 1                      | Day 45 | Day 90 |
| Baseline (Pre-dose)   |                         |        |        |                            |        |        |
| FEV <sub>1.0</sub>    | 0.040                   | 0.056  | 0.056  | 0.0504                     | 0.0504 | 0.869  |
| FVC                   | 0.079                   | 0.036  | 0.028  | 0.268                      | 0.028  | 0.584  |
| FEF <sub>25-75%</sub> | 0.296                   | 0.132  | 0.578  | 0.104                      | 0.040  | 0.496  |
| SGaw                  | 0.108                   | 0.576  | 0.189  | NOT REPORTED BY SPONSOR    |        |        |
| AUC                   |                         |        |        |                            |        |        |
| FEV <sub>1.0</sub>    | 0.028                   | 0.003  | 0.002  | 0.001                      | 0.001  | 0.076  |
| FVC                   | 0.095                   | 0.001  | 0.009  | 0.001                      | 0.001  | 0.019  |
| FEF <sub>25-75%</sub> | 0.027                   | 0.264  | 0.088  | 0.015                      | 0.001  | 0.283  |
| SGaw                  | 0.176                   | 0.128  | 0.057  | NOT REPORTED BY SPONSOR    |        |        |

NOTE:

+ The entries were extracted from Tables 4.1.1-4.4.3 (p. 50-63, Vol. 1.10) reported by the sponsor.

++ The entries were extracted from Tables 4.1.1-4.3.4 (p. 47-57, Vol. 1.12) reported by the sponsor.

In the IIIa10 study, the baseline means for FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> displayed a slight increase in the Atrovent group, while the Alupent group showed a declining tendency. Cross-sectionally, Atrovent baseline means of FEV<sub>1.0</sub> were significantly higher than those for Alupent on days 1 and 45, but the difference was not significant on day 90. Also, on day 45, baseline FVC and FEF<sub>25-75%</sub> were significantly higher in the Atrovent group than in the Alupent group.

In terms of average AUC in FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> Atrovent patients exhibited an increase on day 45 but declined below the day 1 level on day 90, while Alupent patients displayed a longitudinally declining trend. Cross-sectionally, the Atrovent group showed a significantly higher mean ( $p < 0.015$ ) than Alupent in FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> on days 1 and 45. No SGaw significance figures were reported by the sponsor.

In terms of onset of response (at least a 15% increase in FEV<sub>1.0</sub>) in a majority of patients, it was reported as exactly the same in the two treatment groups (onset time of less than 15 minutes on days 1 and 45); on day 90, the Atrovent group had a shorter onset time than Alupent (15 vs 30 minutes). The median duration of response on days 1, 45 and 90 were (5,3,4) and (3,1,0.25) hours for Atrovent and Alupent groups respectively. The median durations were not statistically compared by the sponsor.

In terms of physician's global evaluations of the treatment in COPD patients, only group means scores and raw data were tabulated. No statistical comparison was made. No data were reported on patient's assessment of the treatment.

In the E80-666 study by R. Goldring, M.D., the adjusted means and p-values of statistical comparisons of efficacy data are given below:

| Efficacy Measurements | Atrovent |        | Alupent |       | P-values of Comparisons |        |       |        |        |
|-----------------------|----------|--------|---------|-------|-------------------------|--------|-------|--------|--------|
|                       | Day 1    | Day 45 | Day 90  | Day 1 | Day 45                  | Day 90 | Day 1 | Day 45 | Day 90 |
| Sample Size           | 16       | 15     | 15      | 13    | 12                      | 12     | 29    | 27     | 27     |
| <b>Baseline</b>       |          |        |         |       |                         |        |       |        |        |
| FEV <sub>1.0</sub>    | 1.001    | 0.995  | 0.985   | 0.933 | 0.990                   | 0.925  | 0.623 | 0.974  | 0.682  |
| FVC                   | 2.082    | 2.047  | 2.033   | 1.955 | 1.900                   | 1.888  | 0.593 | 0.524  | 0.555  |
| FEF <sub>25-75%</sub> | 0.409    | 0.437  | 0.381   | 0.391 | 0.435                   | 0.365  | 0.820 | 0.989  | 0.814  |
| SGaw                  | 0.032    | 0.037  | 0.031   | 0.053 | 0.045                   | 0.042  | 0.043 | 0.468  | 0.185  |
| <b>AUC</b>            |          |        |         |       |                         |        |       |        |        |
| FEV <sub>1.0</sub>    | 0.917    | 1.149  | 1.143   | 0.842 | 0.760                   | 0.873  | 0.809 | 0.207  | 0.437  |
| FVC                   | 2.303    | 2.255  | 2.440   | 1.217 | 1.882                   | 1.197  | 0.082 | 0.201  | 0.061  |
| FEF <sub>25-75%</sub> | 0.179    | 0.423  | 0.476   | 0.214 | 0.467                   | 0.481  | 0.903 | 0.866  | 0.983  |
| SGaw                  | 0.098    | 0.098  | 0.137   | 0.043 | 0.057                   | 0.052  | 0.110 | 0.260  | 0.063  |

As shown in the table above, both Atrovent and Alupent groups presented a longitudinal declining trend in pre-dose baseline pulmonary tests, but the groups exhibited an increasing tendency in AUC. However, there were few statistically significant differences between groups observed in either baseline or AUC.

## 2. Bronchial Asthma Study

In the Ila5 study, no statistically significant differences between groups in baseline means for FEV<sub>1.0</sub>, FVC and FEF<sub>25-75%</sub> were observed on days 1, 45 or 90. In terms of AUC for FEV<sub>1.0</sub> and FEF<sub>25-75%</sub>, again no statistically significant treatment difference was seen on each study day. However, the AUC for FVC in the Atrovent group (19.83) was significantly smaller (P=0.004) than that for the Alupent group (20.82) on day 1. The difference was no longer significant on days 45 and 90. The SGaw results were not reported by the sponsor. Longitudinally, both Atrovent and Alupent exhibited a declining trend in every pulmonary function in both pre-dose baseline and areas under the curves.

In the majority of patients, the Alupent group had a much shorter time to onset of response than the Atrovent group (5, 5, and 15 minutes on Alupent versus 120, 120, and 60 minutes on Atrovent on days 1, 45 and 90, respectively). Similarly, Alupent had a longer duration than Atrovent (3, 3, and 1 hours on Alupent versus 1/2, 1/2, and 1 on Atrovent).

In terms of physician's global evaluation of the treatment, only group mean scores and raw data were presented. No statistical comparison was made. No data were reported on patient's evaluation of the treatment.

TABLE 3: ADJUSTED MEANS OF EFFICACY DATA

| <u>Efficacy Measurements</u> | <u>Ila5 Study<sup>+</sup></u> |               |               |                |               |               |
|------------------------------|-------------------------------|---------------|---------------|----------------|---------------|---------------|
|                              | <u>Atrovent</u>               |               |               | <u>Alupent</u> |               |               |
|                              | <u>Day 1</u>                  | <u>Day 45</u> | <u>Day 90</u> | <u>Day 1</u>   | <u>Day 45</u> | <u>Day 90</u> |
| Sample Size                  | 78                            | 70            | 69            | 77             | 73            | 68            |
| Baseline                     |                               |               |               |                |               |               |
| FEV <sub>1.0</sub>           | 2.44                          | 2.41          | 2.41          | 2.32           | 2.22          | 2.22          |
| FVC                          | 3.58                          | 3.55          | 3.45          | 3.39           | 3.30          | 3.28          |
| FEF <sub>25-75%</sub>        | 1.93                          | 1.85          | 1.93          | 1.83           | 1.64          | 1.75          |
| SGaw                         | NOT REPORTED BY THE SPONSOR   |               |               |                |               |               |
| AUC                          |                               |               |               |                |               |               |
| FEV <sub>1.0</sub>           | 14.57                         | 14.27         | 14.46         | 15.12          | 14.22         | 14.00         |
| FVC                          | 19.83                         | 19.58         | 19.46         | 20.82          | 19.93         | 19.77         |
| FEF <sub>25-75%</sub>        | 12.47                         | 12.06         | 12.38         | 13.24          | 11.82         | 11.71         |
| SGaw                         | NOT REPORTED BY THE SPONSOR   |               |               |                |               |               |

NOTE:

+ The entries were extracted from Tables 4.1.1-4.4.3 (p. 45-57, Vol. 1.15) reported by the sponsor.

**TABLE 4: P-VALUES OF STATISTICAL COMPARISON OF EFFICACY DATA  
BETWEEN ATROVENT AND ALUPENT TREATMENTS**

|                                                                                                                 | IIa5 Study <sup>+</sup>     |               |               |
|-----------------------------------------------------------------------------------------------------------------|-----------------------------|---------------|---------------|
|                                                                                                                 | <u>Day 1</u>                | <u>Day 45</u> | <u>Day 90</u> |
| <b>Baseline</b>                                                                                                 |                             |               |               |
| FEV <sub>1.0</sub>                                                                                              | 0.278                       | 0.127         | 0.174         |
| FVC                                                                                                             | 0.192                       | 0.117         | 0.291         |
| FEF <sub>25-75%</sub>                                                                                           | 0.521                       | 0.150         | 0.288         |
| SGaw                                                                                                            | NOT REPORTED BY THE SPONSOR |               |               |
| <b>AUC</b>                                                                                                      |                             |               |               |
| FEV <sub>1.0</sub>                                                                                              | 0.148                       | 0.918         | 0.375         |
| FVC                                                                                                             | 0.004                       | 0.438         | 0.535         |
| FEF <sub>25-75%</sub>                                                                                           | 0.130                       | 0.694         | 0.330         |
| SGaw                                                                                                            | NOT REPORTED BY THE SPONSOR |               |               |
| <b>Onset of <math>\geq 15\%</math> FEV<sub>1</sub> increase<sup>(1)</sup><br/>in Majority of Patients (min)</b> |                             |               |               |
| Atrovent                                                                                                        | 120                         | 120           | 60            |
| Alupent                                                                                                         | 5                           | 5             | 15            |
| <b>Median Duration of <math>\geq 15\%</math><sup>(1)</sup><br/>FEV<sub>1</sub> Increase (hrs)</b>               |                             |               |               |
| Atrovent                                                                                                        | 0.5                         | 0.5           | 1             |
| Alupent                                                                                                         | 3                           | 3             | 1             |

**NOTE:**

<sup>+</sup> The entries were extracted from Tables 4.1.1-4.4.3 (p. 45-57, Vol. 1.15) reported by the sponsor.

(1) Taken from Table 4.1.4, p. 48, Volume 1.15.

**B. Safety Data**

**1. COPD Studies**

In IIb5 studies, the most frequently reported possibly drug related adverse reactions were cardiovascular palpitations (2 on Atrovent, 1 on Alupent), nervousness (1 on Atrovent, 2 on Alupent), dizziness (1 on Atrovent, 3 on Alupent), gastrointestinal distress (2 patients in each group), musculoskeletal tremor (3, all treated with Alupent), dry mouth (2 on Atrovent, 1 on Alupent), and cough (3 on Atrovent, 2 on Alupent). Other possibly drug related reactions such as headache, drowsiness, coordination difficulties, tinnitus, rash, nausea, diarrhea, vomiting, urinary difficulty, congestion, irritation from aerosol, decreased visual acuity and ophthalmological difficulties of accommodation were also reported by one or two of the patients.

Numbers of patients who experienced clinically abnormal lab value changes from initial baselines were as follows: 2 elevations in BUN (1 in each group); 3 elevations in uric acid (2 on Atrovent, 1 on Alupent); 1 elevation in total bilirubin (Atrovent group); 1 decrease in hematocrit and hemoglobin (Atrovent group); and 5 increases in blood glucose (2 on Atrovent and 3 on Alupent). On average, the pulse rate tended to drop 5-8 beats/minute below baseline during

the first 3 hours after dosing of Atrovent, while the Alupent group reportedly dropped fewer than 4 beats/minute. This difference was reported to be statistically significant at the 0.05 level. Average blood pressures also showed a declining tendency in each drug group during the treatment period.

In the IIIa10 study, the most frequently reported possibly drug-related adverse reactions were headache (1 on Atrovent, 2 on Alupent), dizziness (2 on Atrovent, 1 on Alupent), nausea (3 on Atrovent, 2 on Alupent) gastrointestinal distress (3 on Atrovent, 4 on Alupent), respiratory exacerbation of symptoms (4 on Alupent) and cough (3 on Atrovent, 1 on Alupent). Other possibly drug-related reactions such as cardiovascular palpitations, nervousness, coordination difficulties, fatigue, insomnia, weakness, rash, vomiting, change in appetite, constipation, urinary difficulty, musculoskeletal tremor, muscle pain, decreased visual acuity, epistaxis, dry mouth, irritation from aerosol, hoarseness, pleurisy and congestion were each also reported by 1 or 2 patients.

Numbers of patients who experienced a clinically abnormal change in lab values from initial baseline were as follows: 3 elevations in white blood cells (1 on Atrovent, 2 on Alupent), 1 elevation in uric acid (Alupent group), 4 elevations in alkaline phosphatase (2 in each group), 1 elevation in LDH (Atrovent group), 1 decrease in total protein and globulin (Atrovent group), 2 increases in Gamma GT (1 in each group), 1 decrease in hematocrit and hemoglobin (Atrovent group), 1 decrease in phosphorus, 2 decreases in sodium and 1 increase in potassium and chloride (all in the Atrovent group), and 8 increases in glucose (3 on Atrovent, 5 on Alupent). The pulse rate tended to drop 1-5 beats below baseline during the first hour after test dosing. Consequently, blood pressures were also reported to show a declining tendency.

In Goldring's study, no adverse reactions were reported by the sponsor. However, numerous observed laboratory abnormalities included hemotocrit (50% on Atrovent, 23% on Alupent), hemoglobin (94% on Atrovent, 92% on Alupent), RBC (31% on Atrovent, 23% on Alupent), WBC (12.5% on Atrovent, 15% on Alupent), lymphocytes (56% on Atrovent, 33% on Alupent), eosinophil (71% on Atrovent, 100% on Alupent), alkaline phosphatase (31% on Atrovent, 46% on Alupent), and LDH (75% on Atrovent, 100% on Alupent).

## 2. Bronchial Asthma Study

In the IIa5 study, the most frequently reported possibly drug-related adverse reactions were nervousness (5 on Atrovent, 14 on Alupent; nearly significant difference with  $p=.058$ ), dizziness (3 in each group), cardiovascular palpitations (3 on Alupent), headache (4 on Atrovent, 2 on Alupent), nausea (2 on Atrovent, 1 on Alupent), irritation from aerosol (2 in each group), respiratory exacerbation of symptoms (6 on Atrovent, 1 on Alupent) and cough (8 on Atrovent and 2 on Alupent). Other possibly drug-related reactions such as tachycardia, insomnia, weakness, drowsiness, rash, hives, GI distress, musculoskeletal tremor, decreased visual acuity and dry mouth were each also reported by 1 or 2 patients. The pulse rate tended to drop 1-5 beats/minute below baseline between hours 1 and 3 after dosing with Atrovent or Alupent. The blood pressures also showed a declining tendency.

V. REVIEWER'S COMMENTS

A. Statistical Methodology

1. These trials were longitudinal studies (90 days) and were statistically analyzed in a cross-sectional manner. No consideration was given for the repeated measures aspect of the designs. The sponsor should explain how their analysis addresses the possibility that drug tolerance develops over time.

2. There is no indication as to whether reported p-values were one-sided or two-sided.

3. The statistical methodology section was too brief. The statistical model should be specifically defined. For example, to analyze a study of two drug groups (Dj) conducted at four clinics (Ci) using baseline (Xijk) as covariate at a specific day, a statistical model can be given in mathematical form as follows:

$$Y_{ijk} = \mu + \beta X_{ijk} + C_i + D_j + CD_{ij} + E_{ijk}$$

with terms appropriately defined in the model.

The sponsor should explain why Day 1 baseline was used to adjust analyses for pulmonary function parameters on days 45 and 90, rather than the pre-dose baseline for each test day.

4. Demographic data such as age, sex, race, etc., and disease characteristics such as age of onset and baseline severity were tabulated only, but not statistically compared between treatment groups (e.g., see p. 48, Vol. 1.10).

5. The sponsor should statistically compare Atrovent and Alupent in terms of time to onset of response (at least 15% increase over baseline in FEV<sub>1</sub>) and duration of response. Also, physician's and patient's evaluation of the treatment effects should be analyzed statistically.

6. The results of preliminary statistical tests such as tests of clinic by drug interaction terms should also be presented and examined before proceeding to a new statistical model or pooling insignificant terms into residuals.

7. Appendix G provided the SAS output of ANOVA tables resulting from the analysis of efficacy data. Two types of sum of squares (I and IV) were presented. No indication was given as to which type sum of squares was used for hypothesis-testing.

8. Laboratory values were tabulated but were not statistically compared between treatment groups. The proportions of patients in each treatment group with laboratory parameters which were within the normal range before treatment but outside the normal range after treatment should be statistically compared.

9. In IIa5 studies, the efficacy data were divided into day 1 responders or non-responder and statistically analyzed separately. It is inappropriate to draw efficacy conclusions from a statistical analysis conditioned on the patient's response to the drug.

10. During these trials, 85% of COPD patients and 96% of bronchial asthma patients used the concomitant medication theophylline, a potent bronchodilator. Other concomitant medications such as steroids and beta-adrenergic bronchodilators were frequently used during the study period. No consideration for the use of concomitant medication was made in the statistical analysis. The sponsor should address this issue. Details are given in Tables 5 and 6 below.

**TABLE 5: DISTRIBUTION OF PATIENTS TAKING CONCOMITANT MEDICATION IN COPD STUDIES**

| <u>Studies/No. of Patients*</u> | <u>Theophylline</u> |             | <u>Steroids**</u> |             |
|---------------------------------|---------------------|-------------|-------------------|-------------|
|                                 | <u>Atr.</u>         | <u>Alu.</u> | <u>Atr.</u>       | <u>Alu.</u> |
| <b>Multicenter IIb5</b>         |                     |             |                   |             |
| Cugell/N. Gross (21/20)         | 17                  | 19          | 4                 | 8           |
| Emirgil (6/6)                   | 4                   | 4           | 2                 | 1           |
| Niewoehner (14/14)              | 13                  | 11          | 1                 | 0           |
| <u>Plummer (8/8)</u>            | <u>6</u>            | <u>7</u>    | <u>0</u>          | <u>0</u>    |
| Sub-Total                       | 40                  | 41          | 7                 | 9           |
| <b>Multicenter IIIa10</b>       |                     |             |                   |             |
| Ashutosh (10/10)                | 10                  | 9           | 3                 | 3           |
| Bleecker/Britt (7/8)            | 6                   | 8           | 3                 | 2           |
| Cumiskey (9/8)                  | 7                   | 7           | 1                 | 1           |
| G. Gross (8/6)                  | 6                   | 4           | 5                 | 3           |
| N. Gross (16/16)                | 14                  | 16          | 6                 | 2           |
| Lakshminarayan (10/12)          | 7                   | 11          | 2                 | 4           |
| Miller/Kotch (6/6)              | 4                   | 5           | 4                 | 2           |
| Renzetti (4/3)                  | 2                   | 2           | 0                 | 1           |
| Sackner/Wanner (4/5)            | 3                   | 3           | 1                 | 1           |
| <u>Tashkin (9/7)</u>            | <u>9</u>            | <u>7</u>    | <u>2</u>          | <u>1</u>    |
| Sub-Total                       | 68                  | 72          | 27                | 20          |
| Overall Total (132/129)         | 108                 | 113         | 34                | 29          |
| Percent                         | 81.8                | 87.6        | 25.8              | 22.5        |

**NOTE:**

\* Sample sizes on Atrovent and Alupent respectively.

\*\* Included both oral and inhaled steroids.

**TABLE 6: DISTRIBUTION OF PATIENTS TAKING CONCOMITANT MEDICATION  
IN THE BRONCHIAL ASTHMA STUDY**

| <u>Studies/No. of Patients*</u> | <u>Theophylline</u> |             | <u>Steroids**</u> |             |
|---------------------------------|---------------------|-------------|-------------------|-------------|
|                                 | <u>Atr.</u>         | <u>Alu.</u> | <u>Atr.</u>       | <u>Alu.</u> |
| <u>Multicenter IIa5</u>         |                     |             |                   |             |
| Busse/Bush (19/17)              | 19                  | 17          | 5                 | 10          |
| Falliers (15/15)                | 15                  | 15          | 0                 | 0           |
| O'Hallaren (16/17)              | 15                  | 16          | 12                | 12          |
| Storm (16/17)                   | 16                  | 17          | 10                | 7           |
| <u>Weg (15/17)</u>              | <u>15</u>           | <u>13</u>   | <u>4</u>          | <u>1</u>    |
| Total (81/83)                   | 80                  | 78          | 31                | 30          |
| Percent                         | 98.8                | 94.0        | 38.3              | 36.1        |

**NOTE:**

\* Sample sizes on Atrovent and Alupent respectively.

\*\* Included both oral and inhaled steroids.

11. One of the sponsor's claims in this submission was that Atrovent had the potential for maintenance and prevention of the bronchospastic symptoms associated with COPD (statement 12, p. 56, Vol. 1.20). The statistical analysis did not specifically address this question. It is suggested that the sponsor re-analyze the efficacy data of pulmonary functions with emphasis on acute effects as well as prophylactic effects of Atrovent, which the wording of the label tends to imply.

12. For safety data, the statistical analysis of laboratory data can be more meaningfully addressed by grouping lab values in terms of hepatic function (e.g. liver enzymes such as total bilirubin, SGOT, SGPT, and LDH) cardiac, renal and other functions which have a clear medical interpretation.

**B. Missing Tables and Inconsistencies**

**1. COPD Study**

a. In the IIb5 study, the numbers of patients who had clinically abnormal changes in phosphorus levels from initial values were reported as zero in both Atrovent and Alupent groups, as shown in Table 7.1 (p. 71, Vol. 1.10). However, Appendix F26 (Vol. 1.10) showed that there were nine patients (four on Atrovent and five on Alupent) who started with initial phosphorus within normal range, but were elevated outside the normal range at the end of the trial. These abnormal elevations of phosphorus occurred primarily in Plummer's study (50% and 67% of Atrovent and Alupent groups, respectively). Similar situations also occurred in other laboratory values such as white blood cell counts (p. 138, Vol. 1.10), BUN (p. 179, p. 181, Vol. 1.10), LDH (p. 227, p. 231, Vol. 1.10), and alkaline phosphatase (p. 357, Vol. 1.12). Some explanation should be provided.

b. In the IIIa10 study, the tables of adjusted means for SGaw corresponding to Tables 4.1.1-4.1.3 were missing and should be provided (p. 47-49, Vol. 1.12).

c. In Appendix L of the IIIa10 study, the numbers of patients taking concomitant medications were not consistent with patient listings (e.g., pp. 189, 193, 196 and 210 in Vol. 13).

d. Appendix B of Goldring's study showed that patient #1209 dropped out for unknown reasons, but Appendices F14, F15, and F16 showed that he had abnormally high levels of liver enzymes (SGOT, SGPT, and LDH respectively). Was this patient in fact dropped due to abnormal liver enzymes? The creatinine of patient #1211 in Goldring's study was extremely high (100 mg/DL) at the end of the study. Some explanation should be provided for the high frequency of abnormal lab values in Goldring's study, when no adverse reactions were reported.

## 2. Bronchial Asthma Study

a. In the IIa5 study, the reported numbers of treated patients were not consistent. There were 73 patients on Atrovent who completed the 90 days of study as shown in the Abstract Section on p. 22 of Volume 1.15. A total of nine patients on Atrovent dropped out, as shown in Table 1 of page 10. Hence, there should have been 82 patients on Atrovent, but only 81 patients were treated with Atrovent as stated in the same Abstract Section on p. 22. This discrepancy also calls into question the number of patients treated with Alupent.

b. In the IIa5 study, Appendix A (p. 87, Vol. 1.15) showed that no patient treated with Atrovent dropped out due to lack of efficacy in the Busse/Bush study, but Appendix B (p. 89, Vol. 1.15) indicated that patient 902 dropped out on day 16 due to efficacy failure.

## VI. Comments to be conveyed to the firm

All items in reviewer's comments should be conveyed to the firm except tables 5 and 6.

  
Kao-Shing Huang, Ph.D.  
Mathematical Statistician

cc: NDA 19-085  
HFN-160  
HFN-160/Dr. Shanahan  
HFN-224/Dr. Lisook  
✓HFN-713/Dr. Dubey  
HFN-713/Dr. Huang  
Chron  
File: DRU 1.3.2 NDA  
KHuang/cg/plt/ebd/njs/03/28/84/34594/#1955c

Concur: Dr. Johnson *11/23/84*

Dr. Dubey *6/4/3/84*

N-19085

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NDA

19-085

NDA

19-085

MOR

Review and Evaluation of Clinical Data

NDA #19-085

M.O. Review #1

Applicant - Boehringer Ingelheim Ltd.

Submitted: 7/29/83  
Date Completed: 11/16/83

1. General Information

a. Name of Drug

(1) Generic: ipratropium bromide

(2) Trade: ATROVENT<sup>R</sup>INHALER

b. Pharmacologic Category: Bronchodilator

c. Proposed Indications; "ATROVENT<sup>R</sup> (ipratropium bromide) is indicated as a bronchodilator for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis. Some asthmatic patients have been shown to respond to ATROVENT<sup>R</sup>."

d. Dosage form and route of administration;

"The usual dose of ATROVENT<sup>R</sup> is two oral inhalations four times a day. Patients may take additional inhalations as required; however, the total number of inhalations should not exceed 12 in 24 hours." There are 20 mcg Atrovent delivered per whiff. Dosage is 40 mcg four times a day.

ATROVENT<sup>R</sup> is supplied as a metered dose inhaler with or without a mouthpiece. Each 21 gram vial provides sufficient medication for 300 inhalations. Each actuation delivers 18 mcg of ipratropium bromide from the mouthpiece.

e. Related drugs; chemically related to atropine.

2. Manufacturing Controls (Refer to Chemistry Review). It is a quaternary ammonia compound unrelated to any approved bronchodilator since it is an anticholinergic agent.

NOV 22 1985

3. Pharmacology: ipratropium bromide has a higher parenteral efficacy than atropine. This was not the case in enteral administration. Here, the drug was less effective than atropine but did not show the same level of side effects. In the case of aerosol administration, the drug was more effective in preclinical studies as a bronchial spasmolytic agent but produced less of the antisialagogue (drying) effect. No increase in heart rate or changes in EKG were observed at doses a hundred fold those recommended therapeutically. The drug was devoid of CNS significant activity. ATROVENT<sup>R</sup> is a novel anticholinergic with a high degree of bronchospasmolytic activity. When used as a metered aerosol, it is practically devoid of side effects and possesses a wide therapeutic range.

The section in pharmacokinetics has been referred to our Division of Biopharmaceutics for review, evaluation and comments.

For data on safety studies, acute toxicity, subchronic toxicity, chronic toxicity, carcinogenicity, embryotoxicity, teratology and reproduction, mutagenicity, tolerance studies in animals and further details on pharmacology please refer to the pharmacology review.

#### Clinical Studies in the NDA Tabulated

Each clinical study included a protocol as well as a statistical analysis report.

#### Special Studies

- |                             |   |            |
|-----------------------------|---|------------|
| 1. Ventilation/perfusion -  | 2 |            |
| 2. Mucociliary clearance -  | 2 |            |
| 3. Methacholine challenge - | 1 |            |
| 4. Sputum studies -         | 2 |            |
| 5. Ocular studies -         | 3 | = Total 10 |

Dose-Ranging Studies 2 whiffs (40 mcg) 4 x daily.

Asthmatics - 3  
Bronchitics - 2

Controlled Clinical Trials - multiple dose studies

#### Bronchitics (COPD)

Protocol 1 ii-b-5

2 multicenter studies comparing Atrovent with metaprotarenol (Alupent) -

5 studies, 6 principal or co-investigators

Protocol 2 111-a-10 multicenter - 10 studies -  
147 patients on Atrovent and  
147 on metaproterenol

Protocol 3 11-a-5 multicenter in asthmatics 5 centers; 6  
investigators  
71 on Atrovent  
73 on metaproterenol

4 90 day study in 22 asthmatics with added and not  
added theophylline

A. Special Studies

|                                          | <u>#Studies</u> |
|------------------------------------------|-----------------|
| 1. Ventilation - Perfusion studies:      | 2               |
| (a) E-82-318                             |                 |
| (b) E-82-215                             |                 |
| 2. Mucociliary Clearance Studies:        | 2               |
| (a) E-80-735                             |                 |
| (b) E-80-789                             |                 |
| 3. Methacholine challenge                | 1               |
| (a) E-81-22                              |                 |
| 4. Sputum Studies                        | 2               |
| (a) E-81-38                              |                 |
| (b) E-81-166                             |                 |
| 5. Ocular Studies                        |                 |
| (a) E-74-213 }                           |                 |
| (b) E-73-709 }                           | 2               |
| (c) E-82-1861 - single and multiple dose | 1               |
|                                          | 10              |

Total number of special studies = 10

B) Dose - ranging studies

E-79-81 )  
E-79-948 ) Asthmatics  
E-79-961 )

E-80-3 )  
E-79-80) - Bronchitics

C) Multiple Dose Studies in Bronchitics - 2 protocols

1) Protocol II b 5 - two multicenter studies in bronchitics in  
comparison with metaproterenol

5 studies

E-80-664 -  
E-80-665 -  
E-80-668 -  
E-80-667 -  
E-80-666 - ; analyzed separately due to  
misinterpretation of protocol affecting proper washout timing.

2) Protocol III a 10

E-81-1250 -  
E-81-1293 -  
E-81-1153 -  
E-81-1417 -  
E-82-854 -  
E-81-1151 -  
E-81-1177 -  
E-81-1150 -  
E-81-1176 -  
E-81-1154 -

Bronchitics  
10 studies

147 patients were on Atrovent  
140 patients were on metaproterenol

D. Multiple Dose Studies in Asthmatics

Protocol II a 5

E-80-503 -  
E-80-527 -  
E-80-526 -  
E-80-457 -  
E-80-525 -

81 patients on Atrovent  
83 patients on metaproterenol

- E. 90 day study in 22 asthmatics to compare Atrovent in combination with theophylline to theophylline alone.

E-82-1801 -

11 patients on Atrovent plus theophylline  
11 patients on theophylline alone

New NDA 19-085 ATROVENT (ipratropium bromide) bronchodilator inhaler

### CLINICAL STUDIES

#### ABSTRACT

A summary of these "special studies, in the NDA, numbers one and two were the same, to determine the effects of Atrovent in ventilation/perfusion relationships as manifested by changes in arterial blood gases and blood pH" (Dr. Dr. 2) with results showing Atrovent improved pulmonary function in patients with stable airway obstruction without changing blood pH, arterial gases in blood and therefore did not change ventilation/perfusion ratios. 3) Dr. showed in a study of Atrovent on mucociliary clearance in patients with chronic obstructive pulmonary disease that compared to placebo, Atrovent showed a 103.3% increase in pulmonary function whereas these patients on placebo showed a 9.2% mean percent over baseline at 1 hour improvement. 4), Dr. in a study on the effect of Atrovent on tracheal mucous velocity in patients with chronic obstructive pulmonary disease Atrovent produced no significant changes in tracheal mucous velocity compared to baseline and to placebo, although slightly higher values were seen after placebo. 5) Dr. conducted a trial to examine certain biochemical mechanisms which may be responsible for the effects of Atrovent in patients with bronchial asthma with the result that the role of biochemical effect could not be determined. 6) Dr. studied and evaluated the effects of Atrovent (inhaled) on sputum in chronic bronchitis, compared to placebo in which no significant results were noted between the two treatments on mucous. 7) Drs. and conducted the same study as #6 above with results showing higher sputum volume in patients on Atrovent over those on placebo. (Figure 1 noted). Pulmonary function improved in patients on Atrovent over those on placebo. Studies 8, 9, and finally 10, were ophthalmology studies, all of which showed Atrovent produced no clinically significant changes in pupil size, accommodation, visual acuity or intraocular pressure in subjects and in patients with chronic lung disease.

- A. Special Studies - 10 in number.

1)

Atrovent metered dose inhaler (MDI)

Study No.: E-82-318

Protocol Designation: Id<sub>2</sub>

Date: Dec. 2, 1981

Summary items 2 and 3 in the NDA; location Vol. 1.2, part E1, pp. 7-147.  
Reference to complete data item 12, Vol. 1.42.

"Objective: To determine the effects of ATROVENT on ventilation - perfusion relationships as manifested by changes in arterial blood gases and blood pH."

This study planned to evaluate 20 male or female patients, ages 18-65 with reversible bronchospasm, an FEV<sub>1</sub> < 75% of predicted. There were to be 2 test days. Criteria for inclusion were found acceptable, in that patients were to have "relatively stable or mild to moderately severe airway obstruction," and patients must have the stated "reversible bronchospasm shown by a 15% improvement in FEV<sub>1</sub> within 30 minutes following 2 whiffs (0.150 mg) of isoproterenol from a metered dose inhaler".

"Drug administration will be by oral inhalation in which each test dose will be 2 whiffs of ATROVENT, 20 mcg each or

Placebo - 2 whiffs,"

Duration - 2 single test doses of drug 2 weeks apart, or at least 24 hours apart.

Control - double-blind, crossover.

Randomization - by order of Entrance

Other Medication and Habits - inhaled steroids and cromolyn permitted if stabilized. Other anticholinergics will be excluded at least 2 weeks prior to entry; all parenteral or oral bronchodilators excluded for at least 12 hours prior to entry and during test days. All inhaled bronchodilators are excluded at least 8 hours prior to and during testing.

SUMMARY - objective as stated

Number and type cases - 19, 9 male, 10 female, ages 25-75 years. Exclusion criteria were implemented; 3 were excluded for protocol violations and pulmonary function test results. These 3 were not evaluated for efficacy. Sixteen, 7 males, 9 females ages 46-75 completed the study. Diagnosis: Extrinsic asthma, duration 5-70 years. Method of study - randomized, single dose, crossover with at least 24 hours to 2 weeks separation of dosing. Both test days were completed within 2 weeks.

Dose and Route of Administration - oral metered dose inhalation of ATROVENT, 40 mcg (20 mcg/whiff), or placebo, 2 whiffs. Concomitant medication - no patients were on investigational drugs. Of the 16 who completed the study, 5 were on steroids, 10 mg or less daily of prednisone or 20 mg every other day; 9 were on inhaled steroids for which dose had been stabilized 8 hours prior to testing and for duration of test days. Before and after the study PE and lab testing were done. Patients qualified for admission according to requirements stated above. PE, CBC, SMA-12, urinalysis, repeated at end of test days.

Each Test Day - observations involved pulmonary function testing at the same time of each test day for each test dose. Baseline FEV<sub>1</sub> on each test day was within 10% of that on qualification day; FVC and FEV<sub>1</sub> were measured at 15, 30, 60, 120 minutes after drug was given. Pulse rate and blood pressure (BP) were measured at the same time as pulmonary function tests and always after the patient rested 2 minutes. Vital signs were measured before pulmonary function testing. Blood samples were drawn at 15, 30, 60 and 120 min. after drug was given. Blood pH and arterial blood gases were studied for PaCO<sub>2</sub>, PaO<sub>2</sub>, SaO<sub>2</sub>, HCO<sub>3</sub> and measured at the same time as pulmonary function testing was done. Hgb and Hct were measured at baseline, of the 2 study days. Adverse reactions were recorded in detail.

### Results

Efficacy - pulmonary function (FEV<sub>1</sub> and FVC) increased clinically at all test measures within 2 hours after ATROVENT vs placebo evaluation of AUC (area under curve) of change from baseline curves for FEV<sub>1</sub> and FVC showed better results for ATROVENT over placebo. This data has been verified.

"Measurements of blood pH and arterial blood gases showed no marked changes from pretreatment levels or between ATROVENT and placebo". Please refer to statistical evaluation.

Safety - no clinically significant changes in BP or pulse rates or laboratory tests or adverse reaction - gastric distress was reported in a patient on placebo; no others.

Conclusion: Atrovent improved pulmonary function in patients with stable airway obstruction, but did not affect ventilation/perfusion status. This complete clinical trial is found, including case report forms, in volume 1.42 and Vol. 1.2, pp 7-147.

### Special Studies (continued)

2) \_\_\_\_\_

Study No. E82 - 315

Protocol designation - Id<sub>2</sub> (same as study #1 above)

Date - Dec. 2, 1981

Atrovent metered dose inhaler (MDI)

Objective: As in study #1 above.

Protocol Id<sub>2</sub> in Vol. 1.2, pp 148-287 and expanded summary; also in Vol. 1.43.

This study had the same objective and followed the same protocol as that of Dr. \_\_\_\_\_ as described in 1) above, these details will not be repeated here, however; the following details of this study are noted:

Number and types of cases - 13 patients, 11 M, 2 F, ages 29-65 entered; 12 completed the study.

### Results

Efficacy - with the exception of FVC at the 2-hour test, pulmonary function as measured by FEV<sub>1</sub> and FVC increased clinically after Atrovent over placebo. Efficacy evaluation for AUC (area under curve) of change from baseline for both FEV<sub>1</sub> and FVC showed better results for Atrovent vs placebo (verified).

Safety - no adverse reactions; no clinically significant changes in BP, pulse rates, nor lab. values were observed.

Conclusion: Atrovent improved pulmonary function in patients with stable airway obstruction without changing blood pH, arterial gases in blood and therefore did not change ventilation - perfusion ratios.

3)

Study No. E-80-735

Protocol Designation - 1e 3b

Date: July 9, 1980

Title: Effects of Atrovent on mucociliary clearance in patients with chronic obstructive pulmonary disease (COPD).

Location in NDA - Vol. 1.44 pp. 1-93; Vol. 1.2 pp 288-354

**Investigator:**

This study, in introductory remarks, notes that drugs with anti-cholinergic effects depress mucociliary activity in man. The study drug in this NDA, ipratropium bromide, is said to show no such activity in preclinical studies, that "tracheobronchial clearance of isotope-labeled particles before and after inhalation of ipratropium bromide did not show any reduction in clearance in healthy subjects or in-patients with bronchitis. The purpose of this study is to confirm that Atrovent does not depress mucociliary clearance."

The objective as stated is "to determine the effects of administration of Atrovent on mucociliary clearance and pulmonary function in patients with chronic obstructive pulmonary disease" (COPD). The protocol called for studying 20 adults with a diagnosis of chronic bronchitis or bronchial asthma. Duration was 2 test days. Appropriate records on patients were maintained. Inclusion criteria were acceptable, specifically regarding patients with mucous velocities greater than 3mm/minute in the trachea and 1.5 mm.min. in the main bronchs and/or whole lung clearance of 0.3%/min; male or female; ages 18-60. Exclusion criteria are found to be acceptable.

Drug Administration: Oral inhalation, Atrovent 2 whiffs of 20 mcg each or placebo. Duration will be single test doses (2) with at least 24 hours washout period between doses. Both test drugs (2) will be completed in one week.

The study was a crossover, double-blind, randomized design. Other medication and habits are stated and found appropriate for this type study.

Method of Evaluation:

History, PE, prior to admission. Baseline pulmonary function testing were done on admission (body plethysmography) to measure airway resistance (Raw), thoracic gas volume (VTG) and functional residual capacity (FRC), specific conductance (SGaw) were calculated (1/Raw/VTG); FVC, FEV<sub>1</sub>, FEF<sub>25-75</sub> and flow volume loops (HE O<sub>2</sub> vs air) at baseline and 5 minutes after 2 whiffs of Alupent (1.3 mg) from an MDI (metered dose inhaler); diffusing capacity measured by single carbon monoxide method). On each test day tracheal and bronchial mucous velocities were to be measured and specific appropriate data recorded for statistical analyses. Raw, VTG and FRC were measured at baseline and repeated once at 1-2 hours post-drug by body plethysmography and BP and pulse rates recorded at the same times.

### Adverse Reactions

Appropriate provisions were made for recording and evaluation by the investigator. Provision was made for signed informed consent. The expected completion date was set at six months after receipt of supplies.

In the clinical trial 11 males, ages 18-36 were entered, 10 completed the study. One patient, age 34, with a diagnosis of severe bronchial asthma received both test doses but was unable to complete the 4-hour breathing process on either day. His data were not included in the efficacy evaluation. There were 2 intrinsic (mild) and 8 extrinsic asthma cases (5 mild, 3 moderate); duration of disease was from 3 to 24 years. Method of study has already been adequately described, specifically under dose, route and duration of treatment. Administration of test drug was by metered dose inhaler (MDI), as Atrovent 40 mcg (20 mcg/whiff) - 2 whiffs or placebo, 2 whiffs; the aerosols were administered after inhalation of iron oxide particles.

Upon entrance to the study 3 patients were on S-R theophylline, which had been discontinued at least 12 hours prior to test dose administration on each of the 2 test days. One of these patients was also on Alupent tablets, twice a day. Five patients used beta adrenergic aerosol therapy prior to the study when necessary, none within 12 hours of entry. Observations before the study on each test day adhered to the protocol.

### Results:

Efficacy: The SGaw (Specific Airway Conductance), mean percent change over baseline at 1 hour was 103.3% increase after Atrovent and 9.2% after placebo. There were no significant differences in treatment effects in any of the variables measuring radio-tagged iron oxide particles from the lungs. At the end of 4 hours the mean percent of radiosotopically labelled mucous still present in patients receiving Atrovent was approximately 30% and 20% in those receiving placebo.

Safety - There was no significant change in mucociliary clearance velocity, but there was significant improvement in pulmonary function with no adverse reactions reported.

4)

"Effect of Atrovent on tracheal mucus velocity in patients with asthma."

Study No. E-80-789

Protocol Designation: 1e 3c

Date - July 1, 1980

Location in NDA - Vol. 1.44; Vol. 1.2

Objective - "To determine the effects of administration of Atrovent on tracheal mucous velocity and pulmonary function in patients with asthma and to compare these effects with placebo".

Fourteen adults with asthma were to be studied for 2 days. Criteria for inclusion and exclusion of patients were listed and found to be acceptable.

Drug administration - dose as in the previous study described

Type - Double-blind, 2-way crossover study.

Other medications and habits - same as study #3 described earlier; same for method of evaluation.

Plan of study: spirometry on each test day, FVC, FEV<sub>1</sub> and FEF<sub>25-75</sub> measured at baseline, 1/2, 1, and 2 hours after drug has been given. Tracheal mucous velocity was obtained by observation of "The motion of radiopaque discs of teflon mixed with bismuth trioxide deposited in the trachea and observed by a fluoroscopic image intensifier. The doses are blown through the inner channel of a fiberoptic bronchoscope which is located just above the vocal cords. Measurements of tracheal mucous velocity are conducted at baseline, 1/2 and 2 hours after drug administration." Vital signs are recorded at the same intervals as pulmonary function testing.

Number and types of cases - 14 patients, 5 males, 9 females; ages 18-46 entered and completed the study.

#### Results:

Efficacy - Atrovent produced no significant changes in tracheal mucous velocity compared to baseline and to placebo, although slightly higher values were seen after placebo. No clinically important changes in pulmonary function were detected in this study because these were mild cases of asthma and were not required to demonstrate stable or reversible bronchospasm upon entry to the study.

Safety - One patient on Atrovent showed wheezing and dyspnea immediately following measurement of tracheal mucous velocity. No vital signs were abnormal in any other patients.

Conclusion: Atrovent did not produce significant changes in tracheal mucous velocity when compared with placebo.

cromolyn, acetylcysteine, expectorants, and inhaled steroids were excluded. Oral bronchodilators (xanthines and adrenergic agents) permitted but dosage was to remain constant. Aerosol bronchodilators allowed on an "as needed" basis. Daily use was to be recorded.

#### Method of Drug Administration

Route - oral inhalation

Dose - Each test dose was Atrovent - 2 whiffs of 20 mcg each q.i.d. or

Placebo - 2 whiffs q.i.d.

Provision was made for additional whiffs when necessary but limited.

#### Duration of Treatment

7 weeks continuous for each treatment separated by at least a one-week washout period.

Type of Control - double-blind, crossover study

Randomization - adequately described

#### Method of Evaluation

Visits to the laboratory by patients were to be weekly for the 7 weeks of the test period. Each of the two seven week test periods was to be separated by at least a week. During each of the 7 week test periods, patients routinely used either Atrovent or placebo aerosols in a sequence following the randomization code.

#### Pre-Admissions

Adequately described to achieve the objectives of this study. PE, CBC, SMA-12, urinalysis and EKG were repeated also at the end of each 7 week period. FVC and FEV<sub>1</sub> determined at baseline as well as cytology, Gram stains of sputum, and a chest x-ray were also done at baseline.

Observations to be carried out on each of the test days were described and found acceptable. They included the overall change of pulmonary status and any change was ranked by physician and patient by a 15% degree of improvement or worsening from very much better as +7 to very much worse as -7. Medication

history and any deviations each week were reviewed. Current subjective symptoms to be graded were listed and described in detail, in general concerned cough frequency, cough severity, difficulty raising sputum, sputum thickness, severity of dyspnea, character of wheeze, severity of background wheezing (symptom); wheezing attacks, upper respiratory, oropharyngeal and eye symptoms.

Physical findings described in detail in an appropriate manner included vital signs, chest examination for prolongation of the expiratory phase, rales, Ronchi, wheeze and intensity of breath sounds, all to be assessed in detail.

Upper respiratory examination was described adequately in detail in which head, eyes, nose and throat examination were to be carried out to detect any possible irritation from the aerosol and evaluated on a scale of 0 = normal to 6, 7, = markedly present observations for defects in sinus areas, nasal mucosa, oropharyngeal mucosa, conjunctivae. All the above were classified as subjective measurements to be made.

Objective measurements to be made are also described adequately in detail under:

Pulmonary function tests.  
Sputum measurements - 24 hour sample.

Adverse reactions were to be adequately recorded.

Data Submitted Regarding The Actual Conduct Of The Study And The Results Obtained.

The objective was to determine the effect of Atrovent in bronchial mucous production and to determine the effect of long-term use of the drug on bronchial mucosa epithelium and on bronchopulmonary inflammatory response; subjective and objective measurements were made in the long-term use by measuring clinical response to the Atrovent.

\*It should be noted that this NDA concedes certain populations with asthmatic and/or bronchitic bronchospasm respond better than Atrovent to bronchodilators such as sympathomimetic amines and xanthines, but the claim made is that certain sub-populations of these groups of patients respond favorably to Atrovent (Ipratropium bromide) as a bronchodilator.

In this clinical trial there were 19 patients entered, 14 males, 5 females, ages 48 to 86 years. Fifteen of the nineteen patients completed the study.

Inclusion and exclusion criteria mentioned above as satisfactory were followed. Diagnosis of patients was chronic bronchitis (4 mild, 10 moderate, 1 severe). Duration of illness was from 15 to 60 years.

Four patients became dropouts.

- 1 Due to side effect-on placebo, had "light flashes" over one eye.
- 2 Due to factors unrelated to therapy, 1 placebo, and 1 Atrovent patient hospitalized for intercurrent infection and "chest cold" respectively.
- 1 Due to protocol violation.

Method of Study - randomized, double-blind, crossover design with at least one week washout period between treatment crossovers.

Route - oral by metered dose inhaler (MDI)

Dose and Duration of Treatment

Atrovent, 40 mcg/q.i.d. or placebo 2 whiffs q.i.d., continuous treatment for each 7-week treatment period.

Single test doses of Atrovent 40 mcg (20 mcg/whiff) or placebo 2 whiffs upon waking on the first day of each weekly visit to the lab (7 weeks on each treatment) for examination.

Observations on Each Test Day - followed protocol; P.E. including vital signs, chest and upper respiratory examination for prolongation of the expiratory phase, rales, ronchi, wheezes and intensity of breath sounds of area of the lung were auscultated and graded. Use of concomitant medication; observations before and after study and details of upper respiratory examination also adhered to the protocol.

Objective measurements - Sputum sample, 24 hours, and 3 hours after drug administration as called for in protocol.

Subjective measurements - Data from patient diaries, cough frequencies and severity, difficulty in raising sputum and sputum thickness, severity of dyspnea, wheezing including background and the number of attacks. Upper respiratory, oropharyngeal and eye symptoms were recorded and graded for the 24 hour period prior to each test dose.

Safety - Onset and duration of adverse effects were recorded and whether or not, in the physician's opinion, they were drug-related.

Results - "Variables are presented in the statistical report in Appendices A, B, C and are represented by figures and graphs for selected variables. These provided the general pattern of response and also a visual assessment of the difference in the patterns for Atrovent and placebo." These are in volumes 1.3 and 1.4 for Division of Biometrics.

\*Interpretation of these statistical reports will be performed by our Division of Biometrics and will await their review and evaluation.

## Efficacy

### Sputum Parameters

Statistically regarding efficacy, the sponsor states "the only statistically significant difference between treatments on the sputum measurements was a significantly higher ( $< 0.035$ ) 24 - hour volume on placebo. This was due to an increasing sputum volume on placebo rather than a decrease in volume on Atrovent. (Figure 1, Volume 1.3). This will, as said, await review by our Division of Biometrics on this section which is not medical. "Viscosity varied inconsistently from visit to visit and also between Atrovent and placebo" "Stickiness also showed no consistent patterns". "Water Loss Rates showed no consistent patterns over the 7- week administration periods or between treatment groups. There were also no consistent changes in the dry weights of the sputum".

"Bronchopulmonary inflammatory level was unchanged over the 7- week administration period in both treatment groups as noted by the quantitative count of organisms per oil immersion field, sputum, protein, and cytology including both quantitative counts and qualitative differential counts. There were also no changes noted in the bronchial epithelial cells." These data are referenced and may be verified as part of the statistical, not medical report.

### "The Objective Observations"

The pulmonary function test results in this study, and the vital signs recorded on each test day "did not show any trends over the course of the study nor were there any significant differences between the drugs." Reference is made to a table which was interpreted by this reviewer. Mean overall values over 7 weeks showed no marked differences between Atrovent and placebo regarding SVC (L) (Standard vital capacity), VC(L); FEV<sub>1</sub>(L); FEV<sub>25-75%</sub> L/sec.), pulse rate, systolic and diastolic blood pressures. The source data: Appendix B of the Statistical Report.

Regarding chest examination results for rales, ronchi, wheezes, intensity of breath sounds and prolongation of the expiratory phase of respiration were not affected by either form of therapy in the patients studied, 15 on Atrovent, 15 on placebo. Reference is again made to data in the statistical report, "Appendix H, Tables 140 to 144".

### Subjective Evaluation

This was obtained from diaries. No difference in cough frequency was noted except in one patient, nor were there notable differences in cough severity, difficulty in raising sputum, sputum thickness, severity of dyspnea, back-ground wheeze, or the number of wheezing attacks. (Reference for the above is made to the statistical report, variables 17.1 - 20.3, regarding the two

treatment groups, Atrovent and placebo. In the parameters of subjective upper respiratory oropharyngeal and eye symptoms during the 24-hour period prior to their weekly visits, patients' diaries showed no differences between Atrovent or placebo treatment groups.

Safety:

No changes in laboratory findings; no significant changes in ECG tracings over the 15-week period; adverse effects - only after continuous doses and did not interfere with treatment. They were (Table 3) - arrhythmia - 2 on Atrovent; dizziness, 2 on each treatment; rash - 1 on each; itch, 1 on Atrovent; G.I. - reported by 3 on Atrovent and by 2 on placebo; tremor-1 on Atrovent; eye-3 on Atrovent, 1 on placebo; oro-otolaryngeal -2 on each treatment. All refer to the statistical report.

In summary, a multiple-dose, double-blind crossover study comparing Atrovent to placebo over 7 weeks of treatment which showed no clinically significant differences in sputum, flows of any kind, bronchopulmonary inflammatory status, pulmonary function testing, physical findings on chest examination by the physician nor subjective evaluations by patients. Statistically, not medically "the only statistically significant finding was a greater sputum volume on placebo rather than a decrease in volume on Atrovent". This statement by the applicant must await verification by our Division of Biometrics following their review and evaluation of the statistical report in this study.

7)

Study No. E81-166

Protocol Designation II d<sub>2</sub> (same as preceding study).

Date - September 17, 1980

Location in NDA - Vols. 1.5, 1.6, 1.51 - 1.56

In the introduction to this study the applicant stresses the obvious, that is, that "because of the cholinergic/anticholinergic influence on bronchial mucous production, it is important to determine if Atrovent has an effect on this critical component of bronchial function. Since

Atrovent is administered as an inhalant and will be used chronically by patients it is also important to determine the effect of long-term Atrovent on the bronchial mucosal epithelium and the inflammatory response characteristic of the chronic bronchitic". The same applied to the immediately preceding study

The protocol for this study, protocol designation II d2, is the same as the preceding study -Dr. and will not be repeated here. The same applies to the study objective.

Twenty-five patients entered the study, 13 males, 12 females, ages 27-72 years and 23 patients completed it. Inclusion criteria stipulated that patients be ages 18-65, diagnosed as having chronic bronchitis and have a relatively mild to moderately severe, stable airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal. Patients had to have less than 20% response on FEV<sub>1</sub> within 30 minutes of 2 whiffs (1.3 mg) from an Alupent MDI, be unresponsive to a methacholine challenge and had to produce a consistent daily volume of sputum of at least 5 ml/day and the sputum, had to have less than 20% eosinophilia with the predominant inflammatory cell being neutrophil and/or macrophages. Exclusion occurred if patients had a significant systemic disease, active TBC, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to anticholinergic drugs; those with peptic ulcer requiring those drugs were excluded. Pregnant females or those who expected to become so during the study were excluded. Patients with a history of significant asthma, or who had a viral infection or febrile illness within the preceding month were excluded. Two patients dropped from the study; one in the placebo group moved out of State and 1 in the Atrovent group incurred an infection.

Twenty-three patients, 9 males, 14 females, ages 27-72, diagnosed as chronic bronchitics, 3 mild, 15 moderate, 5 severe with duration of illness 1-25 years completed the study.

Route - Oral inhalation by metered dose inhaler (MDI).

Dose and duration of treatment - continuous treatment of Atrovent 40 mcg q.i.d., or placebo 2 whiffs q.i.d. for each 7 week treatment period.

Regarding use of concomitant medication, no other investigational drugs nor anticholinergic drugs were used during this study. Oral steroids were taken by 6 patients and oral beta-adrenergic bronchodilators by the same number, and 9 took aerosols. Theophylline alone or in combination was taken by 15 patients and 1 took a Beta-blocker.

Observations before and after the study adhered to the protocol regarding history, P.E., vital signs, laboratory tests, ECG prior to admission. P.E., CBC, SMA-12, urinalysis, and ECG were performed upon completion of each 7-week treatment period. Cytologic and Gram stains of sputum, and if necessary, chest x-ray, and pulmonary function tests were done at baseline.

At each test day, P.E., chest examination and upper respiratory examination was performed. The chest examination was complete adhering to the protocol regarding prolongation of expiration, rales, ronchi, wheezes, and intensity of breath sounds were noted and graded. There was adherence to the protocol regarding the upper respiratory exam of head, eyes, nose, throat for possible aerosol irritation which was graded on an 8 point scale. Injection, erythema, edema and secretions were noted for nasal and oropharyngeal mucosa, tenderness and local edema for sinus areas and injection, erythema and exudate for the conjunctivae.

There were objective and subjective measurements as called for in the protocol recorded. Objective measurements included pulmonary function testing in the AM at the same time for each test day. Patients took no oral bronchodilators for at least 12 hours and no aerosols for 6 hours prior to laboratory reporting. Patients took a dose, (2 whiffs) of the assigned study medication upon awakening. Pulmonary function tests performed were SVC, FVC, and FEV<sub>1</sub> and FEF<sub>25-75%</sub> concomitantly with pulse and respiratory rates on appearing at the lab and at the end of each of the 7 weeks of treatment. On each test day, as called for, "the patient took 2 sputum samples to the lab containing (1) all the sputum collected over the 24-hour period just prior to the test day and (2) a container with all the sputum collected during the 3-hour period following inhalation of the test dose." The 24-hour, and 3-hour sputums were collected on the first day of each 7-week treatment period with prior use of any bronchodilator agent(s).

The 24-hour sputum sample was used to measure: "Viscosity, dry weight with water loss rates, volume, protein, Gram stain, cytology, qualitative and quantitative (cells/day)."

The 3-hour sputum sample was used to measure:

- (1) Viscosity;
- (2) Volume.

The subjective measurements taken from patient diaries included "cough frequency and severity, difficulty in raising sputum and sputum thickness, severity of dyspnea, wheezing with background and number of attacks." Upper respiratory, oropharyngeal, and eye symptoms, graded, were recorded for the 24-hour period prior to each test dose.

Safety - Complete recording was made.

### Results

Reference is first made to variables presented on the statistical report in Appendics A, B, and C to be reviewed and evaluated by our Division of Biometrics. Regarding efficacy for sputum parameters figure 1 of that report shows a higher sputum volume in 24 hours for 23 patients over 7 weeks of treatment from patients taking Atrovent over those on placebo. Viscosity varied inconsistently on both the 3-hour and 24 hour sputum samples in those on Atrovent and on those on placebo over the 7- week treatment period. (Variable 1.1 to 2.8, 9.1 to 10.8 in the statistical report). No consistent pattern was seen between the two treatments regarding water loss rates by the percent of sputum remaining nor on the dry weight of the sputum. (Statistical report, variables 3.1 to 3.7 inclusive). Bronchopulmonary inflammatory level did not change over the 7- week treatment period in either treatment group.

Objective observations by pulmonary function tests of SVC (standard vital capacity), FVC (forced vital capacity), FEV<sub>1</sub> (forced expiratory volume in the first second of expiration) and FEF<sub>25-75%</sub> (forced mid-expiration flow) was higher in patients administered Atrovent than in those administered placebo (statistical report, variables 22.1 - 22.4 - to be reported upon by our Division of Biometrics). There were no significant changes in pulse rate or blood pressure reported during the study. Physical examination of the chest for rales, ronchi and wheeze showed no consistent differences over time or between treatments. Prolongation of the expiratory phase of respiration was recorded as being slightly higher for Atrovent over-placebo-treated patients, but was not clinically significant.

Subjective Evaluation - These data were from patient diaries and showed no differences between treatment groups for cough frequency, severity, difficulty in raising sputum, severity of dyspnea nor background wheeze clinically. There were no clinically significant differences in the number of patients reporting subjective upper respiratory, oropharyngeal and eye symptoms during the 24- hours prior to the weekly visits. (For statistical evaluation reference is made for the above subjective evaluations statistically to variables 15.1 - 19.2 and variable 3 and Table 5 of the statistical report for this study).

Safety - The investigator found no laboratory findings of clinical significance; the same applies for ECG tracings over the 15-week study. The only adverse reactions reported, occurred after continuous dose study and did not cause discontinuation of treatment. The adverse reactions reported were, of 24 treated patients, one case of headache in a patient on Atrovent, also one case of nausea and one case of "eye-burn" among patients on Atrovent. Among patients on placebo, there was 1 report of headache and nervousness and two reports of irritation from aerosol. No reports of the adverse reactions stated in patients administered Atrovent can be considered serious, and from this study the drug can be considered safe after 7 weeks of continuous administration.

In conclusion, this study demonstrated in a multiple dose, double-blind, crossover type study that when Atrovent was compared to placebo in comparable patients (and crossover 7 weeks), no differences in sputum flow, broncho-pulmonary inflammatory level, physical findings on chest exam, or subjective evaluations by patient or physicians were noted. Reference is made to the statistical report to be verified that "the only statistically significant finding was a greater sputum volume on placebo." Pulmonary function testing at all weeks of the study showed more favorable results in patients administered Atrovent over those administered placebo.

8.)

Study No. E-74-213

Date - 3/28/74; Final Report - 5/26/83

Phase of Study - 1

Locations in the NDA - Vol. 1.7 pp. 1-68;  
Vol. 1.57

Objective of the Study - "To assess the effect of Atrovent on pupil size, accomodation, visual acuity and intraocular pressure in normal volunteers".

This single dose study to compare the effects on the eye of Atrovent vs placebo measured the following variables:

Pupil size  
Accomodation  
Intraocular pressures.

The study was of 2 days duration, placebo controlled, crossover. Ages of subjects were 20-35 years. All subjects had a normal ocular history and showed no abnormal signs upon complete ophthalmologic examination. The examination covered refraction, ocular motility, pupil size, reactivity to light, accomodation, biomicroscopy, tonometry and ophthalmoscopy.

Double-Blind Crossover - Medication

On each of 2 separate days, subjects received single test doses of either Atrovent, 2 inhalations (20 mcg/inhalation) or placebo 2 inhalations. The 2 mcg test doses were administered in random order at least 24 hour apart.

On each of the two test days, the tests noted above were measured before and 1, 3, 5 hours after administration of the test doses.

Adverse experiences on Atrovent included 2 cases of lightheadness, 2 headaches, 1 nausea, 2 blurred vision, 1 post-nasal drainage, 2 tired. On placebo there were reports of lightheadness in 2; headache, 3; diaphoresis in 1; flushing, nausea, blurred vision, dry throat, post-nasal drainage, nervous, 1 in each case and 3 reports of "tired".

Result - no significant differences on pupil size, binocular accommodation, tonometry and visual acuity were obtained on Atrovent as compared to placebo. Statistical analysis was performed.

9)

Study No. E-73-709

Final Report - 5-26-83

Objective of Study - as above in 8) Dr study.

Procedure, method of study, concomitant medication, observations made and times, all the same as #8 above, including medications.

Results - The effects of Atrovent vs. placebo in 30 normal subjects for pupil size determination, binocular determination, tonometry and visual acuity in serial tests following single doses of Atrovent or placebo showed no significant differences between the two treatments except for a slight increase in mean pupil size at 30 minutes following Atrovent. This change was too small to be considered clinically significant.

10)

Study No. E-82-1861

Date of Final Report 7/8/83

Phase of study - I

Site -

Location, in NDA - Vol. 1.7, pp. 286-359; Vols. 1.59, 1.60, 1.61, 1.62

As in the two previously described (8), (9) above, the principal thrust of these investigators was to study the effect of the anticholinergic agent Atrovent (Sch 1000) on the eye.

"Objective: To assess the effect of Atrovent on pupil size, accommodation, visual acuity and intraocular pressure in patients with chronic, reversible bronchitis who were also participating in a multiple-dose study to evaluate the bronchodilator effectiveness and safety of Atrovent." Note - unlike the two previous studies 8), 9) above patients with chronic bronchitis were studied in this trial rather than normal subjects.

Twenty-nine male and female patients, ages 17-67 years entered the bronchodilator study; 28 (1 less) of these patients also participate in the eye study. 4 patients dropped out leaving 24 for analysis in the single-dose study and 5 others were dropped leaving 19 patients who completed the multiple-dose study. Reasons for dropouts are listed in Table 1, 3 due to side effects; 1, factors unrelated to therapy; 2 due to treatment failures; 2 lost to follow-up; 2 for protocol violations, for a total of 9.

The studies were 2 double-blind, three-way, crossover studies: a single-dose study and a 45-day, multiple dose study. For each study patients were randomly assigned to one of six treatment sequences.

#### Medications

Single doses of Atrovent MDI 40 mcg, oral inhalation (20 mcg (whiff), Berotec, 5 mg (2.5 mg/tablet), and a combination of Atrovent MDI 40 mcg, plus Berotec tablets 5 mg. Placebo tablets and placebo aerosol were used to preserve blinding. At least 24 hours separated the 3 test doses which were administered prior to the starting of multiple dose study. Continuous treatment - Atrovent 40 mcg q.i.d. Berotec, 5 mg q.i.d. and Atrovent, 40 mcg plus Berotec, 5 mg q.i.d., each treatment self-administered by the patient for 45 days with evaluation on day 45 of each drug regimen.

#### Concomitant Medication:

No other investigational drugs were used. Of 28 patients entering the study of eye effect, 6 were on oral prednisone, 23 on inhaled steroids, 16 took theophylline.

Observations before the study are described and found acceptable.

Observations on test days in the ophthalmic study measured before and 2 hours after each dose in the single-dose study: pupil diameter of each eye; accommodation (in duplicate) of each eye and both eyes together; intraocular pressure of each eye, and visual acuity of each eye. The same measurements were taken after 45 days of continuous treatment in the multiple-dose study. An ECG was measured at baseline and 1 hour after a test day on day 45. Adverse reactions were recorded.

### Results

Efficacy - pupil diameter decreased from day 1 to day 45 statistically but not of clinical significance for Atrovent. There were no significant differences between drugs at either timepoint on either day of test. Following 45 days of treatment there was "a slight statistically significant increase in pupil diameter of the right eye occurring at 2 hours following test dose, but not clinically significant and remained less than the Day 1 baseline".

#### Accommodation:

Small changes were noted but were not clinically significant in response to test doses or after 45 days of treatment.

#### Intraocular Pressure.

Small clinically insignificant decreases in intraocular pressure two hours after each test dose on both test days.

#### Visual acuity:

No statistical or clinically significant changes in visual acuity occurred.

### Safety

ECG tracings showed no significant changes after Atrovent over the 90-day period.

There were no adverse reactions after Atrovent. After test doses of Berotec, tremor (3 cases) and palpitations (1 case) and after continuous treatment with betrotec, 1 case of tremor was reported. "After administration of the Atrovent/Berotec combination, 1 arrhythmia was reported; 1 conduction disturbance; palpitations and supra-ventricular tachycardia after the test dose was reported and that patient dropped out of the study. Other adverse reactions after the combination test doses were headache (1); tremor (3). After multiple doses of the combination 2 patients had tremor."

Conclusion: In this study small, but not clinically significant eye changes were detected. Contrary to the pharmacologic effect expected from use of anticholinergic agents, pupil diameters and intraocular pressures decreased following test doses of the drug Atrovent.

The statistical report follows to be reviewed by our Division of Biometrics.

Reviewer's Comments - No significant number, nor serious adverse reactions were recorded on any of these ten special studies.

End - "Special Studies" - Ten. Each study protocol, objective, method of study, objective and subjective results obtained and locations on the NDA are identified. Statistical analyses were done. This completes "A) Special Studies".

B) Dose-Ranging Studies - 5 in number

1)

Study No. E-79-81; Location on the NDA-Vols. 1.8 + 1.63.(pp. 1-122).

Objectives:

"To confirm the results of the original dose-ranging studies. To determine the efficacy and optimal dosage of Atrovent when delivered by a metered dose inhaler (MDI) in a single dose, double-blind crossover study in adult patients with asthma. To compare the Atrovent dose response curve with that of isoproterenol and to placebo with particular emphasis on, onset and duration of action, side effects and adverse reactions, including effects on pulse and blood pressure."

Fourteen patients with asthma, reversible airway obstruction (increase in FEV<sub>1</sub> of at least 15% within 30 minutes of 0.150 mg of isoproterenol were called for in the protocol).

Age - 18-65 years: sex-male or female. Females of child-bearing potential were to be excluded. The aim was to study 14 completed patients (at least 4 doses of Atrovent, 2 doses of isoproterenol, 1 dose of placebo).

Appropriate records were kept.

A list of associated diseases which excluded patients was given and found appropriate.

A list of other drugs to be excluded was also stated and found responsible. Some might be administered under appropriate specific conditions such as steroids, and cromolyn, but not within 8 hours of testing procedures.

Type of control - double-blind, single-dose, seven way crossover.

Appropriate randomization.

Observations to be made:

Prior to study admission - history, P.E., Lab tests, ECG, evidence of an increase of at least 15% in FEV<sub>1</sub> after administration of isoproterenol.

Follow-up observations:

- 1) Flow volume curves to be obtained at baseline, 5, 15 and 30 minutes, and 1, 2, 3, 4, 5, 6 hours after drug administration.
- 2) Global evaluation - by physician and patient.
- 3) PE, CBC, ECG, urinalysis at the end of the study.
- 4) Pulse rate, blood pressure on each day at the same time of day, at the same time intervals as the forced expiratory volume, i.e., baseline, 5 minutes, 15 and 30 minutes and: 2, 3, 4, 5, 6 hours post-drug administration
- 5) Lead 2 EKG monitored at 5, 15, 30 minutes and 1, 2, 4 hours post-drug.
- 6) Side effects ) Adequately provided for  
7) Adverse effects or reactions) recording

Dose and Route:

By inhalation

Atrovent 20, 40, 60, 80 mcg or ug from MDI at 20 ug per whiff.  
Isoproterenol 75 and 150 ug from MDI dispensing 75 ug/puff

Placebo

Each dose consisted of 1 inhalation from each of 4 canisters as:

|                      | <u>Atrovent</u> | <u>Isoprotorenol</u> | <u>Placebo</u> | <u>Total</u> |
|----------------------|-----------------|----------------------|----------------|--------------|
| Atrovent 20 ug       | 1               | 0                    | 3              | 4            |
| Atrovent 40 ug       | 2               | 0                    | 2              | 4            |
| Atrovent 60 ug       | 3               | 0                    | 1              | 4            |
| Atrovent 80 ug       | 4               | 0                    | 0              | 4            |
| Isoproterenol 75 ug  | 0               | 1                    | 3              | 4            |
| Isoproterenol 150 ug | 0               | 2                    | 2              | 4            |
| Placebo              | 0               | 0                    | 4              | 4            |

Duration of treatment - until the 7- way crossover was completed with a washout period of at least 24 hours between each dose of drug.

Provision was made for written informed consent.

Summary of study.

Objectives - as stated above.

Cases - 14 patients, ages 22-66 with asthma were admitted and completed the study. All had reversible airway obstruction.

Method of study - as per protocol.

Dose, route, duration of treatment.

Concomitant medication.

Observations before and after the study.

Observations in each rest day.

Adverse reactions.

All of these followed the described protocol and recording.

Results

Efficacy - the adjusted mean area under FEV<sub>1</sub> curve for all doses of Atrovent was greater than for placebo. No significant dose-response relation was seen. The median duration of 15% increase on FEV<sub>1</sub> was 4, 3.5, 1.5 and 3 hours for the 20, 40, 60, 80 mcg doses of Atrovent respectively. This is shown on Table 1, summary of FEV<sub>1</sub> (L) responses in 14 patients, Vol. 1.8, page 8, source is Table 2 of the statistical report to be re evaluated by our Division of Biometrics.

Comparisons among Atrovent 40 mcg, isoproterenol 150 mcg and placebo in Table 2 and Fig 1 of this study showed Atrovent to be significantly better than placebo in AUC (area under the curve) for FEV<sub>1</sub> and for the peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. The areas under the curves are significantly greater than those for isoproterenol for both parameters and the peak effect was equivalent. The time to peak for Atrovent was within 30 minutes for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. Summary is in Table 2 and figure 1, Vol. 1.8, pp. 9, 10. Source - statistical report tables 2, 3, 4, 5, 6. For Atrovent, the onset of 15% increase on FEV<sub>1</sub> occurred within 15 minutes and the onset of 20% increase in FEF<sub>25-75%</sub> occurred within 5 minutes; the duration of effect was 3.5 and 4 hours for the two parameters respectively. For isoproterenol, the onset of effect in FEV<sub>1</sub> and FEF<sub>25-75%</sub> was 5 minutes with duration to 1.5 hours for both parameters. (Table 2) (Fig. 1).

Safety - There were no clinically significant changes in blood pressures seen after any test doses, including placebo. Slight decreases in mean pulse rates were observed after all test doses, including placebo, none clinically significant.

ECG trainings showed no changes, nor did lab findings in the study period. Adverse experience -1 patient complained of dry throat after Atrovent 80 mcg.

Conclusion: No significant dose-response relationship was detected. Based on the results of this and other doses range studies the 40 mcg dose was selected for the multiple-dose studies. Atrovent was better than placebo in AUC for FEV<sub>1</sub> and for peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and better significantly than isoproterenol in AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>.

Reviewer's comment - This section, verified, is followed by the statistical report to be reviewed and evaluated by our Division of Biometrics. Atrovent was shown to increase FEV<sub>1</sub> and FEF<sub>25-75</sub> in percent change over baseline in this clinical trial.

2)

Study No. E-79-948

Locations in the NDA-Vol. 1.8 pp. 123-225  
Vol. 1.64

The protocol for this study was the same as that followed by above 1) same objectives also.

Results -

Efficacy - The adjusted mean area under the FEV<sub>1</sub> curve for all doses of Atrovent was greater than that for placebo and isoproterenol. No significant dose-response relationship was seen. The median duration of 15% increase in FEV<sub>1</sub> was 6 hours after all Atrovent doses. Based on the results of this and other dose-range studies the 40 mcg dose was selected for use in multiple dose studies. Atrovent was significantly better than both isoproterenol and placebo and AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. The peak effect was significantly greater than placebo for both parameters and the time to peak for Atrovent was 60 minutes for both parameters.

Most patients on Atrovent showed the onset of 15% increase in FEV<sub>1</sub> and FEF<sub>25-75%</sub> occurred at 5 minutes, lasting 6 hours. For isoproterenol the onset was also 5 minutes and duration of effect for FEV<sub>1</sub> was 6 hours and for FEF<sub>25-75%</sub> 4 hours. Reference is made to the statistical report and is illustrated in Table 2 and Fig. 1.

Safety - Adverse experiences were reported in 3 patients, 1 of headache after Atrovent, 20 mcg, nervousness after Atrovent, 60 mcg and isoproterenol accompanied by dizziness; a third patient had a headache after Atrovent, 80 mcg.

Summary - no significant dose-response relationship was reported. Atrovent, 40 mcg was significantly better than placebo in AUC in peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for both parameters. The statistical report follows this section to be reviewed by biometrics.

3)

Study No. E-79-961

The same protocol as that in 1) and 2) above was followed.

Objective - same - to determine optimal dosage of Atrovent in adult asthmatics and confirm results of earlier dose-ranging studies on which 40 mcg had been found to be safe and effective in long term trials.

The Results regarding efficacy and safety were the same as for study 2) above, i.e. Atrovent 40 mcg was significantly better than placebo in AUC and peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for both parameters. This section is followed by the statistical report to be reviewed by our Division of Biometrics.

4

Objectives - Dose-ranging studies same as those in 1), 2), 3) above.

Study No. E-80-3; Vol. 1.9 and 1.66.

Protocol - Same as that for 1), 2), 3) above except SGaw was measured.

Results

Efficacy - AUC for all doses of Atrovent was greater than that for placebo and increased linearly with dose (p=0.026 - referenced to statistical report). The median duration of 15% increase in FEV<sub>1</sub> was 2, ① 3, and 3 hours for the 20, ④ 40, 60 and 80 mcg doses of Atrovent respectively. The results of this study also provided a basis for 40 mcg dose of Atrovent in multiple-dose studies. Atrovent was significantly better than placebo in AUC for FEV<sub>1</sub> and SGaw. The AUCs are greater than those for isoproterenol for all 3 parameters,

FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw, but do not reach significance. (Reference is made to the statistical report which follows the clinical portion of this study). Peak effect was higher for isoproterenol in FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw, but not significantly so, the time to peak for Atrovent varies from 60 minutes for FEV<sub>1</sub> and SGaw to 120 minutes for FEF<sub>25-75%</sub>. For Atrovent in most patients the onset of 15% increase in FEV<sub>1</sub> or 20% increase in FEF<sub>25-75%</sub> occurred within 15 minutes and persisted for 1 hour for FEV<sub>1</sub> and 2 hours for FEF<sub>25-75%</sub>. For isoproterenol, the onset in FEV<sub>1</sub> and FEF<sub>25-75%</sub> occurred at 5 minutes and persisted for 1 hour for FEV<sub>1</sub>. Clinically significant median responses in FEF<sub>25-75%</sub> were not seen with isoproterenol.

Safety - Only adverse experiences consisted of increased sputum production reported by 3 patients on isoproterenol (2 after 75 mcg, 1 after 150 mcg) and 1 patient after placebo. Dry mouth/irritation were reported after Atrovent 20 mcg (1 patient) and placebo (1 patient).

Summary - A linear dose - response curve was demonstrated for Atrovent in this study. Based on the results of this and other dose-range studies the 40 mcg dose of Atrovent was selected for use in the multiple-dose studies. Atrovent 40 mcg was significantly better than placebo on AUCs for FEV<sub>1</sub> and SGaw.

Reviewers comment - This section is followed by the referenced statistical report. Atrovent clinically was superior to isoproterenol in raising FEV<sub>1</sub> and FEF<sub>25-75%</sub> from baseline in this clinical trial.

5)

Study No. E 79-80; located in the NDA Vol. 1.9 pp. 137-317 and Vol. 1.67.

Atrovent - metered dose inhaler.

Objectives and protocol - same as those in 1, 2, 3, 4 above.

### Results

Efficacy - the adjusted mean AUC for FEV<sub>1</sub> for all doses of Atrovent was greater than for placebo and increased linearly with dose. The median duration of 15% increase in FEV<sub>1</sub> was 1.6, 5, 5.3 and 4 hours for the 20, 40, 60, 80 mcg doses of Atrovent. Based on the results of this and other dose range studies the 40 mcg dose was selected for use in the multiple-dose studies. All other efficacy parameters showed the same beneficial effect of peak and duration of pulmonary function tests, FEV<sub>1</sub>, FEF<sub>25-75%</sub>, and SGaw as the preceding studies.

### Results

Safety - Adverse experiences were reported in 2 patients (of 14). One complained of headache after each test doses including of placebo; and a second one who had coordination difficulties after Atrovent 40 mcg complained of dizziness after Atrovent 60 mcg and isoproterenol 75 mcg.

Summary - A Linear dose response curve was demonstrated for Atrovent at doses of 40, 60 and 80 mcg and the higher doses offered no significant clinical advantage over the 40 mcg dose. Based on the results of this and other dose-range studies the 40 mcg dose was selected for use in multiple-dose studies Atrovent was significantly better than placebo in AUC and peak effect for FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw and significantly better than isoproterenol in AUC for SGaw. The statistical report follows this clinical section.

Reviewer's Comments - In these five (5) dose-ranging studies by doctors

Atrovent was better than placebo in AUC for FEV<sub>1</sub> and for peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. In two of the five studies a linear dose curve response was seen for Atrovent and in last 2 studies Atrovent was significantly better than placebo in AUC for FEV<sub>1</sub> and SGaw, and in one of these ( ) also for FEF<sub>25-75%</sub>. Adverse reactions were reported in a very small number of patients and were not serious. Thus pulmonary function was increased in those patients with pulmonary disease and these studies formed the basis for the 40 mcg dose of Atrovent in multiple-dose studies. The drug was found to be safe and well tolerated. This completes dose - ranging studies.

### C) Controlled Studies (COPD Patients) - 14 in Number - Chronic Obstructive Pulmonary Disease (COPD)

#### 1) Multicenter Study - Protocol II b 5

Date May 16, 1980; In NDA Vols. 1.10, 1.11; 1.68-1.73

#### Investigators:

a)

b)

c)

d)

Locations in the NDA-

- a) Vols, 1.10, 1.11, 1.68 through 173.
- b) 1.10, 1.11, 1.74, 1.75, 1.76
- c) 1.10, 1.11, 1.77, 1.78, 1.79, 1.80, 1.81
- d) 1.10, 1.11, 1.82, 1.83, 1.84, 1.85

The protocol for this multicenter study:

**Objective:** To determine bronchodilator efficacy of Atrovent MDI, measured after a single dose (day 1) in comparison with Alupent regarding onset, and duration of action, the effects of continuous dosing and patient acceptance of the two drugs. There was a 90-day treatment period.

Four centers took part on this study, a, b, c, d, above admitting 97 patients; 89 males, 8 females, ages 45-79 years with an average of 62.1 years. 83 completed 90 days of treatment. Statistical report was done. Inclusion criteria included ages 40-75 with a diagnosis of chronic obstructive pulmonary disease (COPD) with a history of smoking more than 20 pack years, mild to moderately severe airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal and FEV<sub>1</sub> less than 70% of FVC; total lung capacity (TLC) greater than 80% of predicted normal, reversible bronchospasm shown by a 15% improvement in FEV<sub>1</sub> within 30 minutes following 2 whiffs (0.150 mg) of isoproterenol from an MDI. Exclusions: significant systemic disease, active TBC, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to sympathomimetic amines or anticholinergics. (Atrovent [ipratropium, is an anticholinergic drug]). Pregnant females, or those expecting to become so, were excluded during the study. Patients with a history of asthma, allergic rhinitis, or atopy, or who had a recent viral infection or febrile illness including URI were excluded. Patients with elevated IgE serum levels or total blood eosinophilia above normal were to be excluded; however 19 patients receiving Atrovent and 23 receiving Alupent had elevated IgE levels. An analysis was made to determine what effect, if any, this may have on interpretation of the principal efficacy evaluations. On all test days this analysis gave no evidence of interaction between treatment and

elevated IgE. (See statistical report). Thirteen patients failed to complete the study (3 received treatment for 7 days or less), 7, patients from 8 to 39 days and 3 patients from 40 to 71 days). The distribution of patients lost is shown in Table 1 below (taken from statistical report):

Table 1  
"Distribution of Patients Lost, Dropped or Withdrawn"

|                                | <u>Atrovent</u> | <u>Alupent</u>  |
|--------------------------------|-----------------|-----------------|
| <u>Total</u> lost or withdrawn | 6               | 7               |
| Side effect                    | 2               | 2               |
| Lost to follow-up              | 1               | 1               |
| Lack of efficacy               | 1               | 2               |
| Protocol violations            | 0               | 1               |
| Other (Related illnesses)      | <u>2</u>        | <u>1</u>        |
| Total =                        | 6               | + 7 = <u>13</u> |

The patient's diary was appropriately completed as a recording of daily medications used, daily assessment of wheeze, cough, tightness and shortness of breath and ability to exercise or perform usual activities.

#### Results (COPD-1)

Efficacy - The mean FEV<sub>1</sub> baselines on day 1, before any drug administration were significantly higher for the group randomized to Atrovent than for those randomized to Alupent and remained so when recorded at days 45 and 90. In order to account for this initial difference in baselines all treatment comparisons were adjusted using the day 1 baseline as covariant. This topic was discussed (Meeting 10/17/83 with our Division of Biometrics). The adjusted mean areas under the curves and the adjusted mean peaks for FEV<sub>1</sub>, FEF<sub>25-75%</sub>, FVC and SGaw are greater for Atrovent over Alupent on all test days (Table 2, Vol. 1.10, 19 15). The time profiles are shown for the adjusted means in figures 1-4. The means shown are greater on Atrovent than on Alupent for all parameters at all timepoints except at 15 min. for FEV<sub>1</sub> and FEF<sub>25-75%</sub> on Day 45. Clinically significant improvements on pulmonary function as measured by 15% or greater in FEV<sub>1</sub> and FEF<sub>25-75%</sub> on Day 1 with Atrovent occurred at 15 to 30 minutes, reached a peak in 1 to 2 hours and persisted for 3 to 4 hours in most patients. We are referred to figures 5 and 6 pages 20, 21 for this. In the opinion of this medical reviewer, awaiting statistical evaluation, figure 5, as 20, Vol. 1.10 shows little difference in FEV<sub>1</sub> percent change from baseline on days 1 or 90 but does favor Atrovent over Alupent.

Safety - Mean pulse rates were lower than the baseline by 2 to 8 beats/minute at all timepoints after Atrovent and by 0-3 beats/minute up to 3 hours after Alupent. These are of no clinical significance. There were no lab changes noted in this study nor were there EKG changes over the 90-day period.

Adverse reactions on continuous therapy were 49 on Atrovent and 48 on Alupent. Those on Alupent were slightly more severe than in those on Atrovent.

Initially results of pulmonary function tests in the Atrovent-treated patient group were better than those patients on Alupent and they remained consistently better from day 1 through day 90 for FEV<sub>1</sub>, FEF<sub>25-75%</sub>, FVC, SGaw as well as the mean percent change from baseline over the covered period of observation. While improvement in FVC changes in patients on Atrovent was markedly better than in patients on Alupent, changes in FEV<sub>1</sub>, FEF<sub>25-75%</sub> were not as impressive.

Adjusted mean FEF<sub>25-75%</sub> from day 1-45-90 periods showed no impressive changes for Atrovent over Alupent nor for changes in SGaw. Clinically the recorded changes were not impressive. Statistically, mean adjusted results were impressive to the statistician's report submitted by the sponsor. Final determination of this conclusion will await analysis by our Division of Biometrics. Medically, the results of Atrovent treatment is marginal in degree over the Alupent treatment results in the periods Days 1-45-90.

From a medical viewpoint these studies showed Atrovent to improve adjusted mean FEV<sub>1</sub> on day 1 over Alupent; the same applied to days 45 and day 90; Atrovent also was superior to Alupent in increased FVC on days 1, 45, and 90. For FEF<sub>25-75%</sub>, adjusted mean values on day 1 Atrovent was shown to be superior to Alupent; on day 45, Atrovent was superior to Alupent, hours 1, 2, 3, 4, but not on hours 5 and 6. On day 90 Atrovent was superior to Alupent at all 6 hours. For adjusted SGaw mean on days 1, 45 and 90, no change clinically was seen between Atrovent and Alupent. For FEV<sub>1</sub> mean percent change from baseline, there was little or no clinical difference between the results obtained on Atrovent and on Alupent at days 1 and 90 (day 45 is not charted). For FEF<sub>25-75%</sub> mean percent change from baseline clinically on days 1 and 90 showed Atrovent to be superior to Alupent. (Day 45 not changed).

No serious adverse reactions were report either in number nor type.

The conclusion medically from this multicentric study is that safety and efficacy of Atrovent has been shown to be comparable to that of Alupent from a therapeutic standpoint. Prevention of bronchoconstriction has not been demonstrated in these data.

2) MDI 90-Day Multi-Center Study

iii-a-10 COPD

Protocol - III a 10; location on NDA; Vols 1.12, 1.13, 1.14, 1.86-1.110-1.40

Date - July 6, 1981 - 11 studies

Objective - same as that of 1) above, i.e. to determine efficacy of Atrovent, in comparison with Alupent. Dosing, repeated over 90 days for efficacy and safety and patient acceptance of Atrovent in comparison with Alupent.

Subjects - more than those of 1) above 160-220 with a diagnosis of chronic bronchitis.

Diagnostic criteria for exclusion and inclusion were the same as for 1) above.

Method of Drug Administration, dose and route, as for 1) above including continuous treatment.

Duration of treatment - 2 week washout period and 90 days of continuous therapy.

Method of Randomization. Other medication, and habits, and those bronchodilators which may be administered for control of acute attacks during the 90-day study period, same as 1) above, i.e. SQ epinephrine or Terbutaline, i.v. aminophylline, rectal aminophylline. Patient daily diary recorded type and dosage of all medication taken concomitantly; frequency and dosage daily of study drug.

Method of Evaluation

Pre-admission  
washout period  
Observations to be made - pulmonary function tests  
Physician's global evaluation  
Patient daily diary  
Patient evaluation  
Theophylline level determination  
Adverse reactions

Are all the same as study protocol 1) above.

There are eleven (11) investigators and co-investigators: (1. #11 deleted due to misinterpretation of protocol, Dr.

1)

2)

3)

4)

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7)

8)

9)

10)

11)

The objective, as stated is the same as study 1) above.

Ten centers participated in this study admitting 164 patients, 83 on Atrovent, 81 on Alupent. 129 males and 35 females, ages 37-75 were admitted and 122 patients completed the study with 90 days of treatment. (Thirteen more were in progress when the statistical report was made and 29 had dropped out. Inclusion and exclusion criteria were the same as study 1) above, namely chronic bronchitics and reversible bronchospasm who showed a 15% improvement in FEV<sub>1</sub> within 30 minutes following 0.150 mg of isoproterenol by MDI. Four patients were included in this study who had FEV<sub>1</sub> increases of 14%, 13.6%, 13% and 14% after isoproterenol challenge, and 1 patient whose FEV<sub>1</sub> was 74.5% of his FVC.

Patients were excluded for the same reasons as given in study 1) above.

Twenty-nine patients, as stated failed to complete the study. The reasons for dropout are given in Table I below:

Table I

Reasons for patients lost, dropped or withdrawn.  
(Tables 1 and 2 of the statistical report)

|                                  | <u>Atrovent</u> | <u>Alupent</u> |
|----------------------------------|-----------------|----------------|
| Total Loss, dropped or withdrawn | 8               | 21             |
| Side effect                      | 1               | 4              |
| Loss to follow-up                | 3               | 4              |
| Lack of efficacy                 | 0               | 6              |
| Protocol violation               | 2               | 4              |
| Other                            | 2               | 3              |
|                                  | <u>8</u>        | <u>21</u>      |

The study was double-blind, parallel design following a 2 week washout.

Route - oral inhalation by metered dose inhalation (MDI)

Dose and Duration: continuous treatment with Atrovent, 40 mcg. q.i.d. or Alupent 1500 mcg q.i.d. self-administered by patients for 90 days. Additional whiffs were permitted under rigid conditions found acceptable.

Single test doses of Atrovent 40 mcg (2 whiffs, 20 mcg/whiff or Alupent 1500 mcg, 2 whiffs 750 mcg/whiff) on days 1, 45 and 90.

Concomitant medication was the same as that provided for study 1) above.

Observations before and after the study were also the same as study 1) above including a 2- week washout period prior to the study, and observations on test days 1, 45 and 90, except in this study it was not specified that SGaw would be done.

### Results

Efficacy - In this study, unlike study 1) above the mean FEV<sub>1</sub> baselines on day 1 obtained prior to administration of any treatment were lower (not higher) for the group randomized to Atrovent than for the group randomized to Alupent. In order to account for this difference initially, difference in baselines, all treatment comparisons were adjusted using the day 1 baseline as a covariant. (Refer to statistical report).

The adjusted mean AUC and adjusted mean peaks for  $FEV_1$ , FEF<sub>25-75%</sub> and FVC, as in study 1) above were greater on days 1 and 45 with the exception of FEF<sub>25-75%</sub> day 1 adjusted mean peak reaching statistical difference shown in Table 2, Vol. 1.12, pg 16. Time profiles of the adjusted means shown in figures 1 to 3 are greater for Atrovent than for those on Alupent ( $FEV_1$  day 1, day 45, day 90); adjusted mean FVC on day 1, day 45, day 90; adjusted mean FEF<sub>25-75</sub> on day 1 (close), day 45, day 90.

Clinically significant improvements in pulmonary function as measured by 15% or greater increase in  $FEV_1$  and FEF<sub>25-75%</sub> occurred within 15 minutes, reached a peak in 1-2 hours and persisted for 3-5 hours in most patients on Atrovent. The  $FEV_1$  mean percent change from baseline on day 1 and day 90 was shown to be greater for Atrovent than for Alupent (figure 4), and figure 5 shows the same advantage of Atrovent over Alupent in the FEF<sub>25-75</sub> mean percent change from baseline on day 1 and on day 90.

Reviewer's comment: The above changes are more pronounced in favor of Atrovent over Alupent in this study than those shown in study 1) above.

Safety - The mean pulse rate changes on both drugs, laboratory findings and ECG tracings were the same as those reported in study 1) above, namely none of clinical significance. Adverse reactions were reported by 5 patients after Atrovent (single instances of nervousness, cough, GI distress and 2 with dizziness) and by 3 patients after Alupent, 1 each of headache, dizziness and exacerbation of symptoms, following continuous therapy.

Reviewer's comment - These two multicenter studies may essentially be combined as showing Atrovent produced better pulmonary responses than Alupent, peaking in 1-2 hours and lasting 3-5 hours. This study more definitely than 1) favors Atrovent over Alupent with no significant incidence or severity of side effects.

A separate study in this group of chronic obstructive pulmonary disease (COPD) was conducted by \_\_\_\_\_ it is not included in this review at this point due to a misinterpretation of the protocol by the investigator.

These studies showed Atrovent to be superior to Alupent on results obtained on  $FEV_1$  elevations on adjusted mean  $FEV_1$  on days 1, 45, and 90: the same for adjusted mean FVC on days 1, 45 and 90 and for adjusted mean FEF<sub>25-75</sub> on days 1, 45 and to a lesser degree on day 90 in favor of Atrovent.

On the mean percent change from baseline (not adjusted) for  $FEV_1$  on days 1 and 90, clinically, Atrovent was superior to Alupent. The same applied for the mean percent change from baseline for FEF<sub>25-75</sub> on days 1 and 90. These results were achieved without significant numbers nor severity of side effects.

Concludes controlled studies in chronic obstructive pulmonary disease, 2 of 2 at this point in the NDA.

D. Controlled Studies (Asthmatic Patients)

1. asthma

Multicenter study - II a 5; 5 studies

Location in NDA - Vols. 1.14, 1.16, 1.17, 1.18, 1.112-1.141

Investigators

- 1) ison, WI
- 2)
- 3)
- 4)
- 5)
- 6)

Objective of Multicenter Study

"To determine the efficacy and safety of Atrovent inhaler with emphasis on onset and duration of action, the effects of continuous dosing (persistence of bronchodilating effects; side effects, adverse reactions and laboratory studies), patient acceptance of Atrovent in comparison with Alupent".

Cases, number and type studied:

In these 5 centers 82 males and 82 females (164 total) between ages 13-50 years participated in the studies. 144 completed 90 days of treatment and 4 were in progress when the statistical report was prepared.

Inclusion criteria - acceptable, included patients to be ages 12-50; have a substantiated diagnosis of asthma (Bronchial Asthma Society Criteria); have a relative stable mild to moderately severe airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal, and FEV<sub>1</sub> less than 70% of FVC (forced vital capacity); and have reversible bronchospasm demonstrated by a 15% improvement in FEV<sub>1</sub> within 15 minutes following 2 whiffs. (0.150 mg) of isoproterenol from a MDI (metered dose inhaler). Exclusion criteria were - significant systemic disease, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to sympathomimetic amines or anticholinergics. Pregnant females or those expecting to become pregnant during the study period were excluded. Those who smoked regularly during the past year and patients with chronic sputum production were excluded. Those with a smoking history of less than 10 pack years who had discontinued smoking more than 1 year prior to start of the study were included.

16 patients failed to complete the study:

Reasons

|                    | <u>Atrovent</u> | <u>Alupent</u> |        |
|--------------------|-----------------|----------------|--------|
| Side effects       | 0               | 3              |        |
| Lost to follow-up  | 3               | 2              |        |
| Lack of efficacy   | 6               | 1              |        |
| Protocol violation | 0               | 1              |        |
| TOTAL              | 9               | 7              | + = 16 |

The study was in each case randomized, double-blind, parallel design following a 2 week washout.

The route was oral by metered dose inhalation. (MDI)

Dosage and Duration:

Continuous treatment - Atrovent 40 mcg q.i.d. or Alupent 1500 mcg. q.i.d. self-administered for 90 days. Provisions were made for reasonable additional whiffs and recorded.

Single test doses of Atrovent 40 mcg (20 mcg/whiff) or Alupent 1500 mcg (750 mcg/whiff) were also administered.

Regarding concomitant medication, other investigational drugs or anticholinergics were excluded. Of 16 patients on Atrovent and 20 on Alupent who took oral steroids, 13 and 17 respectively were on minimal doses (10 mg or less of prednisone daily or 20 mg or less every other day). One patient on Atrovent started on prednisone during the study and 1 on Alupent who was not on steroids when the study started, took a course less than 5 days during the study. Their data were used. The data from 2 patients in each group whose steroid dosages varied were not used. 19 patients in each treatment group took inhaled steroids which were permitted if the dose was stabilized during the complete study including the washout period. One patient on Atrovent used steroid by inhalation only during the washout period, this case was included in efficacy analysis. Efficacy data from day 45 of another patient on Atrovent were not used in analysis because of non-stabilization of steroid dosage. Stabilized doses of cromolyn were used by 1 Atrovent patient and 3 Alupent-treated patients. The only medication allowed as a bronchodilator during the 2 week washout period before the study was theophylline, anhydrous. Theophylline tablets were permitted up to 12 hours prior to testing during the study. Short periods of use of inhaled beta-adrenergic bronchodilators were used by 6 patients on Atrovent and 3 on the Alupent group.

Observations Before and After the Study included history, P.E., lab tests, EKG prior to admission. The P.E., CBC, SMA-12, urinalysis and the lab tests and EKG were repeated at the end of the study. Each patient was tested in the A.M. on the qualification day. No bronchodilators were used for at least 12 hours prior to pulmonary function testing. There was, as noted, a 2-week washout period prior to the study. Following qualification, the patient underwent a 2-week washout period during which no beta-adrenergic drugs were taken with the exception of 3 patients assigned to the Atrovent group (2 of the 3 were dropped from the study and the third was continued since 1 whiff of salbutamol inhaler had been used on 4 occasions) and 4 patients assigned to the Alupent (all 4 were dropped from the study).

Observations During the Study: Excluded for at least 12 hours prior to the start and for the duration of the testing period were all bronchodilators, sympathomimetic drugs and antihistamines. Pulmonary function testing was done at the same time of the day in each individual. FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub> and PEFR (peak expiratory flow rate) were determined at baseline, 5, 15 and 30 minutes and 1, 2, 3, 4, 5 and 6 hours after drug administration. Pulse rates and blood pressure readings were taken and recorded at the same intervals as pulmonary function testing. A 2 minute resting period was allowed after pulmonary function testing and pulse and blood pressure determinations were made. At each visit the patient's diary was reviewed and summarized on the case report. The entries on these diaries included a record of daily medications, assessment of wheezing, cough, tightness and shortness of breath and ability to exercise or perform usual activities. Onset, severity and duration of any side effect was recorded as it occurred with the investigator's opinion as to drug relationship.

## Results

Efficacy - Reference is made to the statistical report. The mean percent changes in FEV<sub>1</sub> for all patients showed slight differences if any, favoring Alupent on day 1, Atrovent on day 45 and Atrovent on day 90. The clinical significance of these results is not impressive but rather show both treatment groups to respond to much the same degree. "Patients were later classified as responders or non-responders based on whether or not they achieved at least a 15% increase over baseline in FEV<sub>1</sub> within 2 hours following the test dose on day 1. Forty of 78 patients, or 51% of the patients on Atrovent and 56 of 77 patients or 73% of the patients on Alupent were responders. There were no significantly different areas under the curves or peak effects for any parameter on any test day between the drugs." For FEV<sub>1</sub> and FEF<sub>25-75%</sub> the responses were greater for Alupent on day 1 and for Atrovent on days 45 and 90. On test days the FVC responses were greater, but only slightly for Atrovent on day 1, the same as Alupent on day 45 and less on day 90. "Statistically the only factor which consistently correlated with responses was percent of predicted FEV<sub>1</sub> with greater responses seen in patients whose percent of predicted FEV<sub>1</sub> was lower. further analysis indicated that although all patients had an FEV<sub>1</sub> less than 75% of their predicted normal on the qualification day, many patients had an FEV<sub>1</sub> well in excess of this level at baseline on day 1." (Applicant's wording).

The applicant further states: "Twenty-five patients on Atrovent (mean predicted percent 88.9%) and 27 patients on Alupent (mean percent of predicted 89.1%) had an FEV<sub>1</sub> greater than 75% of predicted on day 1. An analysis was made excluding these patients. It would be expected that this would be a more reliable analysis since the patients who were excluded did not show a response because they did not have a significant degree of bronchospasm. When this was done 90% of the patients on Alupent responded as would be expected, but the percentage of Atrovent responders only increased to 60%." This is not a favorable finding for the Atrovent NDA.

"Analysis of the FEV<sub>1</sub> data on these responders with an FEV<sub>1</sub> less than 75% of their predicted normal at baseline on day 1 was similar to that seen in the responder group as a whole". Reference is made to the statistical report on this multicenter study for the above results and this review should await analysis of the data by our Division of Biometrics. Adjusted PEF<sub>R</sub> (Mean) on days 1, 45 and 90 for responders was superior for Atrovent over Alupent. (Vol. 1.15, p9 19, fig. 7). Persistence of bronchodilating effect was slightly diminished in many cases. (See recommendations.)

Safety - None of the changes in mean pulse rates after Atrovent or Alupent, nor the blood pressure readings were clinically significant. There were no clinically significant changes in laboratory findings, and the same applies for ECG. Adverse experiences after test doses of Atrovent were 2 cases of "nervousness"; 1 case of a patient with "headache and cough" and single cases of headache, dizziness, cough, chest tightness. Following test doses of Alupent one patient had weakness and dizziness and another was nervous. Following continuous treatment there were 4 cases of nervousness after Atrovent and 13 cases after Alupent. These were the most frequently reported adverse experiences. In addition, there were 13 patients with cough or chest tightness (exacerbation of symptoms) after Atrovent, and 2 patients with cough after Alupent treatment.

In summary this multicenter study Atrovent was compared to a beta-adrenergic bronchodilator, Alupent in a 90-day double-blind study in non-smoking asthmatics. The overall data analyzed showed that "only about 50% of these patients responded to Atrovent and therefore, analysis of the onsets, peak effect and duration of action in the group as a whole was inappropriate. In the group who showed a 15% increase in FEV<sub>1</sub> within 2 hours on day 1, significant improvement in pulmonary function occurred within 15 minutes, reached a peak in 1 1/2-2 hours and lasted for 3 to 6 hours in the majority of patients." (Applicant's wording). There were more (73% responders) to Alupent than to Atrovent (51%).

(Statistical Data)

Reviewer comment - Fig. 3, pg 14, Vol. 1.15 shows for adjusted mean FEV<sub>1</sub> on days 1, 45 and 90 for all patients there was little clinical difference in FEV<sub>1</sub> during 6 hours on these days between patients on Atrovent and those on

Alupent. For the adjusted mean FEV<sub>1</sub> day 45 and day 90 there were respective differences at all 6 time intervals on those days clinically favoring Atrovent over Alupent, principally on days 45 and 90 (Vol. 1.15, Fig 4, pg 16). For Figure 5, pg. 17 adjusted mean FVC for responders. Days 1, 45 and 90 shows very little difference between Atrovent and Alupent results achieved. In fig. 6, pg 18, Vol. 1.15, adjusted mean FEF<sub>25-75%</sub>. For responders on days 1, 45, and 90 all 3 days show Atrovent favored over Alupent in this parameter except on day 1 at hour 5 in figure 7, pg 15, Vol. 1.15 adjusted mean PEFr for responders Atrovent is shown to be superior to Alupent in this parameter on all days, 1, 45, and 90.

The adjusted mean FEV<sub>1</sub> on day 1 for all patients showed Alupent to be superior at times 0, 1, 2, then closely parallel to Atrovent at times 3, 4, 5, 6 hours post-drug for that day.

The adjusted mean FEV<sub>1</sub> on day 48 showed no clinical difference between the two drugs in any patients.

The adjusted mean FEV<sub>1</sub> on day 90, after hour 1 showed some superiority in increase in this parameter in the Atrovent Group among all patients.

For responders adjusted mean FEV<sub>1</sub> on days 1 and 45 showed no meaningful clinical difference but on day 90 Atrovent was showed to be superior to Alupent after hour one. The adjusted mean FVC for responders on days 1, 45 and 90 showed no meaningful difference medically between those on Alupent and those on Atrovent.

The adjusted mean FEF<sub>25-75%</sub> for responders on day 1 gave mixed results; on days 45 and day 90 the Atrovent-treated patients showed superiority in this parameter.

For the adjusted PEFr for responders after hour 2 on days 1 and 45 Atrovent was superior to Alupent and was so after hour 1 on day 90.

The final conclusions on these studies will incorporate the review and evaluation of the statistical reports by our Division of Biometrics. Medically, it appears superior results were obtained with the administration of Atrovent to patients with Chronic Obstructive Pulmonary Disease (COPD) than to those with Asthma.

6)

(with theophylline)

Study No. E 82-1801 - Atrovent MDI

Final Report Date - 7/11/83 - Location in NDA Vol. 1.18; 1.138-1.141

Objective - To determine safety and efficacy of Atrovent administered with oral theophylline highlighting the duration of action, persistence of bronchodilating action with repeated dosing, effects of repeated dosing (side effects, adverse reactions and lab. studies) and patient acceptance of Atrovent plus theophylline compared to theophylline alone.

### Cases

Twenty-five adult patients entered the study and 22 completed the study. Patients entered had to be 12 years of age, or older, have a diagnosis of bronchial asthma and a relatively stable mild to moderately severe airway obstruction. All patients had reversible bronchospasm (increase of at least 15% FEV<sub>1</sub> within 30 minutes of 0.150 mg of isoproterenol (2 whiffs) from a metered dose inhaler (MDI).

Those excluded had serious systemic disease including cardiac, renal, hepatic or metabolic, those with prostatic hypertrophy, bladder neck obstruction, glaucoma or hypersensitivity to atropine-like compounds. Also excluded were pregnant women, or those expecting to become so during the study.

Of the 3 who did not complete the study as planned, one patient on Atrovent plus theophylline suffered 2 attacks requiring prednisone; a second on Atrovent plus theophylline felt there was no improvement and the third was admitted to a hospital while on theophylline alone.

Eleven males and eleven females qualified for entry and completed the study. Group I (Atrovent plus theophylline) consisted of 7 males, 4 females with a mean age of 40 and mean duration of illness of 14 years. Group II (Theophylline), 4 males, 7 females had a mean age of 42 years and mean duration of illness was 17 years. In each group Asthma was mild in 4 cases and moderate in 11.

The study was randomized, double-blind, parallel.

The route of drug administration: Atrovent-oral inhalation by MDI.  
Theophylline-oral.

Dose and Duration of Treatment: Throughout the study patients were maintained on therapeutic doses of oral theophylline and treatment was either Atrovent, 40 mcg (20 mcg/whiff) q.i.d. or matching placebo, self administered by the patients for 90 days. Study medications used were recorded by each patient in a diary.

Test doses which were administered while the patient was on maintenance doses of Theophylline were Atrovent 40 mcg on days 1 and 90 for Group I and on days 2 and 91 for Group II, and placebo MDIs, two whiffs, on days 2 and 91 for Group I and on Days 1 and 90 for Group II.

Table I  
Study Design

| <u>Days</u>           | <u>1</u>            | <u>2</u>            | <u>For 90 days</u> | <u>90</u>           | <u>91</u>           |
|-----------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
| Group I <sup>a</sup>  | Atrovent<br>40 mcg  | Placebo<br>2 whiffs | Atrovent           | Atrovent<br>40 mcg  | Placebo<br>2 whiffs |
| Group II <sup>a</sup> | Placebo<br>2 whiffs | Atrovent<br>40 mcg  | Placebo            | Placebo<br>2 whiffs | Atrovent<br>40 mcg  |

a = All patients received maintenance doses of theophylline throughout the study to ensure a theophylline blood level of 10 to 20 mcg/ml.

Concomitant Medication - verified - No patients were on other investigational drugs. One patient on Atrovent and 2 on placebo were on 10 mg or less of prednisone daily or 20 mg every other day. Inhaled steroids were used by 7 patients in the Atrovent Group and 3 in the placebo group. One patient used an Alupent inhaler for an acute attack. One patient in each treatment group used cromolyn which was permitted if a stabilized dose was used throughout the study. One patient in each group had i.v. aminophylline which was allowed for acute attacks. No other similar medications were used.

Observations before and after the study: Following admission to the study, but before administration of test medication, patients not receiving a therapeutic oral dosage of theophylline were administered theophylline alone until optimal blood levels and therapeutic results were obtained. Complete history, P.E. incl. pulse rate and B.P. were performed.

Lab tests included CBC, blood chemistry tests, urinalysis and chest x-ray. These were repeated at the end of the study.

Observations During the Study: Excluded after midnight preceding the pulmonary function test day, were all bronchodilators, including the study drugs Atrovent and Placebo, steroids, and cromolyn. Smoking and drinks containing xanthines were not allowed on pulmonary function testing days.

Pulmonary function tests were done at baseline and at 30 minutes, and 1, 2, 3, 4, 5 and 6 hours after a test dose on days 1, 2, 90 and 91.

Theophylline blood levels were determined at baseline and two hours after test drug administration on days 1, 2, 45, 90 and 91.

ECGs were done at baseline and 1 hour after test drug administration on days 1, 2, 45, 90, 91.

Patients' diaries were reviewed on each visit and the entries summarized on the case report. Patient entries included a record of daily medications used, including investigational drug, the number of acute attacks and peak flow readings.

Side effects and adverse reactions and the investigators' opinion as to drug relationship and severity were recorded.

### Results

Theophylline blood levels between treatment groups were comparable and also between the beginning and end of the study.

### Efficacy

Tabulations show mean changes from baseline of FEV<sub>1</sub> values for the single-dose crossover studies at the beginning and end of the study in data from the statistical report along with the effect due to Atrovent, i.e. Atrovent plus theophylline minus theophylline alone. They show the effects of Atrovent at the beginning and the end of the study were comparable in both groups with somewhat generally greater effects at the end of the study showing no tolerance had developed to the use of Atrovent over the 91-day study. (Table 2, vol. 1.18, pg. 15). "The pooled data using both treatment groups and both test days showed a statistically significant benefit at each time point over 6 hours and the overall benefit was highly significant." (Table 3, Vol. 1.18, pg. 15). It has been shown also that the results to respiratory resistance generally paralleled the FEV<sub>1</sub> results. (These data are detailed in the statistical report and final judgement await review by our Division of Biometrics). The efficacy data are agreeable to this reviewer shown in Tables 2 and 3, Vol. 1.18, pg. 15.

In the study, theophylline, and theophylline plus Atrovent use in asthmatics "there was no evidence, in terms of FEV<sub>1</sub> and respiratory resistance that the acute benefits of adding Atrovent to oral theophylline were any less after 90 days of regular use than they were at the start of the treatment period." The effects or benefits were usually greater at the end of the study indicating there was little or no development of tolerance to Atrovent after three months of regular use. Reported side effects were not clinically significant. The mean daily number of acute attacks were similar in the two patient groups as were the long term subjective assessments and concomitant use of other therapy.

Safety - There were no clinically significant changes in pulse rates or blood pressure readings in this study. Exacerbation of symptoms following test doses of both treatments was the single most frequently reported adverse effect and was reported by 5 patients after placebo and 2 patients after Atrovent. After continuous treatment with Atrovent and Theophylline, adverse effects were: palpitations (1) nausea and dry mouth (2) and cough (1). After multiple doses of theophylline alone there were palpitations (1) and exacerbation of symptoms (1).

In summary this 90 day study on the safety and efficacy of the addition of Atrovent to theophylline was compared to theophylline alone. With the addition of Atrovent there was a "statistically significant effect in FEV<sub>1</sub> demonstrated for Atrovent both at the beginning and end of the 90 day administration period with no increase in side effects."

Medically, awaiting review by our Division of Biometrics, the statements regarding efficacy and safety made above have been substantiated in this NDA. This is demonstrated in vol. 1.18, pg 15 Tables 2 and 3. The drug is safe and effective in cases where added efficacy is shown when Atrovent is used with theophylline as claimed in this study by \_\_\_\_\_, M.D.

It is true that the effects of Atrovent were comparable at the beginning and the end of the study. (Table 2 pg. 14, Vol. 1.18, where the mean changes in baseline FEV<sub>1</sub> (L) (Reference Tables 1, 2, 7, 8 of the statistical report. This pertains to Group 1 (continuous therapy - Atrovent plus theophylline for 90 days) where the Atrovent effect is shown as Atrovent/Theophylline minus theophylline in which that effect is comparable at the beginning of the trial (days 1, 2, and end of trial days 90, 91.)

Reviewers comment - It has been shown above that Atrovent added to theophylline enhanced the bronchodilating effect without the addition of clinically significant adverse reactions. There was improvement in this combination over that of Theophylline, when used alone as a bronchodilator.

This concludes controlled asthma studies.

E. Other Studies (5 types)

E-1. Emergency Atrovent Program - Data Summary

Location in NDA - Vol. 1.18; Vols. 1.142 - 1.150

Objective of this study - to provide Atrovent to patients not responding to other medications. The information obtained from this program concerned safety data not efficacy for Atrovent administered under these conditions.

The results showed the drug to be safe.

There are numerous tables and appendices submitted concerning this program. They are from Appendix A-1; the total number of patients treated was 244. As of September 10, 1982 patient numbers 1-298 had been assigned and of these 298, 44 had no documentation of drug administration = 254 remaining. On 15 patients only the initial report was received, but no documentation that they received Atrovent and one patient died before receiving medication. Therefore this report is based on 244 patients who received Atrovent including 6 patients who died after the cut-off date. Of the 244 patients 86 or 35.5%

withdrew for treatment failure (37 patients - 15.3%); side effects (23 patients - 9.5%); no reason reported (6 patients - 2.5%) and one patient (0.4%) tried withdrawal for 1-2 weeks and since asthma failed to increase she did not restart Atrovent. (Table 1). Distribution by sex was about equal, (50.4% male, 49.6% female). Seventy-six percent of the patients were between 30 and 59 years of age, 88% were caucasian. Bronchial asthma was the most frequently reported use for Atrovent on this emergency basis (71%), Table 2, Vol. 1.18.

Reviewer's comment: Conclusion: This emergency program lends support to safety of the drug.

E-2)

Study No. E-80-854

Protocol - /a/; Location in NDA-Vols. 1.18, 1.151

Date - June 30, 1980

Regarding this study: The applicant states "this protocol was designed as a pilot study to evaluate the effect of Atrovent on cough frequency in patients with chronic idiopathic non-productive cough and or determine sample size for a definitive study in a meeting with Dr. he recommended that we not proceed further with this project because the variability would necessitate a very large patient population and that difficulties in recruitment would preclude much enrollment."

E3) Multicenter Study

Protocol - II c 6

To compare a single dose crossover study of Atrovent with Alupent effect. No meaningful data was obtained.

E-4) \_\_\_\_\_ (E 81-1334)

This "study as submitted concerned a single patient. No meaningful data was obtained. The patient was administered a test \_\_\_\_\_ of Atrovent MDI.

E-5) Clinical Laboratory Studies - Effectiveness

"Clinical laboratory studies related to effectiveness are not applicable for support of this New Drug Application" (wording of applicant - hence, no data).

6) Clinical Laboratory Studies Related to Safety

Location in the NDA - Vol. 1.19, pp 2-161

Summary verified - in general, clinical laboratory tests were performed before and after treatment with Atrovent on 348 patients in double-blind controlled studies, including 173 patients in 90 day studies. Laboratory studies were also obtained on approximately 150 patients in the emergency Atrovent program. These studies included CBC, urinalysis, blood chemistries.

Detailed data on these studies are found in the statistical reports. In general, the laboratory tests remained within normal range. The only tests in the controlled clinical studies in which significant changes were seen were glucose in 12 patients, sodium in 3 and potassium in 2. Elevated glucose was attributed to non-fasting in patients and/or using steroids.

Changes in laboratory values in open-label studies were not clinically significant. ECGs were obtained at baseline and post treatment in 380 patients. In conclusion no laboratory or ECG abnormalities could be attributed to Atrovent and no trends were evident.

E-7 Summary of Clinical Literature Reviewed by the Applicant

Location in the NDA; Vol. 1.19 pages 163-246 with each page containing an abstract of published papers dated 1971-1982 on Atrovent metered dose inhaler. There are eleven (11) special studies; eleven (11) on sputum and mucociliary clearance studies; eleven (11) on dose-ranging studies; thirteen (13) on repetitive-dose (long/short-term) studies; twenty six (26) on single dose studies; seven (7) on studies in experimentally provoked bronchospasm; there are therefore a total of seventy-nine (79) such abstracts. This is followed by forty three (43) pages listing a bibliography on Atrovent. No meaningful information not contained in the NDA elsewhere is included in these sections.

Table 1 Includes Humanitarian use program

| <u>Adverse experience</u>   | <u>Atrovent<br/>(228)</u> | <u>Alupent<br/>(223)</u> |
|-----------------------------|---------------------------|--------------------------|
| <u>Cardiovascular</u>       |                           |                          |
| Palpitations                | 4 (1.8%)                  | 4 (1.8%)                 |
| Tachycardia                 |                           | 1 (0.4%)                 |
| <u>CNS</u>                  |                           |                          |
| Dizziness                   | 6 (2.6%)                  | 7 (3.1%)                 |
| Nervousness                 | 7 (3.1%)                  | 17 (7.6%)                |
| Headache                    | 6 (2.6%)                  | 4 (1.8%)                 |
| Weakness                    |                           | 2 (0.9%)                 |
| Incoordination              | 1 (0.4%)                  | 2 (0.9%)                 |
| Insomnia                    | 1 (0.4%)                  | 1 (0.4%)                 |
| Tinnitus                    |                           | 1 (0.4%)                 |
| Fatigue                     |                           | 1 (0.4%)                 |
| <u>Dermatological</u>       |                           |                          |
| Rash                        | 3 (1.3%)                  | 1 (0.4%)                 |
| Hives                       |                           | 1 (0.4%)                 |
| <u>G.I.</u>                 |                           |                          |
| Nausea                      | 7 (3.1%)                  | 2 (0.9%)                 |
| Vomiting                    |                           | 2 (0.9%)                 |
| G.I. distress               | 6 (2.6%)                  | 7 (0.9%)                 |
| Constipation                | 1 (0.4%)                  |                          |
| Diarrhea                    | 1 (0.4%)                  | 1 (0.4%)                 |
| Change in appetite          |                           | 2 (0.9%)                 |
| <u>G.U.</u>                 |                           |                          |
| Urinary difficulty          | 1 (0.4%)                  | 2 (0.9%)                 |
| <u>Ophthalmological</u>     |                           |                          |
| Decreased visual acuity     | 1 (0.4%)                  | 2 (0.9%)                 |
| Lack of accommodation       |                           | 1 (0.4%)                 |
| <u>Oro-Otolaryngeal</u>     |                           |                          |
| Dry mouth                   | 4 (1.8%)                  | 2 (0.9%)                 |
| Epistaxis                   |                           | 1 (0.4%)                 |
| Irritation from aerosol     | 4 (1.8%)                  | 4 (1.8%)                 |
| Hoarseness                  |                           | 1 (0.4%)                 |
| <u>Respiratory</u>          |                           |                          |
| Cough                       | 14 (6.1%)                 | 2 (1.3%)                 |
| Exacerbation<br>of symptoms | 6 (2.6%)                  | 7 (3.1%)                 |
| Pleurisy                    | 1 (0.4%)                  |                          |
| Congestion                  |                           | 1 (0.4%)                 |
| <u>Musculoskeletal</u>      |                           |                          |
| Tremor                      |                           | 6 (2.7%)                 |
| Muscle pain                 |                           | 1 (0.4%)                 |

Table 2  
Overall incidence of adverse experiences among 452 patients  
treated with Atrovent  
in double-blind, controlled, clinical studies within the recommended dose

| <u>Adverse experience</u>     | <u># of Patients</u> | <u>% of Total Treated</u> |
|-------------------------------|----------------------|---------------------------|
| <u>Cardiovascular</u>         |                      |                           |
| Arrhythmia                    | 2                    | 0.4                       |
| Palpitations                  | 4                    | 0.9                       |
| <u>CNS</u>                    |                      |                           |
| Dizziness                     | 8                    | 1.8                       |
| Nervousness                   | 7                    | 1.5                       |
| Headache                      | 9                    | 2.0                       |
| Coordination difficulties     | 2                    | 0.4                       |
| Insomnia                      | 1                    | 0.2                       |
| <u>Dermatological</u>         |                      |                           |
| Rash                          | 4                    | 0.9                       |
| Itching                       | 1                    | 0.2                       |
| <u>G.I.</u>                   |                      |                           |
| Nausea                        | 10                   | 2.2                       |
| G.I. Distress                 | 8                    | 1.8                       |
| Constipation                  | 2                    | 0.4                       |
| Diarrhea                      | 2                    | 0.4                       |
| Vomiting                      | 1                    | 0.2                       |
| <u>G.U.</u>                   |                      |                           |
| Urinary difficulty            | 1                    | 0.2                       |
| <u>Musculoskeletal</u>        |                      |                           |
| Tremor                        | 3                    | 0.7                       |
| <u>Ophthalmological</u>       |                      |                           |
| Decreased visual acuity       | 1                    | 0.2                       |
| Difficulties in accommodation | 1                    | 0.2                       |
| Other ophthalmological        | 3                    | 0.7                       |
| <u>Oro-Otolaryngeal</u>       |                      |                           |
| Dry mouth                     | 9                    | 2.0                       |
| Irritation from aerosol       | 6                    | 1.3                       |
| <u>Respiratory</u>            |                      |                           |
| Cough                         | 14                   | 3.1                       |
| Exacerbation of symptoms      | 7                    | 1.5                       |
| Pleurisy                      | 1                    | 0.2                       |

This overall section above is followed by a study-by-study detail of the same information and will not be part of this review.

Reviewers comment: Reviewing the data presented regarding adverse experiences and reactions, the benefit/risk ratio indicates the drug to be safe.

Overall conclusions regarding safety and efficacy of Atrovent (ipratropium bromide).

In controlled clinical trials 499 patients were administered Atrovent and 244 patients were enrolled in a humanitarian use protocol establishing the safety and efficacy of Atrovent<sup>R</sup> inhaler.

In 42 asthmatic and 27 bronchitic patients dose-ranging studies confirmed the efficacy of the 40 mcg dose 4 x a day of Atrovent in addition to previously-conducted dose-ranging studies with a similar formulation marketed in Europe.

Multicenter 90 day controlled clinical trials comparing Atrovent inhaler to a standard beta adrenergic bronchodilator metaproterenol showed the following:

Number of patients enrolled

|             | <u>Atrovent</u> | <u>Metaproterenol</u> |
|-------------|-----------------|-----------------------|
| Bronchitics | 147             | 140                   |
| Asthmatics  | 81              | 83                    |
|             | <u>228</u>      | <u>223</u>            |

The response of bronchitics to Atrovent was clearly better than that to metaproterenol over 6 hours testing on days 1, 45 and 90 by pulmonary function testing which included FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw. Onset and duration of increasing improvement was within 15 minutes, lasting 3-5 hours and in some cases, up to 6 hours. The studies in asthmatics did not produce such consistent favorable results with about one-half of the 78 patients on Atrovent responding with a 15% or greater increase in FEV<sub>1</sub>. Atrovent safety was shown in these same 90 day controlled clinical trials by small decreases in pulse rates and blood pressures, not of clinical significance.

The incidence of adverse reactions was low and required cessation of therapy in 3 of 228 patients treated with Atrovent (all secondary to cough). Cough was the most frequent reaction which occurred (in about 6% of patients). Other, less significant adverse reactions reported accounted for 1-3% of adverse reactions.

A 90 day study which was clinically controlled in asthmatics showed the safety and efficacy of Atrovent used with theophylline. Special studies showed Atrovent did not decrease mucociliary clearance from the lungs or tracheal mucous velocities in the trachea, nor dry up secretions from the lung or alter viscosity of sputum showing lack of anticholinergic effects. Atrovent did not affect pupil size, accommodation, visual acuity or intraocular pressure. Also anticholinergic effects were absent in the G.U. system and Atrovent did not affect ventilation/perfusion relationships or lower PaO<sub>2</sub>. (See body of this review - special studies).

The conclusion may be reached that Atrovent is an effective bronchodilator in bronchitics and some asthmatics. It is an anticholinergic bronchodilator with a low incidence of adverse reactions and no significant anticholinergic side effects. In the older bronchitic patient, beta adrenergic bronchodilators may often be ineffective and do have cardiovascular side effects. Therefore Atrovent has been shown to be safe and effective for patients with chronic bronchitis and chronic obstructive pulmonary disease (COPD).

Review and Evaluation of Labeling (CFR 201.56)

The prescribing information part of the submitted labeling is acceptable except for one point. While, on the immediate-container labeling the phrase: "Caution: Federal Law prohibits dispensing without prescription", that statement should also appear in the prescribing information labeling.

The Sections involving "DESCRIPTION" and "CLINICAL PHARMACOLOGY" largely involve the assigned reviewing Chemist and Pharmacologist respectively and our Division of Biopharmaceutics. There are no medical objections to these sections, but should satisfy the assigned reviewers.

The Section titled "INDICATIONS AND USAGE" in which it is claimed the drug "is indicated as a bronchodilator for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis" is unacceptable in that submitted data fails to support the claim for "prevention" as used in this draft labeling section. Also, in this section the sentence reading: "Some asthmatic patients have been shown to respond to Atrovent" is unacceptable in the proposed labeling regarding indications for use of the drug. The data submitted shows, at best, 50% or less of such patients respond to administration of the drug. This may be due to chance alone but definitely is not, as required "substantial evidence of safety and effectiveness (CFR 314.111(a)(5)(ii)).

The Sections titled "WARNINGS," "CONTRAINDICATIONS" are acceptable.

The Section titled "PRECAUTIONS" should have, not presently included, subsections titled "GENERAL," "OVERDOSAGE" (not as submitted "OVERDOSE"). Also under this Section, the statement made in the draft labeling "Acute overdose is unlikely since ..." should be deleted and adherence to the referenced CFR Section regarding labeling for prescription drugs in humans be followed in a non-promotional manner.

The Section titled "DOSAGE and ADMINISTRATION: is acceptable.

The Section "HOW SUPPLIED" is for review by the assigned reviewing chemist.

The separate Section titled "INSTRUCTIONS FOR USE OF METERED-DOSE INHALER" and the accompanying illustrated drawing are acceptable.

Recommendations regarding submitted draft labeling:

Revisions: Those possibly recommended by the assigned reviewing Chemist and the Pharmacologist regarding the Sections titled "Description" and "Clinical Pharmacology." There are no Medical objections to these Sections.

The Section titled INDICATIONS AND USAGE should be revised to read:  
"Atrovent (R) (ipratropium Bromide) is indicated as a bronchodilator for treatment of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, chronic bronchitis. Atrovent (ipratropium bromide) is not indicated in situations requiring rapid response to treatment."

This requires deletions of statements regarding use of Atrovent in "prevention" and in asthmatic patients."

Data submitted on "Emergency use of Atrovent" is found to be totally inconclusive. The sentence recommended under this Section should be added as well as that presently included under the Section titled WARNINGS because of the practical importance of that statement to the prescribing physician.

The Section titled PRECAUTIONS will have a subsection and its content "General."

The Section titled "OVERDOSE" will read "OVERDOSAGE," deleting the phrase "Acute overdosage is unlikely."

Finally the draft labeling should state "Federal law prohibits dispensing without a prescription."

Recommendations:

- 1) The specified labeling revisions should be implemented.
- 2) Providing #1 above is in compliance with the recommendations stated, the NDA may be found medically approvable for safety and efficacy as a bronchodilator for treatment of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis.

The NDA is found to be nonapprovable for use in "prevention" of the above nor for any use in the management of asthmatic patients.

*E. J. Shanahan* (not read)  
E. J. Shanahan, M.D.  
4/17/84

*JWW*  
11/17/80

*OK #1 8/85*

NDA 19-085  
HFN-160  
HFN-340  
R/D E. J. Shanahan, M.D., 11/16/83  
R/D initialed by JWWinkler 7/12/85, PHRussell 7/15/85  
FT-jb, W1337N, D0071N  
FT Revised OLA 8/6/85  
Doc. Room 160

NDA 19-085 Atrovent Inhaler  
Ipratropium Bromide  
Bronchodilator

Boehringer Ingelheim Ltd.  
Submitted: ~~7/17/85~~ 7/2/85  
Reviewed: 8/29/85

1. REVIEW AND EVALUATION OF LABELING

Draft labeling has been submitted as a result of a meeting between representatives of the firm and this Agency held on June 19, 1985. The topic of the meeting was a discussion of labeling for this new NDA.

The submitted, revised draft labeling concerns the sections titled CLINICAL PHARMACOLOGY, INDICATIONS, and USAGE, PRECAUTIONS, and a statement concerning "inclusion of the Rx legend on the insert."

Essentially, the submitted draft labeling reflects the conclusions reached at the noted meeting with one exception, concerning the statement made under the section titled INDICATIONS and USAGE reading "approximately one-half of asthmatic patients have been shown to respond to ATROVENT<sup>R</sup>. (See CLINICAL PHARMACOLOGY)."

RECOMMENDATIONS:

1. The statement, which is the final sentence of the section titled INDICATIONS and USAGE including the words in parenthesis regarding use in asthmatic patients should be deleted from that section of the labeling.
2. Providing 1) above is implemented, the submitted draft labeling should be approved.
3. Adverse experience tables for patients treated in the 90-day controlled clinical trials in support of the Package Inserts: For Atrovent: This tabulation shows that the total number of patients included in the NDA submission was 228 while the number that completed study was 254 - Table 1. This table is a breakdown by body systems showing the number and percentage of patients reporting adverse experiences under each system and, where available, the numbers and percentages for each sign/symptom for each body system. This was for both the 228 patients in the NDA submission and those under "Completed Study."

There are no remarkable differences between the latter two groups, i.e. "NDA submission" and "completed study." The highest incidences reported were in order of frequency:

- |                |                             |
|----------------|-----------------------------|
| 1. Cough       | 4. Exacerbation of Symptoms |
| 2. Nausea      | 5. Dizziness                |
| 3. Nervousness | 6. G.I. Distress            |

NOV 22 1985

Table 2 - "Overall Incidence of Adverse Experiences Among Patients Treated with ALUPENT for 90 days:

Number of patients (%)    NDA Submission = 223  
                                         Completed Study = 249

Incidence in order of frequency was:

1. Nervousness
2. Exacerbation of Symptoms
3. G.I. Distress
4. Tremor

There was no appreciable clinically significant difference between the Atrovent and Alupent treatment groups regarding incidence nor severity of adverse experience reporting except for more cases of insomnia reported in the Alupent-treated patients.

4. "A safety updated section comprising a summary of foreign data from clinical trials and spontaneous post marketing reports as well as a summary of the literature available since the NDA submission." The review of unpublished and published reports of adverse reactions associated with the use of Atrovent MDI from foreign data sources is consistent with the adverse reactions seen in clinical trials and the Atrovent compassionate use program in the United States. The analysis of these data shows Atrovent MDI is safe.

5. Foreign Marketing Status

There is a list of 25 countries where Atrovent Inhaler is currently marketed as well as the dates introduced in each, 1974-1984. The amount distributed for this period of time represents approximately 3 million single doses.

Atrovent has also been approved in France and Italy where marketing has not begun. There are no pending registrations, nor have any registrations been refused.

6. Patient information and exclusivity information is given in this section. Most of the information presented in this section is in the submitted NDA. Exclusivity - date first ANDA could be approved and length of exclusivity period is given as five (5) years from date of NDA approval.

Applicable patent numbers and expiration date of each are given as

CONCLUSION: Sections 2 through 4 above are in compliance with the agreements reached with the meeting with the representatives of the firm held on June 19, 1985.

Addendum dated 11/8/85 to Review of Labeling

Under the section titled CLINICAL PHARMACOLOGY add the following:

In these asthmatic patients who were responders to Atrovent<sup>R</sup> there was no evidence of development of tachyphylaxis.

*E. J. Shanahan*  
E. J. Shanahan, M.D.

NDA 19-035

HFN-160

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DocRm. 160

HFN-160/JWWinkler 11/1/85: NOTE: Will discuss further in-house, probably OK to leave asthma claim in "Indications" section also.

R.D. EJShanahan 11/8/85

R.D. Init by:JWWinkler 11/8/85:PHRussell 11/8/85

shg W2164K 11/14/85

*ok 11/15/85*

NDA 19-085 Atrovent (ipratropium Br.)  
Bronchodilator

Boehringer-Ingelheim  
Submitted: 7/29/83  
Reviewed: 11/16/83  
Date this Addendum: 11/20/86

Addendum to MOR dated 11/16/83

Reference, MOR, page 45, Study

Study No. E-82-1801 - Atrovent MDI

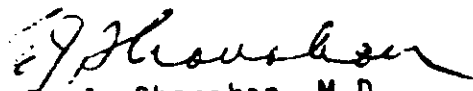
Final report date by Applicant - 7/11/83 - Location in NDA Vol. 1.18;  
1.138-1.141.

Objective - "To determine safety and efficacy of Atrovent by oral inhalation plus Theophylline highlighting the duration of action, persistence of bronchodilating action with repeated dosing, other effects of repeated dosing (side effects, adverse reactions and on lab studies), and patients' acceptance of Atrovent plus Theophylline compare to Theophylline alone."

Twenty five adult asthmatic patients were entered.

The Medical and the Biometrics Reviews differed regarding conclusions. Following further review and discussion (11/19/86) it was determined that the Study was not a valid support for the applicant's conclusions. The Biometrics Review, dated May 10, 1985 stated "The data showed that the addition of Atrovent to Theophylline maintenance treatment failed to provide a significant increase in FEV<sub>1</sub> causing the change from pre-dosing baseline on day 90), or mean peak flow rate (43.3 on Atrovent vs. 44.8 on placebo in the morning, and 37.7 vs. 23.4 in the evening). Furthermore, patients receiving Atrovent in addition to Theophylline treatment reported a higher rate of acute attacks per day (0.187 with Atrovent vs. 0.120 without Atrovent) and a higher mean daily use of Theophylline (4.82 whiffs with Atrovent vs. 4.11 without Atrovent); these differences failed to reach statistical significance at the 5% level."

Conclusion - In this study in Asthmatic patients the statistical report failed to support the Applicant's claims made.

  
E. J. Shanahan, M.D.  
11/20/86

NDA 19-085

HFN-160

HFN-340

HFN-713 Dr. Huang

HFN-160 Dr. Winkler

R/D EJShanahan 11/20/86

R/D Init. by JWinkler 11/21/86, PGWalters 11/21/86

FT OLA 2502N 0156N 12/13/86

Doc. Room 160

DEC 8 1986

N-19085-1

NDA

19-085

Approval

Letter



Labeling



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

NDA 19-085

DEC 29 1986

Food and Drug Administration  
Rockville MD 20857

Boehringer Ingelheim Pharmaceuticals, Inc.  
90 East Ridge  
P.O. Box 368  
Ridgefield, CT 06877

Attention: Ruth Wasserman  
Associate Director, Regulatory Affairs

Gentlemen:

Please refer to your new drug application dated July 29, 1983, submitted pursuant to section 505(b) of the Federal Food, Drug and Cosmetic Act for the preparation Atrovent (ipratropium bromide) Inhalation Aerosol.

We also refer to our letter dated December 19, 1986 which declared this application approvable and to your communications dated December 18, 22 and 23, 1986.

Additional reference is made to a December 23, 1986 verbal commitment by Dr. Wilson of Boehringer Ingelheim to change the Product Title on the carton and immediate container labels to "Atrovent (ipratropium bromide) Inhalation Aerosol" within 6 months or at the next printing, whichever occurs first.

We have completed the review of this application including the draft labeling submitted in your last amendment and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in that draft labeling. Accordingly, the application is approved, effective as of the date of this letter.

The final printed labeling (FPL) must be identical to the draft labeling. Marketing the product with FPL that is not identical to the draft labeling may render the product misbranded and an unapproved new drug. Please submit twelve copies of the FPL to FDA as soon as available. For administrative purposes this submission should be designated "FPL Supplement" to the approved NDA 19-085. Approval of this supplement by FDA is not required before the labeling is used. Should additional information relating to the safety and effectiveness of this drug product become available prior to our receipt of the final printed labeling, revision of that labeling may be required.

NDA 19-085  
Page 2

Please submit one market package of the drug product when it is available.

We remind you that you must comply with the requirements set forth under 21 CFR 314.80 and 314.81 for an approved NDA.

Sincerely yours,

*Paula Botstein MD*

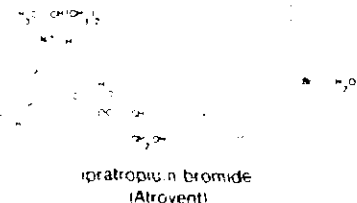
Paula Botstein, M.D.  
Acting Deputy Director (Medical Affairs)  
Office of Drug Research and Review  
Center for Drugs and Biologics

**Pharmacist:**  
Tear off "Instructions for Use" and dispense with container in carton.

**Atrovent®**  
(ipratropium bromide)  
**Inhalation Aerosol**

**Prescribing Information**

**DESCRIPTION** The active ingredient in Atrovent® (ipratropium bromide) Inhalation Aerosol is ipratropium bromide. It is an anticholinergic bronchodilator chemically described as 8-azoniabicyclo[3.2.1]octane, 3-(3-hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methyl-ethyl)-bromide, monohydrate (*endo, syn*)-, (±), a synthetic quaternary ammonium compound, chemically related to atropine



Ipratropium bromide is a white crystalline substance, freely soluble in water and lower alcohols but insoluble in lipophilic solvents such as ether, chloroform, and fluorocarbons.

Atrovent Inhalation Aerosol is an inhalation aerosol for oral administration. The net weight is 14 grams, it yields 200 inhalations. Each actuation of the valve delivers 18 mcg of ipratropium bromide from the mouthpiece. The inert ingredients are: dichlorodifluoromethane, dichlorotetrafluoroethane, and trichlorofluoromethane as propellants and soya lecithin.

**CLINICAL PHARMACOLOGY** Atrovent® (ipratropium bromide) is an anticholinergic (parasympatholytic) agent which, based on animal studies, appears to inhibit vagally mediated reflexes by antagonizing the action of acetylcholine, the transmitter agent released from the vagus nerve. Anticholinergics prevent the increases in intracellular concentration of cyclic guanosine monophosphate (cyclic GMP) which are caused by interaction of acetylcholine with the muscarinic receptor on bronchial smooth muscle.

The bronchodilation following inhalation of Atrovent is primarily a local, site-specific effect, not a systemic one. Much of an inhaled dose is swallowed as shown by fecal excretion studies. Atrovent is not readily absorbed into the systemic circulation either from the surface of the lung or from the gastrointestinal tract as confirmed by blood level and renal excretion studies.

The half-life of elimination is about 2 hours after inhalation or intravenous administration. Autoradiographic studies in rats have shown that Atrovent does not penetrate the blood-brain barrier.

In controlled 90 day studies in patients with bronchospasm associated with chronic obstructive pulmonary disease (chronic bronchitis and emphysema) significant improvements in pulmonary function (FEV<sub>1</sub> and FEF<sub>25-75%</sub>) increases of 15% or more occurred within 15 minutes, reached a peak in 1-2 hours, and persisted for periods of 3 to 4 hours in the majority of patients

200 Dose Size  
Bronchodilator

and up to 6 hours in some patients. In addition, significant increases in Forced Vital Capacity (FVC) have been demonstrated.

Controlled clinical studies have demonstrated that Atrovent® (ipratropium bromide) does not alter either mucociliary clearance or the volume or viscosity of respiratory secretions. In studies without a positive control Atrovent did not alter pupil size, accommodation or visual acuity (See ADVERSE REACTIONS).

Ventilation/perfusion studies have shown no clinically significant effects on pulmonary gas exchange or arterial oxygen tension. Atrovent does not produce clinically significant changes in pulse rate or blood pressure.

**INDICATIONS AND USAGE** Atrovent® (ipratropium bromide) is indicated as a bronchodilator for maintenance treatment of bronchospasm associated with chronic obstructive pulmonary disease, including chronic bronchitis and emphysema.

**CONTRAINDICATIONS** Hypersensitivity to atropine or its derivatives.

**WARNINGS** Atrovent® (ipratropium bromide) is not indicated for the initial treatment of acute episodes of bronchospasm where rapid response is required.

**PRECAUTIONS** **General** Atrovent® (ipratropium bromide) should be used with caution in patients with narrow-angle glaucoma, prostatic hypertrophy or bladder-neck obstruction.

**Information for Patients** Patients should be advised that temporary blurring of vision may result if the aerosol is sprayed into the eyes.

**Drug Interactions** Atrovent has been used concomitantly with other drugs, including sympathomimetic bronchodilators, methylxanthines, steroids and cromolyn sodium, commonly used in the treatment of chronic obstructive pulmonary disease, without adverse drug reactions. There are no formal studies fully evaluating the interactive effects of Atrovent and these drugs with respect to effectiveness.

**Carcinogenesis, Mutagenesis, Impairment of Fertility** Two-year oral carcinogenicity studies in rats and mice have revealed no carcinogenic potential at doses up to 1,250 times the maximum recommended human daily dose for Atrovent. Results of various mutagenicity studies were negative.

Fertility of male or female rats at oral doses up to approximately 10,000 times the maximum recommended human daily dose was unaffected by Atrovent administration. At doses above 18,000 times the maximum recommended human daily dose, increased resorption and decreased conception rates were observed.

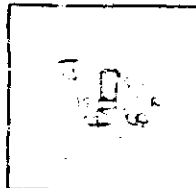
**Pregnancy TERATOGENIC EFFECTS** **Pregnancy Category B.** Oral reproduction studies performed in mice, rats and rabbits (at doses approximately 2,000, 200,000 and 26,000 times the maximum recommended human daily dose, respectively) and inhalation reproduction studies in rats and rabbits (at doses approximately 312 and 375 times the maximum recommended human daily dose, respectively) have demonstrated no evidence of teratogenic effects as a result of Atrovent. However, no adequate or well controlled

**Instructions for Use**

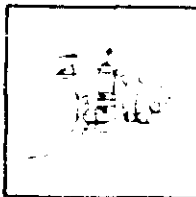
1. Insert metal canister into clear end of mouthpiece.
2. Remove protective cap, invert canister and shake thoroughly.



3. Enclose mouthpiece with the lips. The base of the canister should be held vertically. Keep the eyes closed because temporary blurring of vision may result if the aerosol is sprayed into the eyes.



4. Exhale deeply, then inhale slowly through the mouth and at the same time firmly press once on the upended canister base; continue to inhale deeply.



5. Hold your breath for a few seconds and then remove the mouthpiece from the mouth and exhale slowly. Wait approximately 15 seconds and repeat second inhalation as outlined above.



6. Replace protective cap after use.
7. **Keep the mouthpiece clean. Wash with hot water. If soap is used, rinse thoroughly with plain water.**

studies have been conducted in pregnant women. Because animal reproduction studies are not always predictive of human response, Atrovent\* (ipratropium bromide) should be used during pregnancy only if clearly needed.

**Nursing Mothers** It is not known whether Atrovent is excreted in human milk. Although lipid-insoluble quaternary bases pass into breast milk, it is unlikely that Atrovent would reach the infant to an important extent, especially when taken by aerosol. However, because many drugs are excreted in human milk, caution should be exercised when Atrovent is administered to a nursing woman.

**Pediatric Use** Safety and effectiveness in children below the age of 12 have not been established.

**ADVERSE REACTIONS** Adverse reaction information concerning Atrovent\* (ipratropium bromide) is derived from 90 day controlled clinical trials (N=254), other controlled clinical trials using recommended doses of Atrovent (N=377) and an uncontrolled study (N=1924). Additional information is derived from the foreign post-marketing experience and the published literature.

Adverse reactions occurring in greater than one percent of patients in the 90 day controlled clinical trials appear in the following table:

| Reaction                      | Percent of Patients            |                                   |
|-------------------------------|--------------------------------|-----------------------------------|
|                               | Ipratropium bromide<br>N = 254 | Metaproterenol sulfate<br>N = 249 |
| <b>Cardiovascular</b>         |                                |                                   |
| Palpitations                  | 1.8                            | 1.6                               |
| <b>Central Nervous System</b> |                                |                                   |
| Nervousness                   | 3.1                            | 5.8                               |
| Dizziness                     | 2.4                            | 2.8                               |
| Headache                      | 2.4                            | 2.0                               |
| <b>Dermatological</b>         |                                |                                   |
| Rash                          | 1.2                            | 0.4                               |
| <b>Gastrointestinal</b>       |                                |                                   |
| Nausea                        | 2.8                            | 1.2                               |
| Gastrointestinal distress     | 2.4                            | 2.8                               |
| Vomiting                      | 0                              | 1.2                               |
| <b>Musculoskeletal</b>        |                                |                                   |
| Tremor                        | 0                              | 2.4                               |
| <b>Ophthalmological</b>       |                                |                                   |
| Blurred vision                | 1.2                            | 0.8                               |
| <b>Oro-Otolaryngeal</b>       |                                |                                   |
| Dry mouth                     | 2.4                            | 0.8                               |
| Irritation from aerosol       | 1.6                            | 1.6                               |
| <b>Respiratory</b>            |                                |                                   |
| Cough                         | 5.9                            | 1.2                               |
| Exacerbation of symptoms      | 2.4                            | 3.6                               |

Additional adverse reactions reported in less than one percent of the patients considered possibly due to Atrovent include urinary difficulty, fatigue, insomnia and hoarseness.

The large uncontrolled, open-label study included seriously ill patients. About 7% of patients treated discontinued the program because of adverse events.

Of the 2301 patients treated in the large uncontrolled study and in clinical trials other than the 90 day studies, the most common adverse reactions reported were: dryness of the oropharynx, about 5 in 100; cough, exacerbation of symptoms and irritation from aerosol, each about 3 in 100; headache, about 2 in 100; nausea, dizziness, blurred vision/difficulty in accommodation, and drying of secretions, each about 1 in 100. Less frequently reported adverse reactions that were possibly due to Atrovent\* (ipratropium bromide) include tachycardia, paresthesias, drowsiness, coordination difficulty, itching, hives, flushing, alopecia, constipation, tremor, mucosal ulcers.

Cases of precipitation or worsening of narrow angle glaucoma, acute eye pain and hypotension have been reported.

A case of giant urticaria with positive rechallenge has been reported from the foreign marketing experience (See CONTRAINDICATIONS).

**OVERDOSAGE** Acute overdosage by inhalation is unlikely since Atrovent\* (ipratropium bromide) is not well absorbed systemically after aerosol or oral administration. The oral LD<sub>50</sub> of Atrovent ranged between 1001 and 2010 mg/kg in mice; between 1667 and more than 4000 mg/kg in rats; and between 400 and 1300 mg/kg in dogs.

**DOSAGE AND ADMINISTRATION** The usual dose of Atrovent\* (ipratropium bromide) is two inhalations (36 mcg) four times a day. Patients may take additional inhalations as required; however, the total number of inhalations should not exceed 12 in 24 hours.

**HOW SUPPLIED** Atrovent\* (ipratropium bromide) Inhalation Aerosol is supplied as a metered dose inhaler with mouthpiece. Each 14 gram vial provides sufficient medication for 200 inhalations. NDC 0597-0082-14. Each actuation delivers 12 mcg of ipratropium bromide from the mouthpiece.

Do not store above 30°C (86°F); avoid excessive humidity.

**CAUTION** Federal law prohibits dispensing without prescription.



**Boehringer  
Ingelheim**

Distributed by  
Boehringer Ingelheim  
Pharmaceuticals, Inc.  
Ridgefield, CT 06877

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3M Center, 225-1S-07  
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Revised 12/86  
830070

Pharm

Date of Completion: 3/27/84

NDA 19-085

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original submission dated 7/29/83

APPLICANT: Boehringer Ingelheim Ltd.  
90 East Ridge  
P.O. Box 368  
Ridgefield, CT 06877  
  
(203) 438-0311

NAME OF DRUG: Descriptive: Atrovent Metered Dose Inhaler  
Sch 1000 or Sch 1000 - Br

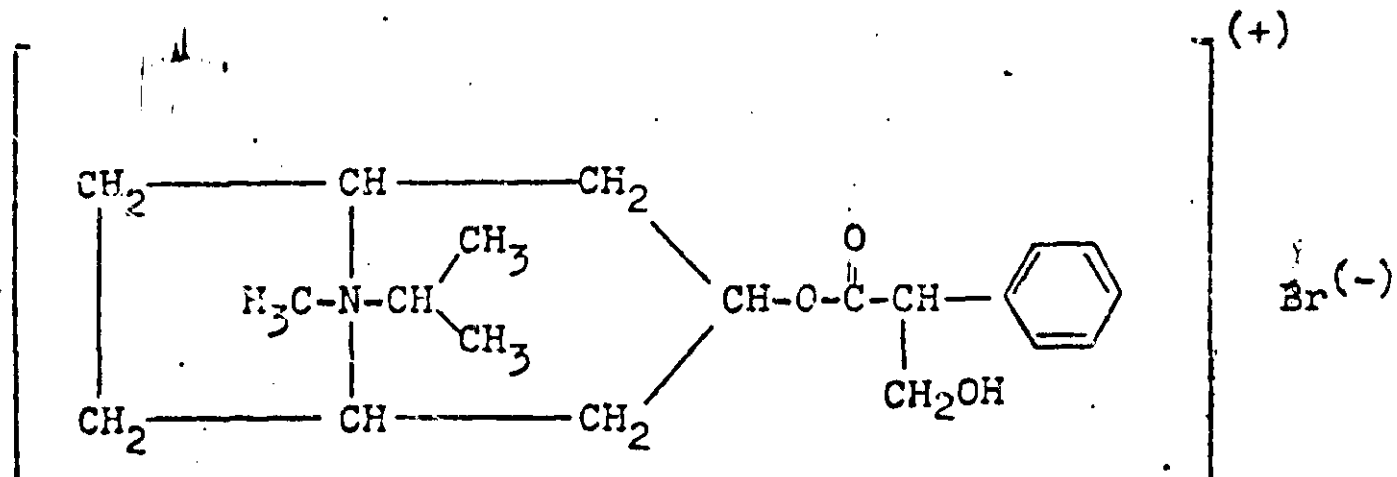
USAN: Ipratropium bromide monohydrate

Chemical: 8-Azoniabicyclo[3.2.1] octane,  
3-(3-hydroxy-1-oxo-2-phenylpro-  
oxy)-8-methyl-8-(1-methyl-  
ethyl)-, bromide, monohydrate  
(endo,syn)-, (+)- or

(8r)-3a-Hydroxy-8-isopropyl-  
1aH, 5aH-tropanium bromide

(+)-tropate monohydrate.

STRUCTURE:



Empirical Formula: C<sub>20</sub>H<sub>30</sub>NO<sub>3</sub>Br·H<sub>2</sub>O

Molecular Weight: 430.4

APR 12 1984

RELATED INDs AND NDAs:

ATROVENT® was studied under IND 9,814.

Drug Master Files referenced in this Application are:

Boehringer Ingelheim Ltd., Danbury, CT - DMF 4006

COMPOSITION OF DRUG:

| <u>Component</u>                                  | <u>% W/W</u> |
|---------------------------------------------------|--------------|
| Ipratropium bromide, micronized                   |              |
| Soya lecithin                                     |              |
| Dichlorodifluoromethane, NF<br>(Propellant 12)    |              |
| Dichlorotetrafluoroethane, NF<br>(Propellant 114) |              |
| Trichloromonofluoromethane, NF<br>(Propellant 11) |              |

\*Nitrogen

\* Removed in processing.

ROUTE OF ADMINISTRATION: Oral inhalation

DOSE: Usual dose is 2 inhalations 4 times a day. Patients may take additional inhalations as required; however, the total number of inhalations should not exceed 12 in 24 hours. Each actuation delivers 20 ug from the mouthpiece. Therefore, the maximum daily dose =  $20 \times 12 = 240$  ug/day = 4.8 ug/kg/day for 50 kg subject.

DOSAGE FORM: Suspension aerosol with Freons for oral inhalation.

CATEGORY: Bronchodilator

CLINICAL INDICATION: Bronchodilator for the treatment of chronic obstructive pulmonary diseases.

COMPARISON DRUG: Atropine sulfate

PRECLINICAL DATA:

GLP STATEMENTS: Yes.

FOREIGN ANIMAL STUDIES: Yes, most studies have been performed at C.H. Boehringer Sohn, West Germany; and some, in Boehringer Ingelheim in Japan. Some were done at  
and some, at

A few were done at

REVIEW OF PRECLINICAL DATA:

- COMMENTS: 1. For the list of preclinical studies, see pages 1 through 12, Volume 1.22.
2. The following review consists of photocopies of pharmacology reviews of from the original submission to 3/23/83 submission (except LD50s) organized according to categories.

PHARMACOLOGY/BIOCHEMISTRY (from the original review of

Anticholinergic Effects

A. Gastrointestinal Tract

(a) Salivary Secretions (stimulated by pilocarpine)

When administered IV, SC, and intraduodenally to dogs atropine was 2, and 5 times respectively, more effective than Sch in inhibiting salivary secretions. Decreased blood pressure was slightly inhibited by Sch but not in a dose-related fashion.

Administered as an aqueous aerosol Sch was slightly less effective than atropine. Maximum inhibition occurred at 10-15 minutes. Heart rate was not consistently altered. Decreased blood pressure produced by AcCh was only slightly inhibited by Sch (max effect at 1 min).

When administered as a metered aerosol, doses of 1-8 mg/animal, Sch effectively inhibited salivary secretion but did not alter BP, heart rate or ECG consistently.

In the mouse Sch was as effective IV but 1/2 as effective PO. In the rat, Sch was 2 times as effective as atropine when given SC but only 1/50 as effective intraduodenally in inhibiting carbachol-induced salivary secretions.

(b) Gastric Secretions:

Volume of gastric secretion was only slightly affected by increasing doses of Sch or atropine but Sch had more effect on volume of gastric acid secretion. Sch was about twice as potent as Atropine when given SC to rats but only 1/10 as potent when given PO.

(c) Intestines:

Following IV administration of Sch to intact dogs, spasmolytic effect appeared immediately whereas with intraduodenal and oral routes it occurred more slowly. Pulse rate increased with increase in dose.

(d) Bladder:

When Sch was administered IV to anesthetized dogs spasmolytic effect was as effective as atropine and produced an immediate response with a maximum in 7 minutes. A non dose-related increase in heart rate was noted. Respiratory rate was not altered. A slight transient decrease in BP occurred. When administered intraduodenally Sch was 1/2 as effective as atropine and onset of action was longer.

(e) Rectum:

In vitro studies on isolated guinea pig rectum indicate that Sch was at least as effective as atropine in preventing AcCh and pilocarpine-induced spasms but had no effect on BaCl<sub>2</sub> or histamine spasms.

B. Eye

In the mouse Sch was as effective as atropine when given SC but only about 1/15 as potent when given PO in producing a mydriatic effect.

C. Bronchi

In guinea pigs IV Sch was twice as effective as atropine in preventing AcCh-induced spasms.

Sch was also twice as effective when given IV to dog. With hi dose (0.3 mcg/kg and higher) spasmolytic effect was noted immediately. Sch was administered to dogs as metered aerosol (25 mcg/dog). Maximum effects occurred at 6-11 minutes. Duration of effect was longer than after IV administration.

Sch was twice as effective as atropine when given to dogs as an aqueous aerosol. After hi dose, effect was noted immediately with a maximum 6-13 minutes. Metered aerosol was effective for slightly longer period than aqueous aerosol.

D. CNS

0.3-10 mg/kg IV to rabbits did not alter arousal/stimulus or prevent carbachol-induced tremors; death occurred at 30 mg/kg.

In mice centrally induced lacrimal and salivary secretions induced by oxotremorine were inhibited by 0.005-0.05 mg/kg Sch but not the tremors.

E. Cardiovascular

When given SC to intact dog Sch was as effective as atropine but only 1/5 as effective when given PO in producing 50% increase in heart rate. In dogs, 0.03-0.1 mg/kg IV had no effect on BP or circulatory reflexes. 0.3 mg/kg produced a slight and transitory decrease in carotid sinus pressure reflex; at 1.0 mg/kg reflex was abolished for a few minutes.

F. Action of Sch 1000 on the Oxotremorine Test in Mice (1/30/75 review)

Sch 1000 at low dose levels given S.C., 0.0046 and 0.05 mg/kg acts as a peripheral anti-cholinergic agent and depresses markedly lacrimal and salivary secretions in mice. The mice had been given 1 mg/kg S.C. Oxotremorine, a drug which produces marked increase in lacrimal and salivary secretions. Sch 1000 had no effect on reducing tremors which are also produced by oxotremorine. Atropine used as the experimental control reduced secretions and tremors at lower than level.

G. Mydriatic Effect of Sch 1000, administered systemically in mice: comparison with atropine -

In a test to determine the mydriatic side effect of Sch 1000, mice were given this drug S.C. and orally at a dose level of 0.1 ml/10g. The results show that the mydriatic efficacy of Sch 1000 is similar to atropine when given S.C., however when Sch 1000 is given orally, it has 10-20 times the mydriatic efficacy of atropine.

H. Action of Subcutaneous and Intraduodenal Sch 1000 on Salivary Secretion in Anesthetized Rats.

Comparison with Atropine

Sch 1000 given S.C. and intraduodenally inhibited salivary secretion markedly at dose levels of 0.032 and 7.0 mg/kg respectively. Atropine inhibited salivary secretions at 0.018 mg/kg S.C. and 0.14 mg/kg intraduodenally. Anesthetized rats, given S.C. injection of carbackol for salivary stimulation were used.

I. Effect of S.C. and Enteral Sch 1000 on Gastric Secretion in the Rat

Comparison with Atropine

The results of this study show that 10-30 mcg/kg of Sch 1000 S.C. at a dose of 3-10 mg/kg stops the secretion of free HCl in the stomach of rats, whereas atropine must be given in dose of 30 to 100 mcg/kg to achieve the same effect. Neither drug had much influence on the volume of gastric juice secretion in the stomach. Atropine, given by enteral administration at a dose level of 0.5 mg/kg and Sch 1000 at a dose level of 5.5 mg/kg given similarly, reduced total acidity volume in the stomach of rats by 50%.

J. Cardiovascular Effects of Oral and Subcutaneous Sch 1000 in the conscious dog: Comparison with atropine.

Purpose of this study was to determine tachycardial side-effects of Sch 1000 and atropine following S.C. and oral administration in dogs. Results showed that a 50% increase in heart rate of dogs is achieved by a 0.011 mg/kg dose of Sch 1000, S.C. and a 0.540 mg/kg dose when drug is given orally. Atropine has the same effect when it is given at a dose of 0.016 mg/kg S.C. and 0.135 mg/kg orally.

K. Action of Sch 1000 administered by inhalation on respiration and Acetylcholine induced Bronchospasm in Anesthetized dogs

A dose of Sch 1000 given I.V. at 0.15 mg/kg inhibited acetylcholine induced bronchospasms by 50% in dogs. An I.V. injection of atropine at 0.27 mg/kg gave similar results.

Sch 1000 given as an aqueous aerosol is twice as effective in inhibiting bronchospasms in the anesthetized dog. The effect is immediate and the maximum effect is 6-13 minutes.

When Sch 1000 is administered by a metered aerosol, a 25 mcg total dose inhibited bronchospasms by 50% in the anesthetized dog.

L. Effect on Salivary Secretion, Blood Pressure and Heart Rate in the Mongrel Dogs

Anesthetized dogs with acetylcholine produced decrease in blood pressures and pilocarpine induced salivary stimulation were used in this study. Results show that 0.01 mg/kg Sch 1000 I.V. reduced salivary flow by 100%, blocked the acetylcholine decrease in blood pressure and cause an increase in heart rate. Atropine at a similar dose produces comparable results.

Sch 1000 given by aqueous aerosol in a concentration of 4% inhibited salivary flow by 50%. However, it had little or no effect on H.R. or h.p. Atropine in a 3% aqueous solution inhibited salivary flow by 50% and completely blocked acetylcholine induced decrease in blood pressure.

A total dose of 2.6 mg of Sch 1000 given as a metered aerosol effectively inhibited salivary flow in dogs.

M. Effect of Metered Aerosol on ECG and Heart Rate of Anesthetized Dogs

Anesthetized dogs were given Sch 1000 at dose levels of 1, 4, and 8 mg by metered aerosol. A 7.5 mg dose of alupent in a metered aerosol was used as the reference control. Results show that Sch 1000 at these dose levels cause a transient decrease in h.r. and then an increase in h.r. for approximately 1-2 minutes. The decrease in h.r. was thought to be due to propellant gas. No pathological changes in the ECG patterns of the dogs were noted. An increase in the h.r. of the dogs receiving alupent was observed.

N. E.E.G. Studies In Rabbits

Pharmacology Research Lab.

Sch 1000 I.V. had no effect on the central nervous system of rabbits.

Other Actions demonstrated in the Pharmacology Screening Program Pharmacology Research Lab.

O. Gastrointestinal System

The effect of Sch 1000 on contractions variously induced in the isolated rectum of the guinea pig were studied in this program.

Sch 1000 was more effective than atropine in inhibiting acetylcholine-induced contractions in the rectums of g.p.'s. It also was more effective in inhibiting pilocarpine induced spasms. Dose levels used were those dosages that inhibited contractions or spasms by 50%, The ED<sub>50</sub> dose. Sch 1000 had little effect on BaCl<sub>2</sub> or histamine contractions.

P. Expectorant Action of Sch 1000

Sch 1000 in milligram doses caused inhibition of bronchial secretions; however, in small doses (micrograms), Sch 1000 caused an increase in bronchial secretions.

Q. BEAGLE DOGS: Cardiotoxicity study by INHALATION: with propellant gases and under hypoxic conditions (Sept. 3, 1973): Translation (Boehinger Sohn).

- a. Under normal ventilation: Dogs(? M &?F of 7-14.5 kg) inhaled 200 whiffs (total 4 mg) of mixture of Sch-1000-Br and freon gases 11, 12, and 114, after pentobarbital (30-40 mg/kg I.V.) and gallamide triethiodide (4 mg/kg I.V.), and ECG were recorded from leads I and II: No pathological changes in ECG were observed. Heart rates were decreased with increased O<sub>2</sub> tension and decreased CO<sub>2</sub> tension.
- b. Under hypoventilation: Hypoxia itself produced more changes on ECG before starting infusion, and the changes became more marked as time went by, as did bradycardia.

R. BEAGLE DOGS: by I.V. infusion:

- a. Under normal ventilation: One dog at 5 mg/kg/min.: after 725 mg/kg, there was a marked bradycardia.  
  
One dog at 10 mg/kg/min: The dog tolerated up to 1550 mg/kg, but with marked bradycardia and hypotension.
- b. Under hypoventilation:  
  
10 mg/kg/min + placebo (containing freons) metered aerosol of 10 puffs every 5 min: Hypoventilation produced repolarization problems on dogs before the administration of infusion, and the effects were exaggerated by the infusion. Animal survived after 1000 mg/kg and 200 puffs.

It was stated that the hypotension and bradycardia produced by Sch 1000-Br is probably due to its ganglionic-blocking activity and curare like effect, but it did not produce any pathological changes on ECG.

**S. IN-VITRO PROTEIN BINDING OF SCH 1000:** (from 11/9/83 review)

Lab. Performing the study: C.H. Boehringer Sohn  
(Dr. Wahl, 9/24/80)

Methods: Equilibrium dialysis with/without  $^{14}\text{C}$ -Sch. 1000 (Batch 8).  
Batch no of cold Sch-1000 (VIII)

Plasma 1 was a mixture of old plasma samples from 22 persons, and was older than 1 week.

Plasma 2 was fresh from 3 male subjects and used separately.

Rat plasma was from several male rats of Chbb: THOM (SPF).  
See pages 1-3 for details.

**RESULTS:**

The Sch 1000 binding to human plasma was reported as an average of 18% (14-20%), to human albumin as 6% (4-10%), and to rat plasma as 8% (6-8%), and there were no significant differences between old and fresh plasma samples.

**II. ADME AND PHARMACOKINETICS:** (from the original review)

**A. Absorption, Metabolism and Excretion Studies**

Rat

After IV administration of 1-6 mg/kg  $\text{IV}^{14}\text{C}$ -Sch, 79% of radioactivity (RA) was excreted in 24 hours (57% urine, 22% feces), 18% was excreted in

bile and less than 1% was exhaled. Elimination from blood was biphasic: 1)  $t/2=4.3$  minutes and 2)  $t/2=1.4$  hours. Tissue distribution, in order of decreasing activity, was GIT, liver, kidney, bladder, heart, and brown fat.

Following PO administration of 1-25 mg/kg of RA drug, seventy-four percent of RA was eliminated in 24 hours (5% urine, 69% feces), 3% in bile and less than 1% was expired. Blood levels could not be determined. Distribution studies indicate drug was found, in order of decreasing activity, in GIT, liver, kidneys, heart, lacrimal glands, and brown fat. At least three metabolites were reported but no details given as to structure.

Dog

After 5 mg/kg  $^{14}\text{C}$ -Sch IV, elimination of RA from blood was biphasic: 1)  $t/2=2.5$  minutes and 2)  $t/2=3-4$  hours. About 38% of RA was eliminated in urine and 1% in feces in 24 hours. One hour after IV dosing about 73% of RA excreted in urine was due to unchanged drug; approximately 8-10 metabolites were present in small amounts. None of metabolites were further identified. Over a 7-day period, 88% was recovered (45% urine, 43% feces).

When the above dose was given PO to two dogs, maximum blood levels were noted after 3 hours. In one dog blood  $t/2$  was 10 hours, in the other, maximum levels were maintained for 15 hours. About 23% of RA was excreted in urine in 24 hours and 54% in feces. Over a 7-day period 98% of RA was eliminated (27% urine, 71% feces). Metabolic pattern was not determined.

Man

After PO administration of 30 mg  $^{14}\text{C}$ -Sch to 7 humans, maximum blood levels were noted 2-4 hours then slowly decreased;  $t/2$  was about 4 hours. Twenty-nine percent of administered RA was recovered in 24 hours (8% urine, 21% feces). In 48 hours, fecal excretion accounted for 89%. Over 6-day period, the total recovery was 97% (9% urine, 89% feces).

Following IV administration of 1 mg.  $^{14}\text{C}$ -Sch to 7 humans a biphasic elimination was noted: 1)  $t/2=5$  minutes and 2)  $t/2=4.2$  hours. In 24 hours, 72% of RA was excreted (70% urine (52% in 4 hours) and 2% feces). Over 6-day period, 78% of RA was accounted for (72% urine, 6% feces). Based on renal excretion following IV and PO administration, absorption is approximately 11% of administered dose.

One hour after IV dosing, urine was examined for metabolites. About 71% of unchanged drug was present, one metabolite accounted for 15% and seven others were present only in small amount (all less than 4%). With time the amount of unchanged drug decreased and major metabolite increased. Other seven metabolites remained about the same.

Four hours<sup>1</sup> following PO administration, qualitative distribution of metabolites was somewhat similar to that noted after IV dosing. Amount of unchanged drug was smaller (24%). Two metabolites found following dosing IV were not seen after PO administration and two found after PO administration were not observed after IV route. Metabolites were not identified structurally.

Five subjects received a total of 555 mcg. of  $^{14}\text{C}$ -Sch in ethanol and freons via inhalation. Peak concentration occurred 3 hours after exposure. Half-life for plasma elimination was about 2 hours. Fifty-one percent of administered RA was accounted for in 24 hours (48% feces, 3% urine); as after PO administration, urinary excretion was very small, over 6 days, 72% was recovered (69% feces, 3% urine). Unchanged drug and three metabolites were found in urine but not further identified.

The absorption of Sch in rat, dog, and man, based on urinary excretion following IV and PO use, was 9, 60 and 11%, respectively. It would appear that absorption in rat and man were comparable but considerably higher in dog. Only small percent of RA was found in feces following IV administration to man. Apparently, drug and/or its metabolites are not secreted into intestine via the bile to a significant extent while the reverse seems to be true for the rat and dog.

Based on the quantitative distribution of unchanged drug and metabolites in the urine following IV administration, the metabolic pattern in the dog (9-11 metabolites) and man (8 metabolites) appears to be somewhat similar. However, since metabolites were not structurally identified, it is not known if they were identical. Sufficient information was not available to make a definite statement about the rat metabolism data.

**B** Pharmacokinetics of <sup>14</sup>C - Sch 1000 Metered Aerosol - Synopsis (1/24/75 review)

Rats Radioactive Sch 1000

# animal/dose level 139M

dose 1 mg/kg

administration and duration

I.V. and oral 15 day observation

Results

Plasma levels could not be obtained in the rat. Excretion of Sch 1000 was fairly rapid with the maximum excretion occurring within 6 hours. 5.0% of the drug was excreted via the urine and 83% was excreted via the fecal route when taken orally and 61.4% via the kidney and 39.0% in the fecal matter when the drug was given I.V.

Dog Radioactive Sch 1000

# animals/dose 2

dose level 5 mg/kg

administration and duration

I.V. and oral, 15 day observation period.

Results:

Following oral administration the maximum plasma value was found within 3 hours. 23.6% of the drug is excreted via the urine when given orally and 72% in the fecal matter while 45% is found in the urine when the drug is given I.V. and 44% in the feces. The half life of the drug when given orally is 3-4 hours.

In man, the metabolism of Sch 1000 is similar to that found in the dog. The maximum concentration of this drug in blood was observed within 3-4 hour. The drug is excreted rapidly 30-70% within the first 24 hours depending on whether the drug is given orally or I.V. when given I.V., 72% is excreted in the urine, and 6.0 in the feces. When taken orally, the drug is excreted 9.0% in the urine and 88.5% in the feces.

C. Pharmacokinetics Using Radioactive Sch 1000 Metered Aerosol

man (age 22-34)

Radioactive Sch 1000

# dose level 4M + 1F

dose 469-619 mcg. average 555 mcg.

administration and duration

Inhalation, 3 puffs observations 144 hours  
Sch 1000 given as a radioactive solution in I.V. studies.

Results:

The maximum plasma levels were reached within 1-3 hours, the half life of this drug in plasma was 1.4 to 4.1 hours. The renal excretion of Sch 1000 was most rapid in the first 24 hours 2.8% and the renal excretion for the 6-15 day test was 3.2%. The fecal excretion was found to be 31.0 to 70.0% in the first 24 hours and 55.5 to 88.6% in this test.

The maximum plasma levels observed for the inhalation of AHR3053 was 92 picograms per gram of plasma.

D. The Excretion and Whole-Blood concentrations of Radioactive Sch 1000 after I.V. administration to monkeys during a chronic Inhalation Toxicity Study of the Non-Radioactive Drug.

Rhesus Monkeys (M. <sup>mulatta</sup> ~~macchatta~~) Sch 1000

#Animals/Dose Levels: 1F and 1M

. Doses: Control and 1 mg/kg

Administration and Duration:

Animals used were on a chronic inhalation study. Animals used were controls and the 1600 mcg/da groups. These animals were given radioactive Sch 1000 I.V., a one shot dose.

Results:

An I.V. dose of Sch 1000 at 1 mg/kg given to the monkeys before the initiation of the chronic inhalation study, and at 3 and 6 months during the inhalation study showed no significant differences in the rates of excretion. A total of 66.7% of the dose was found in the urine of the monkeys in the control group and 62.9% in the 1600 mcg/da group at the 3 months evaluations. At the same evaluations, 25.6% of the dose was found in the fecal matter of the control monkeys and 24.9% in the feces of the 1600 mcg/da monkeys. Results for pre-exposure and at 6 months were similar. Similar results were obtained when the whole blood of these monkeys was evaluated for radioactivity. The radioactivity of the control and experimental monkeys were comparable at pre-exposure, 3 and 6 months. Sponsor suggests that these data show there is no accumulation of drug or inhibition of drug metabolizing enzyme(s) during the administration of Sch 1000. Thin-layer chromatography analysis of the urine show that the drug is excreted unchanged by the monkeys.

E. Species Comparison of Biochemical Findings Sch 100 in Rat, Dog and Man  
(C.H. Boehringer Sohn, Germany) (3/14/77 review)

Rat

Rats, given an oral dose of 100 mg/kg Sch 1000 showed a maximum concentration in the blood within 2-4 hours, drug remained in the blood at high levels for several hours (approximately 7). Rats, given a i.v. dose of Sch 1000 (1mg/kg) had a rapid fall in blood drug concentration during the first 4.3 minutes (50% drop). During the next 3 hours, drug was lost at a slower rate. During the first 24 hours, 4.5% of drug was found in the urine and 76.9% was found in feces when Sch 1000 is given to the rat orally. When given by I.V., 57.1% was found in the urine and 39.5 was noted in the feces. A total of 5 components were found in the urine of rats given Sch 1000 orally. The largest amount of these components was unchanged drug.

Dog

Dogs, given Sch 1000 orally at a dose of 5 mg/kg showed a maximum drug concentration in the blood within 2-4 hours. Large concentrations were noted for up to 10 hours in the blood. 23.2% of oral Sch 1000 was found in the urine of the dog within 24 hours and 72.5% was found in the feces. After I.V. administration 38.4% of the dose was found in the urine and 42.6% in the feces.

g) GUINEA PIGS: ACUTE INHALATION (Boehringer Sohn), 5/12/71:(Ref.29):

(11/2/78 review)  
6 M/group were exposed to aerosol chambers of 4% and 8% aqueous solutions for 30 min., 1, 2, 4, and 6 hours with chamber pressure of 1.5 at. and the air mixture flow of 5-5.9 liters/min. All the animals were kept under observation for 14 days after exposure.

RESULTS:

The results showed that a 4% solution was well tolerated up to a dose of 18 mg/kg/6 hours, assuming 50% uptake. The 8% solution was well tolerated for 30 min and 1 hour, but after 2 hours breathing became troubled and rapid. After 4 hours, coughing was noted, but all animals survived.

When animals were exposed to 4 times of 8% solution, 2 out of 3 died after 4.5-5 hours (199 mg/kg/5 hours), and the third, survived animal, displayed severe respiratory distress and rapid breathing during the test. Macroscopic examination showed no pathological findings, but histopathology revealed slight emphysema and hyperemia of the lungs in two dead ones.

COMMENTS ON ACUTE TOXICITY STUDIES:

IV ED<sub>50</sub> in M and F mice were reported as 17 and 18 respectively in 1/23/83 submission, but Table 1 for Ref. 24 shows 17, 18 and 20.5. Why different?  
2. There was no submission in IND for 1/83 for Ref. 28.

**I: FOUR WEEK ORAL TOXICITY STUDY IN RATS: E83-123:**

Lab Performing the Study: Same as above.

Strain: SD-JCI. Sprague-Dawley

No. of Animals/Level: 15 M + 15 F except that the high dose group had an additional 10 M + 10 F recovery group with 6 weeks untreated period.  
M = 187 + 17 g, F = 154 + 10 g, both 6 weeks old.

Dosage Level: 0, 12, 120, or 1200 mg/kg/day  
Sch 1000 (Batch No. 1/74).

Duration and Route: For 4 weeks by stomach tube in 1.0 ml/100 g in distilled water.

Mortality: Only 1 M died in the mid dose group, but 9 M + 4 F in the high dose group died during the study.

Clinical Signs: Mid dose group (120 mg/kg) showed a slight weakness. Rats in high dose group were very weak with ruffling up of coat, red incrustated eyes and nose, and ataxia, especially during the first week, and they disappeared during the first week of recovery period.

Growth: Body weight gain of treated groups was generally significantly ( $p < 0.01$  in M in two high dose groups and  $p < 0.05$  in F high dose group) depressed from the first week and also dose-related especially in M.

See pages 21 and 31.

Food Consumption: Both M and F of the treated groups had generally less food consumption than respective controls and significantly ( $p < 0.05$ ) less in the high dose group during the first week. But F of the high dose group had significantly higher consumption during weeks 2-4, due to decreased consumption in the control F, and M in the same group had lesser food than control M only during week 3. See page 32.

Water Consumption:

Both M and F of the high dose group had significantly ( $p < 0.01$ ) higher water consumption during weeks 2 to 4 over control group or other 2 groups. See page 33.

COMMENTS: Page numbers for the results of each parameter were missing in the RESULTS section of this study. See pages 14-15.

Hematology:

Some values were significantly different from the control values, but they were reported as "in normal range". See pages 14 and 34-45.

Coagulation Time:

Prothrombin time showed no difference between groups.

Clinical Chemistry:

Although some values were significant, no consistent dose-related or time-related changes were seen. See pages 46-57.

Urinalysis:

No dose-related changes were reported. See pages 58-80.

Organ Weight:

It was stated that "parallel to the decrease of body weight of male and female rats at 1200 mg/kg dose all weights of the main parenchymatous organs, as heart, liver, kidneys and endocrine glands, decrease significantly too. To the contrary, the weight of the adrenals increases in both sexes."

The difference was generally at  $p < 0.01$  in M (see page 81) and  $p < 0.05$  in F (see page 82), and most of them showed a trend of dose-relationship.

Dissection Data:

Group 0 (control)

M 4 necroses of liver  
M 10 hydronephrosis  
F 9 hydrometra

Group 1 (12 mg/kg)

N.A.D.

Group 2 (120 mg/kg)

M 36 kidney infarct  
F 39 hydronephrosis

Group 3 (1200 mg/kg)

M 50 cachexia, obstipatio, edema pulmonum  
M 52 cachexia  
M 53 edema pulmonum  
M 54 meteorism  
M 56 gastric dilatatio, obstipatio of colon,  
edema pulmonum  
M 59 obstipatio, pneumonia  
M 60 cachexia, enteritis catarrhalis, pneumonia  
M 69 cachexia, enteritis catarrhalis, pneumonia  
F 53 enteritis catarrhalis, pneumonia  
F 54 enteritis catarrhalis  
F 68 cachexia, enteritis catarrhalis

Histopathology:

It was reported that "except, the atrophy of male accessory sex glands following loss of body weight at high dosage, no dose-related changes occur."

Necropsy:

Urinary calculi <sup>were</sup> ~~was~~ seen in an F(24) in low dose group and perisplenitis fibrosa in a M (25) in mid dose group.

Histopathology:

No drug induced changes were reported. For the detailed report, see page 17-18 and 92-101.



JK. Toxicity Subacute (from 1/24/75 review)

The Toxicity of Rats to Sch 1000 administered by inhalation over a period of 30 days

Rats ( Charles Rivers)

Sch 1000

# animals/dose level:

10F + 10 M

dose level: 21,600 mcg/da, 14,400 mcg/da and 7,200 mcg/da, plus control and vehicle groups. administration and duration

Inhalation - 20 mcg spray every 5 seconds for 15 minutes. Animals were exposed 15 minutes, 6,4,2 times a day in the high, medium and low dose groups, respectively.

30 day study

Results:

Clinical Signs

Animal behavior appeared normal after an adjustment period.

Mortalities

Nine animals (all groups) died during this study. Animals deaths were not drug-related.

Food Consumption

Food consumption of the animals was normal following an initial drop in consumed food during the first week.

Body Weight

Animals had an initial weight loss during the first week. Animals (majority) gained weight during the final 3 weeks of the study.

Ophthalmoscopy

Examinations carried out pre-exposure and at termination recorded no abnormalities that could be attributed to treatment with Sch 1000.

Laboratory Investigations

Hematology Values obtained pre-exposure and at termination revealed no significant changes due to Sch 1000.

Biochemistry Biochemical values obtained for these animals were within normal limits.

Urinalyses

All values were within normal limits at both the pre-exposure and at termination.

Macroscopic Pathology

No morphological changes were observed which were considered to be drug induced.

Subacute Toxicity Study in Male Dogs

Dogs, beagles

Sch 1000

# animal/dose level

4 M - 6 M in control group.

Dose level: 1.5, 3.0 and 15.0 mg/kg

administration and duration: Oral administration, 7 days weekly for 13 weeks.

Results:

Clinical Signs

All animals survived the tests. Test animals exhibited dryness of mouth, mydriasis and inhibition of lacrimation. Vomiting and diarrhea was seen in several animals of every group.

Body Weight

Dogs in 15.0 mg/kg group showed a slight weight loss. All other dogs had comparable body weights.

Food Consumption

Food Consumption of the dogs in treatment groups was not significantly different from the control group.

Hematology

The erythrocyte sedimentation rate of 2 dogs in the 3.0 mg/kg groups were slightly accelerated during weeks 0 and 6. All other values for all dogs were within normal range.

Biochemistry

No significant differences were noted between the treated groups and the control group.

Urinalysis

Values for urine specimen were within normal limits.

Ophthalmoscopic

Examination of the eyes of these dogs showed that the animals in the 3.0 and 15.0 mg/kg groups had drug induced keratoconjunctivitis sicca. Daily rinsing with 3% boric acid with applications of aureomycin and Vitamin A ointment healed this condition in 5 of the 8 dogs in the abovementioned groups. Histological investigations showed that keratitis sicca is due to a dose-dependent inhibitory effect of Sch 1000 on lacrimation.

Autopsy Findings

Several dogs in the control group as well as the 3.0 and 15.0 mg/kg had slight lipomatosis Cordis, mainly in the right ventricle. The livers of all the dogs in this study had parasitic or bacterial granulomas. In this test, 1 dog in each of the control group and the 1.5 mg/kg group had reduction in spermatogenesis. This condition was also noted in 1 dog of the 3.0 mg/kg and 2 dogs in

the 15.0 mg/kg group. In several of these dogs the reduction was bilateral. In 2 control dogs, 6 dogs of the 1.5 and .3.0 mg/kg groups and 1 dog in the 15.0 mg/kg a partial atrophy of the glandular epithelium of the prostate was observed.

M. Subacute

1) Inhalation Study in the Rat

In this study, 5 samples of the upper and lower trachea, bronchus and lung parenchyma were taken from each of 3 males and 3 females (rat) for electron microscopic examination. However, study can not be evaluated because dose levels, administration and duration are not included in the submission. Apparently, these particulars were submitted earlier; however, I can not locate them.

2) Inhalation Study in Monkeys

Electron Microscopy Report  
(C.H. Boehringer Solm, Germany)

This is another study in which samples were taken from trachea, bronchus, bronchiolus and lung parenchyma of the monkey for electron microscopic examination again, important details of the study are missing.

N. 6-WEEK INHALATION STUDY ON RATS:

No. of Animals/group: 15 M + 15 F (average wt: M=290 g, F=225 g)

Strain: Albino SPF rats of Chbb:THOM(SPF)

Age at the beginning: M=64 days, F=78 days,  $\pm$  2 days

| Dosage Level: | Group         | No. of whiffs/min | Actual dose (mg/l) |
|---------------|---------------|-------------------|--------------------|
|               | 0(Handling)   | -                 | -                  |
|               | 1(propellant) | 36                | -                  |
|               | 2             | 12                | 0.17               |
|               | 3             | 12                | 0.34               |
|               | 4             | 36                | 1.0                |

Duration in chamber: all 120 min.

Duration and Route: 6-weeks by inhalation (5/10/77-6/27/77)

|                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Growth Effect:      | There was no difference. 2 rats from each group except group 2 showed decrease in body weights with unknown reasons.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Food Consumption:   | There was no difference.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Mortality:          | One of group 1 and 2 or group 2 died, but the death seemed to be not drug related. No deaths were seen in groups 3 and 4.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Hematology:         | There were no drug induced changes.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Coagulation Test:   | No test was performed as such.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Clinical Chemistry: | GPT of groups 2 and 4 females and GOT of all females were slightly increased, and glucose of females of three treated groups was decreased, but they were not dose-related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Urinalysis:         | No dose related change was seen.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Organ Weights:      | There was no dose related change.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Gross pathology:    | There were some changes such as pleural adhesion or small brownish-red miliary foci of lung in all groups except group 2, but those changes were not dose related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Histopathology:     | <p>The examination was performed by Prof. K. Paulini of the Pathological Institute, Clinics of the Universtiy of Mainz. There was no dose related change. Common findings were bronchiectases, foam-cell-rich interstitial pneumonia, slight and various eosinophilia of heart muscle fibers, dysatelectases, PAS-positive cytoplasm of interstitial tissue and alveoli, fatty infiltration of hepatocytes, siderosis of spleen reticulum cells, and calcium-iron incrustations of distal tubules of females.</p> <p>Glandular cysts of the gastic mucosa found in 5 rats of group 1 and dilatation of the bladder found in 6 rats of group 3 were distinct. Other findings were numerous, but they were found in 1-3 rats of each group. The single finding of retinal atrophy in group 4 may be worth mentioning.</p> |

○. 13 WEEK INHALATION TOXICITY STUDY IN DOGS WITH SCH 1000-BR "POWDER  
APPROVAL # (11/9/83 review)

Lab. Performing the Study:

C.H. Boehringer Sohn

(Dr. Kollmer, 12/15/79)

Strain:

Beagle dogs

No. of Animals/Level:

3 M + 3 F

(M = 13.5 kg and 14 months, and  
F = 11.5 kg and 13 months in  
average).

Dosage Level:

| Groups | Dose in $\mu\text{g kg}^{-1}$ |        | No. of dogs |   | Animal No.         |
|--------|-------------------------------|--------|-------------|---|--------------------|
|        | nominal                       | actual | m           | f |                    |
| 0      | placebo                       |        | 3           | 3 | 001-003<br>051-053 |
| 1      | 10                            | 7      | 3           | 3 | 101-103<br>151-153 |
| 2      | 50                            | 36     | 3           | 3 | 201-203<br>251-253 |
| 3      | 250                           | 179    | 3           | 3 | 301-303<br>351-353 |

the nominal dose is the intended dose and the total dose administered; the actual dose was calculated by subtracting from the total amount administered the substance residue demonstrated by quantitative analysis in the inhalation apparatus.

"The experimental animals received Sch 1000-BR on inspiration in the form of a powder aerosol at the same time each day (between 8.00 and 15.00 h). Animals in groups 2 and 3 received Sch 1000-BR powder capsules containing 0.2 mg Sch 1000-BR. The fine adjustment of the dosage in accordance with body weight was carried out using capsules containing 0.04 mg Sch 1000-BR in animals from all the treated groups. Control animals received an aerosol with placebo capsules containing only glucose."

Justification of the dose: See pages 10-11.

Batch Nos: 88121 and 88122 for capsules containing 0.2 mg Sch-1000 and 25 mg glucose

88125 for capsules containing 0.04 mg Sch-1000 and 25 mg glucose

78119 for placebo capsule containing 25 mg glucose

Duration and Route: 13 weeks: Started on 4/17/78 for M and on 4/18/78 for F and ended on 7/18/78

The drug was introduced in the form of a powder aerosol directly into the trachea with inspiration. See inhalation apparatus on page 8.

Mortality: None

Growth: There were no significant differences between groups, although F showed a slight trend of depression.

COMMENTS: 1. Some page numbers were missing or illegible.  
2. No group mean at one was given.

Food Consumption: No significant differences between groups were reported.

Clinical Symptoms and Behavior: M and F exhibited dryness of the nose in group 2 (50 ug/kg/day) and dryness of the nose and oral mucosa in group 3 (250 ug/kg/day), and it lasted 1-2 hours in 50 ug/kg/day group and 4-5 hours in 250 ug/kg/day group.

|                          |           |                                                                                                                                                                                                                                                                     |
|--------------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                          |           | Incidence of diarrhea and emesis were reported on pages 023 and 024, but there was no dose or time relationship.                                                                                                                                                    |
| Hematology:              |           | There were no significant differences between groups.                                                                                                                                                                                                               |
|                          | COMMENTS: | Values were presented for weeks -2, 1, 6, and 13.                                                                                                                                                                                                                   |
| Myelogram (Bone marrow): |           | No dose or time related changes were reported for bone marrow investigation. (See page 111-114).                                                                                                                                                                    |
| Coagulation Time:        |           | No significant differences between groups were noted for partial thromboplastine time, thromboplastine time, or thrombin time.                                                                                                                                      |
| Clinical Chemistry:      |           | Na <sup>+</sup> of 3 treated groups was statistically significantly higher than control group, but only at week 6, and without dose-relationship. Cholesterol of 3 treated groups was slightly higher than control group at week 13, but without dose-relationship. |
| Urinalysis:              |           | There were no significant differences between groups.                                                                                                                                                                                                               |
| Organ Weights:           |           | There were no significant differences between groups in selected organ weight, ((F high dose group had larger weight of left ovary, but not significant.))                                                                                                          |
|                          | COMMENTS: | It was reported that the weight of the lung was inadvertently omitted.                                                                                                                                                                                              |

Necropsy:

No - drug induced changes were reported. See pages 42-44 for details of autopsy findings.

Histopathology:

The following summary was submitted on the results of microscopic examination by

“A comparison between the findings in the control animals and in the animals of the groups which received the substance give no indication that the substance has a damaging effect. In my opinion all organ changes observed belong to the spontaneous pathological processes in the dog.

Slight inflammatory processes in the lung, especially those of an interstitial inflammatory nature, were found both in the control animals and in the animals of the dose groups. They are also known to us from control animals or other experiments. Some of the inflammatory changes are, without doubt, of parasitic origin, in other cases in which no parasites are detectable in the section this aetiology is at least probable. Unilateral or bilateral non-purulent pyelonephritis is often found in dogs. The fact that, in this experiment, it was only found in animals of the dose groups, is pure coincidence. In other experiments with dogs of the same age I have encountered it in control animals.

In summary it can be said that the histopathological examination provided no indication that the substance administered by inhalation had any harmful effects.”

statistically significantly smaller in high dose group, but the mean absolute weight of epididymides in high dose group was statistically significantly larger when compared to that of the control group M (p. 36).

In the mean relative organ weights, only the high dose group had statistically significantly heavy epididymides, seminal vesicles, and preputial glands (p. 41). See Table 7 for lesion in M sex organs.

Mean Litter Data:

|                                             | Control  | 5 mg/kg  | 50 mg/kg | 500 mg/kg |
|---------------------------------------------|----------|----------|----------|-----------|
| No. of dams pregnant                        | 20       | 20       | 19       | 14        |
| No. of dams died during pregnancy           | 0        | 0        | 0        | 0         |
| No. of dams with all fetuses dead           | 1        | 0        | 0        | 0         |
| No. of dams with live fetuses               | 19       | 20       | 19       | 14        |
| Total no. of corpora lutea                  | 287      | 310      | 267      | 202       |
| Av. no. of corpora lutea per dam            | 15.1±1.8 | 15.5±1.0 | 15.1±2.2 | 14.4±2.1  |
| Total no. of implantations                  | 265      | 283      | 209      | 169       |
| Av. no. of implantations per dam            | 13.9±1.8 | 14.2±1.7 | 11.0±1.7 | 12.1±2.8  |
| % implantations/corpora lutea <sup>a)</sup> | 92.6     | 91.8     | 94.4     | 83.6      |
| Total no. of fetal deaths                   | 22       | 27       | 18       | 14        |
| early resorptions                           | 15       | 17       | 15       | 13        |
| late resorptions                            | 7        | 5        | 3        | 1         |
| dead fetuses                                | 0        | 5        | 0        | 0         |
| Av. no. of fetal deaths per dam             | 1.2±1.5  | 1.4±1.9  | 0.9±0.9  | 1.0±1.3   |
| % fetal deaths/implantations <sup>a)</sup>  | 8.2      | 10.1     | 10.9     | 8.2       |
| Total no. of live fetuses                   | 243      | 256      | 251      | 155       |
| Av. no. of live fetuses per dam             | 12.8±2.1 | 12.8±2.9 | 13.2±1.6 | 11.1±2.9  |
| % live fetuses/implantations <sup>a)</sup>  | 91.8     | 89.9     | 89.1     | 91.8      |
| Sex ratio (male/female) <sup>a)</sup>       | 0.9      | 1.0      | 1.2      | 1.2       |
| Av. total weight <sup>a)</sup>              | 5.4±0.3  | 5.5±0.2  | 5.4±0.3  | 5.4±0.4   |

a) calculated using litter value as a basic unit

C. Table 1

Group Mean Litter Data  
グループ別胎仔記録

Species : Rat p.o. Substance: Sch 1000 Br  
動物の種類: 白鼠 投与: Sch 1000 Br

|                                            | Control<br>対 照 | 5 mg/kg     | 50 mg/kg    | 500 mg/kg   |
|--------------------------------------------|----------------|-------------|-------------|-------------|
| Number of animals<br>動物数                   | 20             | 20          | 20          | 20          |
| Maternal mortality<br>母体死亡率                | 1              | 0           | 0           | 0           |
| Abortions<br>流産数                           | 0              | 0           | 0           | 0           |
| Natural deliveries<br>自然分娩数                | 0              | 0           | 0           | 0           |
| Total number of pregnant animals<br>妊娠動物数  | 19             | 19          | 19          | 19          |
| Total implantation sites<br>着床数            | 245            | 223         | 263         | 239         |
| Per dam<br>1母体当たり着床数                       | 12.9 ± 3.7     | 11.7 ± 3.2  | 13.8 ± 1.5  | 12.6 ± 2.9  |
| Total viable fetuses<br>生存胎仔数              | 233            | 215         | 255         | 223         |
| Male in %<br>雄比率 (%)                       | 53             | 52          | 50          | 48          |
| Female in %<br>雌比率 (%)                     | 47             | 48          | 50          | 32          |
| In % of implantation sites<br>胎仔生存率 (%)    | 95.1           | 96.4        | 97.0        | 93.3        |
| Per dam<br>1母体当たり生存胎仔数                     | 12.3 ± 4.0     | 11.3 ± 3.6  | 13.4 ± 1.4  | 11.7 ± 3.3  |
| Total resorption sites<br>吸収数              | 12             | 8           | 8           | 16          |
| Early<br>早期吸収数                             | 10             | 6           | 8           | 15          |
| Late<br>晚期吸収数                              | 2              | 2           | 0           | 1           |
| In % of implantation sites<br>胎仔吸収率 (%)    | 4.9            | 3.6         | 3.0         | 6.7         |
| Per dam<br>1母体当たり吸収数                       | 0.6 ± 1.1      | 0.4 ± 0.8   | 0.4 ± 0.8   | 0.8 ± 1.8   |
| Total dead fetuses<br>死亡胎仔数                | 0              | 0           | 0           | 0           |
| In % of implantation sites<br>胎仔死亡率 (%)    | 0              | 0           | 0           | 0           |
| Per dam<br>1母体当たり死亡胎仔数                     | 0              | 0           | 0           | 0           |
| Mean litter weight (g)<br>平均同組胎仔体重 (g)     | 71.6 ± 23.0    | 65.6 ± 20.2 | 76.4 ± 7.9  | 66.8 ± 15.7 |
| Mean viable fetal weight (g)<br>平均胎仔体重 (g) | 5.84 ± 0.39    | 5.90 ± 0.43 | 5.70 ± 0.41 | 5.74 ± 0.49 |

d.

|                                                 | Control<br>对 照 | 5 mg/kg    | 50 mg/kg    | 500 mg/kg   |
|-------------------------------------------------|----------------|------------|-------------|-------------|
| Corpora lutea<br>黄 体 数                          | 263            | 261        | 266         | 282         |
| Pre-implantation-loss %<br>植入前丢失率 (%)           | 6.8            | 14.6       | 1.1         | 15.2        |
| Post-implantation-loss %<br>植入后丢失率 (%)          | 4.9            | 3.7        | 3.0         | 6.7         |
| Total variations<br>总变异数                        | 66             | 67         | 54          | 24          |
| In % of viable fetuses<br>在可存活胎数中 (%)           | 42.0           | 46.9       | 32.0        | 16.2        |
| Per dam<br>每母体                                  | 3.5            | 3.5        | 2.8         | 1.3         |
| Total malformations<br>总畸形数                     | 3              | 1          | 0           | 3           |
| In % of viable fetuses<br>在可存活胎数中 (%)           | 1.3            | 0.5        | 0           | 1.3         |
| Per dam<br>每母体                                  | 0.16           | 0.05       | 0           | 0.16        |
| Spontaneously delivered groups<br>自然分娩组         |                |            |             |             |
| Number of animals<br>动物数                        | 10             | 10         | 10          | 10          |
| Number of pregnant animals<br>妊娠动物数             | 7              | 9          | 10          | 9           |
| Total number of young<br>总仔数                    | 72             | 107        | 120         | 104         |
| At birth<br>出生时                                 |                |            |             |             |
| Viable young per dam<br>每母体可存活仔数                | 10.1 ± 3.3     | 11.8 ± 1.6 | 11.9 ± 2.0  | 11.0 ± 3.5  |
| Dead young per dam<br>每母体死亡仔数                   | 0.1 ± 0.4      | 0.1 ± 0.3  | 0.1 ± 0.3   | 0.6 ± 0.7   |
| Mean litter weight (g)<br>平均窝仔体重 (g)            | 62.8 ± 20.1    | 73.7 ± 8.8 | 73.6 ± 15.4 | 61.6 ± 20.3 |
| Mean weight of viable young (g)<br>平均可存活仔体重 (g) | 6.2 ± 0.7      | 6.3 ± 0.6  | 6.2 ± 0.8   | 5.6 ± 0.7   |
| 21 day<br>21日龄时                                 |                |            |             |             |
| Viable young<br>可存活仔数                           | 63             | 98         | 114         | 88          |
| Viable young per dam<br>每母体可存活仔数                | 9.9 ± 4.2      | 10.9 ± 2.5 | 11.4 ± 2.5  | 9.8 ± 4.8   |
| Litter weight (g)<br>窝仔体重 (g)                   | 371 ± 161      | 455 ± 45   | 447 ± 78    | 369 ± 166   |
| Mean weight of young (g)<br>平均仔体重 (g)           | 41.2 ± 5.0     | 41.7 ± 7.5 | 39.2 ± 4.2  | 37.7 ± 5.3  |
| Mortality of young<br>仔死亡率                      | 7              | 8          | 5           | 11          |
| Variations<br>变异数                               | 2              | 10         | 8           | 0           |
| Malformations<br>畸形数                            | 0              | 0          | 0           | 2           |

NDA 19-085

Date: November 18, 1985

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Amendment Dated September 12, 1985

APPLICANT: Boehringer Ingelheim

DRUG: Atrovent Metered Dose Inhaler

COMMENTS:

This amendment is for manufacturing and controls. The reviewing chemist has requested comment on the safety of two items:

1. The extractable component of the seals, identified as

This chemical is allowed in rubber articles intended for repeated use in the packaging of food products at 25 times the amount found in the sealant for this product. See 21 CFR 177.2600(c)(4)(ii)(b). In considering that only 0.0001% by weight of the chemical was found in the cannister it is concluded that no additional testing of the product for safety is required.

2. The presence of 1.5% propellant 11 extractables in the product.

Due to the technical aspects of testing inhalation products in animals it is not a usual requirement that the final product be used in toxicity testing. It appears that one dog study may have been conducted in which the animals were given the final drug product for 3 months, but it cannot be determined if the aerosol containers were identical to those produced by Riker Labs. Therefore, it is not certain that any animal testing was done using the final drug product which may have contained propellant 11 extractables.

However, assurance of testing for safety is provided in the following review provided by Dr. Wilson for NDA 19-009 (pirbuterol acetate) which utilizes the identical aerosol cannister manufactured by Laboratories:

"The applicant has determined the amount of extractable material from the rubber components of the assembled aerosol cannister. This was accomplished by evaporating the contents of placebo aerosols containing Freon 11 and 12 in the same amounts as found in the commercial aerosol product. The weight of this residue after storage at 30°C for 36 and 48 months was found to be 4 to 9 mg per 25.6 g Freons (165 to 350 ppm). Identification of the leachable materials would be helpful in the safety evaluation.

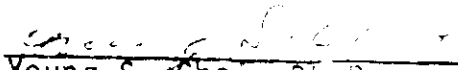
The reviewing chemist questioned the toxicity of the rubber components in the valve for the aerosol container. The components involved are

All ingredients in both rubber compounds with the exception of one minor ingredient are approved for food packaging under the Food Additive Regulations.

Drawings in the present NDA and in . . . The valve is believed to be identical to that tested clinically by . . . Other products marketed in aerosol containers with . . . include

Finally, the most important factor contributing to the safety of the rubber components of the . . . is that the aerosol container was incorporated in six month inhalation studies in dogs and monkeys in the applicant's laboratory."

CONCLUSION: The NDA is approvable from the standpoint of safety testing for the aerosol container.

  
\_\_\_\_\_  
Young S. Choi, Ph.D.  
Pharmacologist

cc: NDA 19-085  
HFN 160, HFN 340  
Doc Room 160  
R/D YSChoi, 11/18/85  
R/D init. JKInscoc, 11/20/85  
FT/jb, W4405P, D3616P, 11/21/85

nmor

NDA 19-085 Atrovent (ipratropium Br.)  
Bronchodilator

Boehringer-Ingelheim  
Submitted: 7/29/83  
Reviewed: 11/16/83  
Date this Addendum: 11/20/86

Addendum to MOR dated 11/16/83

Reference, MOR, page 45, Study Kirk, B.W., M.D., Health Science Center,  
Winnepeg, Manitoba, Canada.

Study No. E-82-1801 - Atrovent MDI

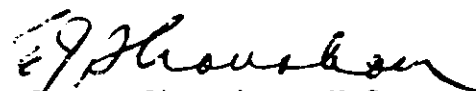
Final report date by Applicant - 7/11/83 - Location in NDA Vol. 1.18;  
1.138-1.141.

Objective - "To determine safety and efficacy of Atrovent by oral inhalation plus Theophylline highlighting the duration of action, persistence of bronchodilating action with repeated dosing, other effects of repeated dosing (side effects, adverse reactions and on lab studies), and patients' acceptance of Atrovent plus Theophylline compare to Theophylline alone."

Twenty five adult asthmatic patients were entered.

The Medical and the Biometrics Reviews differed regarding conclusions. Following further review and discussion (11/19/86) it was determined that the Kirk Study was not a valid support for the applicant's conclusions. The Biometrics Review, dated May 10, 1985 stated "The data showed that the addition of Atrovent to Theophylline maintenance treatment failed to provide a significant increase in FEV<sub>1</sub> causing the change from pre-dosing baseline on day 90), or mean peak flow rate (43.3 on Atrovent vs. 44.8 on placebo in the morning, and 37.7 vs. 23.4 in the evening). Furthermore, patients receiving Atrovent in addition to Theophylline treatment reported a higher rate of acute attacks per day (0.187 with Atrovent vs. 0.120 without Atrovent) and a higher mean daily use of Theophylline (4.82 whiffs with Atrovent vs. 4.11 without Atrovent); these differences failed to reach statistical significance at the 5% level."

Conclusion - In this study in Asthmatic patients the statistical report failed to support the Applicant's claims made.

  
E. J. Shanahan, M.D.  
11/20/86

L NDA 19-085

HFN-160  
HFN-340  
HFN-713 Dr. Huang  
HFN-160 Dr. Winkler  
R/D EJShanahan 11/20/86  
R/D Init. by JWWinkler 11/21/86, PGWalters 11/21/86  
FT OLA 2502N 0156N 12/13/86  
Doc. Room 160

DEC 8 1986

Review and Evaluation of Clinical Data

NDA #19-085

M.O. Review #1

Applicant - Boehringer Ingelheim Ltd.

Submitted: 7/29/83  
Date Completed: 11/16/83

1. General Information

a. Name of Drug

(1) Generic: ipratropium bromide

(2) Trade: ATROVENT<sup>R</sup>INHALER

b. Pharmacologic Category: Bronchodilator

c. Proposed Indications; "ATROVENT<sup>R</sup> (ipratropium bromide) is indicated as a bronchodilator for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis. Some asthmatic patients have been shown to respond to ATROVENT<sup>R</sup>."

d. Dosage form and route of administration;

"The usual dose of ATROVENT<sup>R</sup> is two oral inhalations four times a day. Patients may take additional inhalations as required; however, the total number of inhalations should not exceed 12 in 24 hours." There are 20 mcg Atrovent delivered per whiff. Dosage is 40 mcg four times a day.

ATROVENT<sup>R</sup> is supplied as a metered dose inhaler with or without a mouthpiece. Each 21 gram vial provides sufficient medication for 300 inhalations. Each actuation delivers 18 mcg of ipratropium bromide from the mouthpiece.

e. Related drugs; chemically related to atropine.

2. Manufacturing Controls (Refer to Chemistry Review). It is a quaternary ammonia compound unrelated to any approved bronchodilator since it is an anticholinergic agent.

NOV 22 1985

3. Pharmacology: ipratropium bromide has a higher parenteral efficacy than atropine. This was not the case in enteral administration. Here, the drug was less effective than atropine but did not show the same level of side effects. In the case of aerosol administration, the drug was more effective in preclinical studies as a bronchial spasmolytic agent but produced less of the antisialagogue (drying) effect. No increase in heart rate or changes in EKG were observed at doses a hundred fold those recommended therapeutically. The drug was devoid of CNS significant activity. ATROVENT<sup>R</sup> is a novel anticholinergic with a high degree of bronchospasmolytic activity. When used as a metered aerosol, it is practically devoid of side effects and possesses a wide therapeutic range.

The section in pharmacokinetics has been referred to our Division of Biopharmaceutics for review, evaluation and comments.

For data on safety studies, acute toxicity, subchronic toxicity, chronic toxicity, carcinogenicity, embryotoxicity, teratology and reproduction, mutagenicity, tolerance studies in animals and further details on pharmacology please refer to the pharmacology review.

#### Clinical Studies in the NDA Tabulated

Each clinical study included a protocol as well as a statistical analysis report.

#### Special Studies

- |                             |   |            |
|-----------------------------|---|------------|
| 1. Ventilation/perfusion -  | 2 |            |
| 2. Mucociliary clearance -  | 2 |            |
| 3. Methacholine challenge - | 1 |            |
| 4. Sputum studies -         | 2 |            |
| 5. Ocular studies -         | 3 | = Total 10 |

Dose-Ranging Studies 2 whiffs (40 mcg) 4 x daily.  
Asthmatics - 3  
Bronchitics - 2

Controlled Clinical Trials - multiple dose studies

#### Bronchitics (COPD)

Protocol 1 ii-b-5 2 multicenter studies comparing Atrovent with metaproterenol (Alupent) -  
5 studies, 6 principal or co-investigators

Protocol 2 iii-a-10 multicenter - 10 studies -  
147 patients on Atrovent and  
147 on metaproterenol

Protocol 3 ii-a-5 multicenter in asthmatics 5 centers; 6  
investigators  
71 on Atrovent  
73 on metaproterenol

4 B. W. Kirk 90 day study in 22 asthmatics with added and not  
added theophylline

A. Special Studies

|                                          | <u>#Studies</u> |
|------------------------------------------|-----------------|
| 1. Ventilation - Perfusion studies:      | 2               |
| (a) E-82-318                             |                 |
| (b) E-82-215                             |                 |
| 2. Mucociliary Clearance Studies:        | 2               |
| (a) E-80-735                             |                 |
| (b) E-80-789                             |                 |
| 3. Methacholine challenge                | 1               |
| (a) E-81-22                              |                 |
| 4. Sputum Studies                        | 2               |
| (a) E-81-38                              |                 |
| (b) E-81-166                             |                 |
| 5. Ocular Studies                        |                 |
| (a) E-74-213 }                           |                 |
| (b) E-73-709 }                           | 2               |
| (c) E-82-1861 - single and multiple dose | 1               |
|                                          | <u>10</u>       |

Total number of special studies = 10

B) Dose - ranging studies

E-79-81 )  
E-79-948 )                      Asthmatics  
E-79-961 )

E-80-3 )  
E-79-80) Bronchitics

C) Multiple Dose Studies in Bronchitics - 2 protocols

- 1) Protocol II b 5 - two multicenter studies in bronchitics in comparison with metaproterenol

5 studies

E-80-664 - Cuqell/Gross  
E-80-665 - Emirgil, et al  
E-80-668 - Niewoehner  
E-80-667 - Plummer  
E-80-666 - R. Goldring; analyzed separately due to misinterpretation of protocol affecting proper washout timing.

- 2) Protocol III a 10

E-81-1250 - Ashutosh  
E-81-1293 - Bleecker/Britt  
E-81-1153 - Cumiskey  
E-81-1417 - G. Gross  
E-82-854 - N. Gross  
E-81-1151 - S. Lakshminarayan  
E-81-1177 - Miller/Kotch  
E-81-1150 - Renzetti  
E-81-1176 - Sackner  
E-81-1154 - Tashkin

Bronchitics  
10 studies

147 patients were on Atrovent  
140 patients were on metaproterenol

D. Multiple Dose Studies in Asthmatics

Protocol II a 5

E-80-503 - Busse/Bush  
E-80-527 - Falliers  
E-80-526 - O'Hollaren  
E-80-457 - W. Storms  
E-80-525 - J. Weg

81 patients on Atrovent  
83 patients on metaproterenol

- E. 90 day study in 22 asthmatics to compare Atrovent in combination with theophylline to theophylline alone.

E-82-1801 - B. W. Kirk

11 patients on Atrovent plus theophylline  
11 patients on theophylline alone

New NDA 19-085 ATROVENT (ipratropium bromide) bronchodilator inhaler

CLINICAL STUDIES

ABSTRACT

In summary of these "special studies, in the NDA, numbers one and two were the same, to determine the effects of Atrovent in ventilation/perfusion relationships as manifested by changes in arterial blood gases and blood pH "(Dr. Anderson, #1, Dr. Lyons, 2) with results showing Atrovent improved pulmonary function in patients with stable airway obstruction without changing blood pH, arterial gases in blood and therefore did not change ventilation/perfusion ratios. 3) Dr. Bergofsky showed in a study of Atrovent on mucociliary clearance in patients with chronic obstructive pulmonary disease that compared to placebo, Atrovent showed a 103.3% increase in pulmonary function whereas these patients on placebo showed a 9.2% mean percent over baseline at 1 hour improvement. 4), Dr. A. Wanner in a study on the effect of Atrovent on tracheal mucous velocity in patients with chronic obstructive pulmonary disease Atrovent produced no significant changes in tracheal mucous velocity compared to baseline and to placebo, although slightly higher values were seen after placebo. 5) Dr. Norris conducted a trial to examine certain biochemical mechanisms which may be responsible for the effects of Atrovent in patients with bronchial asthma with the result that the role of biochemical effect could not be determined. 6) Dr. Chodosh studied and evaluated the effects of Atrovent (inhaled) on sputum in chronic bronchitis, compared to placebo in which no significant results were noted between the two treatments on mucous. 7) Drs. Kass, Burgher and Daniels conducted the same study as #6 above with results showing higher sputum volume in patients on Atrovent over those on placebo. (Figure 1 noted). Pulmonary function improved in patients on Atrovent over those on placebo. Studies 8, 9, and finally 10, were ophthalmology studies, all of which showed Atrovent produced no clinically significant changes in pupil size, accommodation, visual acuity or intraocular pressure in subjects and in patients with chronic lung disease.

- A. Special Studies - 10 in number.

- 1) Anderson, William M., M.D., Harrisburg Pennsylvania

Atrovent metered dose inhaler (MDI)

Study No.: E-82-318

Protocol Designation: Id<sub>2</sub>

Date: Dec. 2, 1981

Summary items 2 and 3 in the NDA; location Vol. 1.2, part E1, pp. 7-147.  
Reference to complete data item 12, Vol. 1.42.

"Objective: To determine the effects of ATROVENT on ventilation - perfusion relationships as manifested by changes in arterial blood gases and blood pH."

This study planned to evaluate 20 male or female patients, ages 18-65 with reversible bronchospasm, an FEV<sub>1</sub> < 75% of predicted. There were to be 2 test days. Criteria for inclusion were found acceptable, in that patients were to have "relatively stable or mild to moderately severe airway obstruction," and patients must have the stated "reversible bronchospasm shown by a 15% improvement in FEV<sub>1</sub> within 30 minutes following 2 whiffs (0.150 mg) of isoproterenol from a metered dose inhaler".

"Drug administration will be by oral inhalation in which each test dose will be 2 whiffs of ATROVENT, 20 mcg each or

Placebo - 2 whiffs,"

Duration - 2 single test doses of drug 2 weeks apart, or at least 24 hours apart.

Control - double-blind, crossover.

Randomization - by order of Entrance

Other Medication and Habits - inhaled steroids and cromolyn permitted if stabilized. Other anticholinergics will be excluded at least 2 weeks prior to entry; all parenteral or oral bronchodilators excluded for at least 12 hours prior to entry and during test days. All inhaled bronchodilators are excluded at least 8 hours prior to and during testing.

SUMMARY - objective as stated

Number and type cases - 19, 9 male, 10 female, ages 25-75 years. Exclusion criteria were implemented; 3 were excluded for protocol violations and pulmonary function test results. These 3 were not evaluated for efficacy. Sixteen, 7 males, 9 females ages 46-75 completed the study. Diagnosis: Extrinsic asthma, duration 5-70 years. Method of study - randomized, single dose, crossover with at least 24 hours to 2 weeks separation of dosing. Both test days were completed within 2 weeks.

Dose and Route of Administration - oral metered dose inhalation of ATROVENT, 40 mcg (20 mcg/whiff), or placebo, 2 whiffs. Concomitant medication - no patients were on investigational drugs. Of the 16 who completed the study, 5 were on steroids, 10 mg or less daily of prednisone or 20 mg every other day; 9 were on inhaled steroids for which dose had been stabilized 8 hours prior to testing and for duration of test days. Before and after the study PE and lab testing were done. Patients qualified for admission according to requirements stated above. PE, CBC, SMA-12, urinalysis, repeated at end of test days.

Each Test Day - observations involved pulmonary function testing at the same time on each test day for each test dose. Baseline FEV<sub>1</sub> on each test day was within 10% of that on qualification day; FVC and FEV<sub>1</sub> were measured at 15, 30, 60, 120 minutes after drug was given. Pulse rate and blood pressure (BP) were measured at the same time as pulmonary function tests and always after the patient rested 2 minutes. Vital signs were measured before pulmonary function testing. Blood samples were drawn at 15, 30, 60 and 120 min. after drug was given. Blood pH and arterial blood gases were studied for PaCO<sub>2</sub>, PaO<sub>2</sub>, SaO<sub>2</sub>, HCO<sub>3</sub> and measured at the same time as pulmonary function testing was done. Hgb and Hct were measured at baseline, of the 2 study days. Adverse reactions were recorded in detail.

### Results

Efficacy - pulmonary function (FEV<sub>1</sub> and FVC) increased clinically at all test measures within 2 hours after ATROVENT vs placebo evaluation of AUC (area under curve) of change from baseline curves for FEV<sub>1</sub> and FVC showed better results for ATROVENT over placebo. This data has been verified.

"Measurements of blood pH and arterial blood gases showed no marked changes from pretreatment levels or between ATROVENT and placebo". Please refer to statistical evaluation.

Safety - no clinically significant changes in BP or pulse rates or laboratory tests or adverse reaction - gastric distress was reported in a patient on placebo; no others.

Conclusion: Atrovent improved pulmonary function in patients with stable airway obstruction, but did not affect ventilation/perfusion status. This complete clinical trial is found, including case report forms, in volume 1.42 and Vol. 1.2, pp 7-147.

### Special Studies (continued)

2) Lyons, H.A., M.D., Brooklyn, N.Y.

Study No. E82 - 315

Protocol designation - Id<sub>2</sub> (same as study #1 above)

Date - Dec. 2, 1981

Atrovent metered dose inhaler (MDI)

Objective: As in study #1 above.

Protocol Id<sub>2</sub> in Vol. 1.2, pp 148-287 and expanded summary; also in Vol. 1.43.

This study had the same objective and followed the same protocol as that of Dr. Anderson as described in 1) above, these details will not be repeated here, however; the following details of this study are noted:

Number and types of cases - 13 patients, 11 M, 2 F, ages 29-65 entered; 12 completed the study.

### Results

Efficacy - with the exception of FVC at the 2-hour test, pulmonary function as measured by FEV<sub>1</sub> and FVC increased clinically after Atrovent over placebo. Efficacy evaluation for AUC (area under curve) of change from baseline for both FEV<sub>1</sub> and FVC showed better results for Atrovent vs placebo (verified).

Safety - no adverse reactions; no clinically significant changes in BP, pulse rates, nor lab. values were observed.

Conclusion: Atrovent improved pulmonary function in patients with stable airway obstruction without changing blood pH, arterial gases in blood and therefore did not change ventilation - perfusion ratios.

### 3) Bergofsky, E.H., M.D.

Study No. E-80-735

Protocol Designation - 1e 3b

Date: July 9, 1980

Title: Effects of Atrovent on mucociliary clearance in patients with chronic obstructive pulmonary disease (COPD).

Location in NDA - Vol. 1.44 pp. 1-93; Vol. 1.2 pp 288-354

Investigator: E.H. Bergofsky, M.D., Professor of Medicine, Health Sciences Center, Stony Brook, N.Y.

This study, in introductory remarks, notes that drugs with anti-cholinergic effects depress mucociliary activity in man. The study drug in this NDA, ipratropium bromide, is said to show no such activity in preclinical studies, that "tracheobronchial clearance of isotope-labeled particles before and after inhalation of ipratropium bromide did not show any reduction in clearance in healthy subjects or in-patients with bronchitis. The purpose of this study is to confirm that Atrovent does not depress mucociliary clearance."

The objective as stated is "to determine the effects of administration of Atrovent on mucociliary clearance and pulmonary function in patients with chronic obstructive pulmonary disease" (COPD). The protocol called for studying 20 adults with a diagnosis of chronic bronchitis or bronchial asthma. Duration was 2 test days. Appropriate records on patients were maintained. Inclusion criteria were acceptable, specifically regarding patients with mucous velocities greater than 3mm/minute in the trachea and 1.5 mm.min. in the main bronchs and/or whole lung clearance of 0.3%/min; male or female; ages 18-60. Exclusion criteria are found to be acceptable.

Drug Administration: Oral inhalation, Atrovent 2 whiffs of 20 mcg each or placebo. Duration will be single test doses (2) with at least 24 hours washout period between doses. Both test drugs (2) will be completed in one week.

The study was a crossover, double-blind, randomized design. Other medication and habits are stated and found appropriate for this type study.

Method of Evaluation:

History, PE, prior to admission. Baseline pulmonary function testing were done on admission (body plethysmography) to measure airway resistance (Raw), thoracic gas volume (VTG) and functional residual capacity (FRC), specific conductance (SGaw) were calculated (1/Raw/VTG); FVC, FEV<sub>1</sub>, FEF<sub>25-75</sub> and flow volume loops (HE O<sub>2</sub> vs air) at baseline and 5 minutes after 2 whiffs of Alupent (1.3 mg) from an MDI (metered dose inhaler); diffusing capacity measured by single carbon monoxide method). On each test day tracheal and bronchial mucous velocities were to be measured and specific appropriate data recorded for statistical analyses. Raw, VTG and FRC were measured at baseline and repeated once at 1-2 hours post-drug by body plethysmography and BP and pulse rates recorded at the same times.

### Adverse Reactions

Appropriate provisions were made for recording and evaluation by the investigator. Provision was made for signed informed consent. The expected completion date was set at six months after receipt of supplies.

In the clinical trial 11 males, ages 18-36 were entered, 10 completed the study. One patient, age 34, with a diagnosis of severe bronchial asthma received both test doses but was unable to complete the 4-hour breathing process on either day. His data were not included in the efficacy evaluation. There were 2 intrinsic (mild) and 8 extrinsic asthma cases (5 mild, 3 moderate); duration of disease was from 3 to 24 years. Method of study has already been adequately described, specifically under dose, route and duration of treatment. Administration of test drug was by metered dose inhaler (MDI), as Atrovent 40 mcg (20 mcg/whiff) - 2 whiffs or placebo, 2 whiffs; the aerosols were administered after inhalation of iron oxide particles.

Upon entrance to the study 3 patients were on S-R theophylline, which had been discontinued at least 12 hours prior to test dose administration on each of the 2 test days. One of these patients was also on Alupent tablets, twice a day. Five patients used beta adrenergic aerosol therapy prior to the study when necessary, none within 12 hours of entry. Observations before the study on each test day adhered to the protocol.

### Results:

Efficacy: The SGaw (Specific Airway Conductance), mean percent change over baseline at 1 hour was 103.3% increase after Atrovent and 9.2% after placebo. There were no significant differences in treatment effects in any of the variables measuring radio-tagged iron oxide particles from the lungs. At the end of 4 hours the mean percent of radiosotopically labelled mucous still present in patients receiving Atrovent was approximately 30% and 20% in those receiving placebo.

Safety - There was no significant change in mucociliary clearance velocity, but there was significant improvement in pulmonary function with no adverse reactions reported.

4) Wanner, A., M.D., Chief, Dir. Pulmonary Disease, Mt. Sinai Medical Center, Miami Beach, FLA.

"Effect of Atrovent on tracheal mucus velocity in patients with asthma."

Study No. E-80-789

Protocol Designation: 1e 3c

Date - July 1, 1980

Location in NDA - Vol. 1.44; Vol. 1.2

Objective - "To determine the effects of administration of Atrovent on tracheal mucous velocity and pulmonary function in patients with asthma and to compare these effects with placebo".

Fourteen adults with asthma were to be studied for 2 days. Criteria for inclusion and exclusion of patients were listed and found to be acceptable.

Drug administration - dose as in the previous study described

Type - Double-blind, 2-way crossover study.

Other medications and habits - same as study #3 described earlier; same for method of evaluation.

Plan of study: spirometry on each test day, FVC, FEV<sub>1</sub> and FEF<sub>25-75</sub> measured at baseline, 1/2, 1, and 2 hours after drug has been given. Tracheal mucous velocity was obtained by observation of "The motion of radiopaque discs of teflon mixed with bismuth trioxide deposited in the trachea and observed by a fluoroscopic image intensifier. The doses are blown through the inner channel of a fiberoptic bronchoscope which is located just above the vocal cords. Measurements of tracheal mucous velocity are conducted at baseline, 1/2 and 2 hours after drug administration." Vital signs are recorded at the same intervals as pulmonary function testing.

Number and types of cases - 14 patients, 5 males, 9 females; ages 18-46 entered and completed the study.

#### Results:

Efficacy - Atrovent produced no significant changes in tracheal mucous velocity compared to baseline and to placebo, although slightly higher values were seen after placebo. No clinically important changes in pulmonary function were detected in this study because these were mild cases of asthma and were not required to demonstrate stable or reversible bronchospasm upon entry to the study.

Safety - One patient on Atrovent showed wheezing and dyspnea immediately following measurement of tracheal mucous velocity. No vital signs were abnormal in any other patients.

Conclusion: Atrovent did not produce significant changes in tracheal mucous velocity when compared with placebo.

- 5) Norris, H.G., M.D., National Jewish Hospital and Research, Center, Denver, Colorado.

"To examine certain biochemical mechanisms which may be responsible for the effects of Atrovent on patients with asthma".

Study No. E-81-22

Protocol Design - 1 g 3

Date - October 3, 1980

Location in NDA - Vol. 1.45; Vol. 1.2 pp 419-490

This study attempted to determine if the blockage effects on methacholine-induced bronchospasm "produced by premedication with Atrovent is accompanied by a corresponding block of increase in plasma cGMP and also in urinary cGMP. Simultaneous measurements of plasma and urinary cAMP will be used to evaluate the balance between cyclic nucleotides of opposing effect before and after mecholy challenge." Therefore, there were two objectives of this study, which are:

- (1) To determine whether a single dose of Atrovent can block methacholine - induced bronchospasm in patients with asthma and;
- (2) To examine the role of cAMP, cGMP and prostoglandins as broncho-chemical mediators of the effects of Atrovent in patients with asthma."

Recent studies suggest significant increases in plasma cGMP (cyclic guanosin monophosphate) during the first few minutes after mecholy challenge, coincident with the onset of bronchospasm. Current evidence suggests that the effects of cholinergic agents are exerted in part through an increase in cGMP. Atrovent is an anticholinergic, bronchodilator drug. It has been shown that certain sub-populations of patients with bronchospasm respond better to anticholinergics than to any other class of drug. In certain subpopulations there are multiple mechanisms responsible for regulating bronchomotor tone. Two major factors, are chemical mediators and cholinergic innervation. Both beta adrenergic bronchodilators and theophylline decrease bronchial smooth muscle tone by increasing levels of cyclic AMP (Adenosine monophosphate). Anti-cholinergic bronchodilators theoretically decrease cyclic GMP (guanosin monophosphate), a bronchoconstrictor, but the importance of this mechanism has not been established. Resting bronchial smooth muscle tone is maintained through cholinergic vagal innervation which also acts as a pathway for protective reflex bronchospasm in response to irritating stimuli. The relative importance of these mechanisms differ, not only

between patients with chronic bronchitis and those with asthma, but also between patients with a given diagnosis, especially the asthmatic. In chronic bronchitis, the disease involves lung damage which develops secondary to smoking and other toxic substances. Loss of lung function as a result of this damage is not reversible, but pulmonary function can be improved by anticholinergic drugs which decrease resting smooth muscle tone and block reflex bronchospasm. Asthma is not a disease, but a syndrome of wide ranges of hyperactivity manifest by varying degrees of bronchospasm, mucous plugging, and bronchial wall edema of various etiologies in some asthmatics, such as those with extrinsic atopic disease. Chemical mediators released from a variety of cells and tissues by IgE and other mechanisms are the primary cause of this hyperactivity. In other patients, as with intrinsic asthma, sensitivity to industrial pollutants, and patients with pyogenic asthma, the role of mediators is not as clear. In either case, reflex bronchospasm may be present in varying degrees. The results of this complicated study showed for efficacy that Atrovent effectively blocked bronchospasm following methacholine challenge, while placebo did not in all 18 patients studied at the highest common methacholine dose. The mean percent changes from baseline for Atrovent were higher than those for placebo at all time points. The fall in FEV<sub>1</sub> was greater for placebo than Atrovent in all 18 patients.

No consistent pattern for the results for plasma cAMP, cGMP and thromboxane biochemical parameters were seen in patients administered Atrovent or placebo at the times recorded in this study.

Safety - ECG tests conducted on 10 patients before and after the study were reported as normal. There were no adverse reactions.

Conclusion - This study was designed to determine if a single dose of Atrovent could block methacholine - induced bronchospasm and to examine the role of cAMP and cGMP and thromboxane as biochemical mediators of bronchospasm. Atrovent effectively blocked methacholine induced bronchospasm in all 18 patients tested. "No consistent pattern of response was seen in the biochemical parameters and their role as biochemical mediators could not be stated".

- 6) Chodosh, S., M.D., Pulmonary Section, Ambulatory Care Center, Boston City Hospital, Boston, Mass.

"Evaluation of the effects of inhaled Atrovent on sputum in chronic bronchitis".

Study No: - E-81-38

Protocol - II d 2

Date - September 17, 1980

Location on the NDA - Vols 1.47, 1.48, 1.49, 1.50,  
Vols. 1.3, 1.4

Objectives: comparison of inhaled Atrovent to placebo on:

1. Sputum physical properties
2. Bronchial epithelium
3. Bronchopulmonary inflammatory level
4. Subjective and objective measures of the clinical status after seven weeks of daily administration in patients with stable chronic bronchitis.

In this study it is noted as an anticholinergic, Atrovent's pharmacologic action differs from the xanthine and Beta-Adrenergic Bronchodilators. Because of the cholinergic/anticholinergic influence on bronchial mucous production, it is important to note if Atrovent has an effect on this component of bronchial function. Also, since chronic long term use of inhaled Atrovent is anticipated, it is important to determine the effect of long-term use of Atrovent on bronchial mucosal epithelium and the response in the chronic bronchitic. The seven week duration of administration was to demonstrate the effect of such use of the drug compared to inhalation of placebo aerosol in the same patients in a double-blind study design.

#### PROTOCOL

Subjects - 24 adults, male and/or female with chronic bronchitis. Dropouts were recorded. Statistical analysis was done.

Diagnostic Criteria for inclusion - acceptable;

1. Verified chronic bronchitis
2. Age 18 and over
3. Stable to moderately severe airway obstruction ( $FEV_1 < 75\%$  of predicted normal)
4. Less than 10% eosinophilia in sputum
5. Consistent daily sputum volume of at least 5 ml/day.

Criteria for exclusion - acceptable

Other Medications and Habits - Acceptable; Other investigational drugs excluded. Other anticholinergics excluded. Concomitant oral corticosteroids allowed only if stabilized on minimal doses. Temporary increases in this type drug may be allowed under acceptable conditions described. Antihistamines,

cromolyn, acetylcysteine, expectorants, and inhaled steroids were excluded. Oral bronchodilators (xanthines and adrenergic agents) permitted but dosage was to remain constant. Aerosol bronchodilators allowed on an "as needed" basis. Daily use was to be recorded.

#### Method of Drug Administration

Route - oral inhalation

Dose - Each test dose was Atrovent - 2 whiffs of 20 mcg each q.i.d. or

Placebo - 2 whiffs q.i.d.

Provision was made for additional whiffs when necessary but limited.

#### Duration of Treatment

7 weeks continuous for each treatment separated by at least a one-week washout period.

Type of Control - double-blind, crossover study

Randomization - adequately described

#### Method of Evaluation

Visits to the laboratory by patients were to be weekly for the 7 weeks of the test period. Each of the two seven week test periods was to be separated by at least a week. During each of the 7 week test periods, patients routinely used either Atrovent or placebo aerosols in a sequence following the randomization code.

#### Pre-Admissions

Adequately described to achieve the objectives of this study. PE, CBC, SMA-12, urinalysis and EKG were repeated also at the end of each 7 week period. FVC and FEV<sub>1</sub> determined at baseline as well as cytology, Gram stains of sputum, and a chest x-ray were also done at baseline.

Observations to be carried out on each of the test days were described and found acceptable. They included the overall change of pulmonary status and any change was ranked by physician and patient by a 15% degree of improvement or worsening from very much better as +7 to very much worse as -7. Medication

history and any deviations each week were reviewed. Current subjective symptoms to be graded were listed and described in detail, in general concerned cough frequency, cough severity, difficulty raising sputum, sputum thickness, severity of dyspnea, character of wheeze, severity of background wheezing (symptom); wheezing attacks, upper respiratory, oropharyngeal and eye symptoms.

Physical findings described in detail in an appropriate manner included vital signs, chest examination for prolongation of the expiratory phase, rales, Ronchi, wheeze and intensity of breath sounds, all to be assessed in detail.

Upper respiratory examination was described adequately in detail in which head, eyes, nose and throat examination were to be carried out to detect any possible irritation from the aerosol and evaluated on a scale of 0 = normal to 6, 7, = markedly present observations for defects in sinus areas, nasal mucosa, oropharyngeal mucosa, conjunctivae. All the above were classified as subjective measurements to be made.

Objective measurements to be made are also described adequately in detail under:

Pulmonary function tests.  
Sputum measurements - 24 hour sample.

Adverse reactions were to be adequately recorded.

Data Submitted Regarding The Actual Conduct Of The Study And The Results Obtained.

The objective was to determine the effect of Atrovent in bronchial mucous production and to determine the effect of long-term use of the drug on bronchial mucosa epithelium and on bronchopulmonary inflammatory response; subjective and objective measurements were made in the long-term use by measuring clinical response to the Atrovent.

\*It should be noted that this NDA concedes certain populations with asthmatic and/or bronchitic bronchospasm respond better than Atrovent to bronchodilators such as sympathomimetic amines and xanthines, but the claim made is that certain sub-populations of these groups of patients respond favorably to Atrovent (Ipratropium bromide) as a bronchodilator.

In this clinical trial there were 19 patients entered, 14 males, 5 females, ages 48 to 86 years. Fifteen of the nineteen patients completed the study.

Inclusion and exclusion criteria mentioned above as satisfactory were followed. Diagnosis of patients was chronic bronchitis (4 mild, 10 moderate, 1 severe). Duration of illness was from 15 to 60 years.

Four patients became dropouts.

- 1 Due to side effect-on placebo, had "light flashes" over one eye.
- 2 Due to factors unrelated to therapy, 1 placebo, and 1 Atrovent patient hospitalized for intercurrent infection and "chest cold" respectively.
- 1 Due to protocol violation.

Method of Study - randomized, double-blind, crossover design with at least one week washout period between treatment crossovers.

Route - oral by metered dose inhaler (MDI)

Dose and Duration of Treatment

Atrovent, 40 mcg/q.i.d. or placebo 2 whiffs q.i.d., continuous treatment for each 7-week treatment period.

Single test doses of Atrovent 40 mcg (20 mcg/whiff) or placebo 2 whiffs upon waking on the first day of each weekly visit to the lab (7 weeks on each treatment) for examination.

Observations on Each Test Day - followed protocol; P.E. including vital signs, chest and upper respiratory examination for prolongation of the expiratory phase, rales, ronchi, wheezes and intensity of breath sounds of area of the lung were auscultated and graded. Use of concomitant medication; observations before and after study and details of upper respiratory examination also adhered to the protocol.

Objective measurements - Sputum sample, 24 hours, and 3 hours after drug administration as called for in protocol.

Subjective measurements - Data from patient diaries, cough frequencies and severity, difficulty in raising sputum and sputum thickness, severity of dyspnea, wheezing including background and the number of attacks. Upper respiratory, oropharyngeal and eye symptoms were recorded and graded for the 24 hour period prior to each test dose.

Safety - Onset and duration of adverse effects were recorded and whether or not, in the physician's opinion, they were drug-related.

Results - "Variables are presented in the statistical report in Appendices A, B, C and are represented by figures and graphs for selected variables. These provided the general pattern of response and also a visual assessment of the difference in the patterns for Atrovent and placebo." These are in volumes 1.3 and 1.4 for Division of Biometrics.

\*Interpretation of these statistical reports will be performed by our Division of Biometrics and will await their review and evaluation.

## Efficacy

### Sputum Parameters

Statistically regarding efficacy, the sponsor states "the only statistically significant difference between treatments on the sputum measurements was a significantly higher ( $< 0.035$ ) 24 - hour volume on placebo. This was due to an increasing sputum volume on placebo rather than a decrease in volume on Atrovent. (Figure 1, Volume 1.3). This will, as said, await review by our Division of Biometrics on this section which is not medical. "Viscosity varied inconsistently from visit to visit and also between Atrovent and placebo" "Stickiness also showed no consistent patterns". "Water Loss Rates showed no consistent patterns over the 7- week administration periods or between treatment groups. There were also no consistent changes in the dry weights of the sputum".

"Bronchopulmonary inflammatory level was unchanged over the 7- week administration period in both treatment groups as noted by the quantitative count of organisms per oil immersion field, sputum, protein, and cytology including both quantitative counts and qualitative differential counts. There were also no changes noted in the bronchial epithelial cells." These data are referenced and may be verified as part of the statistical, not medical report.

### "The Objective Observations"

The pulmonary function test results in this study, and the vital signs recorded on each test day "did not show any trends over the course of the study nor were there any significant differences between the drugs." Reference is made to a table which was interpreted by this reviewer. Mean overall values over 7 weeks showed no marked differences between Atrovent and placebo regarding SVC (L) (Standard vital capacity); FVC(L); FEV<sub>1</sub>(L); FEV<sub>25-75%</sub> L/sec.), pulse rate, systolic and diastolic blood pressures. The source data: Appendix B of the Statistical Report.

Regarding chest examination results for rales, ronchi, wheezes, intensity of breath sounds and prolongation of the expiratory phase of respiration were not affected by either form of therapy in the patients studied, 15 on Atrovent, 15 on placebo. Reference is again made to data in the statistical report, "Appendix H, Tables 140 to 144".

### Subjective Evaluation

This was obtained from diaries. No difference in cough frequency was noted except in one patient, nor were there notable differences in cough severity, difficulty in raising sputum, sputum thickness, severity of dyspnea, background wheeze, or the number of wheezing attacks. (Reference for the above is made to the statistical report, variables 17.1 - 20.3, regarding the two

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treatment groups, Atrovent and placebo. In the parameters of subjective upper respiratory oropharyngeal and eye symptoms during the 24-hour period prior to their weekly visits, patients' diaries showed no differences between Atrovent or placebo treatment groups.

Safety:

No changes in laboratory findings; no significant changes in ECG tracings over the 15-week period; adverse effects - only after continuous doses and did not interfere with treatment. They were (Table 3) - arrhythmia - 2 on Atrovent; dizziness, 2 on each treatment; rash - 1 on each; itch, 1 on Atrovent; G.I. - reported by 3 on Atrovent and by 2 on placebo; tremor-1 on Atrovent; eye-3 on Atrovent, 1 on placebo; oro-otolaryngeal -2 on each treatment. All refer to the statistical report.

In summary, a multiple-dose, double-blind crossover study comparing Atrovent to placebo over 7 weeks of treatment which showed no clinically significant differences in sputum, flows of any kind, bronchopulmonary inflammatory status, pulmonary function testing, physical findings on chest examination by the physician nor subjective evaluations by patients. Statistically, not medically "the only statistically significant finding was a greater sputum volume on placebo rather than a decrease in volume on Atrovent". This statement by the applicant must await verification by our Division of Biometrics following their review and evaluation of the statistical report in this study.

7) Kass, I., M.D. Professor of Medicine, University of Nebraska Medical Center

Burgher, L., M.D. Acting Chief, Pulmonary Division, University of Nebraska Medical Center, and

Daniels, W.C., Ph.D., Assistant Professor of Internal Medicine, University of Nebraska Medical Center.

Study No. E81-166

Protocol Designation II d<sub>2</sub> (same as preceding study).

Date - September 17, 1980

Location in NDA - Vols. 1.5, 1.6, 1.51 - 1.56

In the introduction to this study the applicant stresses the obvious, that is, that "because of the cholinergic/anticholinergic influence on bronchial mucous production, it is important to determine if Atrovent has an effect on this critical component of bronchial function. Since

Atrovent is administered as an inhalant and will be used chronically by patients it is also important to determine the effect of long-term Atrovent on the bronchial mucosal epithelium and the inflammatory response characteristic of the chronic bronchitic". The same applied to the immediately preceding study Chodosh, S., M.D. of Boston City Hospital.

The protocol for this study, protocol designation II d2, is the same as the preceding study (Dr. Chodosh) and will not be repeated here. The same applied to the study objective.

Twenty-five patients entered the study, 13 males, 12 females, ages 27-72 years and 23 patients completed it. Inclusion criteria stipulated that patients be ages 18-65, diagnosed as having chronic bronchitis and have a relatively mild to moderately severe, stable airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal. Patients had to have less than 20% response on FEV<sub>1</sub> within 30 minutes of 2 whiffs (1.3 mg) from an Alupent MDI, be unresponsive to a methacholine challenge and had to produce a consistent daily volume of sputum of at least 5 ml/day and the sputum, had to have less than 20% eosinophilia with the predominant inflammatory cell being neutrophil and/or macrophages. Exclusion occurred if patients had a significant systemic disease, active TBC, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to anticholinergic drugs; those with peptic ulcer requiring those drugs were excluded. Pregnant females or those who expected to become so during the study were excluded. Patients with a history of significant asthma, or who had a viral infection or febrile illness within the preceding month were excluded. Two patients dropped from the study; one in the placebo group moved out of State and 1 in the Atrovent group incurred an infection.

Twenty-three patients, 9 males, 14 females, ages 27-72, diagnosed as chronic bronchitics, 3 mild, 15 moderate, 5 severe with duration of illness 1-25 years completed the study.

Route - Oral inhalation by metered dose inhaler (MDI).

Dose and duration of treatment - continuous treatment of Atrovent 40 mcg q.i.d., or placebo 2 whiffs q.i.d. for each 7 week treatment period.

Regarding use of concomitant medication, no other investigational drugs nor anticholinergic drugs were used during this study. Oral steroids were taken by 6 patients and oral beta-adrenergic bronchodilators by the same number, and 9 took aerosols. Theophylline alone or in combination was taken by 15 patients and 1 took a Beta-blocker.

Observations before and after the study adhered to the protocol regarding history, P.E., vital signs, laboratory tests, ECG prior to admission. P.E., CBC, SMA-12, urinalysis, and ECG were performed upon completion of each 7-week treatment period. Cytologic and Gram stains of sputum, and if necessary, chest x-ray, and pulmonary function tests were done at baseline.

At each test day, P.E., chest examination and upper respiratory examination was performed. The chest examination was complete adhering to the protocol regarding prolongation of expiration, rales, ronchi, wheezes, and intensity of breath sounds were noted and graded. There was adherence to the protocol regarding the upper respiratory exam of head, eyes, nose, throat for possible aerosol irritation which was graded on an 8 point scale. Injection, erythema, edema and secretions were noted for nasal and oropharyngeal mucosa, tenderness and local edema for sinus areas and injection, erythema and exudate for the conjunctivae.

There were objective and subjective measurements as called for in the protocol recorded. Objective measurements included pulmonary function testing in the AM at the same time for each test day. Patients took no oral bronchodilators for at least 12 hours and no aerosols for 6 hours prior to laboratory reporting. Patients took a dose, (2 whiffs) of the assigned study medication upon awakening. Pulmonary function tests performed were SVC, FVC, and FEV<sub>1</sub> and FEF<sub>25-75%</sub> concomitantly with pulse and respiratory rates on appearing at the lab and at the end of each of the 7 weeks of treatment. On each test day, as called for, "the patient took 2 sputum samples to the lab containing (1) all the sputum collected over the 24-hour period just prior to the test day and (2) a container with all the sputum collected during the 3-hour period following inhalation of the test dose." The 24-hour, and 3-hour sputums were collected on the first day of each 7-week treatment period with prior use of any bronchodilator agent(s).

The 24-hour sputum sample was used to measure: "Viscosity, dry weight with water loss rates, volume, protein, Gram stain, cytology, qualitative and quantitative (cells/day)."

The 3-hour sputum sample was used to measure:

- (1) Viscosity;
- (2) Volume.

The subjective measurements taken from patient diaries included "cough frequency and severity, difficulty in raising sputum and sputum thickness, severity of dyspnea, wheezing with background and number of attacks." Upper respiratory, oropharyngeal, and eye symptoms, graded, were recorded for the 24-hour period prior to each test dose.

Safety - Complete recording was made.

### Results

Reference is first made to variables presented on the statistical report in Appendices A, B, and C to be reviewed and evaluated by our Division of Biometrics. Regarding efficacy for sputum parameters figure 1 of that report shows a higher sputum volume in 24 hours for 23 patients over 7 weeks of treatment from patients taking Atrovent over those on placebo. Viscosity varied inconsistently on both the 3-hour and 24 hour sputum samples in those on Atrovent and on those on placebo over the 7-week treatment period. (Variable 1.1 to 2.8, 9.1 to 10.8 in the statistical report). No consistent pattern was seen between the two treatments regarding water loss rates by the percent of sputum remaining nor on the dry weight of the sputum. (Statistical report, variables 3.1 to 3.7 inclusive). Bronchopulmonary inflammatory level did not change over the 7-week treatment period in either treatment group.

Objective observations by pulmonary function tests of SVC (standard vital capacity), FVC (forced vital capacity), FEV<sub>1</sub> (forced expiratory volume in the first second of expiration) and FEF<sub>25-75%</sub> (forced mid-expiration flow) was higher in patients administered Atrovent than in those administered placebo (statistical report, variables 22.1 - 22.4 - to be reported upon by our Division of Biometrics). There were no significant changes in pulse rate or blood pressure reported during the study. Physical examination of the chest for rales, ronchi and wheeze showed no consistent differences over time or between treatments. Prolongation of the expiratory phase of respiration was recorded as being slightly higher for Atrovent over placebo-treated patients, but was not clinically significant.

Subjective Evaluation - These data were from patient diaries and showed no differences between treatment groups for cough frequency, severity, difficulty in raising sputum, severity of dyspnea nor background wheeze clinically. There were no clinically significant differences in the number of patients reporting subjective upper respiratory, oropharyngeal and eye symptoms during the 24-hours prior to the weekly visits. (For statistical evaluation reference is made for the above subjective evaluations statistically to variables 15.1 - 19.2 and variable 3 and Table 5 of the statistical report for this study).

Safety - The investigator found no laboratory findings of clinical significance; the same applies for ECG tracings over the 15-week study. The only adverse reactions reported, occurred after continuous dose study and did not cause discontinuation of treatment. The adverse reactions reported were, of 24 treated patients, one case of headache in a patient on Atrovent, also one case of nausea and one case of "eye-burn" among patients on Atrovent. Among patients on placebo, there was 1 report of headache and nervousness and two reports of irritation from aerosol. No reports of the adverse reactions stated in patients administered Atrovent can be considered serious, and from this study the drug can be considered safe after 7 weeks of continuous administration.

In conclusion, this study demonstrated in a multiple dose, double-blind, crossover type study that when Atrovent was compared to placebo in comparable patients (and crossover 7 weeks), no differences in sputum flow, broncho-pulmonary inflammatory level, physical findings on chest exam, or subjective evaluations by patient or physicians were noted. Reference is made to the statistical report to be verified that "the only statistically significant finding was a greater sputum volume on placebo." Pulmonary function testing at all weeks of the study showed more favorable results in patients administered Atrovent over those administered placebo.

8.) Brown, S.I., M.D.

Department of Ophthalmology  
University of Pittsburg  
School of Medicine  
Pittsburg, Pennsylvania

Study No. E-74-213

Date - 3/28/74; Final Report - 5/26/83

Phase of Study - 1

Locations in the NDA - Vol. 1.7 pp. 1-68;  
Vol. 1.57

Objective of the Study - "To assess the effect of Atrovent on pupil size, accomodation, visual acuity and intraocular pressure in normal volunteers".

This single dose study to compare the effects on the eye of Atrovent vs placebo measured the following variables:

Pupil size  
Accomodation  
Intraocular pressures.

The study was of 2 days duration, placebo controlled, crossover. Ages of subjects were 20-35 years. All subjects had a normal ocular history and showed no abnormal signs upon complete ophthalmologic examination. The examination covered refraction, ocular motility, pupil size, reactivity to light, accomodation, biomicroscopy, tonometry and ophthalmoscopy.

Double-Blind Crossover - Medication

On each of 2 separate days, subjects received single test doses of either Atrovent, 2 inhalations (20 mcg/inhalation) or placebo 2 inhalations. The 2 mcg test doses were administered in random order at least 24 hour apart.

On each of the two test days, the tests noted above were measured before and 1, 3, 5 hours after administration of the test doses.

Adverse experiences on Atrovent included 2 cases of lightheadness, 2 headaches, 1 nausea, 2 blurred vision, 1 post-nasal drainage, 2 tired. On placebo there were reports of lightheadness in 2; headache, 3; diaphoresis in 1; flushing, nausea, blurred vision, dry throat, post-nasal drainage, nervous, 1 in each case and 3 reports of "tired".

Result - no significant differences on pupil size, binocular accommodation, tonometry and visual acuity were obtained on Atrovent as compared to placebo. Statistical analysis was performed.

9) Harris, L., M.D.

Cornell University Medical College  
New York Hospital  
New York, N.Y., 10021

Study No. E-73-709

Final Report - 5-26-83

Objective of Study - as above in 8) Dr. Brown's study.

Procedure, method of study, concomitant medication, observations made and times, all the same as #8 above, including medications.

Results - The effects of Atrovent vs. placebo in 30 normal subjects for pupil size determination, binocular determination, tonometry and visual acuity in serial tests following single doses of Atrovent or placebo showed no significant differences between the two treatments except for a slight increase in mean pupil size at 30 minutes following Atrovent. This change was too small to be considered clinically significant.

10) Gauthier, T., M.D., Trottier, M., M.D., and Chaput, R., M.D.

Study No. E-82-1861

Date of Final Report 7/8/83

Phase of study - I

Site - Hospital du Sacre-Coeur, Cartierville, Quebec

Location, in NDA - Vol. 1.7, pp. 286-359; Vols. 1.59, 1.60, 1.61, 1.62

As in the two previously described (8), (9) above, the principal thrust of these investigators was to study the effect of the anticholinergic agent Atrovent (Sch 1000) on the eye.

"Objective: To assess the effect of Atrovent on pupil size, accommodation, visual acuity and intraocular pressure in patients with chronic, reversible bronchitis who were also participating in a multiple-dose study to evaluate the bronchodilator effectiveness and safety of Atrovent." Note - unlike the two previous studies 8), 9) above patients with chronic bronchitis were studied in this trial rather than normal subjects.

Twenty-nine male and female patients, ages 17-67 years entered the bronchodilator study; 28 (1 less) of these patients also participate in the eye study. 4 patients dropped out leaving 24 for analysis in the single-dose study and 5 others were dropped leaving 19 patients who completed the multiple-dose study. Reasons for dropouts are listed in Table 1, 3 due to side effects; 1, factors unrelated to therapy; 2 due to treatment failures; 2 lost to follow-up; 2 for protocol violations, for a total of 9.

The studies were 2 double-blind, three-way, crossover studies: a single-dose study and a 45-day, multiple dose study. For each study patients were randomly assigned to one of six treatment sequences.

#### Medications

Single doses of Atrovent MDI 40 mcg, oral inhalation (20 mcg (whiff), Berotec, 5 mg (2.5 mg/tablet), and a combination of Atrovent MDI 40 mcg, plus Berotec tablets 5 mg. Placebo tablets and placebo aerosol were used to preserve blinding. At least 24 hours separated the 3 test doses which were administered prior to the starting of multiple dose study. Continuous treatment - Atrovent 40 mcg q.i.d. Berotec, 5 mg q.i.d. and Atrovent, 40 mcg plus Berotec, 5 mg q.i.d., each treatment self-administered by the patient for 45 days with evaluation on day 45 of each drug regimen.

#### Concomitant Medication:

No other investigational drugs were used. Of 28 patients entering the study of eye effect, 6 were on oral prednisone, 23 on inhaled steroids, 16 took theophylline.

Observations before the study are described and found acceptable. Observations on test days in the ophthalmic study measured before and 2 hours after each dose in the single-dose study: pupil diameter of each eye; accommodation (in duplicate) of each eye and both eyes together; intraocular pressure of each eye, and visual acuity of each eye. The same measurements were taken after 45 days of continuous treatment in the multiple-dose study. An ECG was measured at baseline and 1 hour after a test day on day 45. Adverse reactions were recorded.

### Results

Efficacy - pupil diameter decreased from day 1 to day 45 statistically but not of clinical significance for Atrovent. There were no significant differences between drugs at either timepoint on either day of test. Following 45 days of treatment there was "a slight statistically significant increase in pupil diameter of the right eye occurring at 2 hours following test dose, but not clinically significant and remained less than the Day 1 baseline".

#### Accommodation:

Small changes were noted but were not clinically significant in response to test doses or after 45 days of treatment.

#### Intraocular Pressure.

Small clinically insignificant decreases in intraocular pressure two hours after each test dose on both test days.

#### Visual acuity:

No statistical or clinically significant changes in visual acuity occurred.

### Safety

ECG tracings showed no significant changes after Atrovent over the 90-day period.

There were no adverse reactions after Atrovent., After test doses of Berotec, tremor (3 cases) and palpitations (1 case) and after continuous treatment with betrotec, 1 case of tremor was reported. "After administration of the Atrovent/Berotec combination, 1 arrhythmia was reported; 1 conduction disturbance; palpitations and supra-ventricular tachycardia after the test dose was reported and that patient dropped out of the study. Other adverse reactions after the combination test doses were headache (1); tremor (3). After multiple doses of the combination 2 patients had tremor."

Conclusion: In this study small, but not clinically significant eye changes were detected. Contrary to the pharmacologic effect expected from use of anticholinergic agents, pupil diameters and intraocular pressures decreased following test doses of the drug Atrovent.

The statistical report follows to be reviewed by our Division of Biometrics.

Reviewer's Comments - No significant number, nor serious adverse reactions were recorded on any of these ten special studies.

End - "Special Studies" - Ten. Each study protocol, objective, method of study, objective and subjective results obtained and locations on the NDA are identified. Statistical analyses were done. This completes "A) Special Studies".

B) Dose-Ranging Studies - 5 in number

- 1) Chervinsky, P., M.D.  
190 Hawthorn Street  
New Bedford, Mass., 02740

Study No. E-79-81; Location on the NDA-Vols. 1.8 + 1.63.(pp. 1-122).

Objectives:

"To confirm the results of the original dose-ranging studies. To determine the efficacy and optimal dosage of Atrovent when delivered by a metered dose inhaler (MDI) in a single dose, double-blind crossover study in adult patients with asthma. To compare the Atrovent dose response curve with that of isoproterenol and to placebo with particular emphasis on, onset and duration of action, side effects and adverse reactions, including effects on pulse and blood pressure."

Fourteen patients with asthma, reversible airway obstruction (increase in FEV<sub>1</sub> of at least 15% within 30 minutes of 0.150 mg of isoproterenol were called for in the protocol).

Age - 18-65 years: sex-male or female. Females of child-bearing potential were to be excluded. The aim was to study 14 completed patients (at least 4 doses of Atrovent, 2 doses of isoproterenol, 1 dose of placebo).

Appropriate records were kept.

A list of associated diseases which excluded patients was given and found appropriate.

A list of other drugs to be excluded was also stated and found responsible. Some might be administered under appropriate specific conditions such as steroids, and cromolyn, but not within 8 hours of testing procedures.

Type of control - double-blind, single-dose, seven way crossover.

Appropriate randomization.

Observations to be made:

Prior to study admission - history, P.E., Lab tests, ECG, evidence of an increase of at least 15% in FEV<sub>1</sub> after administration of isoproterenol.

Follow-up observations:

- 1) Flow volume curves to be obtained at baseline, 5, 15 and 30 minutes, and 1, 2, 3, 4, 5, 6 hours after drug administration.
- 2) Global evaluation - by physician and patient.
- 3) PE, CBC, ECG, urinalysis at the end of the study.
- 4) Pulse rate, blood pressure on each day at the same time of day, at the same time intervals as the forced expiratory volume, i.e., baseline, 5 minutes, 15 and 30 minutes and: 2, 3, 4, 5, 6 hours post-drug administration
- 5) Lead 2 EKG monitored at 5, 15, 30 minutes and 1, 2, 4 hours post-drug.
- 6) Side effects ) Adequately provided for  
7) Adverse effects or reactions) recording

Dose and Route:

By inhalation

Atrovent 20, 40, 60, 80 mcg or ug from MDI at 20 ug per whiff.  
Isoproterenol 75 and 150 ug from MDI dispensing 75 ug/puff

Placebo

Each dose consisted of 1 inhalation from each of 4 canisters as:

|                      | <u>Atrovent</u> | <u>Isoprotorenol</u> | <u>Placebo</u> | <u>Total</u> |
|----------------------|-----------------|----------------------|----------------|--------------|
| Atrovent 20 ug       | 1               | 0                    | 3              | 4            |
| Atrovent 40 ug       | 2               | 0                    | 2              | 4            |
| Atrovent 60 ug       | 3               | 0                    | 1              | 4            |
| Atrovent 80 ug       | 4               | 0                    | 0              | 4            |
| Isoproterenol 75 ug  | 0               | 1                    | 3              | 4            |
| Isoproterenol 150 ug | 0               | 2                    | 2              | 4            |
| Placebo              | 0               | 0                    | 4              | 4            |

Duration of treatment - until the 7- way crossover was completed with a washout period of at least 24 hours between each dose of drug.

Provision was made for written informed consent.

Summary of study.

Objectives - as stated above.

Cases - 14 patients, ages 22-66 with asthma were admitted and completed the study. All had reversible airway obstruction.

Method of study - as per protocol.

Dose, route, duration of treatment.

Concomitant medication.

Observations before and after the study.

Observations in each rest day.

Adverse reactions.

All of these followed the described protocol and recording.

Results

Efficacy - the adjusted mean area under FEV<sub>1</sub> curve for all doses of Atrovent was greater than for placebo. No significant dose-response relation was seen. The median duration of 15% increase on FEV<sub>1</sub> was 4, 3.5, 1.5 and 3 hours for the 20, 40, 60, 80 mcg doses of Atrovent respectively. This is shown on Table 1, summary of FEV<sub>1</sub> (L) responses in 14 patients, Vol. 1.8, page 8, source is Table 2 of the statistical report to be re evaluated by our Division of Biometrics.

Comparisons among Atrovent 40 mcg, isoproterenol 150 mcg and placebo in Table 2 and Fig 1 of this study showed Atrovent to be significantly better than placebo in AUC (area under the curve) for FEV<sub>1</sub> and for the peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. The areas under the curves are significantly greater than those for isoproterenol for both parameters and the peak effect was equivalent. The time to peak for Atrovent was within 30 minutes for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. Summary is in Table 2 and figure 1, Vol. 1.8, pp. 9, 10. Source - statistical report tables 2, 3, 4, 5, 6. For Atrovent, the onset of 15% increase on FEV<sub>1</sub> occurred within 15 minutes and the onset of 20% increase in FEF<sub>25-75%</sub> occurred within 5 minutes; the duration of effect was 3.5 and 4 hours for the two parameters respectively. For isoproterenol, the onset of effect in FEV<sub>1</sub> and FEF<sub>25-75%</sub> was 5 minutes with duration to 1.5 hours for both parameters. (Table 2) (Fig. 1).

Safety - There were no clinically significant changes in blood pressures seen after any test doses, including placebo. Slight decreases in mean pulse rates were observed after all test doses, including placebo, none clinically significant.

ECG trainings showed no changes, nor did lab findings in the study period. Adverse experience -1 patient complained of dry throat after Atrovent 80 mcg.

Conclusion: No significant dose-response relationship was detected. Based on the results of this and other doses range studies the 40 mcg dose was selected for the multiple-dose studies. Atrovent was better than placebo in AUC for FEV<sub>1</sub> and for peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and better significantly than isoproterenol in AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>.

Reviewer's comment - This section, verified, is followed by the statistical report to be reviewed and evaluated by our Division of Biometrics. Atrovent was shown to increase FEV<sub>1</sub> and FEF<sub>25-75</sub> in percent change over baseline in this clinical trial.

2) Schleuter, D.P., M.D.

Medical College of Wisconsin  
Milwaukee County General Hospital  
Milwaukee, Wisconsin

Study No. E-79-948

Locations in the NDA-Vol. 1.8 pp. 123-225  
Vol. 1.64

The protocol for this study was the same as that followed by Dr. Chervinsky, above 1) same objectives also.

Results -

Efficacy - The adjusted mean area under the FEV<sub>1</sub> curve for all doses of Atrovent was greater than that for placebo and isoproterenol. No significant dose-response relationship was seen. The median duration of 15% increase in FEV<sub>1</sub> was 6 hours after all Atrovent doses. Based on the results of this and other dose-range studies the 40 mcg dose was selected for use in multiple dose studies. Atrovent was significantly better than both isoproterenol and placebo and AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. The peak effect was significantly greater than placebo for both parameters and the time to peak for Atrovent was 60 minutes for both parameters.

Most patients on Atrovent showed the onset of 15% increase in FEV<sub>1</sub> and FEF<sub>25-75%</sub> occurred at 5 minutes, lasting 6 hours. For isoproterenol the onset was also 5 minutes and duration of effect for FEV<sub>1</sub> was 6 hours and for FEF<sub>25-75%</sub> 4 hours. Reference is made to the statistical report and is illustrated in Table 2 and Fig. 1.

Safety - Adverse experiences were reported in 3 patients, 1 of headache after Atrovent, 20 mcg, nervousness after Atrovent, 60 mcg and isoproterenol accompanied by dizziness; a third patient had a headache after Atrovent, 80 mcg.

Summary - no significant dose-response relationship was reported. Atrovent, 40 mcg was significantly better than placebo in AUC in peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for both parameters. The statistical report follows this section to be reviewed by biometrics.

3) Wilson, R.H.L., M.D.

Ward H-5  
Laquna Honda Hospital  
San Francisco, CA

Study No. E-79-961

The same protocol as that in 1) and 2) above was followed.

Objective - same - to determine optimal dosage of Atrovent in adult asthmatics and confirm results of earlier dose-ranging studies on which 40 mcg had been found to be safe and effective in long term trials.

The Results regarding efficacy and safety were the same as for study 2) above, i.e. Atrovent 40 mcg was significantly better than placebo in AUC and peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for both parameters. This section is followed by the statistical report to be reviewed by our Division of Biometrics.

4) Nadel, J.A., M.D.

University of California  
San Francisco School of Medicine  
Cardiovascular Research Unit  
San Francisco, CA 94143

Objectives - Dose-ranging studies same as those in 1), 2), 3) above.

Study No. E-80-3; Vol. 1.9 and 1.66.

Protocol - Same as that for 1), 2), 3) above except SGaw was measured.

Results

Efficacy - AUC for all doses of Atrovent was greater than that for placebo and increased linearly with dose ( $p=0.026$  - referenced to statistical report). The median duration of 15% increase in FEV<sub>1</sub> was 2, ① 3, and 3 hours for the 20, ④ 60 and 80 mcg doses of Atrovent respectively. The results of this study also provided a basis for 40 mcg dose of Atrovent in multiple-dose studies. Atrovent was significantly better than placebo in AUC for FEV<sub>1</sub> and SGaw. The AUCs are greater than those for isoproterenol for all 3 parameters.

FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw, but do not reach significance. (Reference is made to the statistical report which follows the clinical portion of this study). Peak effect was higher for isoproterenol in FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw, but not significantly so, the time to peak for Atrovent varies from 60 minutes for FEV<sub>1</sub> and SGaw to 120 minutes for FEF<sub>25-75%</sub>. For Atrovent in most patients the onset of 15% increase in FEV<sub>1</sub> or 20% increase in FEF<sub>25-75%</sub> occurred within 15 minutes and persisted for 1 hour for FEV<sub>1</sub> and 2 hours for FEF<sub>25-75%</sub>. For isoproterenol, the onset in FEV<sub>1</sub> and FEF<sub>25-75%</sub> occurred at 5 minutes and persisted for 1 hour for FEV<sub>1</sub>. Clinically significant median responses in FEF<sub>25-75%</sub> were not seen with isoproterenol.

Safety - Only adverse experiences consisted of increased sputum production reported by 3 patients on isoproterenol (2 after 75 mcg, 1 after 150 mcg) and 1 patient after placebo. Dry mouth/irritation were reported after Atrovent 20 mcg (1 patient) and placebo (1 patient).

Summary - A linear dose - response curve was demonstrated for Atrovent in this study. Based on the results of this and other dose-range studies the 40 mcg dose of Atrovent was selected for use in the multiple-dose studies. Atrovent 40 mcg was significantly better than placebo on AUCs for FEV<sub>1</sub> and SGaw.

Reviewers comment - This section is followed by the referenced statistical report. Atrovent clinically was superior to isoproterenol in raising FEV<sub>1</sub> and FEF<sub>25-75%</sub> from baseline in this clinical trial.

5) Sackner, M., M.D.

Chief Division of Pulmonary Disease  
Mt. Sinai Medical Center  
Miami Beach, FLA 33140

Study No. E 79-80; located in the NDA Vol. 1.9 pp. 137-317 and Vol. 1.67.

Atrovent - metered dose inhaler.

Objectives and protocol - same as those in 1, 2, 3, 4 above.

Results

Efficacy - the adjusted mean AUC for FEV<sub>1</sub> for all doses of Atrovent was greater than for placebo and increased linearly with dose. The median duration of 15% increase in FEV<sub>1</sub> was 1.6, 5, 5.3 and 4 hours for the 20, 40, 60, 80 mcg doses of Atrovent. Based on the results of this and other dose range studies the 40 mcg dose was selected for use in the multiple-dose studies. All other efficacy parameters showed the same beneficial effect of peak and duration of pulmonary function tests, FEV<sub>1</sub>, FEF<sub>25-75%</sub>, and SGaw as the preceding studies.

Results

Safety - Adverse experiences were reported in 2 patients (of 14). One complained of headache after each test doses including of placebo; and a second one who had coordination difficulties after Atrovent 40 mcg complained of dizziness after Atrovent 60 mcg and isoproterenol 75 mcg.

Summary - A Linear dose response curve was demonstrated for Atrovent at doses of 40, 60 and 80 mcg and the higher doses offered no significant clinical advantage over the 40 mcg dose. Based on the results of this and other dose-range studies the 40 mcg dose was selected for use in multiple-dose studies Atrovent was significantly better than placebo in AUC and peak effect for FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw and significantly better than isoproterenol in AUC for SGaw. The statistical report follows this clinical section.

Reviewer's Comments - In these five (5) dose-ranging studies by doctors P. Chervinsky, D.P. Schleuter, R.H.L. Wilson, J.A. Nadel and M. Sackner, Atrovent was better than placebo in AUC for FEV<sub>1</sub> and for peak effect for FEV<sub>1</sub> and FEF<sub>25-75%</sub> and significantly better than isoproterenol in AUC for FEV<sub>1</sub> and FEF<sub>25-75%</sub>. In two of the five studies a linear dose curve response was seen for Atrovent and in last 2 studies Atrovent was significantly better than placebo in AUC for FEV<sub>1</sub> and SGaw, and in one of these (Sackner) also for FEF<sub>25-75%</sub>. Adverse reactions were reported in a very small number of patients and were not serious. Thus pulmonary function was increased in those patients with pulmonary disease and these studies formed the basis for the 40 mcg dose of Atrovent in multiple-dose studies. The drug was found to be safe and well tolerated. This completes dose - ranging studies.

C) Controlled Studies (COPD Patients) - 14 in Number - Chronic Obstructive Pulmonary Disease (COPD)

1) Multicenter Study - Protocol II b 5

Date May 16, 1980; In NDA Vols. 1.10, 1.11; 1.68-1.73

Investigators:

- a) David Cugell, M.D. Head Pulmonary Medicine Department of Medicine, N.W. School of Medicine, Chicago, Illinois, and Nicholas J. Gross, M.D., Section Chief, Hines V.A. Hospital, Hines, Illinois E-80-764
- b) Cemil Emirgil, M.D., Head, Pulmonary Disease Lab. Lawrence J. DeLorenzo, M.D./G. McQuine, M.D. Westchester County Medical Center Valhalla, N.Y. E-80-665

- c) Dennis Niewoehner, M.D., Chief Pulmonary Disease Section  
VA Medical Center  
54th and 48th Ave., S., Minneapolis, Minn. E-80-668
- d) Alan Plummer, M.D., Pulmonary Disease  
Emory University Clinic E-80-667  
Atlanta, GA

7 investigators  
4 centers

Locations in the NDA-

- a) Vols 1.10, 1.11, 1.68 through 173.
- b) 1.10, 1.11, 1.74, 1.75, 1.76
- c) 1.10, 1.11, 1.77, 1.78, 1.79, 1.80, 1.81
- d) 1.10, 1.11, 1.82, 1.83, 1.84, 1.85

The protocol for this multicenter study:

Objective: To determine bronchodilator efficacy of Atrovent MDI, measured after a single dose (day 1) in comparison with Alupent regarding onset, and duration of action, the effects of continuous dosing and patient acceptance of the two drugs. There was a 90-day treatment period.

Four centers took part on this study, a, b, c, d, above admitting 97 patients; 89 males, 8 females, ages 45-79 years with an average of 62.1 years. 83 completed 90 days of treatment. Statistical report was done. Inclusion criteria included ages 40-75 with a diagnosis of chronic obstructive pulmonary disease (COPD) with a history of smoking more than 20 pack years, mild to moderately severe airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal and FEV<sub>1</sub> less than 70% of FVC; total lung capacity (TLC) greater than 80% of predicted normal, reversible bronchospasm shown by a 15% improvement in FEV<sub>1</sub> within 30 minutes following 2 whiffs (0.150 mg) of isoproterenol from an MDI. Exclusions: significant systemic disease, active TBC, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to sympathomimetic amines or anticholinergics. (Atrovent [ipratropium, is an anticholinergic drug]). Pregnant females, or those expecting to become so, were excluded during the study. Patients with a history of asthma, allergic rhinitis, or atopy, or who had a recent viral infection or febrile illness including URI were excluded. Patients with elevated IgE serum levels or total blood eosinophilia above normal were to be excluded; however 19 patients receiving Atrovent and 23 receiving Alupent had elevated IgE levels. An analysis was made to determine what effect, if any, this may have on interpretation of the principal efficacy evaluations. On all test days this analysis gave no evidence of interaction between treatment and

elevated IgE. (See statistical report). Thirteen patients failed to complete the study (3 received treatment for 7 days or less), 7, patients from 8 to 39 days and 3 patients from 40 to 71 days). The distribution of patients lost is shown in Table 1 below (taken from statistical report):

Table 1  
"Distribution of Patients Lost, Dropped or Withdrawn"

|                                | <u>Atrovent</u> | <u>Alupent</u>  |
|--------------------------------|-----------------|-----------------|
| <u>Total</u> lost or withdrawn | 6               | 7               |
| Side effect                    | 2               | 2               |
| Lost to follow-up              | 1               | 1               |
| Lack of efficacy               | 1               | 2               |
| Protocol violations            | 0               | 1               |
| Other (Related illnesses)      | <u>2</u>        | <u>1</u>        |
| Total =                        | 6               | + 7 = <u>13</u> |

The patient's diary was appropriately completed as a recording of daily medications used, daily assessment of wheeze, cough, tightness and shortness of breath and ability to exercise or perform usual activities.

#### Results (COPD-1)

Efficacy - The mean FEV<sub>1</sub> baselines on day 1, before any drug administration were significantly higher for the group randomized to Atrovent than for those randomized to Alupent and remained so when recorded at days 45 and 90. In order to account for this initial difference in baselines all treatment comparisons were adjusted using the day 1 baseline as covariant. This topic was discussed (Meeting 10/17/83 with our Division of Biometrics). The adjusted mean areas under the curves and the adjusted mean peaks for FEV<sub>1</sub>, FEF<sub>25-75%</sub>, FVC and SGaw are greater for Atrovent over Alupent on all test days (Table 2, Vol. 1.10, 19 15). The time profiles are shown for the adjusted means in figures 1-4. The means shown are greater on Atrovent than on Alupent for all parameters at all timepoints except at 15 min. for FEV<sub>1</sub> and FEF<sub>25-75%</sub> on Day 45. Clinically significant improvements on pulmonary function as measured by 15% or greater in FEV<sub>1</sub> and FEF<sub>25-75%</sub> on Day 1 with Atrovent occurred at 15 to 30 minutes, reached a peak in 1 to 2 hours and persisted for 3 to 4 hours in most patients. We are referred to figures 5 and 6 pages 20, 21 for this. In the opinion of this medical reviewer, awaiting statistical evaluation, figure 5, as 20, Vol. 1.10 shows little difference in FEV<sub>1</sub> percent change from baseline on days 1 or 90 but does favor Atrovent over Alupent.

Safety - Mean pulse rates were lower than the baseline by 2 to 8 beats/minute at all timepoints after Atrovent and by 0-3 beats/minute up to 3 hours after Alupent. These are of no clinical significance. There were no lab changes noted in this study nor were there EKG changes over the 90-day period.

Adverse reactions on continuous therapy were 49 on Atrovent and 48 on Alupent. Those on Alupent were slightly more severe than in those on Atrovent.

Initially results of pulmonary function tests in the Atrovent-treated patient group were better than those patients on Alupent and they remained consistently better from day 1 through day 90 for FEV<sub>1</sub>, FEF<sub>25-75%</sub>, FVC, SGaw as well as the mean percent change from baseline over the covered period of observation. While improvement in FVC changes in patients on Atrovent was markedly better than in patients on Alupent, changes in FEV<sub>1</sub>, FEF<sub>25-75%</sub> were not as impressive.

Adjusted mean FEF<sub>25-75%</sub> from day 1-45-90 periods showed no impressive changes for Atrovent over Alupent nor for changes in SGaw. Clinically the recorded changes were not impressive. Statistically, mean adjusted results were impressive to the statistician's report submitted by the sponsor. Final determination of this conclusion will await analysis by our Division of Biometrics. Medically, the results of Atrovent treatment is marginal in degree over the Alupent treatment results in the periods Days 1-45-90.

From a medical viewpoint these studies showed Atrovent to improve adjusted mean FEV<sub>1</sub> on day 1 over Alupent; the same applied to days 45 and day 90; Atrovent also was superior to Alupent in increased FVC on days 1, 45, and 90. For FEF<sub>25-75%</sub>, adjusted mean values on day 1 Atrovent was shown to be superior to Alupent; on day 45, Atrovent was superior to Alupent, hours 1, 2, 3, 4, but not on hours 5 and 6. On day 90 Atrovent was superior to Alupent at all 6 hours. For adjusted SGaw mean on days 1, 45 and 90, no change clinically was seen between Atrovent and Alupent. For FEV<sub>1</sub> mean percent change from baseline, there was little or no clinical difference between the results obtained on Atrovent and on Alupent at days 1 and 90 (day 45 is not charted). For FEF<sub>25-75%</sub> mean percent change from baseline clinically on days 1 and 90 showed Atrovent to be superior to Alupent. (Day 45 not changed).

No serious adverse reactions were report either in number nor type.

The conclusion medically from this multicentric study is that safety and efficacy of Atrovent has been shown to be comparable to that of Alupent from a therapeutic standpoint. Prevention of bronchoconstriction has not been demonstrated in these data.

2) MDI 90-Day Multi-Center Study

iii-a-10 COPD

Protocol - III a 10; location on NDA; Vols 1.12, 1.13, 1.14,  
1.86-1.110-1.40

Date - July 6, 1981 - 11 studies

Objective - same as that of 1) above, i.e. to determine efficacy of Atrovent, in comparison with Alupent. Dosing, repeated over 90 days for efficacy and safety and patient acceptance of Atrovent in comparison with Alupent.

Subjects - more than those of 1) above 160-220 with a diagnosis of chronic bronchitis.

Diagnostic criteria for exclusion and inclusion were the same as for 1) above.

Method of Drug Administration, dose and route, as for 1) above including continuous treatment.

Duration of treatment - 2 week washout period and 90 days of continuous therapy.

Method of Randomization. Other medication, and habits, and those bronchodilators which may be administered for control of acute attacks during the 90-day study period, same as 1) above, i.e. SQ epinephrine or Terbutaline, i.v. aminophylline, rectal aminophylline. Patient daily diary recorded type and dosage of all medication taken concomitantly; frequency and dosage daily of study drug.

Method of Evaluation

Pre-admission  
washout period  
Observations to be made - pulmonary function tests  
Physician's global evaluation  
Patient daily diary  
Patient evaluation  
Theophylline level determination  
Adverse reactions

Are all the same as study protocol 1) above.

There are eleven (11) investigators and co-investigators: (1. #11 deleted due to misinterpretation of protocol, Dr. Goldring)).

- 1) Kunar Ashutosh, M.D., VA Hospital, Syracuse, NY.
- 2) E.R. Blecker, M.D./E.G. Britt, M.D., Division of Pulmonary Medicine, The Johns Hopkins University; The Baltimore City Hospitals, Baltimore, MD.
- 3) J.W. Cumminskey, M.D., Asst. Professor of Medicine, University of California at Davis, VA Medical Center, Martinez, CA.
- 4) Gary W. Gross, M.D. Dallas Tx.
- 5) Nicholas Gross, M.D., Chief pulmonary Medicine, Hines VA Hospital, Hines, IL.
- 6) S. Laksgminarayan, M.D., Chief Pulmonary Medicine, Seattle Veterans Hospital, Seattle, WA.
- 7) M.J. Miller M.D./A Kotch, M.D., Respiratory Therapy Center, Danbury Hospital, Danbury, CT.
- 8) Attilio Renzetti, M.D., University of Utah Medical Center, Salt Lake City, UT.
- 9) Marvin Sackner, M.D., Chief Pulmonary Disease Division, MT. Sinai Medical Center, Miami, Beach, FL.
- 10) D. Tashkin, Professor of Medicine, Department of Medicine, UCLA School of Medicine, Los Angeles, CA.
- 11) Goldring, R., M.D., NYU, Bellevue Hospital New York, N.Y.,  
Misinterpretation of protocol. Note this study was analyzed separately.  
Protocol violation concerning washout period.

The objective, as stated is the same as study 1) above.

Ten centers participated in this study admitting 164 patients, 83 on Atrovent, 81 on Alupent. 129 males and 35 females, ages 37-75 were admitted and 122 patients completed the study with 90 days of treatment. (Thirteen more were in progress when the statistical report was made and 29 had dropped out. Inclusion and exclusion criteria were the same as study 1) above, namely chronic bronchitics and reversible bronchospasm who showed a 15% improvement in FEV<sub>1</sub> within 30 minutes following 0.150 mg of isoproterenol by MDI. Four patients were included in this study who had FEV<sub>1</sub> increases of 14%, 13.6%, 13% and 14% after isoproterenol challenge, and 1 patient whose FEV<sub>1</sub> was 74.5% of his FVC.

Patients were excluded for the same reasons as given in study 1) above.

Twenty-nine patients, as stated failed to complete the study. The reasons for dropout are given in Table I below:

Table I

Reasons for patients lost, dropped or withdrawn  
(Tables 1 and 2 of the statistical report)

|                                  | <u>Atrovent</u> | <u>Alupent</u> |
|----------------------------------|-----------------|----------------|
| Total Loss, dropped or withdrawn | 8               | 21             |
| Side effect                      | 1               | 4              |
| Loss to follow-up                | 3               | 4              |
| Lack of efficacy                 | 0               | 6              |
| Protocol violation               | 2               | 4              |
| Other                            | 2               | 3              |
|                                  | <u>8</u>        | <u>21</u>      |

The study was double-blind, parallel design following a 2 week washout.

Route - oral inhalation by metered dose inhalation (MDI)

Dose and Duration: continuous treatment with Atrovent, 40 mcg. q.i.d. or Alupent 1500 mcg q.i.d. self-administered by patients for 90 days. Additional whiffs were permitted under rigid conditions found acceptable.

Single test doses of Atrovent 40 mcg (2 whiffs, 20 mcg/whiff or Alupent 1500 mcg, 2 whiffs 750 mcg/whiff) on days 1, 45 and 90.

Concomitant medication was the same as that provided for study 1) above.

Observations before and after the study were also the same as study 1) above including a 2- week washout period prior to the study, and observations on test days 1, 45 and 90, except in this study it was not specified that SGaw would be done.

### Results

Efficacy - In this study, unlike study 1) above the mean FEV<sub>1</sub> baselines on day 1 obtained prior to administration of any treatment were lower (not higher) for the group randomized to Atrovent than for the group randomized to Alupent. In order to account for this difference initially, difference in baselines, all treatment comparisons were adjusted using the day 1 baseline as a covariant. (Refer to statistical report).

The adjusted mean AUC and adjusted mean peaks for  $FEV_1$ ,  $FEF_{25-75}$  and FVC, as in study 1) above were greater on days 1 and 45 with the exception of  $FEF_{25-75}$  day 1 adjusted mean peak reaching statistical difference shown in Table 2, Vol. 1.12, pg 16. Time profiles of the adjusted means shown in figures 1 to 3 are greater for Atrovent than for those on Alupent ( $FEV_1$  day 1, day 45, day 90); adjusted mean FVC on day 1, day 45, day 90; adjusted mean  $FEF_{25-75}$  on day 1 (close), day 45, day 90.

Clinically significant improvements in pulmonary function as measured by 15% or greater increase in  $FEV_1$  and  $FEF_{25-75}$  occurred within 15 minutes, reached a peak in 1-2 hours and persisted for 3-5 hours in most patients on Atrovent. The  $FEV_1$  mean percent change from baseline on day 1 and day 90 was shown to be greater for Atrovent than for Alupent (figure 4), and figure 5 shows the same advantage of Atrovent over Alupent in the  $FEF_{25-75}$  mean percent change from baseline on day 1 and on day 90.

Reviewer's comment: The above changes are more pronounced in favor of Atrovent over Alupent in this study than those shown in study 1) above.

Safety - The mean pulse rate changes on both drugs, laboratory findings and ECG tracings were the same as those reported in study 1) above, namely none of clinical significance. Adverse reactions were reported by 5 patients after Atrovent (single instances of nervousness, cough, GI distress and 2 with dizziness) and by 3 patients after Alupent, 1 each of headache, dizziness and exacerbation of symptoms, following continuous therapy.

Reviewer's comment - These two multicenter studies may essentially be combined as showing Atrovent produced better pulmonary responses than Alupent, peaking in 1-2 hours and lasting 3-5 hours. This study more definitely than 1) favors Atrovent over Alupent with no significant incidence or severity of side effects.

A separate study in this group of chronic obstructive pulmonary disease (COPD) was conducted by R. Goldring of N.Y.U. at Bellevue Hospital but is not included in this review at this point due to a misinterpretation of the protocol by the investigator.

These studies showed Atrovent to be superior to Alupent on results obtained on  $FEV_1$  elevations on adjusted mean  $FEV_1$  on days 1, 45, and 90: the same for adjusted mean FVC on days 1, 45 and 90 and for adjusted mean  $FEF_{25-75}$  on days 1, 45 and to a lesser degree on day 90 in favor of Atrovent.

On the mean percent change from baseline (not adjusted) for  $FEV_1$  on days 1 and 90, clinically, Atrovent was superior to Alupent. The same applied for the mean percent change from baseline for  $FEF_{25-75}$  on days 1 and 90. These results were achieved without significant numbers nor severity of side effects.

Concludes controlled studies in chronic obstructive pulmonary disease, 2 of 2 at this point in the NDA.

D. Controlled Studies (Asthmatic Patients)

1. asthma

Multicenter study - II a 5; 5 studies

Location in NDA - Vols. 1.14, 1.16, 1.17, 1.18, 1.112-1.141

Investigators

- 1) W.W. Busse, M.D./R.K. Bush, M.D., University of Wisconsin, Madison, WI
- 2) C. Falliers, M.D., Allergy and Asthma Clinic, Denver, Colorado.
- 3) J.D. O'Hollaren, M.D., Portland Medical Center, Portland, Oregon.
- 4) W.J. Storms, M.D., Allergy Associates, P.C., Colorado Springs, CO
- 5) J.C. Weg, M.D. Professor Internal Medicine Physician in Charge, Pulmonary Division University of Michigan, Ann Arbor, Michigan
- 6) Kirk, B.W., M.D. Health Science Center, Winnipeg, Manitoba, Canada.

Objective of Multicenter Study

"To determine the efficacy and safety of Atrovent inhaler with emphasis on onset and duration of action, the effects of continuous dosing (persistence of bronchodilating effects; side effects, adverse reactions and laboratory studies), patient acceptance of Atrovent in comparison with Alupent".

Cases, number and type studied:

In these 5 centers 82 males and 82 females (164 total) between ages 13-50 years participated in the studies. 144 completed 90 days of treatment and 4 were in progress when the statistical report was prepared.

Inclusion criteria - acceptable, included patients to be ages 12-50; have a substantiated diagnosis of asthma (Bronchial Asthma Society Criteria); have a relative stable mild to moderately severe airway obstruction with an FEV<sub>1</sub> less than 75% of predicted normal, and FEV<sub>1</sub> less than 70% of FVC (forced vital capacity); and have reversible bronchospasm demonstrated by a 15% improvement in FEV<sub>1</sub> within 15 minutes following 2 whiffs. (0.150 mg) of isoproterenol from a MDI (metered dose inhaler). Exclusion criteria were - significant systemic disease, prostatic hypertrophy, bladder neck obstruction, known narrow-angle glaucoma, hypersensitivity to sympathomimetic amines or anticholinergics. Pregnant females or those expecting to become pregnant during the study period were excluded. Those who smoked regularly during the past year and patients with chronic sputum production were excluded. Those with a smoking history of less than 10 pack years who had discontinued smoking more than 1 year prior to start of the study were included.

16 patients failed to complete the study:

Reasons

|                    | <u>Atrovent</u> | <u>Alupent</u> |      |
|--------------------|-----------------|----------------|------|
| Side effects       | 0               | 3              |      |
| Lost to follow-up  | 3               | 2              | -    |
| Lack of efficacy   | 6               | 1              |      |
| Protocol violation | 0               | 1              |      |
| TOTAL              | 9               | 7              | = 16 |

The study was in each case randomized, double-blind, parallel design following a 2 week washout.

The route was oral by metered dose inhalation. (MDI)

Dosage and Duration:

Continuous treatment - Atrovent 40 mcg q.i.d. or Alupent 1500 mcg. q.i.d. self-administered for 90 days. Provisions were made for reasonable additional whiffs and recorded.

Single test doses of Atrovent 40 mcg (20 mcg/whiff) or Alupent 1500 mcg (750 mcg/whiff) were also administered.

Regarding concomitant medication, other investigational drugs or anticholinergics were excluded. Of 16 patients on Atrovent and 20 on Alupent who took oral steroids, 13 and 17 respectively were on minimal doses (10 mg or less of predisone daily or 20 mg or less every other day). One patient on Atrovent started on prednisone during the study and 1 on Alupent who was not on steroids when the study started, took a course less than 5 days during the study. Their data were used. The data from 2 patients in each group whose steroid dosages varied were not used. 19 patients in each treatment group took inhaled steroids which were permitted if the dose was stabilized during the complete study including the washout period. One patient on Atrovent used steroid by inhalation only during the washout period, this case was included in efficacy analysis. Efficacy data from day 45 of another patient on Atrovent were not used in analysis because of non-stabilization of steroid dosage. Stabilized doses of cromolyn were used by 1 Atrovent patient and 3 Alupent-treated patients. The only medication allowed as a bronchodilator during the 2 week washout period before the study was theophylline, anhydrous. Theophylline tablets were permitted up to 12 hours prior to testing during the study. Short periods of use of inhaled beta-adrenergic bronchodilators were used by 6 patients on Atrovent and 3 on the Alupent group.

Observations Before and After the Study included history, P.E., lab tests, EKG prior to admission. The P.E., CBC, SMA-12, urinalysis and the lab tests and EKG were repeated at the end of the study. Each patient was tested in the A.M. on the qualification day. No bronchodilators were used for at least 12 hours prior to pulmonary function testing. There was, as noted, a 2-week washout period prior to the study. Following qualification, the patient underwent a 2-week washout period during which no beta-adrenergic drugs were taken with the exception of 3 patients assigned to the Atrovent group (2 of the 3 were dropped from the study and the third was continued since 1 whiff of salbutamol inhaler had been used on 4 occasions) and 4 patients assigned to the Alupent (all 4 were dropped from the study).

Observations During the Study: Excluded for at least 12 hours prior to the start and for the duration of the testing period were all bronchodilators, sympathomimetic drugs and antihistamines. Pulmonary function testing was done at the same time of the day in each individual. FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub> and PEFR (peak expiratory flow rate) were determined at baseline, 5, 15 and 30 minutes and 1, 2, 3, 4, 5 and 6 hours after drug administration. Pulse rates and blood pressure readings were taken and recorded at the same intervals as pulmonary function testing. A 2 minute resting period was allowed after pulmonary function testing and pulse and blood pressure determinations were made. At each visit the patient's diary was reviewed and summarized on the case report. The entries on these diaries included a record of daily medications, assessment of wheezing, cough, tightness and shortness of breath and ability to exercise or perform usual activities. Onset, severity and duration of any side effect was recorded as it occurred with the investigator's opinion as to drug relationship.

### Results

Efficacy - Reference is made to the statistical report. The mean percent changes in FEV<sub>1</sub> for all patients showed slight differences if any, favoring Alupent on day 1, Atrovent on day 45 and Atrovent on day 90. The clinical significance of these results is not impressive but rather show both treatment groups to respond to much the same degree. "Patients were later classified as responders or non-responders based on whether or not they achieved at least a 15% increase over baseline in FEV<sub>1</sub> within 2 hours following the test dose on day 1. Forty of 78 patients, or 51% of the patients on Atrovent and 56 of 77 patients or 73% of the patients on Alupent were responders. There were no significantly different areas under the curves or peak effects for any parameter on any test day between the drugs." For FEV<sub>1</sub> and FEF<sub>25-75%</sub> the responses were greater for Alupent on day 1 and for Atrovent on days 45 and 90. On test days the FVC responses were greater, but only slightly for Atrovent on day 1, the same as Alupent on day 45 and less on day 90. "Statistically the only factor which consistently correlated with responses was percent of predicted FEV<sub>1</sub> with greater responses seen in patients whose percent of predicted FEV<sub>1</sub> was lower. further analysis indicated that although all patients had an FEV<sub>1</sub> less than 75% of their predicted normal on the qualification day, many patients had an FEV<sub>1</sub> well in excess of this level at baseline on day 1." (Applicant's wording).

The applicant further states: "Twenty-five patients on Atrovent (mean predicted percent 88.9%) and 27 patients on Alupent (mean percent of predicted 89.1%) had an FEV<sub>1</sub> greater than 75% of predicted on day 1. An analysis was made excluding these patients. It would be expected that this would be a more reliable analysis since the patients who were excluded did not show a response because they did not have a significant degree of bronchospasm. When this was done 90% of the patients on Alupent responded as would be expected, but the percentage of Atrovent responders only increased to 60%." This is not a favorable finding for the Atrovent NDA.

"Analysis of the FEV<sub>1</sub> data on these responders with an FEV<sub>1</sub> less than 75% of their predicted normal at baseline on day 1 was similar to that seen in the responder group as a whole". Reference is made to the statistical report on this multicenter study for the above results and this review should await analysis of the data by our Division of Biometrics. Adjusted PEF<sub>R</sub> (Mean) on days 1, 45 and 90 for responders was superior for Atrovent over Alupent. (Vol. 1.15, p9 19, fig. 7). Persistence of bronchodilating effect was slightly diminished in many cases. (See recommendations.)

Safety - None of the changes in mean pulse rates after Atrovent or Alupent, nor the blood pressure readings were clinically significant. There were no clinically significant changes in laboratory findings, and the same applies for ECG. Adverse experiences after test doses of Atrovent were 2 cases of "nervousness"; 1 case of a patient with "headache and cough" and single cases of headache, dizziness, cough, chest tightness. Following test doses of Alupent one patient had weakness and dizziness and another was nervous. Following continuous treatment there were 4 cases of nervousness after Atrovent and 13 cases after Alupent. These were the most frequently reported adverse experiences. In addition, there were 13 patients with cough or chest tightness (exacerbation of symptoms) after Atrovent, and 2 patients with cough after Alupent treatment.

In summary this multicenter study Atrovent was compared to a beta-adrenergic bronchodilator, Alupent in a 90-day double-blind study in non-smoking asthmatics. The overall data analyzed showed that "only about 50% of these patients responded to Atrovent and therefore, analysis of the onsets, peak effect and duration of action in the group as a whole was inappropriate. In the group who showed a 15% increase in FEV<sub>1</sub> within 2 hours on day 1, significant improvement in pulmonary function occurred within 15 minutes, reached a peak in 1 1/2-2 hours and lasted for 3 to 6 hours in the majority of patients." (Applicant's wording). There were more (73% responders) to Alupent than to Atrovent (51%).

(Statistical Data)

Reviewer comment - Fig. 3, pg 14, Vol. 1.15 shows for adjusted mean FEV<sub>1</sub> on days 1, 45 and 90 for all patients there was little clinical difference in FEV<sub>1</sub> during 6 hours on these days between patients on Atrovent and those on

Alupent. For the adjusted mean FEV<sub>1</sub> day 45 and day 90 there were respective differences at all 6 time intervals on those days clinically favoring Atrovent over Alupent, principally on days 45 and 90 (Vol. 1.15, Fig 4, pg 16). For Figure 5, pg. 17 adjusted mean FVC for responders. Days 1, 45 and 90 shows very little difference between Atrovent and Alupent results achieved. In fig. 6, pg 18, Vol. 1.15, adjusted mean FEF<sub>25-75%</sub>. For responders on days 1, 45, and 90 all 3 days show Atrovent favored over Alupent in this parameter except on day 1 at hour 5 in figure 7, pg 15, Vol. 1.15 adjusted mean PEF<sub>R</sub> for responders Atrovent is shown to be superior to Alupent in this parameter on all days, 1, 45, and 90.

The adjusted mean FEV<sub>1</sub> on day 1 for all patients showed Alupent to be superior at times 0, 1, 2, then closely parallel to Atrovent at times 3, 4, 5, 6 hours post-drug for that day.

The adjusted mean FEV<sub>1</sub> on day 48 showed no clinical difference between the two drugs in any patients.

The adjusted mean FEV<sub>1</sub> on day 90, after hour 1 showed some superiority in increase in this parameter in the Atrovent Group among all patients.

For responders adjusted mean FEV<sub>1</sub> on days 1 and 45 showed no meaningful clinical difference but on day 90 Atrovent was showed to be superior to Alupent after hour one. The adjusted mean FVC for responders on days 1, 45 and 90 showed no meaningful difference medically between those on Alupent and those on Atrovent.

The adjusted mean FEF<sub>25-75%</sub> for responders on day 1 gave mixed results; on days 45 and day 90 the Atrovent-treated patients showed superiority in this parameter.

For the adjusted PEF<sub>R</sub> for responders after hour 2 on days 1 and 45 Atrovent was superior to Alupent and was so after hour 1 on day 90.

The final conclusions on these studies will incorporate the review and evaluation of the statistical reports by our Division of Biometrics. Medically, it appears superior results were obtained with the administration of Atrovent to patients with Chronic Obstructive Pulmonary Disease (COPD) than to those with Asthma.

6) Kirk, B.W., M.D. (with theophylline)  
Health Science Centre  
Winnipeg, Manitoba, Canada

Study No. E 82-1801 - Atrovent MDI

Final Report Date - 7/11/83 - Location in NDA Vol. 1.18; 1.138-1.141

Objective - To determine safety and efficacy of Atrovent administered with oral theophylline highlighting the duration of action, persistence of bronchodilating action with repeated dosing, effects of repeated dosing (side effects, adverse reactions and lab. studies) and patient acceptance of Atrovent plus theophylline compared to theophylline alone.

### Cases

Twenty-five adult patients entered the study and 22 completed the study. Patients entered had to be 12 years of age, or older, have a diagnosis of bronchial asthma and a relatively stable mild to moderately severe airway obstruction. All patients had reversible bronchospasm (increase of at least 15% FEV<sub>1</sub> within 30 minutes of 0.150 mg of isoproterenol (2 whiffs) from a metered dose inhaler (MDI).

Those excluded had serious systemic disease including cardiac, renal, hepatic or metabolic, those with prostatic hypertrophy, bladder neck obstruction, glaucoma or hypersensitivity to atropine-like compounds. Also excluded were pregnant women, or those expecting to become so during the study.

Of the 3 who did not complete the study as planned, one patient on Atrovent plus theophylline suffered 2 attacks requiring prednisone; a second on Atrovent plus theophylline felt there was no improvement and the third was admitted to a hospital while on theophylline alone.

Eleven males and eleven females qualified for entry and completed the study. Group I (Atrovent plus theophylline) consisted of 7 males, 4 females with a mean age of 40 and mean duration of illness of 14 years. Group II (Theophylline), 4 males, 7 females had a mean age of 42 years and mean duration of illness was 17 years. In each group Asthma was mild in 4 cases and moderate in 11.

The study was randomized, double-blind, parallel.

The route of drug administration: Atrovent-oral inhalation by MDI.  
Theophylline-oral.

Dose and Duration of Treatment: Throughout the study patients were maintained on therapeutic doses of oral theophylline and treatment was either Atrovent, 40 mcg (20 mcg/whiff) q.i.d. or matching placebo, self administered by the patients for 90 days. Study medications used were recorded by each patient in a diary.

Test doses which were administered while the patient was on maintenance doses of Theophylline were Atrovent 40 mcg on days 1 and 90 for Group I and on days 2 and 91 for Group II, and placebo MDIs, two whiffs, on days 2 and 91 for Group I and on Days 1 and 90 for Group II.

Table I  
Study Design

| Days      | 1                   | 2                   | For 90 days | 90                  | 91                  |
|-----------|---------------------|---------------------|-------------|---------------------|---------------------|
| Group Ia  | Atrovent<br>40 mcg  | Placebo<br>2 whiffs | Atrovent    | Atrovent<br>40 mcg  | Placebo<br>2 whiffs |
| Group IIa | Placebo<br>2 whiffs | Atrovent<br>40 mcg  | Placebo     | Placebo<br>2 whiffs | Atrovent<br>40 mcg  |

a = All patients received maintenance doses of theophylline throughout the study to ensure a theophylline blood level of 10 to 20 mcg/ml.

Concomitant Medication - verified - No patients were on other investigational drugs. One patient on Atrovent and 2 on placebo were on 10 mg or less of prednisone daily or 20 mg every other day. Inhaled steroids were used by 7 patients in the Atrovent Group and 3 in the placebo group. One patient used an Alupent inhaler for an acute attack. One patient in each treatment group used cromolyn which was permitted if a stabilized dose was used throughout the study. One patient in each group had i.v. aminophylline which was allowed for acute attacks. No other similar medications were used.

Observations before and after the study: Following admission to the study, but before administration of test medication, patients not receiving a therapeutic oral dosage of theophylline were administered theophylline alone until optimal blood levels and therapeutic results were obtained. Complete history, P.E. incl. pulse rate and B.P. were performed.

Lab tests included CBC, blood chemistry tests, urinalysis and chest x-ray. These were repeated at the end of the study.

Observations During the Study: Excluded after midnight preceeding the pulmonary function test day, were all bronchodilators, including the study drugs Atrovent and Placebo, steroids, and cromolyn. Smoking and drinks containing xanthines were not allowed on pulmonary function testing days.

Pulmonary function tests were done at baseline and at 30 minutes, and 1, 2, 3, 4, 5 and 6 hours after a test dose on days 1, 2, 90 and 91.

Theophylline blood levels were determined at baseline and two hours after test drug administration on days 1, 2, 45, 90 and 91.

ECGs were done at baseline and 1 hour after test drug administration on days 1, 2, 45, 90, 91.

Patients' diaries were reviewed on each visit and the entries summarized on the case report. Patient entries included a record of daily medications used, including investigational drug, the number of acute attacks and peak flow readings.

Side effects and adverse reactions and the investigators' opinion as to drug relationship and severity were recorded.

### Results

Theophylline blood levels between treatment groups were comparable and also between the beginning and end of the study.

### Efficacy

Tabulations show mean changes from baseline of FEV<sub>1</sub> values for the single-dose crossover studies at the beginning and end of the study in data from the statistical report along with the effect due to Atrovent, i.e. Atrovent plus theophylline minus theophylline alone. They show the effects of Atrovent at the beginning and the end of the study were comparable in both groups with somewhat generally greater effects at the end of the study showing no tolerance had developed to the use of Atrovent over the 91-day study. (Table 2, vol. 1.18, pg. 15). "The pooled data using both treatment groups and both test days showed a statistically significant benefit at each time point over 6 hours and the overall benefit was highly significant." (Table 3, Vol. 1.18, pg. 15). It has been shown also that the results to respiratory resistance generally paralleled the FEV<sub>1</sub> results. (These data are detailed in the statistical report and final judgement await review by our Division of Biometrics). The efficacy data are agreeable to this reviewer shown in Tables 2 and 3, Vol. 1.18, pg. 15.

In the Kirk study, theophylline, and theophylline plus Atrovent use in asthmatics "there was no evidence, in terms of FEV<sub>1</sub> and respiratory resistance that the acute benefits of adding Atrovent to oral theophylline were any less after 90 days of regular use than they were at the start of the treatment period." The effects or benefits were usually greater at the end of the study indicating there was little or no development of tolerance to Atrovent after three months of regular use. Reported side effects were not clinically significant. The mean daily number of acute attacks were similar in the two patient groups as were the long term subjective assessments and concomitant use of other therapy.

Safety - There were no clinically significant changes in pulse rates or blood pressure readings in this study. Exacerbation of symptoms following test doses of both treatments was the single most frequently reported adverse effect and was reported by 5 patients after placebo and 2 patients after Atrovent. After continuous treatment with Atrovent and Theophylline, adverse effects were: palpitations (1) nausea and dry mouth (2) and cough (1). After multiple doses of theophylline alone there were palpitations (1) and exacerbation of symptoms (1).

In summary this 90 day study on the safety and efficacy of the addition of Atrovent to theophylline was compared to theophylline alone. With the addition of Atrovent there was a "statistically significant effect in FEV<sub>1</sub> demonstrated for Atrovent both at the beginning and end of the 90 day administration period with no increase in side effects."

Medically, awaiting review by our Division of Biometrics, the statements regarding efficacy and safety made above have been substantiated in this NDA. This is demonstrated in vol. 1.18, pg 15 Tables 2 and 3. The drug is safe and effective in cases where added efficacy is shown when Atrovent is used with theophylline as claimed in this study by B.W. Kirk, M.D.

It is true that the effects of Atrovent were comparable at the beginning and the end of the study. (Table 2 pg. 14, Vol. 1.18, where the mean changes in baseline FEV<sub>1</sub> (L) (Reference Tables 1, 2, 7, 8 of the statistical report. This pertains to Group 1 (continuous therapy - Atrovent plus theophylline for 90 days) where the Atrovent effect is shown as Atrovent/Theophylline minus theophylline in which that effect is comparable at the beginning of the trial (days 1, 2, and end of trial days 90, 91.)

Reviewers comment - It has been shown above that Atrovent added to theophylline enhanced the bronchodilating effect without the addition of clinically significant adverse reactions. There was improvement in this combination over that of Theophylline, when used alone as a bronchodilator.

This concludes controlled asthma studies.

#### E. Other Studies (5 types)

##### E-1. Emergency Atrovent Program - Data Summary

Location in NDA - Vol. 1.18; Vols. 1.142 - 1.150

Objective of this study - to provide Atrovent to patients not responding to other medications. The information obtained from this program concerned safety data not efficacy for Atrovent administered under these conditions.

The results showed the drug to be safe.

There are numerous tables and appendices submitted concerning this program. They are from Appendix A-1; the total number of patients treated was 244. As of September 10, 1982 patient numbers 1-298 had been assigned and of these 298, 44 had no documentation of drug administration = 254 remaining. On 15 patients only the initial report was received, but no documentation that they received Atrovent and one patient died before receiving medication. Therefore this report is based on 244 patients who received Atrovent including 6 patients who died after the cut-off date. Of the 244 patients 86 or 35.5%

withdrew for treatment failure (37 patients - 15.3%); side effects (23 patients - 9.5%); no reason reported (6 patients - 2.5%) and one patient (0.4%) tried withdrawal for 1-2 weeks and since asthma failed to increase she did not restart Atrovent. (Table 1). Distribution by sex was about equal, (50.4% male, 49.6% female). Seventy-six percent of the patients were between 30 and 69 years of age, 88% were caucasian. Bronchial asthma was the most frequently reported use for Atrovent on this emergency basis (71%), Table 2, Vol. 1.18.

Reviewer's comment: Conclusion: This emergency program lends support to safety of the drug.

E-2) Loudon, R.G., M.D. University of Cincinnati, College of Medicine,  
Cincinnati, Ohio

Study No. E-80-854

Protocol - /a/; Location in NDA-Vols. 1.18, 1.151

Date - June 30, 1980

Regarding this study: The applicant states "this protocol was designed as a pilot study to evaluate the effect of Atrovent on cough frequency in patients with chronic idiopathic non-productive cough and or determine sample size for a definitive study in a meeting with Dr. Loudon he recommended that we not proceed further with this project because the variability would necessitate a very large patient population and that difficulties in recruitment would preclude much enrollment."

E3) Multicenter Study

Protocol - II c 6

To compare a single dose crossover study of Atrovent with Alupent effect. No meaningful data was obtained.

E-4) R. Bone, M.D. (E 81-1334)

This "study as submitted concerned a single patient. No meaningful data was obtained. The patient was administered a test dose of Atrovent MDI.

E-5) Clinical Laboratory Studies - Effectiveness

"Clinical laboratory studies related to effectiveness are not applicable for support of this New Drug Application" (wording of applicant - hence, no data).

6) Clinical Laboratory Studies Related to Safety

Location in the NDA - Vol. 1.19, pp 2-161

Summary verified - in general, clinical laboratory tests were performed before and after treatment with Atrovent on 348 patients in double-blind controlled studies, including 173 patients in 90 day studies. Laboratory studies were also obtained on approximately 150 patients in the emergency Atrovent program. These studies included CBC, urinalysis, blood chemistries.

Detailed data on these studies are found in the statistical reports. In general, the laboratory tests remained within normal range. The only tests in the controlled clinical studies in which significant changes were seen were glucose in 12 patients, sodium in 3 and potassium in 2. Elevated glucose was attributed to non-fasting in patients and/or using steroids.

Changes in laboratory values in open-label studies were not clinically significant. ECGs were obtained at baseline and post treatment in 380 patients. In conclusion no laboratory or ECG abnormalities could be attributed to Atrovent and no trends were evident.

E-7 Summary of Clinical Literature Reviewed by the Applicant

Location in the NDA; Vol. 1.19 pages 163-246 with each page containing an abstract of published papers dated 1971-1982 on Atrovent metered dose inhaler. There are eleven (11) special studies; eleven (11) on sputum and mucociliary clearance studies; eleven (11) on dose-ranging studies; thirteen (13) on repetitive-dose (long/short-term) studies; twenty six (26) on single dose studies; seven (7) on studies in experimentally provoked bronchospasm; there are therefore a total of seventy-nine (79) such abstracts. This is followed by forty three (43) pages listing a bibliography on Atrovent. No meaningful information not contained in the NDA elsewhere is included in these sections.

Table 1 Includes Humanitarian use program

| <u>Adverse experience</u>   | <u>Atrovent<br/>(228)</u> | <u>Alupent<br/>(223)</u> |
|-----------------------------|---------------------------|--------------------------|
| <u>Cardiovascular</u>       |                           |                          |
| Palpitations                | 4 (1.8%)                  | 4 (1.8%)                 |
| Tachycardia                 |                           | 1 (0.4%)                 |
| <u>CNS</u>                  |                           |                          |
| Dizziness                   | 6 (2.6%)                  | 7 (3.1%)                 |
| Nervousness                 | 7 (3.1%)                  | 17 (7.6%)                |
| Headache                    | 6 (2.6%)                  | 4 (1.8%)                 |
| Weakness                    |                           | 2 (0.9%)                 |
| Incoordination              | 1 (0.4%)                  | 2 (0.9%)                 |
| Insomnia                    | 1 (0.4%)                  | 1 (0.4%)                 |
| Tinnitus                    |                           | 1 (0.4%)                 |
| Fatigue                     |                           | 1 (0.4%)                 |
| <u>Dermatological</u>       |                           |                          |
| Rash                        | 3 (1.3%)                  | 1 (0.4%)                 |
| Hives                       |                           | 1 (0.4%)                 |
| <u>G.I.</u>                 |                           |                          |
| Nausea                      | 7 (3.1%)                  | 2 (0.9%)                 |
| Vomiting                    |                           | 2 (0.9%)                 |
| G.I. distress               | 6 (2.6%)                  | 7 (0.9%)                 |
| Constipation                | 1 (0.4%)                  |                          |
| Diarrhea                    | 1 (0.4%)                  | 1 (0.4%)                 |
| Change in appetite          |                           | 2 (0.9%)                 |
| <u>G.U.</u>                 |                           |                          |
| Urinary difficulty          | 1 (0.4%)                  | 2 (0.9%)                 |
| <u>Ophthalmological</u>     |                           |                          |
| Decreased visual acuity     | 1 (0.4%)                  | 2 (0.9%)                 |
| Lack of accommodation       |                           | 1 (0.4%)                 |
| <u>Oro-Otolaryngeal</u>     |                           |                          |
| Dry mouth                   | 4 (1.8%)                  | 2 (0.9%)                 |
| Epistaxis                   |                           | 1 (0.4%)                 |
| Irritation from aerosol     | 4 (1.8%)                  | 4 (1.8%)                 |
| Hoarseness                  |                           | 1 (0.4%)                 |
| <u>Respiratory</u>          |                           |                          |
| Cough                       | 14 (6.1%)                 | 2 (1.3%)                 |
| Exacerbation<br>of symptoms | 6 (2.6%)                  | 7 (3.1%)                 |
| Pleurisy                    | 1 (0.4%)                  |                          |
| Congestion                  |                           | 1 (0.4%)                 |
| <u>Musculoskeletal</u>      |                           |                          |
| Tremor                      |                           | 6 (2.7%)                 |
| Muscle pain                 |                           | 1 (0.4%)                 |

Table 2

Overall incidence of adverse experiences among 452 patients treated with Atrovent in double-blind, controlled, clinical studies within the recommended dose

| <u>Adverse experience</u>     | <u># of Patients</u> | <u>% of Total Treated</u> |
|-------------------------------|----------------------|---------------------------|
| <u>Cardiovascular</u>         |                      |                           |
| Arrhythmia                    | 2                    | 0.4                       |
| Palpitations                  | 4                    | 0.9                       |
| <u>CNS</u>                    |                      |                           |
| Dizziness                     | 8                    | 1.8                       |
| Nervousness                   | 7                    | 1.5                       |
| Headache                      | 9                    | 2.0                       |
| Coordination difficulties     | 2                    | 0.4                       |
| Insomnia                      | 1                    | 0.2                       |
| <u>Dermatological</u>         |                      |                           |
| Rash                          | 4                    | 0.9                       |
| Itching                       | 1                    | 0.2                       |
| <u>G.I.</u>                   |                      |                           |
| Nausea                        | 10                   | 2.2                       |
| G.I. Distress                 | 8                    | 1.8                       |
| Constipation                  | 2                    | 0.4                       |
| Diarrhea                      | 2                    | 0.4                       |
| Vomiting                      | 1                    | 0.2                       |
| <u>G.U.</u>                   |                      |                           |
| Urinary difficulty            | 1                    | 0.2                       |
| <u>Musculoskeletal</u>        |                      |                           |
| Tremor                        | 3                    | 0.7                       |
| <u>Ophthalmological</u>       |                      |                           |
| Decreased visual acuity       | 1                    | 0.2                       |
| Difficulties in accommodation | 1                    | 0.2                       |
| Other ophthalmological        | 3                    | 0.7                       |
| <u>Oro-Otolaryngeal</u>       |                      |                           |
| Dry mouth                     | 9                    | 2.0                       |
| Irritation from aerosol       | 6                    | 1.3                       |
| <u>Respiratory</u>            |                      |                           |
| Cough                         | 14                   | 3.1                       |
| Exacerbation of symptoms      | 7                    | 1.5                       |
| Pleurisy                      | 1                    | 0.2                       |

This overall section above is followed by a study-by-study detail of the same information and will not be part of this review.

Reviewers comment: Reviewing the data presented regarding adverse experiences and reactions, the benefit/risk ratio indicates the drug to be safe.

Overall conclusions regarding safety and efficacy of Atrovent (ipratropium bromide).

In controlled clinical trials 499 patients were administered Atrovent and 244 patients were enrolled in a humanitarian use protocol establishing the safety and efficacy of Atrovent<sup>R</sup> inhaler.

In 42 asthmatic and 27 bronchitic patients dose-ranging studies confirmed the efficacy of the 40 mcg dose 4 x a day of Atrovent in addition to previously-conducted dose-ranging studies with a similar formulation marketed in Europe.

Multicenter 90 day controlled clinical trials comparing Atrovent inhaler to a standard beta adrenergic bronchodilator metaproterenol showed the following:

Number of patients enrolled

|             | <u>Atrovent</u> | <u>Metaproterenol</u> |
|-------------|-----------------|-----------------------|
| Bronchitics | 147             | 140                   |
| Asthmatics  | 81              | 83                    |
|             | <u>228</u>      | <u>223</u>            |

The response of bronchitics to Atrovent was clearly better than that to metaproterenol over 6 hours testing on days 1, 45 and 90 by pulmonary function testing which included FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub> and SGaw. Onset and duration of increasing improvement was within 15 minutes, lasting 3-5 hours and in some cases, up to 6 hours. The studies in asthmatics did not produce such consistent favorable results with about one-half of the 78 patients on Atrovent responding with a 15% or greater increase in FEV<sub>1</sub>. Atrovent safety was shown in these same 90 day controlled clinical trials by small decreases in pulse rates and blood pressures, not of clinical significance.

The incidence of adverse reactions was low and required cessation of therapy in 3 of 228 patients treated with Atrovent (all secondary to cough). Cough was the most frequent reaction which occurred (in about 6% of patients). Other, less significant adverse reactions reported accounted for 1-3% of adverse reactions.

A 90 day study which was clinically controlled in asthmatics showed the safety and efficacy of Atrovent used with theophylline. Special studies showed Atrovent did not decrease mucociliary clearance from the lungs or tracheal mucous velocities in the trachea, nor dry up secretions from the lung or alter viscosity of sputum showing lack of anticholinergic effects. Atrovent did not affect pupil size, accommodation, visual acuity or intraocular pressure. Also anticholinergic effects were absent in the G.U. system and Atrovent did not affect ventilation/perfusion relationships or lower PaO<sub>2</sub>. (See body of this review - special studies).

The conclusion may be reached that Atrovent is an effective bronchodilator in bronchitics and some asthmatics. It is an anticholinergic bronchodilator with a low incidence of adverse reactions and no significant anticholinergic side effects. In the older bronchitic patient, beta adrenergic bronchodilators may often be ineffective and do have cardiovascular side effects. Therefore Atrovent has been shown to be safe and effective for patients with chronic bronchitis and chronic obstructive pulmonary disease (COPD).

Review and Evaluation of Labeling (CFR 201.56)

The prescribing information part of the submitted labeling is acceptable except for one point. While, on the immediate-container labeling the phrase: "Caution: Federal Law prohibits dispensing without prescription", that statement should also appear in the prescribing information labeling.

The Sections involving "DESCRIPTION" and "CLINICAL PHARMACOLOGY" largely involve the assigned reviewing Chemist and Pharmacologist respectively and our Division of Biopharmaceutics. There are no medical objections to these sections, but should satisfy the assigned reviewers.

The Section titled "INDICATIONS AND USAGE" in which it is claimed the drug "is indicated as a bronchodilator for maintenance treatment and prevention of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis" is unacceptable in that submitted data fails to support the claim for "prevention" as used in this draft labeling section. Also, in this section the sentence reading: "Some asthmatic patients have been shown to respond to Atrovent" is unacceptable in the proposed labeling regarding indications for use of the drug. The data submitted shows, at best, 50% or less of such patients respond to administration of the drug. This may be due to chance alone but definitely is not, as required "substantial evidence of safety and effectiveness (CFR 314.111(a)(5)(ii)).

The Sections titled "WARNINGS," "CONTRAINDICATIONS" are acceptable.

The Section titled "PRECAUTIONS" should have, not presently included, subsections titled "GENERAL," "OVERDOSAGE" (not as submitted "OVERDOSE"). Also under this Section, the statement made in the draft labeling "Acute overdosage is unlikely since ..." should be deleted and adherence to the referenced CFR Section regarding labeling for prescription drugs in humans be followed in a non-promotional manner.

The Section titled "DOSAGE and ADMINISTRATION: is acceptable.

The Section "HOW SUPPLIED" is for review by the assigned reviewing chemist.

The separate Section titled "INSTRUCTIONS FOR USE OF METERED-DOSE INHALER" and the accompanying illustrated drawing are acceptable.

Recommendations regarding submitted draft labeling:

Revisions: Those possibly recommended by the assigned reviewing Chemist and the Pharmacologist regarding the Sections titled "Description" and "Clinical Pharmacology." There are no Medical objections to these Sections.

The Section titled INDICATIONS AND USAGE should be revised to read:  
"Atrovent (R) (ipratropium Bromide) is indicated as a bronchodilator for treatment of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, chronic bronchitis. Atrovent (ipratropium bromide) is not indicated in situations requiring rapid response to treatment."

This requires deletions of statements regarding use of Atrovent in "prevention" and in asthmatic patients."

Data submitted on "Emergency use of Atrovent" is found to be totally inconclusive. The sentence recommended under this Section should be added as well as that presently included under the Section titled WARNINGS because of the practical importance of that statement to the prescribing physician.

The Section titled PRECAUTIONS will have a subsection and its content "General."

The Section titled "OVERDOSE" will read "OVERDOSAGE," deleting the phrase "Acute overdosage is unlikely."

Finally the draft labeling should state "Federal law prohibits dispensing without a prescription."

Recommendations:

- 1) The specified labeling revisions should be implemented.
- 2) Providing #1 above is in compliance with the recommendations stated, the NDA may be found medically approvable for safety and efficacy as a bronchodilator for treatment of the bronchospastic symptoms associated with chronic obstructive pulmonary disease, especially chronic bronchitis.

The NDA is found to be nonapprovable for use in "prevention" of the above nor for any use in the management of asthmatic patients.

*E. J. Shanahan* (not read)  
E. J. Shanahan, M.D.  
4/17/84

NDA 19-085  
HFN-160  
HFN-340  
R/D EJShanahan, M.D., 11/16/83  
R/D initialed by JWinkler 7/12/85, PHRussell 7/15/85  
FT-jb, W1337N, D0071  
FT Revised OLA 8/6/85  
Doc. Room 160

*JWinkler*  
11/17/80

*OK 11/8/85*

N-19085-3

Necropsy:

No - drug induced changes were reported. See pages 42-44 for details of autopsy findings.

Histopathology:

The following summary was submitted on the results of microscopic examination by Prof. Dr. J. von Sandersleben, (med. vet.) from Institute of Animal Pathology, University of Munich.

“A comparison between the findings in the control animals and in the animals of the groups which received the substance give no indication that the substance has a damaging effect. In my opinion all organ changes observed belong to the spontaneous pathological processes in the dog.

Slight inflammatory processes in the lung, especially those of an interstitial inflammatory nature, were found both in the control animals and in the animals of the dose groups. They are also known to us from control animals of other experiments. Some of the inflammatory changes are, without doubt, of parasitic origin, in other cases in which no parasites are detectable in the section this aetiology is at least probable. Unilateral or bilateral non-purulent pyelonephritis is often found in dogs. The fact that, in this experiment, it was only found in animals of the dose groups, is pure coincidence. In other experiments with dogs of the same age I have encountered it in control animals.

In summary it can be said that the histopathological examination provided no indication that the substance administered by inhalation had any harmful effects.”

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No - drug induced changes were reported. See pages 42-44 for details of autopsy findings.

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“A comparison between the findings in the control animals and in the animals of the groups which received the substance give no indication that the substance has a damaging effect. In my opinion all organ changes observed belong to the spontaneous pathological processes in the dog.

Slight inflammatory processes in the lung, especially those of an interstitial inflammatory nature, were found both in the control animals and in the animals of the dose groups. They are also known to us from control animals of other experiments. Some of the inflammatory changes are, without doubt, of parasitic origin, in other cases in which no parasites are detectable in the section this aetiology is at least probable. Unilateral or bilateral non-purulent pyelonephritis is often found in dogs. The fact that, in this experiment, it was only found in animals of the dose groups, is pure coincidence. In other experiments with dogs of the same age I have encountered it in control animals.

In summary it can be said that the histopathological examination provided no indication that the substance administered by inhalation had any harmful effects.”

EKG:

The mean heart rates of high dose group were significantly ( $p < 0.01$ ) increased in weeks 6 and 13, 30 minutes and 24 hours after administration, but the PQ, QRS, and QT intervals were within normal limits, without pathology.

Lung Function Mechanism and Acid-Base/Blood Gas:

No significant changes were reported.

Ophthalmology and Histology of Eyes:

No drug induced changes were reported. See pages 39-41 for detailed reports.

P THREE MONTH INHALATION TOXICITY STUDY IN DOGS WITH AEROSOL FORM: (2/22/84 review)  
E83-125:

Lab. Performing the Study: Les Oncins Research and Breeding Center  
Saint-Germain sur l'Arbresle.

Strain: Beagle dogs, approximately 6 month old,  
and 10 kg.

No. of Animals/Level: 3 M + 3 F

Dosage Level: 10 puffs (= 400 ug)/day Sch 1000 or  
propellant (control group). Mean dose  
for M = 50 ug/kg and for F = 34 ug/kg.

Duration and Route: Dogs were treated twice a day, 10  
puffs/day, 6 days/week for 13 weeks via  
a mask while held by two aides using  
the following method from metered  
aerosol containers from Boehringer  
Ingelheim:

" At each administration the animal was held on a table by two aides, a watertight mask of about 1 litre capacity was fixed to its muzzle and the aerosol was introduced into the mask. Following aerosol administration, the animal's head was left in the treated atmosphere for 30 seconds, allowing 10 pulmonary ventilations. The normal values for pulmonary ventilation in beagles are:

- respiratory rate : 15 - 25 per minute
- volume of airflow : 150 - 250 ml for an animal weighing 10 - 14 kg.

The respiratory rate is almost identical in man (20 per min.) who has a forced inspiratory capacity of approx. 3 litres.

The percentage of aerosolized substance reaching the general circulation in a man making one forced inspiration and a dog making 10 successive inspirations-expirations is therefore comparable. "

|                         |                                                                                                                                                                                                                                |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mortality:              | None.                                                                                                                                                                                                                          |
| Clinical Investigation: | No abnormal behavior or clinical signs were observed, and "all tolerated the treatment very well".                                                                                                                             |
| Growth:                 | The body weight of F treated group stayed more or less the same for the first 9 weeks but slightly less than the control F, while the M treated group followed the exactly the same pattern as the control M. See pages 12-14. |

Food Consumption:

Food consumption was less in the F treated group, only week 13; but all other times, it was similar to or more than the respective control group. See page 15.

Hematology:

Some showed slight changes, but they were reported as "within normal limits." See pages 18-19.

Coagulation Time:

Not done.

Clinical Chemistry:

No significant changes were reported.

COMMENTS: Some of control values fluctuated more than treated group. See pages 25-29.

Urinalysis:

No drug-induced changes were reported. See pages 36-38.

Organ Weight:

The absolute weight of heart and liver was slightly smaller in F treated group than control F, but gonad in M treated group was slightly larger than control M. See pages 41-42.

Gross Pathology:

No drug related abnormalities were reported.

Histopathology:

It was stated by a pathologist that "in most of the dogs there were discrete inflammatory lesions at various sites in the respiratory tract. As they also occurred in some of the controls, it seems that they are of little significance. No significant lesion was found in any of the organs studied." For the detailed report on all dogs, see pages 44-51.

EKG:

No signs of myocardial damages were reported. See pages 53-77.

Arterial Blood Pressure:

It was reported that conscious dogs receiving 10 puffs of Sch 1000 in 20 seconds showed "a slight reduction in BP of 10-15 mm Hg," returning to normal within 2 min., but 2 dogs did not show any changes.

COMMENTS: It was stated that "examination of the fundi showed no deterioration.", but it was not clear whether the fundi was meant for eye or not. See on page 5 for protocol and on page 8 for results.

#### CHRONIC TOXICITY STUDIES:

##### A. THE ADMINISTRATION BY INHALATION TO RHESUS MONKEYS OF SCH 1000 FOR 6 MONTHS: (1/30/75 review)

Huntingdon Research Center  
Huntingdon, England

Rhesus Monkeys (M. Mulatta)

Sch 1000

NO. ANIMALS/DOSE LEVEL: 3 F & 3M

DOSE LEVELS: 200, 800 and 1600 mg per day, control and vehicle control

ADMINISTRATION AND DURATION: Inhalation, 2 X daily, 6 days a week, and 1 X the seventh day.

##### RESULTS:

Mortality: One animal in the control group died.

Clinical signs: Animals physical condition and behavior were within normal limits.

Body Weight: Body weight of animals in all groups was similar. Animals gained weight throughout the study.

Food Consumption: Dosing of animals with Sch 1000 seems to have little or no effect on their food consumption.

Ophthalmoscopy: Eyes of the monkeys were examined at pre-dosing, the 12 and 25 weeks. No abnormalities were observed which were due to the administration of this drug. No mydriasis was observed during 2nd or 3rd examination.

Electrocardiography: ECG's taken at pre-dosing, 4, 12, and 26 weeks revealed no biologically significant differences between treated and control groups.

Lung Function Tests: Lung function tests performed at pre-exposure, 8 and 24 weeks showed no significant changes in lung mechanics and ventilation or blood gases.

Estimation of Fluorocarbon-II in the Blood:

The concentration of fluorocarbon-II in the blood was found to be greater as the dose of Sch 1000 was increased. There was a wide variation between individual monkeys.

Biochemistry: Tests carried out at pre-exposure, 4, 12, and 26 weeks show values for all animals were within normal limits except for 2 animals in each of the 600 and 1600 mg dose groups who had elevated SAP's at the 26 week evaluations.

Hematology: Hematology values were within normal limits for all monkeys.

Urinalysis: Urine values for all monkeys were comparable and unremarkable.

Terminal studies: Lung abnormalities were seen in monkeys in the control and treated groups. The most severe lesions were seen in the treated monkeys.

No biologically significant changes were seen in the organ weight of these monkeys. No drug-related changes were seen in the respiratory tracts, livers, or G.I. tracts of these animals when the tissues were examined grossly and histologically.

B. RATS: 26 WEEK INHALATION: (Boehringer Sohn) (11/2/78 review)

|                       |                      |
|-----------------------|----------------------|
| No. of Animals/group: | Group 1: 20 M + 20 F |
|                       | Group 2: 30 M + 30 F |
|                       | Group 3: 20 M + 20 F |
|                       | Group 4: 20 M + 20 F |
|                       | Group 5: 30 M + 30 F |

Body Weight: Body weight of animals in all groups was similar. Animals gained weight throughout the study.

Food Consumption: Dosing of animals with Sch 1000 seems to have little or no effect on their food consumption.

Ophthalmoscopy: Eyes of the monkeys were examined at pre-dosing, the 12 and 25 weeks. No abnormalities were observed which were due to the administration of this drug. No mydriasis was observed during 2nd or 3rd examination.

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Estimation of Fluorocarbon-II in the Blood:

The concentration of fluorocarbon-II in the blood was found to be greater as the dose of Sch 1000 was increased. There was a wide variation between individual monkeys.

Biochemistry: Tests carried out at pre-exposure, 4, 12, and 26 weeks show values for all animals were within normal limits except for 2 animals in each of the 800 and 1600 mg dose groups who had elevated SAP's at the 26 week evaluations.

Hematology: Hematology values were within normal limits for all monkeys.

Urinalysis: Urine values for all monkeys were comparable and unremarkable.

Terminal studies: Lung abnormalities were seen in monkeys in the control and treated groups. The most severe lesions were seen in the treated monkeys.

No biologically significant changes were seen in the organ weight of these monkeys. No drug-related changes were seen in the respiratory tracts, livers, or G.I. tracts of these animals when the tissues were examined grossly and histologically.

B. RATS: 26 WEEK INHALATION: (Boehringer Sohn) (11/2/78 review)

|                       |          |             |
|-----------------------|----------|-------------|
| No. of Animals/group: | Group 1: | 20 M + 20 F |
|                       | Group 2: | 30 M + 30 F |
|                       | Group 3: | 20 M + 20 F |
|                       | Group 4: | 20 M + 20 F |
|                       | Group 5: | 30 M + 30 F |

| <b>Strain:</b>             | Hysterectomy derived, specific pathogen free albino rats, CD strain (Kent, England)                                                                                                                                                                                                                                                                                                                                                    |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|--------------------|---------------------|--|---------------------|-------------------------|--------------|--------------------------|-----------------|--------------------------|---------------|--------------------------|
| <b>Dosage Levels:</b>      | <table border="1"><thead><tr><th><u>Group:</u></th><th><u>(app. dose)</u></th></tr></thead><tbody><tr><td>1: Chamber control:</td><td></td></tr><tr><td>2: Vehicle control:</td><td>6 x 15 min/day (freons)</td></tr><tr><td>3: Low dose:</td><td>2 x 15 " (128 ug+freons)</td></tr><tr><td>4: Medium dose:</td><td>4 x 15 " (256 ug+freons)</td></tr><tr><td>5: High dose:</td><td>6 x 15 " (512 ug+freons)</td></tr></tbody></table> | <u>Group:</u> | <u>(app. dose)</u> | 1: Chamber control: |  | 2: Vehicle control: | 6 x 15 min/day (freons) | 3: Low dose: | 2 x 15 " (128 ug+freons) | 4: Medium dose: | 4 x 15 " (256 ug+freons) | 5: High dose: | 6 x 15 " (512 ug+freons) |
| <u>Group:</u>              | <u>(app. dose)</u>                                                                                                                                                                                                                                                                                                                                                                                                                     |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| 1: Chamber control:        |                                                                                                                                                                                                                                                                                                                                                                                                                                        |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| 2: Vehicle control:        | 6 x 15 min/day (freons)                                                                                                                                                                                                                                                                                                                                                                                                                |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| 3: Low dose:               | 2 x 15 " (128 ug+freons)                                                                                                                                                                                                                                                                                                                                                                                                               |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| 4: Medium dose:            | 4 x 15 " (256 ug+freons)                                                                                                                                                                                                                                                                                                                                                                                                               |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| 5: High dose:              | 6 x 15 " (512 ug+freons)                                                                                                                                                                                                                                                                                                                                                                                                               |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
|                            | * 11(24.4%), 12(52.9%), and 114(22.5%).                                                                                                                                                                                                                                                                                                                                                                                                |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| <b>Duration and Route:</b> | Exposure regime: 1 burst of aerosol in the chamber every 5 seconds for 15 min. (64 ug)                                                                                                                                                                                                                                                                                                                                                 |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| <b>Growth Effect:</b>      | Exposures were divided into a morning and afternoon sessions, and morning only on 7th day.<br>7 days/week for 26 weeks by inhalation. There was dose-related depression in weekly mean body weight gain in male upto about 16% with highest dose group, but it was considered in the normal range. There was no difference in females.                                                                                                 |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| <b>Mortality:</b>          | Total of 32 died, 8 each/group except group 1 with 3 and group 4 with 5.<br>27 of those which died were the result of over compression in the restraining tubes, 4 due to periodic bleeding, and one with broken tail.                                                                                                                                                                                                                 |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |
| <b>Food Consumption:</b>   | The amount consumed expressed as g/kg/group/week was slightly higher in females than males, but no dose related change was seen.<br>There were no differences in water consumption.                                                                                                                                                                                                                                                    |               |                    |                     |  |                     |                         |              |                          |                 |                          |               |                          |

|                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                           |                          |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| Strain:                               | Hysterectomy derived, specific pathogen free albino rats, CD strain (Kent, England)                                                                                                                                                                                                                                                                                                                                                       |                          |
| Dosage Levels:                        | Group:                                                                                                                                                                                                                                                                                                                                                                                                                                    | (app. dose)              |
|                                       | 1: Chamber control:                                                                                                                                                                                                                                                                                                                                                                                                                       |                          |
|                                       | 2: Vehicle control:                                                                                                                                                                                                                                                                                                                                                                                                                       | 6 x 15 min/day (freons)  |
|                                       | 3: Low dose:                                                                                                                                                                                                                                                                                                                                                                                                                              | 2 x 15 " (128 ug+freons) |
|                                       | 4: Medium dose:                                                                                                                                                                                                                                                                                                                                                                                                                           | 4 x 15 " (256 ug+freons) |
|                                       | 5: High dose:                                                                                                                                                                                                                                                                                                                                                                                                                             | 6 x 15 " (512 ug+freons) |
|                                       | * 11(24.4%), 12(52.9%), and 114(22.5%).                                                                                                                                                                                                                                                                                                                                                                                                   |                          |
| Duration and Route:<br>Growth Effect: | Exposure regime: 1 burst of aerosol in the chamber every 5 seconds for 15 min. (64 ug)<br><br>Exposures were divided into a morning and afternoon sessions, and morning only on 7th day.<br><br>7 days/week for 26 weeks by inhalation. There was dose-related depression in weekly mean body weight gain in males upto about 16% with highest dose group, but it was considered in the normal range. There was no difference in females. |                          |
| Mortality:                            | Total of 32 died, 8 each/group except group 1 with 3 and group 4 with 5.<br><br>27 of those which died were the result of over compression in the restraining tubes, 4 due to periodic bleeding, and one with broken tail.                                                                                                                                                                                                                |                          |
| Food Consumption:                     | The amount consumed expressed as g/kg/group/week was slightly higher in females than males, but no dose related change was seen.<br><br>There were no differences in water consumption.                                                                                                                                                                                                                                                   |                          |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hematology:             | No dose-related changes were seen.                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Coagulation Test:       | No test was done.                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Clinical Chemistry:     | There were no differences between groups. Some of values at 6 week were quite low, less than 50% of pre or 12 week values, but no dose-related change was seen.                                                                                                                                                                                                                                                                                               |
| Urinalysis:             | There were no abnormalities.                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Organ Weights:          | Both absolute and relative weights showed no drug related changes. Both males and females showed slightly higher relative weights of adrenal glands without pathology.                                                                                                                                                                                                                                                                                        |
| Macroscopic pathology:  | Severe hydroephritis was noted in one rat each of groups 1, 2, and 5, and clear fluid filled cysts enveloping in one rat each of groups 1, 3, and 4. Other findings seem to be sporadic, and noted in one rat or two.                                                                                                                                                                                                                                         |
| Microscopic Pathology:  | <p>Infiltration of acute inflammatory cells were conspicuous in occasional mucous glands of the trachea in 2 rats of group 2, and 4 rats of group 5.</p> <p>Dystrophic mineralization was recorded in the kidneys of a rat of group 2 and 2 rats of group 5.</p> <p>Testicular atrophy with reduced spermatogenesis was noted in one rat each of group 1 and group 5.</p> <p>These findings and other sporadic findings were considered not drug induced.</p> |
| Ophthalmoscopy:         | No dose related change was noted.                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Surfactant Production:  | <p>Pattle bubble stability ratio test on random samples of rat lung at autopsy and 1 um epon resin sections of lung showed no drug related changes.</p> <p>Analysis on ciliary beat, lung mechanics, and blood gas levels did not show any drug related changes.</p>                                                                                                                                                                                          |
| Plasma fluorocarbon II: | Freon 11 was found to be increased with dose administered.                                                                                                                                                                                                                                                                                                                                                                                                    |

C. Subacute Toxicity: (Boehringer)

Rat (SPF, strain FW 49): 10M, 10F/level

Dose: 0, 6, 30 or 150-200 mg/kg (increased after 14th week).

Duration and Route: Six months, PO (diet).

Mortality: One mid and 1 hi dose, accidentally

Body weight, determined weekly then biweekly, was decreased in mid (14%) and hi dose F (27%) and hi dose M (28%). Food consumption was correspondingly reduced in these animals. Water intake was variable but decreased somewhat in hi dose M. Hematological parameters, determined periodically during the study, were not unusual. Blood glucose and BUN's were essentially normal; however, SAP and SGPT were decreased significantly in treated M and F throughout the study. Terminal serum K and cholesterol were not remarkable. Slight decrease in adrenal weight was noted in mid and hi dose F.

The following histological alterations appeared noteworthy:

|                        | Control | Lo    | Mid   | Hi    |
|------------------------|---------|-------|-------|-------|
| <u>Liver</u>           |         |       |       |       |
| Fatty degeneration     |         |       |       |       |
| cellular               | 14/20   | 11/20 | 13/20 | 6/20  |
| peripheral             | 3/20    | 3/20  | 1/20  | 9/20  |
| periportal             | 4/20    | 7/20  | 7/20  | 12/20 |
| <u>Small Intestine</u> |         |       |       |       |
| dilation               | 3/20    | 2/20  | 6/20  | 15/20 |
| <u>Large Intestine</u> |         |       |       |       |
| dilation               | 0/20    | 11/20 | 16/20 | 12/20 |
| edema                  | 0/20    | 4/20  | 1/20  | 5/20  |
| <u>Bladder</u>         |         |       |       |       |
| dilation               | 4/20    | 3/20  | 10/20 | 16/20 |

Other Comments:

Hypersecretion of Harder's gland in mid and hi dose F after 13th week. Hi dose M and F were hyperactive. Retention of urine and feces was noted in treated animals.

D. Chronic Toxicity: (Boehringer)

Species: Dog (Beagle, age 7-11 month)

Duration and Route:

One year, interim sacrifice at 6 month; PO, powder in capsules, 7 days/week for 1st 93 days then tablets were administered in capsules. Group 1 received tablets throughout the study.

Schedule:

| Group   | Dose (mg/kg) | # Sacrificed<br>- 6 mo. | # Sacrificed<br>- 1 yr. |
|---------|--------------|-------------------------|-------------------------|
| Control | 0            | 2M, 2F                  | 3M, 3F                  |
| 1*      | 1.5          | 3M, 3F                  | -- --                   |
| 2       | 3.0          | 2M, 2F                  | 3M, 3F                  |
| 3       | 15.0         | 2M, 2F                  | 3M, 3F                  |
| 4       | 75.0         | 2M, 2F                  | 3M, 3F                  |

\* This group started about 6 months after experiment began because lacrimal secretions were decreased in treated dogs.

Mortality:

One group 4 dog sacrificed in moribund condition.

Body weight, determined weekly, was decreased in group 3 and 4 dogs in early part of the study. Terminal values approached initial level in group 3 but not 4. Food intake was reduced in a dose-related manner from group 2 on. Water intake was not consistently altered. Raw data were not included for body weight, food consumption, hematology and clinical chemistries, including urinalysis.

Generally, SGPT and SGOT values were said to be increased in groups 3 and 4 dogs during last half of the study and SAP was increased only in group 4. In the latter part of the study, creatinine was reported to be increased in group 3 and decreased in group 4 from early part on. BUN's were increased in group 3 in last half of the study and decreased in group 4 throughout most of the study. Terminal bilirubin and globulin values were increased in group 4 dogs and cholesterol levels were increased midway in the study only. Blood glucose was essentially normal. Other electrolytes were not appreciably altered. Qualitative and microscopic urinalyses, performed on several occasions during the study, were normal. Organ weights were remarkable.

In the dog sacrificed on day 111 of the study, fatty degeneration of hepatic and renal epithelium, involution of thymus and decreased RBC and WBC in bone marrow was noted. In animals sacrificed at 6 months, fatty liver degeneration was observed in 1 group 4 dog and atrophy of germinal epithelium of testes and epididymus was seen in another dog in this group. In dogs sacrificed at one year, atrophy of epithelium of prostate was seen in one dog each in groups 2, 3 and 4. Localized necrosis of gastric mucosa was observed in 2/6 group 4 dogs.

Other Comments:

Emesis was noted in all treated animals from group 2 on. A dose-related decrease in mucosal secretions occurred. Due to decreased salivary secretions at hi dose, food was mixed with water. Decreased conjunctival secretions led to keratoconjunctivitis sicca and animals had to be treated with boric acid, antibiotic and vitamin A and D ointment -- no healing took place. Purulent nasal discharge was also noted occasionally.

E. Chronic Toxicity: (Boehringer)

Rat (SPF, strain FW 49): 35M, 35F/level

Dose: 0, 6, 30 or 150-200 mg/kg (increased during the 14th week).

Duration and Route: Fifty-five weeks, PO (diet).

Mortality: Control, 4: Low, 3: Mid, 7: Hi, 24.

Note: All deaths except one occurred after 14th week.

Hi dose M and F gained 29 and 20% respectively of weight gained by controls. Food consumption was correspondingly decreased in these animals. Water intake, measured several times during the study, was decreased in hi dose M and to a lesser extent in F. Hematological parameters were unremarkable. SAP values were decreased in the middle of the study in hi dose F and to a lesser degree in M when compared to corresponding controls. Blood glucose, BUN, SGPT and serum K and cholesterol values were within normal limits. Terminal microscopic and qualitative urinalysis of hi dose animals were normal. 3/14 hi dose M had distinct increase in prostate weight.

The following histological changes seemed drug-related:

|                                   | % Incidence |    |     |    |
|-----------------------------------|-------------|----|-----|----|
|                                   | Control     | Lo | Mid | Hi |
| Small Intestine<br>dilation       | 4           | 8  | 59  | 66 |
| Large Intestine<br>dilation       | 4           | 30 | 17  | 42 |
| colitis                           | 0           | 0  | 0   | 18 |
| Bladder<br>dilation               | 8           | 8  | 35  | 50 |
| Gastric Mucosa<br>dyscholic cysts | 4           | 22 | 7   | 29 |

Other Comments:

A dose-related hypersecretion of Harder's gland was noted. After 4 months, increased irritability was seen in hi dose animals.

F. Chronic toxicity test in Dogs Using Sch 1000 (1/24/75 review)

Dogs, beagle (7-11 months) Sch 1000

# animals/dose level: 2F + 2 M in 26 week study, 3F+3M in 52 week-study

dose level: 1.5, 3.0, 15.0 and 75.0 mg/kg plus controls.

administration and duration:

7 day - oral administration; duration - 26 and 52 weeks.

Results

Clinical Signs

Only 1 death occurred in this study. Animals in the treated groups had a reduction in secretion of conjunctiva, nasal and oral mucosa. This was dose-dependent and animals in higher dose groups had difficulty eating dry foods.

#### Body Weight

Body weight of dogs was slightly less in the higher dose groups when compared to the weight of the control animals.

#### Food Consumption

The food consumption of the animals in the 3.0, 15.0 and 75.0 mg/kg was below that of the animals in the control group.

#### Hematology

Erythrocyte, hemoglobin and hematocrit values were slightly higher in the treated animals however hematology values for all animals were within normal range.

#### Biochemistry

Dogs in dose levels groups of 15.0 and 75.0 mg/kg had increased SGPT from week 25 to week 52. Creatinine and Urea-N values were slightly confusing in that the 15.0 mg/kg group had increased creatinine and urea-N and the 75 mg/kg group had decreased creatinine and Urea-N values.

#### Urinalysis

No significant differences was noted between groups.

#### Ophthalmoscopic Investigation

A dose dependent keratitis sicca was observed in all dogs receiving dose levels of 3.0 mg/kg or higher. Dogs had difficulty in opening eyelids, minimal lacrimation, cloudiness in corneal epithelium and circumscissured fluorescence - positive epithelial erosions. Histological examinations were used to help clarify and diagnose these conditions.

#### Autopsy Findings

Autopsy findings at the end of 26 weeks showed 1 dog in the 75.0 mg/kg with fatty degeneration of the liver, as well as a reduction of erythropoiesis and leucopoiesis in the bone marrow. Another dog in the same group showed incomplete spermatogenesis with incipient atrophy of germinal epithelium. These conditions were thought to be drug-induced. Three dogs in the 15.0 and 75.0 mg/kg had vacuolations resembling status spongiosus. The 52 week autopsy findings revealed fine-particled fatty degeneration of the myocardial fibers in all dogs. All dogs in this study showed a diffuse fatty degeneration of the liver. In the two highest dose groups, the fatty degeneration was more diffused. Several animals in the 3.0, 15.0 and 75.0 mg/kg groups had bilateral atrophy of the spermatogenic epithelium and cellular interstitial inflammatory infiltrates with incipient fibrosis.

G. Chronic Toxicity Study with Sch 1000 in male dogs for 52 weeks

Dogs, beagle (20/15) Sch 1000

# animals/ dose level: 4 M with 2M for control

dose level: 3, 15 mg/kg Sch 1000, and 0.6 mg/kg atropine sulphate

administration and duration = oral administration, 7 days a week for 52<sup>1/2</sup> weeks.

Results:

The body weight of the dogs in the 15.0 mg/kg dropped sharply during the first weeks, the dogs did gain weight during the study but never reached their original weights.

Food Consumption

Food consumption of the dogs in the 15.0 mg/kg groups was markedly reduced especially during the first few weeks of the study.

Clinical Observations

Some vomiting and diarrhea was noted in the 3.0 and 15.0 mg/kg dogs. Marked inhibition of secretion was observed in the 15.0 mg/kg dogs throughout the entire study. The oral and nasal membranes were found to be very dry with fissures and dogs had to be fed their food as a wet pulp.

Mortality

One dog in the highest dose group of Sch 1000 died on the 15th day of the study.

Hematology

Dogs in the 15.0 mg/kg group had low total erythrocyte, hemoglobin and hematocrit values when evaluations were made during the 52nd week of this study.

Biochemistry One dog in each of the 3.0 and 15.0 had high SPGT-52nd week.  
Urinalysis

No differences between the groups were observed.

Ophthalmoscopic findings

One dog in the 3.0 mg/kg and all the dogs in the 15.0 mg/kg group had doses-dependent keratitis sicca. No pathological changes were noted in the eyes of the dogs receiving atropine sulphate.

Autopsy Findings

All dogs in this study had parasitic or bacterial granulomas of the liver. 3 dogs in the 3.0 mg/kg and 2 dogs in the 15.0 mg/kg group had chronic interstitial epididymitis. Subepithelial lymphohistiocytic infiltration were observed in the tissue of the tongues of several dogs in the 2 groups of dogs dosed with Sch 1000.

18 MONTH ORAL CHRONIC TOXICITY STUDY IN RATS: (10/26/83 review)

Lab. Performing the Study: C.H. Boehringer Sohn in West Germany

Strain: Chbb:THOM (SPF)

No. of Animals/Level: 25 M + 25 F (182.4 g and 142.4 g average weight, and about 43 day old)

Dosage Level: 0, 0.5, 6, 30 mg/kg/day Sch 1000-Br  
Batch No: IV-X and 810783

Autopsy Findings

All dogs in this study had parasitic or bacterial granulomas of the liver. 3 dogs in the 3.0 mg/kg and 2 dogs in the 15.0 mg/kg group had chronic interstitial epididymitis. Subepithelial lymphohistiocytis infiltration were observed in the tissue of the tongues of several dogs in the 2 groups of dogs dosed with Sch 1000.

4. 18 MONTH ORAL CHRONIC TOXICITY STUDY IN RATS: (10/26/83-review)

Lab. Performing the Study: C.H. Boehringer Sohn in West Germany

Strain: Chbb:THOM (SPF)

No. of Animals/Level: 25 M + 25 F (182.4 g and 142.4 g average weight, and about 43 day old)

Dosage Level: 0, 0.5, 6, 30 mg/kg/day Sch 1000-Br  
Batch No: IV-X and 810783

COMMENTS: See pages 437-440 for actual weekly intake of the drug.

The following table indicates the actual amounts of compound Sch-B consumed from the food (deviations from theory in percent):

| Week | G <sub>1</sub> |         | G <sub>2</sub> |        | G <sub>3</sub> |        |
|------|----------------|---------|----------------|--------|----------------|--------|
|      | Male           | Females | Male           | Female | Male           | Female |
| 1    | - 0.18         | +66.35  | - 2.12         | -11.02 | - 7.95         | + 8.57 |
| 12   | - 2.83         | + 5.52  | -23.2          | -20.21 | -18.32         | -11.24 |
| 39   | +17.46         | +22.73  | -17.1          | -10.91 | - 7.18         | - 8.58 |
| 52   | +19.94         | +15.22  | -10.47         | -10.79 | - 9.04         | + 6.70 |
| 65   | -14.06         | + 8.00  | - 4.88         | +12.78 | + 1.40         | - 1.09 |
| 78   | + 7.38         | +19.32  | - 1.58         | - 9.76 | -10.66         | + 9.76 |

Duration and Route:

Orally in food for 78 weeks (from 10/10/77 and necropsy on 4/23/79)

Clinical Standpoint:

"Small abscesses around the mouth" were observed in many rats of all groups, and they were results of *Pasteurella pneumotropica* infection. No abnormal behavior was observed.

Growth:

0.5 mg/kg/day group showed slight but significantly increased body weight gain in both M and F. Six mg/kg/day group also showed a slightly larger gain, but not significant.

COMMENTS:

No Table for group means was submitted for body weight gain.

**Food Consumption:**

General trend was that weekly food consumption in both M and F showed a great increase in first 3-5 weeks, followed by a gradual decrease up to week 33, then the consumption jumped up to a higher level and stayed to the end. However, M in 30 mg/kg/day showed a significantly smaller food consumption throughout the study period.

**COMMENTS:**

No Table for groups means was submitted, but figures were submitted on pages 94 for M and 99 for F.

**Water Consumption:**

Water consumption (every 13 weeks) in M and F of 0.5 mg/kg/day group showed a increase at every point of examination, and it was correlated with the increased body weight gain in this group.

**Hematology:**

Some values were significantly different from the control, but there were no dose-related or time-dependent biologically significant changes.

**Thromboplastime Time:**

Although the values of 30 mg/kg/day group (M and F) were higher (some significantly) than control values, starting from week 6, they were reported to be within normal ranges for untreated rats. (Compare pages 104-263).

**Clinical Chemistry:**

Although some values were significantly different from the control values, only BUN showed a persistent change: BUN in both M and F of 30 mg/kg/day showed significant decrease starting from week 6 to the end, and some of the values in 5.0 mg/kg/day group also showed decrease during the same time.

**Urinalysis:**

No drug-related pathological findings were reported.

**Necropsy:**

Drug-related finding was "coprostitis" in 1 in 6.0 mg/kg/day group and 4 in 30 mg/kg/day group. See pages 48-61.

**COMMENTS:**

This finding was known to be drug-induced from earlier studies.

**Organ Weight:**

No dose-related significant changes were reported. See pages 352-363.

**Food Consumption:**

General trend was that weekly food consumption in both M and F showed a great increase in first 3-5 weeks, followed by a gradual decrease up to week 33, then the consumption jumped up to a higher level and stayed to the end. However, M in 30 mg/kg/day showed a significantly smaller food consumption throughout the study period.

**COMMENTS:**

No Table for groups means was submitted, but figures were submitted on pages 94 for M and 99 for F.

**Water Consumption:**

Water consumption (every 13 weeks) in M and F of 0.5 mg/kg/day group showed a increase at every point of examination, and it was correlated with the increased body weight gain in this group.

**Hematology:**

Some values were significantly different from the control, but there were no dose-related or time-dependent biologically significant changes.

**Thromboplastime Time:**

Although the values of 30 mg/kg/day group (M and F) were higher (some significantly) than control values, starting from week 6, they were reported to be within normal ranges for untreated rats. (Compare pages 104-263).

**Clinical Chemistry:**

Although some values were significantly different from the control values, only BUN showed a persistent change: BUN in both M and F of 30 mg/kg/day showed significant decrease starting from week 6 to the end, and some of the values in 6.0 mg/kg/day group also showed decrease during the same time.

**Urinalysis:**

No drug-related pathological findings were reported.

**Necropsy:**

Drug-related finding was "coprostitis" in 7 in 6.0 mg/kg/day group and 4 in 30 mg/kg/day group. See pages 48-61.

**COMMENTS:**

This finding was known to be drug-induced from earlier studies.

**Organ Weight:**

No dose-related significant changes were reported. See pages 352-363.

Histopathology:

No drug-induced organ damages were reported. However, there were large number of tumors in this study. 13 M + 15 F in the control group had a total 37 tumors, showing 56%, due to concurrent neoplasias (primary multiplicity of tumors): Among these tumor bearing rats, 3 had 2, 1 had 3, and the other had 5 concurrent neoplasias. See pages 64-66.

10 M + 12 F in the 0.5 mg/kg/day group had a total 34 neoplasias (44%); and of these, 5 had 2, 2 with 3, 1 with 4 concurrent neoplasias. See pages 67-70.

6 M + 9 F in 6.0 mg/kg/day group had a total of 27 neoplasias (30%), and of these, 5 had 2, and 1 with 3 concurrent neoplasias. See pages 70-73.

8 M + 9 F in 30.0 mg/kg/day group had a total of 23 neoplasias (34%), and of these, 4 had 2 and 1 with 3 concurrent neoplasias. See pages 73-77.

Among them the following carcinomas were noted: hepatocellular carcinoma of the liver and carcinoma in thyroid and pituitary glands in the control group; lymphosarcoma of thymus, hemangiosarcoma of spleen and salivary gland, malignant ependymoma of cerebrum and pituitary, adenocarcinoma of mamma (2 rats) cystadenocarcinoma of mamma in low dose group; hemangiosarcoma of a lymph node, carcinoma of mamma, lymphosarcoma of thymus, subcutaneous fibrosarcoma in mid dose group; lymphosarcoma of thymus (2 rats), adenocarcinoma of thyroid (2 rats), carcinoma of lung, adenoid uterine carcinoma in high dose group. One rat in high dose group had leiomyoma of the cervix. All those tumors were reported to be due to "normal aging pathology" or "the spontaneous pathology" of this rat strain.

6.0 mg/kg/day group:

"Animal No. 271; died intercurrently; the cause of death was an extensively metastasizing, solid, anaplastic and partially cirrhotic mammary carcinoma."

30.0 mg/kg/day group:

"Four animals died intercurrently. The cause of death in Animal No. 304 was circulatory insufficiency during blood sample collection (pulmonary shock), in an animal with myocarditis.

Animal No. 307 was sacrificed moribund and was found to have moderately severe cystitis. In Animal No. 322, the cause of death was severe hemorrhagic cystitis and prostatitis concomitant with bilateral pyelitis and unilateral perinephritis. Cause of death in Animal No. 354 was hematopyometra."

A. ORAL CARCINOGENICITY STUDY OF SCH 1000-BR IN RATS: (11/25/83 review)

|                       |                                                                                                                                            |
|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| Strain:               | Chbb: THOM(SPF)                                                                                                                            |
| No. of Animals/Level: | 100 M + 100 F for control group<br>50 N + 50 F for treated groups<br>(M = 117.8 g and 34 + 2 days old.<br>F = 117.6 g and 35 + 2 days old) |
| Dosage Level:         | 0.5, 2, or 6 mg/kg/day in food.                                                                                                            |
|                       | Batch No. = IV/X                                                                                                                           |

From the 27th week onwards the substance was dosed consistently in ppm. The doses were set on the basis of the food concentrations in the weeks 14 - 26. From the 27th week of treatment until the 85th the dosage scheme was as follows:

|                  | mg/kg | av. concentr.<br>week 14 - 26<br>in ppm | constant dosage from week 27<br>in ppm |
|------------------|-------|-----------------------------------------|----------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 11.19                                   | 12                                     |
| G <sub>1</sub> ♀ | 0.5   | 9.24                                    | 10                                     |
| G <sub>2</sub> ♂ | 2     | 44.44                                   | 45                                     |
| G <sub>2</sub> ♀ | 2     | 36.70                                   | 37                                     |
| G <sub>3</sub> ♂ | 6     | 132.25                                  | 135                                    |
| G <sub>3</sub> ♀ | 6     | 110.77                                  | 110                                    |

From week 78 of the experiment the rats consumed less food so that the effective intake of substance became less. It was therefore necessary to increase the ppm dosages of Sch 1000-8r in the food. From week 86 onwards the dosages were as follows:

|                  | mg/kg | Constant dosage<br>weeks 27 - 85<br>in ppm | Constant dosage<br>from week 86<br>in ppm |
|------------------|-------|--------------------------------------------|-------------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 12                                         | 15                                        |
| G <sub>1</sub> ♀ | 0.5   | 10                                         | 12                                        |
| G <sub>2</sub> ♂ | 2     | 45                                         | 55                                        |
| G <sub>2</sub> ♀ | 2     | 37                                         | 45                                        |
| G <sub>3</sub> ♂ | 6     | 135                                        | 170                                       |
| G <sub>3</sub> ♀ | 6     | 110                                        | 120                                       |

The control group received the same food without Sch 1000-Br.

**Average Drug Intake:**

"The effective amount of substance ingested did not always correspond with the planned dosages because of the varying food consumption (see pages 021 to 032 / Part II).

On average the following amounts were ingested (given in mg/body weight):

| sex | group 1 | group 2 | group 3 |
|-----|---------|---------|---------|
| m   | 0.49    | 1.87    | 5.59    |
| f   | 0.52    | 1.98    | 5.89    |

**Duration and Route:** 114 weeks mixed in the food as 0.1% "trituration with lactose."

Initiated on 9/28-29/77 and autopsied from 11/23/79 to 12/6/79.

**Mortality:** Total 157 out of 500 died intercurrently or sacrificed moribund as shown in the following Table. There were more F than M, but the difference between F and M was significant ( $p < 0.05$ ) only in low and mid dose groups, and there were no significant differences between groups, and none were reported to be drug related. See pages 055 to 122 in Part II for findings on dead animals.

| groups | dose      | animals used |     | died / sacrificed intercurrently |    | total |
|--------|-----------|--------------|-----|----------------------------------|----|-------|
|        |           | ♂            | ♀   | ♂                                | ♀  |       |
| 0      | control   | 100          | 100 | 25                               | 34 | 59    |
| 1      | 0.5 mg/kg | 50           | 50  | 10                               | 22 | 32    |
| 2      | 2 mg/kg   | 50           | 50  | 8                                | 22 | 30    |
| 3      | 6 mg/kg   | 50           | 50  | 16                               | 20 | 36    |
|        | total     | 250          | 250 | 59                               | 98 | 157   |

**Clinical Observations:** No drug related findings were reported. See page 33 in Part II.

**Growth:** There were no significant differences between groups. See pages 001-004 in Part II for weekly for 26 weeks then every 4 weeks' growth.

**Food Consumption:** No differences between groups were reported for weekly consumption.

**Water Consumption:** M of mid dose consumed less water, but M in low dose group consumed more water than control M. See page 018 in Part II.

F of treated groups consumed dose-dependently more water than the control F most of the time. See page 020 in Part II.

**COMMENTS:** "Every three months the water consumption was determined for one week by measuring out the water and reweighing the volume not consumed. The consumption of drinking water was measured for the first 12 living male and the first 12 living female animals of each group."

**Hematology:**

Leucocyte counts at the end of the study showed an increase in all groups including the control groups, and the differential counts showed "no deviation from normal findings," although percent of monocyte and banded cells were significantly ( $p < 0.05$ ) increased in the high dose M. Monocytes were significantly ( $p < 0.01$ ) lower in the mid dose group M. See pages 034 and 035 in Part II for group means.

**Others Usual Parameters:** Not done.

**Autopsy Findings:**

No dose-related or drug-induced findings were reported, and the changes reported on pages 123-173 in Part II were regarded as "spontaneous" lesions.

**Histopathology:**

The following report was submitted:

"The neoplasias detected are compiled in tables 1 to 7. Table 8 gives a survey of the non-neoplastic lesions. The individual histopathological findings are to be found in the protocols (page 178 to 676) in part II of the report. A list of the abbreviations used in the microscopic diagnoses is given on pages 174 to 177 in part II."

In addition, the following results were also submitted:

Table 2: Frequencies of mammary tumors/100 animals/group

Table 3: Metastasising tumors classified according to groups

Table 4: Benign and malignant tumors and metastases of all animals used/groups and sex

Table 5: Numbers of animals with multiple or solitary primary tumors/group and sex

Table 6: Neoplasms of the rats dying intercurrently or sacrificed moribund

Table 7: Time of the first occurrence of the different types of tumor in rats dying intercurrently or sacrificed moribund

Table 8: Non-neoplastic lesions/group/sex/organ

**Hematology:**

Leucocyte counts at the end of the study showed an increase in all groups including the control groups, and the differential counts showed "no deviation from normal findings," although percent of monocyte and banded cells were significantly ( $p < 0.05$ ) increased in the high dose M. Monocytes were significantly ( $p < 0.01$ ) lower in the mid dose group M. See pages 034 and 035 in Part II for group means.

**Others Usual Parameters:** Not done.

**Autopsy Findings:**

No dose-related or drug-induced findings were reported, and the changes reported on pages 123-173 in Part II were regarded as "spontaneous" lesions.

**Histopathology:**

The following report was submitted:

"The neoplasias detected are compiled in tables 1 to 7. Table 8 gives a survey of the non-neoplastic lesions. The individual histopathological findings are to be found in the protocols (page 178 to 676) in part II of the report. A list of the abbreviations used in the microscopic diagnoses is given on pages 174 to 177 in part II."

In addition, the following results were also submitted:

Table 2: Frequencies of mammary tumors/100 animals/group

Table 3: Metastasising tumors classified according to groups

Table 4: Benign and malignant tumors and metastases of all animals used/groups and sex

Table 5: Numbers of animals with multiple or solitary primary tumors/group and sex

Table 6: Neoplasms of the rats dying intercurrently or sacrificed moribund

Table 7: Time of the first occurrence of the different types of tumor in rats dying intercurrently or sacrificed moribund

Table 8: Non-neoplastic lesions/group/sex/organ

"In the statistical analysis of the types of tumours listed in table 1 with the exact test according to Fisher, no significantly increased incidence was calculated either for the male or the female rats of the three dose groups in comparison to the control group. Concerning the aim of the trial, this means that a 114-weeks administration of Sch 1000-8r induced no increase in tumour frequency in the animals of the three dose groups. Due to the one-sided treatment of the problem by the test, a possible decrease of the tumour incidence could not be checked.

A comparison between the sexes within each group revealed the following significant differences ( $P < 0.05$ ):

1. Adenomas of the adrenal cortex are significantly more frequent in the male animals of groups 0, 1 and 2.
2. Pituitary adenomas are, in all groups, significantly more frequent in female animals.
3. Pituitary carcinomas are significantly more frequent in female control rats.
4. Lipomas of the skin/subcutis are significantly more frequent in male control rats.

Distribution and frequency of the different primary tumours, as well as the more frequent-occurrence of pituitary tumours in female animals or of tumours of the adrenals and of the skin/subcutis in male animals, are typical for this rat strain (TILOV et al., 1976, UEBERBERG and Lützen, 1979). There is, therefore, no influence of the substance on the calculated differences between the sexes. "

The following was the Evaluation on Histopathological Findings submitted by the Sponsor:

"The aim of this 114-week long-term study in the rat was to determine if the substance used, Sch 1000-Br, has a tumourigenic or carcinogenic effect. In order to answer this, the following questions are important from the pathological point of view:

1. Is there an increased occurrence of a certain type of tumour, or of several tumour types, in the treated animals of the three dose groups that also occurred in the control animals?
2. Are types of tumours observed in the treated rats of the three dose groups which do not occur in the control animals?
3. Is the number of malignant tumours increased in the animals treated with Sch 1000-Br in comparison with the control rats?
4. Is the number of multiple tumour carriers or the tumour variety for each individual animal higher in the treated rats of the three dose groups than in control animals?
5. Do certain types of tumour occur earlier in the treated rats than in the control animals of the same age?

When recording the non-neoplastic lesions the following question must also be answered:

6. After this long-term administration of the substance Sch 1000-Br do substance-induced changes occur which had not been observed up to now in previous toxicological experiments in the rat or other species?

Question 1:

In the rats of the three dose groups there was no significant increase in any of the types of tumours detected in comparison with the control animals of the same group.

Within the four experimental groups there were significant sex differences for individual tumour types ( $p < 0.05$ ):

Cortical adenomas of the adrenals were found more frequently in the male animals of groups 0, 1 and 2.

Pituitary adenomas were increased in the female animals of all four experimental groups.

Pituitary carcinomas and lipomas of the skin/subcutis were observed more frequently in the female and male rats of the control group.

Such sex differences are characteristic for the strain of rat used. They are therefore to be evaluated as a spontaneous occurrence independent of the substance.

Question 2:

Some benign and malignant neoplasms at various primary sites occurred only in the rats of the three dose groups (group 1 = 13 tumours, group 2 = 14 tumours, group 3 = 16 tumours). For these tumours no comparable types of tumour were found in the animals of the control group.

The primary sites of these tumours were mainly organs in which the tumor rate is relatively low (e.g. lung,

pancreas, colon, stomach, salivary gland, eyes etc.)  
On the other hand organs were also concerned, the tumour rate of which was high and which showed a broad spectrum of tumours (e.g. mammary gland, uterus, ovary, skin).

These individually occurring tumours are spontaneous tumours, i.e. not substance-induced.

This statement can be supported by the fact that some other types of tumour at various primary sites were found exclusively in the control animals (Group 0 = 33 tumours). If one examines the distribution of these with regard to their primary sites, it is clear that similar conditions exist in the control as in the treated animals. On the one hand there are again organs with low tumour rates (e.g. aorta, colon, bones, brain etc.), and on the other hand, primary sites with a high tumour rate and a wide tumour spectrum.

Furthermore, the investigations into the spontaneous tumour rate of the strain used (TILOV et al., 1976, UEBERBERG and LÜTZEN, 1979) and surveys on the spontaneous tumor rates of other strains of rats (e.g. MACKENZIE and GARNER, 1973) show that, with regard to primary site, frequency and type of tumour, such a wide variation is to be expected in aging rats.

### Question 3

From the statistical analysis it can be seen that after a 2 year administration of Sch 1000-Br the number of malignant tumours in the treated animals is not increased in comparison with the control animals of the same sex respectively. The same applies to the benign neoplasms.

Question 4:

No effect of the substance, in the sense of an increase in the number of multiple tumour carriers in the dose groups, or with regard to the variation in types of tumour, could be established statistically in comparison with the control animals.

Question 5:

Apart from isolated cases the most common tumours occurred in a certain sequence. Tumours of the mammary glands occurred in the first year of life, adenomas of the interstitial cells of Leydig, thymic lymphomas and adenomas of the pituitary occurred most frequently at the beginning of the second year of life, followed by tumours of the adrenal cortex. If the four experimental groups are compared, it becomes clear that, in spite of this difference in timing, the most common neoplasms were observed simultaneously in the control and the treated animals.

Question 6:

No substance-induced, non-neoplastic lesions could be detected on histopathological examination. The findings recorded were inflammatory and degenerative changes and changes due to age. They are distributed among all four experimental groups with varying frequency

and are to be attributed exclusively to the spontaneous pathology of the strain of rat used. Comparable spontaneous findings are already known from previous long-term studies.

In summary it can be said that after administration of Sch 1000-Br, in doses of 0.5 mg/kg, 2.0 mg/kg and 6.0 mg/kg, to rats, for 114 weeks, no tumourigenic or carcinogenic effect was observed. Frequency, types of tumour and time of occurrence of the neoplasms were not affected by the administration of the substance.

Likewise no substance-induced, non-neoplastic lesions could be detected histopathologically.

All histopathological findings obtained are, without exception, to be attributed to the spontaneous pathology of the strain of rat used. ”

**B. ORAL CARCINOGENICITY STUDY OF SCH 1000-BR-IN MICE:**

Strain: Chbb: NMRI (SPF)

No. of Animals/Level: 100 M + 100 F for the control group  
50 M + 50 F for the treated groups  
(M = 31.45 g and 50 ± 2 days old  
F = 25.58 g and 49 ± 2 days old)

Dosage Level: 0.5, 2, or 6 mg/kg/day in food  
Batch No. = IV/X

From the 27th week onwards the substance was dosed consistently in ppm. The doses were set on the basis of the food concentrations in the weeks 14 - 26. From the 27th week of treatment onwards the dosage scheme was as follows:

|                  | mg/kg | av. concentr.<br>week 14 - 26<br>in ppm | constant dosage<br>from week 27<br>in ppm |
|------------------|-------|-----------------------------------------|-------------------------------------------|
| G <sub>1</sub> ♂ | 0.5   | 4.077                                   | 5                                         |
| G <sub>1</sub> ♀ | 0.5   | 2.754                                   | 3                                         |
| G <sub>2</sub> ♂ | 2     | 16.739                                  | 15                                        |
| G <sub>2</sub> ♀ | 2     | 11.032                                  | 12                                        |
| G <sub>3</sub> ♂ | 6     | 51.686                                  | 50                                        |
| G <sub>3</sub> ♀ | 6     | 33.307                                  | 35                                        |

The control group received the same food without Sch 1000-Br.

**Average Drug Intake:**

“The effective amount of substance ingested did not always correspond with the planned dosages, especially towards the end of the experiment, because of the varying food consumption. On average the following amounts were ingested (given in mg/body weight):

| sex | group 1 | group 2 | group 3 |
|-----|---------|---------|---------|
| m   | 0.56    | 1.76    | 5.59    |
| f   | 0.48    | 1.87    | 5.50    |

(see pages 080 - 090/Part II).”

**Duration and Route:** 107 weeks mixed in food.  
Initiated on 6/20-21/77 and  
autopsied on 7/2-6/79.

**Mortality:** Total 170 out of 500 mice died  
intercurrently or were sacrificed  
moribund as shown in the following  
Table.

| groups | dose      | animals used |     | died/sacrificed intercurrently |     |       |
|--------|-----------|--------------|-----|--------------------------------|-----|-------|
|        |           | ♂            | ♀   | ♂                              | ♀   | total |
| 0      | control   | 100          | 100 | 29                             | 43  | 72    |
| 1      | 0.5 mg/kg | 50           | 50  | 13                             | 21  | 34    |
| 2      | 2 mg/kg   | 50           | 50  | 13                             | 21  | 34    |
| 3      | 6 mg/kg   | 50           | 50  | 9                              | 21  | 30    |
| total  |           | 250          | 250 | 64                             | 106 | 170   |

There were more F than M as shown, but the difference between F and M was significant ( $p < 0.05$ ) only in the control and high groups; but there were no significant differences between groups.

For the probable causes of death or illness of these mice, see page 115-161 in Part II.

**Clinical Observation:**

No-drug related findings were reported.  
See pages 091-093 in Part II.

**Growth:**

No differences between groups were reported. See pages 055-061 in Part II.

**Food Consumption:**

No significant differences between groups were reported.

**Water Consumption:**

Only mid dose group M had less water consumption during the second year. See pages 078-079 in Part II.

**Hematology:**

Leucyte count at the end of the study showed no dose-related changes, and there were no significant differences between groups.

"In all experimental groups individual animals showed in some cases considerably increased total leucocyte values (group 0: 2 m, 4 f; group 1: 2 f; group 2: 1 m, 1 f; group 3: 4 m, 3 f). This includes only those animals whose leucocyte values were over 20,000/mm<sup>3</sup>. The differential blood count of these 17 animals showed in 11 cases (nos. 012, 043, 553, 579, 612, 659, 218, 227, 228, 241, 704) a clearly increased percentage of lymphocytes, in 5 cases (nos. 528, 643, 181, 719, 743) an increase in the granulocytes and lymphocytes and in one case (no. 542) an extreme increase in the number of granulocytes. In a total of 8 (nos. 012, 542, 553, 579, 659, 227, 241, 743) of the 17 mice the increased total leucocyte value correlated with a histopathologically detected lymphatic or plasma cell leukemia. In the remaining 9 cases the increased total leucocyte value is a sign of an acute/chronic inflammation. (see pages 094 to 114/Part II)."

COMMENTS: Among the 8 mice with leukemia, 1 M + 3 F belonged to the control group; 1 F, to mid dose group; and 2 M + 1 F, to the high dose group.

Other usual parameters: Not done.

Autopsy Findings: No significant drug-induced changes were reported, and the changes reported on pages 162 to 206 in Part II were regarded as "spontaneous" lesions.

Histopathology: The following reports were submitted.

"In the carcinogenicity study in mice after administration of the substance Sch 1000-Br, the histological investigations were carried out by Prof. Dr. W. Drommer and Prof. Dr. G. Trautwein, Institut für Pathologie of the Tierärztliche Hochschule, Hannover.

The neoplasias observed are summarized in tables 1 - 5. A survey of the non-neoplastic alterations is given in table 6. The individual findings of each experimental animal can be found in the protocols (Pages 211 to 710) in part II of the report."

"In the carcinogenicity study in mice after administration of the substance Sch 1000-Br, the histological investigations were carried out by Prof. Dr. W. Drommer and Prof. Dr. G. Trautwein, Institut für Pathologie of the Tierärztliche Hochschule, Hannover.

The neoplasias observed are summarized in tables 1 - 5. A survey of the non-neoplastic alterations is given in table 6. The individual findings of each experimental animal can be found in the protocols (Pages 211 to 710) in part II of the report."

"Comparing the dose groups with the control group, the statistical analysis reveals only a few significant findings ( $P < 0.05$ ) for the different types of tumour:

1. Lung adenomas are significantly more frequent in the female animals of group 2, in comparison to the female mice of group 0.
2. This is also true for the reticulosarcomatosis.
3. Hepatocellular adenomas are significantly more frequent in the male mice of group 2 than in the male control animals.
4. Adenocarcinomas of the ovary are significantly more frequent in the female animals of group 1 than in the female control animals.

Since no dose-dependency could be established for the tumor frequency, these slight statistical differences are to be considered as having no biological importance. The statistical test treats the one-sided question of an increase in the tumour rate in the dose groups as compared to the control animals. For this reason, no statement can be given on the remarkably low tumour frequency in group 3.

In comparing the sexes the following significant differences ( $P < 0.05$ ) can be recognized:

1. Lung adenomas are in groups 0, 1 and 3 significantly more frequent in male animals.
2. Reticulosarcomatoses (group 2), lymphatic leucoses (group 0, 3), and thymomas (group 3) are significantly more frequent in the female animals of these groups.

3. Hepatocellular adenomas are in group 2 significantly more frequent in male animals.

The results of Stötzer and Notman (1982) confirm a possible influence of the sex concerning the higher frequency of lung adenomas in male animals of all 3 experimental groups in this long-term study. In their investigation of age-dependent occurrence of tumours in the same mouse strain, they showed that lung tumours are more frequent in 27 months old male animals. The significantly higher incidence of the lymphatic leucosis, the reticulosarcomatosis and the thymomas in the female animals are characteristic of this strain (Weisse et al., 1975, Stötzer and Notman, 1982). The significantly higher rate of hepatic cell adenomas in the male animals of group 2 coincides also with the results of Weiss et al. (1975). In their investigation they detected a higher incidence of hepatocellular adenomas in male mice.

In table 1a the metastasising tumours are compiled according to groups. The primary tumours and their organic localisation are listed in table 1, but not the organic metastases (secondary tumours). A statistical evaluation of the data of this table was not carried out for methodical reasons, since the metastases were found in a varying number of organs in different animals. The number of metastasising tumours as well as the total number of organs with metastases show no influence of substance or dose. Metastases were found most frequently in liver, ovary and lung.

In table 2 the benign and malignant tumours are summarized for each group separately according to sex.

A statistical evaluation of the data of these tables cannot be carried out for methodical reasons, since the numbers include animals with two and more benign or malignant tumours.

Considering the total number of tumours the malignant neoplasias predominate (cf. Weisse et al., 1975). This predominance is mainly due to the fact that the number of malignant tumours is in female mice in the different experimental groups up to four times higher than the number of benign tumours. The basis of this is the high rate of lymphatic leucoses and thymomas in the female mice (cf. tab. 1). In the male mice, on the contrary, the number of benign tumours does not differ greatly from that of malignant tumours in the different experimental groups.

A significant increase due to an influence of the substance either of benign or of malignant tumours is not observed for the 3 dose groups for either sex.

Table 3 lists the tumours of the animals which died intercurrently or were sacrificed moribund.

Tumour spectrum and primary site of the most frequently diagnosed neoplasias coincide largely with the findings at the end of the trial (Tab. 1). The dominating tumours are lymphatic leucoses, reticulosarcomatoses, lung adenomas and carcinomas.

Comparing the number of control and dose animals which died intercurrently or were sacrificed moribund, no significant differences are found for either sex in the groups. However, within groups 0 and 3 significantly ( $P < 0.05$ ) more intercurrent deaths are calculated for the female mice.

Significant differences ( $P < 0.05$ ) result for the different types of tumour when comparing the three dose groups with group 0:

1. Adenocarcinomas of the lung are significantly more frequently the cause of disease and death in the female animals of group 3 compared to the female control animals.
2. The reticulosarcomatosis is significantly more often the reason for sacrifice or the cause of death in the female animals of group 2 in comparison to the female animals of group 0.

Just as for the incidence of tumours in all animals used, no substance influence can be detected for the tumour frequency of the animals which died intercurrently or were sacrificed moribund.

A significant ( $P < 0.05$ ) influence of the sex was found in the animals which died intercurrently only for the adenocarcinomas of the lung, i.e. this tumour is more frequent in the male mice than in the female mice of group 0 (cf. Stötzer and Notman, 1982).

In addition, the following results were also submitted:

Table 4: Lists of the animals with 2 or more primary tumors/group and sex.

Table 5: Time of the first occurrence of the different types of tumors in mice which died intercurrently or sacrificed moribund.

Table 6: Non-neoplastic lesions/group/sex/organ.

The following was the "Evaluation on Histopathological Findings" submitted by the consultants:

"This long-term study was to investigate whether the substance Sch 1000-Br has a tumorigenic or cancerogenic effect in the mouse. In order to find an answer to this, several questions are important from the pathological point of view:

1. In the animals of the three dose groups is there an increased occurrence of a tumour type or several tumour types which were also observed in the control animals?
2. Are types of tumour observed in the mice of the three dose groups which do not occur in the control animals?
3. Is the number of malignant tumours increased in the animals treated with Sch 1000-Br in comparison with the control mice?
4. Is the number of multiple tumour carriers or the tumour variety for each individual animal higher in the mice of the three dose groups than in the control group?
5. Do certain tumours occur earlier in the mice treated with the test substances than in the control animals of the same age?

Finally in the diagnosis of the non-neoplastic lesions the following question should be considered:

6. After this long-term administration of Sch 1000-Br is it possible that substance induced organ changes occur in the mouse which had not been observed up to now in the previous toxicological experiments with other animal species?

The following was the "Evaluation on Histopathological Findings" submitted by the consultants:

"This long-term study was to investigate whether the substance Sch 1000-Br has a tumorigenic or cancerogenic effect in the mouse. In order to find an answer to this, several questions are important from the pathological point of view:

1. In the animals of the three dose groups is there an increased occurrence of a tumour type or several tumour types which were also observed in the control animals?
2. Are types of tumour observed in the mice of the three dose groups which do not occur in the control animals?
3. Is the number of malignant tumours increased in the animals treated with Sch 1000-Br in comparison with the control mice?
4. Is the number of multiple tumour carriers or the tumour variety for each individual animal higher in the mice of the three dose groups than in the control group?
5. Do certain tumours occur earlier in the mice treated with the test substances than in the control animals of the same age?

Finally in the diagnosis of the non-neoplastic lesions the following question should be considered:

6. After this long-term administration of Sch 1000-Br is it possible that substance induced organ changes occur in the mouse which had not been observed up to now in the previous toxicological experiments with other animal species?

Question 1:

In comparison to the control animals of the same sex the following types of tumours occurred significantly more frequently in the dose animals:

pulmonary adenomas and reticulosarcomatosis in the female animals of group 2,

hepatocellular adenomas in the male animals of group 2,

adenocarcinomas of the ovary in the female animals of group 1.

Since, with regard to the overall frequency of tumours and, therefore, also to the incidence of the four above named types of neoplasia, no dose-dependency can be detected, these statistical differences are to be interpreted as coincidental, i.e. not substance induced.

Question 2:

In isolated cases benign or malignant tumours with various primary sites occurred only in animals of the three dose groups (group 1 = 5 tumours, group 2 = 11 tumours, group 3 = 6 tumours). In general, organs were concerned of which the tumour rate is very low (e.g. mammary gland, uterus, urinary bladder, intestine). Although the control animals used in this study did not show comparable neoplasias, in our opinion these were spontaneous occurrences, independent of the substance. This statement can be supported by the fact that, certain other types of tumour with various primary sites and low incidence occurred only in the control animals (group 0 = 11 tumours, e.g.

mammary gland, testes, skin, thoracic and abdominal walls). Furthermore, investigations into the spontaneous tumour rate of the strain of mice used (Weisse et al., 1975) and the summarized results of other authors (Benirschke et al., 1978, Turusov et al., 1979, Frith and Ward, 1980), show that, with regard to primary site and tumour frequency, such a wide variation is to be expected in aging mice.

Question 3:

From the statistical analysis it can be seen that after a 2-year administration of Sch 1000-Br the number of malignant tumours in the dose animals is not increased in comparison with the non-treated animals.

Question 4:

With regard to the number of multiple tumour carriers and the multiplicity of tumours in each individual animal there are no statistically significant differences between control and dose animals.

Question 5:

Tumours of individual organs or organ systems appear at different times. Thus, broadly speaking, the lymphatic leucoses are followed by the pulmonary tumours and then the hepatic and ovarian neoplasias. However, it can be seen that these most frequent tumours were to be detected within certain periods simultaneously in control and dose animals.

No substance-induced, non-neoplastic changes could be detected histologically. The inflammatory and degenerative changes observed and those caused by age are exclusively spontaneous lesions which are distributed among the four experimental groups with varying frequency. Type and frequency of the lesions are already known from previous long-term studies with the same strain of mouse.

To summarize these results, it can be said that after oral administration of Sch 1000-Br in dosages of 0.5 mg/kg, 2.0 mg/kg and 6.0 mg/kg for 107 weeks no substance induced neoplasias could be detected. The incidence, type and time of appearance of the tumours were not affected by the substance administered.

Likewise no substance induced, non-neoplastic organic lesions could be observed.

All histopathological findings observed are, without exception, to be attributed to the spontaneous pathology of the strain of mouse used."

**ANTIGENICITY and MUTAGENICITY TESTS: (Report No. 0199)**  
Lab performing study: Taijin Institute for Bio-medical  
Research, Japan

**1. Antigenicity tests:**

Group of 5 M guinea-pigs (Hartley strain, 280-330 g) were sensitized with Sch 1000 (1 mg/ml/kg) with/without Freund's complete adjuvant by I.M. injection every other day for 8 times along with human serum (positive control) and saline control (negative control), both 4 times injections, and they were challenged 2 weeks later from the last day of sensitization by following method:

- a. Active anaphylactic shock reaction: Sch 1000 (1 mg/kg) and human serum (1 mg/kg) were administered to the sensitized animals, and they were observed 3 hours for anaphylactic shock reaction.
- b. Schultz-Dale reaction: Antigenicity was assessed by contraction of isolated ileum from sensitized animals by adding the sensitizing agent in the tissue bath.
- c. Passive cutaneous anaphylaxis test (PCA): Four intradermal injections of 0.1 ml sensitized serum were made on side of the (served) dorsal skin of a normal guinea-pig, and the animals were injected 30 min. later with mixture of 1 ml of Evans blue and sensitizing agents (Sch 1000 0.1 mg or human serum 5 mg) in saline, and the animals were killed 30 min. later, and their inverted skins were examined for the leakage of the dye.
- d. Acute cutaneous anaphylaxis test (ACA): 0.1 ml of 10 ug of Sch 1000 or human serum was injected intradermally on the back (served) of sensitized animals, and the reaction at the injection site was observed 24 hours later.

**RESULTS OF ANTIGENICITY TESTS:** Sch 1000 with/without adjuvant did not show antigenicity in the tests used while human serum did show antigenicity.

**1. MUTAGENICITY TEST:**

Reverse mutation test (Ames test) using *Salmonella typhimurium* TA 98 and TA 100 and aqueous solution of Sch 1000 (0.1 ml of 0.1-10 mg/ml) was used along with AP-2 as a positive control and distilled water as negative control groups, with/without S-9 mixture.

**RESULT:** It was reported as negative, but close examination of data indicated that the results are not conclusive.

**2. MUTAGENICITY STUDY OF THE COMPOUND SCH1000 BR (Ames Test): (9/17/81 rev)**

Lab. Performing the Test: Boehringer, Sohn, Germany

Date of Test: 5/27/80 - 6/27/80 (page 6)

GLP Statements: Not submitted.

**Methods:** 1. Sch 1000 BR (Batch no. 709078/3) was dissolved in DMSO, and the concentrations tested were 25, 250, 2500, 5000, and 10,000 ug/plate (only once for TA 1537) against benzo(a)pyrene(BP) as positive control in most mutagenicity testings; and MNNG, twice; and DENA, once.

2. Sch 1000 was investigated for a point mutagenic effect in reverse mutation tests in a total of 5 *Salmonella typhimurium* strains (TA 1535, TA 1537, TA 1538, TA 98, TA 100) using microsomal enzyme fractions and an NADPH-generating system. See pages 4-8 for details.

**Results:** 1. Due to extremely high spontaneous mutation rate (mean = 560/plate) of TA 100, the test was repeated without metabolic activation. See Table 2.

2. Up to 5000 ug/plate of Sch 1000 had no bacteriostatic effect on strains tested, but 10,000 ug/plate produced a toxic effect on TA 1537, and this concentration was used only once for repeated test in Table 6.

3. There were no differences in number of colonies (mean) between control and Sch for all for strains, TA 1535, TA 1538, TA 98, and TA 100 with/without addition of various activation systems.

~~But, Sch produced dose-dependent increase of revertant colonies in TA 1537, only with addition of Aroclor-induced microsomes fractions. Number of colonies with 5000 ug/plate was 70 while control was only 23, indicating positive mutation, as BP. See Table 5 for details.~~

4. The repeated test with the same system did not show any difference between Sch and control plate with addition of the same Aroclor induced fraction. However, this time the results after addition of phenobarbital induced fraction increased the revertant mutants slightly (average mean 20) from 250 up over the control mean of 14, while the results of studies without any fraction were only 4 -2/plate in all concentrations including control and BP.

3. MUTAGENICITY STUDIES: DOMINANT LETHAL STUDY IN MICE:(11/9/83 review)

A. DOMINANT LETHAL STUDY IN MICE:

IRI Project No. 703287:

Lab. Performing the Study: Inveresk Research International  
Musselburgh, EH21 7UB, Scotland

Methods: See the pages 8-15. Batch No. = 709078.

RESULTS:

1). Dose-Finding Study:

Two M/group of CD-1 mice (11-13 week olds) were dosed by gavage with 100, 250, 500, 1000, or 2000 mg/kg/day, as suspension in distilled water (10-20 ml/kg) for 5 days (7/7-11/80).

Two rats each in 1000 mg/kg/day group and 2000 mg/kg/day groups died on days 6 and 7, and they had signs of Piloerection, hypokinesia, and ataxia as shown in the following table:

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                    | Duration of Clinical Signs |
|-----------|------------------|------------|--------|------------|----------------------------------|-----------------------------------|----------------------------|
|           |                  |            | No.    | Day        |                                  |                                   |                            |
| 1         | 100              | 1<br>2     | 0/2    | K          | 5(2)                             | None                              |                            |
| 2         | 250              | 3<br>4     | 0/2    | K          | 5(2)                             | None                              |                            |
| 3         | 500              | 5<br>6     | 0/2    | K          | 5(2)                             | None                              |                            |
| 4         | 1000             | 7<br>8     | 2/2    | 7(2)       | 5(2)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |
| 5         | 2000             | 9<br>10    | 2/2    | 6(1), 7(1) | 5(2)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |

K = Killed at end of 15 day observation period.

See pages 16-17 for other findings.

2). Main Toxicity Study:

Six M/group of mice were dosed by gavage with 350 to 1772 mg/kg/day for 5 days (7/15-19/80). The following Table shows the results of this study.

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                                    | Duration of Clinical Signs           |
|-----------|------------------|------------|--------|------------|----------------------------------|---------------------------------------------------|--------------------------------------|
|           |                  |            | No.    | Day        |                                  |                                                   |                                      |
| 1         | 350              | 11-16      | 0/6    | K          | 5(6)                             | None                                              |                                      |
| 2         | 525              | 17-22      | 0/6    | K          | 5(6)                             | Piloerection                                      | day 2-6                              |
| 3         | 788              | 23-28      | 2/6    | 6(2), K(4) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia<br>Sedation | day 3-5<br>day 3-7<br>day 5<br>day 5 |
| 4         | 1181             | 29-34      | 6/6    | 6(6)       | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 4-5<br>day 2-5<br>day 4-5        |
| 5         | 1772             | 35-40      | 6/6    | 8(1), 6(5) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 3-5<br>day 3-5<br>day 3-5        |

K = Killed at end of 15 day observation period.

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                    | Duration of Clinical Signs |
|-----------|------------------|------------|--------|------------|----------------------------------|-----------------------------------|----------------------------|
|           |                  |            | No.    | Day        |                                  |                                   |                            |
| 1         | 100              | 1<br>2     | 0/2    | K          | 5(2)                             | None                              |                            |
| 2         | 250              | 3<br>4     | 0/2    | K          | 5(2)                             |                                   |                            |
| 3         | 500              | 5<br>6     | 0/2    | K          | 5(2)                             | None                              |                            |
| 4         | 1000             | 7<br>8     | 2/2    | 7(2)       | 5(2)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |
| 5         | 2000             | 9<br>10    | 2/2    | 6(1), 7(1) | 5(2)                             | Hypokinesia, piloerection, ataxia | day 3-6                    |

K = Killed at end of 15 day observation period.

See pages 16-17 for other findings.

2). Main Toxicity Study:

Six M/group of mice were dosed by gavage with 350 to 1772 mg/kg/day for 5 days (7/15-19/80). The following Table shows the results of this study.

| Group No. | Dose Level mg/kg | Animal No. | Deaths |            | No. of Doses Given (No. of Mice) | Clinical Signs                                    | Duration of Clinical Signs           |
|-----------|------------------|------------|--------|------------|----------------------------------|---------------------------------------------------|--------------------------------------|
|           |                  |            | No.    | Day        |                                  |                                                   |                                      |
| 1         | 350              | 11-16      | 0/6    | K          | 5(6)                             | None                                              |                                      |
| 2         | 525              | 17-22      | 0/6    | K          | 5(6)                             | Piloerection                                      | day 2-6                              |
| 3         | 788              | 23-28      | 2/6    | 6(2), K(4) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia<br>Sedation | day 3-5<br>day 3-7<br>day 5<br>day 5 |
| 4         | 1181             | 29-34      | 6/6    | 6(6)       | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 4-5<br>day 2-5<br>day 4-5        |
| 5         | 1772             | 35-40      | 6/6    | 5(1), 6(5) | 5(6)                             | Hypokinesia<br>Piloerection<br>Ataxia             | day 3-5<br>day 3-5<br>day 3-5        |

K = Killed at end of 15 day observation period.

"From the results of this main toxicity test the LD<sub>50</sub> (5-day) value was calculated as 842 mg/kg with 95% confidence limits of 710-1000 mg/kg. The dose levels used in the main dominant lethal test were as follows:

| <u>Group</u>                     | <u>Dose Level</u> |
|----------------------------------|-------------------|
| Low - 0.05 x LD <sub>50</sub>    | 42 mg/kg          |
| Middle - 0.16 x LD <sub>50</sub> | 135 mg/kg         |
| High - 0.5 x LD <sub>50</sub>    | 421 mg/kg         |

3). Dominant Lethal Study:

After a preliminary pregnancy study with six M/group and F, 15 M/group of mice were selected (see page 21) and dosed by gavage for 5 days with 42, 135, 421 mg/kg/day (10 ml/kg) Sch 1000-BR along with a vehicle control group receiving 10 ml/kg distilled water and a positive control group receiving 100 mg/kg/day ethyl methane sulphonate in 10 ml/kg. Then, 1 M was mated with 2 F virgin mice (8-10 weeks old) in each cage (Day 0) for 5 days (total 8 sets of matings during 8 weeks), and F were separated from the M, killed, and examined.

There were no deaths in any group, and no toxic signs were noted in vehicle control, low, or mid dose groups. Two mice (Nos. 5 and 62) in the high dose group had piloerection and lethargy during dosing, and one in the positive control group (no. 153) had piloerection on day 4.

The following Table showed pregnancy rate for F:

| Week    | Distilled Water<br>10 ml/kg, x 5 | Sch 1000 BR<br>42 mg/kg, x 5 | Sch 1000 BR<br>135 mg/kg, x 5 | Sch 1000 BR<br>421 mg/kg, x 5 | DMS<br>100 mg/kg, x 5 |
|---------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1       | 26/30 87%                        | 22/30 73%                    | 26/30 87%                     | 26/30 87%                     | 24/30 80%             |
| 2       | 29/30 97%                        | 30/30 100%                   | 29/30 97%                     | 26/30 87%                     | 22/29 76%             |
| 3       | 24/30 80%                        | 25/30 83%                    | 27/30 90%                     | 24/30 80%                     | 22/30 73%             |
| 4       | 27/30 90%                        | 25/30 83%                    | 26/30 87%                     | 23/30 77%                     | 24/30 80%             |
| 5       | 24/30 80%                        | 26/30 87%                    | 24/29 83%                     | 21/29 72%                     | 24/30 80%             |
| 6       | 28/30 93%                        | 26/29 90%                    | 24/30 80%                     | 26/30 87%                     | 22/30 73%             |
| 7       | 26/30 87%                        | 25/29 86%                    | 27/29 93%                     | 27/30 90%                     | 24/30 80%             |
| 8       | 27/28 96%                        | 24/28 86%                    | 24/28 86%                     | 24/30 80%                     | 24/30 80%             |
| Overall | 211/238 89%                      | 203/236 86%                  | 207/236 88%                   | 197/239 82%                   | 186/239 78%           |

\* = Actual pregnancies/possible pregnancies

There were no significant differences between 4 groups in mean numbers of implantations per pregnant F, but during mating weeks 1 and 2, the positive control group had a slightly less implantation. See Table 7 on page 34.

"Early deaths" were assessed by 4 methods as the following 4 Tables, but only method 4 was statistically analyzed.

Method 1:

Pregnancies with 2 or More Early Deaths  
Dominant Lethal Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, x 5 | Sch 1000 BR<br>42 mg/kg, x 5 | Sch 1000 BR<br>135 mg/kg, x 5 | Sch 1000 BR<br>421 mg/kg, x 5 | DMS<br>100 mg/kg, x 5 |
|------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1    | 2/26 7.7%                        | 5/22 22.7%                   | 2/26 7.7%                     | 1/26 3.8%                     | 14/24 58.3%           |
| 2    | 1/29 3.4%                        | 3/30 10.0%                   | 3/29 10.3%                    | 2/26 7.7%                     | 11/22 50.0%           |
| 3    | 0/24 0.0%                        | 1/25 4.0%                    | 5/27 18.5%                    | 1/24 4.2%                     | 2/22 9.1%             |
| 4    | 7/27 25.9%                       | 2/25 8.0%                    | 4/26 15.4%                    | 3/23 13.0%                    | 1/24 4.2%             |
| 5    | 3/24 12.5%                       | 3/26 11.5%                   | 2/24 8.3%                     | 1/21 4.8%                     | 5/24 20.8%            |
| 6    | 5/28 17.9%                       | 2/26 7.7%                    | 1/24 4.2%                     | 1/26 3.8%                     | 5/22 22.7%            |
| 7    | 4/26 15.4%                       | 1/25 4.0%                    | 4/27 14.8%                    | 7/27 25.9%                    | 3/24 12.5%            |
| 8    | 1/27 3.7%                        | 1/24 4.2%                    | 2/24 8.3%                     | 1/24 4.2%                     | 1/24 4.2%             |

Method 2:

Estimated Percentage of Implants Recorded as Early Deaths (E)  
Dominant Lethal Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, x 5 | Sch 1000 BR<br>42 mg/kg, x 5 | Sch 1000 BR<br>135 mg/kg, x 5 | Sch 1000 BR<br>421 mg/kg, x 5 | EMS<br>100 mg/kg, x 5 |
|------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1    | 3.0                              | 6.6                          | 3.6                           | 1.5                           | 20.6                  |
| 2    | 2.4                              | 4.6                          | 3.5                           | 2.2                           | 18.2                  |
| 3    | 1.4                              | 2.3                          | 4.9                           | 3.0                           | 3.8                   |
| 4    | 7.0                              | 3.0                          | 4.0                           | 5.3                           | 2.7                   |
| 5    | 2.7                              | 4.1                          | 4.1                           | 2.4                           | 5.4                   |
| 6    | 4.8                              | 3.6                          | 2.0                           | 2.8                           | 5.0                   |
| 7    | 6.0                              | 1.9                          | 6.2                           | 7.6                           | 6.8                   |
| 8    | 3.6                              | 2.9                          | 4.0                           | 3.3                           | 2.3                   |

Method 3:

Mean Number of Live Implants and Late Deaths per Pregnancy  
Dominant Lethal Test  
Sch 1000 BR

| Week | Distilled Water<br>10 ml/kg, x 5 | Sch 1000 BR<br>42 mg/kg, x 5 | Sch 1000 BR<br>135 mg/kg, x 5 | Sch 1000 BR<br>421 mg/kg, x 5 | EMS<br>100 mg/kg, x 5 |
|------|----------------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------|
| 1    | 12.58                            | 11.27                        | 11.58                         | 12.77                         | 8.46                  |
| 2    | 11.66                            | 11.49                        | 11.45                         | 11.92                         | 8.39                  |
| 3    | 12.29                            | 11.88                        | 12.70                         | 12.38                         | 11.68                 |
| 4    | 11.15                            | 11.60                        | 12.31                         | 12.00                         | 11.83                 |
| 5    | 11.96                            | 11.77                        | 12.79                         | 12.10                         | 11.67                 |
| 6    | 11.54                            | 11.77                        | 12.83                         | 11.77                         | 11.64                 |
| 7    | 11.65                            | 12.20                        | 11.93                         | 11.81                         | 11.21                 |
| 8    | 11.85                            | 11.04                        | 10.96                         | 10.96                         | 10.92                 |

Method 4:

Estimates of  $\theta$ , the Expected  
Proportion of Early Deaths  
Extra-Binomial Variation Assumed

| Week | Treatment Group | $\hat{\theta} \pm S.E. \hat{\theta}$<br>$\hat{\theta} = \log_e \theta (1 - \theta)^{-1}$ |
|------|-----------------|------------------------------------------------------------------------------------------|
| 1    | 1               | -3.480 $\pm$ 0.4025                                                                      |
|      | 2               | -2.657 $\pm$ 0.3075                                                                      |
|      | 3               | -3.294 $\pm$ 0.3770                                                                      |
|      | 4               | -4.192 $\pm$ 0.5613                                                                      |
|      | 5               | -1.331 $\pm$ 0.1898*                                                                     |
| 2    | 1               | -3.714 $\pm$ 0.4158                                                                      |
|      | 2               | -3.044 $\pm$ 0.3006                                                                      |
|      | 3               | -3.307 $\pm$ 0.3445                                                                      |
|      | 4               | -3.802 $\pm$ 0.4545                                                                      |
|      | 5               | -1.503 $\pm$ 0.1972*                                                                     |
| 3    | 1               | -4.289 $\pm$ 0.5395                                                                      |
|      | 2               | -3.731 $\pm$ 0.4103                                                                      |
|      | 3               | -2.960 $\pm$ 0.2660                                                                      |
|      | 4               | -3.493 $\pm$ 0.3667                                                                      |
|      | 5               | -3.228 $\pm$ 0.3460                                                                      |
| 4    | 1               | -2.594 $\pm$ 0.3058                                                                      |
|      | 2               | -3.466 $\pm$ 0.4824                                                                      |
|      | 3               | -3.173 $\pm$ 0.3958                                                                      |
|      | 4               | -2.893 $\pm$ 0.3725                                                                      |
|      | 5               | -3.584 $\pm$ 0.5050                                                                      |
| 5    | 1               | -3.597 $\pm$ 0.3948                                                                      |
|      | 2               | -3.163 $\pm$ 0.3102                                                                      |
|      | 3               | -3.153 $\pm$ 0.3106                                                                      |
|      | 4               | -3.350 $\pm$ 0.3728                                                                      |
|      | 5               | -2.855 $\pm$ 0.2801                                                                      |
| 6    | 1               | -2.614 $\pm$ 0.2982                                                                      |
|      | 2               | -1.286 $\pm$ 0.4181                                                                      |
|      | 3               | -3.890 $\pm$ 0.5772                                                                      |
|      | 4               | -3.561 $\pm$ 0.4769                                                                      |
|      | 5               | -2.945 $\pm$ 0.3688                                                                      |
| 7    | 1               | -2.761 $\pm$ 0.2891                                                                      |
|      | 2               | -3.943 $\pm$ 0.5071                                                                      |
|      | 3               | -2.718 $\pm$ 0.2759                                                                      |
|      | 4               | -2.498 $\pm$ 0.2505                                                                      |
|      | 5               | -2.615 $\pm$ 0.2852                                                                      |
| 8    | 1               | -3.291 $\pm$ 0.3222                                                                      |
|      | 2               | -3.500 $\pm$ 0.3892                                                                      |
|      | 3               | -3.171 $\pm$ 0.3337                                                                      |
|      | 4               | -3.389 $\pm$ 0.3709                                                                      |
|      | 5               | -3.772 $\pm$ 0.4463                                                                      |

1 = Distilled water, 10 ml/kg      2 = Sch 1000 BR, 42 mg/kg  
 3 = Sch 1000 BR, 135 mg/kg      4 = Sch 1000 BR, 421 mg/kg  
 5 = EMS, 100 mg/kg

\* = Significantly increased from the control level, P<0.5

See pages 23-25 for discussion on these methods.

B. CYTOGENETIC STUDY IN CHINESE HAMSTERS OF SCH 1000-BR:

IRI Project No. 703308

Lab. Performing the Study: Inveresk Research International.

Methods: See pages 8-11. Batch No. = 709078

RESULTS:

1) Dose-Finding Study:

Two M/group of Chinese hamsters (8-10 weeks old) were dosed orally by gavage with 100 to 2000 mg/kg/day Sch-1000 Br suspended in distilled water (10 ml/kg) for 5 days (8/13-17/80). All treated hamsters had piloerection from day 1 until the death or termination on day 14. One M died on day 7 and one on day 8 after 1000 mg/kg/day, and both M in 2000 mg/kg/day died on day 6, and they also had hypokinesia on day 3 and sedation on day 5, as shown in the following Table. Autopsy showed red lung and red/orange fluid in the gut of dead animals.

| Group No. | Dose Level mg/kg | Animal No. | Deaths |               | No. of Doses Given (No. of Hamsters) | Clinical Signs                                         | Duration: Clinical Signs |
|-----------|------------------|------------|--------|---------------|--------------------------------------|--------------------------------------------------------|--------------------------|
|           |                  |            | No.    | Day           |                                      |                                                        |                          |
| 1         | 100              | 1<br>2     | 0/2    | K             | 5 (2)                                | Piloerection                                           | day 1-14                 |
| 2         | 250              | 3<br>4     | 0/2    | K             | 5 (2)                                | Piloerection                                           | day 1-14                 |
| 3         | 500              | 5<br>6     | 0/2    | K             | 5 (2)                                | Hypokinesia, piloerection, sedation                    | day 1-14                 |
| 4         | 1000             | 7<br>8     | 2/2    | 7(1),<br>8(1) | 5 (2)                                | Hypokinesia, piloerection, sedation, coma, prostration | day 1-7                  |
| 5         | 2000             | 9<br>10    | 2/2    | 6(2)          | 5 (2)                                | Hypokinesia, piloerection, sedation                    | day 1-5                  |

K = Killed at end of 14 day observation period.

See page 19 for body weight.

N-19085-4

2) Main Toxicity Study:

Six M/group of Chinese hamsters were dosed with 400 to 1142 mg/kg/day Sch 1000-Br for 5 days. The following table was the results of this study. One M in 520 mg/kg/day group, 3 in 676 mg/kg/day group, 4 in 879 mg/kg/day group, and 5 in 1142 mg/kg/day died between days, 7 and 10. Autopsy showed no significant pathology in 400 and 1000 mg/kg/day groups, but other groups had red lungs, red/orange gut fluids, pale kidneys, patchy lungs, and they were dose-related.

| Group No. | Animal No. | Dose Level mg/kg | Deaths |                               | No. of Doses Given (No. of Hamsters) | Clinical Signs                                    | Duration of Clinical Signs                            |
|-----------|------------|------------------|--------|-------------------------------|--------------------------------------|---------------------------------------------------|-------------------------------------------------------|
|           |            |                  | No.    | Day                           |                                      |                                                   |                                                       |
| 1         | 11-16      | 400              | 0/6    | K                             | 5(6)                                 | Hypokinesia<br>Piloerection<br>Soiled coat        | on-day 3<br>day 2-10<br>day 6-10                      |
| 2         | 17-22      | 520              | 1/6    | 9(1), K(5)                    | 5(6)                                 | Hypokinesia<br>Piloerection<br>Ataxia             | day 5-6<br>day 3-14<br>day 5-7                        |
| 3         | 23-28      | 676              | 3/6    | 7(1), 9(1),<br>10(1),<br>K(3) | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | days 2-5 and<br>7-8<br>day 4-5<br>day 2-14<br>day 5-8 |
| 4         | 28-34      | 879              | 4/6    | 7(2), 9(1),<br>10(1),<br>K(2) | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | day 3-4<br>day 5-8<br>day 2-14<br>day 4-10            |
| 5         | 35-40      | 1142             | 5/6    | 7(3), 8(2),<br>K(1)           | 5(6)                                 | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | day 3-7<br>day 5-6<br>day 1-14<br>day 3-9             |

K = Killed at end of 14 day observation period.

See page 21 for body weight.

From the results of this study, the LD<sub>50</sub> (5 day) value was calculated as 722 mg/kg/day (538-969 mg/kg for 95% conf. limit), and the dose levels were chosen as 72- and 361 mg/kg/day for cytogenetic study.

3) Cytogenetic Study:

Ten M/group of Chinese hamster (20-32 g, 8-10 weeks old) were dosed by gavage with 72 or 361 mg/kg/day Sch 1000-BR in 10 ml/kg, along with a vehicle control group receiving 10 ml/kg of distilled water and a positive control group receiving 200 mg/kg/day ethylmethane sulphonate for 5 days (9/12-16/80).

Four hours after the last dose, each hamster was injected with 6 mg/kg ip colchicine dissolved in Hank's Balanced Salt Solution, and 2 hours later hamsters were killed by neck dislocation, then bone marrow slides were made from "femora" for metaphase examination of chromosomes. See pages 8-11 for detailed procedure for metaphase cell preparation.

Body weight of positive control group was slightly less than other groups (see page 22).

On M (no. 67) in 72 mg/kg/day group had diarrhea on day 3, and one M (No. 78) in 361 mg/kg/day group was "subdued" on day 3 with closed eyes, and No. 79 was found moribund on the morning of day 5, and it was killed in extremis, but autopsy did not show any specific pathology.

The following Table was the summary of metaphase chromosome analysis:

Cytogenetic Analysis of Chinese Hamster Bone Marrow Cells  
Sch 1000 BR

Chromatid/Chromosomal Aberrations Scored

Males

Sub-acute

Sampling Time: 6

| Group                                    | Number of Spreads Observed | Observed Aberrations |       |         |            |       |         | Miscellaneous                                         |
|------------------------------------------|----------------------------|----------------------|-------|---------|------------|-------|---------|-------------------------------------------------------|
|                                          |                            | Chromatid            |       |         | Chromosome |       |         |                                                       |
|                                          |                            | Gap                  | B w F | B w/o F | Gap        | B w F | B w/o F |                                                       |
| Distilled Water<br>10 ml/kg x 5          | 500                        | 4                    | 1     | 2       | 1          | 0     | 0       |                                                       |
| Sch 1000 BR<br>72 mg/kg x 5              | 500                        | 9                    | 2     | 1       | 3          | 1     | 0       | 1 Chromatid Fragment                                  |
| Sch 1000 BR<br>161 mg/kg x 5             | 450                        | 14                   | 2     | 1       | 0          | 1     | 0       |                                                       |
| Ethyl methanesulphonate<br>200 mg/kg x 5 | 500                        | 29                   | 25    | 0       | 8          | 1     | 0       | 2 Multi Aberrations<br>5 Exchanges<br>1 Translocation |

\* One animal died

B w F = Break with Fragment

B w/o F = Break without Fragment

The following summary was also submitted:

"The quality of the metaphase chromosomes observed was variable. It was possible, however, to read 50 metaphase spreads for each of the 40 animals used in the cytogenetic test (apart from one hamster which died). Details are given in Appendix Table 3.

Chromosomal damage was seen in all 4 groups. Both gaps and breaks were observed. Most damage was seen in the positive control group, 200 mg EMS/kg, in which was observed not only gaps and breaks, but exchanges, multi-aberrations and a translocation. There was little difference in the numbers or frequencies of aberrations between the low and high dose groups of Sch 1000 BR. In

the vehicle control group fewer gaps and breaks were observed than the other groups. There is statistical evidence that Sch 1000 BR does increase the incidence of cells with any type of aberration in both the low and high dose groups. The significance levels were 0.05 and 0.07 respectively. If cells containing gaps only are rejected from analysis, then there were no significant differences between the vehicle control group and the groups dosed with Sch 100 BR. 7)

C. POINT MUTAGENIC ACTIVITY OF SCH 1000-BR IN SALMONELLA TYPHIMURIUM:

Lab. Performing the Study: Boehringer Ingelheim KG in Rhein

NOTE: This study was repetition of earlier report dated 7/10/80.

Methods: Sch 1000-Br was tested from 10 to 10000  $\mu$ g per plate on TA 1535, TA 1537, TA 1538, TA 100, and TA 98 with/without S-9 metabolic activation, along with a vehicle control and respective positive control groups. See pages 1-9 for detailed procedure.

RESULTS: See the following two Tables.

Table 1: Induction of his<sup>+</sup> mutations in *S. typhimurium* by the substance Sch 1000-Br - without metabolic activation with S-9 mix.  
(Experimental part No. MUT 0002/01)

| substance <sup>a</sup> | concentration<br>(µg/plate) | number of his <sup>+</sup> revertants per plate <sup>b, c</sup> |     |        |    |        |     |       |     |      |     |
|------------------------|-----------------------------|-----------------------------------------------------------------|-----|--------|----|--------|-----|-------|-----|------|-----|
|                        |                             | TA1535                                                          |     | TA1537 |    | TA1538 |     | TA100 |     | TA98 |     |
|                        |                             | ■                                                               | ■   | ■      | ■  | ■      | ■   | ■     | ■   | ■    | ■   |
| control                |                             | 29                                                              |     | 17     |    | 10     |     | 191   |     | IF   |     |
|                        |                             | 42                                                              |     | 12     |    | 15     |     | 180   |     | 16   |     |
|                        |                             | 36                                                              | 36  | 20     | 16 | 16     | 14  | 184   | 185 | 13   | 15  |
| Sch 1000-Br            | 10                          | 37                                                              |     | 16     |    | 13     |     | 178   |     | 12   |     |
|                        |                             | 39                                                              |     | 7      |    | 13     |     | 205   |     | 22   |     |
|                        |                             | 30                                                              | 35  | 12     | 12 | 15     | 14  | 207   | 197 | 17   | 17  |
|                        | 100                         | 36                                                              |     | 17     |    | 27     |     | 184   |     | 15   |     |
|                        |                             | 32                                                              |     | 26     |    | 16     |     | 161   |     | 18   |     |
|                        |                             | 33                                                              | 34  | 15     | 19 | 12     | 18  | 176   | 174 | 24   | 19  |
|                        | 1000                        | 37                                                              |     | 10     |    | 14     |     | 174   |     | 14   |     |
|                        |                             | 30                                                              |     | 13     |    | 11     |     | 171   |     | 17   |     |
|                        |                             | 40                                                              | 36  | 11     | 11 | 18     | 14  | 189   | 178 | 23   | 16  |
| 5000                   | 38                          |                                                                 | 16  |        | 20 |        | 160 |       | 20  |      |     |
|                        | 38                          |                                                                 | 21  |        | 11 |        | 218 |       | 16  |      |     |
|                        | 38                          | 38                                                              | 19  | 19     | 15 | 15     | 181 | 186   | 20  | 19   |     |
| 10000                  | 49                          |                                                                 | 17  |        | 16 |        | 168 |       | 17  |      |     |
|                        | 45                          |                                                                 | 13  |        | 13 |        | 179 |       | 14  |      |     |
|                        | 42                          | 45                                                              | 12  | 13     | 16 | 15     | 176 | 174   | 14  | 14   |     |
| 4-NQO                  | 1                           | 171                                                             |     | 42     |    | 258    |     | >3000 |     | 548  |     |
|                        |                             | 179                                                             |     | 54     |    | 221    |     | >3000 |     | 531  |     |
|                        |                             | 150                                                             | 167 | 35     | 45 | 213    | 231 | >3000 |     | 17   | 540 |

<sup>a</sup> control, 50 µl DMSO/plate; 4-NQO, 4-nitroquinoline-1-oxide

<sup>b</sup> titer of the bacterial suspensions (cells/ml): TA1535,  $2.9 \times 10^9$  (290, 292, 281); TA1537,  $2.0 \times 10^9$  (206, 190, 201); TA1538,  $2.2 \times 10^9$  (222, 208, 237); TA100,  $0.5 \times 10^9$  (58, 49, 51); TA98,  $1.5 \times 10^9$  (156, 160, 13)

the data in parentheses give the number of colonies counted per plate.

<sup>c</sup> ■, absolute number of revertants per plate; ■, mean value of the parallel plates; IF, infection

Table 2: Induction of his<sup>+</sup> mutations in *S. typhimurium* by the substance Sch 1000-Br with metabolic activation with S-9 mix.  
(Experimental part No. MUT 0002/02)

| substance <sup>a</sup> | concentration (µg/plate) | number of his <sup>+</sup> |        | revertants per plate <sup>b, c</sup> |        |        |       |      |        |     |        |  |        |  |       |  |      |  |  |
|------------------------|--------------------------|----------------------------|--------|--------------------------------------|--------|--------|-------|------|--------|-----|--------|--|--------|--|-------|--|------|--|--|
|                        |                          | TA1535                     | TA1537 | TA1535                               | TA1537 | TA1538 | TA100 | TA98 | TA1535 |     | TA1537 |  | TA1538 |  | TA100 |  | TA98 |  |  |
| control                |                          | 24                         |        | 23                                   |        | 17     |       | 133  |        | 28  |        |  |        |  |       |  |      |  |  |
|                        |                          | 22                         |        | 20                                   |        | 26     |       | 153  |        | 29  |        |  |        |  |       |  |      |  |  |
|                        |                          | 17                         | 21     | 18                                   | 20     | 20     | 21    | 127  | 138    | 40  | 32     |  |        |  |       |  |      |  |  |
| Sch 1000-Br            | 10                       | 26                         |        | 12                                   |        | 17     |       | 143  |        | 30  |        |  |        |  |       |  |      |  |  |
|                        |                          | 13                         |        | 18                                   |        | 31     |       | 139  |        | 30  |        |  |        |  |       |  |      |  |  |
|                        |                          | 27                         | 22     | 15                                   | 15     | 19     | 22    | 127  | 136    | 34  | 21     |  |        |  |       |  |      |  |  |
|                        | 100                      | 18                         |        | 14                                   |        | 27     |       | 165  |        | 25  |        |  |        |  |       |  |      |  |  |
|                        |                          | 22                         |        | 30                                   |        | 17     |       | 161  |        | 24  |        |  |        |  |       |  |      |  |  |
|                        |                          | 17                         | 20     | 18                                   | 21     | 24     | 23    | 150  | 159    | 28  | 26     |  |        |  |       |  |      |  |  |
|                        | 1000                     | 23                         |        | 17                                   |        | 22     |       | 151  |        | 27  |        |  |        |  |       |  |      |  |  |
|                        |                          | 22                         |        | 21                                   |        | 33     |       | 140  |        | 27  |        |  |        |  |       |  |      |  |  |
|                        |                          | 20                         | 22     | 19                                   | 19     | 29     | 28    | 131  | 141    | 27  | 27     |  |        |  |       |  |      |  |  |
| 5000                   | 14                       |                            | 21     |                                      | 11     |        | 158   |      | 22     |     |        |  |        |  |       |  |      |  |  |
|                        | 27                       |                            | 25     |                                      | 34     |        | 187   |      | 45     |     |        |  |        |  |       |  |      |  |  |
|                        | 25                       | 22                         | 26     | 24                                   | 21     | 22     | 133   | 159  | 24     | 30  |        |  |        |  |       |  |      |  |  |
| 10000                  | 23                       |                            | 16     |                                      | 18     |        | 171   |      | 25     |     |        |  |        |  |       |  |      |  |  |
|                        | 21                       |                            | 25     |                                      | 24     |        | 153   |      | 24     |     |        |  |        |  |       |  |      |  |  |
|                        | 18                       | 21                         | 18     | 20                                   | 27     | 23     | 150   | 198  | 29     | 26  |        |  |        |  |       |  |      |  |  |
| B(a)P                  | 5                        | 18                         |        | 72                                   |        | 55     |       | 640  |        | 193 |        |  |        |  |       |  |      |  |  |
|                        |                          | 20                         |        | 76                                   |        | 56     |       | 558  |        | 186 |        |  |        |  |       |  |      |  |  |
|                        |                          | 24                         | 21     | 74                                   | 74     | 51     | 54    | 572  | 590    | 232 | 208    |  |        |  |       |  |      |  |  |

<sup>a</sup> control, 50 µl DMSO/plate; B(a)P, benzo(a)pyrene

<sup>b</sup> titer of the bacterial suspensions (cells/ml): TA1535,  $3.1 \times 10^9$  (335, 292, 308); TA1537,  $1.5 \times 10^9$  (112, 160, 191); TA1538,  $2.6 \times 10^9$  (282, 254, 252); TA100,  $0.5 \times 10^9$  (44, 50, 40); TA98,  $2.4 \times 10^9$  (243, 256, 229) the data in parentheses give the number of colonies counted per plate.

<sup>c</sup>  $\bar{n}$ , absolute number of revertants per plate;  $\bar{x}$ , mean value of the parallel plates; IF, infection; sterility control(s): S-9 mix was germ-free.

**D. MICRONUCLEUS TEST OF SCH 1000 BR:**

Project NO 703292 and Extension 1-

Lab. Performing the Study: Inveresk Research International

Methods: See pages 8-15.

**RESULTS:**

**1). Two Day LD<sub>50</sub> Study:**

Two M/group of CD-1 mice (8-10 weeks, 35-40 g) were dosed by gavage in the dose-finding study, and 6 M/group were dose for 2 days in the main toxicity study to determine the oral LD<sub>50</sub>. The LD<sub>50</sub> was estimated as 1044 mg/kg from the following Tables. Toxic signs were the same as before and as in the hamster studies.

Two Day LD<sub>50</sub> Study  
Dose Finding Test  
SCH 1000 BR

| Group No. | Dose Level mg/kg | Deaths |                   | No. of Doses Given (No. of Mice) | Clinical Signs                                    | PM Observations (No. of mice)                        | Duration of Clinical Signs |
|-----------|------------------|--------|-------------------|----------------------------------|---------------------------------------------------|------------------------------------------------------|----------------------------|
|           |                  | No.    | Day (No. of Mice) |                                  |                                                   |                                                      |                            |
| 1         | 100              | 0/2    | K                 | 2(2)                             | NAD                                               | NAD                                                  | None                       |
| 2         | 250              | 0/2    | K                 | 2(2)                             | NAD                                               | NAD                                                  | None                       |
| 3         | 500              | 0/2    | K                 | 2(2)                             | Piloerection                                      | NAD                                                  | 2-5 days                   |
| 4         | 1000             | 1/2    | 3(1)<br>K(1)      | 2(2)                             | Hypokinesia<br>Piloerection<br>Ataxia             | Lungs red, kidneys pale.<br>1/2                      | 2-8 days                   |
| 5         | 2000             | 2/2    | 5(1)<br>7(1)      | 2(2)                             | Hypokinesia<br>Sedation<br>Piloerection<br>Ataxia | Lungs pink, kidneys pale, gut contents fluid.<br>2/2 | 2-6 days                   |

NAD = No abnormalities detected.

K = Animals killed at end of 14 day observation period.

Two Day LD<sub>50</sub> Study  
Main Toxicity Test:  
SCH 1000 BR

| Group No. | Dose Level mg/kg | Deaths |                      | No. of Doses Given (No. of Mice) | Clinical Signs                        | PM Observations (No. of Mice)                                                | Duration of Clinical Signs |
|-----------|------------------|--------|----------------------|----------------------------------|---------------------------------------|------------------------------------------------------------------------------|----------------------------|
|           |                  | No.    | Day (No. of Mice)    |                                  |                                       |                                                                              |                            |
| 1         | 350              | 0/6    | K                    | 2(6)                             | Piloerection                          | NAD                                                                          | 2-3 days                   |
| 2         | 525              | 1/6    | K(5)<br>5(1)         | 2(6)                             | Hypokinesia<br>Piloerection           | Autolysis<br>1/6                                                             | 2-5 days                   |
| 3         | 788              | 1/6    | K(5)<br>5(1)         | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Lungs pink,<br>kidneys pale,<br>liver mottled.<br>1/6                        | 2-5 days                   |
| 4         | 1181             | 4/6    | K(2)<br>4(2)<br>5(2) | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Lungs pink,<br>kidneys pale,<br>liver mottled.<br>2/6                        | 2-5 days                   |
| 5         | 1772             | 6/6    | 4(1)<br>5(5)         | 2(6)                             | Hypokinesia<br>Piloerection<br>Ataxia | Gut contents<br>fluid, kidneys<br>pale, liver<br>mottled, auto-<br>lysis 5/6 | 1-4 days                   |

NAD = No abnormalities detected.

K = Animals killed at end of 14 day observation period.

2) Micronucleus Tests:

A total of 4 series of tests were done, and the first 3 tests used a standard micronucleus protocol (project 703292), but the 4th test, (Project 703292, Extension-1) used a modified version of the same protocol, due to positive results in the first 3 tests.

The first study was done in two part, as shown below.

Phase 1:

"A small but significant increase in the frequency of micronuclei was detected when 15 mice were dosed on 2 consecutive days (0 h and 24 h) with 803.5 mg/kg (0.8 x LD<sub>50</sub>) SCH 1000 BR. Since only the 72 h sample from time 0 h was positive, as judged by the number of micronuclei within any one group, Phase 2 was initiated with a probable positive response for this particular time-sample in mind. In Phase 1 the number of micronucleated normochromatic erythrocytes was not significantly increased.

The vehicle control group did not show an increased number of micronuclei."

Phase 2:

"Again, small increases in the numbers of micronuclei were observed when 3 groups of 5 mice were dosed at 0 h and 24 h with: 0.4 x LD<sub>50</sub>, 401.8 mg/kg/day SCH 1000 BR; 0.6 x LD<sub>50</sub>, 602.6 mg/kg/day SCH 1000 BR; and 0.8 x LD<sub>50</sub>, 803.5 mg/kg/day SCH 1000 BR.

Sampling was done at 72 h only from time 0 h.

All 3 groups of mice dosed with the test compound had increased numbers of micronuclei when compared with the vehicle control group of mice. In one dose group (0.4 x LD<sub>50</sub>) there was more than a 2-fold increase: 20 micronuclei per 5000 polychromatic erythrocytes (PCE) from 5 mice (frequency 0.40%), compared to 8 micronuclei per 5000 PCE from 5 mice (frequency 0.16%) for the vehicle control group. The frequencies of micronuclei in the 0.6 x LD<sub>50</sub> and 0.8 x LD<sub>50</sub> dose group were 0.26% and 0.24% respectively. As in Phase 1, the number of micronucleated normochromatic erythrocytes was marginally, but not significantly, increased.

However, the obtained results failed to indicate any correlation between increasing dose levels and severity of response in terms of induced micronuclei in either polychromatic or normochromatic erythrocytes. Consequently, it was decided to repeat the Phase 2 experiment in its entirety in an attempt to resolve the remaining ambiguities."

Phase 2-Repeat Study:

"Again, 3 groups of 5 mice were dosed at 0 h and 24 h with: 0.4 x LD<sub>50</sub>, 401.8 mg/kg/day SCH 1000 BR; 0.6 x LD<sub>50</sub>, 602.6 mg/kg/day SCH 1000 BR; and 0.8 x LD<sub>50</sub>, 803.5 mg/kg/day SCH 1000 BR.

Sampling was carried out, as before, at 72 h from time 0 h.

The 3 groups of mice dosed with SCH 1000 BR showed, as before, slight increases in the frequencies of micronuclei when compared with the vehicle control group. Once more, the 0.4 x LD<sub>50</sub> dose group showed the highest number of micronuclei, i.e. 16/5000 polychromatic erythrocytes (PCE) from 5 mice (frequency 0.32%), compared to 6 micronuclei/5000 PCE from 5 mice for the vehicle control group (frequency 0.12%). The increase in the 0.4 x LD<sub>50</sub> group, however, was largely due to one mouse having an unusually high proportion of micronucleated PCE (10 micronuclei, frequency 1%). None of the other mice in this dose group showed any significant response. When this one mouse was excluded from analysis, the mean frequency for the group was 0.15%. Normal levels of micronuclei were in all dose groups observed when normochromatic erythrocytes were analysed for presence of chromosome fragments.

All animals survived dosing in Phase 1, Phase 2 and the repeat study of Phase 2."

The following Table was the summary of all 3 above studies:

Micronucleus Test  
Number and Mean  $\pm$  Micronucleated Polychromatic Erythrocytes  
SCH 1000 BR

| Phase                                    | Number of mice |      |      | Number of Micronucleated Polychromatic Erythrocytes |              |            | Number of Micronucleated Microchromatic Erythrocytes |      |      |
|------------------------------------------|----------------|------|------|-----------------------------------------------------|--------------|------------|------------------------------------------------------|------|------|
|                                          | 48 h           | 72 h | 96 h | 48 h                                                | 72 h         | 96 h       | 48 h                                                 | 72 h | 96 h |
| Phase 1                                  |                |      |      |                                                     |              |            |                                                      |      |      |
| Vehicle Control<br>Distilled Water       | 5              | 5    | 5    | 9<br>0.18                                           | 11<br>0.22   | 11<br>0.22 | 4                                                    | 2    | 0    |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 BR | 5              | 5    | 5    | 10<br>0.20                                          | 20*<br>0.40  | 10<br>0.20 | 4                                                    | 9    | 7    |
| Phase 2                                  |                |      |      |                                                     |              |            |                                                      |      |      |
| Vehicle Control<br>Distilled Water       |                | 5    |      |                                                     | 8<br>0.16    |            |                                                      | 0    |      |
| 0.4 x LD50<br>401.8 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 20**<br>0.40 |            |                                                      | 9    |      |
| 0.6 x LD50<br>602.6 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 13<br>0.26   |            |                                                      | 5    |      |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 12<br>0.24   |            |                                                      | 5    |      |
| Phase 2 (R)                              |                |      |      |                                                     |              |            |                                                      |      |      |
| Vehicle Control<br>Distilled Water       |                | 5    |      |                                                     | 6<br>0.12    |            |                                                      | 4    |      |
| 0.4 x LD50<br>401.8 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 16**<br>0.32 |            |                                                      | 4    |      |
| 0.6 x LD50<br>602.6 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 10<br>0.20   |            |                                                      | 1    |      |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 BR |                | 5    |      |                                                     | 13<br>0.26   |            |                                                      | 6    |      |

(R) - Phase 2 (Repeat Study). \* = Positive response ( $\geq 17$  micronuclei/one group of 5 mice)  
\*\* = Positive response ( $\geq 16$  micronuclei/3 groups of 5 mice).

Micronucleus Test  
Number and Mean % Micronucleated Polychromatic Erythrocytes  
SCH 1000 DR

|                                          | Number of Mice |      |      | Number of Micronuclei Observed |       |      | Number of Micronucleated Polychromatic Erythrocytes |      |      |
|------------------------------------------|----------------|------|------|--------------------------------|-------|------|-----------------------------------------------------|------|------|
|                                          | 48 h           | 72 h | 96 h | 48 h                           | 72 h  | 96 h | 48 h                                                | 72 h | 96 h |
| Phase 1                                  |                |      |      |                                |       |      |                                                     |      |      |
| Vehicle Control Distilled water          | 5              | 5    | 5    | 9                              | 11    | 11   | 4                                                   | 2    | 4    |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 DR | 5              | 5    | 5    | 10                             | 10    | 10   | 4                                                   | 9    | 7    |
| Phase 2                                  |                |      |      |                                |       |      |                                                     |      |      |
| Vehicle Control Distilled Water          |                | 5    |      |                                | 0.16  |      | 0                                                   |      |      |
| 0.4 x LD50<br>401.8 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 20.00 |      | 9                                                   |      |      |
| 0.6 x LD50<br>602.6 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 13    |      | 5                                                   |      |      |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 12    |      | 5                                                   |      |      |
| Phase 2 (R)                              |                |      |      |                                |       |      |                                                     |      |      |
| Vehicle Control Distilled Water          |                | 5    |      |                                | 6     |      | 4                                                   |      |      |
| 0.4 x LD50<br>401.8 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 16.00 |      | 4                                                   |      |      |
| 0.6 x LD50<br>602.6 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 10    |      | 4                                                   |      |      |
| 0.8 x LD50<br>803.5 mg/kg<br>SCH 1000 DR |                | 5    |      |                                | 11    |      | 6                                                   |      |      |
|                                          |                |      |      |                                | 0.26  |      |                                                     |      |      |

(R) - Phase 2 (Repeat Study).  
\* = Positive response (1/3 micronucleated/group of 5 mice)  
\*\* = Positive response (1/3 micronucleated/3 groups of 5 mice).

The following table was the summary of all 3 above studies:

"On the basis of these test findings, SCH 1000 BR might appear to induce micronuclei at very low and occasionally detectable rates when mice were dosed at levels approximately half that of the LD<sub>50</sub> value. To eliminate any spurious result or false conclusions arising from too small samples of animals, a different protocol was designed which, it was hoped, would provide additional and conclusive information about the possible clastogenic potential of the test compound. The findings from this modified micronucleus test are given below."

Phase 2 - Extension Study (Project 703292, Extension No. 1)

The results of this study were in the following Table and Appendix Table 4.

Micronucleus Test  
Number and Mean % Micronucleated Polychromatic Erythrocytes  
SCH 1000 BR

| Phase 2 - Extension Study                            | Number of Mice | Number of Micronuclei Observed |      | Number of Micronucleated Normochromatic Erythrocyte |
|------------------------------------------------------|----------------|--------------------------------|------|-----------------------------------------------------|
|                                                      |                | % Micronucleated PCE           | 72 h |                                                     |
| Vehicle Control<br>10 ml/kg<br>Distilled Water       | 15             | 19                             | 0.13 | 10                                                  |
| 0.5 x LD <sub>50</sub><br>502.2 mg/kg<br>SCH 1000 BR | 15             | 23                             | 0.15 | 6                                                   |
| Positive Control<br>50 mg/kg<br>Cyclophosphamide     | 15             | 178                            | 1.19 | 139                                                 |

PCE = Polychromatic erythrocyte.

"No increased frequencies of micronuclei were observed when 15 mice were dosed at 0 h and 24 h with  $0.5 \times LD_{50}$ , 502.2 mg/kg/day SCH 1000 BR. The number of micronucleated normochromatic erythrocytes also was not increased. Sampling was done at 72 h only.

The positive control animals, on the other hand, showed huge increases in the numbers of micronuclei both in polychromatic and normochromatic erythrocytes. The frequency values in the 2 types of erythrocytes were 1.19% and 0.93% respectively.

There was nothing to suggest that SCH 1000 BR induced micronuclei in CD-1 mice when tested at a dose level of  $0.5 \times LD_{50}$ . Accordingly the weak positive findings from the 3 previous micronucleus tests were in no way confirmed or sustained by evidence from this extension study.

As before, no animal deaths were recorded during or after dosing with the test substance. "

The following were the conclusions submitted on these series tests:

"There was no strong and reproducible evidence to show that SCH 1000 BR, in these tests, induced micronuclei at rates which were clearly above the levels at which micronuclei can spontaneously occur. The number of micronuclei observed in the first 3 tests with SCH 1000 BR indicated a slight, albeit ambiguous, effect by the test substance particularly at dose levels approximately half the LD<sub>50</sub> value. These findings were, however, not substantiated when a final, more comprehensive micronucleus test using the 0.5 x LD<sub>50</sub> dose level was run.

The pattern of responses in the series of tests was as follows:

| No. of Animals | Dose Level             | Test 1 | Test 2 | Test 3 | Test 4 |
|----------------|------------------------|--------|--------|--------|--------|
| 5              | 0.8 x LD <sub>50</sub> | +      | -      | -      | 0      |
| 5              | 0.6 x LD <sub>50</sub> | 0      | -      | -      | 0      |
| 15             | 0.5 x LD <sub>50</sub> | 0      | 0      | 0      | -      |
| 5              | 0.4 x LD <sub>50</sub> | 0      | +      | +      | 0      |

+ increase in micronuclei  
- no increase in micronuclei  
0 not tested

The + result in Test 3 mice given 0.4 x LD<sub>50</sub> was due entirely to one mouse out of the 5 treated. The minimal increases in micronucleus frequency observed and the lack of any dose related response led to the conclusion that SCH 1000 BR probably was not a clastogen."

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"There was no strong and reproducible evidence to show that SCH 1000 BR, in these tests, induced micronuclei at rates which were clearly above the levels at which micronuclei can spontaneously occur. The number of micronuclei observed in the first 3 tests with SCH 1000 BR indicated a slight, albeit ambiguous, effect by the test substance particularly at dose levels approximately half the LD<sub>50</sub> value. These findings were, however, not substantiated when a final, more comprehensive micronucleus test using the 0.5 x LD<sub>50</sub> dose level was run.

The pattern of responses in the series of tests was as follows:

| No. of Animals | Dose Level             | Test 1 | Test 2 | Test 3 | Test 4 |
|----------------|------------------------|--------|--------|--------|--------|
| 5              | 0.8 x LD <sub>50</sub> | +      | -      | -      | 0      |
| 5              | 0.6 x LD <sub>50</sub> | 0      | -      | -      | 0      |
| 15             | 0.5 x LD <sub>50</sub> | 0      | 0      | 0      | -      |
| 5              | 0.4 x LD <sub>50</sub> | 0      | +      | +      | 0      |

+ increase in micronuclei  
- no increase in micronuclei  
0 not tested

The + result in Test 3 mice given 0.4 x LD<sub>50</sub> was due entirely to one mouse out of the 5 treated. The minimal increases in micronucleus frequency observed and the lack of any dose related response led to the conclusion that SCH 1000 BR probably was not a clastogen."

A. Teratology:

- (1) Mice (Strain, NMRI) were given <sup>dams</sup> PO by intubation at doses of 0, 2 or 10 mg/kg from day 6-15 of pregnancy. Dams were sacrificed on day 18. Fetuses were examined externally: 2/3 were cleared and stained for skeletal abnormalities and 1/3 were examined for visceral defects.

Weight gain of dams was normal. None of the dams died. Number of live fetuses and total number of implantations were decreased and total number of resorptions was increased but these were within physiologic limits. None of fetuses died. Weight of fetuses was comparable in all groups. No malformations, either skeletal or visceral, were reported to occur.

- (2) Pregnant rats were dosed in the same manner as outlined above.

No behavioral effects were seen. None of the dams died. Weight gain of dams was normal. Number of implantation sites, live fetuses, dead fetuses, resorption sites, and mean fetal weight, were comparable in all groups. In hi dose group one fetus had anophthalmia and hydrocephalus interna.

- (3) Due to the unusual abnormality (anophthalmia and hydrocephalus interna) found in the rat study above, study was repeated by Boehringer.

Same schedule followed as outlined above except doses were 0, 10 or 20 mg/kg.

One hi dose dam died. All parameters measured above in the previous study were comparable in treated and control groups.

- (4) Pregnant rabbits (Russian) were given 0, 2 or 10 mg/kg PO by intubation from day 6-18 of pregnancy. Dams were sacrificed on day 9. One-half fetuses were sacrificed and remainder were placed in an incubator for 24 hours. The latter were examined for visceral and skeletal anomalies.

No unusual behavioral effects were noted. No dams died. Weight gain of dams was reduced in treated groups (25 gm., controls, 25 gm., low and 9 gm., Hi). Number of live and dead fetuses and number of resorptions was comparable in all groups but number of implantation sites was lower in hi dose group than controls. All the pups survived the 24 hour incubation period. Average fetal weight was comparable in all groups. No skeletal or visceral anomalies were reported; three fetuses in hi dose groups showed slight alteration in gall bladder which was considered spontaneous.

A. Teratology:

- (1) Mice (Strain, NMRI) were given <sup>dams</sup> PO by intubation ~~at~~ doses of 0, 2 or 10 mg/kg from day 6-15 of pregnancy. Dams were sacrificed on day 18. Fetuses were examined externally: 2/3 were cleared and stained for skeletal abnormalities and 1/3 were examined for visceral defects.

Weight gain of dams was normal. None of the dams died. Number of live fetuses and total number of implantations were decreased and total number of resorptions was increased but these were within physiologic limits. None of fetuses died. Weight of fetuses was comparable in all groups. No malformations, either skeletal or visceral, were reported to occur.

- (2) Pregnant rats were dosed in the same manner as outlined above.

No behavioral effects were seen. None of the dams died. Weight gain of dams was normal. Number of implantation sites, live fetuses, dead fetuses, resorption sites, and mean fetal weight, were comparable in all groups. In hi dose group one fetus had anophthalmia and hydrocephalus interna.

- (3) Due to the unusual abnormality (anophthalmia and hydrocephalus interna) found in the rat study above, study was repeated by Boehringer.

Same schedule followed as outlined above except doses were 0, 10 or 20 mg/kg.

One hi dose dam died. All parameters measured above in the previous study were comparable in treated and control groups.

- (4) Pregnant rabbits (Russian) were given 0, 2 or 10 mg/kg PO by intubation from day 6-18 of pregnancy. Dams were sacrificed on day 9. One-half fetuses were sacrificed and remainder were placed in an incubator for 24 hours. The latter were examined for visceral and skeletal anomalies.

No unusual behavioral effects were noted. No dams died. Weight gain of dams was reduced in treated groups (125 gm., controls, 25 gm., low and 9 gm., Hi). Number of live and dead fetuses and number of resorptions was comparable in all groups but number of implantation sites was lower in hi dose group than controls. All the pups survived the 24 hour incubation period. Average fetal weight was comparable in all groups. No skeletal or visceral anomalies were reported; three fetuses in hi dose groups showed slight alteration in gall bladder which was considered spontaneous.

**B. Teratological Study of the Compound Sch 1000 (Segment II)**

Rabbits, Small Russian Sch 1000

# animals/dose level 13F

Dose levels: 2.0, 10.0 mg/kg plus control

administration and duration

Administered by stomach tube, day 6-18 day of pregnancy casa rean section delivery on 29 day of pregnancy.

**Results:**

Clinical Symptoms

Rabbits behavior was normal during study

Body Weight

Animals Pregnant in Sch 1000 dose groups had slight weight loss.

Spontaneous Births and Abortions

All mothers carried their fetuses until surgery.

Deaths

No deaths occurred during this study.

Conceptions

Conception rates were normal and similar

Implantations

There was a reduced number of implantations in the 10.0 mg/kg group. Difference was not significant.

Living Fetuses

No significant difference was noted in the number of living fetuses between the groups.

Reabsorptions

The number of reabsorptions was similar and unremarkable.

**B. Teratological Study of the Compound Sch 1000 (Segment II)**

Rabbits, ~~Small Russian~~ Sch 1000

# animals/dose level ~~13F~~

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There was a reduced number of implantations in the 10.0 mg/kg group. Difference was not significant.

Living Fetuses

No significant difference was noted in the number of living fetuses between the groups.

Reabsorptions

The number of reabsorptions was similar and unremarkable.

Survival Rate

Survival rate was 100% in all groups

Litter Weight

Litter weight was similar in the 3 groups.

Malformations

No major malformations were noted in the fetuses. Bone staining using Alizarin Red S was carried out as part of the examination.

**C. TESTING OF SCH 1000 FOR FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE IN RATS**  
(SEGMENT I) (1/30/75 review)

Rats

Sch 1000

#Animals/Dose Level: 10 M & 20 F

Dose Levels: 10, 30, and 90 mg/kg

Administration: Added to food, 75 da prior to mating for males and 14 days for females. Treated rats mated with untreated rats in both cases.

Results:

Clinical Signs: Behavior of animals was normal, no mortalities.

Body Weight: Body weight of both the males and females was similar.

Food Consumption: Food consumption was similar in all groups. The rats in the 10.0 mg/kg seems to consume slightly more food than rats in other groups.

Mating Period: There were no significant differences in the conception and implantation rates between the groups.

Weight of Testicles: Macroscopic and histological examinations of the testes gave no indication of damage due to Sch 1000.

Litter data of Pregnant Females:

The percentage of implantations, living fetuses and resorptions was similar in all groups. There were no dead fetuses in any group. The mean fetus weight was slightly higher in the low and high dose groups. Examination with a-eitzarin Red S staining method revealed no major abnormalities in the skeletons of the fetuses.

Results of the Treated Females Clinical Observation:

Normal behavior, no mortalities.

Body Weight: Body weight in these females was normal with a constant weight increase.

Food Intake: Food intake was comparable in all groups.

Data from 14 Day Gestation:

There were no differences in the average number of implantations between the control and the treated animals. The number of resorptions in the 10 and 90 mg/kg groups was higher than resorptions in the control groups.

21 Day Data-Duration of Gestation:

The duration of gestation in these pregnant rats was normal. The mean litter weight of the pups in the 10 mg/kg groups was slightly less than the weight of the pups in the other 3 groups. The differences in weight between the groups was not significant.

Mortality: at birth

Only one pup in the 10 mg/kg group was found dead at birth.

Malformations: No major malformations were observed when pups were examined at autopsy.

Data from Fo Generation:

Mating of Mothers-

Fourteen days after weaning of the pups, the dams were mated for the 2nd time. The conception rate in the control, 30 and 90 mg/kg groups was 100% and 70% in the 10 mg/kg group.

Total Implantations: Total implantations were similar in all groups.

Viable Fetuses: The mean value for viable pups was similar in all groups.

Percent Implantations: The percentage of implantations in the 10 mg/kg group was lower than in the 3 other groups.

Terminal Studies: No serious abnormalities were observed when these pups were examined at autopsy.

D. Testing of the substance - Sch 1000 for its Effects on Rats in the Perinatal and Postnatal Phase (Segment III)

Rats

Sch 1000

#Animals/Dose Level: 20F.

Doses: 10, 30, and 90 mg/kg plus control.

Administration and Duration: Given by stomach tube from day 14 of gestation until the weaning of pups on the 21st day after parturition.

Results:

Clinical Signs: Behavior of Pregnant rats was normal.

Bodyweight: Body weight of the dams was comparable.

Conception Rates: All conception rates were between 90 and 100%.

Duration of Gestation: All the dams delivered their young on the 21st and 22nd days of pregnancy.

Autopsy of Dams:

No abnormalities were observed in the dams when they were examined by gross methods at autopsy.

Data concerning Pups:

Litter Size: The mean number of pups in each litter was within normal limits.

Body Weight of Pups at Birth: The mean body weight of the pups between groups was comparable.

Mortality 0-21 days: The mortality of the pups in the 90 mg/kg groups was higher (16.9%) than in the control (11.9%), the 10 mg/kg (11.0%) and the 30 mg/kg group (8.8%). Difference was not significant.

Finding in 21 days old pups: The mean litter weight of the pups in the control and 90 mg/kg was slightly lower than the weight of the pups in the other 2 groups.

Autopsy Findings - Pups: Examination of pups at autopsy did not reveal any remarkable findings.

Mating of Pups F1 Generation:

Several pups from the control and 90 mg/kg groups were cross mated, i.e. Female pups from control with male pups from 90 mg/kg group. The conception rates were found to be 85 to 92%.

**E. SEGMENT I REPRODUCTION STUDY IN RAT BY ORAL: (12/17/79 review)**

Lab performed study: C. H. Boehringer Ingelheim in JAPAN. The study is done according to the notification No. 529 of 1/31/75 of Japanese National Council of Pharmacy: Methods for reproduction studies of new drug.

No. of Animals/Level: 20 M (7 weeks old, 239±20 g)+20 F (10 weeks old, 193±17 g)

Strain: JCL-Sprague-Dawley

| Dosage Levels: | Group:                | Dose (mg/kg/day): |
|----------------|-----------------------|-------------------|
|                | D <sub>0</sub>        | 0                 |
|                | D <sub>1</sub> (Low)  | 5                 |
|                | D <sub>2</sub> (Mid)  | 50                |
|                | D <sub>3</sub> (High) | 500               |

Duration and Route: Males were treated for 60 days before the mating through copulation (12 to 20 day) and killed after the mating by gastric intubation.

Females were treated from 14 days before mating through day 7 of gestation by gastric intubation. Caesarean section was performed on day 21 of gestation under ether (5/24/76 to 8/4/76). Drug was dissolved in distilled water and administered in 0.5 ml/100 g.

Mortality: None

General Signs: It was reported that except one M (No. 017) of control group all M of control and low dose groups behaved "unobtrusively". No. 017 of control, No. 211(M) and 217(M) of mid dose group, and No. 307(M) of high dose groups had bronchial breathing sounds which started one or two weeks after beginning of treatment through mating, except the M of control group which started toward the end. 3 M of mid dose groups (No. 210, 216, and 217) and all M of high dose groups showed red "injected eyes" or nose starting one or two weeks after beginning of treatment through mating.

All F of high dose group had red incrustated eyes from about 3 or 4 days of treatment through several days and 2 F of mid dose groups had this sign during a few days after the start of treatment.

**COMMENT:**

All the results were given separately, for males in one section and for females in another section, which required more time for review.

**Growth Effect:**

M of high dose group showed depression in mean body weight gain starting around day 15 which became significantly different from controls after about 30 days through 60 days. Mean body weight of F of high dose group showed a decrease (loss) on the third day, but later it became only slightly depressed, and the total group weight gain during pregnancy was very slightly smaller in high dose, and very slightly larger in two low dose groups when compared to control group.

**COMMENT:**

500 mg/kg group in Figure 2 (p. 27) is not correct.

**Food and Water Consumptions:**

Before mating the intake of food in F was significantly depressed in two high dose groups, and it remained slightly lower in high dose during gestation. No dose related differences in water consumption in F were observed, but some of values in two high dose groups were significantly lower than that of control F.

**COMMENT:**

No values for M on food consumption or water consumption was reported.

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**COMMENT:**

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M of high dose group showed depression in mean body weight gain starting around day 15 which became significantly different from controls after about 30 days through 60 days. Mean body weight of F of high dose group showed a decrease (loss) on the third day, but later it became only slightly depressed, and the total group weight gain during pregnancy was very slightly smaller in high dose, and very slightly larger in two low dose groups when compared to control group.

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**COMMENT:**

No values for M on food consumption or water consumption was reported.

| Group Fertility Data:             |                         | Sch 1000 Br | Rat     | p.o.     | Seg. I    |
|-----------------------------------|-------------------------|-------------|---------|----------|-----------|
| <b>Males</b>                      |                         | Control     | 5 mg/kg | 50 mg/kg | 500 mg/kg |
| Number used                       |                         | 20          | 20      | 20       | 20        |
| Mortality                         |                         | 0           | 0       | 0        | 0         |
| Number mated                      |                         | 20          | 20      | 20       | 20        |
| Copulated with female partner     |                         | 20          | 20      | 20       | 19        |
| at first estrus                   |                         | 20          | 20      | 18       | 12        |
| at later period                   |                         | 0           | 0       | 2        | 7         |
| Not copulated with female partner |                         | 0           | 0       | 0        | 1         |
| copulated with substitute         |                         | -           | -       | -        | 0         |
| not copulated with substitute     |                         | -           | -       | -        | 1         |
| With lesions in sex organs        |                         | 7           | 12      | 9        | 7         |
| <b>Females</b>                    |                         |             |         |          |           |
| Number used                       |                         | 20          | 20      | 20       | 20        |
| Mortality before mating           |                         | 0           | 0       | 0        | 0         |
| Estrous cycles before mating      | normal                  | 20          | 20      | 20       | 15        |
|                                   | with spurious pregnancy | 0           | 0       | 0        | 2         |
|                                   | no cycles               | 0           | 0       | 0        | 0         |
| Number mated                      |                         | 20          | 20      | 20       | 20        |
| Estrous cycles during mating      | normal                  | 20          | 20      | 20       | 16        |
|                                   | with spurious pregnancy | 0           | 0       | 0        | 4         |
|                                   | no cycles               | 0           | 0       | 0        | 0         |
| Inseminated from male partner     |                         | 20          | 20      | 20       | 19        |
| pregnant                          |                         | 20          | 20      | 18       | 12        |
| non pregnant                      |                         | 0           | 0       | 2        | 7         |
| Not inseminated from male partner |                         | 0           | 0       | 0        | 1         |
| inseminated from substitute       |                         | -           | -       | -        | 1         |
| pregnant                          |                         | -           | -       | -        | 1         |
| non pregnant                      |                         | -           | -       | -        | 0         |
| not inseminated from substitute   |                         | -           | -       | -        | 0         |
| With lesions in sex organs        |                         | 0           | 0       | 0        | 0         |

Only one M (No. 311) in high dose group failed to inseminate two untreated substitute females, in addition to original F partner (No. 361), that was later inseminated from an untreated substitute M. 2 F of high dose group showed spurious pregnancy before the mating. Two F (one in control and another in high dose group) had no sperm, but their estrus cycles disappeared, and later they were found pregnant. Therefore, the copulation rate for M is 100% for all group except high dose group with 95% (19/20). The pregnancy rate for F is reported as 100% for control and low dose group, 95% for mid dose (19/20), and 75% for high dose (15/20) including the one F that was pregnant by untreated M partner.

**COMMENT:**

- 1) Only 18/20 in mid dose and 13/20 in high dose group F seemed to be pregnant (Table 1, p. 34), that make pregnancy rate of 90% for mid and 65% for high dose group instead of 95% and 75% reported in p. 14.
- 2) It was reported that ovulation and nidation seemed to be slightly affected in F of high dose group.

**Spontaneous Parturition:** No. 358 of high dose and No. 260 of mid dose F on day 21.

**Necropsy:**

At the end of 12 day mating period or after insemination was demonstrated, all M were killed and necropsied: It was found that No. 208 had edematously swollen testes, No. 214 had edematously swollen prostate, and No. 218, hydronephrosis.

Among dams examined after caesarean section No. 064 of control and 259 of mid dose had hydronephrosis, and No. 358 of high dose had petechiae in gastric and intestinal mucosa.

**Weight of Male Sex Organs:**

Mean absolute weight of testes showed a dose-related depression, and it was

statistically significantly smaller in high dose group, but the mean absolute weight of epididymides in high dose group was statistically significantly larger when compared to that of the control group M (p. 46)

In the mean relative organ weights, only the high dose group had statistically significantly heavy epididymides, seminal vesicles, and preputial glands (p. 41). See Table 7 for lesion in M sex organs.

Mean Litter Data:

|                                             | Control  | 5 mg/kg  | 50 mg/kg | 500 mg/kg |
|---------------------------------------------|----------|----------|----------|-----------|
| No. of dams pregnant                        | 20       | 20       | 19       | 14        |
| No. of dams died during pregnancy           | 0        | 0        | 0        | 0         |
| No. of dams with all fetuses dead           | 1        | 0        | 0        | 0         |
| No. of dams with live fetuses               | 19       | 20       | 19       | 14        |
| Total no. of corpora lutea                  | 287      | 310      | 267      | 202       |
| Avg. no. of corpora lutea per dam           | 15.1±1.8 | 15.5±1.0 | 15.1±2.2 | 14.4±2.1  |
| Total no. of implantations                  | 265      | 283      | 269      | 169       |
| Avg. no. of implantations per dam           | 13.9±1.8 | 14.2±1.7 | 14.2±1.7 | 12.1±2.8  |
| % implantations/corpora lutea <sup>a)</sup> | 92.6     | 91.8     | 94.4     | 83.6      |
| Total no. of fetal deaths                   | 22       | 27       | 18       | 14        |
| early resorptions                           | 15       | 17       | 15       | 13        |
| late resorptions                            | 7        | 5        | 3        | 1         |
| dead fetuses                                | 0        | 5        | 0        | 0         |
| Avg. no. of fetal deaths per dam            | 1.2±1.5  | 1.4±1.9  | 0.9±0.9  | 1.0±1.3   |
| % fetal deaths/implantations <sup>a)</sup>  | 8.2      | 10.1     | 10.9     | 8.2       |
| Total no. of live fetuses                   | 243      | 256      | 251      | 155       |
| Avg. no. of live fetuses per dam            | 12.8±2.1 | 12.8±2.9 | 13.2±1.6 | 11.1±2.9  |
| % live fetuses/implantations <sup>a)</sup>  | 91.8     | 89.9     | 89.2     | 91.8      |
| Sex ratio (male/female) <sup>a)</sup>       | 0.9      | 1.0      | 1.2      | 1.2       |
| Avg. total weight <sup>a)</sup>             | 5.4±0.3  | 5.5±0.2  | 5.4±0.3  | 5.4±0.4   |

a) calculated using litter value as a basic unit

**Malformation:** There were no external malformations of fetuses. In the skeletal examination (170/256 of control, 170/256 of low, 167/251 of mid, and 104/155 of high dose fetuses), it was found that skeletal variations noted was not dose related or smallest in high dose group.

About half of fetuses were examined for the visceral malformation, and only one of control seemed to have variation.

**COMMENT:** It was reported by the Sponsor that fetus No. 370-e of high dose had discurved (waved) ribs and another fetus (No. 053) of control had internal hydrocephalus as malformation.

**F. EFFECT OF METERED AEROSOL ON PREGNANCY OF THE RATS: E83-130: (2/22/84)**

**Lab. Performing the Study:** Huntingdon Research Center  
Huntingdon, Cambridshire, England.

**Strain:** Not given.

**Methods:** 20 F/group of pregnant rats were treated (head only) for 15 min with metered aerosol from day 6 to 15 of gestation, daily dose of 0.512, 1.024, or 1.536 mg/kg/day Sch 1000 (Lot No. 30336), along with a sham dosed control group and vehicle dosed control group (Lot. No. 30335). On day 20, the rats were killed by CO<sub>2</sub> euthanasia and examined. Sch 1000 was a suspension in aerosol formulation, and it delivered 0.02 mg Sch 1000 per valve depression, and vehicle contained 24.4% Fluorocarbon 11, 52.9% Fluorocarbon 12, and 22.5% Fluorocarbon 114 propellants. For detailed methods and procedure, see pages 4-5, 8, and 14-16. Particle size was reported to be less than 0.01 mg (see page 14).

**COMMENTS:** (1) Pages 2 and 3 are missing from the submission.

(2) The unit of the particle size as 0.01 mg must be wrong.

**Malformation:** There were no external malformations of fetuses. In the skeletal examination (100% of control, 170/256 of low, 167/251 of mid, and 104/155 of high dose fetuses), it was found that skeletal variations noted was not dose related or smallest in high dose group.

About half of fetuses were examined for the visceral malformation, and only one of control seemed to have variation.

**COMMENT:** It was reported by the Sponsor that fetus No. 370-x of high dose had discurved (waved) ribs and another fetus (No. 053) of control had internal hydrocephalus as malformation.

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**Lab. Performing the Study:** Huntingdon Research Center  
Huntingdon, Cambridshire, England.

**Strain:** Not given.

**Methods:** 20 F/group of pregnant rats were treated (head only) for 15 min with metered aerosol from day 6 to 15 of gestation, daily dose of 0.512, 1.024, or 1.536 mg/kg/day Sch 1000 (Lot No. 30336), along with a sham dosed control group and vehicle dosed control group (Lot. No. 30335). On day 20, the rats were killed by CO<sub>2</sub> euthanasia and examined. Sch 1000 was a suspension in aerosol formulation, and it delivered 0.02 mg Sch 1000 per valve depression, and vehicle contained 24.4% Fluorocarbon 11, 52.9% Fluorocarbon 12, and 22.5% Fluorocarbon 114 propellants. For detailed methods and procedure, see pages 4-5, 8, and 14-16. Particle size was reported to be less than 0.01 mg (see page 14).

**COMMENTS:** (1) Pages 2 and 3 are missing from the submission.

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RESULTS:

1. PARENT:

a. Signs and Mortalities:

Several rats including control group had "stress reaction", consisting of copious urination and increased defecation, particularly during the first 3 days due to "dosing procedure".

There were 3 "accidental deaths," one in sham control group and 2 on high dose group.

b. Bodyweight Changes:

Two control groups showed greater depression than treated groups during first few days of dosing. See pages 9-10.

c. Pregnancy Rate:

No effect was produced by drug treatment. See Table for Litter data below.

2. LITTER DATA:

TABLE 2

Group mean litter data

|                   |                          |                             |       |          |       |
|-------------------|--------------------------|-----------------------------|-------|----------|-------|
| Group:            | 1                        | 2                           | 3     | 4        | 5     |
| Drug:             | SHAM<br>DOSED<br>CONTROL | VEHICLE<br>DOSED<br>CONTROL |       | SCH 1000 |       |
| Dose (mg/kg/day): | -                        | -                           | 0.512 | 1.024    | 1.536 |

| GROUP | NO. OF ANIMALS | PREGNANCY RATE % | VIABLE YOUNG |     |       | RESORPTIONS |      |       | IMPLANT- ATIONS | CORPORA LUTEA | PRE-IMPLANTATION LOSS % | FOETAL LOSS % | LITTER WT. (g) | MEAN FOETAL WEIGHT (g) |
|-------|----------------|------------------|--------------|-----|-------|-------------|------|-------|-----------------|---------------|-------------------------|---------------|----------------|------------------------|
|       |                |                  | ♂            | ♀   | Total | Early       | Late | Total |                 |               |                         |               |                |                        |
| 1     | A=B=17         | 89.5             | 6.5          | 5.2 | 11.7  | 0.6         | 0.1  | 0.7   | 12.4            | 14.8          | 14.2                    | 5.6           | 42.14          | 3.61                   |
| 2     | A=B=20         | 100.0            | 6.6          | 4.4 | 11.0  | 0.9         | -    | 0.9   | 11.9            | 14.8          | 17.9                    | 7.1           | 41.27          | 3.81                   |
| 3     | A=B=18         | 90.0             | 5.7          | 5.0 | 10.7  | 1.1         | 0.1  | 1.2   | 11.8            | 14.0          | 15.5                    | 9.7           | 42.09          | 3.98 <sup>++</sup>     |
| 4     | A=B=18         | 94.7             | 5.7          | 5.6 | 11.2  | 0.4         | -    | 0.4   | 11.7            | 13.7          | 16.5                    | 5.5           | 43.75          | 3.94 <sup>++</sup>     |
| 5     | A=B=18         | 100.0            | 5.5          | 6.1 | 11.6  | 0.7         | 0.1  | 0.7   | 12.3            | 15.3          | 18.6                    | 6.1           | 43.53          | 3.80                   |

Difference from control groups 1 and 2 statistically significant at Wilcoxon test

P<0.01<sup>++</sup>

Mean A:

includes all surviving animals that provided evidence of nidation, including those showing total resorption.

Mean B:

includes all animals bearing viable young at termination.

b. Major and Minor Abnormalities:

Two major malformations were seen.

SCH 1000, 0.512 mg/kg/day

No. 52 one pup showed anasarca and right microphthalmia and anophthalmia. Skeletal examination showed variant sternbrae and centra, and small eye sockets.

SCH 1000, 1.024 mg/kg/day

No. 73 one pup showed hydronephrosis with dilation of all tubular elements and marked pelvic cavitation.

These malformations were not considered to be related to treatment.

The incidence of minor visceral and skeletal anomalies was not adversely affected by treatment.

c. Group Mean Incidence of Abnormalities:

| GROUP    | NUMBER OF YOUNG     |           |        |                             |        |          |                      |        |     |
|----------|---------------------|-----------|--------|-----------------------------|--------|----------|----------------------|--------|-----|
|          | MAJOR MALFORMATIONS |           |        | MINOR ANOMALIES             |        |          |                      |        |     |
|          | Examined            | Total No. | Mean % | Visceral (Wilson technique) |        |          | Skeletal examination |        |     |
| Examined |                     |           |        | Total No.                   | Mean % | Examined | Total No.            | Mean % |     |
| 1        | 199                 | 0         | 0      | 69                          | 0      | 0        | 130                  | 6      | 5.0 |
| 2        | 219                 | 0         | 0      | 63                          | 3      | 5.2      | 156                  | 7      | 4.2 |
| 3        | 192                 | 1         | 0.4    | 59                          | 2      | 2.8      | 132                  | 3      | 2.2 |
| 4        | 202                 | 1         | 0.4    | 65                          | 1      | 1.4      | 136                  | 2      | 1.2 |
| 5        | 208                 | 0         | 0      | 67                          | 0      | 0        | 141                  | 7      | 4.8 |

Young showing major malformations excluded

d. Skeletal Variation:

| GROUP | PERCENTAGE OF YOUNG * WITH |                      |              |
|-------|----------------------------|----------------------|--------------|
|       | EXTRA<br>RIB(S)            | VARIANT STERNEBRA(E) |              |
|       |                            | Normal ribs          | Extra rib(s) |
| 1     | 30.1                       | 26.3                 | 8.8          |
| 2     | 25.3                       | 32.4                 | 17.0         |
| 3     | 16.7                       | 18.3                 | 18.7         |
| 4     | 36.4                       | 12.2 <sup>+</sup>    | 2.0          |
| 5     | 36.9                       | 20.8                 | 6.7          |

\*Young showing major malformations excluded.

+Difference from control groups 1 and 2 statistically significant at Wilcoxon test ( $p < 0.05$ ).

6. ORAL TERATOLOGICAL TESTING IN RATS: E83-126:

Lab. Performing the Study: Boehringer Ingelheim GmbH  
Pharma-Research Japan.

Strain: SD-JCL Sprague-Dawley female albino  
(300 g average body weight)

Methods: 23 F/group of pregnant rats were treated with 1, 10, 100, or 1000 mg/kg Sch 1000 (Batch No. VII) in distilled water (0.2 ml/100 g) along with a control group receiving distilled water from day 6 to day 15 of gestation via stomach tube every morning (day 0 = positive vaginal smear), and on day 21, 20 rats/group delivered fetuses by C section under ether anesthesia, but 3 rats/group were kept for spontaneous delivery, and they were killed on day 43 (the rat that did not deliver was killed on day 28).

RESULTS:

1. PARENT: Caesarean Section Groups:

a. Clinical Symptoms:

Rat No. 13a had bloody vaginal outflow on day 15, and No. 16a on day 13 in 1 mg/kg/day group.

Rat No. 11c had red incrusted eyes and nose from day 15 and No. 13c from day 10 in 100 mg/kg/day group.

Rat No. 4d, 6d and 10d had red incrusted eyes and nose from day 12 and day 13 respectively in 1000 mg/kg/day group.

b. Bodyweight Changes:

Only 1000 mg/kg/day group had significant loss of weight during the dosing period, but it started to gain afterward. See Fig. 1 on pages 15 and 20-25.

c. Mortalities:

10 F died in 1000 mg/kg/day group as the following Table shows:

| <u>Dams/No.</u>      | <u>Day of death</u> | <u>Administration of substance</u> |
|----------------------|---------------------|------------------------------------|
| 17d, 20d             | 10 <sup>th</sup>    | 4 days                             |
| 1d, 3d, 7d, 14d, 15d | 11 <sup>th</sup>    | 5 days                             |
| 16d, 19d             | 12 <sup>th</sup>    | 6 days                             |
| 4d                   | 16 <sup>th</sup>    | 10 days                            |

Therefore, the mortality rate in this group was 50%.

d. Autopsy Findings:

Rat No. 6b in 10 mg/kg/day group (that was not pregnant) had urinary calculi which led to hydronephrosis sinistra and ureter dilation, as shown on page 14.

e. Pregnancy Rate:

10 and 1000 mg/kg/day groups had less pregnancies than other groups as shown below:

| <u>Group:</u>  | <u>Pregnant/total rat</u> |
|----------------|---------------------------|
| D <sub>0</sub> | = 19/20 ( 95 %)           |
| D <sub>1</sub> | = 20/20 (100 %)           |
| D <sub>2</sub> | = 18/20 ( 90 %)           |
| D <sub>3</sub> | = 19/20 ( 95 %)           |
| D <sub>4</sub> | = 18/20 ( 90 %)           |

f. Other Data:

No significant differences between groups were reported for implantation sites/dam, total viable fetus/dam, resorption site/dam. See Tables below for details.

2. GROUP MEAN LITTER DATA:

a.

"A total number of 1108 fetuses was produced and examined for the presence of malformations.

| <u>Group</u><br><u>mg/kg</u> | <u>liv. fet.</u> | <u>♂</u>   | <u>♀</u>   | <u>skeletal exam.</u> | <u>visceral exam.</u> |
|------------------------------|------------------|------------|------------|-----------------------|-----------------------|
| D <sub>1</sub> control       | 242              | 110        | 132        | 161                   | 81                    |
| D <sub>2</sub> 1.0           | 247              | 124        | 123        | 165                   | 82                    |
| D <sub>3</sub> 10.0          | 237              | 118        | 119        | 156                   | 81                    |
| D <sub>4</sub> 100.0         | 266              | 128        | 138        | 178                   | 88                    |
| D <sub>5</sub> 1000.0        | 116              | 47         | 69         | 77                    | 39                    |
| <b>Total:</b>                | <b>1108</b>      | <b>527</b> | <b>581</b> | <b>737</b>            | <b>371</b>            |

b.

|                                           | Control<br>対 照 | 1.0 mg/kg | 10.0 mg/kg | 100.0 mg/kg | 1000.0 mg/kg |
|-------------------------------------------|----------------|-----------|------------|-------------|--------------|
| Number of animals<br>動物数                  | 20             | 20        | 20         | 20          | 20           |
| Maternal mortalities<br>母体死亡数             | 0              | 0         | 0          | 0           | 10           |
| Abortions<br>流産数                          | 0              | 0         | 0          | 0           | 0            |
| Natural deliveries<br>自然分娩数               | 0              | 0         | 0          | 0           | 0            |
| Total number of pregnant animals<br>妊娠動物数 | 19             | 20        | 18         | 19          | 8            |
| Total implantation sites<br>着床数           | 250            | 258       | 247        | 278         | 119          |
| Per dam<br>1母体当たり着床数                      | 13.2±2.6       | 12.9±3.8  | 13.7±4.0   | 14.6±1.8    | 14.9±1.5     |
| Total viable fetuses<br>生存胎仔数             | 242            | 247       | 237        | 266         | 116          |
| Male in %<br>雄性率 (%)                      | 45.5           | 50.2      | 49.8       | 48.1        | 40.5         |
| Female in %<br>雌性率 (%)                    | 54.5           | 49.8      | 50.2       | 51.9        | 59.5         |
| In % of implantation sites<br>胎仔生存率 (%)   | 96.8           | 95.7      | 96.0       | 95.7        | 97.5         |
| Per dam<br>1母体当たり生存胎仔数                    | 12.7±2.8       | 12.4±3.8  | 13.2±3.9   | 14.0±2.1    | 14.5±1.1     |
| Total resorption sites<br>吸収数             | 7              | 11        | 9          | 12          | 3            |
| Early<br>早期吸収数                            | 7              | 10        | 9          | 12          | 2            |
| Late<br>後期吸収数                             | 0              | 1         | 0          | 0           | 1            |
| In % of implantation sites<br>胎仔吸収率 (%)   | 2.8            | 3.9       | 3.6        | 4.7         | 3.5          |
| Per dam<br>1母体当たり吸収数                      | 0.4±0.8        | 0.6±0.6   | 0.5±0.8    | 0.6±0.8     | 0.4±0.1      |
| Total dead fetuses<br>死亡胎仔数               | 1              | 0         | 1          | 0           | 0            |
| In % of implantation sites<br>胎仔死亡率 (%)   | 0.40           | 0         | 0.40       | 0           | 0            |
| Per dam<br>1母体当たり死亡胎仔数                    | 0.05           | 0         | 0.06       | 0           | 0            |
| Mean litter weight (g)<br>平均胎仔体重 (g)      | 70.2±14.4      | 67.6±18.8 | 72.0±20.4  | 76.8±12.2   | 73.9±8.1     |
| Mean viable fetal weights (g)             |                |           |            |             | 5.1±0.1      |

C.

|                                                 | Control<br>対 照 | 1.0 mg/kg | 10.0 mg/kg | 100.0 mg/kg | 1000.0 mg/kg |
|-------------------------------------------------|----------------|-----------|------------|-------------|--------------|
| Corpora lutea<br>黄体数                            | 277            | 310       | 272        | 302         | 123          |
| Pre-implantation-loss %<br>着床前胚子損失率 (%)         | 9.8            | 16.8      | 9.2        | 7.9         | 3.3          |
| Post-implantation-loss %<br>着床後胎仔損失率 (%)        | 3.2            | 3.9       | 4.0        | 4.7         | 2.5          |
| Total variations<br>変異発現胎仔数                     | 34             | 61        | 52         | 10          | 3            |
| In % of viable fetuses<br>変異発現率 (%)             | 14.0           | 24.7      | 21.9       | 15.0        | 2.6          |
| Per dam<br>1母体当り変異発現胎仔数                         | 1.8            | 3.1       | 2.9        | 2.1         | 0.4          |
| Total malformations<br>奇形発現胎仔数                  | 0              | 0         | 0          | 0           | 1            |
| In % of viable fetuses<br>奇形発現率 (%)             | 0              | 0         | 0          | 0           | 0.9          |
| Per dam<br>1母体当り奇形発現胎仔数                         | 0              | 0         | 0          | 0           | 0.13         |
| Spontaneously delivered groups<br>自然分娩群         |                |           |            |             |              |
| Number of animals<br>動物数                        | 3              | 3         | 3          | 3           | 3            |
| Number of pregnant animals<br>妊娠動物数             | 1              | 2         | 2          | 3           | 2            |
| Total number of young<br>産仔数                    |                |           |            |             |              |
| At birth<br>出生時                                 |                |           |            |             |              |
| Viable youngs per dam<br>1母体当り生存仔数              |                |           |            |             |              |
| Dead youngs per dam<br>1母体当り死亡仔数                |                |           |            |             |              |
| Mean litter weight (g)<br>平均同産仔体重 (g)           |                |           |            |             |              |
| Mean weight of viable youngs (g)<br>平均生存仔体重 (g) |                |           |            |             |              |
| 21 day<br>21日目                                  |                |           |            |             |              |
| Viable youngs<br>生存仔数                           | 10             | 20        | 23         | 38          | 5            |
| Viable youngs per dam<br>1母体当り生存仔数              | 10.0           | 10.0      | 11.5       | 12.7        | 2.5          |
| Mean litter weight (g)<br>平均同産仔体重 (g)           |                |           |            |             |              |
| Mean weight of youngs (g)<br>平均生存仔体重 (g)        |                |           |            |             |              |
| Mortality of youngs<br>産後死亡仔数                   |                |           |            |             |              |
| Variations<br>変異発現仔数                            | 0              | 13        | 7          | 15          | 1            |
| Malformations<br>奇形発現仔数                         | 0              | 0         | 0          | 0           | 0            |

d. Observations:

i. Mean viable fetal weight of 1000 mg/kg/day group was significantly smaller than other groups (5.1 vs. 5.5 in all other groups), and one fetus (No. 12a-7) was "runt" in this group (below 65% of the normal fetus weight).

ii. Skeletal Variations:

The following skeletal variations were observed:

| groups         | n   | additional<br>14th costal<br>pair | sternal deformity              |                                 |
|----------------|-----|-----------------------------------|--------------------------------|---------------------------------|
|                |     |                                   | 5 missing<br>of oss.<br>center | splintering<br>of<br>sternebrae |
| D <sub>0</sub> | 161 | 34 (21.1 %)                       | 0                              | 0                               |
| D <sub>1</sub> | 165 | 60 (36.4 %)                       | 0                              | 1                               |
| D <sub>2</sub> | 156 | 52 (33.3 %)                       | 0                              | 0                               |
| D <sub>3</sub> | 178 | 38 (21.3 %)                       | 1                              | 0                               |
| D <sub>4</sub> | 77  | 1 ( 1.3 %)                        | 0                              | 2                               |

iii. Major Malformations:

One fetus (No. 10d-7 in 1000 mg/kg/day group had no tail and an atresia ani.

See Figs. on page 14.

3. Spontaneously Delivered Groups.

a. Conception Rate:

Only 1 out of 3 in control group was pregnant while 2-3 out of 3 were pregnant in the treated groups.

COMMENTS: Group "D<sub>4</sub>" should be added on line 2 on page 11, since it has only 2 pregnancies out of 3 rats as other groups.

b. Bodyweight gain:

It was reported that "the body weight gain of pregnant dams were parallel to the values of the main groups."

c. Mortality:

One of the 3 pregnant dams in 1000 mg/kg/day group (No. 21d) died on day 24 of gestation with 8 fetuses in her uterus.

d. Fetuses:

One F in 1000 mg/kg/day group delivered 3 live youngs but killed and ate them herself 3 days after parturition. Five youngs survived from the 3rd dams in 1000 mg/kg/day group. All other youngs grew up normally.

A total of 96 fetuses was living after 3 weeks and examined for the presence of variations and malformations, but only several additional 14th costal pair were found. See detailed data in Table above.

- COMMENTS: 1. The bottom of the whole report is missing (cut off); therefore, some pages are not complete, especially page 1 of summary.
2. The bottom of the page 10 was cut off; therefore, the exact figures for mean viable fetal weight were missing in the above Table (5.5 was the figure for all by calculation based on the submitted data.)
3. The result section reported that 18 out of 20 F were pregnant in 1000 mg/kg/day group on page 9 but the Table for Group Mean Litter Data above shows only 8 were pregnant in this group, and total implantation sites and total corpora lutea etc. were presented for only these 8 rats (other rats died or not pregnant).

H. ORAL TERATOLOGY STUDY IN RATS: E83-127:

Lab. Performing the Study: The same as above.

Strain: SD-JCL Sprague-Dawley albino F, about 120 days old and 270 g5.

Methods: 30 pregnant F/group of rats were treated by stomach tube at 5, 50, or 500 mg/kg/day Sch 1000 Br (Batch No. 1/74) from day 8 to day 17 of gestation, along with a control group receiving 0.5 ml/100 g of distilled water. Out of these rats, 10/group delivered spontaneously, but all others (20/group) had "C" section on day 21 (Day 0 = positive vaginal smear). 21 day old fetuses were additionally examined by X-rays for skeletal abnormalities. See pages 1-9 for details.

RESULTS:

I. Caesarean Section Group: PARENTS:

a. Clinical Symptoms:

A few had red incrustated eyes and nose in 500 mg/kg/day group.

b. Body Weight:

500 mg/kg/day group did not gain any weight between day 8 and day 11, then started to gain (see page 20).

c. Food and Water Consumption:

Food consumption of treated groups was slightly depressed with trend of dose-relation during the dosing period, but the water consumption showed no dose-related differences between groups. See page 22.

Methods: 30 pregnant F/group of rats were treated by stomach tube at 5, 50, or 500 mg/kg/day Sch 1000 Br (Batch No. 1/74) from day 8 to day 17 of gestation, along with a control group receiving 0.5 ml/100 g of distilled water. Out of these rats, 10/group delivered spontaneously, but all others (20/group) had "C" section on day 21 (Day 0 = positive vaginal smear). 21 day old fetuses were additionally examined by X-rays for skeletal abnormalities. See pages 1-9 for details.

RESULTS:

1. Caesarean Section Group: PARENTS:

a. Clinical Symptoms:

A few had red incrusted eyes and nose in 500 mg/kg/day group.

b. Body Weight:

500 mg/kg/day group did not gain any weight between day 8 and day 11, then started to gain (see page 20).

c. Food and Water Consumption:

Food consumption of treated groups was slightly depressed with trend of dose-relation during the dosing period, but the water consumption showed no dose-related differences between groups. See page 22.

d. Spontaneous Parturition:

Several fetuses were spontaneously delivered short before Caesarean section as in the following table:

| <u>Group</u> | <u>Dam No.</u> | <u>Number of fetuses</u> |
|--------------|----------------|--------------------------|
| Control      | 6              | 12                       |
|              | 10             | 2                        |
|              | 12             | 4                        |
| 5 mg/kg      | 12a            | 14                       |
|              | 4b             | 3                        |
| 50 mg/kg     | 7b             | 11                       |
|              | 8b             | 14                       |
|              | 9b             | 2                        |
|              | 10b            | 11                       |
|              | 17b            | 1                        |
| 500 mg/kg    | 5c             | 5                        |
|              | 15c            | 9                        |

These fetuses were randomly assigned to the implantation sites and remaining fetuses were produced by Caesarean section.

e. Mortality:

One control rat died on day 21 spontaneously.

f. Autopsy Findings:

The following findings were reported from the examination of all dams from C section group: Uterine bleeding in one control rat (15), swelling and focal necroses of liver and splenomegaly in one control rat(20), and perisplenitis adhesiva in one rat in 500 mg/kg/day group.

g. Pregnancy Rate:

20/20 in control group vs. 19/20 (= 95%) in all 3 treated groups.

h. Implantation and Corpora Lutea:

No significant differences between groups in number of implantations/dam or corpora lutea.

2. GROUP MEAN LITTER DATA:

a. Viable Fetuses:

|                | group<br>(mg/kg) | living<br>fet. | ♂♂  | ♀♀  | skeletal<br>exam. | visceral<br>exam. |
|----------------|------------------|----------------|-----|-----|-------------------|-------------------|
| D <sub>0</sub> | control          | 233            | 123 | 110 | 157               | 76                |
| D <sub>1</sub> | 5                | 215            | 112 | 103 | 143               | 72                |
| D <sub>2</sub> | 50               | 255            | 128 | 127 | 169               | 86                |
| D <sub>3</sub> | 500              | 223            | 108 | 115 | 148               | 75                |
|                | Total:           | 926            | 471 | 455 | 617               | 309               |

C. Table 1

Group Mean Litter Data  
グループ別胎仔数

Species: Rat p.o. Substrate: Sch 000 Br  
動物の種類: Rat p.o. 物質: Sch 000 Br

|                                            | Control<br>対 照 | 5 mg/kg     | 50 mg/kg    | 500 mg/kg   |
|--------------------------------------------|----------------|-------------|-------------|-------------|
| Number of animals<br>動物数                   | 20             | 20          | 20          | 20          |
| Maternal mortality<br>母体死亡数                | 1              | 0           | 0           | 0           |
| Abortions<br>流産数                           | 0              | 0           | 0           | 0           |
| Natural deliveries<br>自然分娩数                | 0              | 0           | 0           | 0           |
| Total number of pregnant animals<br>妊娠動物数  | 19             | 19          | 19          | 19          |
| Total implantation sites<br>着床数            | 245            | 223         | 263         | 239         |
| Per dam<br>1母体当たり着床数                       | 12.9 ± 3.7     | 11.7 ± 3.2  | 13.8 ± 1.5  | 12.6 ± 2.9  |
| Total viable fetuses<br>生存胎仔数              | 233            | 215         | 255         | 223         |
| Male in %<br>雄比率 (%)                       | 53             | 52          | 50          | 48          |
| Female in %<br>雌比率 (%)                     | 47             | 48          | 50          | 52          |
| In % of implantation sites<br>胎仔生存率 (%)    | 95.1           | 96.4        | 97.0        | 93.3        |
| Per dam<br>1母体当たり生存胎仔数                     | 12.3 ± 4.0     | 11.3 ± 3.6  | 13.4 ± 1.4  | 11.7 ± 3.3  |
| Total resorption sites<br>吸収数              | 12             | 8           | 8           | 16          |
| Early<br>早期吸収数                             | 10             | 6           | 8           | 15          |
| Late<br>晚期吸収数                              | 2              | 2           | 0           | 1           |
| In % of implantation sites<br>胎仔吸収率 (%)    | 4.9            | 3.6         | 3.0         | 6.7         |
| Per dam<br>1母体当たり吸収数                       | 0.6 ± 1.1      | 0.4 ± 0.8   | 0.4 ± 0.8   | 0.8 ± 1.8   |
| Total dead fetuses<br>死亡胎仔数                | 0              | 0           | 0           | 0           |
| In % of implantation sites<br>胎仔死亡率 (%)    | 0              | 0           | 0           | 0           |
| Per dam<br>1母体当たり死亡胎仔数                     | 0              | 0           | 0           | 0           |
| Mean litter weight (g)<br>平均同群胎仔体重 (g)     | 71.6 ± 23.0    | 66.8 ± 20.2 | 76.4 ± 7.9  | 66.8 ± 15.7 |
| Mean viable total weight (g)<br>平均胎仔体重 (g) | 5.84 ± 0.39    | 5.90 ± 0.43 | 5.70 ± 0.41 | 5.74 ± 0.49 |

C. Table 1

Group Mean Litter Data  
グループ別胎仔記録

Species: Rat p.o. Substrate: Sch 1000 Br  
動物の種類: Rat p.o. 物質: Sch 1000 Br

|                                            | Control<br>対照 | 5 mg/kg     | 50 mg/kg    | 500 mg/kg   |
|--------------------------------------------|---------------|-------------|-------------|-------------|
| Number of animals<br>動物数                   | 20            | 20          | 20          | 20          |
| Maternal mortality<br>母体死亡数                | 1             | 0           | 0           | 0           |
| Abortions<br>流産数                           | 0             | 0           | 0           | 0           |
| Natural deliveries<br>自然分娩数                | 0             | 0           | 0           | 0           |
| Total number of pregnant animals<br>妊娠動物数  | 19            | 19          | 19          | 19          |
| Total implantation sites<br>着床数            | 245           | 223         | 263         | 239         |
| Per dam<br>1母体当たり着床数                       | 12.9 ± 3.7    | 11.7 ± 3.2  | 13.8 ± 1.5  | 12.6 ± 2.9  |
| Total viable fetuses<br>生存胎仔数              | 233           | 215         | 255         | 223         |
| Male in %<br>雄仔率 (%)                       | 53            | 52          | 50          | 48          |
| Female in %<br>雌仔率 (%)                     | 47            | 48          | 50          | 52          |
| In % of implantation sites<br>胎仔生存率 (%)    | 95.1          | 96.4        | 97.0        | 93.3        |
| Per dam<br>1母体当たり生存胎仔数                     | 12.3 ± 4.0    | 11.3 ± 3.6  | 13.4 ± 1.4  | 11.7 ± 3.3  |
| Total resorption sites<br>吸収数              | 12            | 8           | 8           | 16          |
| Early<br>早期吸収数                             | 10            | 6           | 8           | 15          |
| Late<br>後期吸収数                              | 2             | 2           | 0           | 1           |
| In % of implantation sites<br>胎仔吸収率 (%)    | 4.9           | 3.6         | 3.0         | 6.7         |
| Per dam<br>1母体当たり吸収数                       | 0.6 ± 1.1     | 0.4 ± 0.8   | 0.4 ± 0.8   | 0.8 ± 1.8   |
| Total dead fetuses<br>死亡胎仔数                | 0             | 0           | 0           | 0           |
| In % of implantation sites<br>胎仔死亡率 (%)    | 0             | 0           | 0           | 0           |
| Per dam<br>1母体当たり死亡胎仔数                     | 0             | 0           | 0           | 0           |
| Mean litter weight (g)<br>平均胎仔体重 (g)       | 71.6 ± 23.0   | 66.8 ± 20.2 | 76.4 ± 7.9  | 66.8 ± 19.7 |
| Mean viable total weight (g)<br>平均胎仔体重 (g) | 5.84 ± 0.39   | 5.90 ± 0.43 | 5.70 ± 0.41 | 5.74 ± 0.49 |

d.

|                                                  | Control<br>对照 | 5 mg/kg    | 50 mg/kg    | 500 mg/kg         |
|--------------------------------------------------|---------------|------------|-------------|-------------------|
| Corpora lutea<br>黄体数                             | 263           | 261        | 266         | 282               |
| Pre-implantation loss %<br>孕前期胚胎丢失率 (%)          | 6.8           | 14.6       | 1.1         | 19.2              |
| Post-implantation loss %<br>孕后期胚胎丢失率 (%)         | 4.9           | 3.7        | 3.0         | 6.7               |
| Total variations<br>总变异数                         | 66            | 67         | 54          | 24                |
| In % of viable fetuses<br>在可存活胎鼠中 (%)            | 42.0          | 46.9       | 32.0        | 16.2 <sup>m</sup> |
| Per dam<br>每母鼠                                   | 3.5           | 3.5        | 2.8         | 1.3               |
| Total malformations<br>总畸形数                      | 3             | 1          | 0           | 3                 |
| In % of viable fetuses<br>在可存活胎鼠中 (%)            | 1.3           | 0.5        | 0           | 1.3               |
| Per dam<br>每母鼠                                   | 0.16          | 0.05       | 0           | 0.16              |
| Spontaneously delivered groups<br>自然分娩组          |               |            |             |                   |
| Number of animals<br>动物数                         | 10            | 10         | 10          | 10                |
| Number of pregnant animals<br>妊娠动物数              | 7             | 9          | 10          | 9                 |
| Total number of young<br>总仔鼠数                    | 72            | 107        | 120         | 104               |
| At Birth<br>出生时                                  |               |            |             |                   |
| Viable young per dam<br>每母鼠可存活仔鼠数                | 10.1 ± 3.3    | 11.8 ± 1.6 | 11.9 ± 2.0  | 11.0 ± 3.5        |
| Dead young per dam<br>每母鼠死仔鼠数                    | 0.1 ± 0.4     | 0.1 ± 0.3  | 0.1 ± 0.3   | 0.6 ± 0.7         |
| Mean litter weight (g)<br>每窝仔鼠平均体重 (g)           | 62.8 ± 20.1   | 73.7 ± 8.8 | 73.6 ± 15.4 | 61.6 ± 20.3       |
| Mean weight of viable young (g)<br>可存活仔鼠平均体重 (g) | 6.2 ± 0.7     | 6.3 ± 0.6  | 6.2 ± 0.8   | 5.6 ± 0.7         |
| 11 <sup>th</sup> day<br>11日                      |               |            |             |                   |
| Number of animals<br>动物数                         | 63            | 98         | 114         | 88                |
| Number of pregnant animals<br>妊娠动物数              | 9.9 ± 4.2     | 10.9 ± 2.5 | 11.4 ± 2.5  | 9.8 ± 4.8         |
| Total number of young<br>总仔鼠数                    | 371 ± 161     | 455 ± 45   | 447 ± 78    | 369 ± 166         |
| Mean litter weight (g)<br>每窝仔鼠平均体重 (g)           | 41.2 ± 5.0    | 41.7 ± 7.5 | 39.2 ± 4.2  | 37.7 ± 5.3        |
| Number of animals<br>动物数                         | 7             | 8          | 5           | 11                |
| Number of pregnant animals<br>妊娠动物数              | 2             | 10         | 8           | 0                 |
| Number of animals<br>动物数                         |               | 0          | 0           | 2                 |

e. Observations:

- i. Mean viable fetal weight of 500 mg/kg/day group was slightly but significantly ( $p < 0.01$ ) smaller than that of control group, but it was reported to be within the background for normal data (see page 70,  $5.55 \pm 0.51$  g).

COMMENTS: Mean body weight of viable youngs in 50 and 500 mg/kg/day groups on day 21 after birth was also highly significantly ( $p < 0.01$ ) smaller than that of control group, showing retarded weight gains in these two groups in spontaneously delivered group. See below.

There were no stillbirths or "runts" in the fetuses.

ii. Skeletal Variations:

|                | n   | lumbar ribs (%) | lumbarization | splintering or missing of sternal centers | misshaped vertebral body | others | Total    |
|----------------|-----|-----------------|---------------|-------------------------------------------|--------------------------|--------|----------|
| D <sub>0</sub> | 157 | 64(40.8)        | 2(1.3)        | 0                                         | 2 (1.3)                  | 0      | 66(42.0) |
| D <sub>1</sub> | 143 | 64(44.8)        | 0             | 1(0.7)                                    | 1 (0.7)                  | 1(0.7) | 67(46.9) |
| D <sub>2</sub> | 169 | 29(29.0)        | 4(2.4)        | 1(0.6)                                    | 2 (1.2)                  | 1(0.6) | 34(32.0) |
| D <sub>3</sub> | 148 | 15(10.1)**      | 2(1.4)        | 0                                         | 4 (2.7)                  | 3(2.0) | 24(16.2) |

\* Several fetuses have shown two or more variations.

As shown above, the rate of fetuses with lumbar ribs in 500 mg/kg group was significantly smaller than control, and the decrease was dose-related.

fff. Malformations: skeletal:

The following report was submitted:

Control

5-x Vertebraschisis  
8-x Vertebraschisis  
10-x Vertebraschisis

D<sub>1</sub> (50 mg/kg)

18a-x Discurvatio costae

D<sub>2</sub> (50 mg/kg)

N.A.D.

D<sub>3</sub> (500 mg/kg)

1c-x Vertebraschisis  
5e-x Vertebraschisis  
13e-x Discurvatio costae

3. Spontaneously Delivered Group:

Dams: Clinical symptoms and body weight gain of pregnant dams paralleled about to that of C section group above.

500 mg/kg/day group actually lost some weight during day 8 and day 11 before starting to gain again. See Fig. on page 21 and Table on page 49.

At autopsy no abnormalities were noted.

Pups:

a. At Birth:

Pups (of viable young) in 500 mg/kg/day group had slight but significantly ( $p < 0.01$ ) smaller birth weight, and the increased number of dead young per dam was produced in this group (0.1 in others vs. 0.6.).

b. During Weaning Period:

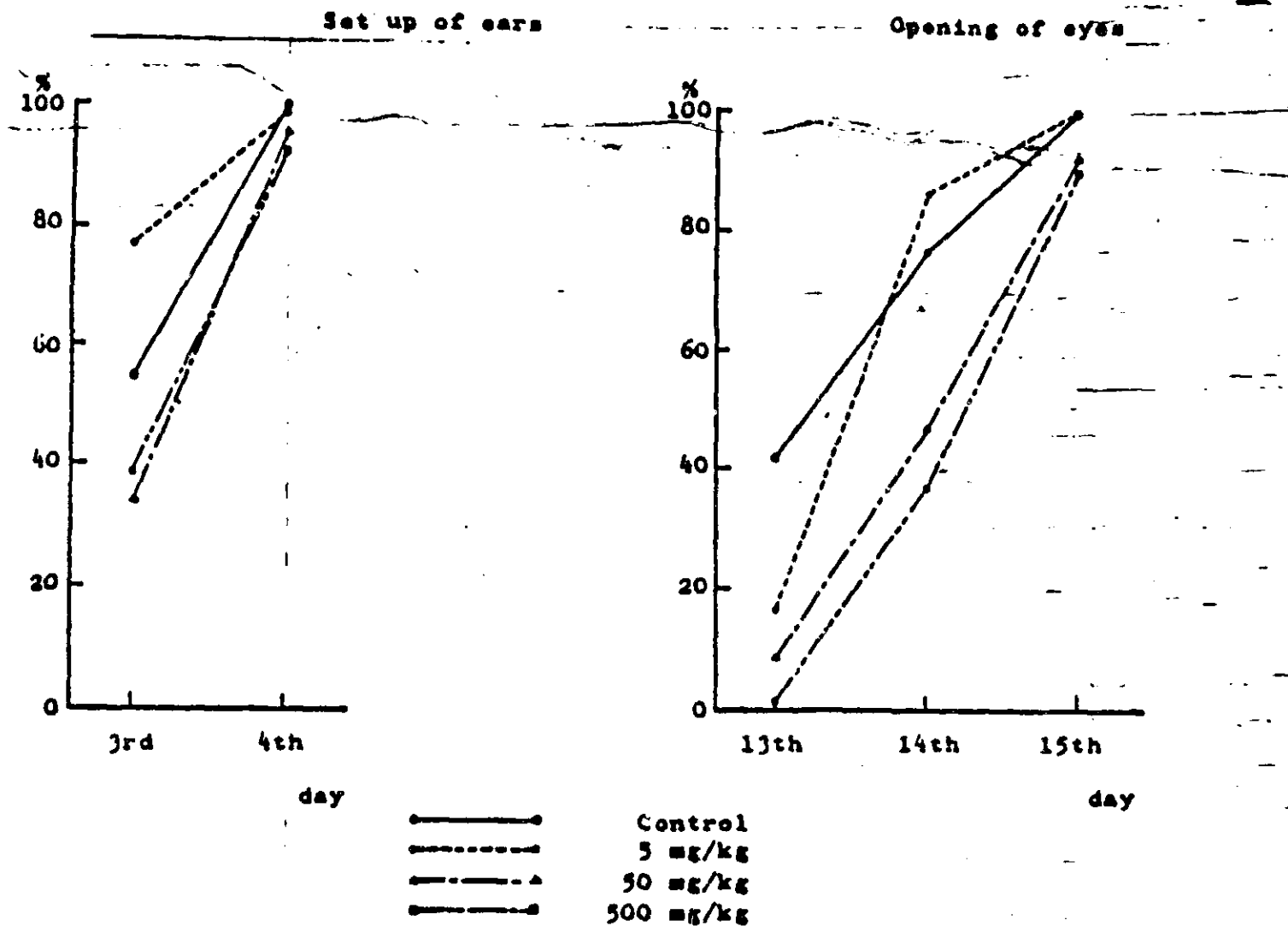
i. Pups from 50 and 500 mg/kg/day group were slightly smaller than those of control but not significant. See Graph on page 23 for the growth curves, and group mean body weight in Table below.

| Group<br>#   | Day after birth       |         |          |          |                        |
|--------------|-----------------------|---------|----------|----------|------------------------|
|              | 1                     | 4       | 7        | 14       | 21                     |
| Control<br># | 6.2±0.7               | 9.2±1.8 | 13.1±1.6 | 23.6±3.5 | 41.2±5.0               |
| 5<br>mg/kg   | 6.3±0.6               | 9.6±1.0 | 14.1±1.4 | 27.5±4.7 | 41.7±7.5               |
| 50<br>mg/kg  | 6.2±0.8               | 9.3±1.2 | 12.9±1.5 | 24.9±3.5 | 39.2±4.2 <sup>**</sup> |
| 500<br>mg/kg | 5.6±0.7 <sup>**</sup> | 8.5±1.1 | 12.2±1.5 | 24.0±2.7 | 37.7±5.3 <sup>**</sup> |

ii. Ear and Eye Openings:

It was reported that "the setting up of ears and the opening of eyes seemed to be dose-dependently retarded for about one day; however, no background data are existing in case of this new investigation method." See Graph 5 and Table 12 below:

Graph 5 Development of Ears and Eyes of the Pups  
Sch 1000 Br Rats p.o.



| Day         | No. of pups, the ears of which were setting up |                   | No. of pups, the eyes of which opened |                  |                   |
|-------------|------------------------------------------------|-------------------|---------------------------------------|------------------|-------------------|
|             | 3th                                            | 4th               | 13th                                  | 14th             | 15th              |
| Control (%) | 36/66<br>(54.3)                                | 64/64<br>(100.0)  | 26/63<br>(41.3)                       | 48/63<br>(76.2)  | 63/63<br>(100.0)  |
| 5 mg/kg     | 75/98<br>(76.5)                                | 97/98<br>(99.0)   | 16/98<br>(16.3)                       | 84/98<br>(85.7)  | 98/98<br>(100.0)  |
| 50 mg/kg    | 39/114<br>(34.2)                               | 109/114<br>(95.6) | 10/114<br>(8.8)                       | 52/114<br>(45.6) | 105/114<br>(92.1) |
| 500 mg/kg   | 35/90<br>(38.9)                                | 81/88<br>(92.1)   | 1/88<br>(1.1)                         | 32/88<br>(36.3)  | 79/88<br>(89.7)   |

iii. Locomotion and Behavior:

No significant difference between groups was reported for open-field test as shown in Table below:

Results of Open-field Test\*

Sch 1000 Br p.o.

Rats

| Group   |       | loco-<br>motion |  | rearing<br>up |  | preening |
|---------|-------|-----------------|--|---------------|--|----------|
| Control |       | 32.5±12.4       |  | 1.8±1.8       |  | 0.5±0.6  |
| 5       | mg/kg | 28.8±10.6       |  | 2.0±1.9       |  | 0.6±0.7  |
| 50      | mg/kg | 29.8±12.0       |  | 1.8±1.8       |  | 0.7±0.6  |
| 500     | mg/kg | 33.1±17.2       |  | 1.6±1.7       |  | 0.5±0.5  |

- Makrolon<sup>(R)</sup> cages, Type III (floor space approximately 700 cm<sup>2</sup>), were used, the floor of which was divided into 15 equal squares. The individual pups were individually placed in the center and the movements were registered during the period of one minute.

locomotion: Number of squares, crossed per minute  
 rearing up: Number per minute  
 preening: Number of wiping the face by forelimbs

c. 21st Day:

- i. Many pups died during weaning: 7 in control, 8, 5, and 11 in 5, 50, and 500 mg/kg/day group respectively, but others grew up normally.
- ii. A total of 363 pups alive after 3 weeks, and among them the following variations were found with two malformations in 500 mg/kg/day group.

|                | n=  | additional<br>sternbrae<br>(%) | additional<br>lumbar vertebrae<br>(%) |
|----------------|-----|--------------------------------|---------------------------------------|
| D <sub>0</sub> | 63  | 2 (3.2)                        | 0                                     |
| D <sub>1</sub> | 98  | 9 (9.2)                        | 1 (1.0)                               |
| D <sub>2</sub> | 114 | 8 (7.0)                        | 0                                     |
| D <sub>3</sub> | 88  | 1 (1.1)                        | 0                                     |

Two malformations in 500 mg/kg/day group were a case of nanism with absence of one thoracic vertebra and three ribs, vertebraschisis, deformity of lumbar vertebrae and discurvatio of spinal column seen in a pup(21c-x), and discurvatio costae seen in the second pup(25c-x).

- COMMENTS: 1) A total of 7 malformations was reported for C section group above; therefore, a total of 9 malformations was found in this rat study.
- 2) Line 9 from the bottom of page 17: 7 should be changed to 9, due to 2 more in 500 mg/kg/day group. See top of the page 18.
- iii. Significantly ( $p < 0.01$ ) smaller mean body weight was seen in 50 and 500 mg/kg/day group. See Table above.

c. 21st Day:

- i. Many pups died during weaning: 7 in control, 8, 5, and 11 in 5, 50, and 500 mg/kg/day group respectively, but others grew up normally.
- ii. A total of 363 pups alive after 3 weeks, and among them the following variations were found with two malformations in 500 mg/kg/day group.

|                | n=  | additional<br>sternbrae<br>(%) | additional<br>lumbar vertebrae<br>(%) |
|----------------|-----|--------------------------------|---------------------------------------|
| D <sub>0</sub> | 63  | 2 (3.2)                        | 0                                     |
| D <sub>1</sub> | 98  | 9 (9.2)                        | 1 (1.0)                               |
| D <sub>2</sub> | 114 | 8 (7.0)                        | 0                                     |
| D <sub>3</sub> | 88  | 1 (1.1)                        | 0                                     |

Two malformations in 500 mg/kg/day group were a case of nanism with absence of one thoracic vertebra and three ribs, vertebraschisis, deformity of lumbar vertebrae and discurvatio of spinal column seen in a pup(21c-x), and discurvatio costae seen in the second pup(25c-x).

- COMMENTS: 1) A total of 7 malformations was reported for C section group above; therefore, a total of 9 malformations was found in this rat study.
- 2) Line 9 from the bottom of page 17: 7 should be changed to 9, due to 2 more in 500 mg/kg/day group: See top of the page 18.
- iii. Significantly ( $p < 0.01$ ) smaller mean body weight was seen in 50 and 500 mg/kg/day group. See Table above.

I. ORAL TERATOLOGY STUDY IN RABBITS: -E83-128:

Lab. Performing the Study: See above, but according to recommendations of the National Council of Pharmacy, Japan.

Strain: Himalayan (small Russian breed), about 2 kg and 4 month old.

Methods: 15 pregnant F/group of rabbits were treated with 5, 25, or 125 mg/kg/day Sch 1000-Br (Batch No. 1/74) from day 6 to day 18 of gestation via stomach tube along with a control group receiving distilled water. They were killed by air embolism on day 29, about 24 hours before parturition, and examined. See pages 1-6 for detailed information.

RESULTS:

1. PARENT:

a. Clinical Symptoms:

Several dams in 125 mg/kg/day group had misshaped feces, and the amount was markedly diminished.

b. Body Weight:

All 3 treated groups did not gain any weight during the first 6 days treatment, then started to gain slowly during the next 6 days treatment period. 5 and 125 mg/kg/day groups gained much less than the other groups during day 24 and day 29. See Graph 1 on page 14 and Tables 2 and 3.

c. Food and Water Consumption:

Food consumption of 25 and 125 mg/kg/day groups was distinctly (but not significantly) decreased during the dosing period. Water consumption did not show dose related changes. See page 15 and Table 4 on page 24.

d. Spontaneous Parturition:

One dam in the control group and one on 125 mg/kg/day group aborted during the last week of gestation as shown below:

e. Pregnancy Rate:

100% in control group (15/15), the low dose groups with 86% (13/15) and 73% in high dose group (11/15 including aborted one). See Table 1 below.

COMMENTS: On page 8, it was reported that the high dose had 80% (12/15), but it must be a mistake, since all other figures are based only 10 pregnant dams in Table 1 below.

f. Mortality:

One in 5 mg/kg/day group died "a couple of days" before the drug treatment, and at autopsy "caudopositis of left kidney and bleeding into the gastric wall have been found" in this dam (6a).

g. Autopsy Finding After Caesarean Section on All Dams:

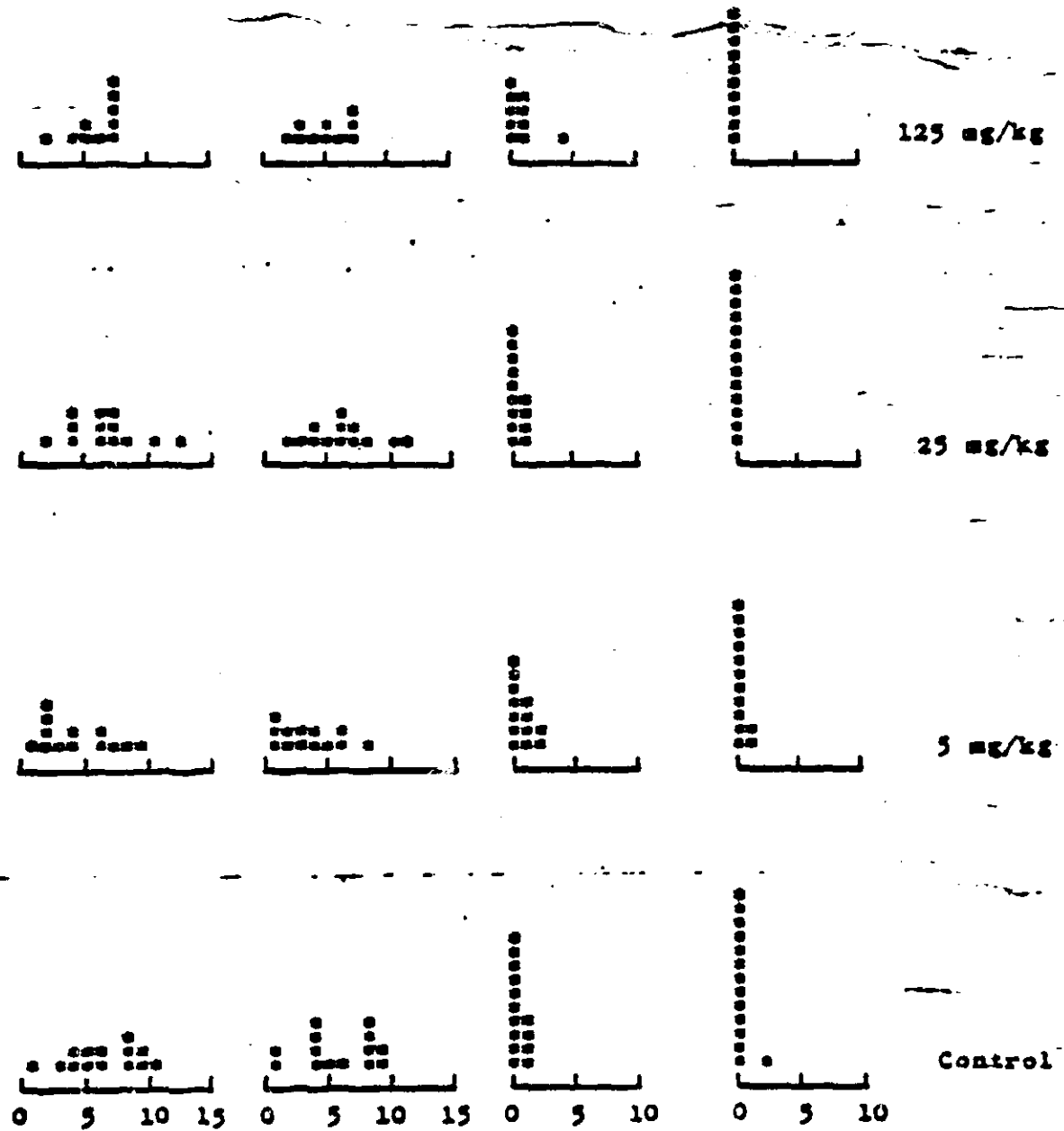
Only one dam (11b) in 25 mg/kg/day group had "hydrothorax, ascites, and adipositas hepatica."

h. Implantation:

Number of implantation sites/dam was lowest in 5 mg/kg/day group, followed by the 125 mg/kg/day group, that was slightly lower than control group. See Table 1 and Graph 3 below for details.

COMMENTS: Mistake: Table 4 under Implantations on page 8 is wrong, since it contains food and water consumption only.

Graph 3 Litter Distribution of Each Parameter



Implantations Viable fetuses Resorptions Dead fetuses

COMMENTS: Mistakes: Graph 2 on lines 13, 7, and 2 from the bottom of page 8 should be changed to Graph 3.

i. Resorption:

Number of resorptions was significantly ( $p < 0.05$ ) larger in 5 and 125 mg/kg/day groups than control group.

j. Corpora Lutea:

Number of corpora lutea was greater than the total number of implantation.

Preimplantation loss was greater in 5 mg/kg/day group than other groups, and post-implantation loss was significantly ( $p < 0.05$ ) greatest in this 5 mg/kg/day group followed by 125 mg/kg/day group, then the control group. See Table 1 below.

2. LITTER DATA:

a. Viable Fetuses:

Number of live fetuses per implantation sites and per dam was significantly ( $p < 0.05$ ) lower in 5 mg/kg/day than control group, followed by 125 mg/kg/day group, then control group.

b. Dead Fetuses:

A total of 4 still born were observed; 2 in control and 2 in 5 mg/kg/day group, as shown in the above Graph 3 but these fetuses had no external and skeletal malformations.

c. GROUP MEAN LITTER DATA: Table 1.

|                                             | Control<br>对照组 | 5 mg/kg    | 25 mg/kg   | 125 mg/kg  |
|---------------------------------------------|----------------|------------|------------|------------|
| Number of animals<br>动物数                    | 15             | 15         | 15         | 15         |
| Maternal mortality<br>母体死亡率                 | 0              | 1          | 0          | 0          |
| Abortions<br>流产数                            | 1              | 0          | 0          | 1          |
| Natural deliveries<br>自然分娩数                 | 0              | 0          | 0          | 0          |
| Total number of pregnant animals<br>妊娠动物数   | 14             | 13         | 13         | 10         |
| Total implantation sites<br>着床数             | 86             | 56         | 83         | 57         |
| Per dam<br>1母体着床数                           | 6.1 ± 2.6      | 4.3 ± 2.6  | 6.4 ± 2.7  | 5.7 ± 1.7  |
| Total viable fetuses<br>生存胎仔数               | 80             | 46         | 79         | 49         |
| Male in %<br>雄性率 (%)                        | 46.3           | 43.5       | 46.8       | 46.9       |
| Female in %<br>雌性率 (%)                      | 53.7           | 56.5       | 53.1       | 53.1       |
| In % of implantation sites<br>胎仔生存率 (%)     | 93.0           | 82.1*      | 95.1       | 86.0       |
| Per dam<br>1母体生存胎仔数                         | 5.7 ± 2.6      | 3.5 ± 2.2* | 6.1 ± 2.6  | 4.9 ± 1.9  |
| Total resorption sites<br>吸收数               | 4              | 8          | 4          | 8          |
| Early<br>早期吸收数                              | 4              | 8          | 4          | 7          |
| Late<br>晚期吸收数                               | 0              | 0          | 0          | 1          |
| In % of implantation sites<br>胎仔吸收率 (%)     | 4.7            | 14.3*      | 4.8        | 14.0*      |
| Per dam<br>1母体吸收数                           | 0.3 ± 0.5      | 0.6 ± 0.8  | 0.3 ± 0.5  | 0.8 ± 1.2  |
| Total dead fetuses<br>死亡胎仔数                 | 2              | 2          | 0          | 0          |
| In % of implantation sites<br>胎仔死亡率 (%)     | 2.3            | 3.6        | 0          | 0          |
| Per dam<br>1母体死亡胎仔数                         | 0.1 ± 0.5      | 0.2 ± 0.4  | 0          | 0          |
| Mean litter weight (g)<br>平均胎仔体重 (g)        | 210 ± 79       | 153 ± 89   | 209 ± 63   | 177 ± 55   |
| Mean viable (total weight (g)<br>平均胎仔体重 (g) | 36.7 ± 5.7     | 39.7 ± 4.5 | 34.4 ± 6.7 | 36.1 ± 6.8 |
| Corpora lutea<br>黄体数                        | 126            | 99         | 101        | 68         |
| Pre-implantation loss %<br>着床前胎仔丢失率 (%)     | 31.7           | 43.4       | 21.7       | 16.2       |
| Post-implantation loss %<br>着床后胎仔丢失率 (%)    | 7.0            | 17.9*      | 4.8        | 14.0       |
| Total variations<br>迟发及假胎仔数                 | 0              | 0          | 0          | 1          |
| In % of viable fetuses<br>迟发及假胎仔率 (%)       | 0              | 0          | 0          | 2.0        |
| Per dam<br>1母体迟发及假胎仔数                       | 0              | 0          | 0          | 0.1        |
| Conceptions<br>胎仔数                          | 0              | 0          | 1          | 0          |
| Losses<br>(%)                               | 0              | 0          | 1.3        | 0          |
| Per dam                                     | 0              | 0          | 0.1        | 0          |

N-19085-5

d. Weight of Fetuses:

No differences between groups in mean viable fetal birth weight were shown, but there were several fetuses with the weight below 65% of the normal fetal weight ("runt") in 25 and 125 mg/kg/day groups as shown in the following, but they had no other abnormalities.

|           |      |        |   |                       |
|-----------|------|--------|---|-----------------------|
| Fetus No. | 2b-6 | 16.0 g | ♀ | } 25 mg/kg/day group  |
|           | 3b-3 | 21.6   | ♂ |                       |
|           | 3b-5 | 23.7   | ♂ |                       |
|           | 3b-6 | 22.4   | ♀ |                       |
|           | 3b-7 | 22.5   | ♂ |                       |
|           | 5b-6 | 23.2   | ♂ | } 125 mg/kg/day group |
|           | 5c-5 | 22.8   | ♀ |                       |

e. Variations:

One fetus in 125 mg/kg/day group had asymmetrical sternebrae.

f. Malformations:

Among 254 fetuses examined, one malformation occurred in 25 mg/kg/day group (11b-6 ♀, 20.7 g) with pseudotruncus aortae (dextropositio et dilatatio aortae, atresia pulmonalis, agenesis septi ventriculorum). See Fig. 1 and 2 on page 13.

COMMENTS: It was stated that the deformed heart found in 25 mg/kg/day group was seen before in the strain of animals used, and the 1.3% malformation among viable fetuses are within normal range. See background control data on Himalayan rabbits on page 41.

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|           |      |        |   |                       |
|-----------|------|--------|---|-----------------------|
| Fetus No. | 2b-6 | 16.0 g | ♀ | } 25 mg/kg/day group  |
|           | 3b-3 | 21.6   | ♂ |                       |
|           | 3b-5 | 23.7   | ♂ |                       |
|           | 3b-6 | 22.4   | ♀ |                       |
|           | 3b-7 | 22.5   | ♂ |                       |
|           | 5b-6 | 23.2   | ♂ |                       |
|           | 5c-5 | 22.8   | ♀ | - 125 mg/kg/day group |

e. Variations:

One fetus in 125 mg/kg/day group had asymmetrical sternebrae.

f. Malformations:

Among 254 fetuses examined, one malformation occurred in 25 mg/kg/day group (11b-6 ♀, 20.7 g) with pseudotruncus aortae (dextropositio et dilatatio aortae, atresia pulmonalis, agenesia septi ventriculorum). See Fig. 1 and 2 on page 13.

COMMENTS: It was stated that the deformed heart found in 25 mg/kg/day group was seen before in the strain of animals used, and the 1.3% malformation among viable fetuses are within normal range. See background control data on Himalayan rabbits on page 41.

**I. INHALATION TERATOLOGY STUDY IN RABBITS: E83-131:**

Lab. Performing the Study: Huntingdon Research Center  
Huntingdon, England.

Strain: New Zealand White rabbits.

Methods:

Preliminary Study:

Mature does (6 F/group) that successfully completed coitus with males of proven fertility were injected iv with 10 i.u. luteinizing hormone to ensure that ovulation took place (Day 0 = day of mating), then they were administered daily dose of Sch 1000 using a modified tracheal tube and metered aerosol can with standard concentration for no less than 2 min, each dose being given at the third inspiration, 30 metered doses/rabbit/session, 4 sessions/day from day 6 to day 18 of pregnancy, along with vehicle control group.

Blood samples (1 ml/rabbit) were taken from central ear vein of 3 rabbits receiving Sch 1000 at 0.5, 1, 4, and 12 min after the first dosing (on day 6) for the determination of Freon II.

On day 29 of pregnancy, rabbits were killed by cervical dislocation and examined.

The dose of Sch 1000 was estimated to be 1.8 mg/kg that was based on the assumption that at least 75% of material is ejected from the end of the delivery tube which is sited just above the trachea, and in - vitro tests with the apparatus showed a delivery rate at the end of the tube between 70-80% of the maximal.

Main Teratology Study:

Methods were the same as above preliminary study with the following exceptions and modifications:

- a. No. of Animals/Level: 13 pregnant F  
b. Dosage Level:

| Group | Treatment            | Dosing schedule<br>(No. of metered doses/<br>rabbit/session) | (No. of<br>sessions/day) | No. of rabbit<br>? |
|-------|----------------------|--------------------------------------------------------------|--------------------------|--------------------|
| 1     | Control (sham)       | -                                                            | 4                        | 13                 |
| 2     | Control (propellant) | 30                                                           | 4                        | 13                 |
| 3     | Sch 1000, 0.3mg/kg   | 5                                                            | 4                        | 13                 |
| 4     | Sch 1000, 0.9mg/kg   | 15                                                           | 4                        | 13                 |
| 5     | Sch 1000, 1.8mg/kg   | 30                                                           | 4                        | 13                 |

- c. Blood samples collected from 5 rabbits of Groups 2 and 5, 30 seconds after administration of the first dosing on days 6 and 18.

See detailed procedures on pages 4-6.

RESULTS:

1. Preliminary Study:

- a. Signs: Transient tachypnea was observed in the majority of rabbits in both groups immediately after dosing.

Freon II was detected in all animals immediately after dosing, and the level declined rapidly during the subsequent 12-15 min, although there were individual variations. See page 20 for blood levels.

COMMENTS: Time of blood collection was not same as indicated in Methods for 3 rabbits.

b. Body Weight:

No difference between two groups in weight gain. See Fig. 1 on page 10.

- a. No. of Animals/Level: 13 pregnant F  
b. Dosage Level:

| Group | Treatment            | Dosing schedule                           |                          | No. of rabbit<br>9 |
|-------|----------------------|-------------------------------------------|--------------------------|--------------------|
|       |                      | (No. of metered doses/<br>rabbit/session) | (No. of<br>sessions/day) |                    |
| 1     | Control (sham)       | -                                         | 4                        | 13                 |
| 2     | Control (propellant) | 30                                        | 4                        | 13                 |
| 3     | Sch 1000, 0.3mg/kg   | 5                                         | 4                        | 13                 |
| 4     | Sch 1000, 0.9mg/kg   | 15                                        | 4                        | 13                 |
| 5     | Sch 1000, 1.8mg/kg   | 30                                        | 4                        | 13                 |

- c. Blood samples collected from 5 rabbits of Groups 2 and 5, 30 seconds after administration of the first dosing on days 6 and 18.

See detailed procedures on pages 4-6.

RESULTS:

1. Preliminary Study:

- a. Signs: Transient tachypnea was observed in the majority of rabbits in both groups immediately after dosing.

Freon II was detected in all animals immediately after dosing, and the level declined rapidly during the subsequent 12-15 min, although there were individual variations. See page 20 for blood levels.

COMMENTS: Time of blood collection was not same as indicated in Methods for 3 rabbits.

b. Body Weight:

No difference between two groups in weight gain. See Fig. 1 on page 10.

c. Litter Data:

- i. All 6 control rabbits and 5 treated rabbits were pregnant (100% vs. 83%) and had viable youngs, and only one in treated group was not pregnant.
- ii. Treatment did not produce adverse effect on litter size, mean fetal weight, implantation, and no major malformation was produced. Slightly higher fetal loss was noted in the treated group (6.2% vs. 8.5%). See Table 2 on page 13. The incidence of minor skeletal abnormalities were reported to be normal limits. See Table 3 on page 14 and Table 4 on page 15.

2. MAIN TERATOLOGY STUDY:

a. Signs and Mortalities:

- i. "Several" rabbits in 1.8 mg/kg/day group showed transient anorexia during dosing period.
- ii. One rabbit (No. 47) was sacrificed on day 26 with apparent middle ear disease with tilting of the head to one side, muscular incoordination, and marked nystagmus of right eye. Liver and renal cortex were congested at autopsy, with 8 implantation.

iii. Freon Analysis:

Apparently much higher levels were presented in Sch 1000 treated group than vehicle control group on both days as shown in the following Table:

**GROUP 2 : Propellant:**

| PARENT NUMBER | Blood levels of Freon 11 (ppm), 30 seconds after administration of the first dosing session on day |      |
|---------------|----------------------------------------------------------------------------------------------------|------|
|               | 6                                                                                                  | 18   |
| 22            | 13.0                                                                                               | 8.5  |
| 23            | 8.0                                                                                                | 2.5  |
| 24            | 1.0                                                                                                | <1.0 |
| 25            | 9.0                                                                                                | <1.0 |
| 26            | 11.0                                                                                               | 2.0  |

**GROUP 5 : 1.8 mg/kg/day:**

|    |       |      |
|----|-------|------|
| 61 | 14.0  | 11.0 |
| 62 | 5.0   | 3.0  |
| 63 | 23.0  | 29.0 |
| 64 | 200.0 | 26.0 |
| 65 | 33.0  | 23.0 |

**b. Body Weight Change of Dams with Viable Youngs:**

Retardation of weight gain occurred in 1.8 mg/kg/day group during dosing period (See Fig. 2 on page 11), but the weight of this group was comparable to the control group by day 28. 0.3 mg/kg/day group also had some retardation.

COMMENTS: It was stated that lower weight gain on the high dose group could not be conclusively related to treatment, since the same dose in the preliminary study showed comparable weight gain as the control group.

**c. Pregnancy Rate:**

First 4 groups had 92%, and the 1.8 mg/kg/day group had 85%, but the difference between groups was not significant.

**GROUP 2 : Propellant:**

| PARENT NUMBER | Blood levels of Freon 11 (ppm), 30 seconds after administration of the first dosing session on day |      |
|---------------|----------------------------------------------------------------------------------------------------|------|
|               | 6                                                                                                  | 18   |
| 22            | 13.0                                                                                               | 8.5  |
| 23            | 8.0                                                                                                | 2.5  |
| 24            | 1.0                                                                                                | <1.0 |
| 25            | 9.0                                                                                                | <1.0 |
| 26            | 11.0                                                                                               | 2.0  |

**GROUP 5 : 1.8 mg/kg/day:**

|    |       |      |
|----|-------|------|
| 61 | 14.0  | 11.0 |
| 62 | 5.0   | 3.0  |
| 63 | 23.0  | 29.0 |
| 64 | 200.0 | 26.0 |
| 65 | 33.0  | 23.0 |

**b. Body Weight Change of Dams with Viable Youngs:**

Retardation of weight gain occurred in 1.8 mg/kg/day group during dosing period (See Fig. 2 on page 11), but the weight of this group was comparable to the control group by day 28. 0.3 mg/kg/day group also had some retardation.

COMMENTS: It was stated that lower weight gain on the high dose group could not be conclusively related to treatment, since the same dose in the preliminary study showed comparable weight gain as the control group.

**c. Pregnancy Rate:**

First 4 groups had 92%, and the 1.8 mg/kg/day group had 85%, but the difference between groups was not significant.

d. Pre-implantation Loss:

Preimplantation loss of all treated groups was higher than control or vehicle control group, but it was stated as "within the Lab. Standard Range" and not significant.

2. LITTER DATA:

Two rabbits in 1.8 mg/kg/day group and one in 0.9 mg/kg/day group aborted. It was stated that 2 in 1.8 mg/kg/day group had previously been bled for Freon analysis, and this influence and "since the frequency of total litter losses was in a range previously encountered in control groups, the following comparison were restricted to mean B values (i.e. only litters with viable young)". See Table below.

a. Litter Size and Fetal Loss:

Litter size and fetal loss were not significantly different between group (B values only). See Table below and Appendix 9.

COMMENTS: Fetal loss of two treated groups was much higher than sham control or vehicle control group and dose related (see A values : 15.3% for sham control, 14.9% for vehicle control, but 13.6%, 21.3%, and 29.1% for low, mid, and high dose groups for A values).

b. Litter and Mean Pup Weight:

Slightly lower mean pup weight in treated groups was not significant.

Group mean litter data - teratology study

|              |                                 |   |          |     |     |
|--------------|---------------------------------|---|----------|-----|-----|
| Group        | 1                               | 2 | 3        | 4   | 5   |
| Drug         | CONTROL<br>Sham      Propellant |   | SCH 1000 |     |     |
| Dose (mg/kg) | -                               | - | 0.3      | 0.9 | 1.8 |

| GROUP | NO. OF ANIMALS | PREGNANCY RATE % | VIABLE YOUNG |     |       | RESORPTIONS |      |       | ABORTIONS | IMPLANTATIONS | CORPORA LUTEA | PRE-IMPLANTATION LOSS % | FOETAL LOSS % | LITTER WT. (g) | MEAN FOETAL WEIGHT (g) |
|-------|----------------|------------------|--------------|-----|-------|-------------|------|-------|-----------|---------------|---------------|-------------------------|---------------|----------------|------------------------|
|       |                |                  | ♂            | ♀   | Total | Early       | Late | Total |           |               |               |                         |               |                |                        |
| 1     | A=8-12         | 92               | 4.0          | 3.3 | 7.3   | 0.6         | 0.8  | 1.3   | -         | 8.7           | 8.9           | 2.4                     | 15.3          | 319.7          | 44.6                   |
| 2     | A=8-12         | 92               | 3.8          | 3.6 | 7.4   | 1.1         | 0.3  | 1.4   | -         | 8.8           | 9.6           | 7.6                     | 14.9          | 319.5          | 44.1                   |
| 3     | A=8-11         | 92               | 3.4          | 3.6 | 7.0   | 0.8         | 0.5  | 1.3   | -         | 8.3           | 9.5           | 12.6                    | 13.6          | 311.0          | 44.0                   |
| 4     | A=11           | 92               | -            | -   | 6.8   | 0.5         | 0.6  | 1.2   | 0.5       | 8.5           | 9.9           | 14.0                    | 21.3          | -              | -                      |
|       | B=10           |                  | 4.2          | 3.3 | 7.5   | 0.6         | 0.7  | 1.3   | -         | 8.8           | 10.2          | 14.0                    | 13.4          | 296.4          | 40.3                   |
| 5     | A=11           | 85               | -            | -   | 6.3   | 0.4         | 0.5  | 0.9   | 0.8       | 8.0           | 9.1           | 13.0                    | 29.1          | -              | -                      |
|       | B=9            |                  | 4.4          | 3.2 | 7.7   | 0.4         | 0.7  | 1.1   | -         | 8.8           | 9.8           | 9.7                     | 13.3          | 321.7          | 41.9                   |

Group mean values were then calculated from individual observations in two ways.

Mean A: includes all surviving animals that provided evidence of nidation, including those aborting or exhibiting total resorption

Mean B: includes all animals bearing viable young at termination

Mean B has more meaning when there is a small number of animals per group, in which case the mean values would be unduly influenced by the presence of a single animal aborting or exhibiting total resorption. Mean A is a more accurate index when there is a large number of animals per group or when several animals abort or exhibit total resorption.

c. Major and Minor Malformations:

Four major malformations occurred, 2 in 0.3 mg/kg/day group and 2 in control group. But similar abnormalities have been ~~occurred~~ spontaneously among control youngs; therefore, not drug related. See pages 40-52 for detailed descriptions of malformations and minor variations in Appendix 10. No significant differences between groups were reported for incidence of minor visceral and skeletal anomalies.

d. Skeletal Variations:

No significant differences between groups were shown, although there were higher incidence of both 12 and 13-ribbed pups with variant sternbrae in treated pups as shown in the following Table:

Group mean incidence of skeletal variants - teratology study

| Group        | 1                          | 2                    | 3            | 4   | 5  |
|--------------|----------------------------|----------------------|--------------|-----|----|
| Drug         | CONTROL                    |                      | SCH 1000     |     |    |
|              | Sham                       | Propellant           |              |     |    |
| Dose (mg/kg) | -                          | -                    | 0.3          | 0.9 | 1. |
| GROUP        | PERCENTAGE OF YOUNG * WITH |                      |              |     |    |
|              | EXTRA RIB(S)               | VARIANT STERNEBRA(E) |              |     |    |
|              |                            | Normal ribs          | Extra rib(s) |     |    |
| 1            | 40.7                       | 7.6                  | 0.0          |     |    |
| 2            | 44.0                       | 14.2                 | 12.9         |     |    |
| 3            | 35.8                       | 18.3                 | 2.8          |     |    |
| 4            | 22.4                       | 21.3                 | 10.7         |     |    |
| 5            | 48.1                       | 20.0                 | 12.5         |     |    |

\*Young showing major malformations excluded.

COMMENTS: A mistake on line 2 on page 9: The propellant control group should be changed to sham control group since two major malformations were seen in group 1 instead of group 2 on pages 40 and 41.

K. ORAL PERI-AND POST NATAL REPRODUCTION STUDY IN RATS: E83-132  
(SEGMENT III):

Lab. Performing the Study: Boehringer Ingelheim GmbH  
Pharma-Research, Japan: (according to  
Jap. Council of Pharmacy methods).

Strain: SD-JCL Sprague-Dawley, about 11 weeks  
old and  $22.9 \pm 2.2$  g.

Methods: 20 pregnant F/group of rats were treated by gastric intubation with 5, 50, or 500 mg/kg/day sch 1000-Br (Batch No. VIII) from day 17 of gestation through lactation until day 21 after parturition along with a vehicle control group receiving distilled water (0.5 ml/100 g). 8 pups out of each litter were nursed until day 21, and one M and one F per litter were mated at the age of 12 weeks for the evaluation of their reproductive capability (Segment III). See pages 1-9 for detailed procedure.

RESULTS:

1. PARENTS:

a. General Observations:

One (No. 256) in 50 mg/kg/day group became weak after dosing during pregnancy. In 500 mg/kg/day group, one (No. 354) had weakness, and 3 (No.s 351, 360, 367) had red incrustated eyes during pregnancy. After delivers, other 3 (No.s 355, 359, 365) had red incrustated eyes and No. 354 had prostration.

b. Body Weight Gain:

Total group mean weight gain during gestation period was depressed in 50 mg/kg/day group ( $P < 0.05$ ) and 500 mg/kg/day group ( $p < 0.01$ , see Fig 1 on page 24 and Table 2-1 on page 32), but after parturition, the group mean gain was smallest in 50 mg/kg/day group especially first 5 days. See page 24 and Table 2-2 on page 33.

COMMENTS: The second paragraph on body weight under results section on page 10 was not accurate strictly speaking.

c. Food and Water Consumption:

No significant differences between groups showed in both parameters. See Fig. 2 on page 25 and Table 4 on page 42.

d. Mortality and Necropsy:

Two rats (Nos 359 and 356) died in 500 mg/kg/day group on the first and day 18 from accidental drug delivery.

No. 256 in 50 mg/kg/day group became progressively weak after dosing on day 21 and died on the following morning before delivery, with 14 ripe fetuses in the uterine horns. Shrinking of spleen and hypertrophy of adrenals were seen in this rat at autopsy.

No. 357 in 500 mg/kg/day group died after delivery of only one fetus without remarkable signs. Congestion of lungs was seen in this rat at autopsy.

Two (Nos 354 and 356) in 500 mg/kg/day group suffered from fibrinous pneumonia and loss of body weight and died on day 6 after parturition.

e. Conception Rate:

All three treated groups had 100% (20/20) pregnancy, but the control had 95% (19/20--No. 058 in this group did not deliver and had no implantation sites in her uteri).

f. Gestation Length:

Except 4 control and 2 in 500 mg/kg/day group, which delivered on day 23, all others delivered on day 22 of gestation (exactly between 12 noon on day 21 and 12 noon on day 22). See Table 7.

2. LITTER DATA:

a. At Birth:

- i. The number of viable pups per dam was slightly decreased with increased number of dead pups in 500 mg/kg/day group.

ii. Group mean fetal body weight was slightly but significantly ( $p < 0.01-0.05$ ) smaller in 50 mg/kg/day group and 500 mg/kg/day group. See Table below.

b. During the Nursing Period:

i. Mortality:

After reduction of litter size to maximum 8 pups per litter, the mortality was still significantly higher in 500 mg/kg/day group where a total of 23 pups died during the first week. All pups died in 6 litters (No. 167 in 5 mg/kg/day group, No. 254 in 50 mg/kg/day group, No.s 354, 359, 364, 365 in 500 mg/kg/day group), and they were eaten by their mothers (missed the next morning).

Table 5

Group Mean Number of Pups  
Sch 1000-BR Rat p.o. Seg. III

|           |        | day 0     |           | viable pups after selection |           |            |           | viability     |
|-----------|--------|-----------|-----------|-----------------------------|-----------|------------|-----------|---------------|
|           |        | viabiles  | deads     | day 0                       | day 7     | day 14     | day 21    | at day 21 (%) |
| Control   | total  | 110       |           | 73                          | 72        | 71         | 70        |               |
|           | ♂ mean | 5.8 ± 2.4 | 13        | 3.8 ± 0.8                   | 3.8 ± 0.9 | 3.7 ± 0.9  | 3.7 ± 0.9 | 95.9          |
|           | total  | 111       | 0.7 ± 2.3 | 78                          | 78        | 78         | 78        |               |
|           | ♀ mean | 5.8 ± 2.1 |           | 4.1 ± 0.9                   | 4.1 ± 0.9 | 4.1 ± 0.9  | 4.1 ± 0.9 | 100.0         |
| 5 mg/kg   | total  | 124       |           | 78                          | 74        | 72         | 71        |               |
|           | ♂ mean | 6.2 ± 1.6 | 1         | 3.9 ± 0.4                   | 3.7 ± 1.1 | 3.6 ± 1.0  | 3.6 ± 1.1 | 91.0          |
|           | total  | 119       | 0.1 ± 0.2 | 82                          | 76        | 76         | 76        |               |
|           | ♀ mean | 6.0 ± 1.3 |           | 4.1 ± 0.4                   | 3.8 ± 1.1 | 3.8 ± 1.1  | 3.8 ± 1.1 | 92.7**        |
| 50 mg/kg  | total  | 113       |           | 77                          | 73        | 71         | 71        |               |
|           | ♂ mean | 5.9 ± 2.4 | 10        | 4.1 ± 1.0                   | 3.8 ± 1.3 | 3.7 ± 1.4  | 3.7 ± 1.4 | 92.2          |
|           | total  | 94        | 0.5 ± 0.9 | 74                          | 68        | 67         | 67        |               |
|           | ♀ mean | 4.9 ± 2.1 |           | 3.9 ± 1.0                   | 3.6 ± 1.2 | 3.5 ± 1.3  | 3.5 ± 1.3 | 90.5**        |
| 100 mg/kg | total  | 94        |           | 73                          | 57        | 57         | 57        |               |
|           | ♂ mean | 4.9 ± 2.0 | 29        | 3.8 ± 1.0                   | 3.0 ± 1.8 | 3.0 ± 1.89 | 3.0 ± 1.8 | 78.1**        |
|           | total  | 86        | 1.5 ± 2.9 | 69                          | 58        | 58         | 57        |               |
|           | ♀ mean | 4.5 ± 2.0 |           | 3.6 ± 1.2                   | 3.1 ± 1.9 | 3.1 ± 1.9  | 3.0 ± 1.9 | 82.6**        |

Table 6

Group Mean Body Weight of Pups  
Sch 1000-BR Rat p.o. Seg. III

| dose      | sex | day 0       | after selection |              |              |              |
|-----------|-----|-------------|-----------------|--------------|--------------|--------------|
|           |     | day 0       | day 7           | day 14       | day 21       |              |
| Control   | ♂   | 6.2 ± 0.9   | 6.3 ± 1.0       | 16.3 ± 2.4   | 31.6 ± 4.7   | 51.2 ± 6.6   |
|           | ♀   | 5.9 ± 0.7   | 6.0 ± 0.7       | 15.9 ± 2.2   | 30.7 ± 4.1   | 49.5 ± 6.2   |
| 5 mg/kg   | ♂   | 6.2 ± 0.5   | 6.3 ± 0.5       | 16.7 ± 2.1   | 33.1 ± 3.0   | 53.4 ± 5.5   |
|           | ♀   | 6.0 ± 0.6   | 6.0 ± 0.6       | 16.0 ± 2.2   | 30.3 ± 7.9   | 52.0 ± 5.3   |
| 50 mg/kg  | ♂   | 5.9 ± 0.7*  | 5.9 ± 0.7**     | 15.2 ± 2.6** | 30.4 ± 2.8   | 48.9 ± 4.6*  |
|           | ♀   | 5.6 ± 0.7** | 5.5 ± 0.7**     | 13.9 ± 2.1** | 30.1 ± 2.9   | 46.0 ± 4.2** |
| 500 mg/kg | ♂   | 5.8 ± 0.9** | 5.8 ± 0.9**     | 15.2 ± 1.9** | 27.3 ± 3.1** | 45.1 ± 5.2** |
|           | ♀   | 5.6 ± 0.8*  | 5.6 ± 0.8**     | 13.9 ± 1.9** | 27.3 ± 3.1** | 43.2 ± 5.1** |

ii. General Observations:

- a) One control fetus and one fetus in 50 mg/kg/day group had red incrustated eyes, without any other abnormal signs.
- b) 15 M + 16 F fetuses in 500 mg/kg/day group had red incrustated eyes, either unilateral or bilateral, that was observed after opening of eyelids and gradually disappeared until weaning. Two among these 31 pups suffered from a mild purulent inflammation (histologically confirmed) of lacrimal glands (see photo 1).

iii. Body Weight:

Body weight gain was slightly but significantly ( $p < 0.01 - 0.05$ ) retarded in both sexes in 50 and 500 mg/kg/day groups, with a trend of dose-relationship. See Fig. 3 on page 26.

iv. Setting up of Pinnae and Opening of Eyes:

Setting up of pinnae of 3 treated groups was slightly retarded, but without dose-relationship, but opening of eyes of 3 treated groups was slightly but significantly ( $p < 0.01-0.05$ ) retarded with a dose-relationship, as shown in Fig. 4 below and Table 9 on pages 61-62.

v. Swimming Test:

No significant differences between groups were shown in development of swimming ability. See Fig. 5a on page 28 and Table 10.

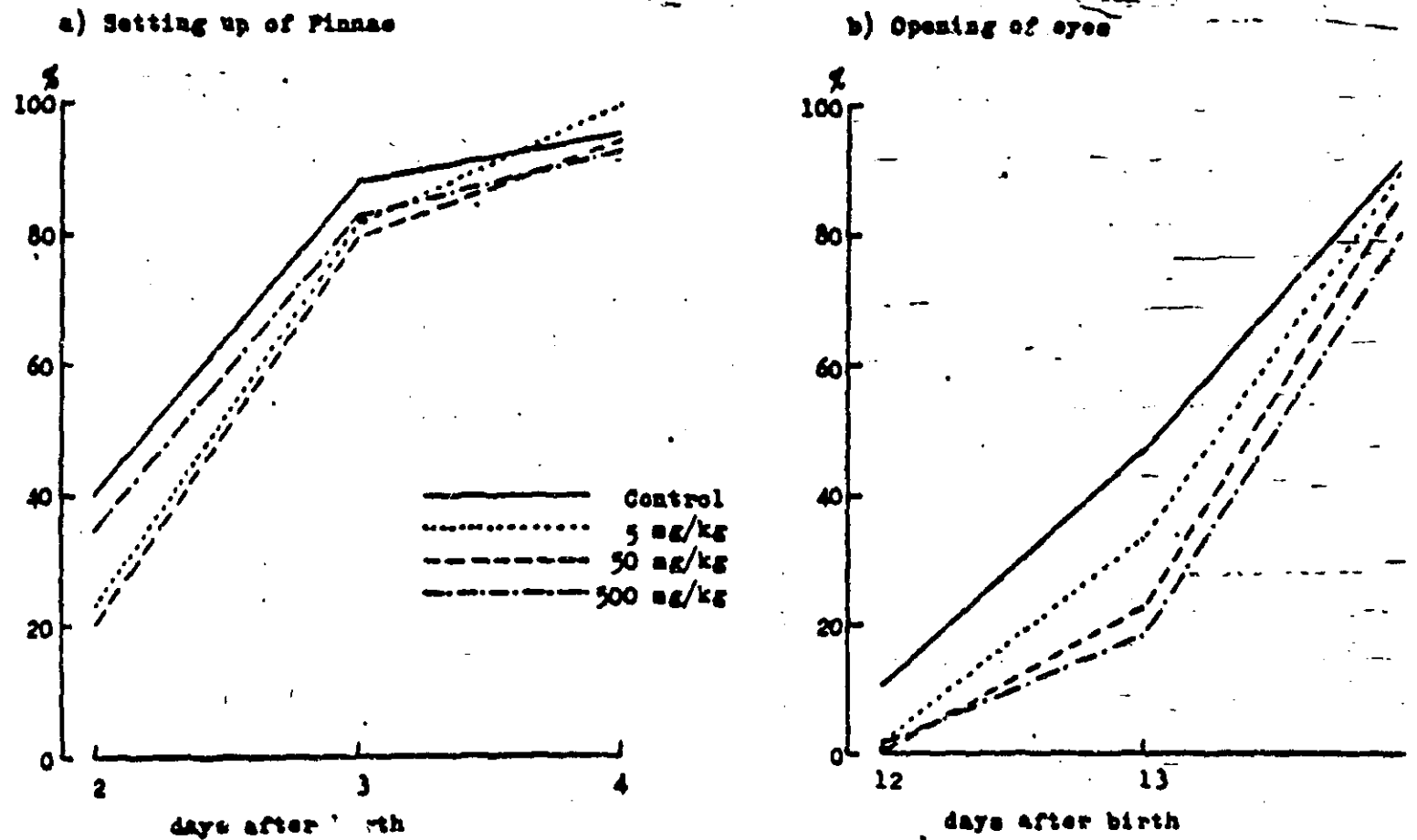
vi. Open-Field Test:

Behavior studies in "open-field" as locomotion, rearing, or preening showed no significant differences between groups.

Figure 4

Development of Pinnac and Eyes of the Pups

Seb 1000-BE Rat p.o. Seg. III



vii. Variations

The following skeletal variations were observed on day 21:

| Group n =          | Sternebrae<br>additives (%) | Costae<br>additives | Lumbarisatio<br>vertebrae<br>sacralis | Total (%) |
|--------------------|-----------------------------|---------------------|---------------------------------------|-----------|
| D <sub>0</sub> 148 | 8 (5.4)                     | 4 (2.7)             | 3 (2.0)                               | 12 (8.1)  |
| D <sub>1</sub> 147 | 15 (10.2)                   | 1 (0.7)             | 3 (2.0)                               | 15 (10.2) |
| D <sub>2</sub> 138 | 2 (1.4)                     | 0                   | 3 (2.2)                               | 3 (2.2)   |
| D <sub>3</sub> 114 | 0                           | 1 (0.9)             | 7 (6.1)                               | 2 (1.8)   |

viii. Malformations

The following abnormalities were reported:

|           |                          |                                          |
|-----------|--------------------------|------------------------------------------|
| Control   | No. 057-X <sub>1</sub> ♀ | Hydrocephalus internus                   |
|           | 057-X <sub>2</sub> ♀     | Aplasia costae dextrae XIII              |
|           | 063-X <sub>1</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>2</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>1</sub> ♀     | Hydrocephalus internus                   |
|           | 066-X <sub>1</sub> ♂     | Hypoplasia testis dextri                 |
| 5 mg/kg   | N.A.D.                   |                                          |
| 50 mg/kg  | N.A.D.                   |                                          |
| 500 mg/kg | No. 353-X <sub>1</sub> ♀ | pes varus extremitatis anterior sinister |
|           | 360-X <sub>1</sub> ♂     |                                          |
|           | 370-X <sub>1</sub> ♀     | Hydrocephalus internus                   |

By that, the malformation rate at day 21 was as follows:

|                |           |         |
|----------------|-----------|---------|
| D <sub>0</sub> | control   | : 4.7 % |
| D <sub>1</sub> | 5 mg/kg   | : 0.0 % |
| D <sub>2</sub> | 50 mg/kg  | : 1.4 % |
| D <sub>3</sub> | 500 mg/kg | : 2.6 % |

3. REPRODUCTION CAPACITY OF F<sub>1</sub> OFFSPRINGS:

a. Clinical Signs and Mortality:

No abnormal signs or mortality were observed.

b. Body Weight:

Body weight gain was slightly but significantly ( $p < 0.01-0.05$ ) retarded in F of 50 mg/kg/day group and M + F of 500 mg/kg/day group from week 3 through week 11 after weaning as shown in Table 12 below: See also Table 6 above.

|           |                          |                                          |
|-----------|--------------------------|------------------------------------------|
| Control   | No. 057-X <sub>1</sub> ♀ | Hydrocephalus internus                   |
|           | 057-X <sub>2</sub> ♀     | Aplasia costae dextrae XIII              |
|           | 063-X <sub>1</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>2</sub> ♂     | Hydrocephalus internus                   |
|           | 063-X <sub>1</sub> ♀     | Hydrocephalus internus                   |
|           | 066-X <sub>1</sub> ♂     | Hypoplasia testis dextri                 |
| 5 mg/kg   | N.A.D.                   |                                          |
| 50 mg/kg  | N.A.D.                   |                                          |
| 500 mg/kg | No. 353-X <sub>1</sub> ♀ | pes varus extremitatis anterior sinister |
|           | 360-X <sub>1</sub> ♂     |                                          |
|           | 370-X <sub>1</sub> ♀     | Hydrocephalus internus                   |

By that, the malformation rate at day 21 was as follows:

|                |           |         |
|----------------|-----------|---------|
| D <sub>0</sub> | control   | : 4.7 % |
| D <sub>1</sub> | 5 mg/kg   | : 0.0 % |
| D <sub>2</sub> | 50 mg/kg  | : 1.4 % |
| D <sub>3</sub> | 500 mg/kg | : 2.6 % |

3. REPRODUCTION CAPACITY OF F<sub>1</sub> OFFSPRINGS:

a. Clinical Signs and Mortality:

No abnormal signs or mortality were observed.

b. Body Weight:

Body weight gain was slightly but significantly ( $p < 0.01-0.05$ ) retarded in F of 50 mg/kg/day group and M + F of 500 mg/kg/day group from week 3 through week 11 after weaning as shown in Table 12 below: See also Table 6 above.

Table 12. Mean Body Weight Development of the Offspring (F1) after Weaning

Sch 1000-BR Est r. s. Rec. III

mean ± S.D.

| Dose      | Sex | n  | Age of week                |                            |                              |                              |                              |                              |                              |                             |                             |
|-----------|-----|----|----------------------------|----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-----------------------------|-----------------------------|
|           |     |    | 3                          | 4                          | 5                            | 6                            | 7                            | 8                            | 9                            | 10                          | 11                          |
| Control   | ♂   | 19 | 50.7<br>±8.2               | 91.0<br>±11.4              | 145.1<br>±15.1               | 205.3<br>±19.0               | 262.3<br>±26.2               | 317.6<br>±30.7               | 359.5<br>±33.9               | 396.3<br>±36.9              | 424.8<br>±40.8              |
|           | ♀   | 19 | 51.0<br>±5.9               | 87.9<br>±8.3               | 130.8<br>±10.1               | 165.1<br>±11.8               | 187.4<br>±11.8               | 207.7<br>±12.0               | 225.6<br>±12.4               | 238.9<br>±12.8              | 252.0<br>±13.6              |
| 5 mg/kg   | ♂   | 19 | 54.1<br>±6.7               | 94.5<br>±7.2               | 149.1<br>±11.4               | 211.5<br>±17.8               | 269.7<br>±23.5               | 324.9<br>±25.5               | 367.1<br>±29.6               | 405.2<br>±28.5              | 435.1<br>±34.3              |
|           | ♀   | 19 | 52.2<br>±6.3               | 89.0<br>±5.7               | 131.5<br>±8.1                | 168.9<br>±10.7               | 193.6<br>±13.2               | 217.4<br>±15.9               | 237.0<br>±18.8               | 252.2<br>±19.1              | 266.3<br>±21.4              |
| 50 mg/kg  | ♂   | 18 | 49.7<br>±4.4               | 89.3<br>±6.6               | 143.9<br>±11.7               | 211.6<br>±20.7               | 265.6<br>±21.1               | 319.2<br>±26.2               | 362.1<br>±30.5               | 396.2<br>±34.2              | 425.9<br>±37.2              |
|           | ♀   | 18 | 45.4<br>±5.1               | 78.7 <sup>**</sup><br>±7.5 | 120.1 <sup>**</sup><br>±8.7  | 152.1 <sup>**</sup><br>±9.5  | 175.5 <sup>**</sup><br>±11.7 | 195.2 <sup>**</sup><br>±12.0 | 213.9 <sup>*</sup><br>±14.7  | 227.0 <sup>*</sup><br>±17.7 | 239.3 <sup>*</sup><br>±18.4 |
| 500 mg/kg | ♂   | 14 | 47.5<br>±5.6               | 83.2 <sup>*</sup><br>±7.6  | 135.3<br>±20.7               | 185.9 <sup>*</sup><br>±21.4  | 235.4 <sup>**</sup><br>±26.8 | 282.3 <sup>**</sup><br>±33.4 | 332.1 <sup>*</sup><br>±33.6  | 369.7<br>±38.3              | 394.4 <sup>*</sup><br>±37.9 |
|           | ♀   | 14 | 44.2 <sup>**</sup><br>±5.2 | 76.2 <sup>**</sup><br>±7.8 | 112.1 <sup>**</sup><br>±12.7 | 145.7 <sup>**</sup><br>±13.7 | 170.2 <sup>**</sup><br>±15.7 | 192.1 <sup>**</sup><br>±16.7 | 207.9 <sup>**</sup><br>±18.4 | 224.2 <sup>*</sup><br>±18.4 | 237.4 <sup>*</sup><br>±19.5 |

c. Copulation Rate and Pregnancy Rate:

Copulation rate of the control group was 100%, but 3 treated groups had 94-95%. However, 2 females in control group and one in 5 mg/kg/day group were not pregnant as shown on the Table 16 below:

Table 16 Group Fertility Data  
Sch 1000-8R Seg. III - F<sub>1</sub>

|                                           | Control   | 5 mg/kg   | 50 mg/kg  | 500 mg/kg |
|-------------------------------------------|-----------|-----------|-----------|-----------|
| Couples for mating                        | 19        | 19        | 18        | 17        |
| Females not inseminated                   | 0         | 1         | 1         | 1         |
| Couples copulated<br>(copulation rate)    | 19 (100%) | 18 (95 %) | 17 (94 %) | 15 (94 %) |
| at 1 <sup>st</sup> estrus<br>in 5 days    | 14        | 16        | 16        | 13        |
| at 1 <sup>st</sup> estrus<br>after 5 days | 2         | 0         | 0         | 0         |
| at 2 <sup>nd</sup> estrus                 | 1         | 0         | 0         | 0         |
| at estrus after<br>pseud-pregnancy        | 1         | 2         | 0         | 2         |
| at diestrus                               | 1         | 0         | 0         | 0         |
| sperm-negative<br>but pregnant            | 0         | 0         | 1         | 1         |
| non-pregnant                              | 2         | 1         | 0         | 0         |
| pregnant<br>(conception rate)             | 17 (89 %) | 17 (94 %) | 16 (94 %) | 15 (94 %) |

At autopsy, three non-pregnant females did not show any abnormal findings.

d. Final Tests on F<sub>1</sub> Offsprings (Autopsy):

Males were killed and necropsied after copulation; and females, at Caesarean section. One control M(No. 059) had atrophy of seminiferal tubule in one testis, and one F in 500 mg/kg/day group (No. 351), bilateral hydronephrosis; and one in 50 mg/kg/day group, multiple anemic splenic infarcts.

e. Corpora Lutea:

The number of corpora lutea per dam was decreased significantly ( $p < 0.01$ ) in 500 mg/kg/day group. See Table for group mean litter data below.

f. Implantation:

The number of implantation sites per dam was also significantly ( $p < 0.01$ ) decreased in 500 mg/kg/day group.

g. LITTER DATA:

A total of 784 fetuses (F<sub>2</sub>) was produced and examined as in the following Table:

|                | Group<br>(mg/kg) | living<br>fetuses | ♂   | ♀   | skeletal<br>exam. | visceral<br>exam. |
|----------------|------------------|-------------------|-----|-----|-------------------|-------------------|
| D <sub>0</sub> | Control          | 230               | 100 | 130 | 154               | 79                |
| D <sub>1</sub> | 5                | 203               | 92  | 111 | 135               | 68                |
| D <sub>2</sub> | 50               | 197               | 103 | 94  | 132               | 65                |
| D <sub>3</sub> | 500              | 154               | 88  | 66  | 102               | 52                |
| total:         |                  | 784               | 383 | 401 | 520               | 264               |

h. Group Mean Litter Data of F<sub>2</sub> generation:

Table 17 Group Mean Litter Data  
Sch 1000-BR Rat p.o. Seg. III - F<sub>2</sub>

|                                        | Control   | 5 mg/kg   | 50 mg/kg  | 500 mg/kg |
|----------------------------------------|-----------|-----------|-----------|-----------|
| No. of litters                         | 17        | 17        | 16        | 13        |
| Total no. Corpora lutea                | 270       | 262       | 242       | 174       |
| Avg. no. Corpora lutea                 | 15.9±2.1  | 15.4±2.2  | 15.1±2.0  | 13.4±2.5  |
| Range no. Corpora lutea                | 13-21     | 12-20     | 12-19     | 8-19      |
| Total no. implantation sites           | 248       | 249       | 224       | 164       |
| Avg. no. implantation sites            | 14.6±1.4  | 14.6±2.3  | 14.0±2.2  | 12.6±1.9  |
| Range no. implantation sites           | 12-17     | 10-20     | 10-18     | 8-16      |
| Ratio (implantations/Corpora lutea) %  | 91.9      | 95.0      | 92.6      | 94.3      |
| Total no. resorptions                  | 18        | 38        | 26        | 9         |
| Early                                  | 14        | 36        | 17        | 9         |
| Late                                   | 4         | 2         | 9         | 0         |
| Avg. no. resorptions                   | 1.1±1.6   | 2.2±2.4   | 1.6±1.7   | 0.7±0.8   |
| Range no. resorptions                  | 0-6       | 0-7       | 0-5       | 0-2       |
| Ratio (resorptions/implantations) %    | 7.3       | 15.3      | 11.6      | 5.5       |
| Total no. dead fetuses                 | 0         | 0         | 1         | 1         |
| Avg. no. dead fetuses                  | 0         | 0         | 0.1±0.3   | 0.1±0.3   |
| Range no. dead fetuses                 | 0-0       | 0-0       | 0-1       | 0-1       |
| Total no. viable fetuses               | 230       | 203       | 197       | 154       |
| Avg. no. viable fetuses                | 13.5±2.3  | 11.9±3.9  | 12.3±3.1  | 11.8±2.2  |
| Range no. viable fetuses               | 8-18      | 4-20      | 6-17      | 6-14      |
| Sex ratio (male:female)                | 100:130   | 92:111    | 103:94    | 88:66     |
| Ratio (viable fetuses/implantations) % | 92.7      | 81.5      | 87.9      | 93.9      |
| Avg. weight of viable fetuses (gms)    | 5.39±0.50 | 5.42±0.47 | 5.33±0.55 | 5.48±0.45 |
| Range weight of viable fetuses (gms)   | 3.7-8.2   | 3.9-6.3   | 3.7-6.8   | 4.2-6.5   |
| No. variations                         | 58        | 56        | 37        | 40        |
| Variation rate (%)                     | 38.4      | 41.5      | 28.0      | 39.2      |
| No. malformations (skeletal)           | 3         | 3         | 9         | 2         |
| Malformation rate (%)                  | 1.3       | 1.5       | 4.6       | 1.3       |

i. Dead Fetuses:

Two dead fetuses were reported;  
One in 50 mg/kg/day group and one in 500 mg/kg/day group.

h. Group Mean Litter Data of F<sub>2</sub> generation:

Table 17 Group Mean Litter Data  
Sch 1000-BR Rat p.c. Seg. III - F<sub>2</sub>

|                                        | Control   | 5 mg/kg   | 50 mg/kg  | 500 mg/kg |
|----------------------------------------|-----------|-----------|-----------|-----------|
| No. of litters                         | 17        | 17        | 16        | 13        |
| Total no. Corpora lutea                | 270       | 262       | 242       | 174       |
| Avg. no. Corpora lutea                 | 15.9±2.1  | 15.4±2.2  | 15.1±2.0  | 13.4±2.5  |
| Range no. Corpora lutea                | 13-21     | 12-20     | 12-19     | 8-19      |
| Total no. implantation sites           | 248       | 249       | 224       | 164       |
| Avg. no. implantation sites            | 14.6±1.4  | 14.6±2.3  | 14.0±2.2  | 12.6±1.9  |
| Range no. implantation sites           | 12-17     | 10-20     | 10-18     | 8-16      |
| Ratio (implantations/Corpora lutea) %  | 91.9      | 95.0      | 92.6      | 94.3      |
| Total no. resorptions                  | 18        | 38        | 26        | 9         |
| Early                                  | 14        | 36        | 17        | 9         |
| Late                                   | 4         | 2         | 9         | 0         |
| Avg. no. resorptions                   | 1.1±1.6   | 2.2±2.4   | 1.6±1.7   | 0.7±0.8   |
| Range no. resorptions                  | 0-6       | 0-7       | 0-5       | 0-2       |
| Ratio (resorptions/implantations) %    | 7.3       | 15.3      | 11.6      | 5.5       |
| Total no. dead fetuses                 | 0         | 0         | 1         | 1         |
| Avg. no. dead fetuses                  | 0         | 0         | 0.1±0.3   | 0.1±0.3   |
| Range no. dead fetuses                 | 0-0       | 0-0       | 0-1       | 0-1       |
| Total no. viable fetuses               | 230       | 203       | 197       | 154       |
| Avg. no. viable fetuses                | 13.5±2.3  | 11.9±3.9  | 12.3±3.1  | 11.8±2.2  |
| Range no. viable fetuses               | 8-18      | 4-20      | 6-17      | 6-14      |
| Sex ratio (male:female)                | 100:130   | 92:111    | 103:94    | 98:66     |
| Ratio (viable fetuses/implantations) % | 92.7      | 81.5      | 87.9      | 93.9      |
| Avg. weight of viable fetuses (gms)    | 5.39±0.50 | 5.42±0.47 | 5.33±0.55 | 5.48±0.45 |
| Range weight of viable fetuses (gms)   | 3.7-8.2   | 3.9-6.3   | 3.7-6.8   | 4.2-6.5   |
| No. variations                         | 38        | 56        | 37        | 40        |
| Variation rate (%)                     | 38.4      | 41.5      | 28.0      | 39.2      |
| No. malformations (skeletal)           | 3         | 3         | 9         | 2         |
| Malformation rate (%)                  | 1.3       | 1.5       | 4.6       | 1.3       |

i. Dead Fetuses:

Two dead fetuses were reported;  
One in 50 mg/kg/day group and one in 500 mg/kg/day group.

j. Viable Fetuses:

The number of viable fetuses per dam was slightly but significantly smaller in 500 mg/kg/day group. The percentage of implantation yielding viable fetuses was significantly smaller in two low dose groups. See Table above.

k. Weight of Fetuses:

The average weight of viable fetuses showed no differences between groups.

l. Skeletal Variations:

The following report was submitted, but it was stated that "the variation rate was within normal range".

Skeletal Variations in F<sub>2</sub> - offspring

| Dose      | n   | Costae<br>additivae<br>(%) | Costae<br>cervicales | Deformatio<br>vertebrae | Lumbalisatio<br>vertebrae<br>sacralis | Sternebra-<br>schisis | Total No. of<br>fetuses with<br>variations |
|-----------|-----|----------------------------|----------------------|-------------------------|---------------------------------------|-----------------------|--------------------------------------------|
| Control   | 151 | 55<br>(36.4)               | 0                    | 3<br>(2.0)              | 2<br>(1.3)                            | 0                     | 58<br>(38.4)                               |
| 5 mg/kg   | 135 | 53<br>(39.3)               | 1<br>(0.7)           | 2<br>(1.5)              | 5<br>(3.7)                            | 0                     | 56<br>(41.5)                               |
| 50 mg/kg  | 132 | 33 <sup>*</sup><br>(25.0)  | 0                    | 4<br>(3.0)              | 1<br>(0.8)                            | 1<br>(0.8)            | 37<br>(28.0)                               |
| 500 mg/kg | 102 | 38<br>(37.3)               | 0                    | 3<br>(2.9)              | 6<br>(5.9)                            | 1<br>(1.0)            | 40<br>(39.2)                               |

Mean Number of ossification centers in F<sub>2</sub> - offspring

|           | Fore phalanx |              | Hind phalanx |              | Caudal<br>vertebrae |
|-----------|--------------|--------------|--------------|--------------|---------------------|
|           | Right        | Left         | Right        | Left         |                     |
| Control   | 11.0<br>±2.0 | 10.8<br>±2.0 | 10.5<br>±2.0 | 10.3<br>±2.0 | 7.2<br>±1.4         |
| 5 mg/kg   | 11.0<br>±2.1 | 10.6<br>±2.2 | 10.4<br>±2.3 | 10.3<br>±2.3 | 7.1<br>±1.3         |
| 50 mg/kg  | 11.7<br>±1.9 | 11.6<br>±1.7 | 11.1<br>±2.4 | 11.1<br>±2.4 | 7.3<br>±1.6         |
| 500 mg/kg | 11.2<br>±1.9 | 10.8<br>±2.1 | 10.6<br>±2.5 | 10.7<br>±2.5 | 7.0<br>±1.1         |

k. Malformations:

The following abnormalities were reported:

Control

051 - X<sub>1</sub>      Synostosis sternebrae III-V  
051 - X<sub>2</sub>      Synostoses sternebrae I-V et deformatio  
                  costae  
051 - X<sub>3</sub>      Synostoses arcuum vertebrarum  
                  thoraciarum dextrarum III-VII et  
                  sinistrarum II-VII, vertebraschisis  
                  thoracii IX et XIII, hemivertebra  
                  thoracii XII et deformatio costae

2.55/kg

154 - X<sub>1</sub>      Vertebraschisis thoracii XII  
161 - X<sub>1</sub>      Synostosis sternebrae III-V  
161 - X<sub>2</sub>      Synostosis sternebrae III-V

50.55/kg

266 - X<sub>1</sub>      Synostosis sternebrae III-V et  
                  deformatio costae  
266 - X<sub>2</sub>      Synostoses sternebrae II, III et V et  
                  deformatio costae  
266 - X<sub>3</sub>      Deformatio costae  
266 - X<sub>4</sub>      Deformatio costae  
267 - X<sub>1</sub>      Vertebraschisis thoracii XII  
267 - X<sub>2</sub>      Synostoses sternebrae III-V  
267 - X<sub>3</sub>      Vertebraschisis thoracii XIII  
267 - X<sub>4</sub>      Vertebraschisis thoracii XIII et  
                  synostosis sternebrae II-V  
267 - X<sub>5</sub>      Vertebraschisis thoracii XIII

500.55/kg

356 - 9        Hernia umbilicalis  
362 - X<sub>1</sub>      Synostosis sternebrae IV et V et  
                  deformatio costae

k. Malformations:

The following abnormalities were reported:

Control

- 051 - X<sub>1</sub> Synostosis sternebrae III-V  
051 - X<sub>2</sub> Synostoses sternebrae I-V et deformatio costae  
051 - X<sub>3</sub> Synostoses arcuum vertebrarum thoracarum dextrarum III-VII et sinistrarum II-VII, vertebraschisis thoracii IX et XIII, hemivertebra thoracii XII et deformatio costae

2 mg/kg

- 154 - X<sub>1</sub> Vertebraschisis thoracii XII  
161 - X<sub>1</sub> Synostosis sternebrae III-V  
161 - X<sub>2</sub> Synostosis sternebrae III-V

30 mg/kg

- 266 - X<sub>1</sub> Synostosis sternebrae III-V et deformatio costae  
266 - X<sub>2</sub> Synostoses sternebrae II, III et V et deformatio costae  
266 - X<sub>3</sub> Deformatio costae  
266 - X<sub>4</sub> Deformatio costae  
267 - X<sub>1</sub> Vertebraschisis thoracii XII  
267 - X<sub>2</sub> Synostoses sternebrae III-V  
267 - X<sub>3</sub> Vertebraschisis thoracii XIII  
267 - X<sub>4</sub> Vertebraschisis thoracii XIII et synostosis sternebrae II-V  
267 - X<sub>5</sub> Vertebraschisis thoracii XIII

500 mg/kg

- 356 - 9 Hernia umbilicalis  
362 - X<sub>1</sub> Synostosis sternebrae IV et V et deformatio costae

In addition to above abnormalities, a few edematous fetuses were noted at Caeserean section, although it was stated that "it was not ascertained whether the skeletal malformations occurred in edematous fetuses or others." See below:

**Control**

051 - 3 Anasarka  
051 - 7 Anasarka

**2 mg/kg**

168 - 6 Edema

**20 mg/kg**

266 - 4 Edema, pes varus extremitatis anterioris  
265 - 5 Edema et pes varus extremitatis anterioris  
266 - 6 Edema et pes varus extremitatis inferioris sinistri  
267 - 7 Edema et pes varus extremitatis anterioris  
266 -12 Edema et pes varus extremitatis anterioris  
266 -14 Edema et pes varus extremitatis anterioris

**200 mg/kg**

N. A. D.

DRUG INTERACTION OF THE 1165a( $\alpha$ -adrenergic agent) and SCH 1000-BR:  
32 DAY INTRAVENOUS TOXICITY STUDY IN BEAGLE DOGS: E83-133:

Lab. Performing the Study: C.H. Boehringer Sohr. Ingelheim an Rhein.

Strain: Pure-bred Beagles, (M about 12 kg,  
F = 12 kg, 10 month old)

Test Substances: Sch 1000-Br, Batch VIII.  
Th 1165a(1-(3,5-dihydroxyphenyl)  
2-[[1-(4-hydroxybenzyl)-ethyl]-  
amino]-ethanol hydrobromide, Batch  
60901

Dosage Levels:

| Group | Dose $\mu\text{g}/\text{kg}$ |          | total | Volume administered<br>ml/kg | Number of animals |     |
|-------|------------------------------|----------|-------|------------------------------|-------------------|-----|
|       | Th 1165a                     | Sch 1000 |       |                              | serial no.        | sex |
| 0     | 0.9% NaCl solution           |          |       | 0.25                         | 001 - 003         | m   |
|       |                              |          |       |                              | 051 - 053         | f   |
| 1     | 3.32                         | 1.32     | 4.64  | 0.25                         | 101 - 103         | m   |
|       |                              |          |       |                              | 151 - 153         | f   |
| 2     | 20                           | 8        | 28    | 0.25                         | 201 - 203         | m   |
|       |                              |          |       |                              | 251 - 253         | f   |
| 3     | 60                           | 24       | 84    | 0.25                         | 301 - 303         | m   |
|       |                              |          |       |                              | 351 - 353         | f   |
| 4     | 60                           | -        | 60    | 0.25                         | 401 - 403         | m   |
|       |                              |          |       |                              | 451 - 453         | f   |
| 5     | -                            | 24       | 24    | 0.25                         | 501 - 503         | m   |
|       |                              |          |       |                              | 551 - 553         | f   |

Duration and Route: Twice daily, 7 days a week for 4 weeks  
(11/11/76-12/13/76), intravenously into  
the antebrachial cephalic vein, while  
animal fasting.

COMMENTS: No rate of infusion was given.

**Reason for the Dosage:** The ratio of the two substances in the combination-tested was 1 part Sch 1000-Br to 2.5 parts Th 1165a, and that is also the ratio intended for man. See page 7 for detailed reasons.

**Mortality:** None.

**Clinical Signs:** After the injection, all in group 3 (highest dose combination) and group 5 (Sch 1000-Br) showed dryness of mucosa in the nose and mouth that lasted for 45-75 min. Two (Nos. 252 and 253) in group 2 (mid dose combination) also had the same symptoms as above.

Dog no. 453 in group 4 (Th 1165a) showed increased lacrimation from the 3rd day, and both eyes had a purulent conjunctivitis on 5th day that subsided in the 4th week. But the increased lacrimation persisted until the end of the study, but no treatment was given.

See Tables 013-015 for other symptoms.

**Body Weight:** There were no significant differences between groups in body weight gains. See Fig. 1 and 2.

**COMMENTS:** No page numbers for the report except the first 3 pages of the summary of the study.

**Food Intake:** There were no differences between groups except M in group 5 had slightly smaller consumption.

**Hematology:** No dose-related or time related significant changes were reported.

**Coagulation Time:** No significant differences between groups were reported for thrombin time and partial thrombin time.

**Clinical Chemistry:** LDH in groups 3 and 4 was highest at weeks 2 and 4, then followed by group 5 that was also higher than respective control values.

HCDH ( $\alpha$ -hydroxybutyrate dehydrogenase) in weeks 2 and 4 was dose-relatedly increased in groups 1, 2, and 3, and that in group 4 was also higher than respective control group at week 4 (control value was smallest).

COMMENTS: It was stated that "no important shift in the  $\alpha$ -HBDH/LDH ratio was found."

CK in groups 1, 2, and 3 showed dose-related increase in week 2 and 4, and that in group 4 was highest that was followed by group 5 on both weeks 2 and 4.

CK Isoenzymes: It was reported that "slight increase in this enzyme consisted mainly of a rise in the muscle-specific CK MM band, not the cardiospecific isoenzyme CK-MB".

Cholesterol in groups 3 and 4 was slightly higher than control group in weeks 2 and 4.

AP in groups 1, 2, and 3 was higher than respective control values in weeks 2 and 4, but without dose-relationship, and that in group 4 was highest in both times.

K<sup>+</sup> in groups 2, 3, and 4 was slightly higher than control values in weeks 2 and 4.

COMMENTS: There were some animals with unusually high values in the report submitted.

Urinalysis: No significant differences between groups were reported.

Organ Weight: There were no dose-related changes in absolute weight of selected organs, although kidneys and thyroid of all treated groups were generally slightly smaller than respective control values.

Weight of testis in group 5 was smaller than control group.

COMMENTS: Many of figures in Table were not legible due to poor copies.

Necropsy Findings: The following report had been submitted:

Group 0 (controls)

Animal No. 001 m Spleen: peripheral haematoma, 0.2 cm in diameter  
Animal No. 002 m Kidneys, bilaterally: slight loss of substance when  
the capsula removed.  
Animal No. 003 m No pathological findings macroscopically.  
Animal No. 051 f Lung: isolated parasitic granulomas in all lobes.  
Animal No. 052 f Injection site: slight haemorrhage.  
Animal No. 053 f Lung: isolated parasitic granulomas in all the lobes.

Group 1:

Animal No. 101 m Liver: a grey-brown elevated calcified focus, 0.5 cm  
in diameter, in the left lateral lobe.  
Animal No. 102 m No pathological findings macroscopically.  
Animal No. 103 m Tonsils: tonsillitis.  
Animal No. 151 f No pathological findings macroscopically.  
Animal No. 152 f Thymus: multiple patchial haemorrhages in the  
parenchyma (probably due to the killing).  
Animal No. 153 f Lung: isolated parasitic granulomas in all the lobes.

Group 2:

Animal No. 201 m Injection site: slightly bloodstained  
Animal No. 202 m Injection site: slightly bloodstained  
Animal No. 203 m Spleen: two small peripheral haematomas.  
Animal No. 251 f No pathological findings macroscopically.  
Animal No. 252 f No pathological findings macroscopically.  
Animal No. 253 f Lung: a few parasitic granulomas in all lobes.  
Spleen: a small peripheral haematoma.  
Liver: clearly outlined lobules.

Group 3:

Animal No. 301 m No pathological findings on macroscopic examination.  
Animal No. 302 m Lung: isolated parasitic granulomas with peripheral  
emphysema in all the lobes; in the right  
diaphragmatic lobe there was a focus of  
pneumonia, 0.5 cm in diameter.  
Animal No. 303 m No pathological findings on macroscopic examination.  
Animal No. 351 f No pathological findings on macroscopic examination.  
Animal No. 352 f No pathological findings on macroscopic examination.  
Animal No. 353 f Lung: isolated parasitic granulomas in all the lobes.

Group 4 (Th 1165a)

|                |   |                                                                                                                                                                                                                            |
|----------------|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Animal No. 401 | m | No pathological findings on macroscopic examination.                                                                                                                                                                       |
| Animal No. 402 | m | Liver: very marked gray-yellow outlining of lobules,<br><del>fatty liver suspected.</del><br>Spleen: two small peripheral haematomas, and one raised<br>focus, 0.3 cm in diameter, in the natural<br>colour of the spleen. |
| Animal No. 403 | m | Lung: isolated parasitic granulomas in all lobes.                                                                                                                                                                          |
| Animal No. 451 | f | No pathological findings on macroscopic examination.                                                                                                                                                                       |
| Animal No. 452 | f | Lung: isolated parasitic granulomas in all lobes,<br>mild peripheral emphysema, probably due to the<br>killing.                                                                                                            |
| Animal No. 453 | f | No pathological findings on macroscopic examination.                                                                                                                                                                       |

Group 5 (Sch 1000-BR)

|                |   |                                                                                              |
|----------------|---|----------------------------------------------------------------------------------------------|
| Animal No. 501 | m | Lung: isolated parasitic granulomas in all lobes.                                            |
| Animal No. 502 | m | No pathological findings on macroscopic examination.                                         |
| Animal No. 503 | m | Thyroid, right: aplasia.<br>Muscles in nape of neck: gray-white discolouration in<br>places. |
| Animal No. 551 | f | Thyroid, right: bloodstained, due to killing.                                                |
| Animal No. 552 | f | No pathological findings on macroscopic examination.                                         |
| Animal No. 553 | f | Lung: isolated parasitic granulomas in all lobes.                                            |

Histopathology:

Histopathological examinations of beagles  
(excluding eyes) were carried out by Herr Prof.  
Dr. med. vet. J. von Sandersleben at Institute  
of Animal Pathology of the University of Munich,  
and the following summary of his findings was  
submitted, in addition to detailed report on  
each dog.

"When the findings from the controls are first compared with the findings from the animals in the groups given Th 1165a or Sch 1000 alone, the following are the results:

- 1) With Sch 1000 alone (Group 5) there were no indications of substance-related changes.
- 2) With Th 1165a alone (Group 4) one animal (402) showed a marked centrolobular or intermediate fatty change in the liver cells; in another (403) only isolated groups of cells showed fatty change. It is at least doubtful whether this finding was due to the substance.

In the animals in the highest group of the combination (Group 3), which received the same amount of Th 1165, the fatty change in the liver was no more severe. I have seen slight degrees of fatty change of individual liver cells or small groups of liver cells (as in one case each from Groups 1 - 4) in controls in other study series. It is also questionable whether the vacuolar/hydropic degeneration of the convoluted renal tubules in one case in Group 4 (403) was connected with the administration of the substance. Here again, this finding does not appear at the highest dosage in the combination. Out of 12 animals which received the highest dosage of Th 1165a alone or combination the renal abnormality appeared in only one. A connection with the substance therefore seems highly improbable. When the findings in the animals which were given the substances in combination are compared with those of the controls or of the animals given Th 1165 or Sch 1000 alone, the following statement can be made:

- 1) In the highest group of the combination (Group 3) there were some very conspicuous scars in the left papillary muscle in one dog (202). This finding should be interpreted as being due to the substance. However, it must be emphasized that it appeared in only one out of six animals. The inconspicuous small flecks of fatty change in a few myocardial fibres, seen in two animals in Group 1 and in one in Group 2, was certainly due to chance. The glycogen content in the livers of the animals in the combination group 3 seems to be somewhat lower on average than in all the other groups. This was probably due to the experiment.

2) The only other findings to be discussed are those in the testes of some males. I have seen relatively sharply outlined areas of atrophy of the germinal epithelium in the testes, as in one animal in Group 4 and 5, in controls in other study series. The confirmation that this change is a spontaneous pathological process comes from the fact that it did not occur in the highest group of the combination (Group 3). The other findings in the testes of the animals in Groups 1 and 2 should also be regarded as chance findings, as they show no dose-response relationship.

In none of the groups were there any changes at the injection sites which were not also present in the controls. Quantitatively also there were no differences. All the other findings in the dogs which are not discussed in detail here are caused, in my opinion, by spontaneous pathology.

To summarise, it can be said that the myocardial scars found in one dog in highest combination group were due to the substance. The slight glycogen depletion of the liver also points in this direction. It seems at least improbable that the severe fatty change in the liver in one case from Group 4 (Th 1165 alone) and one case of vacuolar-hydropic degeneration of the tubules of the renal cortex in the same group were due to the substance. »

Long Term Pharmacology: Electrocardiogram:

Heart Rate:

a. First Day of the Study:

There were no differences between groups before the dosing. One min after the morning dosing, the average heart rate of group 1 was markedly increased, and that in groups 2 and 3 was highly significantly ( $p < 0.01$ ) increased over the control value. One hour later, group 1 returned to control value, but groups 2 and 3 still had highly significantly increased heart rates.

Before the second dosing in the afternoon and 6 hours after the morning dose (above), there were no differences between groups in the average heart rates. But one min after the second dosing, all 3 groups (1,2,3) showed highly significantly ( $p < 0.01$ ) increased heart rates over the control value. One hour later, the heart rate of group 1 returned to control value, but that in groups 2 and 3 was still very significantly higher than control value. 18 hours later, the heart rate of all 3 groups was very similar to control group.

b. 2nd Week of the Study:

Before the dosing, all groups had similar heart rates. But one min after the 1st dosing in the morning, the heart rate of groups 1, 2, and 3 was very significantly ( $p < 0.01$ ) higher than that of control group. One hour later, the heart rate of group 1 was still slightly elevated; that of group 2, significantly so ( $p < 0.05$ ); and that of group 3 highly significantly ( $p < 0.01$ ) so.

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Before the afternoon dosing, all groups had similar heart rates to control group. The results of one min after the second dosing were the same as the first dosing, as reported above, so were the results of one hour and 18 hour after the dosing.

a. First Day of the Study:

There were no differences between groups before the dosing. One min after the morning dosing, the average heart rate of group 1 was markedly increased, and that in groups 2 and 3 was highly significantly ( $p < 0.01$ ) increased over the control value. One hour later, group 1 returned to control value, but groups 2 and 3 still had highly significantly increased heart rates.

Before the second dosing in the afternoon and 6 hours after the morning dose (above), there were no differences between groups in the average heart rates. But one min after the second dosing, all 3 groups (1,2,3) showed highly significantly ( $p < 0.01$ ) increased heart rates over the control value. One hour later, the heart rate of group 1 returned to control value, but that in groups 2 and 3 was still very significantly higher than control value. 18 hours later, the heart rate of all 3 groups was very similar to control group.

b. 2nd Week of the Study:

Before the dosing, all groups had similar heart rates. But one min after the 1st dosing in the morning, the heart rate of groups 1, 2, and 3 was very significantly ( $p < 0.01$ ) higher than that of control group. One hour later, the heart rate of group 1 was still slightly elevated; that of group 2, significantly so ( $p < 0.05$ ); and that of group 3 highly significantly ( $p < 0.01$ ) so.

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Before the afternoon dosing, all groups had similar heart rates to control group. The results of one min after the second dosing were the same as the first dosing, as reported above, so were the results of one hour and 18 hour after the dosing.

**COMMENTS:** Nothing was reported for groups 4 and 5.

c. 4th Week of the Study:

The results of 1st and the 2nd dosing were exactly the same as the 2nd week's results described above.

PQ, QRS, AND QT INTERVALS:

It was reported that the group means of EKG variables were within normal limits for all groups.

ECG Tracings:

It was reported that drug treatment did not produce any pathological ECG tracings.

Ophthalmological Findings:

A complete reports on ophthalmoscopic examination and histological examination of the eyes of all dogs have been submitted, but no drug related lesions were found.

EVALUATION\*

Pharmacology:

Atrovent is a synthetic quaternary ammonium compound derived by quaternization of the atropine molecule. Atrovent has anticholinergic activity quite similar to atropine but the precise subcellular mechanism of action as a bronchospasmolytic agent has not been completely elucidated. The compound acts in part by vagal inhibition, preventing reflex bronchoconstriction, and in part by a local effect on the bronchus.

The anticholinergic and spasmolytic activities of atrovent have been demonstrated in numerous animal screening models and comparisons made with other anticholinergic and spasmolytic agents. The drug has greater antispasmodic efficacy than atropine in isolated organ bath preparations. Parenterally, it is more effective than atropine in its ability to inhibit gastric secretions but less effective on salivary secretion. Following enteral administration, the drug was less effective than atropine as antisecretory and antispasmodic agents. When used as an aerosol, the compound appeared to adhere to the oral and pharyngeal mucosa and was then swallowed. When used as a broncholytic agent by aerosol administration, it was more effective than atropine without producing the atropine-like side effects such as the antisialogogue effect. The ED<sub>50</sub> was approximately 25 micrograms per animal for the bronchospasmolytic effect whereas the ED<sub>50</sub> for the inhibition of salivary secretion was about 100 times higher (2 to 3 mg per animal).

In studies with anesthetized beagles using inhalations of 100 to 200 puffs of 40 mcg, there were no significant changes in heart rate or any other pharmacological changes in the EKG.

The drug is relatively free of significant central nervous system activity.

Pharmacokinetics:

The biochemical and pharmacokinetic parameters with Atrovent have been investigated and include the determination of absorption, distribution, excretion, and metabolism in the rat, dog, monkey, and man. The drug pharmacokinetically is a quaternary ammonium compound. The pharmacokinetics of the drug are biphasic.

\*This evaluation has utilized extensively the wording as found in the summary section for preclinical studies. Appropriate modifications have been made where the reviewer differed regarding interpretation of the data.

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\*This evaluation has utilized extensively the wording as found in the summary section for preclinical studies. Appropriate modifications have been made where the reviewer differed regarding interpretation of the data.

After parenteral administration in the rat, the compound is rapidly absorbed and has a disposition half-life of about 5 minutes. The second phase has a half-life of 4 hours. Approximately 60% of the drug is excreted in the urine within the first 24 hours and an additional 20% in the feces. That part of the drug excreted in the feces is believed to be the result of the drug reaching the intestinal lumen through direct secretion through the intestinal wall. Tissue distribution studies verify a high level of the drug in the small and large intestines during the first 6-12 hours after administration. After intravenous administration, approximately 40% of the unchanged active drug is recovered in the urine. After oral administration, a major part of the administered dose (70%) is accounted for in fecal excretion. The drug did not penetrate the blood/brain barrier, either following intravenous or oral administration. By the oral route, approximately 26% of the unchanged drug was recovered in the urine the first 8 hours after drug administration.

In man, the comparative pharmacokinetic studies after the inhalation and oral routes indicated that the curves were comparable except for the low peak plasma levels following inhalation. However, the half-life for elimination of the drug from plasma after oral administration was within the same range as the half-life after inhalation. The drug was found to be present in plasma within 2 minutes following inhalation. The peak values for renal excretion of 2-3 hours were comparable for the oral and inhalation routes. The similarity between the absorption-distribution patterns following oral administration and inhalation suggests the inhaled drug is being swallowed. Low blood level and the pharmacodynamic effects do not correlate because bronchodilation occurs despite the much lower blood levels after inhalation. The bronchodilating effect is probably due to a local effect in the lung as well as particulate distribution over the oral cavity resulting in buccal absorption.

Fecal excretion, both by oral administration and inhalation, account for the majority of the drug.

Parenterally, an important excretion route was bile.

Comparative determinations of excretory patterns following initial dosage and that obtained after 13 or 26 weeks of subchronic or chronic administration in rats and monkeys, indicated that there was little difference between the urinary excretion and plasma level when compared to the values obtained pre-exposure.

The drug shows no evidence of accumulating in the body. There do not appear to be major species differences in metabolism, since the principal excretory product is unchanged drug.

Toxicology and Pathology:

The safety evaluation studies conducted with Atrovent in a variety of animal species have shown that the drug has a relatively wide margin of safety and compares favorably with other anticholinergic or bronchospasmolytic agents.

In acute toxicity studies, the ranges of the LD<sub>50</sub>'s after intravenous or oral administration were 12 to 20 mg/kg and 400 to greater than 4000 mg/kg, respectively. In the case of the inhalation route, monkeys tolerated dosages of 500-1000 micrograms per day for a 7-day period. These dosages, on a microgram/kilogram basis, are several hundredfold the maximum proposed daily administration in man (4.8 mcg/kg). The major signs and symptoms of toxicity were related to the anticholinergic action of the drug. The therapeutic index of Atrovent compares favorably with atropine and is even more favorable by the aerosol inhalation route of administration.

Subchronic toxicity studies ranging from four weeks to three months have been conducted subcutaneously, orally, and by the inhalation route in the rat, dog, rabbit, and monkey. In four-week or one-month oral studies in the rat, rabbit, and dog, dosages in excess of 300 mg/kg produced death. At lower dosages (30-150 mg/kg) anticholinergic effects as evidenced by dryness around the nose, eyes, and oral mucosa, were observed. Dosages in the range of 3-40 mg/kg did not produce any significant signs of anticholinergic effects. Even at lethal dosages of 300-1300 mg/kg, there were no significant pathological findings or clinical laboratory findings that could not be explained by the anticholinergic action of the drug.

Aerosol exposure of rats for 4-6 weeks at dosages up to 750 times the human daily dose produced no toxic effects. In the monkey, exposures up to 6 weeks and dosages up to 800 mcg/day showed no evidence of anticholinergic effects and no effects on electrocardiogram, neurological parameters, or any of the biochemical measurements. There was no evidence of significant changes demonstrated upon macroscopic or histopathological examination. There were also no effects on the ciliary beat rate; however, there was some evidence of improved mucociliary transport.

In a 13-week dog oral study, at dosages of 1.5, 3 and 15 mg/kg, the anticholinergic action of the drug was manifested by inhibition of lacrimation. In the case of the two highest levels, keratoconjunctivitis sicca was present. However, the ophthalmological and histopathological examinations did not show any toxic damage to the eye.

Two, three-month dog aerosol head only exposure studies were conducted. In one study, the dogs were exposed to approximately 400 mcg/day (about 8-9 times the proposed maximum human dose on mcg/kg basis) without any adverse effects being observed. In the second study, the drug was administered as a powdered aerosol, twice daily, at dosages which were equivalent to 7, 36 and 179 mcg/kg. The highest dose was equivalent to about 40 times the maximal human dose.

No significant effects on pulmonary function or neurological examinations were observed. The electrocardiographic results showed a tachycardia at the highest dose level which was present at the time of the 6 and 13 week evaluations. There was also a dryness of the nose and oral mucosa present, which lasted for 4-5 hours in the high dose level animals but only for 1-2 hours in the intermediate dose level. There were no hematological, ophthalmological or biochemical changes observed. The pathological findings, even at the high dose level, were negative.

Chronic toxicity studies by the oral route of 26 and 52 weeks duration were conducted in purebred beagle dogs. Dosage levels were 1.5, 3.0, 15.0 and 75 mg/kg for the 6-month study. For the one-year study, the levels were identical except that the 1.5 mg/kg level was eliminated. There was a slight decrease in body weight gain in both the 3.0 and 15.0 mg/kg dose levels, with a sharp decrease being present at the highest dose level of 75 mg/kg. The weight reduction was also accompanied by a decreased food consumption. All of the levels showed an increased frequency of emesis except the 1.5 mg/kg dose. At all the levels but the 1.5 mg/kg dose level, there was a dose dependent reduction in secretion of the conjunctiva, nasal and oral mucosa. Keratoconjunctivitis sicca was histologically evident at the three highest dose levels; however, there was no damage to the retina. There were no toxic effects on the hematopoietic system, whereas the clinical chemical parameters showed that the high dose animals had a shift in the albumin/globulin ratio towards higher globulin levels. The most prominent finding during the course of both the six-month and one-year studies, was the antisecretory effects related to anticholinergic action of the drug. There was a sporadic atrophy of the spermatogenic epithelium found in the treated animals which was not dose related. It was considered that definite evidence of toxicity was observed at the two highest dosage levels of 15 and 75 mg/kg, particularly in the one-year study.

Three chronic toxicity studies, ranging in duration from 26- to 78-weeks, by dietary admixture, were conducted in rats. The dosages employed were 6, 30 and 150 mg/kg for the 26- and 55-week studies. For the 78-week study, the high dose was 30 mg/kg. The lowest dose level was 0.5 mg/kg, a level which is approximately 100 times the maximum human therapeutic dose (12 whiffs at 0.02 mg/whiff or 4.8 mcg/kg daily). There was a marked reduction in the rate of body weight gain in males at the highest dosage level of 150 mg/kg which was accompanied by a reduction in food and water consumptions. There was a hypersecretion of the Harder's glands beginning at the 6 mg/kg dose level and increasing in severity through the 150 mg/kg dose level. Constipation was evident in both the male and female animals at the 30 and 150 mg/kg dose levels.

The hematological values were within the normal range, whereas there was a reduction in blood urea nitrogen in the 30 and 150 mg/kg groups. Coprostasis was observed at all of the dosage levels except 0.5 mg/kg. This effect, as well as the secondary inflammatory changes observed in the intestine are related to the anticholinergic action of the drug. The extensive histopathological examinations did not show any drug related organ damage at dosages up to 30 mg/kg. Therefore, Atrovent, when administered by dietary admixture to rats for 78 weeks at a dosage of 6 mg/kg of body weight, did not cause any toxicologically significant effects.

Six-month rat and monkey head-only aerosol exposure studies revealed no effects on any of the parameters studied related to the exposure of Atrovent. These included observations for effects on body weight, food consumption, normal surfactant pattern, ophthalmological effects, electrocardiographic effects, hematological, clinical chemistry and urinary parameters, ciliary beat, lung mechanics and blood gases, tracheal mucociliary transport, gross pharmacological signs, and gross and microscopic pathology, including organ weights. In the case of the rat study, the dosages employed were equivalent to 128, 256 and 384 mcg/rat per day. For the monkeys, the dosage levels were equivalent to 200, 800 and 1600 mcg/day. Therefore, Atrovent did not produce any adverse effects at dosages several hundredfold those employed clinically in the six-month aerosol inhalation studies.

Two-year carcinogenicity studies were conducted in rats and mice at dosage levels of 0.5, 2 and 6 mg/kg by dietary admixture. This route of administration was justified by the demonstrated absorption orally of the drug in the rodent within 2-3 hours after oral administration, as well as the greatly exaggerated dose administration in relationship to the bronchospasmolytic dose in man. There were no adverse effects observed on the hematological parameters or in the extensive gross and microscopic examination of the tissues. There were no drug induced non-neoplastic or neoplastic lesions that were different from the spontaneous changes normally found in the strain of rat or mouse utilized. There were also no differences in latency period between the controls and Atrovent treated groups for onset of tumor formation. Therefore, there was no evidence of carcinogenicity found following 2-year administration of Atrovent to rats and mice.

The effect of the drug on fertility and reproductive capacity (Segment I) was investigated following oral administration in rats. By dietary admixture, dosages as high as 90 mg/kg did not adversely affect fertility. Higher dosages of 500 mg/kg produced manifestation of the anticholinergic action of the drug.

Teratological studies of the Segment II type were performed in mice, rats and rabbits at oral dosages as high as 1000 mg/kg. This dose level was found to be toxic to the dams and fetuses. At a dosage of 500 mg/kg, a slight evidence of the anticholinergic activity of the drug, manifested by red, encrusted eyes and nose was observed. The same dosage produced a slight reduction in viable fetal weight. The weight of the pups at birth and weaning was also slightly reduced at the 50 and 500 mg/kg dose levels. However, there were no effects on any of the usual parameters or visceral or skeletal malformations observed. In the case of the rabbits, dosages as high as 125 mg/kg did not produce any embryotoxicity or teratogenic effects. There was evidence of the anticholinergic action of the drug at the dosage level of 125 mg/kg, as evidenced by misshaped feces, which were also sharply reduced in quantity. Therefore, Atrovent is not teratogenic at dosages several thousand times those recommended for clinical use. In addition, rat and rabbit aerosol-exposures at dosages as high as 1.5 mg/kg per day and 1.8 mg/kg per day (nose only), respectively, did not produce any effects on litter size, pup weight, pregnancy, embryonic or fetal development. Furthermore, there were no embryotoxic effects observed.

Peri- and postnatal rat studies (Segment III) were performed at dosages of 5, 10, 30, 50, 90 and 500 mg/kg orally. Dosages of 50 and 500 mg/kg produced a lower body weight gain and lower fetal weights. At 90 mg/kg there was a higher pup loss which was not statistically significant. Both the 50 and 500 mg/kg dosage levels produced a retardation of the growth of the pups up to weeks 11 while at the 500 mg/kg level, the anticholinergic activity and an increase in mortality were observed. The parameters measured in the F<sub>1</sub> offspring were comparable to the control values.

The mutagenic potential of the drug was studied in the Ames test at concentrations of 10-10,000 micrograms per plate, with and without S-9 liver cell activation against *S. typhimurium* and also with the NaDPH generating system and was found to be negative.

In the mouse micronucleus test there was no dose response nor reproducible evidence to demonstrate that the drug was clastogenic, based upon the induction of micronuclei in either polychromatic or normochromatic erythrocytes at dose levels of at least 0.5 x the LD<sub>50</sub>.

There was no evidence that Atrovent given at dosages of 42-421 mg/kg per day produced a mutagenic action in the male mouse dominant lethal assay. The cytogenetic assay in hamsters did not generate any clearcut evidence that there were significant differences between the vehicle control groups and groups dosed with Atrovent.

NDA 19-085  
Page 190

The various techniques that e used to study the antigenic activity of the drug which included botitive and passive anaphylactic assays, did not show any evidence of seization or allergenicity. The drug was not well tolerated when injd repeatedly subcutaneously over a five-day period.

**CONCLUSIONS:**

It is concluded that Atroven reasonably safe for human use by aerosol inhalation as proposed based preclinical data.

**RECOMMENDATION:**

This NDA is considered apprce based on preclinical data.

*Young S. Choi*  
\_\_\_\_\_  
Young S. Choi, Ph.D.  
Pharmacologist

cc:  
NDA 19-085  
HFN-160, HFN-220  
HFN-160/YSChoi:  
R/D init. by JKInscoc:  
doc rm 160  
f/t jb

NDA 19-085  
Page 190

The various techniques that were used to study the antigenic activity of the drug which included both active and passive anaphylactic assays, did not show any evidence of sensitization or allergenicity. The drug was not well tolerated when injected repeatedly subcutaneously over a five-day period.

**CONCLUSIONS:**

It is concluded that Atrovent is reasonably safe for human use by aerosol inhalation as proposed based on preclinical data.

**RECOMMENDATION:**

This NDA is considered approvable based on preclinical data.

*Young S. Choi*  
Young S. Choi, Ph.D.  
Pharmacologist

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HFN-160, HFN-220  
HFN-160/YSChoi:  
R/D init. by JKInscoc:  
doc rm 160  
f/t jb

NDA

19-085

Microbiology

Revs

NDA 19-085

Division of Surgical-Dental Drug Products

Microbiologist's Review No. 2

December 19, 1985

A. 1. NDA: 19-085

Applicant: Boehringer Ingelheim, Ltd.  
90 East Ridge, P.O. Box 368  
Ridgefield, CT 06877

2. Product Name:

Proprietary: Atrovent Inhaler

Non proprietary: Ipratropium bromide

3. Dosage Form and Route of Administration:

Aerosol inhalation. Rx

4. Pharmacological Category and/or Principal Indication:

Anticholinergic bronchodilator used to treat bronchospasm.

B. 1. Initial Submission: July 29, 1983

2. Amendments:

(a) September 12, 1985 - Review No. 1

(b) December 6, 1985 - Subject of Review No. 2

C. Remarks:

1. The amendment addresses deficiencies noted in Microbiologist's initial review and discussed with Ms. Ruth Wasserman in telephone conversations held on December 2 and 6, 1985.

2. The proposed procedures and specifications for microbiological control of product for lot release has been provided. The applicant has agreed to perform these microbial assays on all batches of the subject drug at the time of release.

JAN - 4 1986

NDA 19-085  
Page 2

D. Conclusions:

The application is approvable for microbiological control of the subject drug.

  
Vivian Greenman

cc:

NDA 19-085

HFN-160

HFN-160/Doc Rm

HFN-160/VGreenman:12/19/85

R/D init. by CPHoiberg:12/20/85/PHRussell:12/20/85

f/t deg: 12/20/85 w2206X/D0028

NDA

19-085

MOR

NDA 19-085 Atrovent Inhaler  
Ipratropium Bromide  
Bronchodilator

Boehringer Ingelheim Ltd.  
Submitted: 7/17/85 1/2/81 -  
Reviewed: 8/29/85

1. REVIEW AND EVALUATION OF LABELING

Draft labeling has been submitted as a result of a meeting between representatives of the firm and this Agency held on June 19, 1985. The topic of the meeting was a discussion of labeling for this new NDA.

The submitted, revised draft labeling concerns the sections titled CLINICAL PHARMACOLOGY, INDICATIONS, and USAGE, PRECAUTIONS, and a statement concerning "inclusion of the Rx legend on the insert."

Essentially, the submitted draft labeling reflects the conclusions reached at the noted meeting with one exception, concerning the statement made under the section titled INDICATIONS and USAGE reading "approximately one-half of asthmatic patients have been shown to respond to ATROVENT<sup>R</sup>. (See CLINICAL PHARMACOLOGY)."

RECOMMENDATIONS:

1. The statement, which is the final sentence of the section titled INDICATIONS and USAGE including the words in parenthesis regarding use in asthmatic patients should be deleted from that section of the labeling.
2. Providing 1) above is implemented, the submitted draft labeling should be approved.
3. Adverse experience tables for patients treated in the 90-day controlled clinical trials in support of the Package Inserts: For Atrovent: This tabulation shows that the total number of patients included in the NDA submission was 228 while the number that completed study was 254 - Table I. This table is a breakdown by body systems showing the number and percentage of patients reporting adverse experiences under each system and, where available, the numbers and percentages for each sign/symptom for each body system. This was for both the 228 patients in the NDA submission and those under "Completed Study."

There are no remarkable differences between the latter two groups, i.e. "NDA submission" and "completed study." The highest incidences reported were in order of frequency:

- |                |                             |
|----------------|-----------------------------|
| 1. Cough       | 4. Exacerbation of Symptoms |
| 2. Nausea      | 5. Dizziness                |
| 3. Nervousness | 6. G.I. Distress            |

NOV 22 1985

Table 2 - "Overall Incidence of Adverse Experiences Among Patients Treated with ALUPENT for 90 days:

Number of patients (%)    NDA Submission = 223  
Completed Study = 249

Incidence in order of frequency was:

1. Nervousness
2. Exacerbation of Symptoms
3. G.I. Distress
4. Tremor

There was no appreciable clinical significance between the Atrovent and Alupent treatment groups regarding incidence nor severity of adverse experiences except for more cases of insomnia reported in the treated patients.

4. "A safety updated section comprising a summary of foreign data from clinical trials and spontaneous post marketing reports as well as a summary of the literature available since the NDA submission." The review of unpublished and published reports of adverse reactions associated with the use of Atrovent MDI from foreign data sources is consistent with the adverse reactions seen in clinical trials and the Atrovent compassionate use program in the United States. The analysis of these data shows Atrovent MDI is safe.

5. Foreign Marketing Status

There is a list of 25 countries where Atrovent Inhaler is currently marketed as well as the dates introduced in each, 1974-1984. The amount distributed for this period of time represents approximately 3 million single doses.

Atrovent has also been approved in France and Italy where marketing has not begun. There are no pending registrations, nor have any registrations been refused.

6. Patient information and exclusivity information is given in this section. Most of the information presented in this section is in the submitted NDA. Exclusivity - date first ANDA could be approved and length of exclusivity period is given as five (5) years from date of NDA approval.

Applicable patent numbers and expiration date of each are given as

3,681,500 - August 1, 1989

4,385,048 - May 24, 2000

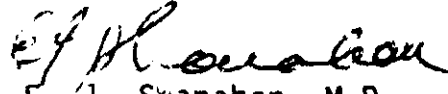
CONCLUSION: Sections 2 through 4 above are in compliance with the agreements reached with the meeting with the representatives of the firm held on June 19, 1985.

Page 3

Addendum dated 11/8/85 to Review of Labeling

Under the section titled CLINICAL PHARMACOLOGY add the following:

In these asthmatic patients who were responders to Atrovent<sup>R</sup> there was no evidence of development of tachyphylaxis.

  
E. J. Shanahan, M.D.

MDA 19-085

HFN-160

HFN-340

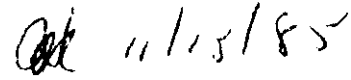
DocRm. 160

HFN-160/JWWinkler 11/1/85: NOTE: Will discuss further in-house, probably OK to leave asthma claim in "Indications" section also.

R.D. EJShanahan 11/8/85

R.D. Init by:JWWinkler 11/8/85:PHRussell 11/8/85

shg W2164K 11/14/85



NDA

19-085

Chemist Revs

DIVISION OF SURGICAL-DENTAL DRUG PRODUCTS  
Chemist's Review #8

A. 1. NDA 19-085

Date Completed: 10/23/85

APPLICANT: Boehringer Ingelheim, Ltd.  
ADDRESS: 90 East Ridge, P.O. Box 368  
Ridgefield, CT 06877

2. PRODUCT NAME(s):

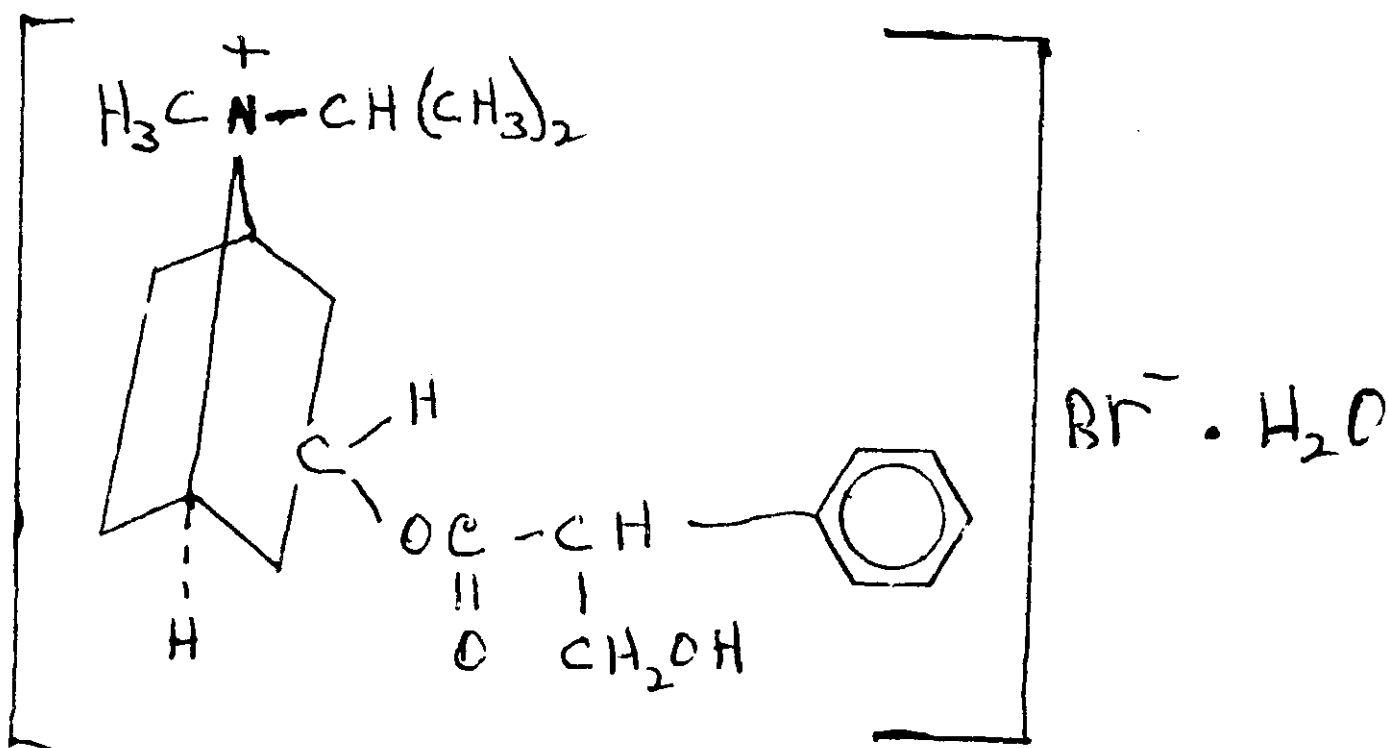
Proprietary: Atrovent Inhaler  
Non-proprietary: Ipratropium bromide  
Code Name and/or Number: Sch 1000

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Rx; Aerosol Inhalation

4. PHARMACOLOGICAL CATEGORY AND/OR PRINCIPAL INDICATION:

Anticholinergic bronchodilator used to treat bronchospasm.

5. STRUCTURAL FORMULA AND CHEMICAL NAME:



OCT 26 1985

- B. 1. INITIAL SUBMISSION: July 29, 1983
2. AMENDMENT: October 11, 1985; Rec'd by Chemist on October 21, 1985.
4. RELATED DOCUMENTS:
- Telecon between O. H. Riggleman and Dr. C. Kumkumian (HFN-102) dated September 24, 1985.

C. REMARKS

1. The labeling deficiencies have been resolved. The fluorochlorohydrocarbons will be identified by their specific names where space permits (PI, container carton).
2. The term "Inhaler" has been permitted in the name. The term "Inhalation Aerosol" appears in the third paragraph of the description section.
3. The storage statement has been revised from "Store at room temperature. Avoid excessive humidity." to "Do not store above 30°C (86°F). Avoid excessive humidity."
4. The consultation reviews from microbiology, pharmacology, and biometrics are still out.

E.

D. CONCLUSIONS AND/OR RECOMMENDATIONS:

From a chemistry standpoint, the NDA can be approved.

The consultative reviews from biometrics, pharmacology, and microbiology should concur before approval is granted.

Draft labeling only has been reviewed. MV is in progress. EIR's are satisfactory; an update has been requested by HFN-322.

*O. H. Riggleman*  
O. H. Riggleman

NDA 19-085  
HFN-160  
DocRm. 160  
R.D. OHRiggleman 10/24/85  
R.D. Init by:CPHoiberg 10/24/85  
shg W2147K 10/25/85

DRAFT OF CHEMIST'S PART, LETTER TO APPLICANT

1. Final chemistry review is awaiting consultation reviews from pharmacology, microbiology, and biometrics.

NDA 19-085

Division of Surgical-Dental Drug Products

Microbiologist's Review No. 2

December 19, 1985

- A. 1. NDA: 19-085
- Applicant: Boehringer Ingelheim, Ltd.  
90 East Ridge, P.O. Box 368  
Ridgefield, CT 06877
2. Product Name:
- Proprietary: Atrovent Inhaler
- Non proprietary: Ipratropium bromide
3. Dosage Form and Route of Administration:
- Aerosol inhalation. Rx
4. Pharmacological Category and/or Principal Indication:
- Anticholinergic bronchodilator used to treat bronchospasm.
- B. 1. Initial Submission: July 29, 1983
2. Amendments:
- (a) September 12, 1985 - Review No. 1
- (b) December 6, 1985 - Subject of Review No. 2
- C. Remarks:
1. The amendment addresses deficiencies noted in Microbiologist's initial review and discussed with Ms. Ruth Wasserman in telephone conversations held on December 2 and 6, 1985.
2. The proposed procedures and specifications for microbiological control of product for lot release has been provided. The applicant has agreed to perform these microbial assays on all batches of the subject drug at the time of release.

JAN - 4 1986

NDA 19-085  
Page 2

D. Conclusions:

The application is approvable for microbiological control of the subject drug.

  
Vivian Greenman

cc:  
NDA 19-085  
HFN-160  
HFN-160/Doc Rm  
HFN-160/VGreenman:12/19/85  
R/D init. by CPHoiberg:12/20/85/PHKusell:12/20/85  
f/t deg: 12/20/85 w2206X/D0028

N 19085 6

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1910 A, 1910

ND 17 1944 ND 17 1944

Doxylamine Succinate  
Unisom<sup>R</sup>, Capsules 25 mg  
NDA 19-440  
Reviewer: Nhan Le Tran, R.Ph., Ph.D.  
Wang [REDACTED]  
(1-S, 1-D)

Pfizer Pharmaceuticals  
New York, NY  
Submission Date:  
March 5, 1985

OCT 8 1985

Review of One (1) Bioavailability Study  
and One (1) Dissolution Study

I. Background:

Doxylamine Succinate is presently marketed by Pfizer Pharmaceuticals (Leeming Division) as an OTC Nighttime Sleep Aid (25mg Doxylamine Succinate Tablets under the trade name UNISOM<sup>R</sup>).

II. Purpose:

The objective of this study is to compare the bioavailability of a single dose of the commercially available Unisom<sup>R</sup> tablet preparation (Doxylamine Succinate, 25 mg) with that of an experimental Unisom capsule (Doxylamine Succinate, 25 mg).

III. Bioavailability Study:

This study was conducted as follows:

1. Principal Investigator: M.A. Zinny, M.D.
2. Study site: Medical and Technical Research Ass. Boston, MA
3. Study plan:

Sixteen healthy male volunteers, aged 19 to 28 years, participated in the study. All were healthy, active, ambulatory adults with no evidence of medical disease and taking no other medications. The screening procedure included: medical history, physical examination, and laboratory profile. (App I, Table 1).

Subjects participated in a single-dose 2-way crossover study, with one week elapsing between trials. On one occasion, a single 25-mg dose of doxylamine succinate as the commercial Unisom tablet was administered with 240 ml of water. On the other occasion, 25 mg of doxylamine succinate as the experimental Unisom capsule was administered. The sequence of the trials was randomized. Subjects fasted for 8 hours prior to and for 2 hours after drug administration. (App I, Table 2)

Venous blood samples were drawn into heparinized tubes prior to the dose and at the following post-dosage times: .5 hours, 1, 2, 3, 4, 8, 12, and 24 hours. Blood samples were centrifuged, and the plasma separated and frozen until the time of assay.

#### 4. Analytical Procedure:

Plasma samples were assayed using a [REDACTED] gas-chromatograph equipped with a nitrogen-phosphorus detector and automatic sampler. The column was coiled glass, 10 ft in length x by 2 mm internal diameter, packed with [REDACTED] or [REDACTED]. The carrier gas was ultra-high purity [REDACTED] mixed with [REDACTED]. Operating temperatures were: injection port [REDACTED]°C; column, [REDACTED]°C; detector, [REDACTED]°C.

Pure standards of doxylamine succinate and brompheniramine maleate (internal standard) were obtained from their manufacturers. Standards of each were prepared by dissolving the 100 mg of base in 100 ml of [REDACTED]. Sequential dilutions were made to [REDACTED] µg/ml. The solutions were stored in the dark in glass-stoppered bottles at [REDACTED]°C.

Brompheniramine (100 ng) was added to a series of 15-ml tubes. Standards for doxylamine were prepared by adding the following amounts of doxylamine base to consecutive tubes: [REDACTED] ng. One ml of drug-free control plasma is added to each of these calibration tubes. To all other tubes, containing only the internal standard, is added 1 ml of "unknown" plasma. All samples from a given subject's pair of trials were extracted and analyzed on the same day using the same calibration standards.

#### IV. Results:

##### 1. Analytical Methodology:

The firm has provided data to validate the assay procedure. The method appears to be specific (Appendix II, Fig. 1). No interference with the drug or with internal standard was observed. Standard curve as shown in Appendix II, Fig. 2 appears to be linear from [REDACTED] ng/mL range. The method appears to be precise (i.e., % CV was 10% at [REDACTED] ng/mL and 4% at [REDACTED] ng/mL) and accurate (i.e. CV% of calibration slope 1.8% (Appendix II, Table 1).

##### 2. Pharmacokinetics:

Individual plasma concentration-time data were presented in Table 1, Appendix III. Data show that the time to reach maximum concentration ( $T_{max}$ ) appears to be similar for tablet and capsule ( $T_{max}$ =2-3 hours). Data on individual pharmacokinetic parameters of capsule and tablet are given in Appendix III, Table 2. Results of ratio analysis are presented in App III, Table 3. The results indicate that 81% and 87% of the subjects lie within 0.75-1.25 for  $C_{max}$  and AUC (both 0-24 and 0- $\infty$ ) respectively; indicating small intra subject variation.

ANOVA performed on AUC 0- $\infty$ ,  $T_{max}$ ,  $C_{max}$  did not indicate significant difference between 2 dosage forms (Appendix III, table 4). Power to detect a 20% difference between capsule and tablet in all parameters exceed 80% (see Attachment 1).

3. Comments:

- a. In order to accurately estimate elimination half-life and  $AUC_{0-\infty}$  samples should be taken over a period of about 3-5  $t_{1/2}$ . In this submission,  $t_{1/2}$  was reported as 11 hours and last sampling time was 24 hours, the estimation of  $t_{1/2}$  may be not accurate, and  $AUC_{24-\infty}$  extrapolated is large. Only due to the fact that both  $AUC_{0-24}$  and  $AUC_{0-\infty}$  indicate bioequivalence is the flaw in inadequate sampling time overcome. The firm should understand the extreme importance of adequate sampling time.

V. Recommendation:

The firm has demonstrated the bioavailability of the capsule to be equivalent to the currently marketed tablet. The Division of biopharmaceutics recommends that the bioavailability study be accepted as fulfilling Bioavailability/Bioequivalence requirement.

VI. In-Vitro Dissolution:

The firm has provided dissolution data on twelve (12) individual capsules of doxylamine succinate using USP Method for the tablets (USP XX, Method II, paddle speed: 100 rpm and 0.5L water, 37°C as dissolution medium). All capsules passed the USP specification i.e.,  $Q=75\%$  in 60 minutes (Attachment I).

Since there are discrepancies between the USP and FDA methods, it was suggested that a dissolution study using the FDA conditions be submitted. The FDA dissolution conditions for doxylamine succinate tablet are as follows:

USP XX, Method II, paddle speed 50 rpm 0.9L 0.1N HCl at 37°C.

Data are shown in Attachment II. Results on twelve (12) individual capsules suggest a specification can be set for the capsules as  $Q=$  [redacted] in 60 minutes; using FDA method (50 RPM).

VII. Recommendation:

The dissolution study is found acceptable to the Division of Biopharmaceutics. For quality control purposes a paddle speed of 50 rpm must be utilized rather than 100 rpm which is unacceptable to the Division.

Overall Recommendation:

The Division of Biopharmaceutics recommends that the Bioavailability study and related dissolution data be accepted as fulfilling the Bioavailability/Bioequivalence requirements, provided that the firm agrees to the following dissolution conditions in their quality control: Dissolution is to be done at 50 rpm in 900 mL SGF (w/o enzyme) at 37°C using USP Method II. The drug product should meet the specification of  $Q=$  [redacted] in 60 minutes.

The firm should be informed of the above recommendation.



Nhan Le Tran, R.Ph., Ph.D.  
Pharmacokinetics Evaluation Branch

RD Initialed by Paul L. Hepp, Pharm.D.

FT Initialed by C.T. Viswanathan, Ph.D. CTV 10/8/85

cc: NDA 19-440 orig., HFN-120, HFN-226(Tran), Drug, Chron, and FOI files.

NLT:smj:kek:dea: [REDACTED] 8-02-85

APP I. Table 1

MEDICAL & TECHNICAL RESEARCH ASSOCIATES, INC.

PFIZER, INC. - UNISOM

DEMOGRAPHICS

| Name  | Subject Number | Age | Date of Birth | Weight (lbs.) | Height (in.) | Race |
|-------|----------------|-----|---------------|---------------|--------------|------|
| R. M. | 1              | 20  | 7/19/64       | 157           | 69           | C    |
| A. S. | 2              | 21  | 6/24/63       | 135           | 70.5         | C    |
| A. M. | 3              | 26  | 3/1/58        | 178           | 70           | C    |
| C. V. | 4              | 28  | 9/6/56        | 165           | 65           | C    |
| D. D. | 5              | 28  | 5/24/56       | 154           | 72           | C    |
| A. K. | 6              | 19  | 11/17/64      | 136           | 67.5         | C    |
| D. M. | 7              | 24  | 7/3/60        | 135           | 67           | C    |
| M. R. | 8              | 20  | 9/15/64       | 143           | 68.5         | C    |
| B. W. | 9              | 25  | 4/23/59       | 163           | 70.5         | C    |
| J. J. | 10             | 23  | 6/26/61       | 143           | 67           | C    |
| J. Q. | 11             | 28  | 12/2/53       | 148           | 68.5         | C    |
| C. W. | 12             | 28  | 1/12/56       | 157           | 67           | C    |
| R. D. | 13             | 20  | 1/16/64       | 141           | 67.5         | C    |
| J. D. | 14             | 25  | 12/16/58      | 173           | 72           | C    |
| R. M. | 15             | 22  | 2/19/62       | 175           | 72           | C    |
| O. C. | 16             | 21  | 1/26/63       | 146           | 72           | C    |
| J. V. | 17             | 27  | 9/10/57       | 175           | 70           | C    |

DRUG C/T

App I - Tab 2

MEDICAL & TECHNICAL RESEARCH ASSOCIATES, INC.

PFIZER, INC.

"Bioavailability of Doxylamine from Commercial  
Unison or from Experimental Unison:

DOSING SCHEDULE

PERIOD I

PERIOD II

| <u>SUBJECT</u> | <u>DATE</u> | <u>DOSE</u> | <u>TIME</u> | <u>DATE</u>      | <u>DOSE</u> | <u>TIME</u> |
|----------------|-------------|-------------|-------------|------------------|-------------|-------------|
| #01 R M        | 9/11/84     | A           | 0800        | 9/18/84          | B           | 0800        |
| #02 A S        | 9/11/84     | B           | 0801        | 9/18/84          | A           | 0801        |
| #03 A M        | 9/11/84     | A           | 0802        | 9/18/84          | B           | 0802        |
| #04 C W        | 9/11/84     | B           | 0803        | 9/18/84          | A           | 0803        |
| #05 D D        | 9/11/84     | A           | 0804        | 9/18/84          | B           | 0804        |
| #06 A X        | 9/11/84     | B           | 0805        | 9/18/84          | A           | 0805        |
| #07 D M        | 9/18/84     | A           | 0806        | 9/25/84          | B           | 0800        |
| #08 M H        | 9/18/84     | A           | 0807        | 9/25/84          | B           | 0801        |
| #09 B W        | 9/18/84     | B           | 0808        | Failed to return |             |             |
| #10 J J        | 9/18/84     | B           | 0809        | 9/25/84          | A           | 0802        |
| #11 J Q        | 9/18/84     | A           | 0810        | 9/25/84          | B           | 0803        |
| #12 C V        | 9/18/84     | B           | 0811        | 9/25/84          | A           | 0804        |
| #13 B O        | 9/18/84     | B           | 0812        | 9/25/84          | A           | 0805        |
| #14 J D        | 9/25/84     | B           | 0806        | 10/2/84          | A           | 0800        |
| #15 R M        | 9/25/84     | A           | 0807        | 10/2/84          | B           | 0801        |
| #16 C G        | 10/2/84     | A           | 0802        | 10/8/84          | B           | 0800        |
| #17 J F        | 10/2/84     | B           | 0803        | 10/8/84          | A           | 0801        |

A = Capsule

B = Tablet

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11/11/11

Table A

TABLE 4

SUMMARY OF KINETIC RESULTS

|                                 | Mean ( $\pm$ SE) Values |                  |                  |                  | Value of F from ANOVA |                      |
|---------------------------------|-------------------------|------------------|------------------|------------------|-----------------------|----------------------|
|                                 | Tablet                  |                  | Capsule          |                  | Treatment             | Treatment x sequence |
| Peak plasma level<br>(ng/ml)    | 99( $\pm$ 6)            | SD 24<br>%CV 24% | 100( $\pm$ 6)    | SD 24<br>%CV 24% | 0.04 (NS)             | 1.62 (NS)            |
| Time of peak<br>(hr after dose) | 2.4( $\pm$ .2)          | .8 33            | 2.6( $\pm$ .2)   | .8 31            | 0.64 (NS)             | 0.07 (NS)            |
| Elimination half-life<br>(hr)   | 10.1( $\pm$ .4)         | 1.6 16%          | 10.8( $\pm$ .7)  | 2.8 26           | 1.56 (NS)             | 0.19 (NS)            |
| 24-hr AUC<br>(ng/ml x hr)       | 1169( $\pm$ 87)         | 348 30%          | 1153( $\pm$ 78)  | 312 27           | 0.09 (NS)             | 0.03 (NS)            |
| Total AUC<br>(ng/ml x hr)       | 1493( $\pm$ 122)        | 488 33%          | 1525( $\pm$ 126) | 504 33%          | 0.28 (NS)             | 0.14 (NS)            |

2 pages purged

## Doxylamine Succinate Tablets

» Doxylamine Succinate Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**Reference standard**—*USP Doxylamine Succinate Reference Standard*—Dry in vacuum over phosphorus pentoxide for 5 hours before using.

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**Dissolution** (711)—

Medium: water; 500 mL.

Apparatus 2: 100 rpm.

Time: 60 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  dissolved from ultraviolet absorbances at the wavelength of maximum absorbance at about 262 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, in comparison with a Standard solution having a known concentration of USP Doxylamine Succinate RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask containing 65 mL of 0.1 N hydrochloric acid. Shake frequently during a 10-minute period, dilute with 0.1 N hydrochloric acid to volume, and mix. Allow the insoluble material to settle, and filter, discarding the first 20 mL of filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with 0.1 N hydrochloric acid to provide a solution containing approximately 25 µg of doxylamine succinate per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Doxylamine Succinate RS in the same medium having a known concentration of about 25 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 262 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  in the Tablet by the formula  $(TC/D)(A_L/A_S)$ , in which  $T$  is the labeled quantity, in mg, of doxylamine succinate in the Tablet,  $C$  is the concentration, in µg per mL, of USP Doxylamine Succinate RS in the Standard solution,  $D$  is the concentration, in µg per mL, of doxylamine succinate in the solution from the Tablet, based on the labeled quantity per Tablet and the extent of dilution, and  $A_L$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

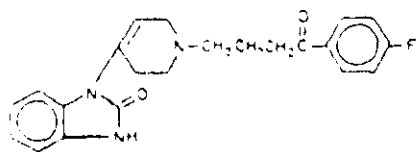
**Assay**—Proceed with Doxylamine Succinate Tablets as directed under *Salts of Organic Nitrogenous Bases* (501), determining the absorbance at the wavelength of maximum absorbance at about 262 nm. Calculate the quantity, in mg, of  $C_{17}H_{22}N_2O \cdot C_4H_6O_4$  in the portion of Tablets taken by the formula  $0.05C(A_L/A_S)$ , in which  $C$  is the concentration, in µg per mL, of USP Doxylamine Succinate RS in the *Standard Preparation*.

**Dried Aluminum Hydroxide Gel**—see Aluminum Hydroxide Gel, Dried

**Dried Ferrous Sulfate**—see Ferrous Sulfate, Dried

**Dried Sodium Phosphate**—see Sodium Phosphate, Dried

## Droperidol



$C_{22}H_{22}FN_3O_2$  379.43

2H-Benzimidazol-2-one, 1-[1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-

1-[1-[3-(*p*-Fluorobenzoyl)propyl]-1,2,3,6-tetrahydro-4-pyridinyl]-2-benzimidazolone {548-73-2}

» Droperidol, dried in vacuum at 70° for 4 hours, contains not less than 98.0 percent and not more than 102.0 percent of  $C_{22}H_{22}FN_3O_2$ .

**Packaging and storage**—Preserve in tight, light-resistant containers under nitrogen, in a cool place.

**Reference standards**—*USP Droperidol Reference Standard*—Dry in vacuum at 70° for 4 hours before using. *USP 4,4'-Bis[1,2,3,6-tetrahydro-4-(2-oxo-1-benzimidazoliny)-1-pyridyl]butyrophenone Reference Standard*—Keep container tightly closed and protected from light. Dry in vacuum at 70° for 4 hours before using.

**Identification**—

**A:** The infrared absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the wavelengths as that of a similar preparation of USP Droperidol RS.

**B:** In a 125-mL separator, combine 15.0 mL of a 1 in 670 solution of Droperidol in chloroform, 10 mL of pH 6.0 phosphate buffer (see *Buffer Solutions* in the section, *Reagents, Indicators and Solutions*), 15 mL of water, and 10 mL of freshly prepared sodium bisulfite solution (1 in 100). Shake for 15 minutes; discard the aqueous layer. Transfer 5.0 mL of the chloroform solution to a glass-stoppered conical flask containing 50.0 mL of citric acid solution (1.9 in 100), insert the stopper, and shake for 5 minutes. The ultraviolet absorption spectrum of the citric acid solution exhibits maxima and minima at the same wavelengths as the similar solution of USP Droperidol RS, and the respective absorptivities, calculated on the dried basis, at the wavelength of maximum absorbance at about 246 nm do not differ by more than 3.0%.

**Melting range, Class I** (741): between 147° and 150°.

**Loss on drying** (731)—Dry it in vacuum at 70° for 4 hours; it contains not more than 5.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Heavy metals, Method II** (231): 0.002%.

**4,4'-Bis[1,2,3,6-tetrahydro-4-(2-oxo-1-benzimidazoliny)-1-pyridyl]butyrophenone**—Dissolve 30.0 mg in 70 mL of isopropyl alcohol in a 100-mL volumetric flask. Add 10.0 mL of 0.1 N hydrochloric acid, dilute with isopropyl alcohol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP 4,4'-Bis[1,2,3,6-tetrahydro-4-(2-oxo-1-benzimidazoliny)-1-pyridyl]butyrophenone RS in the same medium, a concentration of 4.5 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 330 nm, with a suitable spectrophotometer, using a 1 in 10 solution of 0.1 N hydrochloric acid in isopropyl alcohol as the blank; the absorbance of the test solution does not exceed that of the Standard solution, corresponding to more than 1.5%.

**Assay**—Dissolve about 240 mg of Droperidol, previously dried accurately weighed, in 50 mL of glacial acetic acid, add naphtholbenzene TS, and titrate with 0.1 N perchloric acid. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.94  $C_{22}H_{22}FN_3O_2$ .

## Droperidol Injection

» Droperidol Injection is a sterile solution of Droperidol in Water for Injection, prepared with the addition of Lactic Acid. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{22}FN_3O_2$ , as the lactate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

**Reference standard**—*USP Droperidol Reference Standard*—Dry in vacuum at 70° for 4 hours before using.

**Identification**—The ultraviolet absorption spectrum of the preparation, prepared as directed in the *Assay*, exhibits maxima and minima at the same wavelengths as that of the Standard.

7 C O M P O S I T I O N

Minisom Tablets Composition

| <u>Ingredient</u>                    | <u>Wt./Wt.%</u> | <u>Mg. per Tablet</u> | <u>Function</u> |
|--------------------------------------|-----------------|-----------------------|-----------------|
| Doxylamine Succinate, N.F. (16.0913) | 16.0913*        | 24.94                 | Active          |

100.0000      155.00\*

\*Actual tablet weight is adjusted for doxylamine succinate blend assay to achieve 25 mg of doxylamine succinate per tablet, according to the following equation:

$$\text{tablet weight (mg.)} = \frac{25}{\% \text{ doxylamine succinate in blend}} \times 100$$

Minisom Tablets  
Representative Batch Formula

| <u>Ingredient</u>          | <u>Wt./Wt.%</u> | <u>Mg. per Tablet</u> | <u>Gm. per Batch</u> |
|----------------------------|-----------------|-----------------------|----------------------|
| Doxylamine Succinate, N.F. | 16.0913         | 24.94                 |                      |

100.0000      155.00

**New England  
Medical Center Hospitals**

1796 Boston Dispensary  
1894 Floating Hospital for Infants and Children  
1938 Pratt Clinic  
1946 New England Center Hospital  
1958 Rehabilitation Institute



**Tufts University**

1893 School of Medicine  
1899 School of Dental Medicine  
1979 School of Veterinary Medicine  
1980 Sackler School of Graduate  
Biomedical Sciences

---

**Division of Clinical Pharmacology**

Tufts-New England Medical Center  
171 Harrison Avenue  
Box 1007  
Boston, Massachusetts 02111  
Telephone: (617) 956-6997

16 August 1985

Michael J. Farris  
Pfizer Consumer Products  
100 Jefferson Road  
Parsippany, NJ 07054

Re: Study 84-LP664-01

Dear Mr. Farris:

Herewith enclosed is the additional statistical data requested in your letter of July 30. This does not involve additional cost.

Let me know if anything further is needed.

Sincerely,

David J. Greenblatt, M.D.

DJG/flw

---

David J. Greenblatt, M.D.  
Jerold S. Harmatz, B.A.  
Division of Clinical Pharmacology  
Tufts-New England Medical Center

RE: Response to request for additional information described in memo of July 25, 1983, from Dr. Aterno to Mr. Oppenheimer (Study 84-LP-664-01; NDA 19-440)

Item 1

The statistical procedure was analysis of variance for repeated measures, testing the significance of treatment (tablet vs capsule), sequence, and treatment-sequence interaction. Program 2V of the BMDP statistical package was used for these calculations (Dixon WJ, ed.: BMDP Statistical Software, 1981; Berkeley, U of California Press).

The accompanying Tables S-1 through S-5 provide all requested details of these calculations.

Item 2

Statistical power for detecting a 20% difference between preparations was calculated using the method of B.J. Winer (Statistical Principles in Research Design. New York, McGraw Hill, 1962, p. 104). For each of the parameters in Tables S-1 through S-5 (refer also to Table 4 of the original report), the statistical power exceed 0.87.

TABLES S-1

DETAILS OF ANALYSIS OF VARIANCE:  
PEAK PLASMA CONCENTRATION (PKL)

BMDP2V DOXYLAMINE

ANALYSIS OF VARIANCE FOR 1-ST  
DEPENDENT VARIABLE - PKLT PKLC

| SOURCE |       | SUM OF<br>SQUARES | DEGREES OF<br>FREEDOM | MEAN<br>SQUARE | F      | TAIL<br>PROB. |
|--------|-------|-------------------|-----------------------|----------------|--------|---------------|
|        | MEAN  | 317405.28125      | 1                     | 317405.28125   | 368.04 | 0.0000        |
|        | SEQ   | 81.28125          | 1                     | 81.28125       | 0.09   | 0.7634        |
| 1      | ERROR | 12073.93750       | 14                    | 862.42411      |        |               |
|        | PKL   | 9.03125           | 1                     | 9.03125        | 0.04   | 0.8455        |
|        | PS    | 371.28125         | 1                     | 371.28125      | 1.62   | 0.2238        |
| 2      | ERROR | 3208.18750        | 14                    | 229.15625      |        |               |

TABLES S-1

DETAILS OF ANALYSIS OF VARIANCE:  
PEAK PLASMA CONCENTRATION (PKL)

BMDP2V DOXYLAMINE

ANALYSIS OF VARIANCE FOR 1-ST  
DEPENDENT VARIABLE - PKLT PKLC

| SOURCE |       | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE  | F      | TAIL PROB. |
|--------|-------|----------------|--------------------|--------------|--------|------------|
|        | MEAN  | 317405.28125   | 1                  | 317405.28125 | 368.04 | 0.0000     |
|        | SEQ   | 81.28125       | 1                  | 81.28125     | 0.09   | 0.7634     |
| 1      | ERROR | 12073.93750    | 14                 | 862.42411    |        |            |
|        | PKL   | 9.03125        | 1                  | 9.03125      | 0.04   | 0.8455     |
|        | PS    | 371.28125      | 1                  | 371.28125    | 1.62   | 0.2238     |
| 2      | ERROR | 3208.18750     | 14                 | 229.15625    |        |            |

TABLES S-2

DETAILS OF ANALYSIS OF VARIANCE:  
TIME OF PEAK CONCENTRATION (PKT)

BMDP2V DOXYLAMINE

| ANALYSIS OF VARIANCE FOR 1-ST<br>DEPENDENT VARIABLE - PKTT      PKTC |        |                   |                       |                |        |               |
|----------------------------------------------------------------------|--------|-------------------|-----------------------|----------------|--------|---------------|
|                                                                      | SOURCE | SUM OF<br>SQUARES | DEGREES OF<br>FREEDOM | MEAN<br>SQUARE | F      | TAIL<br>PROB. |
|                                                                      | MEAN   | 195.03125         | 1                     | 195.03125      | 367.12 | 0.0000        |
|                                                                      | SEQ    | 0.03125           | 1                     | 0.03125        | 0.06   | 0.8119        |
| 1                                                                    | ERROR  | 7.43750           | 14                    | 0.53125        |        |               |
|                                                                      | PKT    | 0.28125           | 1                     | 0.28125        | 0.64   | 0.4384        |
|                                                                      | PS     | 0.03125           | 1                     | 0.03125        | 0.07   | 0.7942        |
| 2                                                                    | ERROR  | 6.18750           | 14                    | 0.44196        |        |               |

TABLE S-3

DETAILS OF ANALYSIS OF VARIANCE  
ELIMINATION HALF-LIFE (T12B)

| BRDF2V DOXYLAMINE                |        |                |                    |             |        |            |
|----------------------------------|--------|----------------|--------------------|-------------|--------|------------|
| ANALYSIS OF VARIANCE FOR 1-ST    |        |                |                    |             |        |            |
| DEPENDENT VARIABLE - T12BT T12BC |        |                |                    |             |        |            |
|                                  | SOURCE | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE | F      | TAIL PROB. |
|                                  | MEAN   | 3510.10143     | 1                  | 3510.10143  | 491.94 | 0.0000     |
|                                  | SEQ    | 9.92155        | 1                  | 9.92155     | 1.39   | 0.2580     |
| 1                                | ERROR  | 99.89353       | 14                 | 7.13525     |        |            |
|                                  | T12B   | 4.37389        | 1                  | 4.37389     | 1.56   | 0.2315     |
|                                  | TS     | 0.54190        | 1                  | 0.54190     | 0.19   | 0.6664     |
| 2                                | ERROR  | 39.13511       | 14                 | 2.79537     |        |            |

TABLES S-4

DETAILS OF ANALYSIS OF VARIANCE:  
 AREA UNDER 24-HR CURVE (AUC 24)

BMDP2V DOXYLAMINE

ANALYSIS OF VARIANCE FOR 1-ST  
 DEPENDENT VARIABLE - AUC24T AUC24C

|   | SOURCE | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE    | F      | TAIL PROR. |
|---|--------|----------------|--------------------|----------------|--------|------------|
|   | MEAN   | 43142760.50000 | 1                  | 43142760.50000 | 218.19 | 0.0000     |
|   | SE     | 196251.12500   | 1                  | 196251.12500   | 0.99   | 0.3360     |
| 1 | ERROR  | 2768208.37500  | 14                 | 197729.16964   |        |            |
|   | AUC24  | 1891.12500     | 1                  | 1891.12500     | 0.03   | 0.7636     |
|   | AS     | 578.00000      | 1                  | 578.00000      | 0.03   | 0.8678     |
| 2 | ERROR  | 281448.87500   | 14                 | 20103.49107    |        |            |

TABLE S-5

DETAILS OF ANALYSIS OF VARIANCE:  
TOTAL AREA UNDER CURVE (AUC)

BMDP2V DOXYLAMINE

| ANALYSIS OF VARIANCE FOR 1-ST       |        |                |                    |                |        |            |
|-------------------------------------|--------|----------------|--------------------|----------------|--------|------------|
| DEPENDENT VARIABLE - AUCT      AUCC |        |                |                    |                |        |            |
|                                     | SOURCE | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE    | F      | TAIL PROB. |
|                                     | MEAN   | 72869483.29626 | 1                  | 72869483.29626 | 161.42 | 0.0000     |
|                                     | SEQ    | 581259.67321   | 1                  | 581259.67321   | 1.29   | 0.2755     |
| 1                                   | ERROR  | 6320107.30390  | 14                 | 451436.23599   |        |            |
|                                     | AUC    | 8560.12332     | 1                  | 8560.12332     | 0.28   | 0.6034     |
|                                     | AS     | 4253.42108     | 1                  | 4253.42108     | 0.14   | 0.7135     |
| 2                                   | ERROR  | 424287.12619   | 14                 | 30306.22330    |        |            |

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NDHS 19847,

19857 + 19558

1310 0151

Ciprofloxacin IV 200mg, 400mg  
Cipro<sup>®</sup>  
NDA 19-847, 19-857, 19-858  
Reviewer: Suresh Mallikaarjun, Ph.D.  
[REDACTED]

Fung

Miles  
Pharmaceutical Division  
West Haven, CT 06516  
Submission Dates:  
September 24, 1990 DEC 13 1990  
November 1, 1990  
[REDACTED]

### Review of A New Drug Application

The sponsor submitted NDAs 19-847, 19-857, 19-858 for ciprofloxacin on April 25, 1988. Pharmacokinetic data for the three NDAs was reviewed and was found to be adequate by the Division of Biopharmaceutics. However, the clinical division determined that the sponsor needed to demonstrate that the 200 mg IV was comparable to the approved 250 mg and the 400 mg IV was comparable to the 500 mg p.o., for approvability of the IV NDAs. The sponsor has now submitted the following 3 additional studies in support of the NDA:

- i) A single dose, dose proportionality study of 200, 300 and 400 mg IV,
- ii) A single dose crossover study of oral 500 and 750 mg, and IV 300 and 400 mg doses, and
- iii) A multiple dose study of 2 regimens, a) 200 mg IV followed by 500 mg PO and b) 400 IV followed by 750 PO.

NDA 19-857 is for 0.2% of ciprofloxacin in 5% Dextrose, and NDA 19-858 for ciprofloxacin in 0.9% NaCl.

All references i.e. appendices, page numbers, etc, unless otherwise specified, refer to the sponsor's documents.

#### I. Study D89-021-01: Dose proportionality study of single doses of 200, 300 and 400 mg IV Ciprofloxacin: Vol 2 of 7.

Study Design: The single dose, randomized, three way crossover study had 12 subjects receiving 200, 300 and 400 mg IV doses of ciprofloxacin (200 ml) over a 30 minute period, with a 7 day washout period between doses. Serum samples were obtained at 0, 0.5, 1.0, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 16 and 24 hours after the start of the infusion. Urine was collected for 5 intervals from 0 to 24 hours after the start of the infusion. Samples were frozen prior to analysis by HPLC for ciprofloxacin. The analytical validation information was reviewed and found to be satisfactory.

Data Analysis: The AUC(0-24), AUC(0-∞), T<sub>1/2</sub>, C<sub>max</sub>, CL<sub>r</sub>, CL<sub>s</sub>, and X<sub>u</sub> were calculated (pg 06-00015). The AUCs, C<sub>max</sub> and X<sub>u</sub> were analyzed for dose proportionality, and CL<sub>r</sub> and T<sub>1/2</sub> for equivalence across the 3 doses. Analysis of variance was performed by the sponsor on the dose normalized parameters and the 90% C.I. determined (Appendix 2, pg 06-00112). This reviewer constructed plots of AUC, and C<sub>max</sub> versus Dose, and performed linear regression on the data. The 90% C.I. were also calculated for the AUC and C<sub>max</sub>

between the 400 and 200 mg doses. The sponsor excluded data from subject # 1002 due to extravasation of the IV solution.

**Results:** The concentration-time data is listed in Appendix 4 (pg 06-00157). The individual and mean parameters are listed in Appendix 6. The linear regression indicated that the AUC(0-24) and the Cmax were proportional between 200 to 400 mg IV. This was confirmed by the two one-sided test which showed that the AUC(0-24) (90% C.I. 90.7 - 111.6) and Cmax (83.8 - 117.4) of the 200 mg and 400 mg IV doses were proportional. The following table lists the mean parameters.

| <u>Dose</u><br><u>(mg)</u> | <u>AUC(0-∞)</u><br><u>(mg·h/L)</u> | <u>AUC/Dose</u><br><u>(h/L)×100</u> | <u>Cmax</u><br><u>(mg/L)</u> | <u>Cmax/Dose</u><br><u>(1/L)×100</u> | <u>t1/2</u><br><u>(hrs)</u> |
|----------------------------|------------------------------------|-------------------------------------|------------------------------|--------------------------------------|-----------------------------|
| 200                        | 5.7                                | 2.84                                | 3.5                          | 1.72                                 | 6.7                         |
| 300                        | 9.2                                | 3.07                                | 5.5                          | 1.84                                 | 5.5                         |
| 400                        | 11.3                               | 2.83                                | 6.9                          | 1.71                                 | 5.4                         |

**Urine:** The mean amount of unchanged ciprofloxacin recovered in the urine was 51.2%, 51.7%, and 53.5% for the 200, 300 and 400 mg doses. The corresponding renal clearances were 334, 299 and 323 ml/min. The urinary ciprofloxacin concentrations for the 200 mg dose in the 12-24 hour period ranged from 5.6 to 27.4 mcg/ml, with the corresponding urine volumes being 1300 and 610 ml.

II. Study D89-050-01: Dose equivalency of single doses of 300 mg IV, 400 mg IV, 500 mg PO and 750 mg PO. Vol. 3 of 7

**Study Design:** The single dose, randomized, four way crossover study had 12 subjects receiving 300 mg IV, 400 mg IV, 500 mg PO and 750 mg PO doses (250 mg tablets, Batch No. 15487) of ciprofloxacin with a 7 day washout period between doses. The 300 and 400 mg of ciprofloxacin was administered IV in a volume of 200 ml over 1 hour (pg 06-00350). Serum samples were obtained at frequent intervals up to 24 hours (pg 06-00351). Urine was collected for 2 intervals from 0-12, and 12-24 hours after the start of the infusion. Samples were frozen prior to analysis by HPLC for ciprofloxacin. The analytical validation information was reviewed and found to be satisfactory.

**Data Analysis:** The AUC(0-24), AUC(0-∞), T<sub>1/2</sub>, Cmax, CL<sub>r</sub>, CL<sub>s</sub>, and X<sub>u</sub> were calculated (pg 06-00353). The AUCs, Cmax and X<sub>u</sub> were analyzed for dose proportionality within the IV and PO routes, and for equivalence between each IV and PO dose. Analysis of variance was performed by the sponsor on the dose normalized parameters and the 90% C.I. determined (Appendix 2, pgs. 06-00354 - 355). The sponsor excluded subject # 1002 from

between the 400 and 200 mg doses. The sponsor excluded data from subject # 1002 due to extravasation of the IV solution.

**Results:** The concentration-time data is listed in Appendix 4 (pg 06-00157). The individual and mean parameters are listed in Appendix 6. The linear regression indicated that the AUC(0-24) and the C<sub>max</sub> were proportional between 200 to 400 mg IV. This was confirmed by the two one-sided test which showed that the AUC(0-24) (90% C.I. 90.7 - 111.6) and C<sub>max</sub> (83.8 - 117.4) of the 200 mg and 400 mg IV doses were proportional. The following table lists the mean parameters.

| <u>Dose</u><br><u>(mg)</u> | <u>AUC(0-∞)</u><br><u>(mg·h/L)</u> | <u>AUC/Dose</u><br><u>(h/L)×100</u> | <u>C<sub>max</sub></u><br><u>(mg/L)</u> | <u>C<sub>max</sub>/Dose</u><br><u>(1/L)×100</u> | <u>t<sub>1/2</sub></u><br><u>(hrs)</u> |
|----------------------------|------------------------------------|-------------------------------------|-----------------------------------------|-------------------------------------------------|----------------------------------------|
| 200                        | 5.7                                | 2.84                                | 3.5                                     | 1.72                                            | 6.7                                    |
| 300                        | 9.2                                | 3.07                                | 5.5                                     | 1.84                                            | 5.5                                    |
| 400                        | 11.3                               | 2.83                                | 6.9                                     | 1.71                                            | 5.4                                    |

**Urine:** The mean amount of unchanged ciprofloxacin recovered in the urine was 51.2%, 51.7%, and 53.5% for the 200, 300 and 400 mg doses. The corresponding renal clearances were 334, 299 and 323 ml/min. The urinary ciprofloxacin concentrations for the 200 mg dose in the 12-24 hour period ranged from 5.6 to 27.4 mcg/ml, with the corresponding urine volumes being 1300 and 610 ml.

## II. Study D89-050-01: Dose equivalency of single doses of 300 mg IV, 400 mg IV, 500 mg PO and 750 mg PO. Vol. 3 of 7

**Study Design:** The single dose, randomized, four way crossover study had 12 subjects receiving 300 mg IV, 400 mg IV, 500 mg PO and 750 mg PO doses (250 mg tablets, Batch No. 15487) of ciprofloxacin with a 7 day washout period between doses. The 300 and 400 mg of ciprofloxacin was administered IV in a volume of 200 ml over 1 hour (pg 06-00350). Serum samples were obtained at frequent intervals up to 24 hours (pg 06-00351). Urine was collected for 2 intervals from 0-12, and 12-24 hours after the start of the infusion. Samples were frozen prior to analysis by HPLC for ciprofloxacin. The analytical validation information was reviewed and found to be satisfactory.

**Data Analysis:** The AUC(0-24), AUC(0-∞), T<sub>1/2</sub>, C<sub>max</sub>, CL<sub>r</sub>, CL<sub>e</sub>, and X<sub>e</sub> were calculated (pg 06-00353). The AUCs, C<sub>max</sub> and X<sub>e</sub> were analyzed for dose proportionality within the IV and PO routes, and for equivalence between each IV and PO dose. Analysis of variance was performed by the sponsor on the dose normalized parameters and the 90% C.I. determined (Appendix 2, pgs. 06-00354 - 355). The sponsor excluded subject # 1002 from

the analysis due to serum values being persistently elevated after the 300 mg infusion. This was found to be justified, since the C<sub>max</sub> in this subject after the 300 mg infusion (19.75 µg/ml) was much higher than that after 400 mg IV (12.14 µg/ml).

**Results:** The concentration-time data is listed in Appendix 4. The individual and mean parameters are listed in Appendix 6. The two one-sided test indicated that the AUC(0-24) of the 400 mg IV and the 500 mg PO (90% C.I. 97.0 - 111.6), and the C<sub>max</sub> of the 400 mg IV and 750 mg PO doses (88.5 - 116.7) were equivalent. The 90% C.I. of the AUC and C<sub>max</sub> of the 300 mg and 400 mg doses indicated that the doses were proportional (90.9 - 109.6). The average bioavailability for the 500 and 750 mg doses was about 78%. The following table lists the mean parameters.

| <u>Dose</u><br><u>(mg)</u> | <u>AUC(0-24h)</u><br><u>(mg·h/L)</u> | <u>AUC(0-24h)/Dose</u><br><u>(h/L)×100</u> | <u>C<sub>max</sub></u><br><u>(mg/L)</u> | <u>C<sub>max</sub>/Dose</u><br><u>(1/L)×100</u> |
|----------------------------|--------------------------------------|--------------------------------------------|-----------------------------------------|-------------------------------------------------|
| 300 IV                     | 8.33                                 | 2.78                                       | 3.27                                    | 1.09                                            |
| 400 IV                     | 11.1                                 | 2.77                                       | 4.00                                    | 1.00                                            |
| 500 PO                     | 10.6                                 | 2.13                                       | 2.79                                    | 0.56                                            |
| 750 PO                     | 16.5                                 | 2.20                                       | 3.90                                    | 0.52                                            |

**Urine:** The mean amount of unchanged ciprofloxacin recovered in the urine was 61.8%, and 55.8% for the 300 and 400 mg doses. The corresponding renal clearances were 382 and 349 ml/min. The urinary ciprofloxacin concentrations in the 12-24 hour period for the 300 mg dose ranged from 8.3 to 53.4 mcg/ml, with the corresponding urine volumes being 1155 and 220 ml.

### III. Study D90-012-01: Dose equivalency of multiple doses of 200 mg IV, 400 mg IV, 500 mg PO and 750 mg PO. Vol. 4 of 4

**Study Design:** The randomized, crossover, sequential study had 13 subjects enrolled. The following regimens were studied:

- 1) 200 mg IV q 12 h for 6 doses, followed by 500 mg PO q 12 h for 5 doses, and
- 2) 400 mg IV q 12 h for 6 doses, followed by 750 mg PO q 12 h for 5 doses.

Each of these regimens was separated by a washout period of a week. There were no washout periods between the IV and the oral treatments within each regimen. The oral doses were given as 250 mg tablets, Batch No. KQ-9-33.

The IV doses were administered in a volume of 200 ml over 1 hour (pg 06-00708). Serum samples were obtained at frequent intervals up to 12 hours (pg 06-00709) for the 1st and 5th doses. Additional serum samples were drawn at 16 and 24 hours for the last oral doses. Urine was collected for 4 intervals from 0-12 hours, after the start of the infusion (06-00709). Samples were frozen prior to analysis by HPLC for ciprofloxacin.

**Data Analysis:** The AUC(0-24), AUC(0-∞), T<sub>1/2</sub>, C<sub>max</sub>, CL<sub>r</sub>, CL<sub>e</sub>, and X<sub>u</sub> were calculated (pg 06-00353). The AUCs, C<sub>max</sub> and X<sub>u</sub> were analyzed for dose proportionality within the IV and PO routes, and for equivalence between each IV and PO dose. Analysis of variance was performed by the sponsor on the dose normalized AUC and C<sub>max</sub> of Dose 5, and the 90% C.I. determined (Appendix 2, pgs. 06-00354 - 355). The following comparisons were undertaken:

- i) Dose proportionality between the 200 and 400 mg IV doses.
- ii) Equivalence of the AUC and C<sub>max</sub> between Dose 1 and Dose 5.
- ii) Equivalence of AUC between 400 mg IV and 500 mg PO, and of C<sub>max</sub> between 400 mg IV and 750 mg PO.

Subjects #1002 and #1014 withdrew from the study due to adverse effects and their data were excluded from the final analysis.

**Results:** The concentration-time data is listed in Appendix 4. The individual and mean parameters are listed in Appendix 6.

- i) The two one-sided test of the 200 mg and 400 mg doses indicated that the doses were proportional with reference to the AUC (98 - 119.5).
- ii) The AUC after Day 1 was equivalent to that after Day 5 (98.4 - 117.9), and so was the C<sub>max</sub> (89.7 - 111.6).
- iii) The AUC(0-24) of the 400 mg IV and 500 mg PO doses were equivalent (90% C.I. 81.1 - 103.5) by the two one-sided test. The same test indicated that the C<sub>max</sub> of the 400 mg IV and 750 mg PO doses were inequivalent by a very small margin (90.0 - 120.3).

The average bioavailability for the 500 and 750 mg oral doses was about 88%. The following table lists the mean parameters.

| <u>Dose Regimen</u> | <u>AUC<sub>0-12h</sub></u><br><u>(mg·L/h)</u> | <u>AUC<sub>0-12h</sub>/Dose</u><br><u>(L/h) x 100</u> | <u>C<sub>max</sub></u><br><u>(mg/L)</u> | <u>C<sub>max</sub>/Dose</u><br><u>(1/L) x 100</u> |
|---------------------|-----------------------------------------------|-------------------------------------------------------|-----------------------------------------|---------------------------------------------------|
| 200mg iv q 12 h     | 5.81                                          | 2.91                                                  | 2.10                                    | 1.05                                              |
| 400mg iv q 12 h     | 12.7                                          | 3.18                                                  | 4.59                                    | 1.15                                              |
| 500mg po q 12 h     | 13.8                                          | 2.76                                                  | 2.99                                    | 0.60                                              |
| 750mg po q 12 h     | 19.6                                          | 2.62                                                  | 4.36                                    | 0.58                                              |

**Urine:** The mean amount of unchanged ciprofloxacin recovered in the urine was 61.8%, and 55.8% for the 300 and 400 mg doses. The corresponding renal clearances were 382 and 349 ml/min. The urinary ciprofloxacin concentrations in the 12-24 hour period for the 300 mg dose ranged from 8.3 to 53.4 mcg/ml, with the corresponding urine volumes being 1155 and 220 ml.

**Statistical Analyses:** The sponsor performed the statistical analyses of all the studies using the dose adjusted AUC and C<sub>max</sub>. However, except for the 1st dose proportionality study,

it was felt by this reviewer that since the availability of the IV and oral forms were not the same, a dose adjusted comparison would not be appropriate. On consultation with Dr. Tsae Lien Lin, of the Division of Biometrics, this reviewer was advised that the sponsor's analyses were correct, since the variance of the parameters at different doses was not homogenous, and this heterogeneity would be corrected for by the analysis on dose adjusted parameters.

Also, in order to verify the sponsor's 90% C.I., the estimate, and standard error of the estimate of parameters prior to adjustment for the dose were required, which the sponsor calculated using certain equations, the appropriateness of which was confirmed by Dr. Tsae Lien Lin. The 90% C.I.s when calculated using the adjusted and unadjusted parameters were only marginally different, which is to be expected.

### Overall Conclusions:

Dose Proportionality of IV Doses: The single dose study clearly demonstrates dose proportionality in the AUC and Cmax between the 200 mg, 300 mg and the 400 mg doses. The multiple dose study also confirms this proportionality in the AUC and Cmax between 200 mg and 400 mg doses. The AUC and Cmax of the 400 mg were compared between Day 1 and Day 5 and were found to be equivalent.

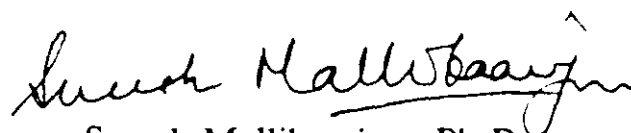
Dose Equivalency: The AUC of the 400 mg IV was equivalent to that of the 500 mg tablet (90% C.I. 81.1 - 103.3). The Cmax of the 400 mg IV was marginally higher than that of the 500 mg tablet (90.0 - 120.3). Although this is not equivalent in the strict sense of the term, for all practical purposes, the Cmax can be considered equivalent, since the magnitude of the differences in the means is quite small, approximately 5%.

Since the 200 mg IV is proportional to the 400 mg IV, it can be concluded, by inference, that the AUC of a 200 mg IV dose would be equivalent to that after a 250 mg oral tablet.

Recommendation for NDA 19-847: From the Division of Biopharmaceutics standpoint, the AUC after a 200 mg, and 400 mg IV dose can be considered to be equivalent to that after a 250 and 500 mg oral tablet respectively. Also, the Cmax after a 400 mg IV dose can be considered to be equivalent to that after a 750 mg oral tablet.

Recommendation for NDA 19-857 and 19-858: The pharmacokinetic and biopharmaceutic information for Cipro<sup>R</sup> IV has been submitted in NDA 19-847, reviewed and found to be satisfactory by the Division of Biopharmaceutics. Pharmacokinetically, there would be no difference between IV administrations of ciprofloxacin diluted just prior to use as in NDA 19-847, and a prepackaged ciprofloxacin solution as in NDAs 19-857 and 19-858. Therefore, a waiver of pharmacokinetic and biopharmaceutic information for NDA 19-857, 0.2% ciprofloxacin in 5% Dextrose solution, and NDA 19-858, 0.2% ciprofloxacin in 0.9%

Sodium Chloride solution, is recommended by the Division of Biopharmaceutics.



Suresh Mallikaarjun, Ph.D.  
Pharmacokinetic Evaluation Branch

FT initialed by C.T. Viswanathan, Ph.D. CTV ~~6/19/90~~ 12/10/90

cc: NDA 19-847, 18-857, 19-858 Original, HFD-520, HFD-426 (Mallikaarjun), HFD-344(Turner), Review, Drug, Chron and HFD-19 (FOI) files.

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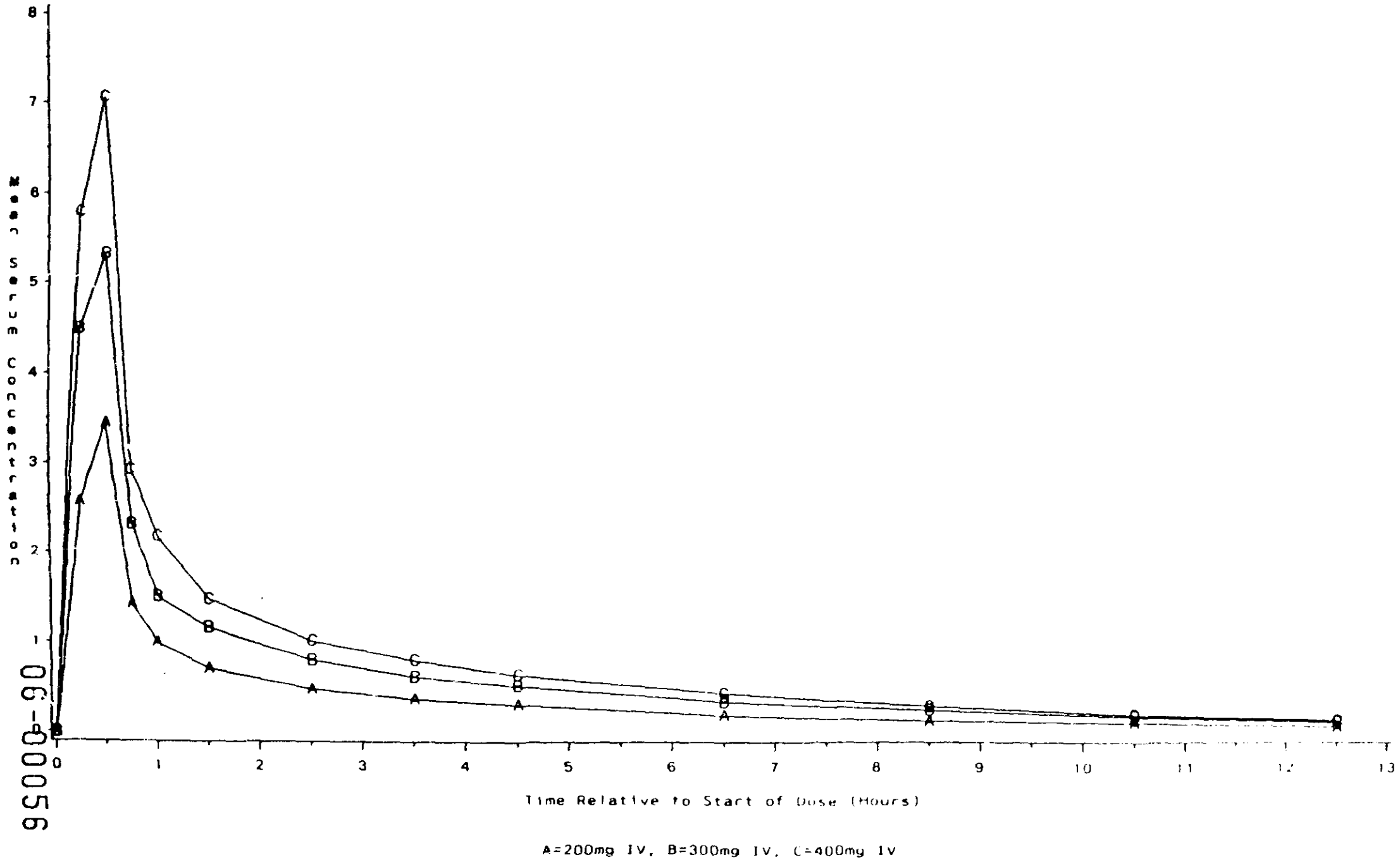
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Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study D89-021-01  
Pharmacokinetics and Tolerance of I.V. Doses of Ciprofloxacin in Healthy Volunteers

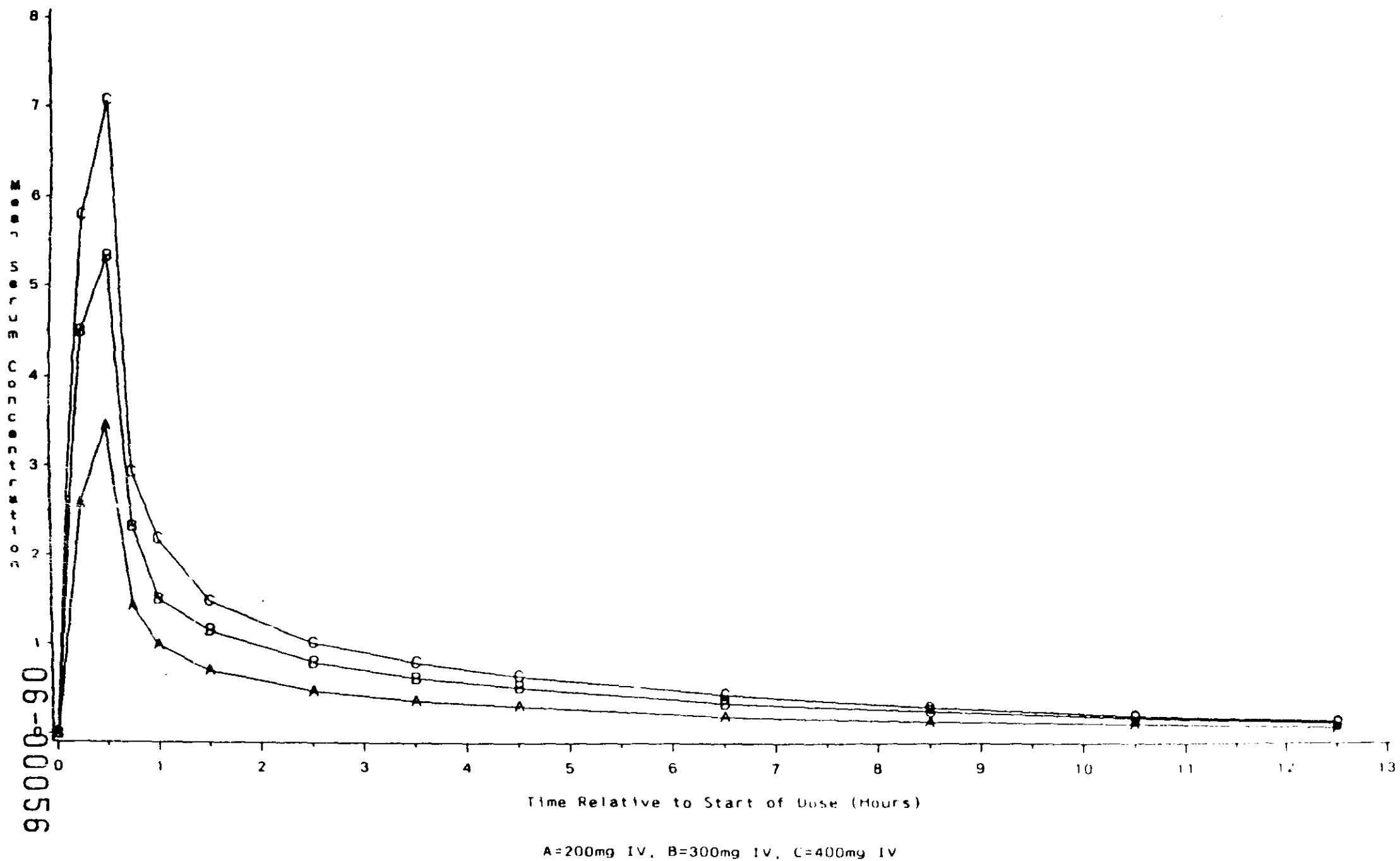
Figure 1A: Mean Serum Ciprofloxacin Concentration (mg/L) versus Time



CP 1/5 T Plate  
000045

Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study DB9-021-01  
Pharmacokinetics and Tolerance of I.V. Doses of Ciprofloxacin in Healthy Volunteers

Figure 1A: Mean Serum Ciprofloxacin Concentration (mg/L) versus Time



CP 4/5 T Plate  
060045

TABLE 4: RESULTS OF STATISTICAL ANALYSIS OF PHARMACOKINETIC VARIABLES

| VARIABLE                 | LS MEANS |        |        | RATIO   | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|--------------------------|----------|--------|--------|---------|----------------|-----------------|-------|--------------|-------|-------|
|                          | 200MG    | 300MG  | 400MG  |         |                | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-24)<br>(mg*h/L)    | 5.46     | 8.95   | 11.05  | NA      |                |                 |       |              |       |       |
| AUC(0-24)/DOSE<br>(h/L)  | 0.0273   | 0.0298 | 0.0276 | 300/200 | 1.093          | 0.993           | 1.204 | 0.000        | 0.056 | 0.127 |
|                          |          |        |        | 400/300 | 0.926          | 0.837           | 1.022 | 0.011        | 0.000 | 0.194 |
|                          |          |        |        | 400/200 | 1.012          | 0.912           | 1.122 | 0.001        | 0.005 | 0.848 |
| AUC(0-inf)<br>(mg*h/L)   | 5.68     | 9.20   | 11.31  | NA      |                |                 |       |              |       |       |
| AUC(0-inf)/DOSE<br>(h/L) | 0.0284   | 0.0307 | 0.0283 | 300/200 | 1.079          | 0.973           | 1.198 | 0.000        | 0.047 | 0.218 |
|                          |          |        |        | 400/300 | 0.922          | 0.827           | 1.027 | 0.018        | 0.000 | 0.210 |
|                          |          |        |        | 400/200 | 0.996          | 0.890           | 1.113 | 0.002        | 0.005 | 0.946 |
| Cmax<br>(mg/L)           | 3.45     | 5.51   | 6.85   | NA      |                |                 |       |              |       |       |
| Cmax/DOSE<br>(1/L)       | 0.0172   | 0.0184 | 0.0171 | 300/200 | 1.066          | 0.911           | 1.249 | 0.003        | 0.105 | 0.490 |
|                          |          |        |        | 400/300 | 0.933          | 0.790           | 1.097 | 0.063        | 0.007 | 0.467 |
|                          |          |        |        | 400/200 | 0.994          | 0.838           | 1.176 | 0.020        | 0.034 | 0.951 |
| HALF-LIFE<br>(h)         | 6.69     | 5.52   | 5.39   |         | 0.825          | 0.757           | 0.899 | 0.268        | 0.000 | 0.001 |
|                          |          |        |        | 400/300 | 0.975          | 0.884           | 1.076 | 0.001        | 0.001 | 0.665 |
|                          |          |        |        | 400/200 | 0.805          | 0.734           | 0.881 | 0.453        | 0.000 | 0.000 |
| CLr<br>(mL/min)          | 334.39   | 298.82 | 320.84 | 300/200 | 0.894          | 0.792           | 1.007 | 0.065        | 0.000 | 0.120 |
|                          |          |        |        | 400/300 | 1.074          | 0.946           | 1.219 | 0.000        | 0.073 | 0.343 |
|                          |          |        |        | 400/200 | 0.959          | 0.850           | 1.081 | 0.009        | 0.002 | 0.556 |
| AmtUR(0-24)/DOSE<br>(%)  | 51.20    | 51.71  | 52.96  | 300/200 | 1.010          | 0.925           | 1.103 | 0.000        | 0.002 | 0.849 |
|                          |          |        |        | 400/300 | 1.024          | 0.936           | 1.120 | 0.000        | 0.003 | 0.651 |
|                          |          |        |        | 400/200 | 1.034          | 0.945           | 1.132 | 0.000        | 0.005 | 0.526 |

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO < 80%. P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO > 120%. P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H0: TRUE RATIO = 100%.

STATISTICAL SUMMARY

07000-90

000029

TABLE 3A

Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study DB9-021-01  
 Pharmacokinetics and Tolerance of I.V. Doses of Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables: Summary Statistics

| LABEL                                        | MEAN        | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|-------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=200mg_Cipro_IV ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 77.72725000 | 2.48710235        | 12 | 8.61557527         | 11.084 | 64.32600000   | 91.05300000   |
| Normalized Dose/BW (mg/kg)                   | 2.60297688  | 0.08533353        | 12 | 0.29560402         | 11.356 | 2.19652290    | 3.10916270    |
| AUC(0-12) (mg*h/L)                           | 4.96324737  | 0.38687046        | 12 | 1.34015859         | 27.002 |               |               |
| AUC(0-12) / Dose (h/L)                       | 0.02481624  | 0.00193435        | 12 | 0.00670079         | 27.002 |               |               |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 1.89288646  | 0.11279139        | 12 | 0.39072064         | 20.642 |               |               |
| AUC(0-24) (mg*h/L)                           | 5.45887650  | 0.41488754        | 12 | 1.43721260         | 26.328 |               |               |
| AUC(0-24) / Dose (h/L)                       | 0.02729438  | 0.00207444        | 12 | 0.00718606         | 26.328 |               |               |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 2.08358548  | 0.12241006        | 12 | 0.42404090         | 20.352 |               |               |
| AUC(0-inf) (mg*h/L)                          | 5.68059167  | 0.42163262        | 12 | 1.46057825         | 25.712 |               |               |
| AUC(0-inf) / Dose (h/L)                      | 0.02840296  | 0.00210816        | 12 | 0.00730289         | 25.712 |               |               |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 2.16971445  | 0.12608752        | 12 | 0.43677997         | 20.131 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.44583333  | 0.32672725        | 12 | 1.13181639         | 32.846 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.01722917  | 0.00163364        | 12 | 0.00565908         | 32.846 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 1.31243537  | 0.10525584        | 12 | 0.36461692         | 27.782 |               |               |
| Time of Peak Conc. (h)                       | 0.50000000  | 0.00000000        | 12 | 0.00000000         | 0.000  |               |               |
| Elimination Half-Life (h)                    | 6.69233333  | 0.28683461        | 12 | 0.99362425         | 14.847 |               |               |
| ----- Treatment Regimen=300mg_Cipro_IV ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 77.72725000 | 2.48710235        | 12 | 8.61557527         | 11.084 | 64.32600000   | 91.05300000   |
| Normalized Dose/BW (mg/kg)                   | 3.90446532  | 0.12800030        | 12 | 0.44340603         | 11.356 | 3.29478436    | 4.66374405    |
| AUC(0-12) (mg*h/L)                           | 8.17847545  | 0.49752461        | 12 | 1.72347579         | 21.073 |               |               |
| AUC(0-12) / Dose (h/L)                       | 0.02726158  | 0.00165842        | 12 | 0.00574492         | 21.073 |               |               |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 2.11973201  | 0.15275755        | 12 | 0.52916768         | 24.964 |               |               |
| AUC(0-24) (mg*h/L)                           | 8.94949633  | 0.56519164        | 12 | 1.95788129         | 21.877 |               |               |
| AUC(0-24) / Dose (h/L)                       | 0.02983165  | 0.00188397        | 12 | 0.00652627         | 21.877 |               |               |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 2.32050994  | 0.17355413        | 12 | 0.60120914         | 25.908 |               |               |
| AUC(0-inf) (mg*h/L)                          | 9.19613133  | 0.59007691        | 12 | 2.04408638         | 22.228 |               |               |
| AUC(0-inf) / Dose (h/L)                      | 0.03065378  | 0.00196692        | 12 | 0.00681362         | 22.228 |               |               |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 2.38468463  | 0.18084069        | 12 | 0.62645054         | 26.270 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 5.50916667  | 0.45175157        | 12 | 1.56491335         | 28.406 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.01836389  | 0.00150584        | 12 | 0.00521638         | 28.406 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 1.43880853  | 0.13978147        | 12 | 0.48421721         | 33.654 |               |               |
| Time of Peak Conc. (h)                       | 0.41666667  | 0.03553345        | 12 | 0.12309149         | 29.542 |               |               |
| Elimination Half-Life (h)                    | 5.52366667  | 0.15974347        | 12 | 0.55336760         | 10.018 |               |               |

MEAN

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VALUES

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TABLE 3A (Continued)

Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study 089-021-01  
 Pharmacokinetics and Tolerance of I V Doses of Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables. Summary Statistics

| LABEL                                        | MEAN        | STD ERROR<br>OF MEAN | N  | STANDARD<br>DEVIATION | C V    | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|----------------------------------------------|-------------|----------------------|----|-----------------------|--------|------------------|------------------|
| ----- Treatment Regimen=400mg_Cipro_IV ----- |             |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 76.88645455 | 2.56407670           | 11 | 8.50408035            | 11.061 | 64.32600000      | 91.05300000      |
| Normalized Dose/BW (mg/kg)                   | 5.26113412  | 0.17691481           | 11 | 0.58676003            | 11.153 | 4.39304581       | 6.21832540       |
| AUC(0-12) (mg*h/L)                           | 10.46159509 | 0.29168237           | 11 | 0.96740098            | 9.247  |                  |                  |
| AUC(0-12) / Dose (h/L)                       | 0.02615399  | 0.00072921           | 11 | 0.00241850            | 9.247  |                  |                  |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 2.00032277  | 0.05923097           | 11 | 0.19644690            | 9.821  |                  |                  |
| AUC(0-24) (mg*h/L)                           | 11.33493831 | 0.32415003           | 11 | 1.07508401            | 9.485  |                  |                  |
| AUC(0-24) / Dose (h/L)                       | 0.02833735  | 0.00081038           | 11 | 0.00268771            | 9.485  |                  |                  |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 2.16788628  | 0.06763085           | 11 | 0.22430614            | 10.347 |                  |                  |
| AUC(0-inf) (mg*h/L)                          | 11.60011818 | 0.32519259           | 11 | 1.07854182            | 9.298  |                  |                  |
| AUC(0-inf) / Dose (h/L)                      | 0.02900030  | 0.00081298           | 11 | 0.00269635            | 9.298  |                  |                  |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 2.22620145  | 0.08640811           | 11 | 0.29321653            | 13.171 |                  |                  |
| C <sub>MAX</sub> (mg/L)                      | 7.09909091  | 0.22631215           | 11 | 0.75059249            | 10.573 |                  |                  |
| C <sub>MAX</sub> / Dose (1/L)                | 0.01774773  | 0.00056578           | 11 | 0.00187648            | 10.573 |                  |                  |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 1.35878071  | 0.04583816           | 11 | 0.15534459            | 11.433 |                  |                  |
| Time of Peak Conc. (h)                       | 0.47727273  | 0.02272727           | 11 | 0.07537784            | 15.793 |                  |                  |
| Elimination Half-Life (h)                    | 5.38936364  | 0.18416876           | 11 | 0.61081868            | 11.334 |                  |                  |

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TABLE 3A (Continued)

Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study DR9-021-01  
 Pharmacokinetics and Tolerance of I.V. Doses of Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables. Summary Statistics

| LABEL                                        | MEAN        | STD ERROR<br>OF MEAN | N  | STANDARD<br>DEVIATION | C V    | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|----------------------------------------------|-------------|----------------------|----|-----------------------|--------|------------------|------------------|
| ----- Treatment Regimen=400mg_Cipro_IV ----- |             |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 76.88645455 | 2.56407670           | 11 | 8.50408035            | 11.061 | 64.32600000      | 91.05300000      |
| Normalized Dose/BW (mg/kg)                   | 5.26113412  | 0.17691481           | 11 | 0.58676003            | 11.153 | 4.39304581       | 6.21832540       |
| AUC(0-12) (mg*h/L)                           | 10.46159509 | 0.29168237           | 11 | 0.96740098            | 9.247  |                  |                  |
| AUC(0-12) / Dose (h/L)                       | 0.02615399  | 0.00072921           | 11 | 0.00241850            | 9.247  |                  |                  |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 2.00032277  | 0.05923097           | 11 | 0.19644690            | 9.821  |                  |                  |
| AUC(0-24) (mg*h/L)                           | 11.33493831 | 0.32415003           | 11 | 1.07508401            | 9.485  |                  |                  |
| AUC(0-24) / Dose (h/L)                       | 0.02833735  | 0.00081038           | 11 | 0.00268771            | 9.485  |                  |                  |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 2.16788628  | 0.06763085           | 11 | 0.22430614            | 10.347 |                  |                  |
| AUC(0-inf) (mg*h/L)                          | 11.60011818 | 0.32519259           | 11 | 1.07854182            | 9.298  |                  |                  |
| AUC(0-inf) / Dose (h/L)                      | 0.02900030  | 0.00081298           | 11 | 0.00269635            | 9.298  |                  |                  |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 2.22620145  | 0.08840811           | 11 | 0.29321653            | 13.171 |                  |                  |
| C <sub>MAX</sub> (mg/L)                      | 7.09909091  | 0.22631215           | 11 | 0.75059249            | 10.573 |                  |                  |
| C <sub>MAX</sub> / Dose (1/L)                | 0.01774773  | 0.00056578           | 11 | 0.00187648            | 10.573 |                  |                  |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 1.35878071  | 0.04683816           | 11 | 0.15534459            | 11.433 |                  |                  |
| Time of Peak Conc. (h)                       | 0.47727273  | 0.02272727           | 11 | 0.07537784            | 15.793 |                  |                  |
| Elimination Half-Life (h)                    | 5.38936364  | 0.18416876           | 11 | 0.61081868            | 11.334 |                  |                  |

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TABLE 3B

Ciprofloxacin IV (BAY q 3939) Pharmacokinetics Study 089-021-01  
 Pharmacokinetics and Tolerance of I.V. Doses of Ciprofloxacin in Healthy Volunteers  
 Table 3B. Derived Pharmacokinetic Variables. Summary Statistics

| LABEL                                        | MEAN         | STD ERROR<br>OF MEAN | N  | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|----------------------------------------------|--------------|----------------------|----|-----------------------|--------|------------------|------------------|
| ----- Treatment Regimen=200mg_Cipro_IV ----- |              |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 77.72725000  | 2.48710235           | 12 | 8.6155753             | 11.084 | 64.3260000       | 91.0530000       |
| Normalized Dose/BW (mg/kg)                   | 2.60297688   | 0.08533353           | 12 | 0.2956040             | 11.356 | 2.1965229        | 3.1091627        |
| Total Clearance (mL/min)                     | 625.81104566 | 49.42211766          | 12 | 171.2032376           | 27.357 |                  |                  |
| Total Clearance/BW (mL/(min*kg))             | 8.00489282   | 0.51813130           | 12 | 1.7948595             | 22.422 |                  |                  |
| 12Hr Amount Excreted (mg)                    | 95.17930833  | 2.85806312           | 12 | 9.9006211             | 10.402 |                  |                  |
| 12Hr Amt. Excreted / Dose(%)                 | 47.58965417  | 1.42903156           | 12 | 4.9503105             | 10.402 |                  |                  |
| 24Hr Amount Excreted (mg)                    | 102.40540833 | 2.45782235           | 12 | 8.5141464             | 8.314  |                  |                  |
| 24Hr Amt. Excreted / Dose(%)                 | 51.20270417  | 1.22891118           | 12 | 4.2570732             | 8.314  |                  |                  |
| Renal Clearance (mL/min)                     | 334.38542668 | 28.66386817          | 12 | 99.2945520            | 29.695 |                  |                  |
| Renal Clearance/BW (mL/(min*kg))             | 4.28696648   | 0.32463425           | 12 | 1.1245660             | 26.232 |                  |                  |
| ----- Treatment Regimen=300mg_Cipro_IV ----- |              |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 77.72725000  | 2.48710235           | 12 | 8.61557527            | 11.084 | 64.3260000       | 91.0530000       |
| Normalized Dose/BW (mg/kg)                   | 3.90446532   | 0.12800030           | 12 | 0.44340603            | 11.356 | 3.2947844        | 4.6637441        |
| Total Clearance (mL/min)                     | 561.86930152 | 26.47010451          | 12 | 91.69513177           | 16.320 |                  |                  |
| Total Clearance/BW (mL/(min*kg))             | 7.30496201   | 0.38900836           | 12 | 1.34756449            | 18.447 |                  |                  |
| 12Hr Amount Excreted (mg)                    | 145.12088333 | 3.98855537           | 12 | 13.81676110           | 9.521  |                  |                  |
| 12Hr Amt. Excreted / Dose(%)                 | 48.37362778  | 1.32951846           | 12 | 4.60558703            | 9.521  |                  |                  |
| 24Hr Amount Excreted (mg)                    | 155.12870000 | 4.05780155           | 12 | 14.05663691           | 9.061  |                  |                  |
| 24Hr Amt. Excreted / Dose(%)                 | 51.70956667  | 1.35260052           | 12 | 4.68554564            | 9.061  |                  |                  |
| Renal Clearance (mL/min)                     | 298.81606791 | 16.41913170          | 12 | 56.87754063           | 19.034 |                  |                  |
| Renal Clearance/BW (mL/(min*kg))             | 3.89528716   | 0.24536086           | 12 | 0.84995495            | 21.820 |                  |                  |
| ----- Treatment Regimen=400mg_Cipro_IV ----- |              |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 76.88645455  | 1.407670             | 11 | 8.50408035            | 11.061 | 64.3260000       | 91.0530000       |
| Normalized Dose/BW (mg/kg)                   | 5.26113412   | 0.17691481           | 11 | 0.58676003            | 11.153 | 4.3930458        | 6.2183254        |
| Total Clearance (mL/min)                     | 579.47040662 | 17.09853748          | 11 | 56.70943329           | 9.786  |                  |                  |
| Total Clearance/BW (mL/(min*kg))             | 7.60964153   | 0.31203903           | 11 | 1.03491637            | 13.600 |                  |                  |
| 12Hr Amount Excreted (mg)                    | 197.70513636 | 12.377031            | 11 | 40.80698964           | 20.634 |                  |                  |
| 12Hr Amt. Excreted / Dose(%)                 | 49.44128409  | 3.07594258           | 11 | 10.20174741           | 20.634 |                  |                  |
| 24Hr Amount Excreted (mg)                    | 211.07799091 | 12.24503229          | 11 | 40.61217766           | 19.240 |                  |                  |
| 24Hr Amt. Excreted / Dose(%)                 | 52.76949773  | 3.06125807           | 11 | 10.15304442           | 19.240 |                  |                  |
| Renal Clearance (mL/min)                     | 312.73888353 | 19.00377066          | 11 | 63.02837687           | 20.154 |                  |                  |
| Renal Clearance/BW (mL/(min*kg))             | 4.08428071   | 0.22610930           | 11 | 0.74991971            | 18.361 |                  |                  |

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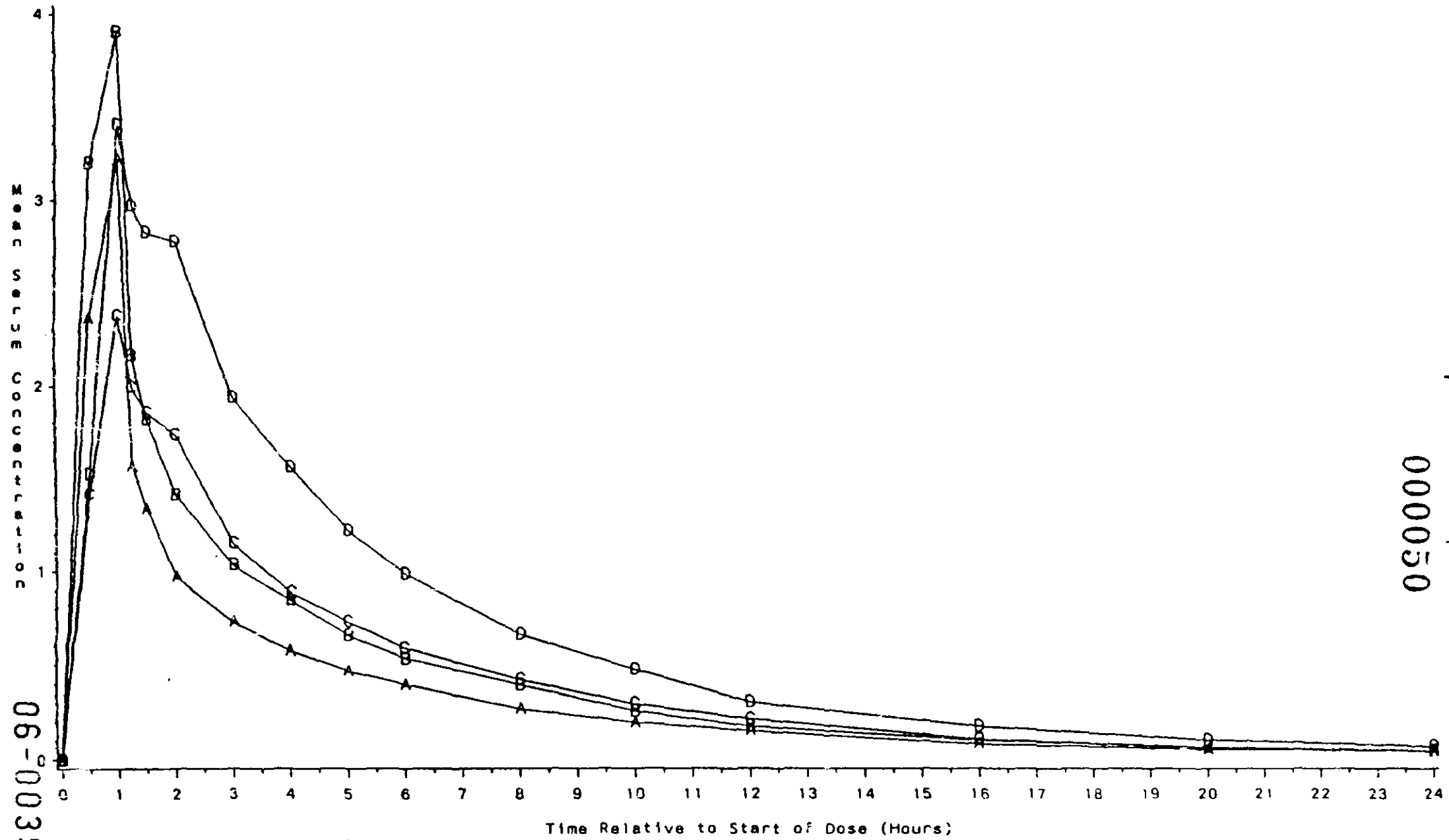
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Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) Pharmacokinetics Study D89-050-01  
Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers

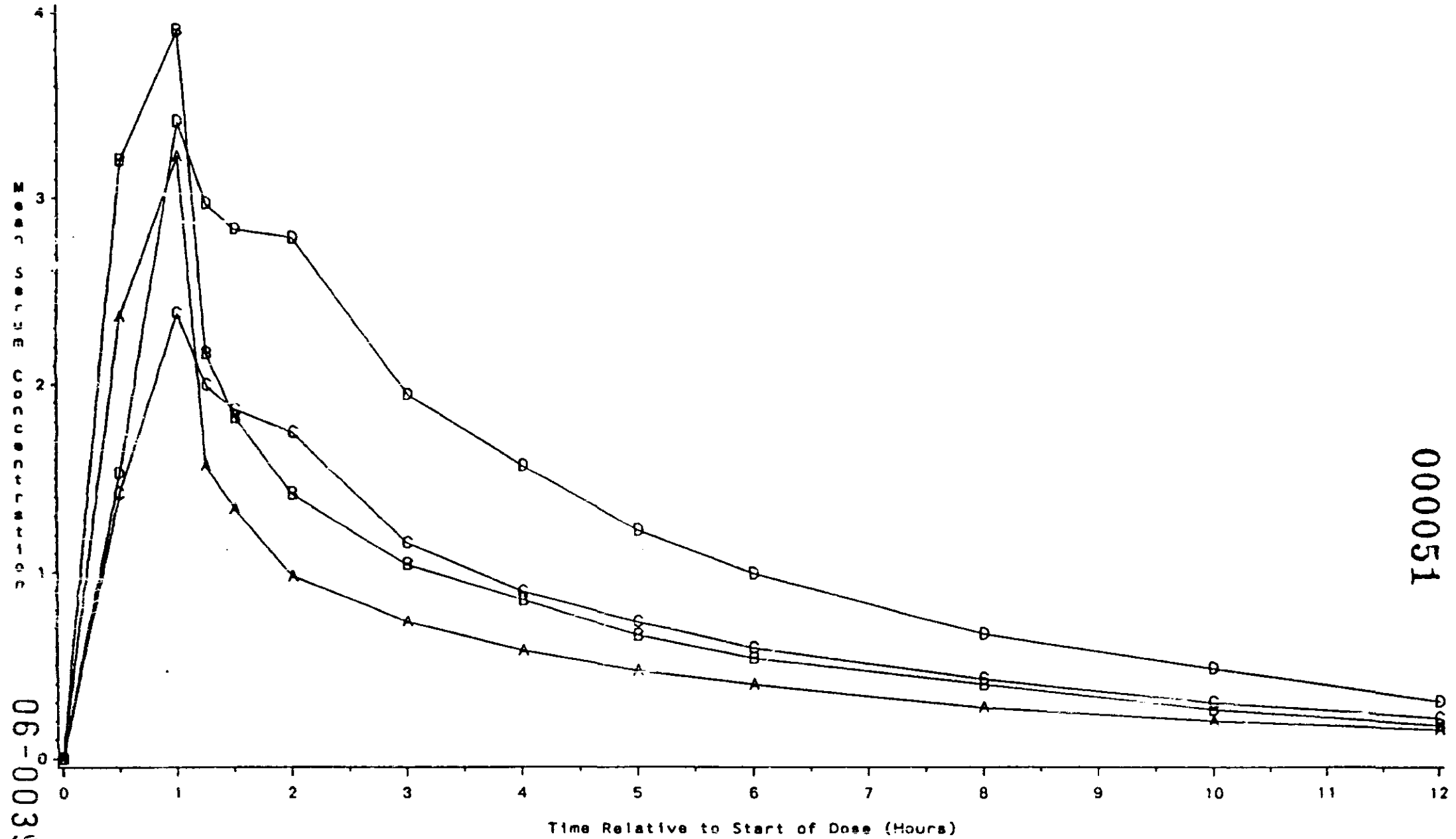
Figure 1: Mean Serum Ciprofloxacin Concentration (mg/L) versus Time (excluding subject 1002)



A=300mg IV, B=400mg IV, C=500mg PO, D=750mg PO

Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) Pharmacokinetics Study DB9-050-01  
Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers

Figure 1: Mean Serum Ciprofloxacin Concentration (mg/L) versus Time (excluding subject 1002)



A=300mg IV, B=400mg IV, C=500mg PO, D=750mg PO

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06-00399

CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 PHARMACOKINETIC DETERMINATION OF DOSE EQUIVALENCY BETWEEN IV AND ORAL CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL D89-050

TABLE 4: RESULTS OF STATISTICAL ANALYSIS OF PHARMACOKINETIC VARIABLES  
 EXCLUDING SUBJECT 1002

| VARIABLE                 | LS MEANS |        |        |        | RATIO           | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|--------------------------|----------|--------|--------|--------|-----------------|----------------|-----------------|-------|--------------|-------|-------|
|                          | 300IV    | 400IV  | 500PO  | 750PO  |                 |                | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-24h)<br>(mg*h/L)   | 8.33     | 11.09  | 10.63  | 16.49  | 400IV/500PO     | 1.043          | 0.937           | 1.165 | 0.000        | 0.020 | 0.512 |
|                          |          |        |        |        | 400IV/750PO     | 0.672          | 0.605           | 0.749 | 0.000        | 0.000 | 0.000 |
|                          |          |        |        |        | 300IV/500PO     | 0.784          | 0.704           | 0.875 | 0.625        | 0.000 | 0.001 |
|                          |          |        |        |        | 300IV/750PO     | 0.505          | 0.455           | 0.563 | 1.000        | 0.000 | 0.000 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.026          | 0.952           | 1.108 | 0.000        | 0.001 | 0.565 |
| AUC(0-24h)/DOSE<br>(h/L) | 0.0278   | 0.0277 | 0.0213 | 0.0220 | 400IV/300IV     | 0.998          | 0.909           | 1.096 | 0.000        | 0.001 | 0.975 |
|                          |          |        |        |        | 750PO/500PO     | 1.035          | 0.917           | 1.167 | 0.001        | 0.023 | 0.633 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.780          | 0.722           | 0.841 | NA           | NA    | NA    |
| AUC(0-inf)<br>(mg*h/L)   | 8.62     | 11.33  | 10.87  | 16.90  | 400IV/500PO     | 1.042          | 0.933           | 1.167 | 0.000        | 0.022 | 0.534 |
|                          |          |        |        |        | 400IV/750PO     | 0.670          | 0.602           | 0.749 | 0.994        | 0.000 | 0.000 |
|                          |          |        |        |        | 300IV/500PO     | 0.793          | 0.711           | 0.888 | 0.553        | 0.000 | 0.002 |
|                          |          |        |        |        | 300IV/750PO     | 0.510          | 0.458           | 0.570 | 1.000        | 0.000 | 0.000 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.031          | 0.954           | 1.115 | 0.000        | 0.002 | 0.503 |
| AUC(0-inf)/DOSE<br>(h/L) | 0.0287   | 0.0283 | 0.0217 | 0.0220 | 400IV/300IV     | 0.985          | 0.895           | 1.084 | 0.001        | 0.001 | 0.794 |
|                          |          |        |        |        | 750PO/500PO     | 1.036          | 0.916           | 1.173 | 0.001        | 0.027 | 0.627 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.776          | 0.717           | 0.838 | NA           | NA    | NA    |
| Cmax<br>(mg/L)           | 3.27     | 4.00   | 2.79   | 3.90   | 400IV/500PO     | 1.431          | 1.257           | 1.643 | 0.000        | 0.987 | 0.000 |
|                          |          |        |        |        | 400IV/750PO     | 1.026          | 0.896           | 1.189 | 0.002        | 0.041 | 0.755 |
|                          |          |        |        |        | 300IV/500PO     | 1.171          | 1.032           | 1.342 | 0.000        | 0.376 | 0.041 |
|                          |          |        |        |        | 300IV/750PO     | 0.840          | 0.735           | 0.971 | 0.275        | 0.000 | 0.053 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.551          | 1.412           | 1.712 | 0.000        | 1.000 | 0.000 |
| Cmax/DOSE<br>(1/L)       | 0.0109   | 0.0100 | 0.0056 | 0.0052 | 400IV/300IV     | 0.917          | 0.839           | 1.001 | 0.007        | 0.000 | 0.103 |
|                          |          |        |        |        | 750PO/500PO     | 0.929          | 0.782           | 1.102 | 0.075        | 0.009 | 0.471 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.516          | 0.467           | 0.567 | NA           | NA    | NA    |
| HALF-LIFE<br>(h)         | 5.67     | 4.68   | 4.59   | 4.58   | 400IV/500PO     | 1.018          | 0.881           | 1.177 | 0.004        | 0.032 | 0.836 |
|                          |          |        |        |        | 400IV/750PO     | 1.021          | 0.883           | 1.181 | 0.004        | 0.035 | 0.806 |
|                          |          |        |        |        | 300IV/500PO     | 1.233          | 1.081           | 1.411 | 0.000        | 0.635 | 0.011 |
|                          |          |        |        |        | 300IV/750PO     | 1.237          | 1.085           | 1.416 | 0.000        | 0.651 | 0.010 |
|                          |          |        |        |        | 400IV/300IV     | 0.825          | 0.723           | 0.940 | 0.344        | 0.000 | 0.018 |
|                          |          |        |        |        | 750PO/500PO     | 0.997          | 0.861           | 1.154 | 0.009        | 0.020 | 0.965 |

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO < 80%.  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO > 120%.  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H0: TRUE RATIO = 100%.

(2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF DOSE-PROPORTIONALITY.  
 SEE APPENDIX II FOR DETAILS.

STATISTICAL SUMMARY

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000033

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY Q 9867)  
 PHARMACOKINETIC DETERMINATION OF DOSE EQUIVALENCY BETWEEN IV AND ORAL CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL DB9-050

TABLE 4: RESULTS OF STATISTICAL ANALYSIS OF PHARMACOKINETIC VARIABLES  
 EXCLUDING SUBJECT 1002

| VARIABLE                 | LS MEANS |        |        |        | RATIO           | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|--------------------------|----------|--------|--------|--------|-----------------|----------------|-----------------|-------|--------------|-------|-------|
|                          | 300IV    | 400IV  | 500PO  | 750PO  |                 |                | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-24h)<br>(mg*h/L)   | 8.33     | 11.09  | 10.63  | 16.49  | 400IV/500PO     | 1.043          | 0.937           | 1.165 | 0.000        | 0.020 | 0.512 |
|                          |          |        |        |        | 400IV/750PO     | 0.672          | 0.605           | 0.749 | 0.994        | 0.000 | 0.000 |
|                          |          |        |        |        | 300IV/500PO     | 0.784          | 0.704           | 0.875 | 0.625        | 0.000 | 0.001 |
|                          |          |        |        |        | 300IV/750PO     | 0.505          | 0.455           | 0.563 | 1.000        | 0.000 | 0.000 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.026          | 0.952           | 1.108 | 0.000        | 0.001 | 0.565 |
| AUC(0-24h)/DOSE<br>(h/L) | 0.0278   | 0.0277 | 0.0213 | 0.0220 | 400IV/300IV     | 0.998          | 0.909           | 1.096 | 0.000        | 0.001 | 0.975 |
|                          |          |        |        |        | 750PO/500PO     | 1.035          | 0.917           | 1.167 | 0.001        | 0.023 | 0.633 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.780          | 0.722           | 0.841 | NA           | NA    | NA    |
| AUC(0-inf)<br>(mg*h/L)   | 8.62     | 11.33  | 10.87  | 16.90  | 400IV/500PO     | 1.042          | 0.933           | 1.167 | 0.000        | 0.022 | 0.534 |
|                          |          |        |        |        | 400IV/750PO     | 0.670          | 0.602           | 0.749 | 0.994        | 0.000 | 0.000 |
|                          |          |        |        |        | 300IV/500PO     | 0.793          | 0.711           | 0.888 | 0.553        | 0.000 | 0.002 |
|                          |          |        |        |        | 300IV/750PO     | 0.510          | 0.458           | 0.570 | 1.000        | 0.000 | 0.000 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.031          | 0.954           | 1.115 | 0.000        | 0.002 | 0.508 |
| AUC(0-inf)/DOSE<br>(h/L) | 0.0287   | 0.0283 | 0.0217 | 0.0225 | 400IV/300IV     | 0.985          | 0.895           | 1.084 | 0.001        | 0.001 | 0.794 |
|                          |          |        |        |        | 750PO/500PO     | 1.036          | 0.916           | 1.173 | 0.001        | 0.027 | 0.627 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.776          | 0.717           | 0.838 | NA           | NA    | NA    |
| Cmax<br>(mg/L)           | 3.27     | 4.00   | 2.79   | 3.90   | 400IV/500PO     | 1.431          | 1.257           | 1.643 | 0.000        | 0.987 | 0.000 |
|                          |          |        |        |        | 400IV/750PO     | 1.026          | 0.896           | 1.189 | 0.002        | 0.041 | 0.755 |
|                          |          |        |        |        | 300IV/500PO     | 1.171          | 1.032           | 1.342 | 0.000        | 0.376 | 0.041 |
|                          |          |        |        |        | 300IV/750PO     | 0.840          | 0.735           | 0.971 | 0.275        | 0.000 | 0.053 |
|                          |          |        |        |        | 200IV/250PO (2) | 1.551          | 1.412           | 1.712 | 0.000        | 1.000 | 0.000 |
| Cmax/DOSE<br>(1/L)       | 0.0109   | 0.0100 | 0.0056 | 0.0052 | 400IV/300IV     | 0.917          | 0.839           | 1.001 | 0.007        | 0.000 | 0.103 |
|                          |          |        |        |        | 750PO/500PO     | 0.929          | 0.782           | 1.102 | 0.075        | 0.009 | 0.471 |
|                          |          |        |        |        | AVGPO/AVGIV     | 0.516          | 0.467           | 0.567 | NA           | NA    | NA    |
| HALF-LIFE<br>(h)         | 5.67     | 4.68   | 4.59   | 4.58   | 400IV/500PO     | 1.018          | 0.881           | 1.177 | 0.004        | 0.032 | 0.836 |
|                          |          |        |        |        | 400IV/750PO     | 1.021          | 0.883           | 1.181 | 0.004        | 0.035 | 0.806 |
|                          |          |        |        |        | 300IV/500PO     | 1.233          | 1.081           | 1.411 | 0.000        | 0.635 | 0.011 |
|                          |          |        |        |        | 300IV/750PO     | 1.237          | 1.085           | 1.416 | 0.000        | 0.651 | 0.010 |
|                          |          |        |        |        | 400IV/300IV     | 0.825          | 0.723           | 0.940 | 0.344        | 0.000 | 0.018 |
|                          |          |        |        |        | 750PO/500PO     | 0.997          | 0.861           | 1.154 | 0.009        | 0.020 | 0.969 |

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO < 80%.  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO > 120%.  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H0: TRUE RATIO = 100%.

(2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF DOSE-PROPORTIONALITY. SEE APPENDIX II FOR DETAILS.

STATISTICAL SUMMARY

06-00381

000033

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY O 9867)  
 PHARMACOKINETIC DETERMINATION OF DOSE EQUIVALENCY BETWEEN IV AND ORAL CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL D89-050

TABLE 4: RESULTS OF STATISTICAL ANALYSIS OF PHARMACOKINETIC VARIABLES  
 EXCLUDING SUBJECT 1002

| VARIABLE                    | LS MEANS |        |        |        | RATIO       | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|-----------------------------|----------|--------|--------|--------|-------------|----------------|-----------------|-------|--------------|-------|-------|
|                             | 300IV    | 400IV  | 500PO  | 750PO  |             |                | LOWER           | UPPER | P80          | P120  | P100  |
| Xu(0-24h)<br>(mg)           | 185.46   | 223.29 | 189.25 | 279.90 | 400IV/500PO | 1.180          | 0.828           | 1.775 | 0.036        | 0.469 | 0.440 |
|                             |          |        |        |        | 400IV/750PO | 0.798          | 0.559           | 1.207 | 0.505        | 0.052 | 0.335 |
|                             |          |        |        |        | 300IV/500PO | 0.980          | 0.699           | 1.463 | 0.162        | 0.184 | 0.924 |
|                             |          |        |        |        | 300IV/750PO | 0.663          | 0.471           | 0.995 | 0.797        | 0.014 | 0.097 |
| Xu(0-24h)/DOSE<br>(%)       | 61.82    | 55.82  | 37.85  | 37.32  | 400IV/300IV | 0.903          | 0.677           | 1.194 | 0.235        | 0.047 | 0.537 |
|                             |          |        |        |        | 750PO/500PO | 0.986          | 0.626           | 1.549 | 0.213        | 0.226 | 0.956 |
| Cl <sub>r</sub><br>(ml/min) | 382.25   | 349.08 | 300.70 | 290.81 | 400IV/500PO | 1.161          | 0.788           | 1.753 | 0.057        | 0.443 | 0.514 |
|                             |          |        |        |        | 400IV/750PO | 1.200          | 0.810           | 1.834 | 0.045        | 0.501 | 0.433 |
|                             |          |        |        |        | 300IV/500PO | 1.271          | 0.880           | 1.902 | 0.021        | 0.603 | 0.275 |
|                             |          |        |        |        | 300IV/750PO | 1.314          | 0.905           | 1.990 | 0.016        | 0.658 | 0.222 |
|                             |          |        |        |        | 400IV/300IV | 0.913          | 0.639           | 1.290 | 0.260        | 0.093 | 0.654 |
|                             |          |        |        |        | 750PO/500PO | 0.967          | 0.622           | 1.495 | 0.228        | 0.197 | 0.894 |

06-00382

000034

- (1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO < 80%.  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO > 120%.  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 100%.  
 (2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF DOSE-PROPORTIONALITY.  
 SEE APPENDIX II FOR DETAILS.

CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 PHARMACOKINETIC DETERMINATION OF DOSE EQUIVALENCY BETWEEN IV AND ORAL CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL D89-050

TABLE 4: RESULTS OF STATISTICAL ANALYSIS OF PHARMACOKINETIC VARIABLES  
 EXCLUDING SUBJECT 1002

| VARIABLE                    | LS MEANS |        |        |        | RATIO       | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|-----------------------------|----------|--------|--------|--------|-------------|----------------|-----------------|-------|--------------|-------|-------|
|                             | 300IV    | 400IV  | 500PO  | 750PO  |             |                | LOWER           | UPPER | P80          | P120  | P100  |
| Xu(0-24h)<br>(mg)           | 185.46   | 223.29 | 189.25 | 279.90 | 400IV/300PO | 1.180          | 0.828           | 1.775 | 0.036        | 0.469 | 0.440 |
|                             |          |        |        |        | 400IV/750PO | 0.798          | 0.559           | 1.207 | 0.505        | 0.052 | 0.335 |
|                             |          |        |        |        | 300IV/500PO | 0.980          | 0.699           | 1.463 | 0.162        | 0.184 | 0.924 |
|                             |          |        |        |        | 300IV/750PO | 0.663          | 0.471           | 0.995 | 0.797        | 0.014 | 0.097 |
| Xu(0-24h)/DOSE<br>(%)       | 61.82    | 55.82  | 37.85  | 37.32  | 400IV/300IV | 0.903          | 0.677           | 1.194 | 0.235        | 0.047 | 0.537 |
|                             |          |        |        |        | 750PO/500PO | 0.986          | 0.626           | 1.549 | 0.213        | 0.226 | 0.956 |
| Cl <sub>r</sub><br>(ml/min) | 382.25   | 349.08 | 300.70 | 290.81 | 400IV/500PO | 1.161          | 0.788           | 1.753 | 0.057        | 0.443 | 0.514 |
|                             |          |        |        |        | 400IV/750PO | 1.200          | 0.810           | 1.834 | 0.045        | 0.501 | 0.433 |
|                             |          |        |        |        | 300IV/500PO | 1.271          | 0.880           | 1.902 | 0.021        | 0.603 | 0.275 |
|                             |          |        |        |        | 300IV/750PO | 1.314          | 0.905           | 1.990 | 0.016        | 0.658 | 0.222 |
|                             |          |        |        |        | 400IV/300IV | 0.913          | 0.639           | 1.290 | 0.260        | 0.093 | 0.654 |
|                             |          |        |        |        | 750PO/500PO | 0.967          | 0.622           | 1.495 | 0.228        | 0.197 | 0.894 |

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO < 80%.  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO > 120%.  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 100%.  
 (2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF DOSE-PROPORTIONALITY.  
 SEE APPENDIX II FOR DETAILS.

06-00382

000034

Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) PK Study D89-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN        | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|-------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=300mg_Cipro_IV ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 4.09514714  | 0.10705556        | 11 | 0.35506314         | 8.670  | 3.67917586    | 4.97933576    |
| AUC(O-12) (mg*h/L)                           | 7.37873527  | 0.38843270        | 11 | 1.28828551         | 17.459 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.02459578  | 0.00129478        | 11 | 0.00429429         | 17.459 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.81422478  | 0.10707999        | 11 | 0.35514414         | 19.576 |               |               |
| AUC(O-24) (mg*h/L)                           | 8.28179948  | 0.45153420        | 11 | 1.49756951         | 18.083 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02760600  | 0.00150511        | 11 | 0.00499190         | 18.083 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 2.03716384  | 0.12507408        | 11 | 0.41482379         | 20.363 |               |               |
| AUC(O-inf) (mg*h/L)                          | 8.58221566  | 0.46038500        | 11 | 1.52692429         | 17.792 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02860739  | 0.00153462        | 11 | 0.00508975         | 17.792 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 2.11040606  | 0.12707002        | 11 | 0.42144359         | 19.970 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.22181819  | 0.20141237        | 11 | 0.66800925         | 20.734 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.01073939  | 0.00067137        | 11 | 0.00222670         | 20.734 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.78914385  | 0.04933102        | 11 | 0.16361247         | 20.733 |               |               |
| Time of Peak Conc. (h)                       | 0.95454545  | 0.04545455        | 11 | 0.15075567         | 15.793 |               |               |
| Elimination Half-Life (h)                    | 5.70871154  | 0.51373759        | 11 | 1.70387481         | 29.847 |               |               |
| ----- Treatment Regimen=400mg_Cipro_IV ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 5.46019618  | 0.14274075        | 11 | 0.47341752         | 8.670  | 4.90556782    | 6.63911434    |
| AUC(O-12) (mg*h/L)                           | 10.05712686 | 0.36781642        | 11 | 1.21990905         | 12.130 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.02514282  | 0.00091954        | 11 | 0.00304977         | 12.130 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.85595806  | 0.08535705        | 11 | 0.28309732         | 15.253 |               |               |
| AUC(O-24) (mg*h/L)                           | 11.11767813 | 0.45058776        | 11 | 1.49443055         | 13.442 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02779420  | 0.00112647        | 11 | 0.00373608         | 13.442 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 2.05277939  | 0.10303596        | 11 | 0.34173162         | 16.647 |               |               |
| AUC(O-inf) (mg*h/L)                          | 11.36310482 | 0.47860878        | 11 | 1.58736575         | 13.969 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02840776  | 0.00119652        | 11 | 0.00396841         | 13.969 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 2.09851407  | 0.10885818        | 11 | 0.36104174         | 17.205 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.98000001  | 0.18506019        | 11 | 0.61377523         | 15.421 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00995000  | 0.00046265        | 11 | 0.00153444         | 15.421 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.73372925  | 0.03984705        | 11 | 0.13215773         | 18.012 |               |               |
| Time of Peak Conc. (h)                       | 0.90909091  | 0.06098367        | 11 | 0.20225996         | 22.249 |               |               |
| Elimination Half-Life (h)                    | 4.66829826  | 0.13171936        | 11 | 0.43686370         | 9.358  |               |               |

MEAN PARAMETER VALUES

06-00376

000028

Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) PK Study D89-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN        | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|-------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=500mg_Cipro_PO ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 6.82524523  | 0.17842594        | 11 | 0.59177190         | 8.670  | 6.13195977    | 8.29889293    |
| AUC(O-12) (mg*h/L)                           | 9.31195104  | 0.66623341        | 11 | 2.20964626         | 23.729 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.01862390  | 0.00133247        | 11 | 0.00441929         | 23.729 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.37767454  | 0.10978761        | 11 | 0.36412432         | 26.430 |               |               |
| AUC(O-24) (mg*h/L)                           | 10.46997683 | 0.75492187        | 11 | 2.50379258         | 23.914 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02093995  | 0.00150984        | 11 | 0.00500759         | 23.914 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 1.54928766  | 0.12443853        | 11 | 0.41271592         | 26.639 |               |               |
| AUC(O-inf) (mg*h/L)                          | 10.72297681 | 0.76896047        | 11 | 2.55035336         | 23.784 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02144595  | 0.00153792        | 11 | 0.00510071         | 23.784 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 1.58680811  | 0.12693186        | 11 | 0.42098534         | 26.530 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 2.71727271  | 0.25149766        | 11 | 0.83412339         | 30.697 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00543455  | 0.00050300        | 11 | 0.00166825         | 30.697 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.40206056  | 0.03977764        | 11 | 0.13192751         | 32.813 |               |               |
| Time of Peak Conc. (h)                       | 1.09090909  | 0.10804422        | 11 | 0.35834214         | 32.848 |               |               |
| Elimination Half-Life (h)                    | 4.64957975  | 0.16798448        | 11 | 0.55714150         | 11.983 |               |               |
| ----- Treatment Regimen=750mg_Cipro_PO ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384            | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 10.23736784 | 0.26763891        | 11 | 0.88765785         | 8.670  | 9.19793966    | 12.44833939   |
| AUC(O-12) (mg*h/L)                           | 14.57386503 | 1.19679235        | 11 | 3.96931117         | 27.236 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.01943182  | 0.00159572        | 11 | 0.00529241         | 27.236 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.42741380  | 0.12127791        | 11 | 0.40223334         | 28.179 |               |               |
| AUC(O-24) (mg*h/L)                           | 16.36739613 | 1.37724548        | 11 | 4.56780651         | 27.908 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02182319  | 0.00183633        | 11 | 0.00609041         | 27.908 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 1.60428117  | 0.14104217        | 11 | 0.46778397         | 29.158 |               |               |
| AUC(O-inf) (mg*h/L)                          | 16.78681553 | 1.43225400        | 11 | 4.75024912         | 28.297 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02238242  | 0.00190967        | 11 | 0.00633367         | 28.297 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 1.64590867  | 0.14721490        | 11 | 0.48825658         | 29.665 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.77000001  | 0.45808096        | 11 | 1.51928267         | 40.299 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00502667  | 0.00061077        | 11 | 0.00202571         | 40.299 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.36853609  | 0.04383503        | 11 | 0.14538434         | 39.449 |               |               |
| Time of Peak Conc. (h)                       | 1.29545455  | 0.11588681        | 11 | 0.38435306         | 29.669 |               |               |
| Elimination Half-Life (h)                    | 4.64213930  | 0.18870753        | 11 | 0.62587206         | 13.482 |               |               |

06-00377

000029

Ciprofloxacin IV/PD (BAY q 3939/BAY o 9867) PK Study D89-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN        | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|-------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=500mg_Cipro_PD ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 6.82524523  | 0.17842594        | 11 | 0.59177190         | 8.670  | 6.13195977    | 8.29889293    |
| AUC(0-12) (mg*h/L)                           | 9.31195104  | 0.66623341        | 11 | 2.20964626         | 23.729 |               |               |
| AUC(0-12) / Dose (h/L)                       | 0.01862390  | 0.00133247        | 11 | 0.00441929         | 23.729 |               |               |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 1.37767454  | 0.10978761        | 11 | 0.36412432         | 26.430 |               |               |
| AUC(0-24) (mg*h/L)                           | 10.46997683 | 0.75492187        | 11 | 2.50379258         | 23.914 |               |               |
| AUC(0-24) / Dose (h/L)                       | 0.02093995  | 0.00150984        | 11 | 0.00500759         | 23.914 |               |               |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 1.54928766  | 0.12443853        | 11 | 0.41271592         | 26.639 |               |               |
| AUC(0-inf) (mg*h/L)                          | 10.72297681 | 0.76896047        | 11 | 2.55035336         | 23.784 |               |               |
| AUC(0-inf) / Dose (h/L)                      | 0.02144595  | 0.00153792        | 11 | 0.00510071         | 23.784 |               |               |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 1.58680811  | 0.12693186        | 11 | 0.42098534         | 26.530 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 2.71727271  | 0.25149766        | 11 | 0.83412339         | 30.697 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00543455  | 0.00050300        | 11 | 0.00166825         | 30.697 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.40206056  | 0.03977764        | 11 | 0.13192751         | 32.813 |               |               |
| Time of Peak Conc. (h)                       | 1.09090909  | 0.10804422        | 11 | 0.35834214         | 32.848 |               |               |
| Elimination Half-Life (h)                    | 4.64957975  | 0.16798448        | 11 | 0.55714150         | 11.983 |               |               |
| ----- Treatment Regimen=750mg_Cipro_PD ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 10.23786784 | 0.26763891        | 11 | 0.88765785         | 8.670  | 9.19793966    | 12.44833939   |
| AUC(0-12) (mg*h/L)                           | 14.57386503 | 1.19679235        | 11 | 3.96931117         | 27.236 |               |               |
| AUC(0-12) / Dose (h/L)                       | 0.01943182  | 0.00159572        | 11 | 0.00529241         | 27.236 |               |               |
| AUC(0-12) / (Dose/BW) (kg*h/L)               | 1.42741380  | 0.12127791        | 11 | 0.40223334         | 28.179 |               |               |
| AUC(0-24) (mg*h/L)                           | 16.36739613 | 1.37724548        | 11 | 4.56780651         | 27.908 |               |               |
| AUC(0-24) / Dose (h/L)                       | 0.02182319  | 0.00183633        | 11 | 0.00609041         | 27.908 |               |               |
| AUC(0-24) / (Dose/BW) (kg*h/L)               | 1.60428117  | 0.14104217        | 11 | 0.46778397         | 29.158 |               |               |
| AUC(0-inf) (mg*h/L)                          | 16.78681563 | 1.43225400        | 11 | 4.75024912         | 28.297 |               |               |
| AUC(0-inf) / Dose (h/L)                      | 0.02238242  | 0.00190967        | 11 | 0.00633367         | 28.297 |               |               |
| AUC(0-inf) / (Dose/BW) (kg*h/L)              | 1.64590867  | 0.14721490        | 11 | 0.48825658         | 29.665 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.77000001  | 0.45808096        | 11 | 1.51928267         | 40.299 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00502667  | 0.00061077        | 11 | 0.00202571         | 40.299 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.36853609  | 0.04383503        | 11 | 0.14538434         | 39.449 |               |               |
| Time of Peak Conc. (h)                       | 1.29545455  | 0.11588681        | 11 | 0.38435306         | 29.669 |               |               |
| Elimination Half-Life (h)                    | 4.64213930  | 0.18870753        | 11 | 0.62587206         | 13.482 |               |               |

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Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) PK Study DB9-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3A: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN        | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|-------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=500mg_Cipro_PO ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 6.82524523  | 0.17842594        | 11 | 0.59177190         | 8.670  | 6.13195977    | 8.29889293    |
| AUC(O-12) (mg*h/L)                           | 9.31195104  | 0.66623341        | 11 | 2.20964626         | 23.729 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.01862390  | 0.00133247        | 11 | 0.00441929         | 23.729 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.37767454  | 0.10978761        | 11 | 0.36412432         | 26.430 |               |               |
| AUC(O-24) (mg*h/L)                           | 10.46997683 | 0.75492187        | 11 | 2.50379258         | 23.914 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02093995  | 0.00150984        | 11 | 0.00500759         | 23.914 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 1.54928766  | 0.12443853        | 11 | 0.41271592         | 26.639 |               |               |
| AUC(O-inf) (mg*h/L)                          | 10.72297681 | 0.76896047        | 11 | 2.55035336         | 23.784 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02144595  | 0.00153792        | 11 | 0.00510071         | 23.784 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 1.58680811  | 0.12693186        | 11 | 0.42098534         | 26.530 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 2.71727271  | 0.25149766        | 11 | 0.83412339         | 30.697 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00543455  | 0.00050300        | 11 | 0.00166825         | 30.697 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.40206056  | 0.03977764        | 11 | 0.13192751         | 32.813 |               |               |
| Time of Peak Conc. (h)                       | 1.09090909  | 0.10804422        | 11 | 0.35834214         | 32.848 |               |               |
| Elimination Half-Life (h)                    | 4.64957975  | 0.16798448        | 11 | 0.55714150         | 11.983 |               |               |
| ----- Treatment Regimen=750mg_Cipro_PO ----- |             |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455 | 1.76198516        | 11 | 5.84384366         | 7.928  | 60.24900000   | 81.54000000   |
| Normalized Dose/BW (mg/kg)                   | 10.23786784 | 0.26763891        | 11 | 0.88765785         | 8.670  | 9.19793966    | 12.44833939   |
| AUC(O-12) (mg*h/L)                           | 14.57386503 | 1.19679235        | 11 | 3.96931117         | 27.236 |               |               |
| AUC(O-12) / Dose (h/L)                       | 0.01943182  | 0.00159572        | 11 | 0.00529241         | 27.236 |               |               |
| AUC(O-12) / (Dose/BW) (kg*h/L)               | 1.42741380  | 0.12127791        | 11 | 0.40223334         | 28.179 |               |               |
| AUC(O-24) (mg*h/L)                           | 16.36739613 | 1.37724548        | 11 | 4.56780651         | 27.908 |               |               |
| AUC(O-24) / Dose (h/L)                       | 0.02182319  | 0.00183633        | 11 | 0.00609041         | 27.908 |               |               |
| AUC(O-24) / (Dose/BW) (kg*h/L)               | 1.60428117  | 0.14104217        | 11 | 0.46778397         | 29.158 |               |               |
| AUC(O-inf) (mg*h/L)                          | 16.78681563 | 1.43225400        | 11 | 4.75024912         | 28.297 |               |               |
| AUC(O-inf) / Dose (h/L)                      | 0.02238242  | 0.00190967        | 11 | 0.00633367         | 28.297 |               |               |
| AUC(O-inf) / (Dose/BW) (kg*h/L)              | 1.64590867  | 0.14721490        | 11 | 0.48825658         | 29.665 |               |               |
| C <sub>MAX</sub> (mg/L)                      | 3.77000001  | 0.45808096        | 11 | 1.51928267         | 40.299 |               |               |
| C <sub>MAX</sub> / Dose (1/L)                | 0.00502667  | 0.00061077        | 11 | 0.00202571         | 40.299 |               |               |
| C <sub>MAX</sub> / (Dose/BW) (kg/L)          | 0.36853609  | 0.04383503        | 11 | 0.14538434         | 39.449 |               |               |
| Time of Peak Conc. (h)                       | 1.29545455  | 0.11588681        | 11 | 0.38435306         | 29.669 |               |               |
| Elimination Half-Life (h)                    | 4.64213930  | 0.18870753        | 11 | 0.62587206         | 13.482 |               |               |

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17SEP90

CIPROFLOXACIN(BAY Q 3939)  
 PHARMACOKINETICS & TOLERANCE OF IV DOSES OF CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL DB9-021

TABLE 1A: SUMMARY OF DEMOGRAPHICS: CONTINUOUS VARIABLES

| SEQUENCE      | N  | AGE (YEARS) |     |     |     | HEIGHT (INS.) |     |      |      | WEIGHT (LBS.) |      |       |       |
|---------------|----|-------------|-----|-----|-----|---------------|-----|------|------|---------------|------|-------|-------|
|               |    | MEAN        | STD | MIN | MAX | MEAN          | STD | MIN  | MAX  | MEAN          | STD  | MIN   | MAX   |
| 200, 300, 400 | 4  | 24.3        | 2.2 | 22  | 27  | 69.5          | 1.3 | 68.0 | 71.0 | 162.0         | 15.7 | 146.0 | 176.0 |
| 400, 200, 300 | 4  | 24.5        | 4.1 | 20  | 30  | 73.0          | 1.4 | 71.0 | 74.0 | 192.3         | 7.8  | 182.0 | 201.0 |
| 300, 400, 200 | 4  | 24.8        | 4.1 | 21  | 30  | 70.9          | 2.8 | 67.0 | 73.0 | 160.5         | 12.7 | 142.0 | 171.0 |
| ALL           | 12 | 24.5        | 3.3 | 20  | 30  | 71.1          | 2.3 | 67.0 | 74.0 | 171.6         | 19.0 | 142.0 | 201.0 |

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18SEP90

CIPROFLOXACIN(BAY Q 3939)  
 PHARMACOKINETIC DETERMINATION OF DOSE EQUIVALENCY BETWEEN I.V. AND ORAL CIPROFLOXACIN IN HEALTHY VOLUNTEERS  
 PROTOCOL 089-050

TABLE 1A: SUMMARY OF DEMOGRAPHICS: CONTINUOUS VARIABLES

| SEQUENCE           | N  | AGE (YEARS) |     |     |     | HEIGHT (INS.) |     |      |      | WEIGHT (LBS.) |      |       |       |
|--------------------|----|-------------|-----|-----|-----|---------------|-----|------|------|---------------|------|-------|-------|
|                    |    | MEAN        | STD | MIN | MAX | MEAN          | STD | MIN  | MAX  | MEAN          | STD  | MIN   | MAX   |
| 300, 400, 500, 750 | 3  | 23.0        | 3.5 | 21  | 27  | 70.3          | 0.6 | 70.0 | 71.0 | 162.7         | 3.2  | 159.0 | 165.0 |
| 500, 300, 750, 400 | 3  | 29.3        | 1.5 | 27  | 30  | 69.8          | 2.3 | 67.5 | 72.0 | 167.0         | 13.1 | 152.0 | 176.0 |
| 400, 750, 300, 500 | 3  | 30.0        | 3.6 | 27  | 34  | 71.0          | 2.0 | 69.0 | 73.0 | 170.0         | 9.5  | 161.0 | 180.0 |
| 750, 500, 400, 300 | 3  | 28.3        | 5.9 | 24  | 35  | 68.5          | 1.8 | 66.5 | 70.0 | 144.3         | 12.7 | 133.0 | 158.0 |
| ALL                | 12 | 27.4        | 4.3 | 21  | 35  | 69.9          | 1.8 | 66.5 | 73.0 | 161.0         | 13.7 | 133.0 | 180.0 |

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19SEP90

CIPROFLOXACIN(BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 1A: SUMMARY OF DEMOGRAPHICS: CONTINUOUS VARIABLES

| SEQUENCE         | N  | AGE (YEARS) |     |     |     | HEIGHT (INS.) |     |      |      | WEIGHT (LBS.) |      |       |       |
|------------------|----|-------------|-----|-----|-----|---------------|-----|------|------|---------------|------|-------|-------|
|                  |    | MEAN        | STD | MIN | MAX | MEAN          | STD | MIN  | MAX  | MEAN          | STD  | MIN   | MAX   |
| 200/500, 400/750 | 7  | 29.6        | 6.9 | 20  | 39  | 69.6          | 2.7 | 66.0 | 73.5 | 167.6         | 24.3 | 131.0 | 202.0 |
| 400/750, 200/500 | 6  | 29.5        | 6.7 | 21  | 38  | 69.6          | 1.6 | 67.2 | 72.0 | 158.8         | 13.8 | 146.0 | 185.0 |
| ALL              | 13 | 29.5        | 6.5 | 20  | 39  | 69.6          | 2.2 | 66.0 | 73.5 | 163.6         | 19.9 | 131.0 | 202.0 |

05-00728

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Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) PK Study D89-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3B: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN         | STD ERROR OF MEAN | N  | STANDARD DEVIATION | C.V.   | MINIMUM VALUE | MAXIMUM VALUE |
|----------------------------------------------|--------------|-------------------|----|--------------------|--------|---------------|---------------|
| ----- Treatment Regimen=300mg_Cipro_IV ----- |              |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455  | 1.76198516        | 11 | 5.8438437          | 7.928  | 60.2490000    | 81.5400000    |
| Normalized Dose/BW (mg/kg)                   | 4.09514714   | 0.10705556        | 11 | 0.3550631          | 8.670  | 3.6791759     | 4.9793358     |
| Total Clearance (mL/min)                     | 602.80210169 | 38.26879393       | 11 | 126.9232306        | 21.056 |               |               |
| Total Clearance/BW (mL/(min*kg))             | 8.23303603   | 0.56813200        | 11 | 1.8842807          | 22.887 |               |               |
| 12Hr Amount Excreted (mg)                    | 175.86390000 | 23.15009343       | 11 | 76.7801738         | 43.659 |               |               |
| 12Hr Amt. Excreted / Dose(%)                 | 58.52130000  | 7.71669781        | 11 | 25.5933913         | 43.659 |               |               |
| 24Hr Amount Excreted (mg)                    | 187.54967273 | 23.15348143       | 11 | 76.7914105         | 40.945 |               |               |
| 24Hr Amt. Excreted / Dose(%)                 | 62.51655758  | 7.71782714        | 11 | 25.5971368         | 40.945 |               |               |
| Renal Clearance (mL/min)                     | 388.12382408 | 54.27087044       | 11 | 179.9961143        | 46.376 |               |               |
| Renal Clearance/BW (mL/(min*kg))             | 5.29728991   | 0.75180359        | 11 | 2.4934504          | 47.070 |               |               |
| ----- Treatment Regimen=400mg_Cipro_IV ----- |              |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455  | 1.76198516        | 11 | 5.8438437          | 7.928  | 60.2490000    | 81.5400000    |
| Normalized Dose/BW (mg/kg)                   | 5.46019618   | 0.14274075        | 11 | 0.4734175          | 8.670  | 4.9055678     | 6.639114      |
| Total Clearance (mL/min)                     | 596.97266510 | 24.62136137       | 11 | 81.6598175         | 13.679 |               |               |
| Total Clearance/BW (mL/(min*kg))             | 8.17013595   | 0.44912980        | 11 | 1.4895950          | 18.232 |               |               |
| 12Hr Amount Excreted (mg)                    | 213.86546364 | 43.29559508       | 11 | 143.5952440        | 67.143 |               |               |
| 12Hr Amt. Excreted / Dose(%)                 | 53.46636591  | 10.82389877       | 11 | 35.8988110         | 67.143 |               |               |
| 24Hr Amount Excreted (mg)                    | 228.00341818 | 43.56444395       | 11 | 144.4869148        | 63.371 |               |               |
| 24Hr Amt. Excreted / Dose(%)                 | 57.00085455  | 10.89111099       | 11 | 36.1217287         | 63.371 |               |               |
| Renal Clearance (mL/min)                     | 357.89673324 | 82.50712317       | 11 | 273.6451701        | 76.459 |               |               |
| Renal Clearance/BW (mL/(min*kg))             | 4.95765080   | 1.15886197        | 11 | 3.8435103          | 77.527 |               |               |
| ----- Treatment Regimen=500mg_Cipro_PO ----- |              |                   |    |                    |        |               |               |
| Body Weight (kg)                             | 73.71545455  | 1.76198516        | 11 | 5.8438437          | 7.928  | 60.2490000    | 81.5400000    |
| Normalized Dose/BW (mg/kg)                   | 6.82524523   | 0.17842594        | 11 | 0.5917719          | 8.670  | 6.1319598     | 8.298893      |
| Total Clearance (mL/min)                     | 817.13209042 | 56.94231082       | 11 | 188.8562797        | 23.112 |               |               |
| Total Clearance/BW (mL/(min*kg))             | 11.09596935  | 0.88328501        | 11 | 2.9295250          | 26.166 |               |               |
| 12Hr Amount Excreted (mg)                    | 173.46932727 | 25.31942397       | 11 | 83.9750292         | 48.409 |               |               |
| 12Hr Amt. Excreted / Dose(%)                 | 34.69386545  | 5.06388479        | 11 | 16.7950058         | 48.409 |               |               |
| 24Hr Amount Excreted (mg)                    | 193.97732727 | 26.72660097       | 11 | 88.6421073         | 45.697 |               |               |
| 24Hr Amt. Excreted / Dose(%)                 | 38.79546545  | 5.34532019        | 11 | 17.7284215         | 45.697 |               |               |
| Renal Clearance (mL/min)                     | 310.74839793 | 42.99101420       | 11 | 142.5850635        | 45.884 |               |               |
| Renal Clearance/BW (mL/(min*kg))             | 4.24467024   | 0.59761575        | 11 | 1.9820672          | 46.695 |               |               |

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Ciprofloxacin IV/PO (BAY q 3939/BAY o 9867) PK Study 089-050-01  
 Pharmacokinetic Determination of Dose Equivalency between I.V. and Oral Ciprofloxacin in Healthy Volunteers  
 Table 3B: Derived Pharmacokinetic Variables: Summary Statistics (Excluding Subject 1002)

| LABEL                                        | MEAN         | STD ERROR<br>OF MEAN | N  | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|----------------------------------------------|--------------|----------------------|----|-----------------------|--------|------------------|------------------|
| ----- Treatment Regimen=750mg_Cipro_PO ----- |              |                      |    |                       |        |                  |                  |
| Body Weight (kg)                             | 73.71545455  | 1.76198516           | 11 | 5.8438437             | 7.928  | 60.2490000       | 81.540000        |
| Normalized Dose/BW (mg/kg)                   | 10.23786784  | 0.26763891           | 11 | 0.8876578             | 8.670  | 9.1979397        | 12.448339        |
| Total Clearance (mL/min)                     | 824.11807100 | 96.26493818          | 11 | 319.2746804           | 38.741 |                  |                  |
| Total Clearance/BW (mL/(min*kg))             | 11.17580987  | 1.22414170           | 11 | 4.0600187             | 36.329 |                  |                  |
| 12Hr Amount Excreted (mg)                    | 237.55743636 | 23.59238061          | 11 | 78.2470744            | 32.938 |                  |                  |
| 12Hr Amt. Excreted / Dose(%)                 | 31.67432485  | 3.14565075           | 11 | 10.4329433            | 32.938 |                  |                  |
| 24Hr Amount Excreted (mg)                    | 266.15866364 | 27.45364286          | 11 | 91.0534325            | 34.210 |                  |                  |
| 24Hr Amt. Excreted / Dose(%)                 | 35.48782182  | 3.66048571           | 11 | 12.1404577            | 34.210 |                  |                  |
| Renal Clearance (mL/min)                     | 279.59452890 | 34.11160702          | 11 | 113.1354015           | 40.464 |                  |                  |
| Renal Clearance/BW (mL/(min*kg))             | 3.83644522   | 0.47838061           | 11 | 1.5866090             | 41.356 |                  |                  |

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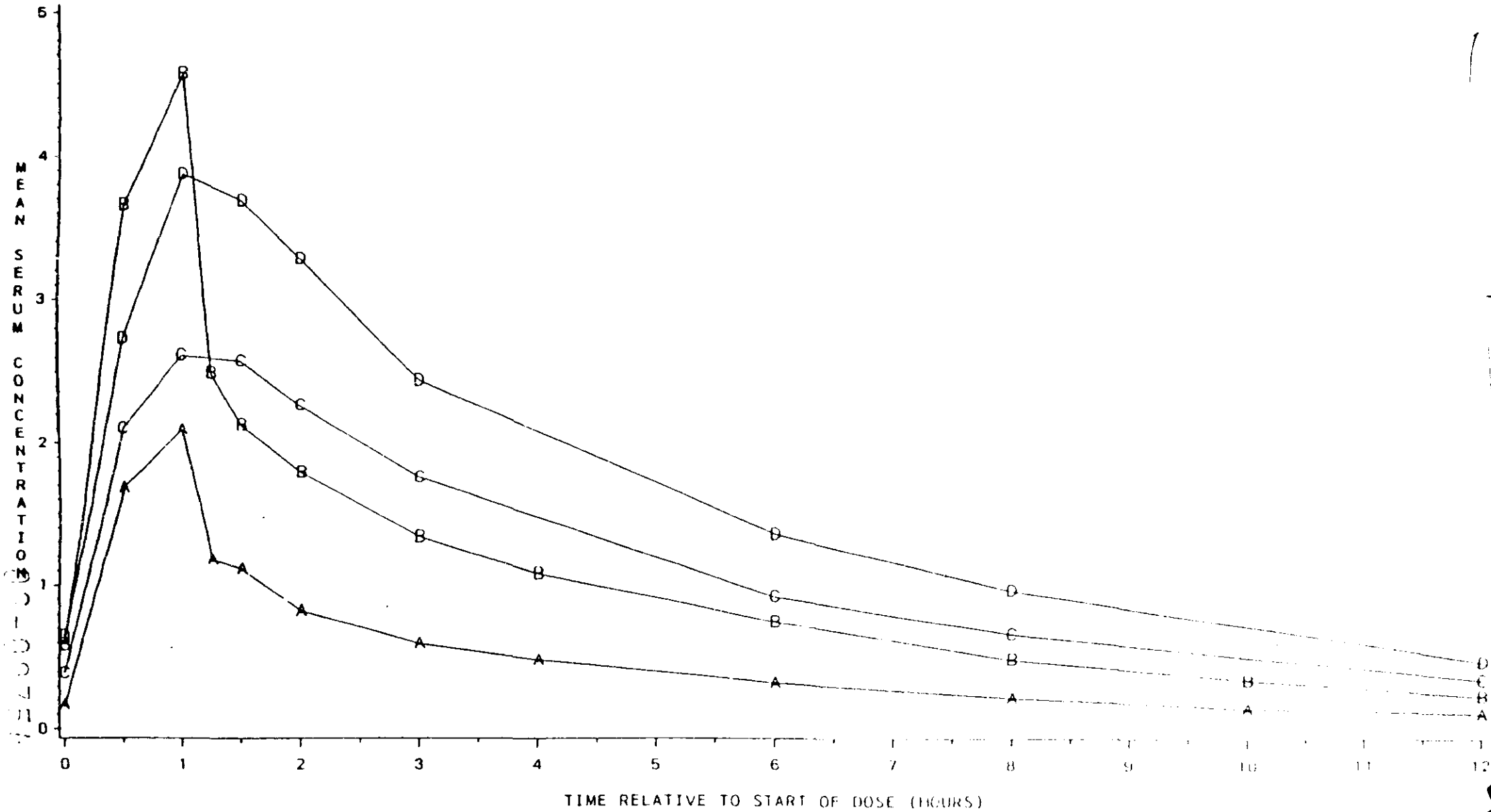
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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 1A: MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 5

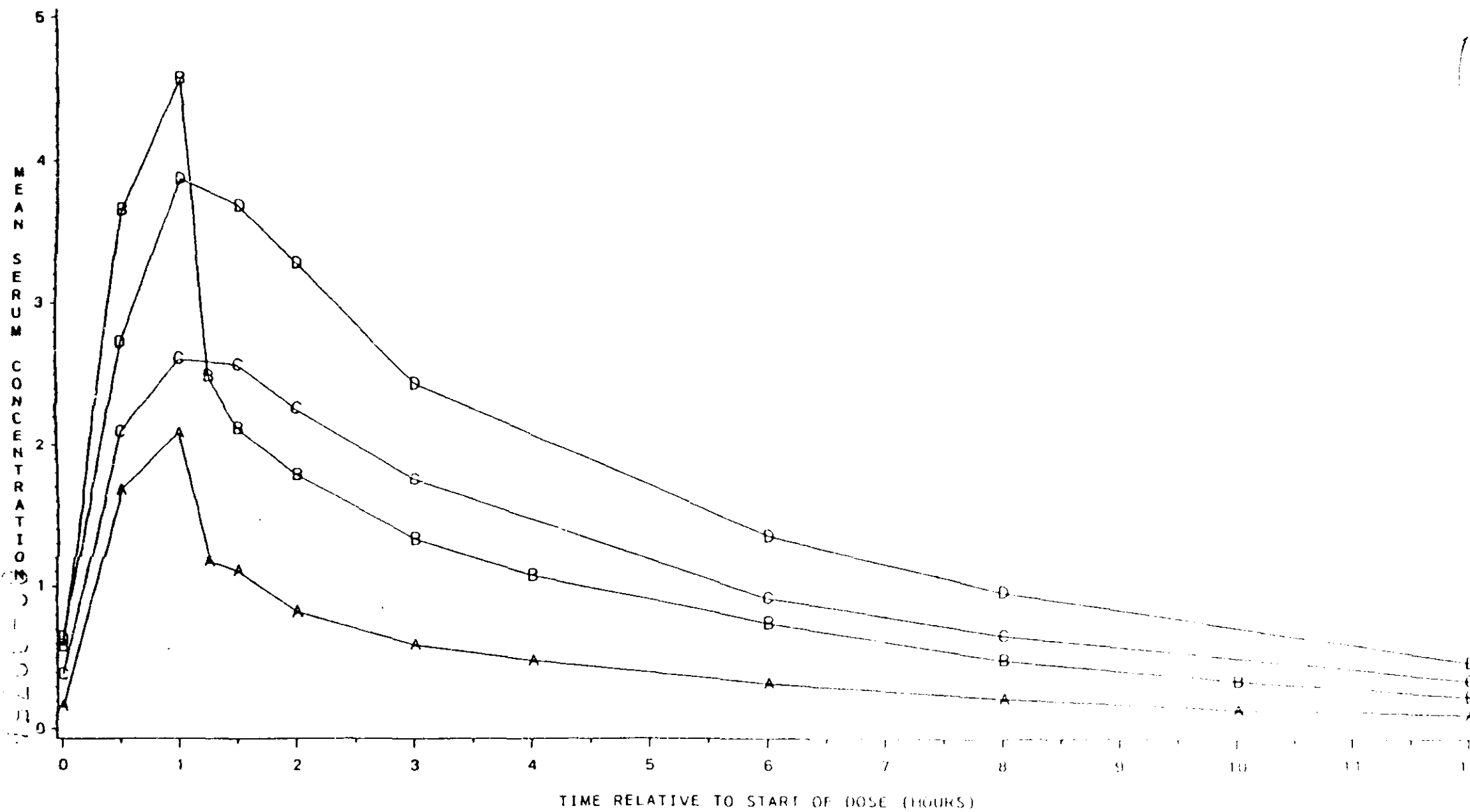


A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

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CIPROFLOXACIN IV/PO (BAY Q 3939/BAY Q 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 1A. MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 5



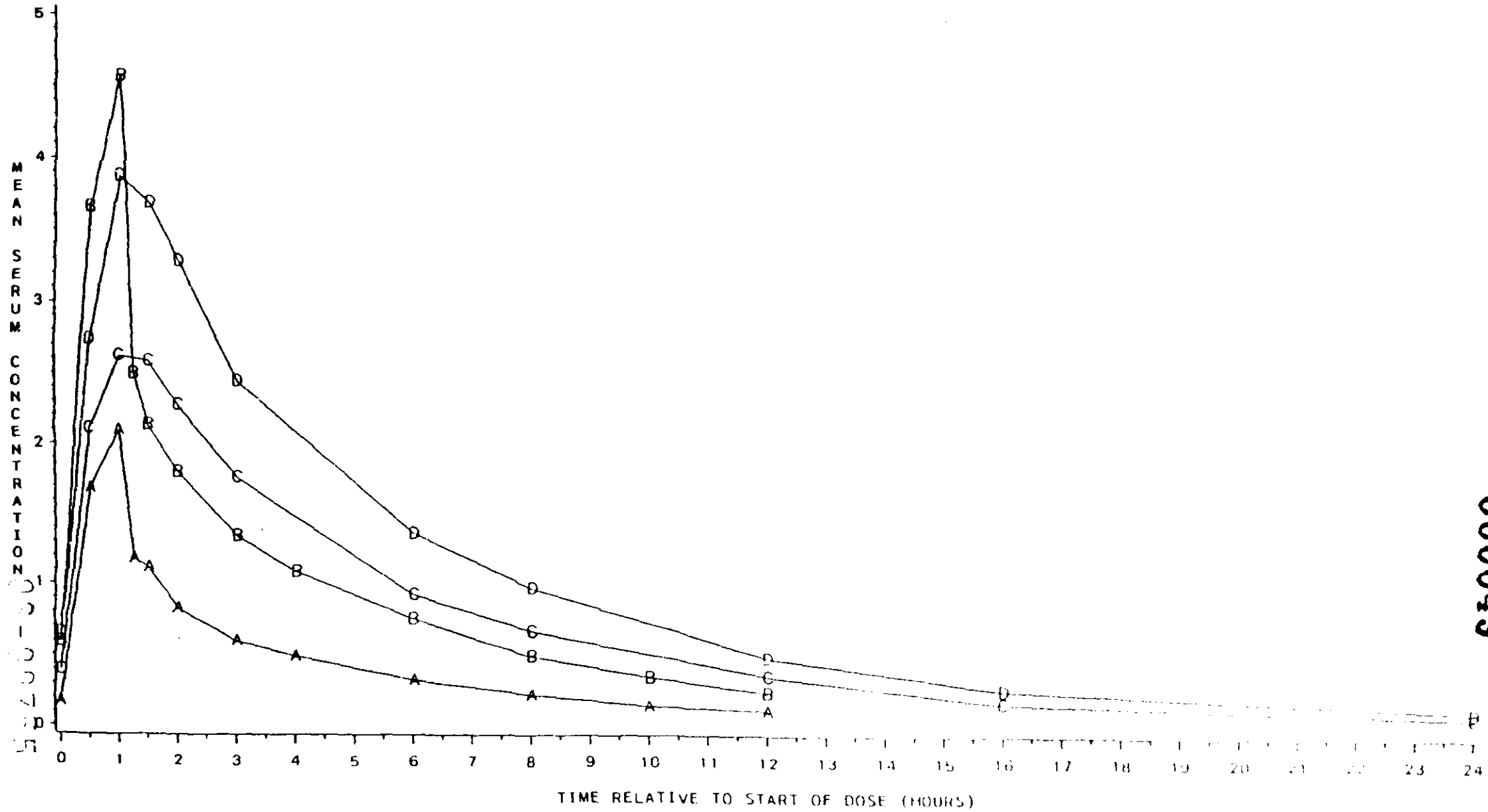
A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

*Cp vs T plus*

000048

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY O 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 1A: MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 5

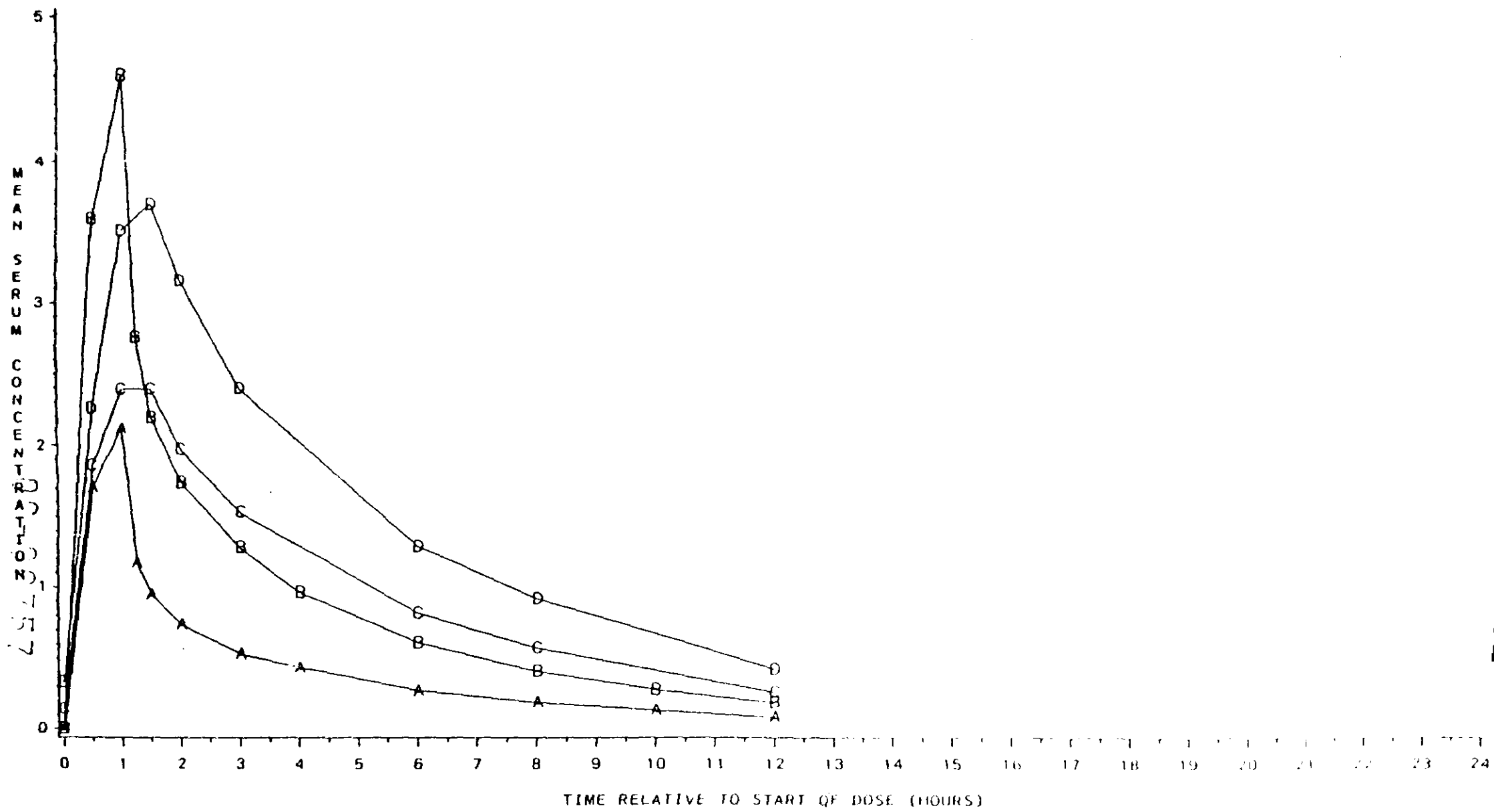


A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

000049

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY O 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 18: MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 1

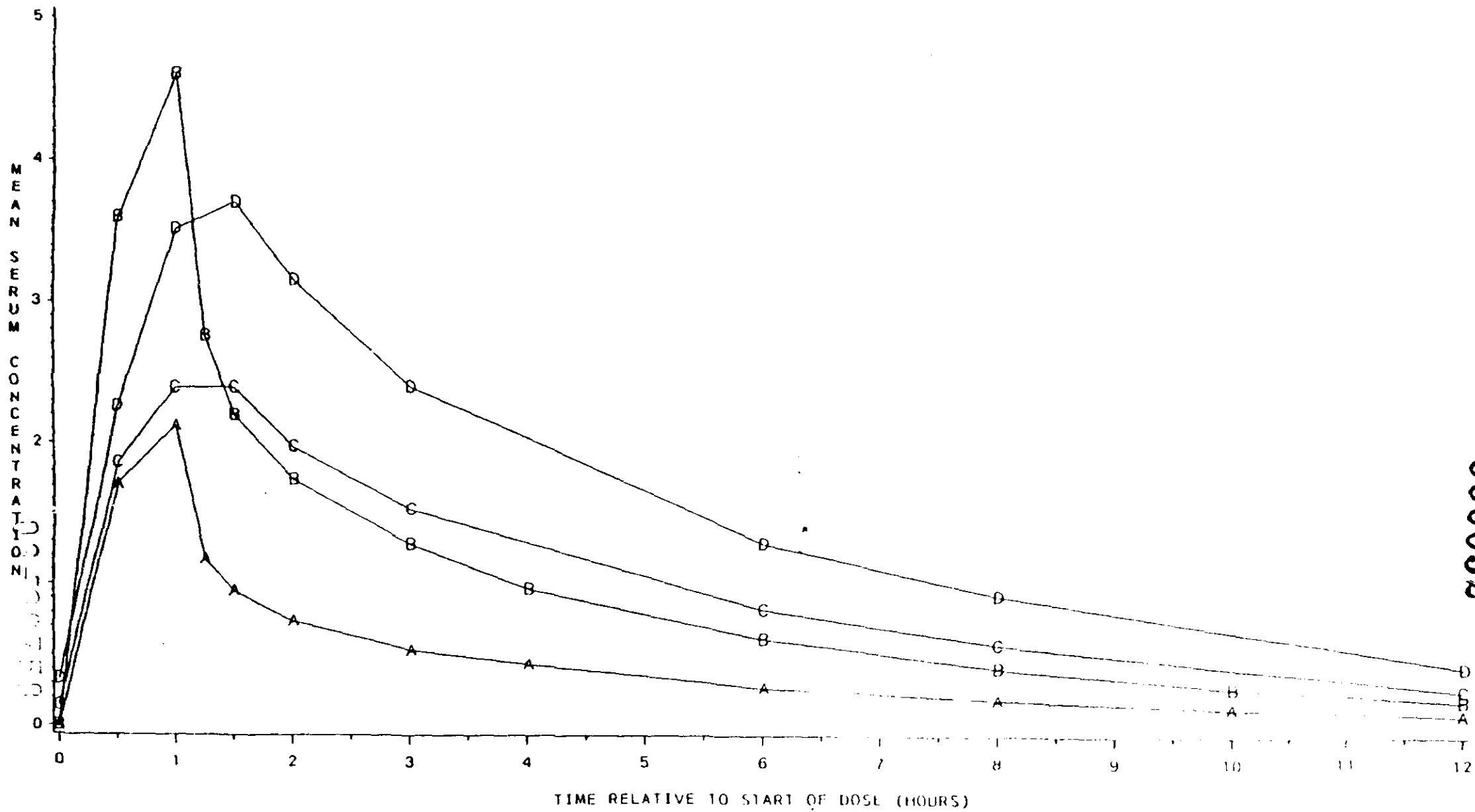


A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

000051

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY D 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 1B: MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 1

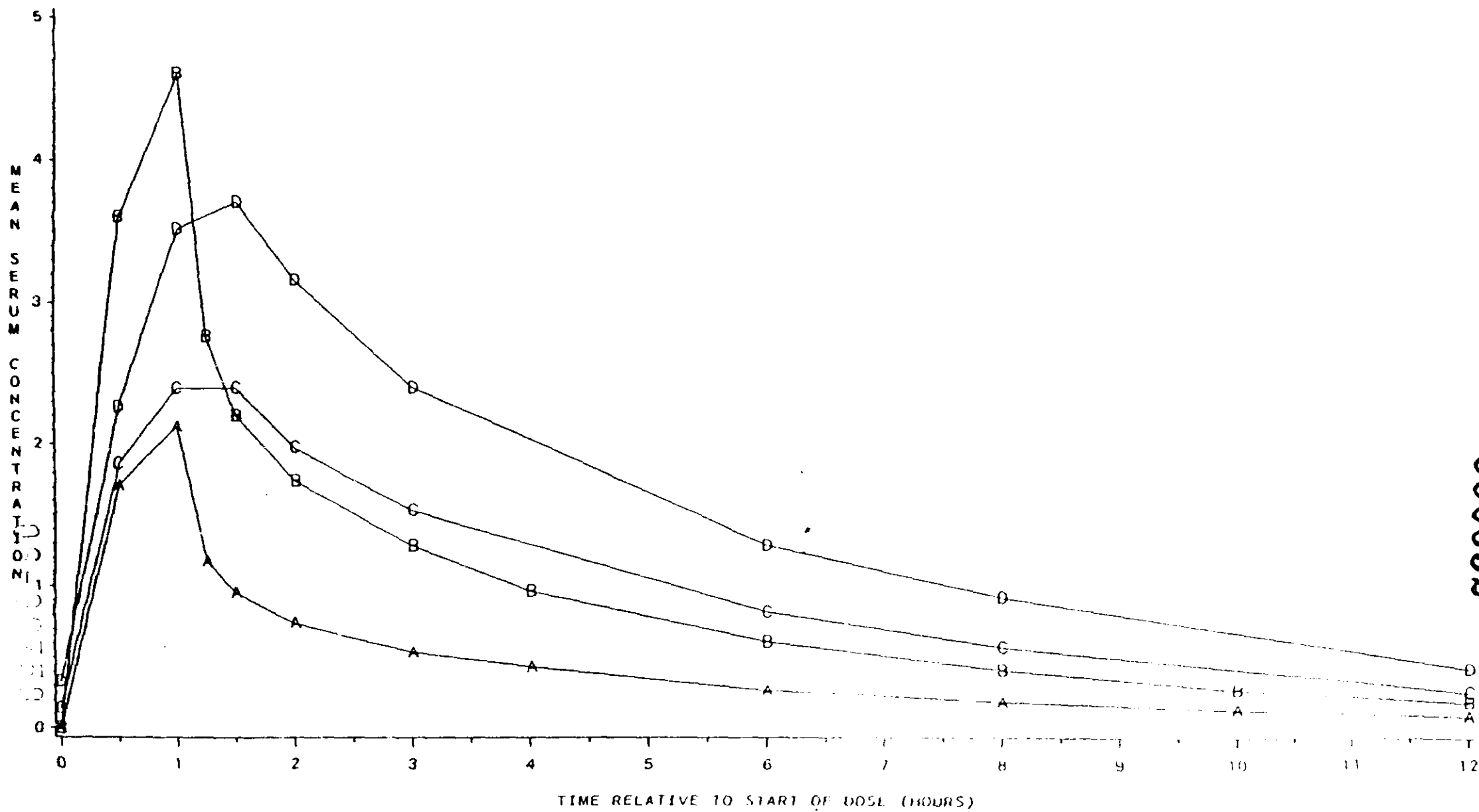


A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

000052

CIPROFLOXACIN IV/PO (BAY Q 3939/BAY O 9867)  
STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
PROTOCOL D90-012

FIGURE 1B: MEAN SERUM CIPROFLOXACIN CONCENTRATION (mg/L) VERSUS TIME  
EXCLUDING SUBJECTS 1002 AND 1014  
DOSING=DOSE 1



A=200mg IV, B=400mg IV, C=500mg PO, D=750mg PO

000052

CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 4A: RESULTS OF STATISTICAL ANALYSIS OF AUC(0-12h), C<sub>max</sub>, X<sub>u</sub>(0-12h), AND CL<sub>r</sub> AFTER THE FIFTH IV AND ORAL DOSES  
 EXCLUDING SUBJECTS 1002 AND 1014

| VARIABLE                           | LS MEANS |        |        |        | RATIO           | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|------------------------------------|----------|--------|--------|--------|-----------------|----------------|-----------------|-------|--------------|-------|-------|
|                                    | 200IV    | 400IV  | 500PO  | 750PO  |                 |                | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-12h)<br>(mg*h/L)             | 5.81     | 12.74  | 13.80  | 19.63  | 400IV/500PO     | 0.923          | 0.823           | 1.038 | 0.022        | 0.000 | 0.255 |
|                                    |          |        |        |        | 400IV/750PO     | 0.649          | 0.577           | 0.732 | 0.936        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/500PO     | 0.421          | 0.373           | 0.476 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/750PO     | 0.296          | 0.262           | 0.336 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/250PO (2) | 0.906          | 0.833           | 0.987 | 0.009        | 0.000 | 0.060 |
| AUC(0-12h)/DOSE<br>(h/L)           | 0.0291   | 0.0318 | 0.0276 | 0.0262 | 400IV/200IV     | 1.096          | 0.979           | 1.228 | 0.000        | 0.092 | 0.177 |
|                                    |          |        |        |        | 750PO/500PO     | 0.949          | 0.834           | 1.077 | 0.016        | 0.002 | 0.486 |
|                                    |          |        |        |        | AVGPO/AVGIV     | 0.883          | 0.811           | 0.960 | NA           | NA    | NA    |
| C <sub>max</sub><br>(mg/L)         | 2.10     | 4.59   | 2.99   | 4.36   | 400IV/500PO     | 1.533          | 1.331           | 1.789 | 0.000        | 0.997 | 0.000 |
|                                    |          |        |        |        | 400IV/750PO     | 1.051          | 0.910           | 1.231 | 0.001        | 0.081 | 0.570 |
|                                    |          |        |        |        | 200IV/500PO     | 0.702          | 0.607           | 0.821 | 0.919        | 0.000 | 0.001 |
|                                    |          |        |        |        | 200IV/750PO     | 0.481          | 0.415           | 0.565 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/250PO (2) | 1.489          | 1.343           | 1.661 | 0.000        | 0.999 | 0.000 |
| C <sub>max</sub> /DOSE<br>(1/L)    | 0.0105   | 0.0115 | 0.0060 | 0.0058 | 400IV/200IV     | 1.092          | 0.988           | 1.209 | 0.000        | 0.063 | 0.146 |
|                                    |          |        |        |        | 750PO/500PO     | 0.972          | 0.805           | 1.173 | 0.045        | 0.034 | 0.799 |
|                                    |          |        |        |        | AVGPO/AVGIV     | 0.537          | 0.482           | 0.596 | NA           | NA    | NA    |
| X <sub>u</sub> (0-12h)<br>(mg)     | 124.23   | 242.44 | 272.40 | 372.23 | 400IV/500PO     | 0.890          | 0.796           | 0.995 | 0.058        | 0.000 | 0.086 |
|                                    |          |        |        |        | 400IV/750PO     | 0.651          | 0.577           | 0.737 | 0.995        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/500PO     | 0.456          | 0.412           | 0.500 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/750PO     | 0.334          | 0.298           | 0.375 | 1.000        | 0.000 | 0.000 |
| X <sub>u</sub> (0-12h)/DOSE<br>(%) | 62.11    | 60.61  | 54.48  | 49.61  | IV/200IV        | 0.976          | 0.878           | 1.083 | 0.002        | 0.001 | 0.693 |
|                                    |          |        |        |        | 750PO/500PO     | 0.911          | 0.806           | 1.027 | 0.047        | 0.000 | 0.195 |
| CL <sub>r</sub><br>(ml/min)        | 377.03   | 346.30 | 351.98 | 353.35 | 400IV/500PO     | 0.984          | 0.811           | 1.185 | 0.033        | 0.040 | 0.883 |
|                                    |          |        |        |        | 400IV/750PO     | 1.039          | 0.848           | 1.271 | 0.019        | 0.010 | 0.748 |
|                                    |          |        |        |        | 200IV/500PO     | 1.071          | 0.906           | 1.269 | 0.003        | 0.111 | 0.489 |
|                                    |          |        |        |        | 200IV/750PO     | 1.131          | 0.936           | 1.363 | 0.001        | 0.211 | 0.251 |
|                                    |          |        |        |        | 400IV/200IV     | 0.918          | 0.762           | 1.093 | 0.008        | 0.000 | 0.433 |
|                                    |          |        |        |        | 750PO/500PO     | 0.947          | 0.784           | 1.130 | 0.000        | 0.009 | 0.621 |

STATISTICAL SUMMARY

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 80%  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 120%  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 100%  
 (2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF NORMALITY  
 SEE APPENDIX II FOR DETAILS.

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 4A: RESULTS OF STATISTICAL ANALYSIS OF AUC(0-12h), C<sub>max</sub>, X<sub>u</sub>(0-12h), AND CL<sub>r</sub> AFTER THE FIFTH IV AND ORAL DOSES  
 EXCLUDING SUBJECTS 1002 AND 1014

| VARIABLE                           | LS MEANS |        |        |        | RATIO           | RATIO ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|------------------------------------|----------|--------|--------|--------|-----------------|----------------|-----------------|-------|--------------|-------|-------|
|                                    | 200IV    | 400IV  | 500PO  | 750PO  |                 |                | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-12h)<br>(mg*h/L)             | 5.81     | 12.74  | 13.60  | 19.63  | 400IV/500PO     | 0.923          | 0.823           | 1.038 | 0.022        | 0.000 | 0.255 |
|                                    |          |        |        |        | 400IV/750PO     | 0.649          | 0.577           | 0.732 | 0.936        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/500PO     | 0.421          | 0.373           | 0.476 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/750PO     | 0.296          | 0.262           | 0.336 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/250PO (2) | 0.906          | 0.833           | 0.987 | 0.009        | 0.000 | 0.060 |
| AUC(0-12h)/DOSE<br>(h/L)           | 0.0291   | 0.0318 | 0.0276 | 0.0262 | 400IV/200IV     | 1.096          | 0.979           | 1.228 | 0.060        | 0.092 | 0.177 |
|                                    |          |        |        |        | 750PO/500PO     | 0.949          | 0.834           | 1.077 | 0.016        | 0.002 | 0.486 |
|                                    |          |        |        |        | AVGPO/AVGIV     | 0.883          | 0.811           | 0.960 | NA           | NA    | NA    |
| C <sub>max</sub><br>(mg/L)         | 2.10     | 4.59   | 2.99   | 4.36   | 400IV/500PO     | 1.533          | 1.331           | 1.789 | 0.000        | 0.997 | 0.000 |
|                                    |          |        |        |        | 400IV/750PO     | 1.051          | 0.910           | 1.231 | 0.001        | 0.081 | 0.570 |
|                                    |          |        |        |        | 200IV/500PO     | 0.702          | 0.607           | 0.821 | 0.919        | 0.000 | 0.001 |
|                                    |          |        |        |        | 200IV/750PO     | 0.481          | 0.415           | 0.565 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/250PO (2) | 1.489          | 1.343           | 1.661 | 0.000        | 0.959 | 0.000 |
| C <sub>max</sub> /DOSE<br>(1/L)    | 0.0105   | 0.0115 | 0.0060 | 0.0058 | 400IV/200IV     | 1.092          | 0.988           | 1.209 | 0.060        | 0.063 | 0.146 |
|                                    |          |        |        |        | 750PO/500PO     | 0.972          | 0.805           | 1.173 | 0.045        | 0.034 | 0.799 |
|                                    |          |        |        |        | AVGPO/AVGIV     | 0.537          | 0.482           | 0.596 | NA           | NA    | NA    |
| X <sub>u</sub> (0-12h)<br>(mg)     | 124.23   | 242.44 | 272.40 | 372.23 | 400IV/500PO     | 0.890          | 0.796           | 0.995 | 0.058        | 0.000 | 0.086 |
|                                    |          |        |        |        | 400IV/750PO     | 0.651          | 0.577           | 0.737 | 0.995        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/500PO     | 0.456          | 0.412           | 0.506 | 1.000        | 0.000 | 0.000 |
|                                    |          |        |        |        | 200IV/750PO     | 0.334          | 0.298           | 0.375 | 1.000        | 0.000 | 0.000 |
| X <sub>u</sub> (0-12h)/DOSE<br>(%) | 62.11    | 60.61  | 54.48  | 49.03  | IV/200IV        | 0.976          | 0.878           | 1.083 | 0.002        | 0.001 | 0.693 |
|                                    |          |        |        |        | 750PO/500PO     | 0.911          | 0.806           | 1.027 | 0.045        | 0.000 | 0.195 |
| CL <sub>r</sub><br>(ml/min)        | 377.03   | 346.30 | 351.98 | 333.35 | 400IV/500PO     | 0.984          | 0.813           | 1.185 | 0.033        | 0.040 | 0.883 |
|                                    |          |        |        |        | 400IV/750PO     | 1.039          | 0.848           | 1.271 | 0.013        | 0.116 | 0.748 |
|                                    |          |        |        |        | 200IV/500PO     | 1.071          | 0.906           | 1.263 | 0.003        | 0.111 | 0.489 |
|                                    |          |        |        |        | 200IV/750PO     | 1.131          | 0.916           | 1.361 | 0.001        | 0.291 | 0.251 |
|                                    |          |        |        |        | 400IV/200IV     | 0.518          | 0.762           | 1.139 | 0.113        | 0.009 | 0.430 |
|                                    |          |        |        |        | 750PO/500PO     | 0.947          | 0.784           | 1.139 | 0.000        | 0.019 | 0.621 |

STATISTICAL SUMMARY

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 80%  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 120%  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 100%  
 (2): THE RATIO ESTIMATE AND THE CORRESPONDING CONFIDENCE LIMITS AND TESTS ARE BASED ON AN ASSUMPTION OF LOG-NORMALITY  
 SEE APPENDIX II FOR DETAILS.

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 4B: RESULTS OF STATISTICAL ANALYSIS OF AUC(0-12h), C<sub>max</sub>, X<sub>u</sub>(0-12h), AND CL<sub>r</sub> AFTER FIRST AND FIFTH IV DOSES  
 EXCLUDING SUBJECTS 1002 AND 1014

| VARIABLE                           | LS MEANS     |              |              |              | RATIO                        | RATIO ESTIMATE | 90% CONF LIMITS |                | P-VALUES (1)   |                |                |
|------------------------------------|--------------|--------------|--------------|--------------|------------------------------|----------------|-----------------|----------------|----------------|----------------|----------------|
|                                    | 200IV DOSE 1 | 200IV DOSE 5 | 400IV DOSE 1 | 400IV DOSE 5 |                              |                | LOWER           | UPPER          | P80            | P120           | P100           |
| AUC(0-12h)<br>(mg*h/L)             | 5.24         | 5.79         | 11.69        | 12.72        | NA                           |                |                 |                |                |                |                |
| AUC(0-12h)/DOSE<br>(h/L)           | 0.0262       | 0.0290       | 0.0292       | 0.0318       | 200(DOSE5/1)<br>400(DOSE5/1) | 1.105<br>1.088 | 0.987<br>0.983  | 1.238<br>1.206 | 0.000<br>0.000 | 0.113<br>0.058 | 0.143<br>0.168 |
| C <sub>max</sub><br>(mg/L)         | 2.14         | 2.10         | 4.60         | 4.57         | NA                           |                |                 |                |                |                |                |
| C <sub>max</sub> /DOSE<br>(1/L)    | 0.0107       | 0.0105       | 0.0115       | 0.0114       | 200(DOSE5/1)<br>400(DOSE5/1) | 0.982<br>0.994 | 0.871<br>0.890  | 1.106<br>1.109 | 0.004<br>0.001 | 0.004<br>0.004 | 0.794<br>0.923 |
| X <sub>u</sub> (0-12h)/DOSE<br>(%) | 72.21        | 62.66        | 62.16        | 60.62        | 200(DOSE5/1)<br>400(DOSE5/1) | 0.868<br>0.975 | 0.671<br>0.719  | 1.112<br>1.306 | 0.293<br>0.135 | 0.018<br>0.118 | 0.339<br>0.885 |
| CL <sub>r</sub><br>(ml/min)        | 470.67       | 381.31       | 369.55       | 340.00       | 200(DOSE5/1)<br>400(DOSE5/1) | 0.810<br>0.920 | 0.637<br>0.674  | 1.018<br>1.233 | 0.463<br>0.216 | 0.003<br>0.007 | 0.128<br>0.633 |

(1) P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO < 80%  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H<sub>0</sub>: TRUE RATIO > 120%  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H<sub>0</sub>: TRUE RATIO = 100%

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CIPROFLOXACIN IV/PO (BAY O 3939/BA, O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 4C: RESULTS OF STATISTICAL ANALYSIS OF AUC(0-24h), AUC(0-inf), AND HALF-LIFE AFTER THE FIFTH ORAL DOSE  
 EXCLUDING SUBJECTS 1002 AND 1014

| VARIABLE                 | LS MEANS |        | RATIO       | RATIO<br>ESTIMATE | 90% CONF LIMITS |       | P-VALUES (1) |       |       |
|--------------------------|----------|--------|-------------|-------------------|-----------------|-------|--------------|-------|-------|
|                          | 500PO    | 750PO  |             |                   | LOWER           | UPPER | P80          | P120  | P100  |
| AUC(0-24h)<br>(mg*h/L)   | 15.79    | 22.47  | NA          |                   |                 |       |              |       |       |
| AUC(0-24h)/DOSE<br>(h/L) | 0.0316   | 0.0300 | 750PO/500PO | 0.949             | 0.836           | 1.075 | 0.018        | 0.004 | 0.460 |
| AUC(0-inf)<br>(mg*h/L)   | 16.34    | 23.33  | NA          |                   |                 |       |              |       |       |
| AUC(0-inf)/DOSE<br>(h/L) | 0.0327   | 0.0311 | 750PO/500PO | 0.952             | 0.840           | 1.077 | 0.016        | 0.004 | 0.483 |
| HALF-LIFE<br>(h)         | 5.33     | 5.58   | 750PO/500PO | 1.047             | 0.969           | 1.133 | 0.000        | 0.000 | 0.305 |

(1): P80 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO < 80%  
 P120 IS THE P-VALUE FROM THE ONE-SIDED TEST OF H0: TRUE RATIO > 120%  
 P100 IS THE P-VALUE FROM THE TWO-SIDED TEST OF H0: TRUE RATIO = 100%

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3A: DERIVED PHARMACOKINETIC VARIABLES SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=IV | DOSING=DOSE 1 | DOSE=200             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 2.7076   | 11            | 0.1040               | 0.3449                | 12.737 | 2.1858           | 3.3727           |
| AUC(0-12h)                  | (mg*h/L)      | 5.2053   | 11            | 0.3064               | 1.0161                | 19.521 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0260   | 11            | 0.0015               | 0.0051                | 19.521 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 1.9339   | 11            | 0.1100               | 0.3649                | 18.868 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 2.1291   | 11            | 0.1372               | 0.4550                | 21.370 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0106   | 11            | 0.0007               | 0.0023                | 21.370 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.7938   | 11            | 0.0542               | 0.1798                | 22.645 |                  |                  |
| t <sub>max</sub>            | (h)           | 0.9545   | 11            | 0.0455               | 0.1508                | 15.793 |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 143.2611 | 11            | 20.9640              | 69.5298               | 48.534 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 71.6305  | 11            | 10.4820              | 34.7649               | 48.534 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 469.2758 | 11            | 63.7026              | 211.2777              | 45.022 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 6.1347   | 11            | 0.6661               | 2.2093                | 36.013 |                  |                  |
|                             |               | ROUTE=IV | DOSING=DOSE 1 | DOSE=400             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 5.4152   | 11            | 0.2080               | 0.6897                | 12.737 | 4.3716           | 6.7454           |
| AUC(0-12h)                  | (mg*h/L)      | 11.6453  | 11            | 0.7459               | 2.4739                | 21.243 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0291   | 11            | 0.0019               | 0.0062                | 21.243 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 2.1424   | 11            | 0.0865               | 0.2870                | 13.395 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 4.5909   | 11            | 0.2286               | 0.7581                | 16.513 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0115   | 11            | 0.0006               | 0.0019                | 16.513 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.8532   | 11            | 0.0435               | 0.1441                | 16.892 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.0000   | 11            | 0.0000               | 0.0000                | 0.000  |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 249.1706 | 11            | 22.8170              | 75.6755               | 30.371 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 62.2927  | 11            | 5.7043               | 18.9189               | 30.371 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 371.5892 | 11            | 38.7007              | 128.3558              | 34.542 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.9389   | 11            | 0.5063               | 1.6792                | 33.999 |                  |                  |

MEAN PARAMETER VALUES

000035

CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3B: DERIVED PHARMACOKINETIC VARIABLES: SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=IV | DOSING=DOSE 5 | DOSE=200             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 2.7076   | 11            | 0.1040               | 0.3449                | 12.737 | 2.1858           | 3.3727           |
| AUC(0-12h)                  | (mg*h/L)      | 5.7555   | 11            | 0.3868               | 1.2830                | 22.291 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0288   | 11            | 0.0019               | 0.0064                | 22.291 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 2.1403   | 11            | 0.1515               | 0.5024                | 23.473 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 2.0900   | 11            | 0.1098               | 0.3641                | 17.422 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0104   | 11            | 0.0005               | 0.0018                | 17.422 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.7776   | 11            | 0.0442               | 0.1466                | 18.848 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.0000   | 11            | 0.0000               | 0.0000                | 0.000  |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 124.1705 | 11            | 6.3897               | 21.1921               | 17.067 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 62.0853  | 11            | 3.1948               | 10.5960               | 17.067 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 379.9126 | 11            | 37.3510              | 123.8792              | 32.607 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 5.0598   | 11            | 0.4208               | 1.3956                | 27.583 |                  |                  |
| -----                       |               |          |               |                      |                       |        |                  |                  |
|                             |               | ROUTE=IV | DOSING=DOSE 5 | DOSE=400             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 5.4152   | 11            | 0.2080               | 0.6897                | 12.737 | 4.3716           | 6.7454           |
| AUC(0-12h)                  | (mg*h/L)      | 12.6774  | 11            | 0.9656               | 3.2024                | 25.260 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0317   | 11            | 0.0024               | 0.0080                | 25.260 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 2.3278   | 11            | 0.1306               | 0.4330                | 18.601 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 4.5618   | 11            | 0.3137               | 1.0405                | 22.809 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0114   | 11            | 0.0008               | 0.0026                | 22.809 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.8453   | 11            | 0.0540               | 0.1791                | 21.185 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.0000   | 11            | 0.0000               | 0.0000                | 0.000  |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 242.0388 | 9             | 14.3233              | 42.9700               | 17.753 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 60.5097  | 9             | 3.5808               | 10.7425               | 17.753 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 350.4899 | 9             | 51.9862              | 155.9586              | 44.497 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.5583   | 9             | 0.5502               | 1.6506                | 36.211 |                  |                  |

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CIPROFLOXACIN IV/PO (BAY Q 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3C: DERIVED PHARMACOKINETIC VARIABLES: SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V    | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=PO | DOSING=DOSE 1 | DOSE=500             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 6.7690   | 11            | 0.2600               | 0.8622                | 12.737 | 5.4645           | 8.4317           |
| AUC(O-12h)                  | (mg*h/L)      | 11.8473  | 11            | 0.9683               | 3.2115                | 27.107 |                  |                  |
| AUC(O-12h)/DOSE             | (h/L)         | 0.0237   | 11            | 0.0019               | 0.0064                | 27.107 |                  |                  |
| AUC(O-12h)/(DOSE/BW)        | (h*kg/L)      | 1.7429   | 11            | 0.1111               | 0.3686                | 21.146 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 2.8573   | 11            | 0.2774               | 0.9200                | 32.199 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0057   | 11            | 0.0006               | 0.0018                | 32.199 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4171   | 11            | 0.0300               | 0.0995                | 23.852 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.2273   | 11            | 0.2273               | 0.7538                | 61.419 |                  |                  |
| X <sub>u</sub> (O-12h)      | (mg)          | 261.8688 | 11            | 12.4610              | 41.3285               | 15.732 |                  |                  |
| X <sub>u</sub> (O-12h)/DOSE | (%)           | 52.3738  | 11            | 2.4922               | 8.2657                | 15.782 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 390.7145 | 11            | 34.0074              | 112.7897              | 28.868 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 5.1894   | 11            | 0.3655               | 1.2122                | 23.360 |                  |                  |
| <hr/>                       |               |          |               |                      |                       |        |                  |                  |
|                             |               | ROUTE=PO | DOSING=DOSE 1 | DOSE=750             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 10.1536  | 11            | 0.3899               | 1.2932                | 12.737 | 8.1967           | 12.6476          |
| AUC(O-12h)                  | (mg*h/L)      | 18.3329  | 11            | 1.8586               | 6.1644                | 33.624 |                  |                  |
| AUC(O-12h)/DOSE             | (h/L)         | 0.0244   | 11            | 0.0025               | 0.0082                | 33.624 |                  |                  |
| AUC(O-12h)/(DOSE/BW)        | (h*kg/L)      | 1.7872   | 11            | 0.1296               | 0.4299                | 24.053 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 4.1618   | 11            | 0.2941               | 0.9755                | 23.141 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0055   | 11            | 0.0004               | 0.0013                | 23.441 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4095   | 11            | 0.0218               | 0.0723                | 17.669 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.2727   | 11            | 0.1236               | 0.4101                | 32.222 |                  |                  |
| X <sub>u</sub> (O-12h)      | (mg)          | 376.6659 | 11            | 20.2165              | 67.0506               | 17.801 |                  |                  |
| X <sub>u</sub> (O-12h)/DOSE | (%)           | 50.2221  | 11            | 2.6955               | 8.9401                | 17.801 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 364.6525 | 11            | 28.9704              | 96.0940               | 26.352 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.8287   | 11            | 0.3111               | 1.0318                | 21.368 |                  |                  |

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3D: DERIVED PHARMACOKINETIC VARIABLES: SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=PO | DOSING=DOSE 5 | DOSE=500             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 6.7690   | 11            | 0.2600               | 0.8622                | 12.737 | 5.4645           | 8.4317           |
| AUC(0-12h)                  | (mg*h/L)      | 13.6554  | 11            | 1.1952               | 3.9642                | 29.030 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0273   | 11            | 0.0024               | 0.0079                | 29.030 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 2.0264   | 11            | 0.1698               | 0.5633                | 27.798 |                  |                  |
| AUC(0-24h)                  | (mg*h/L)      | 15.5503  | 11            | 1.3848               | 4.5927                | 29.535 |                  |                  |
| AUC(0-24h)/DOSE             | (h/L)         | 0.0311   | 11            | 0.0028               | 0.0092                | 29.535 |                  |                  |
| AUC(0-24h)/(DOSE/BW)        | (h*kg/L)      | 2.3104   | 11            | 0.2025               | 0.6717                | 29.071 |                  |                  |
| AUC(0-inf)                  | (mg*h/L)      | 16.0895  | 11            | 1.4365               | 4.7642                | 29.610 |                  |                  |
| AUC(0-inf)/DOSE             | (h/L)         | 0.0322   | 11            | 0.0029               | 0.0095                | 29.610 |                  |                  |
| AUC(0-inf)/(DOSE/BW)        | (h*kg/L)      | 2.3921   | 11            | 0.2126               | 0.7052                | 29.478 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 2.9691   | 11            | 0.2555               | 0.8473                | 28.537 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0059   | 11            | 0.0005               | 0.0017                | 28.537 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4422   | 11            | 0.0380               | 0.1261                | 28.522 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.2273   | 11            | 0.1561               | 0.5179                | 42.196 |                  |                  |
| HALF-LIFE                   | (h)           | 5.3148   | 11            | 0.2051               | 0.6803                | 12.800 |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 272.2606 | 11            | 11.2576              | 37.3371               | 13.714 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 54.4521  | 11            | 2.2515               | 7.4674                | 13.714 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 354.8548 | 11            | 29.7205              | 98.5716               | 27.778 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.7539   | 11            | 0.3647               | 1.2096                | 25.445 |                  |                  |

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3D: DERIVED PHARMACOKINETIC VARIABLES: SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=PO | DOSING=DOSE 5 | DOSE=500             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 6.7690   | 11            | 0.2600               | 0.8622                | 12.737 | 5.4645           | 8.4317           |
| AUC(0-12h)                  | (mg*h/L)      | 13.6554  | 11            | 1.1952               | 3.9642                | 29.030 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0273   | 11            | 0.0024               | 0.0079                | 29.030 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 2.0264   | 11            | 0.1698               | 0.5633                | 27.798 |                  |                  |
| AUC(0-24h)                  | (mg*h/L)      | 15.5503  | 11            | 1.3848               | 4.5927                | 29.535 |                  |                  |
| AUC(0-24h)/DOSE             | (h/L)         | 0.0311   | 11            | 0.0028               | 0.0092                | 29.535 |                  |                  |
| AUC(0-24h)/(DOSE/BW)        | (h*kg/L)      | 2.3104   | 11            | 0.2025               | 0.6717                | 29.071 |                  |                  |
| AUC(0-inf)                  | (mg*h/L)      | 16.0895  | 11            | 1.4365               | 4.7642                | 29.610 |                  |                  |
| AUC(0-inf)/DOSE             | (h/L)         | 0.0322   | 11            | 0.0029               | 0.0095                | 29.610 |                  |                  |
| AUC(0-inf)/(DOSE/BW)        | (h*kg/L)      | 2.3921   | 11            | 0.2126               | 0.7052                | 29.478 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 2.9691   | 11            | 0.2555               | 0.8473                | 28.537 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0059   | 11            | 0.0005               | 0.0017                | 28.537 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4422   | 11            | 0.0380               | 0.1261                | 28.522 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.2273   | 11            | 0.1561               | 0.5179                | 42.196 |                  |                  |
| HALF-LIFE                   | (h)           | 5.3148   | 11            | 0.2051               | 0.6803                | 12.800 |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 272.2606 | 11            | 11.2576              | 37.3371               | 13.714 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 54.4521  | 11            | 2.2515               | 7.4674                | 13.714 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 354.8548 | 11            | 29.7205              | 98.5716               | 27.778 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.7539   | 11            | 0.3647               | 1.2096                | 25.445 |                  |                  |

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CIPROFLOXACIN IV,PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3D: DERIVED PHARMACOKINETIC VARIABLES. SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=PO | DOSING=DOSE 5 | DOSE=750             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 10.1536  | 11            | 0.3899               | 1.2932                | 12.737 | 8.1967           | 12.6476          |
| AUC(0-12h)                  | (mg*h/L)      | 19.5153  | 11            | 1.5054               | 4.9929                | 25.585 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0260   | 11            | 0.0020               | 0.0067                | 25.585 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 1.9098   | 11            | 0.0933               | 0.3093                | 16.197 |                  |                  |
| AUC(0-24h)                  | (mg*h/L)      | 22.3584  | 11            | 1.6653               | 5.5231                | 24.702 |                  |                  |
| AUC(0-24h)/DOSE             | (h/L)         | 0.0298   | 11            | 0.0022               | 0.0074                | 24.702 |                  |                  |
| AUC(0-24h)/(DOSE/BW)        | (h*kg/L)      | 2.1897   | 11            | 0.1025               | 0.3400                | 15.526 |                  |                  |
| AUC(0-inf)                  | (mg*h/L)      | 23.2304  | 11            | 1.6825               | 5.5803                | 24.022 |                  |                  |
| AUC(0-inf)/DOSE             | (h/L)         | 0.0310   | 11            | 0.0022               | 0.0074                | 24.022 |                  |                  |
| AUC(0-inf)/(DOSE/BW)        | (h*kg/L)      | 2.2759   | 11            | 0.1021               | 0.3387                | 14.882 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 4.3173   | 11            | 0.3549               | 1.1770                | 27.263 |                  |                  |
| C <sub>max</sub> /DOSE      | (1/L)         | 0.0058   | 11            | 0.0005               | 0.0016                | 27.263 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4223   | 11            | 0.0243               | 0.0806                | 19.088 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.1364   | 11            | 0.1185               | 0.3931                | 34.595 |                  |                  |
| HALF-LIFE                   | (h)           | 5.6178   | 11            | 0.2710               | 0.8988                | 16.000 |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 370.2406 | 10            | 25.6198              | 81.0170               | 21.882 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 49.3654  | 10            | 3.4160               | 10.8023               | 21.882 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 345.3544 | 10            | 27.4907              | 86.9333               | 25.172 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.5217   | 10            | 0.3366               | 1.0643                | 27.538 |                  |                  |

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CIPROFLOXACIN IV/PO (BAY O 3939/BAY O 9867)  
 STEADY-STATE PHARMACOKINETICS OF CIPROFLOXACIN DURING SEQUENTIAL INTRAVENOUS/ORAL DOSING  
 PROTOCOL D90-012

TABLE 3D: DERIVED PHARMACOKINETIC VARIABLES, SUMMARY STATISTICS  
 EXCLUDING SUBJECTS 1002 AND 1014

| LABEL                       |               | MEAN     | N             | STD ERROR<br>OF MEAN | STANDARD<br>DEVIATION | C.V.   | MINIMUM<br>VALUE | MAXIMUM<br>VALUE |
|-----------------------------|---------------|----------|---------------|----------------------|-----------------------|--------|------------------|------------------|
|                             |               | ROUTE=PO | DOSING=DOSE 5 | DOSE=750             |                       |        |                  |                  |
| BODY WEIGHT                 | (kg)          | 74.9518  | 11            | 2.8537               | 9.4646                | 12.628 | 59.3000          | 91.5000          |
| DOSE/WEIGHT                 | (mg/kg)       | 10.1536  | 11            | 0.3899               | 1.2932                | 12.737 | 8.1967           | 12.6476          |
| AUC(0-12h)                  | (mg*h/L)      | 19.5153  | 11            | 1.5054               | 4.9929                | 25.585 |                  |                  |
| AUC(0-12h)/DOSE             | (h/L)         | 0.0260   | 11            | 0.0020               | 0.0067                | 25.585 |                  |                  |
| AUC(0-12h)/(DOSE/BW)        | (h*kg/L)      | 1.9098   | 11            | 0.0933               | 0.3093                | 16.197 |                  |                  |
| AUC(0-24h)                  | (mg*h/L)      | 22.3584  | 11            | 1.6653               | 5.5231                | 24.702 |                  |                  |
| AUC(0-24h)/DOSE             | (h/L)         | 0.0298   | 11            | 0.0022               | 0.0074                | 24.702 |                  |                  |
| AUC(0-24h)/(DOSE/BW)        | (h*kg/L)      | 2.1897   | 11            | 0.1025               | 0.3400                | 15.526 |                  |                  |
| AUC(0-inf)                  | (mg*h/L)      | 23.2304  | 11            | 1.6825               | 5.5803                | 24.022 |                  |                  |
| AUC(0-inf)/DOSE             | (h/L)         | 0.0310   | 11            | 0.0022               | 0.0074                | 24.022 |                  |                  |
| AUC(0-inf)/(DOSE/BW)        | (h*kg/L)      | 2.2759   | 11            | 0.1021               | 0.3387                | 14.882 |                  |                  |
| C <sub>max</sub>            | (mg/L)        | 4.3173   | 11            | 0.3549               | 1.1770                | 27.263 |                  |                  |
| C <sub>max</sub> /DOSE      | (h/L)         | 0.0058   | 11            | 0.0005               | 0.0016                | 27.263 |                  |                  |
| C <sub>max</sub> /(DOSE/BW) | (kg/L)        | 0.4223   | 11            | 0.0243               | 0.0806                | 19.088 |                  |                  |
| t <sub>max</sub>            | (h)           | 1.1364   | 11            | 0.1185               | 0.3931                | 34.595 |                  |                  |
| HALF-LIFE                   | (h)           | 5.6178   | 11            | 0.2710               | 0.8988                | 16.000 |                  |                  |
| X <sub>u</sub> (0-12h)      | (mg)          | 370.2406 | 10            | 25.6198              | 81.0170               | 21.882 |                  |                  |
| X <sub>u</sub> (0-12h)/DOSE | (%)           | 49.3654  | 10            | 3.4160               | 10.8023               | 21.882 |                  |                  |
| RENAL CLEARANCE             | (mL/min)      | 345.3544 | 10            | 27.4907              | 86.9333               | 25.172 |                  |                  |
| Cl <sub>r</sub> /(DOSE/BW)  | (mL/(min*kg)) | 4.5217   | 10            | 0.3366               | 1.0643                | 23.538 |                  |                  |

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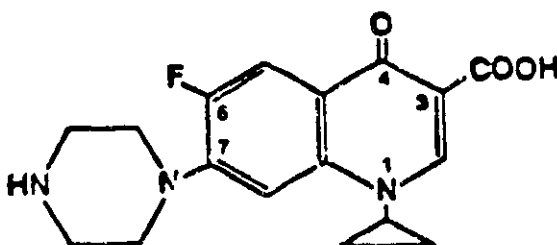
Ciprofloxacin IV  
Cipro<sup>R</sup> IV  
IV solution 100, 200 and 300 mg  
NDA 19-847  
Reviewer: Suresh Mallikaarjun, Ph.D.  
5S,20

Miles  
Pharmaceutical Division  
West Haven, CT 06516  
Submission Dates:  
April 25, 1988  
October 25, 1989  
February 14, 1990  
March 29, 1990, April 11, 1990  
April 16, 1990, June 4, 1990.

Review of a New Drug Application

JUN 13 1990

Ciprofloxacin is a synthetic, broad spectrum antibacterial agent, which belongs to the class of fluoroquinolones and has the following structure:



It is soluble in dilute hydrochloric acid and practically insoluble in water and ethanol. It has a molecular weight of 331.4 and pKas of 6.5 and 8.9. Cipro<sup>R</sup> IV solutions are available as 1.0% concentrates intended for dilution prior to administration, and as 0.2% ready-for-use infusion solutions in either 0.9% NaCl Injection or 5% Dextrose solution. All formulas contain lactic acid as a solubilizing agent and HCl for pH adjustment. The exact formula is listed in Appendix A.

An oral tablet formulation of ciprofloxacin has been approved and is currently marketed in 250, 500 and 750 mg strengths. It is to be noted that the Division of Biopharmaceutics did not review any studies for the oral ciprofloxacin tablets (See Appendix B).

The following studies were submitted in support of this NDA:

| <u>No.</u> | <u>Study</u>    | <u>Type</u>                                |
|------------|-----------------|--------------------------------------------|
| 1.         | D84-025-01      | Single Dose - 100, 150, 200 mg IV.         |
| 2.         | D85-008-01      | Renal Impairment - 200 mg IV.              |
| 3.         | D86-061-01      | Hepatic Impairment - 200 mg IV.            |
| 4.         | Bayer-Yakuhin   | Multiple Dose - 100, 200, 300 mg IV.       |
| 5.         | BAY o 9867/0504 | Vaginal Tissue distribution - 500 mg po.   |
| 6.         | BAY o 9867/0170 | Prostatic Tissue distribution - 500 mg po. |

7. Literature Article Distribution in the Eye - 750 mg po

Study I - D84-025-01

Title: Safety and Pharmacokinetics of Intravenous Ciprofloxacin at Doses of 100, 150 and 200 mg.

Objective: The purpose of this study was to determine the safety and pharmacokinetics of single intravenous doses of 100, 150 and 200 mg of ciprofloxacin in healthy volunteers.

Clinical Investigators: Stephen Zimmer, M.D.  
Michael Dudley, Pharm.D.

Study Location: Roger Williams General Hospital, Providence, Rhode Island.

Study Design and Dosing:

Twelve healthy male volunteers, with an average age of 24.3 years (range of 20 to 37 years) and an average weight of 78.5 kg (range of 62 to 92.5 kgs), were involved in this dose escalating, three way crossover study. Nine subjects were assigned to the drug treatment group and three subjects to the placebo group. The subjects fasted after midnight prior to the day of the dosing and 4 hours after the administration of the dose. The appropriate volume of a 10mg/ml ciprofloxacin ampul (volume=10 ml, Batch No: Bay q 3939) was withdrawn and diluted with normal saline to a final volume of 100 ml. The drug was administered as an IV infusion over 30 minutes. After the 100 ml infusion, an additional 75 ml saline was infused to ensure complete delivery of the dose. The subjects received 100 mg, 150 mg and 200 mg of ciprofloxacin at weekly intervals. The placebo consisted of the vehicle without ciprofloxacin.

Sample collection: Blood samples (5-7 ml) were obtained at 0, 15 minutes, 30 minutes (end of infusion), 35 minutes, 40 minutes, 50 minutes and 1, 1.25, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5, 16.5, 20.5 and 24.5 hours after start of the infusion. Additional blood samples were collected at 35 minutes, 1.5 and 6.5 hours after the start of the infusion for serum bactericidal assay. Urine samples were collected and pooled during the following intervals after the start of the infusion: 0-1.5, 1.5-2.5, 2.5-3.5, 3.5-4.5, 4.5-6.5, 6.5-8.5, 8.5-10.5, 10.5-12.5, 12.5-16.5, 16.5-20.5, 20.5-24.5, 24.5-36.5 and 36.5-58.5 hours. Saliva samples were collected from each volunteer before dosing and at 1 hour after cessation of the infusion.

Analytical method: Serum, urine and saliva samples were assayed for ciprofloxacin by a reversed-phase HPLC procedure. The analytical validation provided was satisfactory.

Parameter evaluation: The pharmacokinetic parameters calculated were AUC and AUMC by the trapezoidal rule, clearance as Dose/AUC. Volume of

7. Literature Article Distribution in the Eye - 750 mg po

Study I - D84-025-01

Title: Safety and Pharmacokinetics of Intravenous Ciprofloxacin at Doses of 100, 150 and 200 mg.

Objective: The purpose of this study was to determine the safety and pharmacokinetics of single intravenous doses of 100, 150 and 200 mg of ciprofloxacin in healthy volunteers.

Clinical Investigators: Stephen Zimmer, M.D.  
Michael Dudley, Pharm.D.

Study Location: Roger Williams General Hospital, Providence, Rhode Island.

Study Design and Dosing:

Twelve healthy male volunteers, with an average age of 24.3 years (range of 20 to 37 years) and an average weight of 78.5 kg (range of 62 to 92.5 kgs), were involved in this dose escalating, three way crossover study. Nine subjects were assigned to the drug treatment group and three subjects to the placebo group. The subjects fasted after midnight prior to the day of the dosing and 4 hours after the administration of the dose. The appropriate volume of a 10mg/ml ciprofloxacin ampul (volume=10 ml, Batch No: Bay q 3939) was withdrawn and diluted with normal saline to a final volume of 100 ml. The drug was administered as an IV infusion over 30 minutes. After the 100 ml infusion, an additional 25 ml saline was infused to ensure complete delivery of the dose. The subjects received 100 mg, 150 mg and 200 mg of ciprofloxacin at weekly intervals. The placebo consisted of the vehicle without ciprofloxacin.

Sample collection: Blood samples (5-7 ml) were obtained at 0, 15 minutes, 30 minutes (end of infusion), 35 minutes, 40 minutes, 50 minutes and 1, 1.25, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5, 16.5, 20.5 and 24.5 hours after start of the infusion. Additional blood samples were collected at 35 minutes, 1.5 and 6.5 hours after the start of the infusion for serum bactericidal assay. Urine samples were collected and pooled during the following intervals after the start of the infusion: 0-1.5, 1.5-2.5, 2.5-3.5, 3.5-4.5, 4.5-6.5, 6.5-8.5, 8.5-10.5, 10.5, 12.5, 12.5-16.5, 16.5-20.5, 20.5-24.5, 24.5-36.5 and 36.5-58.5 hours. Saliva samples were collected from each volunteer before dosing and at 1 hour after cessation of the infusion.

Analytical method: Serum, urine and saliva samples were assayed for ciprofloxacin by a reversed-phase HPLC procedure. The analytical validation provided was satisfactory.

Parameter evaluation: The pharmacokinetic parameters calculated were AUC and AUMC by the trapezoidal rule, clearance as Dose/AUC. Volume of

distribution at steady state  $V_{dss}$  from  $Cl \cdot MRT$  and renal clearance from  $A_e (0-48)/AUC (0-48)$ , where  $A_e$  is the amount of drug excreted in the urine.

Statistical analysis: Statistical comparisons were made using repeated measures of analysis of variance.

RESULTS: The mean serum concentration-time profiles are in Figures 1-3 and the mean pharmacokinetic parameters in Tables 1-2. The mean parameters after 100, 150 and 200 mg IV infusion are listed below:

| <u>Dose</u><br>mg |      | <u>AUC</u><br>mg/hr/L | <u>Cmax</u><br>mg/L | <u>Cl<sub>s</sub></u><br>ml/min/kg | <u>Cl<sub>renal</sub></u><br>ml/min/kg | <u>V<sub>dss</sub></u><br>L/kg | <u>T<sub>1/2</sub></u><br>hrs. |
|-------------------|------|-----------------------|---------------------|------------------------------------|----------------------------------------|--------------------------------|--------------------------------|
| 100               | Mean | 2.24                  | 1.37                | 9.60                               | 4.61                                   | 2.17                           | 4.1                            |
|                   | S.D. | 0.54                  | 0.57                | 2.09                               | 1.14                                   | 0.45                           | 0.9                            |
| 150               | Mean | 3.36                  | 2.04                | 9.57                               | 4.40                                   | 2.40                           | 4.6                            |
|                   | S.D. | 0.74                  | 0.51                | 2.02                               | 1.16                                   | 0.61                           | 1.2                            |
| 200               | Mean | 5.17                  | 3.18                | 8.15                               | 3.80                                   | 2.00                           | 4.2                            |
|                   | S.D. | 0.87                  | 0.63                | 1.21                               | 0.87                                   | 0.39                           | 0.6                            |

The renal clearance was approximately 47% of the total clearance and therefore, both the renal and nonrenal clearance are significant in ciprofloxacin elimination.

Salivary concentrations at 1 hour post-infusion ranged from 0.15 mcg/ml to 0.26 mcg/ml after 100 mg and 200 mg doses respectively.

According to the sponsor's statistical analysis, no significant differences were observed in the  $Cl_s$ ,  $T_{1/2}$ ,  $V_{dss}$  and renal clearance between doses. The sponsor also stated that the AUC and  $C_{max}$  increased proportionally with increasing dose.

Conclusion:

The pharmacokinetics of ciprofloxacin as determined by comparison of clearance,  $V_{dss}$  and half-life, are linear between 100 mg to 200 mg.

Study #2 - D85-008-01:

Title: Effect of renal impairment on the pharmacokinetics of intravenous ciprofloxacin.

Objective: The purpose of this study was to determine the effects of renal impairment on ciprofloxacin pharmacokinetics.

Clinical Investigators: George Drusano, M.D.

Study Location: Univ. of Maryland Hospital, 10 S. Pine Street, Baltimore, MD.

Study Design and Dosing: Thirty two subjects were enrolled in the study. The subjects were divided into groups 2, 3 or 4 depending on their creatinine clearance values. Five subjects were assigned to group 2 ( $Cl_{cr}$  60-100), 11 subjects to group 3 ( $Cl_{cr}$  10-59) and 8 subjects to group 4 ( $Cl_{cr}$  less than 10). Eight subjects with normal renal function ( $Cl_{cr}$  >100) were assigned to Group 1.  $Cl_{cr}$  is creatinine clearance in ml/min/1.73m<sup>2</sup>. Each subject was given a single IV dose of 200 mg ciprofloxacin (Batch No. 974272) as either a 30 minute infusion (for groups 2-4) or a 10 minute infusion (for group 1).

Sample collection: Serum samples were collected before the start of the infusion, and at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after the end of the infusion. Urine samples were collected and pooled according to the following intervals, 0-2, 2-4, 4-8, 8-12 and 12-24 hours after dosing. A urine sample was also collected before the start of the infusion.

Analytical method: Ciprofloxacin was assayed using an HPLC with a diode array detector. Detailed information on the assay validation was not provided by the sponsor. Metabolites were apparently measured by the sponsor but the data presented is very sparse.

Parameter evaluation: The AUC(0-inf) was calculated by the trapezoidal rule and extrapolation, the serum clearance,  $Cl_s$ , from Dose/AUC and the renal clearance,  $Cl_r$ , by  $X_u/AUC$ . The nonrenal clearance was estimated by the difference between the systemic and the renal clearances. The volume of distribution was estimated by  $Cl_s/\beta$ . No statistical analyses of the parameters were reported by the sponsor.

Results: The mean concentration-time profiles are in Figure 3 and the mean parameter values in Table 3. The parameter values for ciprofloxacin are reported below:

| <u>Group</u> | <u><math>Cl_{cr}</math></u> |      | <u><math>Cl_s</math></u><br>L/hr/1.73m <sup>2</sup> | <u><math>Cl_r</math></u><br>L/hr/1.73m <sup>2</sup> | <u><math>T_{1/2}</math></u><br>hr. | <u><math>Vd_{area}</math></u><br>L/Kg |
|--------------|-----------------------------|------|-----------------------------------------------------|-----------------------------------------------------|------------------------------------|---------------------------------------|
| 1            | >100                        | Mean | 26.8                                                | 16.4                                                | 4.27                               | 2.49                                  |
|              |                             | S.D. | 5.7                                                 | 3.5                                                 | .84                                | .46                                   |
| 2            | 60-100                      | Mean | 26.3                                                | 11.9                                                | 6.12                               | 3.19                                  |
|              |                             | S.D. | 10.3                                                | 5.3                                                 | 1.61                               | 1.26                                  |
| 3            | 10-59                       | Mean | 15.0                                                | 4.5                                                 | 7.70                               | 2.38                                  |
|              |                             | S.D. | 3.8                                                 | 2.6                                                 | 1.22                               | .62                                   |
| 4            | <10                         | Mean | 15.4                                                | 0.0                                                 | 8.55                               | 2.73                                  |
|              |                             | S.D. | 4.3                                                 |                                                     | 3.27                               | .92                                   |

AUC and  $C_{max}$  values were not provided in the submission. Since no statistical tests were performed by the sponsor, only qualitative conclusions can be made with reference to the pharmacokinetic parameters of the various groups. The mean renal clearance decreased and the mean

half-life increased from Group 1 to Group 4. The  $Vd_{area}$  was essentially unchanged. Although the systemic clearance decreased from Group 1 to Group 3, the clearance of Group 4 was greater than Group 3. This may be an artifact or it may indicate increased nonrenal clearance.

Conclusion:

The labeling should include a statement to the effect that the dose should be adjusted in renally impaired patients.

Study #3 - D86-061-01:

Title: Pharmacokinetics of ciprofloxacin in hepatic impairment following oral and intravenous administration.

Objective: To determine the effect of hepatic impairment on the pharmacokinetics of ciprofloxacin after the following treatments:

- i) A single 200 mg intravenous dose of ciprofloxacin infused over 30 minutes.
- ii) A single 750 mg dose of ciprofloxacin given orally.
- iii) An oral dosage regimen consisting of nine 750 mg doses administered every 12 hours.

Clinical Investigator: Kenneth C. Lassiter, M.D.

Study Location: Clinical Pharmacology Associates  
2060 N.W. 22nd Avenue  
Miami, FL 33142

Study Design and Dosing: Fourteen subjects were enrolled in this open label parallel design study. Subjects were divided into two groups, Group I consisting of seven subjects with biopsy proven chronic hepatic disease and Group II comprising seven normal healthy volunteers, each of whom were matched to one of the Group I subjects with respect to age, height and weight. The cirrhotic subjects ranged in age from 44 to 65 years (mean = 52.3), and in weight from 130 to 205 pounds (mean = 168.6). The normal volunteers ranged in age from 48 to 68 years (mean = 54.9), and in weight from 143 to 214 pounds (mean = 168.6).

| <u>Phase</u>            | <u>Dose</u>                        | <u>Frequency</u> | <u>No. of doses</u> | <u>Duration of phase</u> |
|-------------------------|------------------------------------|------------------|---------------------|--------------------------|
| Phase I                 | 750 mg p.o.                        | q 12h            | 9 doses             | 5 days                   |
| Washout period - 7 days |                                    |                  |                     |                          |
| Phase II                | 200 mg. IV infusion for 30 minutes | - 1 dose         |                     | 24 hours                 |

Sample collection:

Blood: For Phase I, blood samples (5-7 ml) were collected immediately prior to and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after the 1st and the last doses. Samples were also collected immediately prior to and

1.5 hours after each of the 7 interim oral doses. For Phase II, samples were drawn at 0, 0.25, 0.5, 0.583, 0.666, 0.75, 1, 1.5, 2.5, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5 and 24.5 hours after the start of the infusion.

Urine: Urine was collected for a 12 hour period following the first and last oral doses, and after the intravenous dose. The total volume of the urine was recorded and a 50 ml aliquot stored.

Analytical method: Serum and urine samples were assayed for ciprofloxacin by a reversed-phase HPLC procedure.

Parameter evaluation: AUC(0-t) was calculated by the trapezoidal rule, AUC(0-inf) by the addition of AUC(0-t) and  $C_{last}/K_{el}$ . MRT and  $Vd_{ss_{iv}}$ ,  $Cl_s$ ,  $Cl_r$  and  $f$  were also calculated.

Statistical analysis: Statistical comparisons between the pharmacokinetic parameters of the cirrhotic and normal volunteers were performed by the sponsor using the paired t-test.

RESULTS: Tables 4-5 contain individual parameters and urine data. Figures 4-6 contain mean serum concentration profiles after oral and iv dosing. The following table is an overall summary of the pharmacokinetic parameters of ciprofloxacin:

Phase I: Oral dosing

|           | <u>Dose</u> |       | <u>C<sub>max</sub></u><br>mcg/ml | <u>T<sub>max</sub></u><br>hr | <u>AUC(0-t)</u><br>mcg/ml/hr | <u>AUC(0-inf)</u><br>mcg/ml/hr | <u>F</u> | <u>K<sub>el</sub></u><br>hr <sup>-1</sup> | <u>T<sub>1/2</sub></u><br>hr |
|-----------|-------------|-------|----------------------------------|------------------------------|------------------------------|--------------------------------|----------|-------------------------------------------|------------------------------|
| Cirrhotic | 1st         | Mean  | 3.71                             | 1.29                         | 16.06                        | 18.38                          | 62.7     | 0.292                                     | 3.5                          |
|           |             | %C.V. | 21.9                             | 61.9                         | 30.7                         | 38.5                           | 16.9     | 27.1                                      | 35.3                         |
| Normal    | 1st         | Mean  | 3.49                             | 1.00                         | 14.45                        | 16.18                          | 68.9     | 0.189                                     | 3.7                          |
|           |             | %C.V. | 19.6                             | 37.8                         | 28.3                         | 28.7                           | 25.9     | 11.3                                      | 11.1                         |
| Cirrhotic | 9th         | Mean  | 4.15                             | 1.14                         | 21.04                        | -                              | -        | 0.189                                     | 3.96                         |
|           |             | %C.V. | 28.9                             | 38.5                         | 31.3                         |                                |          | 27.1                                      | 27.4                         |
| Normal    | 9th         | Mean  | 3.67                             | 1.14                         | 17.27                        | -                              | -        | 0.195                                     | 3.67                         |
|           |             | %C.V. | 35.0                             | 45.1                         | 32.0                         |                                |          | 19.2                                      | 15.6                         |

Phase II: IV dosing

|           |       | <u>V<sub>ss</sub></u> | <u>Cl<sub>s</sub></u> | <u>AUC(0-t)</u><br>mcg/ml/hr | <u>AUC(0-inf)</u><br>mcg/ml/hr | <u>Cl<sub>r</sub></u> | <u>K<sub>el</sub></u> | <u>T<sub>1/2</sub></u> |
|-----------|-------|-----------------------|-----------------------|------------------------------|--------------------------------|-----------------------|-----------------------|------------------------|
| Cirrhotic | Mean  | 147.8                 | 28.19                 | 7.35                         | 7.71                           | 28.19                 | 0.145                 | 5.3                    |
|           | %C.V. | 25.9                  | 26.7                  | 29.5                         | 31.1                           | 45.3                  | 40.3                  | 25.8                   |
| Normal    | Mean  | 146.2                 | 33.29                 | 6.12                         | 6.41                           | 17.19                 | 0.165                 | 4.9                    |
|           | %C.V. | 34.1                  | 24.0                  | 27.5                         | 27.0                           | 56.7                  | 44.0                  | 32.4                   |

The sponsor analysis using the paired t-test indicated no statistically

1.5 hours after each of the 7 interim oral doses. For Phase II, samples were drawn at 0, 0.25, 0.5, 0.583, 0.666, 0.75, 1, 1.5, 2.5, 3.5, 4.5, 6.5, 8.5, 10.5, 12.5 and 24.5 hours after the start of the infusion.

Urine: Urine was collected for a 12 hour period following the first and last oral doses, and after the intravenous dose. The total volume of the urine was recorded and a 50 ml aliquot stored.

Analytical method: Serum and urine samples were assayed for ciprofloxacin by a reversed-phase HPLC procedure.

Parameter evaluation: AUC(0-t) was calculated by the trapezoidal rule, AUC(0-inf) by the addition of AUC(0-t) and  $C_{ast}/K_e$ . MRT and  $V_{dss_{iv}}$ , Cls, Clr and F were also calculated.

Statistical analysis: Statistical comparisons between the pharmacokinetic parameters of the cirrhotic and normal volunteers were performed by the sponsor using the paired t-test.

RESULTS: Tables 4-5 contain individual parameters and urine data. Figures 4-6 contain mean serum concentration profiles after oral and iv dosing. The following table is an overall summary of the pharmacokinetic parameters of ciprofloxacin:

Phase I: Oral dosing

|            | <u>Dose</u> |       | <u>C<sub>max</sub></u><br>mcg/ml | <u>T<sub>max</sub></u><br>hr | <u>AUC(0-t)</u><br>mcg/ml/hr | <u>AUC(0-inf)</u><br>mcg/ml/hr | <u>F</u> | <u>K<sub>el</sub></u><br>hr <sup>-1</sup> | <u>T<sub>1/2</sub></u><br>hr |
|------------|-------------|-------|----------------------------------|------------------------------|------------------------------|--------------------------------|----------|-------------------------------------------|------------------------------|
| Cirrhrotic | 1st         | Mean  | 3.71                             | 1.29                         | 16.06                        | 18.38                          | 62.7     | 0.292                                     | 3.5                          |
|            |             | %C.V. | 21.9                             | 61.9                         | 30.7                         | 38.5                           | 16.9     | 27.1                                      | 35.3                         |
| Normal     | 1st         | Mean  | 3.49                             | 1.00                         | 14.45                        | 16.18                          | 68.9     | 0.189                                     | 3.7                          |
|            |             | %C.V. | 19.6                             | 37.8                         | 28.3                         | 28.7                           | 25.9     | 11.3                                      | 11.1                         |
| Cirrhrotic | 9th         | Mean  | 4.15                             | 1.14                         | 21.04                        | -                              | -        | 0.189                                     | 3.96                         |
|            |             | %C.V. | 28.9                             | 38.5                         | 31.3                         |                                |          | 27.1                                      | 27.4                         |
| Normal     | 9th         | Mean  | 3.67                             | 1.14                         | 17.27                        | -                              | -        | 0.195                                     | 3.67                         |
|            |             | %C.V. | 35.0                             | 45.1                         | 32.0                         |                                |          | 19.2                                      | 15.6                         |

Phase II: IV dosing

|            |       | <u>V<sub>ss</sub></u> | <u>Cl<sub>s</sub></u> | <u>AUC(0-t)</u><br>mcg/ml/hr | <u>AUC(0-inf)</u><br>mcg/ml/hr | <u>Cl<sub>r</sub></u> | <u>K<sub>el</sub></u> | <u>T<sub>1/2</sub></u> |
|------------|-------|-----------------------|-----------------------|------------------------------|--------------------------------|-----------------------|-----------------------|------------------------|
| Cirrhrotic | Mean  | 147.8                 | 28.19                 | 7.35                         | 7.71                           | 28.19                 | 0.145                 | 5.3                    |
|            | %C.V. | 25.9                  | 26.7                  | 29.5                         | 31.1                           | 45.3                  | 40.3                  | 25.8                   |
| Normal     | Mean  | 146.2                 | 33.29                 | 6.12                         | 6.41                           | 17.19                 | 0.165                 | 4.9                    |
|            | %C.V. | 34.1                  | 24.0                  | 27.5                         | 27.0                           | 56.7                  | 44.0                  | 32.4                   |

The sponsor analysis using the paired t-test indicated no statistically

significant differences between any of the parameters after the first oral dose. No statistical tests seem to have been performed by the sponsor on parameters after the 9th oral dose or the IV dose. On a visual inspection of parameter values after the 9th oral dose and the IV dose, it appeared that the parameter values were comparable between the normals and cirrhotics. Although the Cmax, AUC(0-t) and AUC(0-inf) were higher for the cirrhotic patients as compared to the normal subjects after the 9th oral dose, it appears that this difference was due to very low values from subject #9 in the normal group.

Metabolites: The Cmax and AUC(0-t) of the metabolite M3 was, on an average, twice as high in normals as compared to the cirrhotic patients after the 1st and 9th oral doses and also after the IV dose. The parameters of M1 and M2 were comparable between the two groups. The mean recoveries of the metabolites M1, M2 and M3 ranged from 0.3% to 3.8% of the administered dose and were also not very different between the cirrhotic and the normal subjects.

Conclusion: The clinical pharmacology section should include the a statement indicating that the pharmacokinetics of ciprofloxacin were not significantly altered in cirrhotic patients as compared to normal volunteers.

Study #4 - Bayer Yakuhin Study:

Title: Bayer Yakuhin Study (Yasunaga: 100, 200, 300 mg b.i.d. multiple doses).

Objective: To investigate the safety and pharmacokinetics of BAY q 3939 after continuous administration by intravenous drip infusion in healthy male adult volunteers.

Clinical Investigators: K. Yasunaga, Y. Sakakibara, Junichi Azuma, Hisato Harada.

Study Location: Osaka Clinical Pharmacological Institute, 12-11, 4-chome, Kasuga, Suita-shi, Osaka Pref.

Study Design and Dosing: Twenty six subjects comprised of four groups of six male volunteers participated in this open label, parallel design study. The average weight of the subjects was 64.2 kg (range 55.0 - 81.5 kg) and the average age was 21.7 years (range 20 - 24 years). The subjects were assigned to the following treatments of IV ciprofloxacin:

Treatment I: 100 mg administered twice daily as an infusion over 1 hour for 7 days. The second daily dose was given 8 hours (at 5 pm) after the first daily dose (at 8 am) and a 16 hour interval separated the afternoon dose from the next morning's dose. On Day 7, only a single morning dose was given.

Treatment II: 200 mg administered in the same manner and duration as for

Treatment I.

Treatment III: 200 mg given as an infusion over 2 hours twice daily for 7 days. The dosing schedule was the same as for Treatment I. Only a single morning dose was given on Day 7.

Treatment IV: 300 mg given as an infusion for 2 hours twice daily for 7 days. The dosing schedule was the same as for Treatment I. Only a single morning dose was given on Day 7.

Sample collection: Plasma samples were drawn at 0, 0.5, 1, 2, 2.25, 2.5, 3, 5, 8, 10, 11 and 23 for 200 mg and 300 mg, and at 0, 0.5, 1, 1.25, 1.5, 2, 3, 5, 8, 9, 10 and 23 hours for the 100 mg dose. All these samples were drawn on the 1st, 4th and 7th day of dosing. A sample at 7 hrs was drawn for the 100 mg dose on the 7th day instead of the sample at 9 hours.

Urine samples were collected over the following intervals on study days 1, 4 and 7: 0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-24 hrs, following the initial daily dose. On the other study days, a 4-8 hr sample was collected.

Analytical method: A HPLC method with fluorescence detection was used to measure ciprofloxacin in plasma and urine. The assay validation provided was satisfactory.

Parameter evaluation: The following parameters were calculated: AUC(0-8) and AUC(0-inf) using the log trapezoidal rule, Cmax, T1/2, total clearance (Cl), amount excreted in the urine (Xu) and renal clearance (Clr) from  $Xu(0-24) / AUC(0-inf)$ .

Statistical analysis: The sponsor performed an analysis of variance on the untransformed and log transformed parameters, AUC(0-inf), Cmax and Clr on Day 1 and 7. The 90% confidence intervals for AUC, Cmax and Clr were calculated comparing the 1 hour infusions (100 and 200 mg), the 2 hour infusions (200 and 300 mg) and the 1hr and 2 hr infusions of 200 mg. Only the ratio of AUC/Dose was analyzed using the unchanged parameter.

#### RESULTS:

The means of some parameters after sampling on Day 7 are listed below. A complete listing of individual subject parameters are in Tables 6-9.

| Total Dose | <u>100 mg</u> | <u>200 mg</u> | <u>200 mg</u> | <u>300 mg</u> |
|------------|---------------|---------------|---------------|---------------|
| Duration   | <u>1 hr</u>   | <u>1 hr</u>   | <u>2 hr</u>   | <u>2 hr</u>   |
| AUC(0-inf) | 2.97          | 5.90          | 6.64          | 11.38         |
| mcg*hr/ml  | 0.29          | 0.74          | 1.16          | 1.82          |
| Cmax       | 1.24          | 2.04          | 1.69          | 2.56          |
| mcg/ml     | 0.14          | 0.07          | 0.27          | 0.31          |

|                  |       |       |       |       |
|------------------|-------|-------|-------|-------|
| Cl <sub>s</sub>  | 34.35 | 34.30 | 31.21 | 26.88 |
| L/hr             | 4.88  | 3.68  | 6.15  | 3.68  |
| Cl <sub>r</sub>  | 22.29 | 24.91 | 23.35 | 19.26 |
| L/hr             | 6.08  | 2.72  | 7.2   | 2.38  |
| T <sub>1/2</sub> | 3.09  | 3.88  | 3.55  | 4.75  |
| hr               | 0.51  | 1.08  | 1.31  | 0.40  |

The systemic clearances was calculated by the reviewer. The individual concentration-time data are listed in Tables 10-13 and in Figures 7-16. The sponsor's statistical analysis are listed in Tables 14-15. Individual urine data is listed in Tables 16-23. The sponsor stated that the analysis of variance indicated that AUCs are not significantly different between doses for any infusion duration and day of administration. However, the sponsor's 90% confidence intervals for the dose normalized AUC on Day 1 (95.6 - 131.0) and Day 7 (97.5 - 134.7) revealed significant differences between the 300 and the 200 mg doses. Comparisons made by the sponsor using the 90% C.I. between the 300 and 100 mg doses for the AUC also showed significant differences on Day 1 (94.7 - 129.5) and on Day 7 (108.5 - 150.5). The dose normalized C<sub>max</sub> was significantly different on Days 1 and 7 for the 1 hr infusions (100 and 200 mg), but not for the 2 hour infusions (200 and 300 mg). The renal clearance was significantly different only between the 1 hr infusions (100 and 200 mg) on Day 1.

Conclusion: The pharmacokinetics of IV ciprofloxacin appear to be linear between 100 and 200 mg doses. There is a trend towards nonlinearity at the 300 mg dose as compared to the 100 and 200 mg doses. This may be due to inherent nonlinearity of ciprofloxacin or it could be due to demographic differences between the groups, since the study had a parallel design.

Study #5 : Vaginal Tissue Distribution Study: BAY o 9867/0504

Summary: A study to determine distribution of ciprofloxacin in vaginal tissues was performed in 10 women, below 65 years of age, hospitalized for gynecological surgery. The women were divided into two groups, which differed only in the sampling schedule:

| <u>Group</u> | <u>N</u> | <u>Blood sampling</u><br><u>(post-dose)</u> | <u>Tissue sampling</u><br><u>(post-dose)</u> |
|--------------|----------|---------------------------------------------|----------------------------------------------|
| I            | 5        | 0, 1, 2, 3, 4, 5, 6, 7 and 8 hrs            | Either 3 or 4 hr                             |
| II           | 5        | 0, 6, 7 and 8 hrs                           | 8 hr.                                        |

After administration of a single oral dose of 500 mg ciprofloxacin, serum and tissue samples were collected and analyzed for ciprofloxacin by an HPLC method. The table on the next page lists the tissues sampled and the mean ciprofloxacin concentrations in the tissues. The serum concentrations are in mcg/ml and the tissue concentrations in mcg/gm of

tissue.

| Time of sampling | Serum | Fallopian tube | Ovary | Endometrium | Uterus | Fat  | Skin | Muscle | Connective tissue |
|------------------|-------|----------------|-------|-------------|--------|------|------|--------|-------------------|
| 4 h              | 1.32  | 3.06           | 6.01  | 5.58        | 4.74   | 1.40 | 2.37 | 7.94   | 3.04              |
| 8 h              | 0.47  | 1.58           | 3.28  | 1.86        | 1.69   | 0.29 | 2.80 | 3.46   | 1.73              |

Conclusion: A statement to the effect that vaginal tissue concentrations, in general are higher than the corresponding serum concentrations, should be included in the labeling.

Study #6: Prostatic Tissue Distribution Study BAY o 9867/0170

Title: Diffusion of Ciprofloxacin into Human Prostatic Tissue after Oral and Intravenous Application.

Summary: Intraprostatic concentrations of ciprofloxacin were assessed in 19 patients undergoing transurethral resection. Nine patients were administered 500 mg po, and 10 patients 100 mg iv bolus of ciprofloxacin. Serum samples and prostatic tissue samples were drawn at 30 and 60 minutes after the dose and analyzed for ciprofloxacin using a microbial assay. The serum and prostatic tissue concentrations were as follows:

| Sampling time             | <u>After 500 mg po</u> |        | <u>After 100 mg iv</u> |        |
|---------------------------|------------------------|--------|------------------------|--------|
|                           | 30 min                 | 60 min | 30 min                 | 60 min |
| Serum (mcg/ml)            | 0.62                   | 0.65   | 0.55                   | 0.78   |
| Prostatic Tissue (mcg/ml) | 1.04                   | 1.43   | 0.48                   | 0.52   |

Conclusion: A statement to the effect that prostatic tissue concentrations, in general are higher than the corresponding serum concentrations, should be included in the labeling.

Study #7: Literature Article - Ciprofloxacin Distribution in the Eye

Title: Penetration of ciprofloxacin into the human eye.

Summary: Twenty patients received one of the following doses of ciprofloxacin prior to surgery:

| <u>Regimen</u>    | <u>No. of Patients</u> | <u>Frequency</u> |
|-------------------|------------------------|------------------|
| A) 1 X 750 mg po  | 3                      | -                |
| B) 2 X 750 mg po, | 3                      | q 12 h           |
| C) 3 X 750 mg po  | 3                      | q 12 h           |
| D) 1 X 400 mg iv  | 3                      | -                |
| E) 1 X 600 mg iv  | 6                      | -                |
| F) 3 X 200 mg iv  | 3                      | q 8 h            |

The IV doses were administered over 30 minutes. Pairs of samples were

drawn from the serum and from either the anterior or vitreous chamber at times ranging between 80 to 150 minutes after the last dose. Ciprofloxacin in the the tissue and serum samples were analyzed by HPLC method with fluorescence detection.

Since sequential samples were not drawn, plasma and tissue concentration profiles could not be determined. The tissue/plasma concentration ratios in the anterior chamber ranged from 0.1 to 0.35 after the IV regimens and 0.03 to 0.22 after oral administration. The tissue/plasma concentration ratio in the vitreous chamber ranged from 0.1 to 0.3 after the 3 X 200 mg IV regimen.

Conclusion: A statement to the effect that ciprofloxacin is present in measurable concentrations in the anterior and vitreous chambers of the eye should be included in the labeling.

Comments:

1. The sponsor was requested to, and provided, information on the solubility of ciprofloxacin in water, as a function of pH. It can be seen from Table 24 that the solubility at pH 5 is 36 mcg/ml, and between pH 6-8 is very low, 0.01 to 0.07 mcg/ml. Typically the concentrations in the urine ranged from approximately 7 to 158 mcg/ml after a 100 mg dose, and 25 to 520 mcg/ml after a 300 mg dose. This indicates a strong probability of ciprofloxacin crystallizing out of the urine, when the urine pH is between 6 to 8, and good probability when the pH is between 5 and 6. Therefore, safety issues regarding crystalluria, and precautions to avoid it such as water loading, need to be addressed by the reviewing medical officer.

2. From the Bayer-Yakuhin study, the pharmacokinetics of IV ciprofloxacin appear to be linear between 100 and 200 mg doses. There is a trend towards nonlinearity at the 300 mg dose as compared to the 200 mg dose. The systemic clearance of the 300 mg dose decreased by 12-14% and the terminal half-life increased by 12 to 33%, as compared to the 200 mg dose. The systemic clearances were not significantly different by analysis of variance but the sponsor's 90% C.I. showed differences between the doses. It is suggested that this information be incorporated into the labeling.

3. In study D86-061-01, the systemic clearance in cirrhotics was entirely due to renal clearance, which was surprisingly not very different from the systemic clearance of the normal subjects. This indicates that in a hepatically impaired patient, the renal clearance compensates almost entirely for the lack of metabolic clearance.

4. The labeling needs to be amended to reflect the presence of ciprofloxacin in various tissues such as the vagina, prostate, and the anterior and vitreous chambers of the eye.

5. The systemic clearances on Day 7 were lower by 12-14% as compared to Day 1, for the 1-hr and 2-hr 200 mg, and 300 mg doses.

6. In the renal impairment study (D85-008-01), the systemic clearance of Group 4 was greater than Group 3, although Group 4 had no renal clearance. This may indicate increased nonrenal clearance in Group 4 as compared to Group 3.

7. In the hepatic impairment study (D86-061-01), it is not clear if the data on metabolites M1, M2 and M3 are provided to complete the pharmacokinetic profile of ciprofloxacin or if the metabolite(s) are active. In either case, since the total excretion of the metabolites is, on an average, less than 4% individually, and less than 10% collectively, the disparities in the M3 metabolite between the two groups may not be significant.

Recommendation: From the Division of Biopharmaceutics' standpoint, the bioavailability requirements for NDA 19-847, Cipro<sup>R</sup> IV (ciprofloxacin IV) 100, 200 and 300 mg IV solution have been fulfilled. The labeling needs to be changed to reflect Comments 1-5.

Please send Comments 1-7 and the Recommendation to the reviewing medical officer.

  
Suresh Mallikaarjun, Ph.D.  
Pharmacokinetic Evaluation Branch

FT initialled by C.T. Viswanathan, Ph.D. CTV 618190

cc: NDA 19-847 Original, HFD-520, HFD-426 (Mallikaarjun),  
HFD-443(Turner), Review, Drug, Chron and HFD-19 (FOI) files.

SUMMARY OF CHEMISTRY, MANUFACTURING, AND CONTROL

APPENDIX

(A)

determination of the subject compounds. The identity of ciprofloxacin is accomplished by a combination of thin-layer chromatography and infrared spectroscopy.

2. DRUG PRODUCT

1. Composition and Dosage Form

Ciprofloxacin solutions for intravenous use are available as 1.0% concentrates which are intended for dilution prior to administration and as ready-for-use 0.2% infusion products in either 0.9% Sodium Chloride Injection or 5% Dextrose Injection. (Each of the three formulations is the subject of a separate New Drug Application.) The products have the following compositions:

Composition of 1.0% Concentrate Solution

| <u>Ingredient</u>    | <u>Amount (mg/100 ml)</u> |
|----------------------|---------------------------|
| Ciprofloxacin        | 1000                      |
| Lactic Acid, 20% W/W |                           |
| Hydrochloric Acid    |                           |
| Water for Injection  | to make                   |

Composition of 0.2% Infusion Solutions

| <u>Ingredient</u>          | <u>Amount (mg/100 ml)</u> |         |
|----------------------------|---------------------------|---------|
| Ciprofloxacin (BAY q 3939) | 200                       | 200     |
| Lactic Acid, 20% W/W       |                           |         |
| Dextrose, Hydrous          |                           |         |
| Sodium Chloride            |                           |         |
| Hydrochloric Acid          |                           |         |
| Water for Injection        |                           | to make |

\*Sufficient quantity for pH adjustment to specified value.

Miles Inc.  
Pharmaceutical Division  
400 Morgan Lane  
West Haven, CT 06516

PAGE

02-00042

001-000-12  
(Rev. 10/87)

PHARMACEUTICAL PHARM

SUMMARY OF CHEMISTRY, MANUFACTURING, AND CONTROL

APPENDIX (A)

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Ciprofloxacin solutions for intravenous use are available as 1.0% concentrates which are intended for dilution prior to administration and as ready-for-use 0.2% infusion products in either 0.9% Sodium Chloride Injection or 5% Dextrose Injection. (Each of the three formulations is the subject of a separate New Drug Application.) The products have the following compositions:

Composition of 1.0% Concentrate Solution

| <u>Ingredient</u>    | <u>Amount (mg/100 ml)</u> |
|----------------------|---------------------------|
| Ciprofloxacin        | 1000                      |
| Lactic Acid, 20% W/W |                           |
| Hydrochloric Acid    |                           |
| Water for Injection  | to make                   |

Composition of 0.2% Infusion Solutions

| <u>Ingredient</u>          | <u>Amount (mg/100 ml)</u> |     |
|----------------------------|---------------------------|-----|
| Ciprofloxacin (BAY q 3939) | 200                       | 200 |
| Lactic Acid, 20% W/W       |                           |     |
| Dextrose, Hydrous          |                           |     |
| Sodium Chloride            |                           |     |
| Hydrochloric Acid          |                           |     |
| Water for Injection        | to make                   |     |

\*Sufficient quantity for pH adjustment to specified value.

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Pharmaceutical Division  
400 Morgan Lane  
West Haven, CT 06516

PAGE

02-00042

02-000-12  
(Rev. 10/87)

PHARMACEUTICAL PHARM

SUMMARY OF CHEMISTRY, MANUFACTURING, AND CONTROL

APPENDIX

(A)

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Composition of 1.0% Concentrate Solution

| <u>Ingredient</u>    | <u>Amount (mg/100 ml)</u> |
|----------------------|---------------------------|
| Ciprofloxacin        | 1000                      |
| Lactic Acid, 20% W/W |                           |
| Hydrochloric Acid    |                           |
| Water for Injection  | to make                   |

Composition of 0.2% Infusion Solutions

| <u>Ingredient</u>          | <u>Amount (mg/100 ml)</u> |         |
|----------------------------|---------------------------|---------|
| Ciprofloxacin (BAY q 3939) | 200                       | 200     |
| Lactic Acid, 20% W/W       |                           |         |
| Dextrose, Hydrous          |                           |         |
| Sodium Chloride            |                           |         |
| Hydrochloric Acid          |                           |         |
| Water for Injection        |                           | to make |

\*Sufficient quantity for pH adjustment to specified value.

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02-00042

001-000-12  
(REV. 10/87)

PHARMACEUTICAL PHARM

APPENDIX (B)

*Drug*

Ciprofloxacin HCL  
100, 250 500, and 750mg tablets  
NDA 19-537  
Reviewer: Ko-Yu Lo  
Wang #3900X  
1-N

Miles  
400 Morgan Lane  
West Haven, Connecticut 06516  
Submission Date:  
February 1, 1986

Review of a Submission

This submission contains the biopharmaceutic portion of NDA 19-537 (Attachment 1) which has not been requested by the NDE Division (HFD-520) for a bioreview. In a recent telephone conversation (7/8/88) with the CSO (Veronika Sabritzky) from HFD-520 it was learned that a review from the Division of Biopharmaceutics (DB) for this NDA is not required. Therefore, DB is returning these jackets to HFD-520 without conducting a formal review of this submission.

*Ko-yu Lo 7/11/88*  
Ko-Yu Lo, Ph.D.

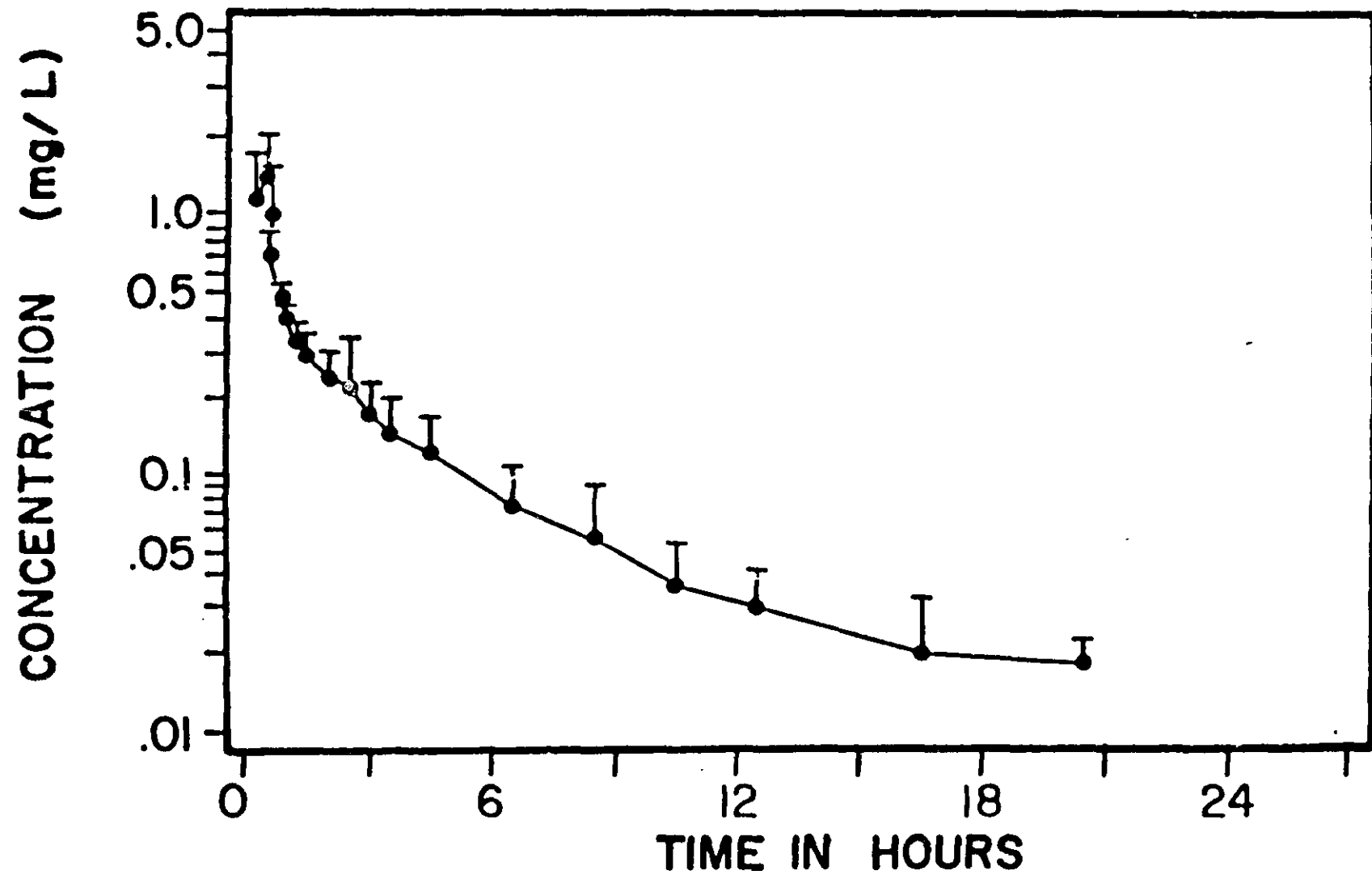
Pharmacokinetics Evaluation Branch

RD Initialed by John P. Hunt (7/11/88)  
FT Initialed by C.T. Viswanathan, Ph.D. CTV 7/11/88

cc: NDA 19-537 Orig., HFN-520, HFD-426 (Lo, Hunt), HFD-344 (Turner), Drug, Chron, and FOI files

KYL:mo:3900X:7/8/88

MEAN (+SD) SERUM CIPROFLOXACIN CONCENTRATIONS IN 9 VOLUNTEERS FOLLOWING A SINGLE 100 mg IV INFUSION

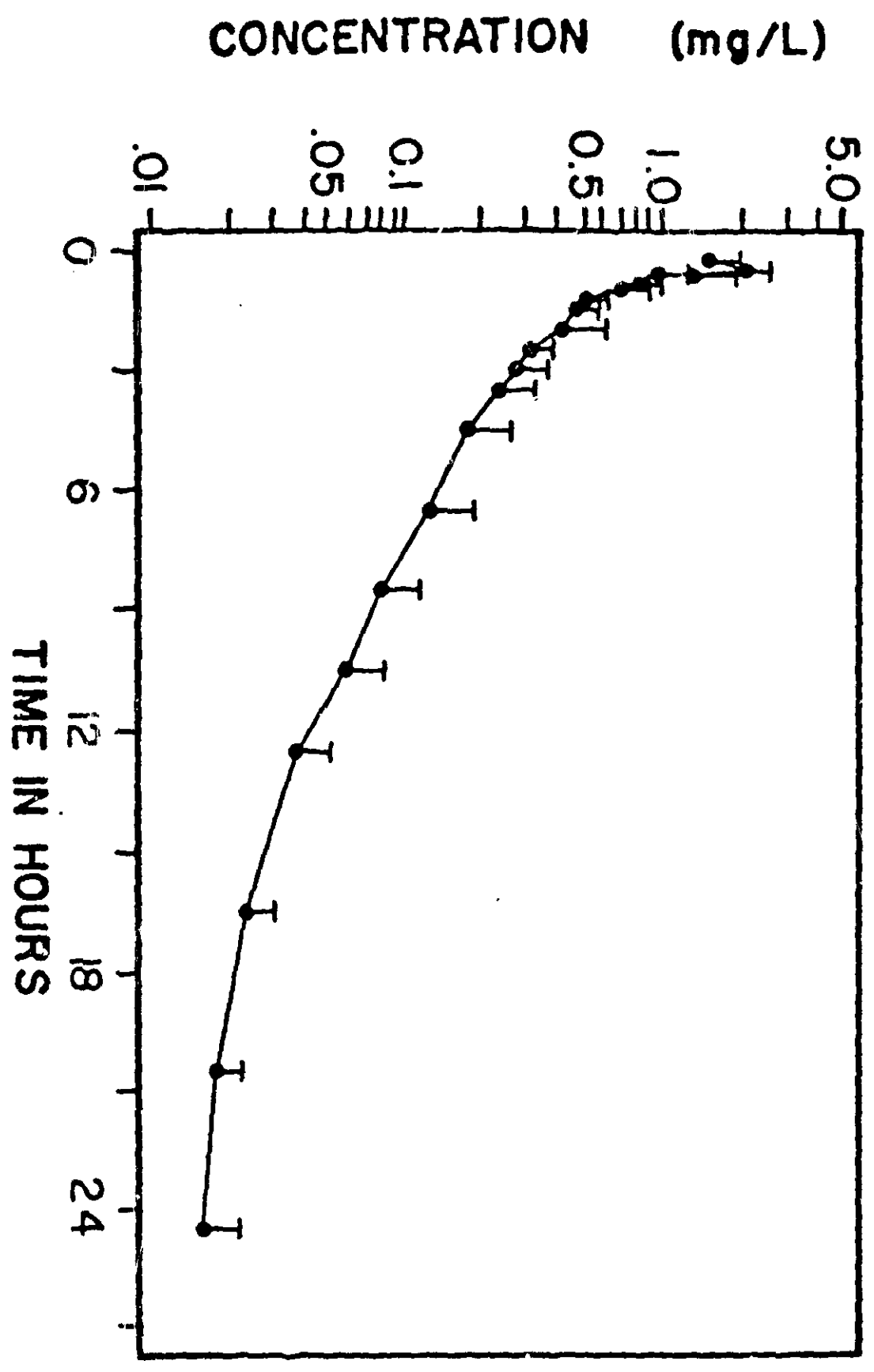


05-2-0023

Fig 19  
①

MEAN (+SD) SERUM CIPROFLOXACIN CONCENTRATIONS IN  
9 VOLUNTEERS FOLLOWING A SINGLE 150 mg IV INFUSION

05-2-0024



MEAN (+SD) SERUM CIPROFLOXACIN CONCENTRATIONS IN 9 VOLUNTEERS FOLLOWING A SINGLE 200mg IV INFUSION

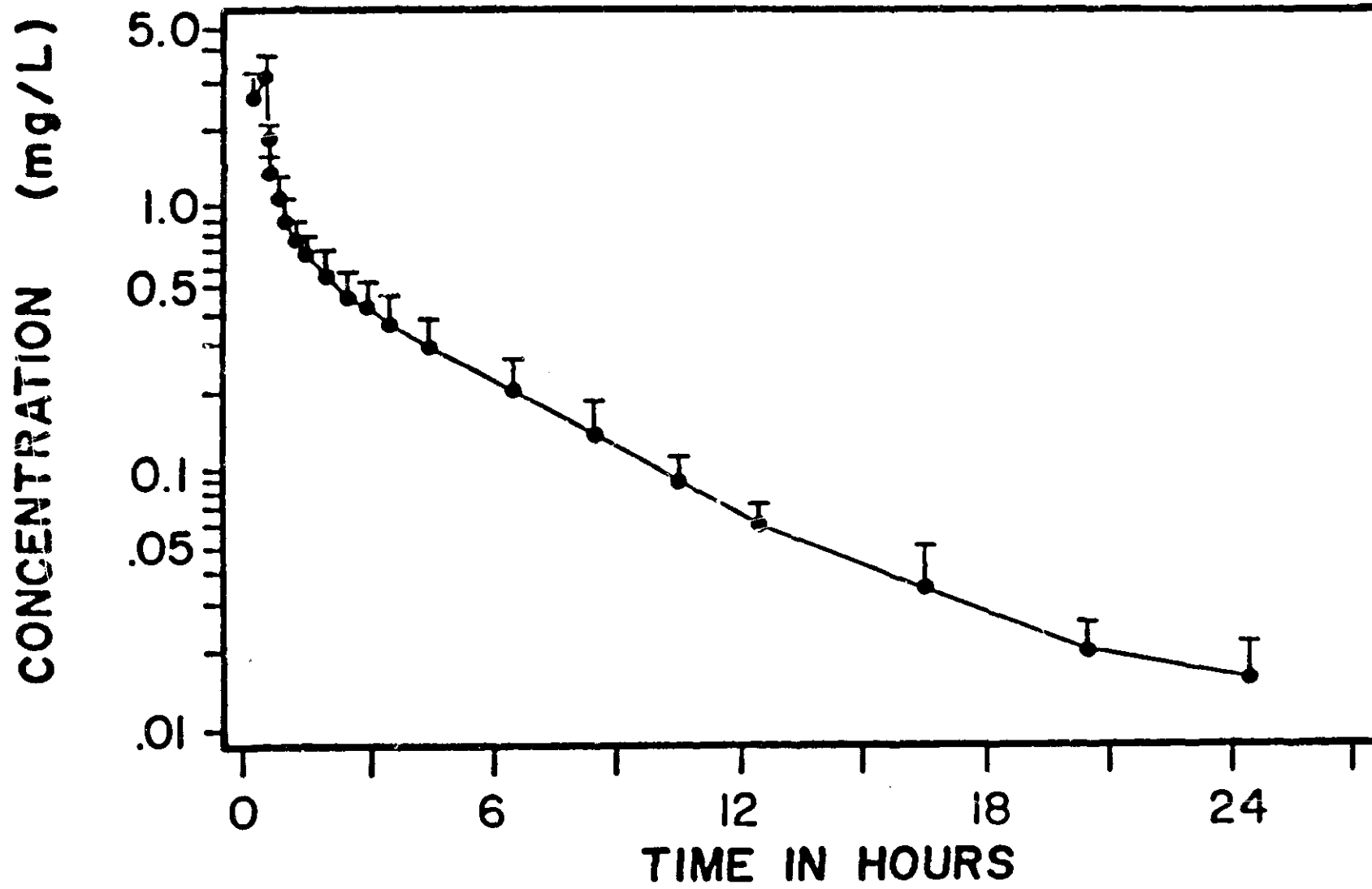


Fig 5, 21

TABLE 3

SERUM PHARMACOKINETICS OF INTRAVENOUS CIPROFLOXACIN IN 9 VOLUNTEERS\*

| DOSE | AUC<br>(MG-HR/L) | PEAK<br>CONCENTRATION<br>(MG/L) | TOTAL BODY<br>CLEARANCE <sup>†</sup><br>(ML/MIN/KG) | VOLUME OF<br>DISTRIBUTION AT<br>STEADY-STATE <sup>†</sup><br>(L/KG) | T <sub>1/2</sub> <sup>†</sup><br>(HRS) | MEAN <sup>†</sup><br>RESIDENCE<br>TIME<br>(HRS) |
|------|------------------|---------------------------------|-----------------------------------------------------|---------------------------------------------------------------------|----------------------------------------|-------------------------------------------------|
| 100  | 2.24 ± 0.54      | 1.37 ± 0.57                     | 9.60 ± 2.09                                         | 2.17 ± 0.15                                                         | 4.1 ± 0.9                              | 3.9 ± 1.8                                       |
| 150  | 3.36 ± 0.74      | 2.04 ± 0.51                     | 9.57 ± 2.02                                         | 2.40 ± 0.61                                                         | 4.6 ± 1.2                              | 4.2 ± 0.8                                       |
| 200  | 5.17 ± 0.87      | 3.18 ± 0.63                     | 8.15 ± 1.21                                         | 2.00 ± 0.39                                                         | 4.2 ± 0.6                              | 4.1 ± 0.5                                       |

\* VALUES ARE MEAN ± SD.

† NO EFFECT OF DOSE ON THESE PARAMETERS (P > 0.05, ANOVA).

05-2-0015

TABLE 3  
11

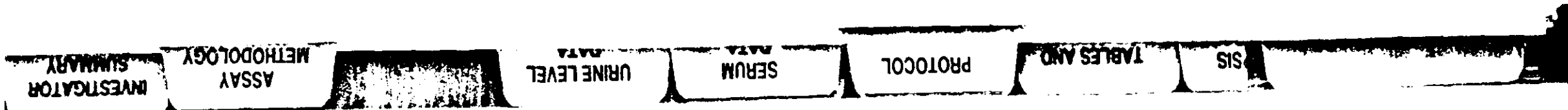


TABLE 3

SERUM PHARMACOKINETICS OF INTRAVENOUS CIPROFLOXACIN IN 9 VOLUNTEERS\*

| DOSE | AUC<br>(MG-HR/L) | PEAK<br>CONCENTRATION<br>(MG/L) | TOTAL BODY<br>CLEARANCE <sup>†</sup><br>(ML/MIN/KG) | VOLUME OF<br>DISTRIBUTION AT<br>STEADY-STATE <sup>†</sup><br>(L/KG) | T <sub>1/2</sub> <sup>†</sup><br>(HRS) | MEAN <sup>†</sup><br>RESIDENCE<br>TIME<br>(HRS) |
|------|------------------|---------------------------------|-----------------------------------------------------|---------------------------------------------------------------------|----------------------------------------|-------------------------------------------------|
| 100  | 2.24 ± 0.54      | 1.37 ± 0.57                     | 9.60 ± 2.09                                         | 2.17 ± 0.45                                                         | 4.1 ± 0.9                              | 3.9 ± 1.8                                       |
| 150  | 3.36 ± 0.74      | 2.04 ± 0.51                     | 9.57 ± 2.02                                         | 2.40 ± 0.61                                                         | 4.6 ± 1.2                              | 4.2 ± 0.8                                       |
| 200  | 5.17 ± 0.87      | 3.18 ± 0.63                     | 8.15 ± 1.21                                         | 2.00 ± 0.39                                                         | 4.2 ± 0.6                              | 4.1 ± 0.5                                       |

\* VALUES ARE MEAN ± SD.

<sup>†</sup> NO EFFECT OF DOSE ON THESE PARAMETERS (P > 0.05, ANOVA).

05-2-0015

TABLE 3

11

TABLE 4

CLEARANCE OF CIPROFLOXACIN FOLLOWING A SINGLE DOSE IN 9 VOLUNTEERS\*

| DOSE | TOTAL SERUM<br>CLEARANCE<br>(ML/MIN/KG) | % EXCRETED<br>UNCHANGED | RENAL CLEARANCE<br>(ML/MIN/KG) | RENAL CLEARANCE:<br>CREATININE CLEARANCE | NON RENAL<br>CLEARANCE<br>(ML/MIN/KG) |
|------|-----------------------------------------|-------------------------|--------------------------------|------------------------------------------|---------------------------------------|
| 100  | 9.60 ± 2.09                             | 48.1 ± 5.6              | 4.61 ± 1.14                    | 3.41 ± 0.78                              | 4.99 ± 1.18                           |
| 150  | 9.57 ± 2.02                             | 45.8 ± 5.9              | 4.40 ± 1.16                    | 3.26 ± 0.92                              | 5.17 ± 1.11                           |
| 200  | 8.15 ± 1.21                             | 46.4 ± 6.9              | 3.80 ± 0.87                    | 2.86 ± 0.86                              | 4.34 ± 0.71                           |

\* VALUES ARE MEAN ± SD. THERE IS NO EFFECT OF DOSE ON THESE PARAMETERS (P > 0.05, ANOVA).

05-2-0016

TABLE 4

12

TABLE 4

CLEARANCE OF CIPROFLOXACIN FOLLOWING A SINGLE DOSE IN 9 VOLUNTEERS\*

| DOSE | TOTAL SERUM<br>CLEARANCE<br>(ML/MIN/KG) | % EXCRETED<br>UNCHANGED | RENAL CLEARANCE<br>(ML/MIN/KG) | RENAL CLEARANCE:<br>CREATININE CLEARANCE | NON RENAL<br>CLEARANCE<br>(ML/MIN/KG) |
|------|-----------------------------------------|-------------------------|--------------------------------|------------------------------------------|---------------------------------------|
| 100  | 9.60 ± 2.09                             | 48.1 ± 5.6              | 4.61 ± 1.14                    | 3.41 ± 0.78                              | 4.99 ± 1.18                           |
| 150  | 9.57 ± 2.02                             | 45.8 ± 5.9              | 4.40 ± 1.16                    | 3.26 ± 0.92                              | 5.17 ± 1.11                           |
| 200  | 8.15 ± 1.21                             | 46.4 ± 6.9              | 3.80 ± 0.87                    | 2.86 ± 0.86                              | 4.34 ± 0.71                           |

\* VALUES ARE MEAN ± SD. THERE IS NO EFFECT OF DOSE ON THESE PARAMETERS (P > 0.05, ANOVA).

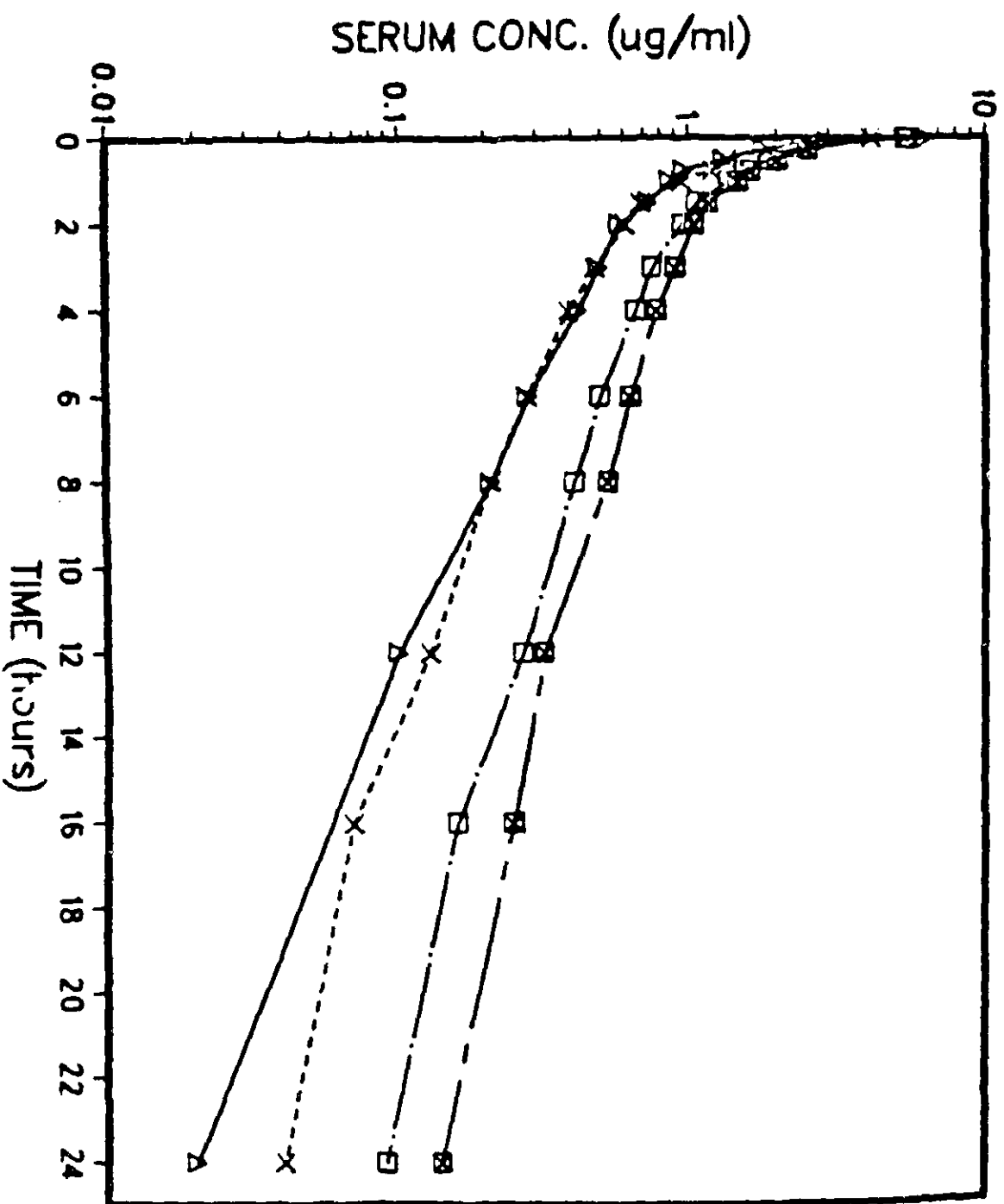
05-2-0016

TABLE 2

12

SERUM CONCENTRATIONS OF CIPROFLOXACIN  
(STUDY D85-008-01)

FIGURE 1



Legend  
 Δ GROUP 1  
 X GROUP 2  
 □ GROUP 3  
 ⊠ GROUP 4

05-2-0902

600000

FF  
 (3)

TABLE (3)

000007

Table 2  
Model Independent Pharmacokinetic Parameters for  
200 mg of Ciprofloxacin Administered to Patients  
with Varying Degrees of Renal Dysfunction

| GROUP | V <sub>AREA</sub><br>L/KG | SCL<br>L/Hr/1.73m <sup>2</sup> | RCL<br>L/Hr/1.73m <sup>2</sup> | T <sub>1/2</sub><br>Hr | RCL/SCL<br>%      |
|-------|---------------------------|--------------------------------|--------------------------------|------------------------|-------------------|
| I     | 2.49<br>±<br>0.46         | 26.8<br>±<br>5.7               | 16.4<br>±<br>3.5               | 4.27<br>±<br>0.84      | 61.6<br>±<br>8.7  |
| II    | 3.19<br>±<br>1.26         | 26.3<br>±<br>10.3              | 11.9<br>±<br>5.3               | 6.12<br>±<br>1.61      | 44.9<br>±<br>11.9 |
| III   | 2.38<br>±<br>0.62         | 15.0<br>±<br>3.8               | 4.5<br>±<br>2.6                | 7.70<br>±<br>1.22      | 29.2<br>±<br>13.2 |
| IV    | 2.73<br>±<br>0.92         | 15.4<br>±<br>4.3               | 0.0                            | 8.55<br>±<br>3.27      | 0.0               |

V<sub>area</sub> = Volume of distribution in the post-distributional phase;  
SCL = Serum clearance; RCL = Renal clearance; T<sub>1/2</sub> = Terminal  
half life.

Values are mean ± standard deviation.

05-2-0900

TABLE 9. INDIVIDUAL CIPROFLOXACIN PHARMACOKINETIC PARAMETERS FOR THE 200 MG IV DOSE

| CIRRHOTIC |                    |                    |                       |                      |                     |                        |         |                       |      |
|-----------|--------------------|--------------------|-----------------------|----------------------|---------------------|------------------------|---------|-----------------------|------|
| SUBJECTS  | AUC <sub>0-t</sub> | AUC <sub>0-∞</sub> | Ke <sub>1</sub> (1/h) | t <sub>1/2</sub> (h) | V <sub>ss</sub> (L) | V <sub>ss</sub> (L/kg) | Cl(L/h) | Cl <sub>r</sub> (L/h) | MRT  |
| 1         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 2         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 3         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 4         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 5         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 6         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 7         |                    |                    |                       |                      |                     |                        |         |                       |      |
| MEAN      | 7.35               | 7.71               | 0.1446                | 5.34                 | 147.80              | 1.97                   | 28.19   | 13.66                 | 5.51 |
| STD       | 2.17               | 2.40               | 0.0583                | 1.38                 | 38.33               | 0.55                   | 7.53    | 6.19                  | 1.56 |
| CV%       | 29.5               | 31.1               | 40.3                  | 25.8                 | 25.9                | 28.1                   | 26.7    | 45.3                  | 28.3 |
| NORMAL    |                    |                    |                       |                      |                     |                        |         |                       |      |
| SUBJECTS  | AUC <sub>0-t</sub> | AUC <sub>0-∞</sub> | Ke <sub>1</sub> (1/h) | t <sub>1/2</sub> (h) | V <sub>ss</sub> (L) | V <sub>ss</sub> (L/kg) | Cl(L/h) | Cl <sub>r</sub> (L/h) | MRT  |
| 8         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 9         |                    |                    |                       |                      |                     |                        |         |                       |      |
| 10        |                    |                    |                       |                      |                     |                        |         |                       |      |
| 11        |                    |                    |                       |                      |                     |                        |         |                       |      |
| 12        |                    |                    |                       |                      |                     |                        |         |                       |      |
| 13        |                    |                    |                       |                      |                     |                        |         |                       |      |
| 14        |                    |                    |                       |                      |                     |                        |         |                       |      |
| MEAN      | 6.12               | 6.41               | 0.1648                | 4.89                 | 146.17              | 1.90                   | 33.29   | 17.19                 | 4.64 |
| STD       | 1.68               | 1.73               | 0.0725                | 1.58                 | 49.88               | 0.60                   | 7.99    | 9.76                  | 1.70 |
| CV%       | 27.5               | 27.0               | 44.0                  | 32.4                 | 34.1                | 31.8                   | 24.0    | 56.7                  | 36.6 |

05-2-1179

Table 4

TABLE 11. CUMULATIVE URINARY RECOVERY OF CIPROFLOXACIN AND METABOLITES (0-12 HRS) AFTER A 200 MG. IV DOSE.

| CIRRHOTIC<br>SUBJECTS | CIPROFLOXACIN |           | M1        |            | M2        |            | M3        |            |           |
|-----------------------|---------------|-----------|-----------|------------|-----------|------------|-----------|------------|-----------|
|                       | AMOUNT(mg)    | % OF DOSE | Cir(1/hr) | AMOUNT(mg) | % OF DOSE | AMOUNT(mg) | % OF DOSE | AMOUNT(mg) | % OF DOSE |
| 1                     |               |           |           |            |           |            |           |            |           |
| 2                     |               |           |           |            |           |            |           |            |           |
| 3                     |               |           |           |            |           |            |           |            |           |
| 4                     |               |           |           |            |           |            |           |            |           |
| 5                     |               |           |           |            |           |            |           |            |           |
| 6                     |               |           |           |            |           |            |           |            |           |
| 7                     |               |           |           |            |           |            |           |            |           |
| MEAN                  | 90.78         | 45.39     | 13.67     | 1.22       | 0.61      | 10.61      | 5.31      | 6.40       | 3.20      |
| STD                   | 29.60         | 14.80     | 6.19      | 0.81       | 0.40      | 4.17       | 2.08      | 3.43       | 1.72      |
| CVX                   | 32.6          | 32.6      | 45.3      | 66.0       | 66.0      | 39.3       | 39.3      | 53.6       | 53.6      |

| NORMAL<br>SUBJECTS | CIPROFLOXACIN |           | M1        |            | M2        |            | M3        |            |           |
|--------------------|---------------|-----------|-----------|------------|-----------|------------|-----------|------------|-----------|
|                    | AMOUNT(mg)    | % OF DOSE | Cir(1/hr) | AMOUNT(mg) | % OF DOSE | AMOUNT(mg) | % OF DOSE | AMOUNT(mg) | % OF DOSE |
| 8                  |               |           |           |            |           |            |           |            |           |
| 9                  |               |           |           |            |           |            |           |            |           |
| 10                 |               |           |           |            |           |            |           |            |           |
| 11                 |               |           |           |            |           |            |           |            |           |
| 12                 |               |           |           |            |           |            |           |            |           |
| 13                 |               |           |           |            |           |            |           |            |           |
| 14                 |               |           |           |            |           |            |           |            |           |
| MEAN               | 96.65         | 48.33     | 17.19     | 1.30       | 0.65      | 9.68       | 4.84      | 14.34      | 7.17      |
| STD                | 38.94         | 19.47     | 9.76      | 0.52       | 0.26      | 6.81       | 3.41      | 10.67      | 5.34      |
| CVX                | 40.3          | 40.3      | 56.7      | 40.0       | 40.0      | 70.4       | 70.4      | 74.4       | 74.4      |

05-2-0181

TABLE 11  
⑤

# EFFECT OF CIRRHOSIS ON CIPROFLOXACIN

MEAN DATA (NORMAL GROUP)

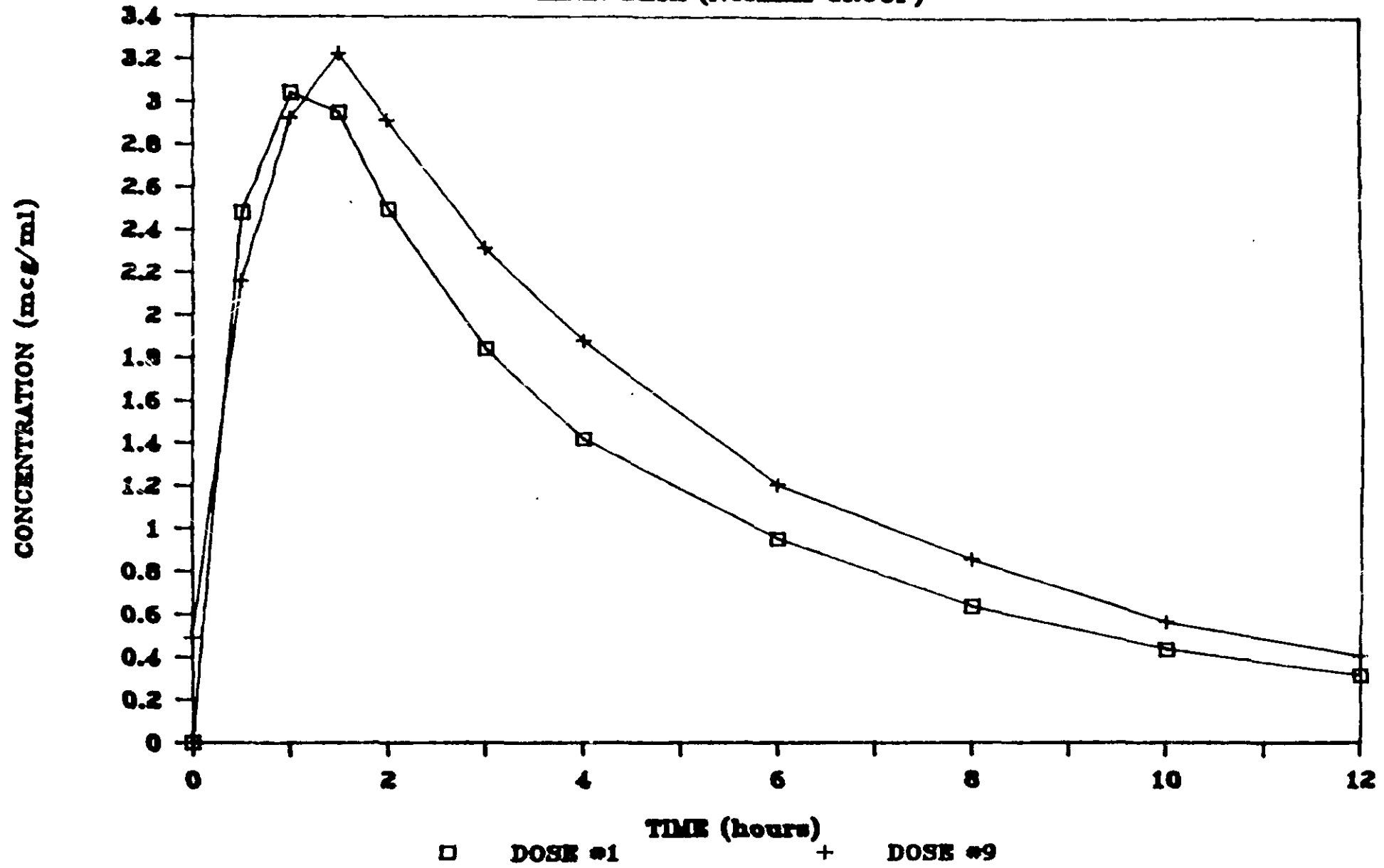


Figure 9. Mean ciprofloxacin serum concentrations following the first and ninth 750 mg oral dose.

05-2-0190

Fig 9  
④

# EFFECT OF CIRRHOSIS ON CIPROFLOXACIN

MEAN DATA (CIRRHOTIC GROUP)

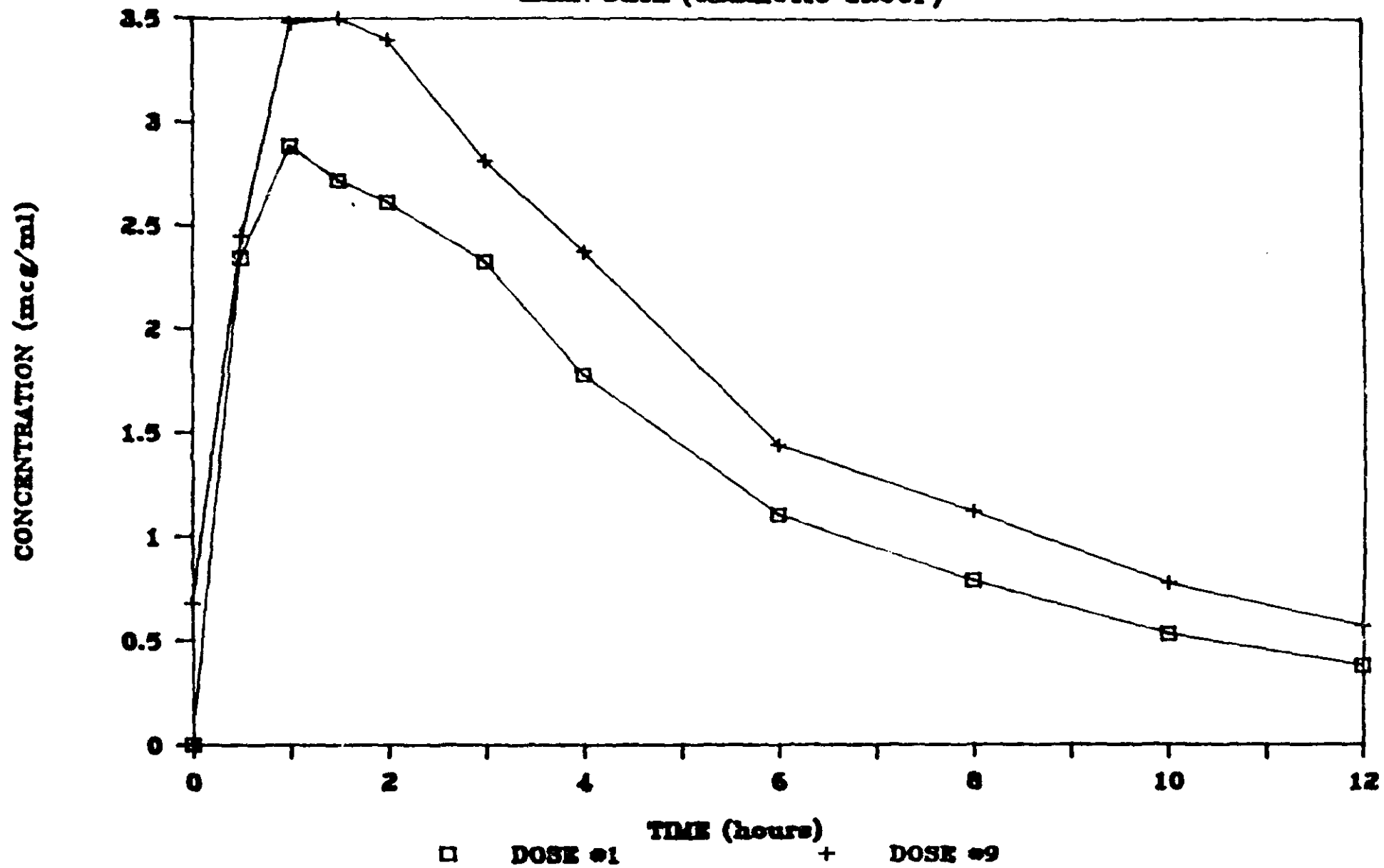


Figure 10. Mean ciprofloxacin concentrations following the first and ninth 750 mg oral dose.

05-2-0191

20

# EFFECT OF CIRRHOSIS ON CIPROFLOXACIN

200 MG IV INFUSION

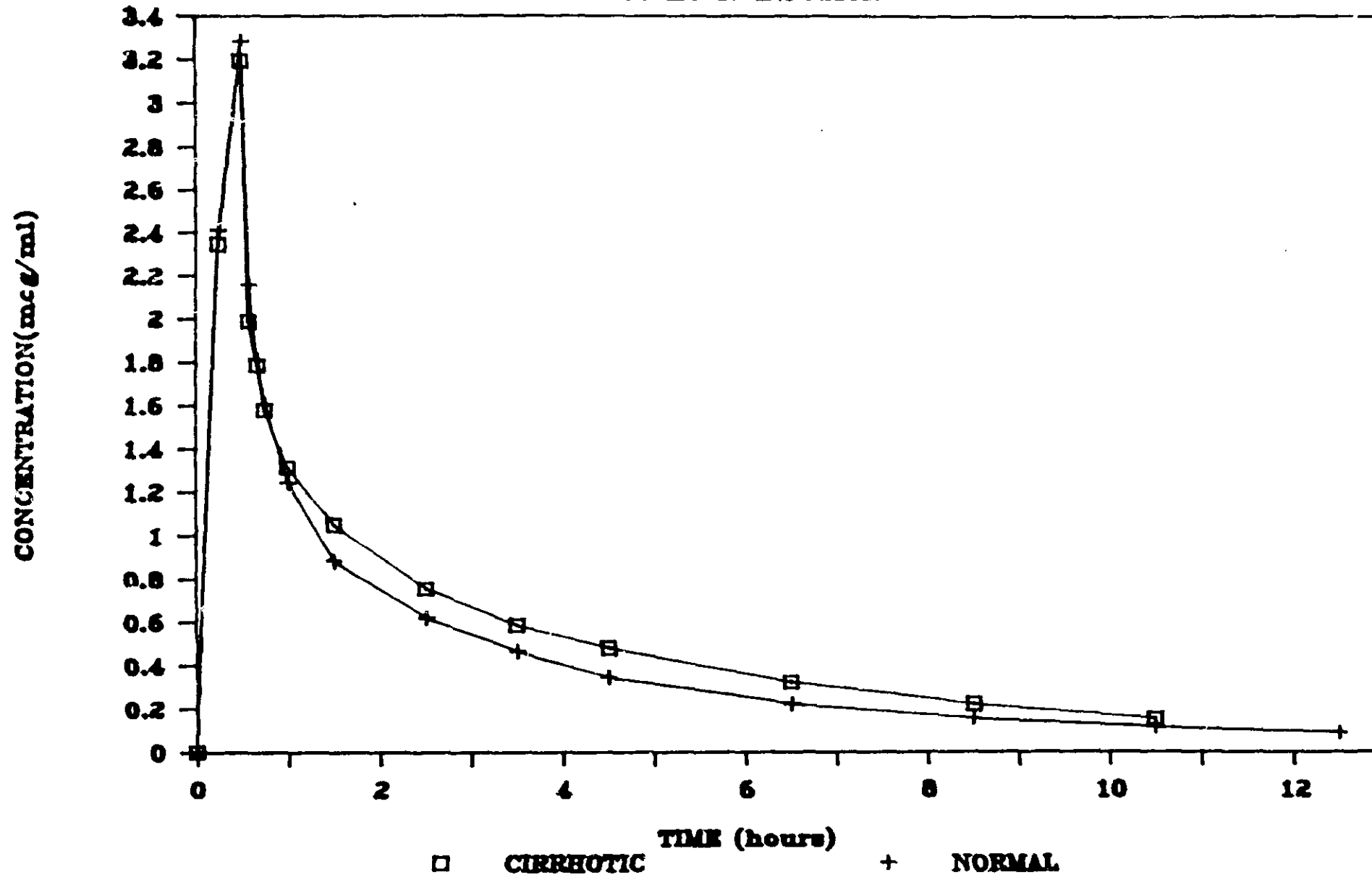


Figure 11. Mean ciprofloxacin serum concentrations following a 30 minute intravenous infusion.

05-2-0192

LENG

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuhin Ciprofloxacin Study

Dose = 100 mg as 1 Hour Infusion

DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>Cmax</u><br><u>(<math>\mu\text{g/ml}</math>)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-8)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|-------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 1              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| 2              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| 3              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| 4              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| 5              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| 6              | —                                                           | —                                                                            | —                                                   | —                           | —                             | —                           |
| Mean           | 2.48                                                        | 2.94                                                                         | 1.51                                                | 3.38                        | 53.8                          | 21.83                       |
| S.D.           | 0.18                                                        | 0.39                                                                         | 0.20                                                | 1.13                        | 3.8                           | 2.56                        |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>Cmax</u><br><u>(<math>\mu\text{g/ml}</math>)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-24)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|-------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------|--------------------------------|-----------------------------|
| 1              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| 2              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| 3              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| 4              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| 5              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| 6              | —                                                           | —                                                                            | —                                                   | —                           | —                              | —                           |
| Mean           | 2.69                                                        | 2.97                                                                         | 1.24                                                | 3.09                        | 64.93                          | 22.29                       |
| S.D.           | 0.29                                                        | 0.45                                                                         | 0.14                                                | 0.51                        | 16.84                          | 6.08                        |

P.B.C. (7)

000005

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuhiin Ciprofloxacin Study

Dose = 200 mg as 1 Hour Infusion

DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>Cmax</u><br><u>(<math>\mu</math>g/ml)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-8)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 7              |                                                      |                                                                       |                                              |                             |                               |                             |
| 8              |                                                      |                                                                       |                                              |                             |                               |                             |
| 9              |                                                      |                                                                       |                                              |                             |                               |                             |
| 11             |                                                      |                                                                       |                                              |                             |                               |                             |
| 12             |                                                      |                                                                       |                                              |                             |                               |                             |
| Mean           | 4.27                                                 | 5.11                                                                  | 2.01                                         | 3.21                        | 113.9                         | 26.86                       |
| S.D.           | 0.40                                                 | 0.69                                                                  | 0.09                                         | 0.60                        | 6.9                           | 3.08                        |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>Cmax</u><br><u>(<math>\mu</math>g/ml)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-24)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------|-----------------------------|--------------------------------|-----------------------------|
| 7              |                                                      |                                                                       |                                              |                             |                                |                             |
| 8              |                                                      |                                                                       |                                              |                             |                                |                             |
| 9              |                                                      |                                                                       |                                              |                             |                                |                             |
| 11             |                                                      |                                                                       |                                              |                             |                                |                             |
| 12             |                                                      |                                                                       |                                              |                             |                                |                             |
| Mean           | 5.34                                                 | 5.90                                                                  | 2.04                                         | 3.88                        | 145.3                          | 24.91                       |
| S.D.           | 0.88                                                 | 0.74                                                                  | 0.07                                         | 1.08                        | 4.8                            | 2.72                        |

\* AUC (0-23h)

T.D.B.C. (7)

000005

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuhin Ciprofloxacin Study

Dose = 200 mg as 1 Hour Infusion

DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>AUC(0-<math>\infty</math>)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>Cmax</u><br>( $\mu\text{g/ml}$ ) | <u>t1/2</u><br>(hrs) | <u>Xu(0-8)</u><br>(mg) | <u>Clr</u><br>(L/hr) |
|----------------|---------------------------------------------|--------------------------------------------------------------|-------------------------------------|----------------------|------------------------|----------------------|
| 7              |                                             |                                                              |                                     |                      |                        |                      |
| 8              |                                             |                                                              |                                     |                      |                        |                      |
| 9              |                                             |                                                              |                                     |                      |                        |                      |
| 11             |                                             |                                                              |                                     |                      |                        |                      |
| 12             |                                             |                                                              |                                     |                      |                        |                      |
| Mean           | 4.27                                        | 5.11                                                         | 2.01                                | 3.21                 | 113.9                  | 26.86                |
| S.D.           | 0.40                                        | 0.69                                                         | 0.09                                | 0.60                 | 6.9                    | 3.08                 |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>AUC(0-<math>\infty</math>)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>Cmax</u><br>( $\mu\text{g/ml}$ ) | <u>t1/2</u><br>(hrs) | <u>Xu(0-24)</u><br>(mg) | <u>Clr</u><br>(L/hr) |
|----------------|---------------------------------------------|--------------------------------------------------------------|-------------------------------------|----------------------|-------------------------|----------------------|
| 7              |                                             |                                                              |                                     |                      |                         |                      |
| 8              |                                             |                                                              |                                     |                      |                         |                      |
| 9              |                                             |                                                              |                                     |                      |                         |                      |
| 11             |                                             |                                                              |                                     |                      |                         |                      |
| 12             |                                             |                                                              |                                     |                      |                         |                      |
| Mean           | 5.34                                        | 5.90                                                         | 2.04                                | 3.88                 | 145.3                   | 24.91                |
| S.D.           | 0.88                                        | 0.74                                                         | 0.07                                | 1.08                 | 4.8                     | 2.72                 |

\* AUC (0-23h)

000006

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuhin Ciprofloxacin Study

Dose = 200 mg as 2 Hour Infusion

DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>AUC(0-<math>\infty</math>)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>Cmax</u><br>( $\mu\text{g/ml}$ ) | <u>t1/2</u><br>(hrs) | <u>Xu(0-8)</u><br>(mg) | <u>Clr</u><br>(L/hr) |
|----------------|---------------------------------------------|--------------------------------------------------------------|-------------------------------------|----------------------|------------------------|----------------------|
| 13             |                                             |                                                              |                                     |                      |                        |                      |
| 14             |                                             |                                                              |                                     |                      |                        |                      |
| 15             |                                             |                                                              |                                     |                      |                        |                      |
| 16             |                                             |                                                              |                                     |                      |                        |                      |
| 18             |                                             |                                                              |                                     |                      |                        |                      |
| Mean           | 4.97                                        | 5.85                                                         | 1.74                                | 3.07                 | 110.6                  | 22.97                |
| S.D.           | 0.80                                        | 1.16                                                         | 0.22                                | 0.41                 | 7.3                    | 5.08                 |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>AUC(0-<math>\infty</math>)</u><br>( $\mu\text{g-hr/ml}$ ) | <u>Cmax</u><br>( $\mu\text{g/ml}$ ) | <u>t1/2</u><br>(hrs) | <u>Xu(0-24)</u><br>(mg) | <u>Clr</u><br>(L/hr) |
|----------------|---------------------------------------------|--------------------------------------------------------------|-------------------------------------|----------------------|-------------------------|----------------------|
| 13             |                                             |                                                              |                                     |                      |                         |                      |
| 14             |                                             |                                                              |                                     |                      |                         |                      |
| 15             |                                             |                                                              |                                     |                      |                         |                      |
| 16             |                                             |                                                              |                                     |                      |                         |                      |
| 18             |                                             |                                                              |                                     |                      |                         |                      |
| Mean           | 6.05                                        | 6.64                                                         | 1.69                                | 3.55                 | 141.3                   | 23.35                |
| S.D.           | 1.47                                        | 1.34                                                         | 0.27                                | 1.31                 | 28.1                    | 7.2                  |

\* AUC (0-23h)

000008

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuhin Ciprofloxacin Study

Dose = 200 mg as 2 Hour Infusion

DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>Cmax</u><br><u>(<math>\mu\text{g/ml}</math>)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-8)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|-------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 13             |                                                             |                                                                              |                                                     |                             |                               |                             |
| 14             |                                                             |                                                                              |                                                     |                             |                               |                             |
| 15             |                                                             |                                                                              |                                                     |                             |                               |                             |
| 16             |                                                             |                                                                              |                                                     |                             |                               |                             |
| 18             |                                                             |                                                                              |                                                     |                             |                               |                             |
| Mean           | 4.97                                                        | 5.85                                                                         | 1.74                                                | 3.07                        | 110.6                         | 22.97                       |
| S.D.           | 0.80                                                        | 1.16                                                                         | 0.22                                                | 0.41                        | 7.3                           | 5.08                        |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu\text{g-hr/ml}</math>)</u> | <u>Cmax</u><br><u>(<math>\mu\text{g/ml}</math>)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-24)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|-------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------|--------------------------------|-----------------------------|
| 13             |                                                             |                                                                              |                                                     |                             |                                |                             |
| 14             |                                                             |                                                                              |                                                     |                             |                                |                             |
| 15             |                                                             |                                                                              |                                                     |                             |                                |                             |
| 16             |                                                             |                                                                              |                                                     |                             |                                |                             |
| 18             |                                                             |                                                                              |                                                     |                             |                                |                             |
| Mean           | 6.05                                                        | 6.64                                                                         | 1.69                                                | 3.55                        | 141.3                          | 23.35                       |
| S.D.           | 1.47                                                        | 1.34                                                                         | 0.27                                                | 1.31                        | 28.1                           | 7.2                         |

\* AUC (0-23h)

Individual Subject Pharmacokinetic  
Parameters From Bayer Yakuin Ciprofloxacin Study

Dose = 300 mg as 2 Hour Infusion

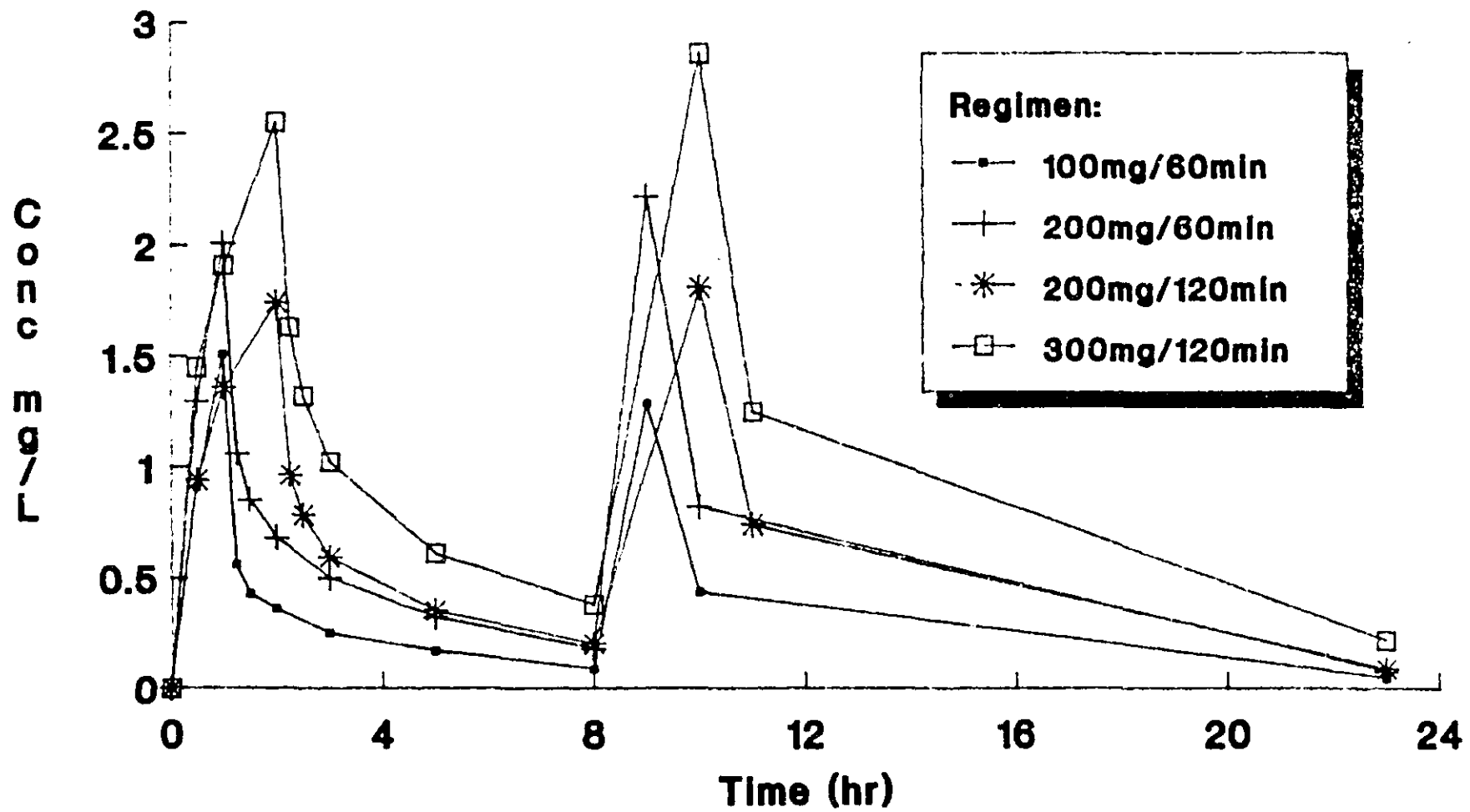
DAY 1

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>Cmax</u><br><u>(<math>\mu</math>g/ml)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-8)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 19             |                                                      |                                                                       |                                              |                             |                               |                             |
| 20             |                                                      |                                                                       |                                              |                             |                               |                             |
| 21             |                                                      |                                                                       |                                              |                             |                               |                             |
| 22             |                                                      |                                                                       |                                              |                             |                               |                             |
| 23             |                                                      |                                                                       |                                              |                             |                               |                             |
| 24             |                                                      |                                                                       |                                              |                             |                               |                             |
| Mean           | 7.93                                                 | 9.79                                                                  | 2.55                                         | 3.43                        | 154.1                         | 19.65                       |
| S.D.           | 0.82                                                 | 1.46                                                                  | 0.28                                         | 0.76                        | 9.2                           | 2.67                        |

DAY 7 (13th Dose)

| <u>Subject</u> | <u>AUC(0-8h)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>AUC(0-<math>\infty</math>)</u><br><u>(<math>\mu</math>g-hr/ml)</u> | <u>Cmax</u><br><u>(<math>\mu</math>g/ml)</u> | <u>t1/2</u><br><u>(hrs)</u> | <u>Xu(0-24)</u><br><u>(mg)</u> | <u>Clr</u><br><u>(L/hr)</u> |
|----------------|------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------|-----------------------------|--------------------------------|-----------------------------|
| 19             |                                                      |                                                                       |                                              |                             |                                |                             |
| 20             |                                                      |                                                                       |                                              |                             |                                |                             |
| 21             |                                                      |                                                                       |                                              |                             |                                |                             |
| 22             |                                                      |                                                                       |                                              |                             |                                |                             |
| 23             |                                                      |                                                                       |                                              |                             |                                |                             |
| 24             |                                                      |                                                                       |                                              |                             |                                |                             |
| Mean           | 11.01                                                | 11.38                                                                 | 2.56                                         | 4.75                        | 215.9                          | 19.26                       |
| S.D.           | 1.67                                                 | 1.82                                                                  | 0.31                                         | 0.40                        | 14.1                           | 2.38                        |

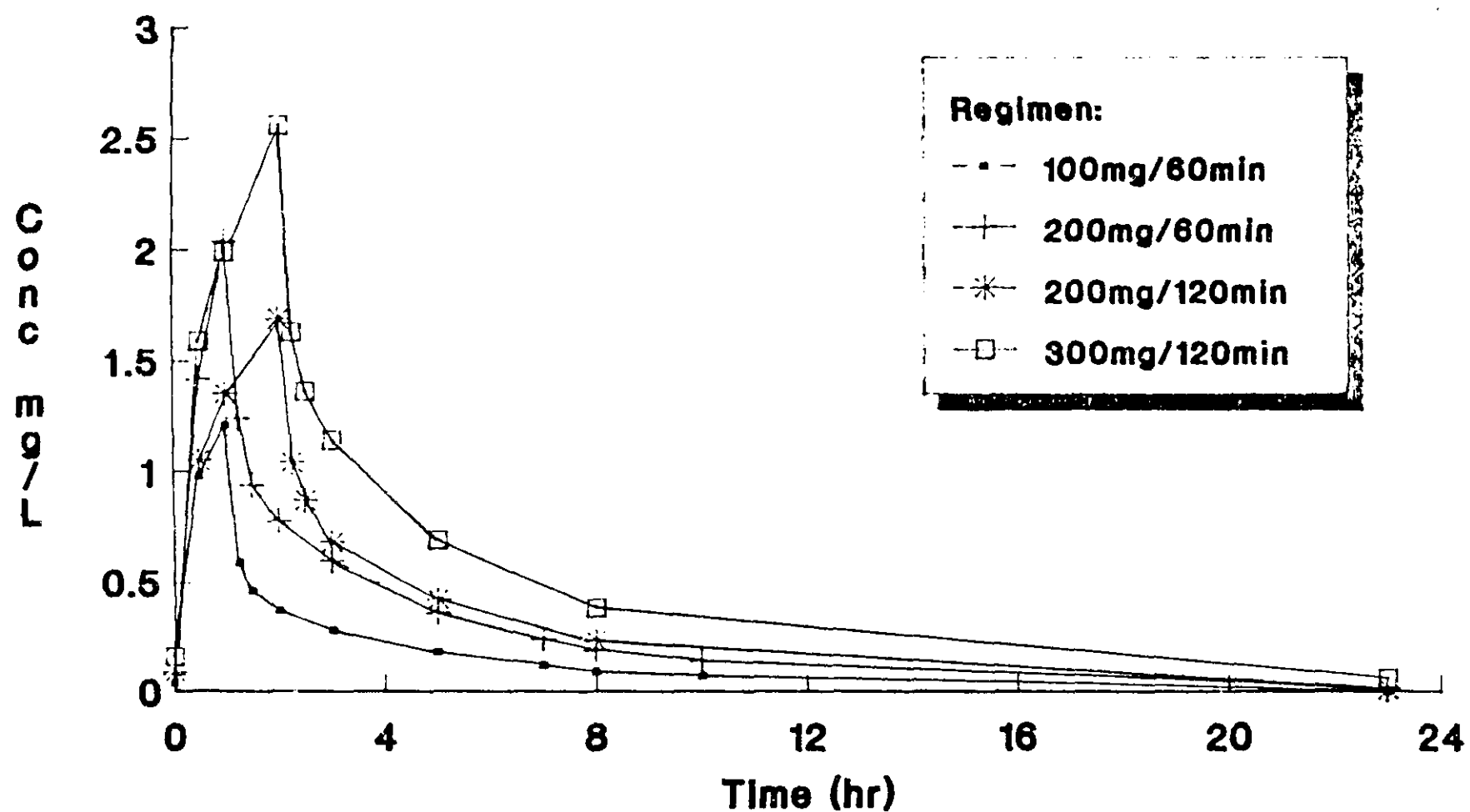
# Mean Plasma Ciprofloxacin Concs (mg/L) During and After Two Intravenous Infusions. Day 1



MQL: 0.0255 mg/L  
Dose Administration: 9am & 5pm  
Bayer/Yakuhin Study

000014  
7

# Mean Plasma Ciprofloxacin Concs (mg/L) During and After The Thirteenth Intravenous Infusion. Day 7

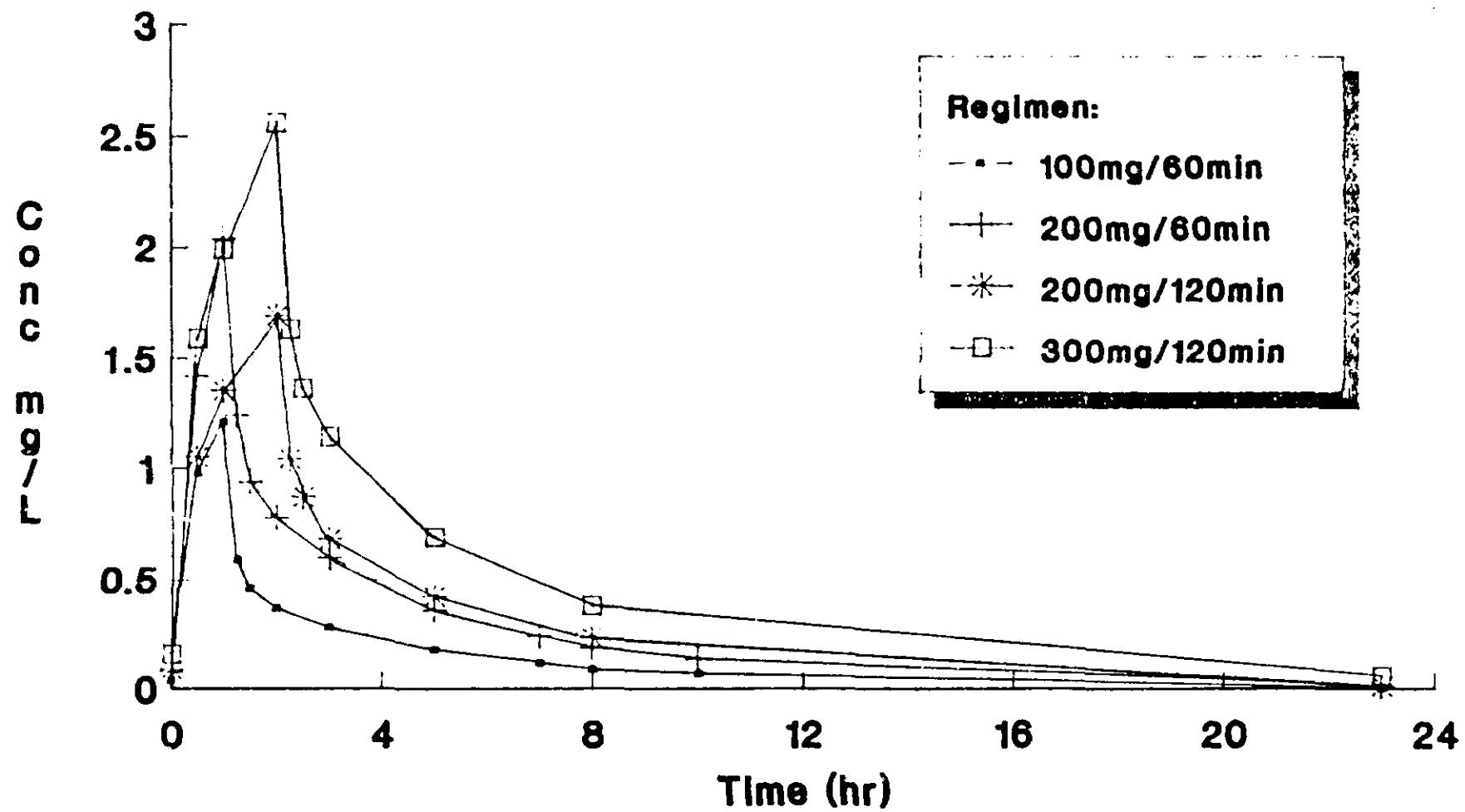


MQL: 0.0255 mg/L  
Dose Administration: 9am & 5pm  
Bayer/Yakuhin Study

000015

Figure 7

# Mean Plasma Ciprofloxacin Concs (mg/L) During and After The Thirteenth Intravenous Infusion. Day 7



MQL: 0.0255 mg/L  
Dose Administration: 9am & 5pm  
Bayer/Yakuhin Study

Figure 7  
000015

Purged

Q v. J. J. J. J.

Prasad

Pungod

Purple

Pringed

ponged

ponged

penrod

Plasma concentrations of Ciprofloxacin after multiple  
 intravenous drip infusion  
 — 100mg IVD(60min.) x 2/day x 7days —

(ug/ml)

| Day | Subject                                      | Time after the initiation of the first administration (hour) |              |              |              |              |              |              |              |              |              |              |              |              |
|-----|----------------------------------------------|--------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|     |                                              | 0                                                            | 0.5          | 1            | 1.25         | 1.5          | 2            | 3            | 5            | 7            | 8            | 9            | 10           | 23           |
| 1st | No.1<br>No.2<br>No.3<br>No.4<br>No.5<br>No.6 |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                                | N.D.<br>-                                                    | 0.91<br>0.19 | 1.51<br>0.20 | 0.56<br>0.05 | 0.43<br>0.05 | 0.36<br>0.05 | 0.25<br>0.02 | 0.17<br>0.03 | /            | 0.09<br>0.02 | 1.29<br>0.19 | 0.44<br>0.08 | 0.05<br>0.01 |
| 4th | No.1<br>No.2<br>No.3<br>No.4<br>No.5<br>No.6 |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                                | 0.05<br>0.01                                                 | 0.91<br>0.18 | 1.38<br>0.12 | 0.61<br>0.07 | 0.45<br>0.05 | 0.35<br>0.05 | 0.25<br>0.04 | 0.17<br>0.04 | /            | 0.09<br>0.02 | 1.43<br>0.14 | 0.44<br>0.07 | 0.04<br>0.02 |
| 7th |                                              |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                                | 0.04<br>0.02                                                 | 0.98<br>0.23 | 1.21<br>0.16 | 0.59<br>0.07 | 0.46<br>0.07 | 0.37<br>0.03 | 0.28<br>0.04 | 0.18<br>0.04 | 0.12<br>0.03 | 0.09<br>0.03 | /            | 0.07<br>0.02 | N.D.<br>-    |

N.D. : < 0.025ug/ml

NRU-1-NR8N

000078

Plasma concentrations of Ciprofloxacin after multiple  
intravenous drip infusion  
— 200mg IVD(60min.) x 2/day x 7days —

(µg/ml)

4/12

| Day | Subject                                   | Time after the initiation of the first administration (hour) |              |              |              |              |              |              |              |              |              |              |              |              |
|-----|-------------------------------------------|--------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|     |                                           | 0                                                            | 0.5          | 1            | 1.25         | 1.5          | 2            | 3            | 5            | 7            | 8            | 9            | 10           | 23           |
| 1st | No. 7<br>No. 8<br>No. 9<br>No.11<br>No.12 |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | N.D.<br>-                                                    | 1.30<br>0.14 | 2.01<br>0.09 | 1.06<br>0.12 | 0.85<br>0.10 | 0.68<br>0.07 | 0.50<br>0.06 | 0.32<br>0.06 | /            | 0.18<br>0.04 | 2.22<br>0.15 | 0.82<br>0.13 | 0.08<br>0.03 |
| 4th | No. 7<br>No. 8<br>No. 9<br>No.11<br>No.12 |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.10<br>0.03                                                 | 1.41<br>0.12 | 2.16<br>0.30 | 1.11<br>0.09 | 0.86<br>0.07 | 0.75<br>0.09 | 0.54<br>0.08 | 0.35<br>0.06 | /            | 0.21<br>0.04 | 1.96<br>0.14 | 0.90<br>0.03 | 0.08<br>0.02 |
| 7th | No. 7<br>No. 8<br>No. 9<br>No.11<br>No.12 |                                                              |              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.08<br>0.05                                                 | 1.42<br>0.14 | 2.04<br>0.07 | 1.24<br>0.14 | 0.94<br>0.08 | 0.78<br>0.06 | 0.60<br>0.10 | 0.36<br>0.05 | 0.24<br>0.05 | 0.19<br>0.04 | /            | 0.14<br>0.02 | 0.01<br>0.02 |

N.D. : < 0.0255µg/ml

07-1-0081

000079

11

Plasma concentrations of Ciprofloxacin after multiple drip infusion

— 200mg IVD(120min.) x 2/day x 7days —

( µg/ml )

5/12

| Day | Subject                                   | Time after the initiation of the first administration (hour) |              |              |              |              |              |              |              |              |              |              |              |
|-----|-------------------------------------------|--------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|     |                                           | 0                                                            | 0.5          | 1            | 2            | 2.25         | 2.5          | 3            | 5            | 8            | 10           | 11           | 23           |
| 1st | No.13<br>No.14<br>No.15<br>No.16<br>No.18 |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | N.D.<br>-                                                    | 0.94<br>0.22 | 1.36<br>0.19 | 1.74<br>0.22 | 0.96<br>0.11 | 0.78<br>0.11 | 0.59<br>0.11 | 0.35<br>0.08 | 0.20<br>0.06 | 1.81<br>0.19 | 0.74<br>0.15 | 0.09<br>0.03 |
| 4th | No.13<br>No.14<br>No.15<br>No.16<br>No.18 |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.08<br>0.03                                                 | 0.93<br>0.20 | 1.28<br>0.20 | 1.72<br>0.25 | 1.01<br>0.20 | 0.79<br>0.16 | 0.63<br>0.14 | 0.39<br>0.10 | 0.21<br>0.08 | 1.73<br>0.24 | 0.78<br>0.13 | 0.09<br>0.04 |
| 7th |                                           |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.09<br>0.03                                                 | 1.05<br>0.12 | 1.35<br>0.15 | 1.69<br>0.27 | 1.04<br>0.11 | 0.87<br>0.13 | 0.68<br>0.13 | 0.42<br>0.10 | 0.23<br>0.06 | /            | /            | 0.01<br>0.02 |

N.D. : < 0.0255µg/ml

07-1-0082

000000

12

Plasma concentrations of Ciprofloxacin after multiple drip infusion

— 200mg IVD(120min.) x 2/day x 7days —

( µg/ml )

5/12

| Day | Subject                                   | Time after the initiation of the first administration (hour) |              |              |              |              |              |              |              |              |              |              |              |
|-----|-------------------------------------------|--------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|     |                                           | 0                                                            | 0.5          | 1            | 2            | 2.25         | 2.5          | 3            | 5            | 8            | 10           | 11           | 23           |
| 1st | No.13<br>No.14<br>No.15<br>No.16<br>No.18 |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | N.D.<br>-                                                    | 0.94<br>0.22 | 1.36<br>0.19 | 1.74<br>0.22 | 0.96<br>0.11 | 0.78<br>0.11 | 0.59<br>0.11 | 0.35<br>0.08 | 0.20<br>0.06 | 1.81<br>0.19 | 0.74<br>0.15 | 0.09<br>0.03 |
| 4th | No.13<br>No.14<br>No.15<br>No.16<br>No.18 |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.09<br>0.03                                                 | 0.93<br>0.20 | 1.28<br>0.20 | 1.72<br>0.25 | 1.01<br>0.20 | 0.79<br>0.16 | 0.53<br>0.14 | 0.39<br>0.10 | 0.21<br>0.08 | 1.73<br>0.24 | 0.78<br>0.13 | 0.09<br>0.04 |
| 7th | No.13<br>No.14<br>No.15<br>No.16<br>No.18 |                                                              |              |              |              |              |              |              |              |              |              |              |              |
|     | Mean<br>±S.D.                             | 0.09<br>0.03                                                 | 1.05<br>0.12 | 1.35<br>0.15 | 1.69<br>0.27 | 1.04<br>0.11 | 0.87<br>0.13 | 0.68<br>0.13 | 0.42<br>0.10 | 0.23<br>0.06 | /            | /            | 0.01<br>0.02 |

N.D. : <0.0255µg/ml

07-1-0082

000006

12

Plasma concentrations of Ciprofloxacin after multiple administration

- 300mg d.i. (120min.) x 2/day x 7 days -

(µg/ml)

| Day | Subject No.   | Time after the initiation of the first administration (hr) |               |               |               |               |               |               |               |               |               |               |               |
|-----|---------------|------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|     |               | 0.0                                                        | 0.5           | 1.0           | 2.0           | 2.25          | 2.5           | 3.0           | 5.0           | 8.0           | 10            | 11            | 23            |
| 1st | 1             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 2             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 3             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 4             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 5             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 6             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | Mean<br>±S.D. | N.D.                                                       | 1.45<br>±0.22 | 1.91<br>±0.30 | 2.55<br>±0.28 | 1.63<br>±0.16 | 1.32<br>±0.14 | 1.02<br>±0.12 | 0.61<br>±0.11 | 0.38<br>±0.09 | 2.86<br>±0.27 | 1.25<br>±0.20 | 0.22<br>±0.10 |
| 4th | 1             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 2             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 3             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 4             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 5             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 6             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | Mean<br>±S.D. | 0.13<br>±0.05                                              | 1.40<br>±0.15 | 1.87<br>±0.23 | 2.49<br>±0.28 | 1.55<br>±0.15 | 1.36<br>±0.18 | 1.13<br>±0.17 | 0.70<br>±0.12 | 0.40<br>±0.10 | 2.63<br>±0.25 | 1.26<br>±0.17 | 0.19<br>±0.08 |
| 7th | 1             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 2             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 3             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 4             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 5             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | 6             |                                                            |               |               |               |               |               |               |               |               |               |               |               |
|     | Mean<br>±S.D. | 0.16<br>±0.06                                              | 1.59<br>±0.25 | 1.99<br>±0.35 | 2.56<br>±0.31 | 1.63<br>±0.17 | 1.36<br>±0.18 | 1.14<br>±0.17 | 0.69<br>±0.16 | 0.38<br>±0.09 | --            | --            | 0.06<br>±0.02 |

N.D. <0.0255µg/ml

07-1-0083

000031

13

Statistical Analysis Table 1  
 Bayer-Yakuhin Ciprofloxacin Pharmacokinetics Study  
 Summary of Statistical Analysis

| Variable | Day | Geometric means* |          |          |          | Dose Comparison | Ratio estimate in % (90% CI) | p-value: equality  |       |
|----------|-----|------------------|----------|----------|----------|-----------------|------------------------------|--------------------|-------|
|          |     | 100 1 hr         | 200 1 hr | 200 2 hr | 300 2 hr |                 |                              |                    |       |
| AUC(0-∞) | 1   |                  | 0.0292   | 0.0254   | 0.0287   | 0.0323          | 200/100 1 hr                 | 86.9( 73.7,102.4)  | 0.155 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 112.5( 95.4,132.6) | 0.230 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 113.3( 95.5,134.5) | 0.222 |
|          | 7   |                  | 0.0294   | 0.0293   | 0.0326   | 0.0376          | 200/100 1 hr                 | 99.7( 84.0,118.4)  | 0.979 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 115.1( 96.9,136.6) | 0.172 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 111.3( 93.0,133.1) | 0.314 |
| Cmax     | 1   |                  | 0.0150   | 0.0101   | 0.0086   | 0.0085          | 200/100 1 hr                 | 67.0( 59.6, 75.4)  | 0.001 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 97.9( 87.0,110.1)  | 0.754 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 85.9( 75.9, 97.1)  | 0.045 |
|          | 7   |                  | 0.0123   | 0.0102   | 0.0083   | 0.0085          | 200/100 1 hr                 | 82.6( 72.6, 93.8)  | 0.018 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 101.6( 89.4,115.5) | 0.829 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 81.9( 71.7, 93.5)  | 0.018 |
| Clr      | 1   |                  | 21.69    | 26.67    | 22.45    | 19.48           | 200/100 1 hr                 | 123.0(103.8,145.6) | 0.048 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 86.8( 73.3,102.8)  | 0.164 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 84.1( 70.5,100.4)  | 0.108 |
|          | 7   |                  | 21.15    | 24.75    | 21.10    | 19.12           | 200/100 1 hr                 | 117.0( 85.3,158.7) | 0.383 |
|          |     |                  |          |          |          |                 | 300/200 2 hr                 | 90.6( 66.8,122.9)  | 0.581 |
|          |     |                  |          |          |          |                 | 2 hr/1 hr 200                | 85.3( 62.0,117.2)  | 0.396 |

\* AUC(0-∞) and Cmax were divided by dose prior to analysis.

000043

Telle  
 (14)

N

19085

10

Statistical Analysis Table 2  
 Bayer-Yakuhin Ciprofloxacin Pharmacokinetics Study  
 Summary of Analysis of AUC without Log Transform

| Day | Mean*    |          |          |          | Dose Comparison | Ratio estimate in % (90% CI) | p-value: equality |
|-----|----------|----------|----------|----------|-----------------|------------------------------|-------------------|
|     | 100 1 hr | 200 1 hr | 200 2 hr | 300 2 hr |                 |                              |                   |
| 1   | 0.0294   | 0.0256   | 0.0292   | 0.0326   | 200/100 1 hr    | 86.9(72.4,103.5)             | 0.180             |
|     |          |          |          |          | 300/200 2 hr    | 111.6(95.6,131.0)            | 0.235             |
|     |          |          |          |          | 2 hr/1 hr 200   | 114.4(95.2,138.0)            | 0.220             |
| 7   | 0.0297   | 0.0295   | 0.0332   | 0.0380   | 200/100 1 hr    | 99.4(81.8,120.2)             | 0.954             |
|     |          |          |          |          | 300/200 2 hr    | 114.3(97.5,134.7)            | 0.164             |
|     |          |          |          |          | 2 hr/1 hr 200   | 112.6(93.2,136.5)            | 0.290             |

\* AUC was divided by dose prior to analysis.

000046

Takuma  
 (15)

16

Urine Data after 100 mg 1-Hour Infusions

000035

APPENDIX 4

APPENDIX 5

| Subj<br>no.     | Method       | Unit        | Day 1 |       |       |       |       |        | Day 2   | Day 2   | Day 3 | Day 3  |         |       |  |  |
|-----------------|--------------|-------------|-------|-------|-------|-------|-------|--------|---------|---------|-------|--------|---------|-------|--|--|
|                 |              |             | 0hr   | 0-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr | Total | 0-12hr | 12-24hr | Total |  |  |
| 1               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| 2               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| 3               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| 4               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| 5               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| 6               | Urine volume | (ml)        |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | HPLC         | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
|                 | Urine conc.  | (ug/ml)     |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Excretion       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Total excretion |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| HPLC            |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| Cip             |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| mean (ug)       |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |
| SD              |              |             |       |       |       |       |       |        |         |         |       |        |         |       |  |  |

total excretion

Urine Data after 100 mg 1-Hour Infusions (Continued)

000037

| Subject No. | Method       | Unit    | Day 7 |       |       |       |        |         |         | Total | ML   |       |        |
|-------------|--------------|---------|-------|-------|-------|-------|--------|---------|---------|-------|------|-------|--------|
|             |              |         | 0-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr |       |      |       |        |
| 1           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
| 2           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
| 3           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
| 4           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
| 5           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
| 6           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |      |       |        |
|             |              |         |       |       |       |       |        |         |         |       |      |       |        |
|             | Urine conc.  | (ug/ml) |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     |       |       |       |       |        |         |         |       |      |       |        |
|             | Excretion    |         |       |       |       |       |        |         |         |       |      |       |        |
|             | NPLC         | Cip     | mean  | (ug)  | 31.22 | 11.86 | 6.63   | 4.88    | 3.27    | 1.99  | 4.88 | 64.92 | 716.28 |
|             |              |         | SD    | (ug)  | 14.37 | 1.89  | 1.76   | 1.23    | .84     | .72   | 1.16 | 18.43 | 109.97 |

Total excretion

Table ~~17~~ 18

000038

Urine Data after 200 mg 1-Hour Infusions

| Subject No. | Method       | Unit | Day 1   |       |       |       |       |        |         |         |       |       | Day 2 |       |        |         | Day 2   |       |       |       |       |        |
|-------------|--------------|------|---------|-------|-------|-------|-------|--------|---------|---------|-------|-------|-------|-------|--------|---------|---------|-------|-------|-------|-------|--------|
|             |              |      | 0hr     | 0-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr | Total | 0-2hr | 2-4hr | 4-6hr | 8-10hr | 10-12hr | 12-24hr | Total |       |       |       |        |
| 7           | Urine volume | (ml) |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Urine conc.  |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Excretion    |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
| 8           | Urine volume | (ml) |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Urine conc.  |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Excretion    |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
| 9           | Urine volume | (ml) |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Urine conc.  |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Excretion    |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
| 11          | Urine volume | (ml) |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Urine conc.  |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Excretion    |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
| 12          | Urine volume | (ml) |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Urine conc.  |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | HPLC         | Cip  | (ug)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             | Excretion    |      |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |        |
|             |              |      | HPLC    | Cip   | mean  | (ug)  | .00   | 70.97  | 20.69   | 15.00   | 8.36  | 78.15 | 27.22 | 42.16 | 261.34 | 72.24   | 22.23   | 24.34 | 79.51 | 29.01 | 46.21 | 273.54 |
|             |              |      |         |       | SD    |       | .00   | 6.66   | 2.42    | .00     | .70   | 5.59  | 2.09  | 2.59  | 10.69  | 0.35    | 1.26    | 3.72  | 7.47  | 1.95  | 6.00  | 10.26  |

Total excretion

PLC Cip: (1.0 ug/ml)

Table ~~18~~ 18

000038

Urine Data after 200 mg 1-Hour Infusions

| Subject No. | Method       | Unit    | Day 1 |       |       |       |        |         |         |       |       |       | Day 2 |        |         |         | Day 2 |       |       |       |       |        |
|-------------|--------------|---------|-------|-------|-------|-------|--------|---------|---------|-------|-------|-------|-------|--------|---------|---------|-------|-------|-------|-------|-------|--------|
|             |              |         | 0-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr | Total | 0-2hr | 2-4hr | 4-6hr | 8-10hr | 10-12hr | 12-24hr | Total |       |       |       |       |        |
| 7           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             | Urine conc.  | (mg)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| Excretion   |              |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| 8           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             | Urine conc.  | (mg)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| Excretion   |              |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| 9           | Urine volume | (ml)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             | Urine conc.  | (mg)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| Excretion   |              |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| 11          | Urine volume | (ml)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             | Urine conc.  | (mg)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| Excretion   |              |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| 12          | Urine volume | (ml)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              | (ug/ml) |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             | Urine conc.  | (mg)    |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
| Excretion   |              |         |       |       |       |       |        |         |         |       |       |       |       |        |         |         |       |       |       |       |       |        |
|             |              |         | HPLC  | Cip   | mean  | (mg)  | .00    | 70.97   | 20.69   | 13.80 | 8.36  | 78.15 | 27.22 | 42.16  | 261.34  | 72.24   | 22.23 | 24.34 | 79.51 | 29.01 | 46.21 | 273.54 |
|             |              |         |       |       | SD    |       | .00    | 6.66    | 2.42    | .80   | .70   | 5.59  | 2.09  | 2.59   | 10.69   | 8.35    | 1.26  | 3.72  | 7.47  | 1.95  | 6.08  | 10.26  |

Total excretion

PLC Cip) : (1.0 ug/ml

Table 19

Urine Data after 200 mg 1-Hour Infusions (Continued)

000051

Table Excretion concentration in urine after drug administration  
(BAY q 3939 : continuous multi dose)

| Subject No. | Method       | Unit        | Day 7 |       |           |       |        |         |         | Day 7 | ALL   |      |        |         |
|-------------|--------------|-------------|-------|-------|-----------|-------|--------|---------|---------|-------|-------|------|--------|---------|
|             |              |             | 0-2hr | 2-4hr | 4-6hr     | 6-8hr | 8-10hr | 10-12hr | 12-24hr | Total | Total |      |        |         |
| 7           | Urine volume | (ml)        |       |       |           |       |        |         |         |       |       |      |        |         |
|             | NPLC         | Cip (ug/ml) |       |       |           |       |        |         |         |       |       |      |        |         |
|             | Urine conc.  | (mg)        |       |       |           |       |        |         |         |       |       |      |        |         |
| Excretion   |              |             |       |       |           |       |        |         |         |       |       |      |        |         |
| 8           | Urine volume | (ml)        |       |       |           |       |        |         |         |       |       |      |        |         |
|             | NPLC         | Cip (ug/ml) |       |       |           |       |        |         |         |       |       |      |        |         |
|             | Urine conc.  | (mg)        |       |       |           |       |        |         |         |       |       |      |        |         |
| Excretion   |              |             |       |       |           |       |        |         |         |       |       |      |        |         |
| 9           | Urine volume | (ml)        |       |       |           |       |        |         |         |       |       |      |        |         |
|             | NPLC         | Cip (ug/ml) |       |       |           |       |        |         |         |       |       |      |        |         |
|             | Urine conc.  | (mg)        |       |       |           |       |        |         |         |       |       |      |        |         |
| Excretion   |              |             |       |       |           |       |        |         |         |       |       |      |        |         |
| 11          | Urine volume | (ml)        |       |       |           |       |        |         |         |       |       |      |        |         |
|             | NPLC         | Cip (ug/ml) |       |       |           |       |        |         |         |       |       |      |        |         |
|             | Urine conc.  | (mg)        |       |       |           |       |        |         |         |       |       |      |        |         |
| Excretion   |              |             |       |       |           |       |        |         |         |       |       |      |        |         |
| 12          | Urine volume | (ml)        |       |       |           |       |        |         |         |       |       |      |        |         |
|             | NPLC         | Cip (ug/ml) |       |       |           |       |        |         |         |       |       |      |        |         |
|             | Urine conc.  | (mg)        |       |       |           |       |        |         |         |       |       |      |        |         |
| Excretion   |              |             |       |       |           |       |        |         |         |       |       |      |        |         |
|             |              |             | NPLC  | Cip   | mean (ug) | 73.31 | 25.65  | 15.72   | 10.94   | 6.47  | 4.02  | 9.16 | 145.27 | 1761.39 |
|             |              |             |       |       | SD        | 10.06 | 1.54   | 1.30    | 1.21    | .85   | 1.04  | .60  | 5.40   | 63.12   |

total excretion

Table ~~19~~ ~~20~~ 20  
000032

Urine Data after 200 mg 2-Hour Infusions

| Method | Unit        | Day 1 |       |       |       |       |        |         |         |       |       | Day 2  |       | Day 2  |         |       |       |        |
|--------|-------------|-------|-------|-------|-------|-------|--------|---------|---------|-------|-------|--------|-------|--------|---------|-------|-------|--------|
|        |             | 0hr   | 1-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr | Total | 0-2hr | 2-4hr  | 4-8hr | 8-10hr | 10-24hr | Total |       |        |
| ug     | (ml)        |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| c.     |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (mg)    |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| ug     | (ml)        |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| c.     |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (mg)    |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| ug     | (ml)        |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (mg)    |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| ug     | (ml)        |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (ug/ml) |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip (mg)    |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| .....  |             |       |       |       |       |       |        |         |         |       |       |        |       |        |         |       |       |        |
| HPLC   | Cip         | mean  | (mg)  | .00   | 60.58 | 28.17 | 13.88  | 8.05    | 66.09   | 32.66 | 42.42 | 251.85 | 68.77 | 28.91  | 26.61   | 78.17 | 82.03 | 276.48 |
|        | SD          |       |       | .00   | 7.34  | 2.29  | .85    | 1.74    | 8.89    | 1.75  | 2.72  | 18.45  | 4.57  | 1.05   | 1.68    | 5.77  | 6.50  | 8.30   |

1 ug/ml

APPENDIX 4

APPENDIX 5



Table ~~21~~ **22**

Urine Data after 300 mg 2-Hour Infusions (Continued)

000039

| Subject No. | Method       | Unit        | Day 7 |       |       |       |        |         |         | Total | All Total |       |        |
|-------------|--------------|-------------|-------|-------|-------|-------|--------|---------|---------|-------|-----------|-------|--------|
|             |              |             | 0-2hr | 2-4hr | 4-6hr | 6-8hr | 8-10hr | 10-12hr | 12-24hr |       |           |       |        |
| 19          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| 20          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| 21          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| 22          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| 23          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| 24          | Urine volume | (ml)        |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug/ml) |       |       |       |       |        |         |         |       |           |       |        |
|             | Urine conc.  | (ug/ml)     |       |       |       |       |        |         |         |       |           |       |        |
|             | HPLC         | Cip (ug)    |       |       |       |       |        |         |         |       |           |       |        |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |
| HPLC Cip    |              |             | Mean  | (ug)  | 80.0  | 45.3  | 26.2   | 18.3    | 12.4    | 8.7   | 17.0      | 215.9 | 2549.6 |
|             |              |             | SD    | (ug)  | 5.3   | 3.0   | 2.2    | 3.8     | 1.4     | 1.5   | 4.3       | 15.5  | 169.6  |
|             | Excretion    |             |       |       |       |       |        |         |         |       |           |       |        |

*Drug*

Ciprofloxacin IV 100mg, 200mg  
0.2% Cipro<sup>R</sup> IV in 5% Dextrose, 50 & 100 ml  
NDA 19-857  
Reviewer: Suresh Mallikaarjun, Ph.D.  
10

Miles  
Pharmaceutical Division  
West Haven, CT 06516  
Submission Dates:  
April 25, 1988

Review of A New Drug Application

JUL 25 1990

The present submission (NDA 19-857) is for 0.2% solution of ciprofloxacin packaged in 50 and 100 ml PVC bags containing 5% Dextrose. The sponsor has requested a waiver of pharmacokinetic and biopharmaceutic requirements for NDA 19-857. Pertinent pharmacokinetic and biopharmaceutic information for ciprofloxacin IV, Cipro<sup>R</sup>, was submitted by the sponsor in NDA 19-847 (Submission Dates: April 25 1988, October 25 1989, February 14 1990, March 29 1990, April 11 1990, April 16 1990, June 4 1990) and was reviewed by the Division of Biopharmaceutics. The review of NDA 19-847 concluded that the sponsor had fulfilled the pharmacokinetic and biopharmaceutic requirements for Cipro<sup>R</sup> IV. The only difference between NDA 19-847 and the present NDA 19-857 is that, in the former, 1% ciprofloxacin was packaged in a 10 ml ampul, diluted with normal saline and infused into the subjects, while in the latter 0.2% ciprofloxacin is prepackaged in a solution of 5% Dextrose. The draft labeling for Cipro<sup>R</sup> contains both the 1% ampul, and the 0.2% prepackaged solution.

Recommendation: The pharmacokinetic and biopharmaceutic information for Cipro<sup>R</sup> has been submitted in NDA 19-847, reviewed and found to be satisfactory by the Division of Biopharmaceutics. Pharmacokinetically, there would be no difference between IV administrations of ciprofloxacin diluted just prior to use as in NDA 19-847, and a prepackaged ciprofloxacin solution as in NDA 19-857. Therefore, a waiver of such information for 0.2% ciprofloxacin in 5% Dextrose solution, NDA 19-857, is recommended by the Division of Biopharmaceutics, according to 21 CFR 320.22 (e).

Please convey the Recommendation to the medical reviewer.

*Suresh Mallikaarjun*  
Suresh Mallikaarjun, Ph.D.  
Pharmacokinetic Evaluation Branch

FT initialed by C.T. Viswanathan, Ph.D. CTV 7/24/90

cc: NDA 19-857 Original, HFD-520, HFD-426 (Mallikaarjun), HFD-443(Turner), Review, Drug, Chron and HFD-19 (FOI) files.

Ciprofloxacin IV 100mg, 200mg  
0.2% Cipro<sup>R</sup> IV in 0.9% NaCl, 50 & 100 ml  
NDA 19-858  
Reviewer: Suresh Mallikaarjun, Ph.D.  
10

Miles  
Pharmaceutical Division  
West Haven, CT 06516  
Submission Dates:  
April 25, 1988

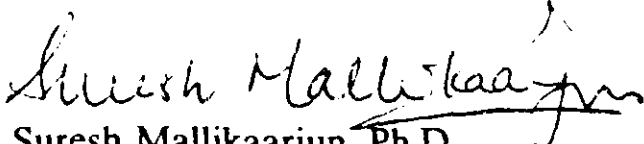
Review of A New Drug Application

JUL 25 1988

The present submission (NDA 19-858) is for 0.2% solution of ciprofloxacin packaged in 50 and 100 ml PVC bags containing 0.9% Sodium Chloride. The sponsor has requested a waiver of pharmacokinetic and biopharmaceutic requirements for NDA 19-858. Pertinent pharmacokinetic and biopharmaceutic information for ciprofloxacin IV, Cipro<sup>R</sup>, was submitted by the sponsor in NDA 19-847 (Submission Dates: April 25 1988, October 25 1989, February 14 1990, March 29 1990, April 11 1990, April 16 1990, June 4 1990) and was reviewed by the Division of Biopharmaceutics. The review of NDA 19-847 concluded that the sponsor had fulfilled the pharmacokinetic and biopharmaceutic requirements for Cipro<sup>R</sup> IV. The only difference between NDA 19-847 and the present NDA 19-858 is that, in the former, 1% ciprofloxacin was packaged in a 10 ml ampul, diluted with normal saline and infused into the subjects, while in the latter 0.2% ciprofloxacin is prepackaged in a solution of 0.9% Sodium Chloride. The draft labeling for Cipro<sup>R</sup> contains both the 1% ampul, and the 0.2% prepackaged solution.

Recommendation: The pharmacokinetic and biopharmaceutic information for Cipro<sup>R</sup> has been submitted in NDA 19-847, reviewed and found to be satisfactory by the Division of Biopharmaceutics. Pharmacokinetically, there would be no difference between IV administrations of ciprofloxacin diluted just prior to use as in NDA 19-847, and a prepackaged ciprofloxacin solution as in NDA 19-858. Therefore, a waiver of such information for 0.2% ciprofloxacin in 0.9% Sodium Chloride solution, NDA 19-858, is recommended by the Division of Biopharmaceutics, according to 21 CFR 320.22 (e).

Please convey the Recommendation to the medical reviewer.

  
Suresh Mallikaarjun, Ph.D.  
Pharmacokinetic Evaluation Branch

FT initialed by C.T. Viswanathan, Ph.D. CTV 7/24/88

cc: NDA 19-858 Original, HFD-520, HFD-426 (Mallikaarjun), HFD-443(Turner), Review, Drug, Chron and HFD-19 (FOI) files.

NDFA 1978  
1310 KV

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES  
Public Health Service  
Food and Drug Administration  
Center for Drugs and Biologics  
Office of Drug Standards

DATE : AUG 10 1988

TO : Raymond J. Lipicky, M.D.  
Acting Director,  
Division of Cardio-Renal Drug Products (HFD-110)

FROM : C.T. Viswanathan, Ph.D.  
Branch Chief, Division of Biopharmaceutics (HFD-226)

SUBJECT : Biopharmaceutics Recommendation of Approval of NDA 17<sup>9</sup>-778  
Prinzide (Lisinopril/HCTZ ) 20/12.5, 20/25mg  
Merck, Sharp & Dohme Research Laboratory

Background

This NDA provides for combination lisinopril and Hydrochlorothiazide in fixed ratio. Both agents are currently approved and marketed products.

Discussion

The sponsor conducted a formal bioequivalence study (single entity vs combination) and a drug interaction study (references #8 & 9 of the NDA).

In a single-dose study (study 105-01, Williams, ref #9), completed in 24 healthy volunteers, bioequivalence of lininopril and HCTZ from PrinzideR and single-entity products was demonstrated based on current guidelines for acceptance (90% confidence intervals).

The drug interaction study was conducted by Williams (study #77, ref #8) under steady-state conditions. The data showed that concomitant administration of lisinopril and HCTZ has little effect on lisinopril disposition but decreases extent of HCTZ absorption (mean difference about 15%) based on urinary recovery.

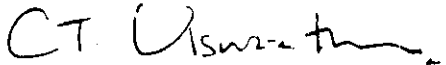
Conclusion

The sponsor had adequately demonstrated the bioequivalence of 20/12.5 and 20/25 PrinzideR Tablets to its components administered separately, and accordingly, is in compliance with 21 CFR section 320.25, part G.

Recommendation

The Division of Biopharmaceutics recommends that the biopharmaceutics portion of NDA 19,778 be accepted for 20/12.5 and 20/25 PrinzideR Tablets.

The Division of Biopharmaceutics recommends that the Q specification for HClZ be 80% rather than the 75% value proposed by the sponsor. If necessary, this can be accomplished by extending the sampling time from 30 to 45 minutes.

  
C.T. Viswanathan, Ph.D.

Prepared by Gene D. Mason, Pharm.D.  
RD Initialed by Mei-Ying Huang, Ph.D. 7/27/88

cc: NDA 19,778 Orig, HFD-110(2), HFD-226(Mason, Viswanathan),  
HFD-344(Turner), Chron, Drug and FOI Files

GDM;gdm; PC, 7/21/88

lisinopril/hydrochlorothiazide  
PrinzideR Tablets; 20/12.5, 20/25 mgs  
NDA 19,778  
Reviewer: Gene D. Mason, Pharm.D.  
PC  
2S, 1D, 30

MERCK, SHARP & DOHME RES LABS  
Sumneytown Pike  
West Point, PA 19486  
Submission Date:  
September 30, 1987  
June 13, 1988  
August 8, 1988  
AUG 10 1988

Review of New Drug Combination Product

I BACKGROUND

This NDA provides for combination lisinopril (the subject of approved MSD NDA 19,558, PrinivilR) and hydrochlorothiazide (HCTZ) in fixed ratio combination. The proposed indications for this product are hypertension and congestive heart failure.

II FORMULATION

The proposed tablet strengths are lisinopril/HCTZ, 20/12.5 and 20/25 mgs. The composition of market-image PrinzideR Tablets is given in Table 1 of this review.

III DISSOLUTION

The proposed dissolution methods and specifications are below.

USP Apparatus II (paddle), 900 ml 0.1N HCL, 50 rpm, 37 degrees C

|                             |    |         |
|-----------------------------|----|---------|
| Q Specification: Lisinopril | at | minutes |
| HCTZ                        | at | minutes |

The amount of Lisinopril & HCTZ dissolved is determined by simultaneous HPLC analysis with detection at 215 nm.

The sponsor indicated that lisinopril and HCTZ are sufficiently soluble in water to allow use of this as the dissolution medium. However, acidic medium was chosen by the sponsor because calcium phosphate dibasic is practically insoluble in water but soluble in acid. The biopharmaceutics reviewer finds that HCTZ is also reported to be practically insoluble in water (The Merck Index, 9th Ed). It was reported that sink conditions using 0.1 N HCL are adequately met for the highest tablet strength of PrinzideR.

The dissolution results for market-image PrinzideR Tablets used in BE study #105-01 are shown in Table 2 of this review. These lots met the proposed Q specification for Lisinopril & HCTZ in the 20/12.5 & 20/25 mg Prinzide Tablets. Table 5 of this review shows individual tablet results for products used in pivotal clinical studies in this NDA. These tablets/capsules also meet the proposed dissolution specification. See comment #3 of this review for further discussion.

#### IV STUDIES REVIEWED

| <u>Ref(Study#)</u> | <u>Investigator</u> | <u>Vol/pg</u>                 | <u>Design</u>     | <u>Objective</u> |
|--------------------|---------------------|-------------------------------|-------------------|------------------|
| 8 (77)             | Williams            | 1.10/pg 5-00084               | Multi-dose, xover | Intxn            |
|                    |                     | A) Lis 2 x 20 mg PO x 7 days  |                   |                  |
|                    |                     | B) HCTZ 1 x 25 mg PO x 7 days |                   |                  |
|                    |                     | C) A) + B) x 7 days           |                   |                  |
| 9 (105-01)         | Williams            | 1.10/pg 5-00201               | Single-Dose       | BE               |
|                    |                     | A) separate 1 x 20/12.5 mg    |                   |                  |
|                    |                     | B) combo 1 x 20/12.5 mg       |                   |                  |
|                    |                     | C) combo 1 x 20/6.5 mg        |                   |                  |
|                    |                     | D) combo 1 x 20/25.0 mg       |                   |                  |

INDIVIDUAL STUDY DESCRIPTIONS CAN BE FOUND IN APPENDIX I OF THIS REVIEW.

TABLES AND GRAPHS CAN BE FOUND IN APPENDIX II OF THIS REVIEW.

#### V RESULTS

##### Ref #8 - Williams (study #77)

The multiple-dose data (rather than the first-dose data) from this drug interaction study is discussed here because steady-state conditions are pertinent to the clinical situation. A qualitative approach was utilized by the reviewer to evaluate this study. With respect to lisinopril, it is concluded that C<sub>max</sub> and T<sub>max</sub> were similar for single and combo treatment. Although statistically significant, the clinical meaning of the 7% difference in mean C<sub>max</sub> observed between single and combo treatment is not known. Urinary recovery of lisinopril was statistically equivalent in this comparison based on 90% confidence intervals of about 85-107%. With respect to HCTZ, C<sub>max</sub> and T<sub>max</sub> were similar for single vs combo treatment. Urinary recovery of HCTZ was significantly lower (about 15% between means) in the combo group compared to the control group. Thus, concomitant administration of lisinopril and HCTZ has little effect on lisinopril disposition but decreases extent of HCTZ absorption (mean difference about 15%) based on urinary recovery.

Ref #9 - Williams (study #105-01)

The results of this single-dose cross-over study conducted in 24 healthy subjects are shown below. Refer to Appendix I of this review for complete details.

|                            |      | Tx A<br>20/12.5<br>Separate | Tx B<br>20/12.5<br>Combo | Tx C<br>20/6.25<br>Combo | Tx D<br>20/25<br>Combo |
|----------------------------|------|-----------------------------|--------------------------|--------------------------|------------------------|
| <u>LISINAPRIL</u>          |      |                             |                          |                          |                        |
| C <sub>max</sub>           | mean | 119.3                       | 121.7                    | 120.9                    | 114.3                  |
|                            | S.D. | 30                          | 29.1                     | 39                       | 33.3                   |
|                            | %CV  | 25.1%                       | 23.9                     | 32.2                     | 29.1%                  |
|                            | n    | 24                          | 24                       | 24                       | 24                     |
| T <sub>max</sub>           | mean | 6.8                         | 6.9                      | 6.9                      | 6.8                    |
|                            | S.D. | 1.3                         | 1.0                      | 1.6                      | 1.2                    |
|                            | C.V. | 19.1%                       | 14.5%                    | 23.2%                    | 17.6%                  |
|                            | n    | 24                          | 24                       | 24                       | 24                     |
| Urine Recovery (% of dose) |      |                             |                          |                          |                        |
|                            | mean | 37.2                        | 38.6                     | 36                       | 36.7                   |
|                            | S.D. | 12.7                        | 13.8                     | 12.4                     | 12.0                   |
|                            | %CV  | 34.1%                       | 35.8%                    | 34.4%                    | 32.7%                  |
|                            | n    | 22                          | 24                       | 24                       | 24                     |
| <u>HCTZ</u>                |      |                             |                          |                          |                        |
| C <sub>max</sub>           | mean | 74                          | 76.3                     | 38.2                     | 148.4                  |
|                            | S.D. | 21.3                        | 17.0                     | 8.6                      | 27.2                   |
|                            | %CV  | 28.8%                       | 22.3%                    | 22.5%                    | 18.3%                  |
|                            | n    | 24                          | 24                       | 24                       | 24                     |
| T <sub>max</sub>           | mean | 2.3                         | 1.8                      | 1.75                     | 1.9                    |
|                            | S.D. | 0.6                         | 0.8                      | 0.5                      | 0.7                    |
|                            | %CV  | 26.1%                       | 44.4%                    | 28.6%                    | 36.8%                  |
|                            | n    | 24                          | 24                       | 24                       | 24                     |
| AUC (mg*hr/L)              |      |                             |                          |                          |                        |
|                            | mean | 0.465                       | 0.513                    | 0.206                    | 1.02                   |
|                            | S.D. | 0.12                        | 0.13                     | 0.15                     | 0.23                   |
|                            | %CV  | 25.8%                       | 25.3%                    | 24.3%                    | 22.5%                  |
|                            | n    | 23                          | 23                       | 23                       | 24                     |
| Urine Recovery (% of dose) |      |                             |                          |                          |                        |
|                            | mean | 66.4                        | 71.9                     | 65.8                     | 70.0                   |
|                            | S.D. | 12.2                        | 12.0                     | 9.7                      | 9.0                    |
|                            | %CV  | 18.4%                       | 16.7%                    | 14.7%                    | 12.9%                  |
|                            | n    | 23                          | 23                       | 23                       | 24                     |

Relative Bioavailability

|                   | Tx B/A<br>20/12.5 mg<br>(% in urine combo / % in urine sep entity) | Tx C/A<br>20/6.25 mg | Tx D/A<br>20/25 mg |
|-------------------|--------------------------------------------------------------------|----------------------|--------------------|
| <u>LISINAPRIL</u> |                                                                    |                      |                    |
| mean              | 1.08                                                               | 1.01                 | 1.03               |
| S.D.              | 0.32                                                               | 0.29                 | 0.25               |
| % CV              | 29.7%                                                              | 28.9%                | 24.3%              |
| Median            | 1.04                                                               | 0.934                | 1.035              |
| Range             | 0.622-1.88                                                         | 0.705-1.72           | 0.641-1.51         |
| n                 | 22                                                                 | 22                   | 22                 |
| <u>HCTZ</u>       |                                                                    |                      |                    |
| mean              | 1.12                                                               | 1.02                 | 1.1                |
| S.D.              | 0.23                                                               | 0.24                 | 0.26               |
| % CV              | 20.5%                                                              | 23.3%                | 23.6%              |
| Median            | 1.08                                                               | 1.01                 | 1.03               |
| Range             | 0.64-1.58                                                          | 0.633-1.75           | 0.82-1.86          |
| n                 | 22                                                                 | 22                   | 23                 |

Statistical Evaluation\*

|                                                                    | SAS GLM           | Power | 90% Confidence Intervals |                    |                  |
|--------------------------------------------------------------------|-------------------|-------|--------------------------|--------------------|------------------|
|                                                                    |                   |       | B vs A<br>(12.5 H)       | C vs A<br>(6.25 H) | D vs A<br>(25 H) |
| <u>Lisinopril</u>                                                  |                   |       |                          |                    |                  |
| C <sub>max</sub>                                                   | NS<br>(p=0.6)     | 97%   | 97-114%                  | 93-110%            | 85-102%          |
| T <sub>max</sub>                                                   | NS<br>(p=0.98)    | 97%   | 87-105%                  | 90-107%            | 88-105%          |
| U <sub>Recov</sub>                                                 | NS<br>(p=0.7)     | 89%   | 94-115%                  | 88-108%            | 89-110%          |
| <u>HCTZ (C<sub>max</sub> &amp; AUC normalized to 12.5 mg dose)</u> |                   |       |                          |                    |                  |
| C <sub>max</sub>                                                   | NS<br>(p=0.83)    | 99%   | 95-110%                  | 96-111%            | 92-108%          |
| T <sub>max</sub>                                                   | SIG<br>(p=0.008)  | 74%   | 70-95%                   | 65-90%             | 65-91%           |
| AUC                                                                | SIG<br>(p=0.0001) | 99%   | 102-116%                 | 86-101%            | 105-120%         |
| U <sub>Recov</sub>                                                 | NS<br>(p=0.067)   | 99%   | 102-117%                 | 92-107%            | 99-113%          |
| R <sub>nCl</sub>                                                   | SIG               | 99%   | 91-105%                  | 103-117%           | 88-102%          |

\* Statistical evaluation performed by reviewer using PC-SAS GLM & FDA STATS Software (see comment #1 of this review)

With respect to lisinopril, it is concluded that the combo and separate entity treatments are bio-equivalent in Cmax, Tmax and Urinary Recovery parameters. This conclusion is based on 90% Confidence Intervals of the difference between treatment means as a % of the reference mean. For HCTZ, the parameters Cmax (dose adjusted), AUC (dose adjusted) and Urinary Recovery satisfied the 80-120% acceptable guideline. The parameter Tmax was significantly shorter for the combo treatments compared to the separate entities given concomitantly. However, Tmax was similar among the treatments, and thus acceptable to this reviewer. In conclusion, study ref #9 demonstrates bio-equivalence between single entities given concomitantly and the combination tablet.

#### V ASSAY METHODS

The sponsor quantitated lisinopril in serum using RIA methods previously described and reviewed in NDA 19,558 for lisinopril tablets. Quality control statistics and a representative standard curve were provided in this submission. The limit of accurate measurement in urine was ~~0.5~~ ng/ml to ~~1.0~~ ng/ml, and in serum was ~~0.5~~ to ~~1.0~~ ng/ml. It was reported that HCTZ does not interfere with measurement of lisinopril. This method was found to be acceptable.

HCTZ was measured in plasma and urine by the investigator (Williams) in refs #8 & #9. At both times, HPLC methodology was utilized. The methods was not identical, and the sponsor provided documentation for each. The assay in ref #8 was found acceptable in NDA 19,558 (lisinopril), thus, representative validation data was not reproduced in this review. For data in reference #9 (formal BE study), the sponsor provided the results of spiked samples (intra- & inter-day precision), typical standard curves for plasma and urine, and results of control samples analyzed during execution of the assay. This information is included in Tables 9-13 to 9-18 of this review. Based on the information provided, the assay is acceptable.

#### VI OVERALL CONCLUSION

The sponsor has adequately demonstrated the bioequivalence of 20/12.5 and 20/12.5 PrinzideR Tablets to its components administered separately, and the combination tablet is in compliance with CFR section 320.25, part G.

With respect to lisinopril, it is concluded that the combo and separate entity treatments are bio-equivalent in Cmax, Tmax and Urinary Recovery parameters. This conclusion is based on 90% Confidence Intervals of the difference between treatment means as a % of the reference mean. For HCTZ, the parameters Cmax (dose adjusted), AUC (dose adjusted) and Urinary Recovery satisfied the 80-120% acceptable guideline. The parameter Tmax was significantly shorter for the combo treatments compared to the separate entities given concomitantly. However, Tmax was similar among the treatments, and thus acceptable to this reviewer. In conclusion, study ref #9 demonstrates bio-equivalence between single entities given concomitantly and the combination tablet.

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#### VI OVERALL CONCLUSION

The sponsor has adequately demonstrated the bioequivalence of 20/12.5 and 20/25 PrinzideR Tablets to its components administered separately, and accordingly, is in compliance with CFR section 320.25, part G.

## VII COMMENTS

1. The statistical evaluation of study reference #9 (Williams study #105-01) produced significant residual effects in some parameters. Also, the biopharmaceutics reviewer was unable to reproduce the sponsor's statistical results. Appropriately, this reviewer consulted with the Division of Biometrics for advice on the acceptability of the statistical evaluation provided by the sponsor. In consultation with Don Schuirmann, it was recommended that the sponsor's output be used with caution and that this reviewer evaluate the data using SAS-GLM procedures and, for parameters with residual effects, utilize appropriate ESTIMATE statements in the evaluation. The unequal residual effects (thought to be due to inadequate washout) can be compensated for by using the GLM estimate statement in the evaluation. A formal consult from the Division of Biometrics is to follow, but at this time, the reviewer found this study acceptable based on his evaluation of the data.

2. Upon request by the reviewer, the sponsor provided individual subject lisinopril and HCTZ concentrations for reference #9 (study 105-01). Inspection of the data showed that all subjects in periods 2 and 3 had non-zero control values for lisinopril measured in serum. All subjects in period 1 had lisinopril values below the limit of the assay. This is consistent with inadequate washout (1-2 weeks) between treatments. Values reported were generally less than 2 ng/ml, and substantially less than peak lisinopril concentrations reported. In most cases, control urine samples for lisinopril were below the limits of the assay. In contrast, control HCTZ values measured in plasma and urine were ideally below the limits of the assay for all subjects in all periods. These findings do not invalidate the results of this study for lisinopril (refer to comment #1 above).

3. It is recommended that the Q specification for HCTZ be  rather than the  value proposed by the sponsor. If necessary, this can be accomplished by extending the sampling time from 30 to 45 minutes. The sponsor will need to provide individual tablet data from at least 2 different lots (12 tablets per lot) of each proposed tablet strength before the proposed HCTZ dissolution specification can be accepted. The lots utilized in BE study #105-01 (ref #9) should be utilized in the evaluation.

4. The following comment pertains to the proposed labeling.

a. Reference #8 showed that coadministration of lisinopril and HCTZ result in a 15% decrease in extent of HCTZ absorption under steady-state conditions. To eliminate the need to interpret the "little, or no effect" statement used to describe this interaction in the labeling, it is recommended that the sponsor include in the labeling the actual values observed.

5. The reviewer notes that the extent of lisinopril availability, as measured by urinary recovery, was about 37% in study #105-01, compared to about 23% in study #77, and 27% in NDA 19-558 (lisinopril). Within each study the values reported are consistent between treatments. Apart from being a cross-study comparison, the reason for this difference is not obvious.

6. The sponsor should be informed of the need to provide individual tablet data for all future biopharmaceutic submissions containing dissolution data. The current policy of the Division of Biopharmaceutics is to request the dissolution results of 12 individual tablets.

7. The 20/6.25 mg tablet strength is not the subject of this NDA at this time. Thus, the recommendation for approval applies only to the 20/12.5 and 20/25 mg Prinzide Tablet strengths.

#### VII RECOMMENDATION

The Division of Biopharmaceutics recommends that the biopharmaceutics portion of NDA 19-778, PrinzideR Tablets, be accepted as demonstration of in-vivo bioequivalence between the proposed marketed tablet and individual components administered separately.

The Division of Biopharmaceutics recommends that the dissolution Q specification for hydrochlorothiazide be set at ~~10~~ rather than ~~10~~ as proposed by the sponsor (refer to comment #3 of this review).

Comments #3 & 6 (pertaining to dissolution) should be forwarded to the sponsor.

Comment #4 (pertaining to labeling) is brought to the attention of HFD-110 and should be forwarded to the sponsor.

*Gene D. Mason Pharm.D. 8/9/88*  
Gene D. Mason, Pharm.D.  
Pharmacokinetic Evaluation Branch

RD Initialed by Mei-Ying Huang, Ph.D. 7/27/88  
FT Initialed by CT Viswanathan, Ph.D. CTV 8/9/88

cc: NDA 19-778, Orig., HFD-110(2), HFD-226(Mason), HFD-344(Turner), Chron, Drug & FOI Files

GDM:gdm, PC; 7/21/88, 7/26/88, 8/9/88

TABLE: 1

Composition of market-image\* tablet formulations used in bioequivalence study.

| Ingredient<br>(amount in mg) | PRINZIDE |      | PRINIVIL | HydroDIURIL |
|------------------------------|----------|------|----------|-------------|
|                              |          |      |          |             |
| Lisinopril, anhydrous        | 20.0     | 20.0 | 20.0     | -           |
| Hydrochlorothiazide, USP     | 12.5     | 6.25 | 25.0     | - 25.0      |

TABLE: 2

Product dissolution summary.

| Reference No. | Dosage Form                             | Formulation No.                 | Test Conditions | Mean %            |              |          |                  |
|---------------|-----------------------------------------|---------------------------------|-----------------|-------------------|--------------|----------|------------------|
|               |                                         |                                 |                 | Dissolution       | S            | CV       | n Range          |
| 8             | Lisinopril<br>20mg tablet               | 0521-OCT-<br>012-C002           | *               | 102               | 1.07         | 6        | 101-104          |
|               | HCTZ<br>25mg tablet                     | CO-421-OCT-<br>023-BU2          | *               | 104               | 0.50         | 6        | 103-104          |
| 9             | Lisinopril<br>20mg tablet               | 0521-OCT-<br>012-C014           | *               | 102               | 1.09         | 12       | 100-104          |
|               | HCTZ<br>12.5mg<br>half-tablet           | CO521-OCT-<br>048-C001<br>(MSD) | *               | 82                | 9.51         | 12       | 69-95            |
|               | Lisinopril/HCTZ<br>20/12.5mg<br>tablet  | 0521-<br>OCT-001-<br>G002       | *               | (L) 100<br>(H) 98 | 1.44<br>2.29 | 12<br>12 | 98-102<br>95-101 |
|               | Lisinopril/HCTZ<br>20/6.25 mg<br>tablet | 0521A-<br>OCT-001-<br>P002      | *               | (L) 102<br>(H) 94 | 1.51<br>3.30 | 12<br>12 | 100-104<br>90-99 |
|               | Lisinopril/HCTZ<br>20/25 mg<br>tablet   | 0521A-<br>OCT-001-<br>H002      | *               | (L) 100<br>(H) 91 | 2.38<br>6.53 | 12<br>12 | 96-104<br>84-99  |

\*900 ml 0.1N HCl at 37°C with 50 rpm paddle agitation in USP Apparatus II sampled at 30 minutes

TABLE: 3

Formulations used in pivotal clinical studies.

| Study                                                                                                        | Study No. | Formulations <sup>a</sup>                  |
|--------------------------------------------------------------------------------------------------------------|-----------|--------------------------------------------|
| <u>Bioavailability</u>                                                                                       |           | SE                                         |
| Bioavailability of Lisinopril and Hydrochlorothiazide                                                        | 70-77     | FT, RT                                     |
| Bioavailability of Lisinopril/Hydrochlorothiazide                                                            | 105-01    | AT, BT, CT, FT, IT <sup>b</sup><br>(M) (M) |
| <u>Safety, Tolerance and Efficacy</u>                                                                        |           | SE                                         |
| Lisinopril and Hydrochlorothiazide Given Alone and Concomitantly for Mild to Moderate Essential Hypertension | 20-02     | BC, JT (M)<br>Combo SE                     |
| Lisinopril, Hydrochlorothiazide and Combination for Mild to Moderate Essential Hypertension                  | 002       | BT, CT, NT<br>SE C-caps                    |
| Ratios of Lisinopril/Hydrochlorothiazide Given Once-A-Day                                                    | 007       | AC, CC, DC, EC, FC<br>SE                   |
| Ratios of Lisinopril/Hydrochlorothiazide Given to Patients Not Adequately Treated with Lisinopril Alone      | 532       | HT, KT, MT, PT, ST, TT<br>(MRO)            |
| Lisinopril Given to Aged                                                                                     | 540       | DT, ET, FT, LT, OT, ST<br>SE               |
| Lisinopril Given to Patients with Severe Hypertension                                                        | 546       | ET, FT, GT, HT, OT, QT, TT<br>(M) (M)      |
| Dose Response                                                                                                | 1         | Refer to NDA 19-558                        |
| Lisinopril Versus Atenolol                                                                                   | 3 and 509 | Refer to NDA 19-558                        |
| Lisinopril Versus Nifedipine                                                                                 | 506       | Refer to NDA 19-558                        |

pivotal efficacy {  
Exposure (safety) }

<sup>a</sup> See Tables C-4a through C-4d.  
<sup>b</sup> A half tablet provided the 12.5 mg dose.

TABLE: 4 (a, b, c, d)

a) Formulations used in pivotal clinical studies. Capsule formulations.

| Ingredients (mg)    | AC   | BC   | CC   | DC   | EC   | FC   |
|---------------------|------|------|------|------|------|------|
| Lisinopril          | 20.0 | 20.0 | ---  | 20.0 | 20.0 | 20.0 |
| Hydrochlorothiazide | ---  | ---  | 12.5 | 6.25 | 12.5 | 25.0 |

b) Formulations used in pivotal clinical studies. Tablet formulations. (Proposed Market-Image Tabs)

| Ingredients (mg)    | AT   | BT   | CT   |
|---------------------|------|------|------|
| Lisinopril          | 20.0 | 20.0 | 20.0 |
| Hydrochlorothiazide | 6.25 | 12.5 | 25.0 |

(Table 4, cont)

Formulations used in pivotal clinical studies.  
Separate-entity market-image formulations.

c).

| <u>Ingredients (mg)</u> | <u>DT</u> | <u>ET</u> | <u>FT</u> | <u>GT</u> | <u>HT</u> | <u>IT*</u> | <u>JT</u> |
|-------------------------|-----------|-----------|-----------|-----------|-----------|------------|-----------|
| Lisinopril              | 5.00      | 10.0      | 20.0      | 40.0      | 80.0      | ---        | ---       |
| Hydrochlorothiazide     | ---       | ---       | ---       | ---       | ---       | 25.0       | 50.0      |

\* A half tablet provided the 12.5 mg dose.

d).

Formulations used in pivotal clinical studies  
Variant tablet formulations.

| <u>Ingredients (mg)</u> | <u>KT</u> | <u>LT</u> | <u>MT</u> | <u>NI</u> | <u>OT</u> | <u>PT</u> | <u>QT</u> | <u>RT</u> | <u>ST</u> | <u>TT</u> | <u>UT</u> |
|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Lisinopril              | 40.0      | 40.0      | ---       | ---       | ---       | ---       | ---       | ---       | ---       | ---       | 20.0      |
| Hydrochlorothiazide     | ---       | ---       | 6.25      | 12.5      | 12.5      | 12.5      | 25.0      | 25.0      | 25.0      | 50.0      | 12.5      |

TABLE: 5

| Study No.       | Cl    | Formula          | Formula Designation*      | Lisinopril/HCTZ (mg) | Method         | % Dissolution     |     |
|-----------------|-------|------------------|---------------------------|----------------------|----------------|-------------------|-----|
|                 |       |                  |                           |                      |                | Individual Values | Avg |
| 20-02           | CH518 | 0521 DFC001D001  | BC                        | 20/0 capsule         | A              | L                 | 93  |
|                 |       | 0521 DFC001D002  | BC                        | 20/0 capsule         | A              | L                 | 93  |
|                 |       | 0152 OCT008A02   | JT (Marketed)             | 0/50 tablet          | B              | H                 | 95  |
| 002             | CM710 | 0521 OCT012C008  | FT (Marketed)             | 20/0 tablet          | A              | L                 | 99  |
|                 | CL479 | C0521A0CT005C013 | BT < Proposed<br>Marketed | 20/12.5 tablet       | A              | L                 | 105 |
|                 |       |                  |                           |                      | A              | H                 | 105 |
|                 | CK954 | 0521 OCT005C010  | FT (Marketed)             | 20/0 tablet          | A              | L                 | 103 |
|                 |       | C0521 OCT007C001 | NT                        | 0/12.5 tablet        | A              | H                 | 101 |
|                 | CL477 | 0521 OCT012C002  | FT (Marketed)             | 20/0 tablet          | A              | L                 | 102 |
|                 |       | C0521 OCT007C003 | NT                        | 0/12.5 tablet        | A              | H                 | 98  |
|                 | CM711 | 0521A0CT002G001  | BT < Proposed<br>Marketed | 20/12.5 tablet       | A              | L                 | 100 |
|                 |       |                  |                           |                      | A              | H                 | 86  |
|                 | 007   | CL961            | C0521 DFC001I002          | CC                   | 0/12.5 capsule | A                 | H   |
| B               |       |                  |                           |                      |                | H                 | 103 |
| A               |       |                  |                           |                      |                | L                 | 100 |
| A               |       |                  |                           |                      |                | L                 | 101 |
| A               |       |                  |                           |                      |                | H                 | 67  |
| B               |       |                  |                           |                      |                | H                 | 100 |
| 0521ADFC001B001 |       |                  | EC                        | 20/12.5 capsule      | A              | L                 | 98  |
|                 |       |                  |                           |                      | A              | H                 | 55  |
|                 |       |                  |                           |                      | B              | H                 | 101 |
| 0521ADFC001C001 |       |                  | FC                        | 20/25 capsule        | A              | L                 | 100 |
|                 |       |                  |                           |                      | A              | H                 | 56  |
|                 |       |                  |                           |                      | B              | H                 | 100 |

\* Formula designation corresponds to Item 3.I.D.

A-USP Apparatus II (Paddles), 50 RPM, 900 mL, 0.1N HCl, 30 min.

B-USP Apparatus I (Baskets), 100 RPM, 900 mL, 0.1N HCl, 60 min.

0571C

**TABLE: 8-1**

MEAN BIOAVAILABILITY VARIABLES  
HYDROCHLOROTHIAZIDE

| URINARY RECOVERY VARIABLES<br>(UNITS)        | DAY | TREATMENT                      |      |      |                                       |      |      | TREATMENT<br>COMPARISON<br>B VS C |
|----------------------------------------------|-----|--------------------------------|------|------|---------------------------------------|------|------|-----------------------------------|
|                                              |     | B = HCTZ 25 MG<br>Q 24 H ALONE |      |      | C = HCTZ 25 MG +<br>LISN 25 MG Q 24 H |      |      |                                   |
|                                              |     | N                              | MEAN | S.D. | N                                     | MEAN | S.D. |                                   |
| URINARY RECOVERY (0-24 HRS)<br>(% DOSE)      | 1   | 14                             | 58.8 | 18.4 | 15                                    | 55.7 | 14.2 | NS                                |
|                                              | 2   | 14                             | 60.3 | 13.1 | 14                                    | 58.6 | 21.7 | NS                                |
|                                              | 3   | 15                             | 62.9 | 17.3 | 15                                    | 58.9 | 14.5 | NS                                |
|                                              | 4   | 15                             | 60.3 | 12.4 | 15                                    | 52.0 | 14.8 | NS                                |
|                                              | 5   | 15                             | 61.9 | 17.5 | 15                                    | 54.3 | 16.8 | NS                                |
|                                              | 6   | 14                             | 69.1 | 19.4 | 15                                    | 48.5 | 18.6 | *                                 |
|                                              | 7   | 45                             | 65.7 | 19.2 | 15                                    | 56.5 | 20.3 | NS                                |
| TOTAL RECOVERY (% DOSE)                      | 1-7 | 12                             | 63.6 | 7.0  | 14                                    | 54.6 | 11.9 | *                                 |
| AVERAGE STEADY STATE RECOVERY<br>(% DOSE)    | 3-7 | 15                             | 63.8 | 6.6  | 15                                    | 54.1 | 12.6 | *                                 |
| SERUM VARIABLES                              |     |                                |      |      |                                       |      |      |                                   |
| OBSERVED MAXIMUM SERUM CONC.<br>(NG/ML)      | 1   | 15                             | 132  | 45.0 | 14                                    | 123  | 35.4 | NS                                |
|                                              | 7   | 15                             | 149  | 45.1 | 15                                    | 127  | 39.0 | NS                                |
| TIME TO OBSERVED MAXIMUM<br>SERUM CONC. (HR) | 1   | 15                             | 2.3  | 0.7  | 14                                    | 2.3  | 0.7  | NS                                |
|                                              | 7   | 15                             | 2.1  | 0.5  | 15                                    | 2.3  | 0.7  | NS                                |

\* SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS, P<0.05.

**TABLE: B-1**

MEAN BIOAVAILABILITY VARIABLES  
HYDROCHLOROTHIAZIDE

| URINARY RECOVERY VARIABLES<br>(UNITS)  | DAY | TREATMENT                      |      |      |                                       |      |      | TREATMENT<br>COMPARISON<br>B VS C |
|----------------------------------------|-----|--------------------------------|------|------|---------------------------------------|------|------|-----------------------------------|
|                                        |     | B = HCTZ 25 MG<br>Q 24 H ALONE |      |      | C = HCTZ 25 MG +<br>LISM 25 MG Q 24 H |      |      |                                   |
|                                        |     | N                              | MEAN | S.D. | N                                     | MEAN | S.D. |                                   |
| <b>URINARY RECOVERY (0-24 HRS)</b>     |     |                                |      |      |                                       |      |      |                                   |
| (% DOSE)                               | 1   | 14                             | 58.8 | 18.4 | 15                                    | 55.7 | 14.2 | NS                                |
|                                        | 2   | 14                             | 60.3 | 13.1 | 14                                    | 58.6 | 21.7 | NS                                |
|                                        | 3   | 15                             | 62.9 | 17.3 | 15                                    | 58.9 | 14.5 | NS                                |
|                                        | 4   | 15                             | 60.3 | 12.4 | 15                                    | 52.0 | 14.8 | NS                                |
|                                        | 5   | 15                             | 61.9 | 17.5 | 15                                    | 54.3 | 16.8 | NS                                |
|                                        | 6   | 14                             | 69.1 | 19.4 | 15                                    | 48.5 | 18.6 | *                                 |
|                                        | 7   | 45                             | 65.7 | 19.2 | 15                                    | 56.5 | 20.3 | NS                                |
| TOTAL RECOVERY (% DOSE)                | 1-7 | 12                             | 63.6 | 7.0  | 14                                    | 54.6 | 11.9 | *                                 |
| AVERAGE STEADY STATE RECOVERY (% DOSE) | 3-7 | 15                             | 63.9 | 6.6  | 15                                    | 54.1 | 12.6 | *                                 |
| <b>SERUM VARIABLES</b>                 |     |                                |      |      |                                       |      |      |                                   |
| OBSERVED MAXIMUM SERUM CONC.           | 1   | 15                             | 132  | 45.0 | 14                                    | 123  | 35.4 | NS                                |
| (NG/ML)                                | 7   | 15                             | 149  | 45.1 | 15                                    | 127  | 39.0 | NS                                |
| TIME TO OBSERVED MAXIMUM               | 1   | 15                             | 2.3  | 0.7  | 14                                    | 2.3  | 0.7  | NS                                |
| SERUM CONC. (HR)                       | 7   | 15                             | 2.1  | 0.5  | 15                                    | 2.3  | 0.7  | NS                                |

\* SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS, P<0.05.

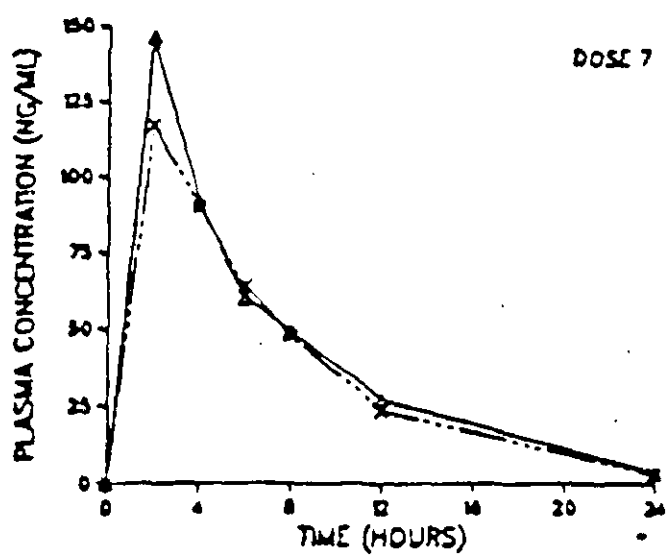
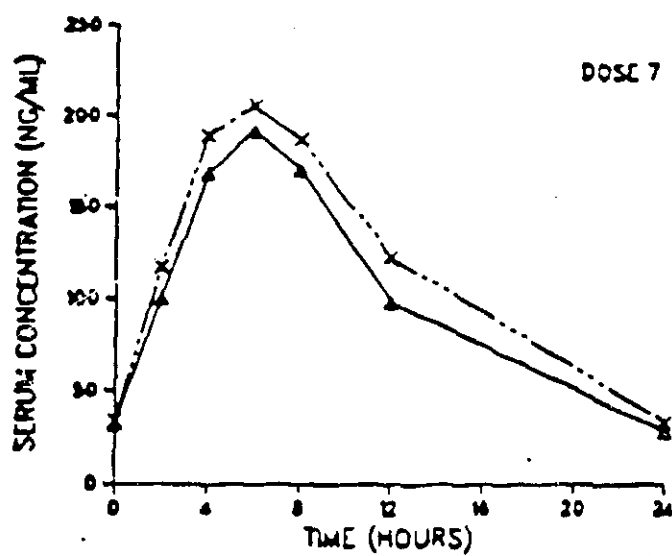
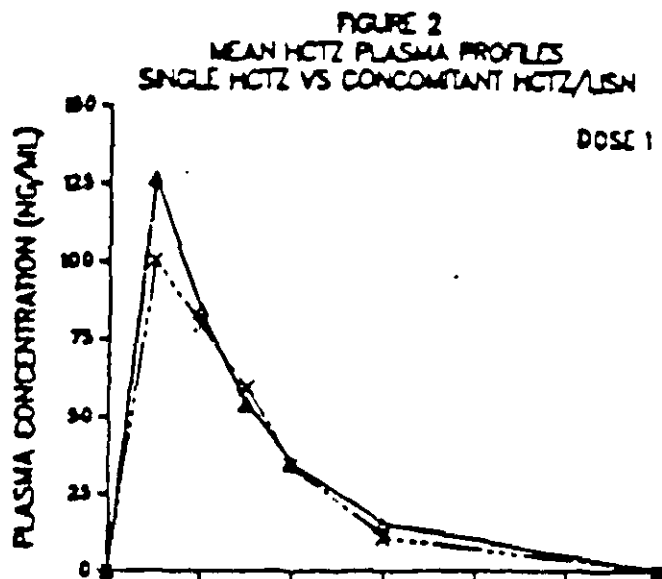
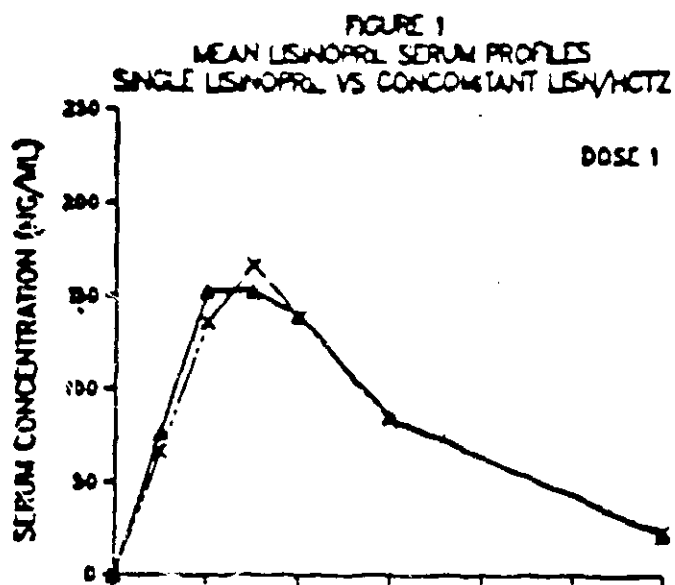
TABLE: 8-2

MEAN BIOAVAILABILITY VARIABLES  
LISINAPRIL

| URINARY RECOVERY VARIABLES<br>(UNITS)        | DAY | TREATMENT                      |      |      |                                       |      |      | TREATMENT<br>COMPARISON<br>A VS C |
|----------------------------------------------|-----|--------------------------------|------|------|---------------------------------------|------|------|-----------------------------------|
|                                              |     | A = LISN 40 MG<br>Q 24 H ALONE |      |      | C = LISN 40 MG +<br>HCTZ 25 MG Q 24 H |      |      |                                   |
|                                              |     | N                              | MEAN | S.D. | N                                     | MEAN | S.D. |                                   |
| URINARY RECOVERY (0-24 HRS)<br>(% DOSE)      | 1   | 12                             | 24.2 | 10.6 | 15                                    | 21.7 | 11.9 | NS                                |
|                                              | 2   | 14                             | 24.3 | 14.8 | 14                                    | 24.7 | 10.9 | NS                                |
|                                              | 3   | 15                             | 22.7 | 15.3 | 15                                    | 22.3 | 12.2 | NS                                |
|                                              | 4   | 15                             | 25.9 | 14.3 | 15                                    | 22.1 | 11.3 | *                                 |
|                                              | 5   | 14                             | 23.1 | 13.3 | 15                                    | 22.7 | 11.5 | NS                                |
|                                              | 6   | 15                             | 20.1 | 8.0  | 15                                    | 22.2 | 16.6 | NS                                |
|                                              | 7   | 15                             | 28.5 | 16.2 | 15                                    | 25.5 | 11.5 | NS                                |
| TOTAL RECOVERY (% DOSE)                      | 1-7 | 11                             | 25.5 | 11.6 | 14                                    | 23.2 | 9.8  | NS                                |
| AVERAGE STEADY STATE RECOVERY<br>(% DOSE)    | 3-7 | 15                             | 23.9 | 10.2 | 15                                    | 22.9 | 10.2 | NS                                |
| SERUM VARIABLES                              |     |                                |      |      |                                       |      |      |                                   |
| OBSERVED MAXIMUM SERUM CONC.<br>(NG/ML)      | 1   | 14                             | 165  | 93.8 | 15                                    | 175  | 85.0 | NS                                |
|                                              | 7   | 15                             | 196  | 108  | 15                                    | 210  | 119  | NS                                |
| TIME TO OBSERVED MAXIMUM<br>SERUM CONC. (HR) | 1   | 14                             | 5.1  | 1.5  | 15                                    | 6.0  | 1.3  | NS                                |
|                                              | 7   | 15                             | 5.5  | 0.9  | 15                                    | 5.7  | 1.3  | NS                                |

\* SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS, P<0.05.

**FIGURE: 8-1**



**TREATMENT**  
 Δ LISINAPRIL  
 X LISINAPRIL PLUS HCTZ

**TREATMENT**  
 Δ HCTZ  
 X HCTZ PLUS LISINAPRIL

07/2/81

Table Q-1

Dosing Allocation Schedule

| Subject No. (SEQ) | Period 1 | Period 2 | Period 3 | Period 4 |   |
|-------------------|----------|----------|----------|----------|---|
| 1                 | 2        | B        | C        | A        | D |
| 2                 | 1        | A        | B        | D        | C |
| 3                 | 3        | C        | D        | B        | A |
| 4                 | 4        | D        | A        | C        | B |
| 5                 | 1        | A        | B        | D        | C |
| 6                 | 4        | D        | A        | C        | B |
| 7                 | 3        | C        | D        | B        | A |
| 8                 | 2        | B        | C        | A        | D |
| 9                 | 3        | C        | D        | B        | A |
| 10                | 1        | A        | B        | D        | C |
| 11                | 2        | B        | C        | A        | D |
| 12                | 4        | D        | A        | C        | B |
| 13                | 4        | D        | A        | C        | B |
| 14                | 1        | A        | B        | D        | C |
| 15                | 3        | C        | D        | B        | A |
| 16                | 2        | B        | C        | A        | D |
| 17                | 1        | A        | B        | D        | C |
| 18                | 2        | B        | C        | A        | D |
| 19                | 4        | D        | A        | C        | B |
| 20                | 3        | C        | D        | B        | A |
| 21                | 4        | D        | A        | C        | B |
| 22                | 1        | A        | B        | D        | C |
| 23                | 2        | B        | C        | A        | D |
| 24                | 3        | C        | D        | B        | A |

Table Q-2

Dosage Formulations

|                     |                                                                                                                                              |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Hydrochlorothiazide | 12.5 mg half-tablet (HydroDIURIL)<br>Formulation No. C0521-OCT-048-C001<br>Assayed at 13.0 mg per half-tablet<br>Dissolved 82% in 30 minutes |
| Lisinopril          | 20 mg tablet<br>Formulation No. 0521-OCT-012-C014<br>Assayed at 19.54 mg per tablet<br>Dissolved 102% in 30 minutes                          |
| Lisinopril/HCTZ     | 20/12.5 mg tablet<br>Formulation No. 0521-OCT-001-C002<br>Assayed at 20.1/12.5 mg per tablet<br>Dissolved 100%/98% in 30 minutes             |
| Lisinopril/HCTZ     | 20/6.25 mg tablet<br>Formulation No. 0521A-OCT-001-F002<br>Assayed at 21.0/6.5 mg per tablet<br>Dissolved 102%/94% in 30 minutes             |
| Lisinopril/HCTZ     | 20/25 mg tablet<br>Formulation No. 0521A-OCT-001-R002<br>Assayed at 19.9/25.7 mg per tablet<br>Dissolved 98%/91% in 30 minutes               |

A = 12.5 mg HCTZ + 20 mg lisinopril separately but concomitantly  
 B = 12.5 mg HCTZ/20 mg lisinopril combination  
 C = 6.25 mg HCTZ/20 mg lisinopril combination  
 D = 25.0 mg HCTZ/20 mg lisinopril combination

Table Q-3

Peak Serum Concentrations of Lisinopril Following  
Dosing Concomitantly (A) and in Combination (B,C,D) with HCTZ

| Subject No. | C <sub>max</sub> (ng/ml) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>20 mg          | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      |                      |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 119                      | 122                  | 121                  | 114                  |
| S.D.        | 30                       | 29                   | 39                   | 33                   |

Table Q-4

Times of Peak Serum Lisinopril Following  
Dosing Concomitantly (A) and in Combination (B,C,D) with HCTZ

| Subject No. | T <sub>max</sub> (hours) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>20 mg          | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      |                      |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 7                        | 7                    | 7                    | 7                    |
| S.D.        | 1                        | 1                    | 1                    | 1                    |

\* Excluded from mean (differs by more than 4 S.D.)

Table 9-3

Peak Serum Concentrations of Lisinopril Following  
Dosing Concomitantly (A) and in Combination (B,C,D) with HCTZ

| Subject No. | C <sub>max</sub> (ng/ml) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>20 mg          | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      |                      |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 119                      | 122                  | 121                  | 114                  |
| S.D.        | 30                       | 29                   | 39                   | 33                   |

Table 9-4

Times of Peak Serum Lisinopril Following  
Dosing Concomitantly (A) and in Combination (B,C,D) with HCTZ

| Subject No. | T <sub>max</sub> (hours) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>20 mg          | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      |                      |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 7                        | 7                    | 7                    | 7                    |
| S.D.        | 1                        | 1                    | 1                    | 1                    |

\* Excluded from mean (differs by more than 4 S.D.)

**Table 9-5**  
**Total Urinary Recovery of Lisinopril Following**  
**Dosing Concomitantly (A) and in Combination (B,C,D) with HCTZ**

| Subject No. | % of Dose in Urine |                      |                      |                      |
|-------------|--------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>20 mg    | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                    |                      |                      |                      |
| 2           |                    |                      |                      |                      |
| 3           |                    |                      |                      |                      |
| 4           |                    |                      |                      |                      |
| 5           |                    |                      |                      |                      |
| 6           |                    |                      |                      |                      |
| 7           |                    |                      |                      |                      |
| 8           |                    |                      |                      |                      |
| 9           |                    |                      |                      |                      |
| 10          |                    |                      |                      |                      |
| 11          |                    |                      |                      |                      |
| 12          |                    |                      |                      |                      |
| 13          |                    |                      |                      |                      |
| 99          |                    |                      |                      |                      |
| 15          |                    |                      |                      |                      |
| 16          |                    |                      |                      |                      |
| 17          |                    |                      |                      |                      |
| 18          |                    |                      |                      |                      |
| 19          |                    |                      |                      |                      |
| 20          |                    |                      |                      |                      |
| 21          |                    |                      |                      |                      |
| 22          |                    |                      |                      |                      |
| 23          |                    | *                    |                      |                      |
| 24          |                    |                      |                      |                      |
| Mean        | 37.2               | 36.9                 | 36.0                 | 36.7                 |
| S.D.        | 12.7               | 11.3                 | 12.4                 | 12.0                 |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

**Table 9-6**  
**Ratios of Lisinopril Urinary Recovery Following Administration of the**  
**Combination Dosage Forms to Recovery of the Separate Entity**  
**Administered Concurrently with HCTZ as a Reference**

| Subject No.    | $\left(\frac{B}{A}\right)$<br>20/12.5 | % Dose in Urine (Combination)<br>% Dose in Urine (Separate Entity) |                                    |
|----------------|---------------------------------------|--------------------------------------------------------------------|------------------------------------|
|                |                                       | $\left(\frac{C}{A}\right)$ 20/6.25                                 | $\left(\frac{D}{A}\right)$ 20/25.0 |
| 1              |                                       |                                                                    |                                    |
| 2              |                                       |                                                                    |                                    |
| 3              |                                       |                                                                    |                                    |
| 4              |                                       |                                                                    |                                    |
| 5              |                                       |                                                                    |                                    |
| 6              |                                       |                                                                    |                                    |
| 7              |                                       |                                                                    |                                    |
| 8              |                                       |                                                                    |                                    |
| 9              |                                       |                                                                    |                                    |
| 10             |                                       |                                                                    |                                    |
| 11             |                                       |                                                                    |                                    |
| 12             |                                       |                                                                    |                                    |
| 13             |                                       |                                                                    |                                    |
| 99             |                                       |                                                                    |                                    |
| 15             |                                       |                                                                    |                                    |
| 16             |                                       |                                                                    |                                    |
| 17             |                                       |                                                                    |                                    |
| 18             |                                       |                                                                    |                                    |
| 19             |                                       |                                                                    |                                    |
| 20             |                                       |                                                                    |                                    |
| 21             |                                       |                                                                    |                                    |
| 22             |                                       |                                                                    |                                    |
| 23             |                                       |                                                                    |                                    |
| 24             |                                       |                                                                    |                                    |
| Geometric Mean | .960                                  | .975                                                               | 1.00                               |
| S.D.           | .183                                  | .29                                                                | .25                                |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-7

Peak Plasma Concentrations of HCTZ Following Dosing  
Concomitantly (A) and in Combination (B,C,D) with 20 mg Lisinopril

| Subject No.                  | C <sub>max</sub> (ng/ml) |                      |                      |                      |
|------------------------------|--------------------------|----------------------|----------------------|----------------------|
|                              | Dose A<br>12.5 mg        | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1                            |                          |                      |                      |                      |
| 2                            |                          |                      |                      |                      |
| 3                            |                          |                      |                      |                      |
| 4                            |                          |                      |                      |                      |
| 5                            |                          |                      |                      |                      |
| 6                            |                          |                      |                      |                      |
| 7                            |                          |                      |                      |                      |
| 8                            | *                        |                      |                      |                      |
| 9                            | *                        |                      |                      |                      |
| 10                           |                          |                      |                      |                      |
| 11                           | *                        |                      |                      |                      |
| 12                           |                          |                      |                      |                      |
| 13                           |                          |                      |                      |                      |
| 99                           |                          |                      |                      |                      |
| 15                           |                          |                      |                      |                      |
| 16                           |                          |                      |                      |                      |
| 17                           |                          |                      |                      |                      |
| 18                           |                          |                      |                      |                      |
| 19                           |                          |                      |                      |                      |
| 20                           |                          |                      |                      |                      |
| 21                           |                          |                      |                      |                      |
| 22                           |                          |                      |                      |                      |
| 23                           |                          |                      |                      |                      |
| 24                           |                          |                      |                      |                      |
| Mean                         | 67.5                     | 76.3                 | 38.2                 | 148                  |
| S.D.                         | 12.7                     | 17.0                 | 8.6                  | 27                   |
| Normalized to<br>Lowest Dose | 33.8                     | 39.7                 | 38.2                 | 37.4                 |

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-8

Times of Peak Plasma HCTZ Following Dosing  
Concomitantly (A) and in Combination (B,C,D) with 20 mg Lisinopril

| Subject No. | T <sub>max</sub> (hours) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>12.5 mg        | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      |                      |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 2                        | 2                    | 2                    | 2                    |
| S.D.        | 1                        | 1                    | 1                    | 1                    |

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-9

Total Urinary Recovery of HCTZ Following Dosing  
Concomitantly (A) and in Combination (B,C,D) with 20 mg Lisinopril

| Subject No. | % of Dose in Urine |                      |                      |                      |
|-------------|--------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>12.5 mg  | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                    |                      |                      |                      |
| 2           |                    |                      |                      |                      |
| 3           |                    |                      |                      |                      |
| 4           |                    |                      |                      |                      |
| 5           |                    |                      |                      |                      |
| 6           |                    |                      |                      |                      |
| 7           |                    |                      |                      |                      |
| 8           |                    |                      |                      |                      |
| 9           |                    |                      |                      |                      |
| 10          |                    |                      |                      |                      |
| 11          |                    |                      |                      |                      |
| 12          |                    | *                    |                      |                      |
| 13          |                    |                      |                      |                      |
| 99          |                    |                      |                      |                      |
| 15          |                    |                      |                      |                      |
| 16          |                    |                      |                      |                      |
| 17          |                    |                      |                      |                      |
| 18          |                    |                      |                      |                      |
| 19          |                    |                      |                      |                      |
| 20          |                    |                      |                      |                      |
| 21          |                    |                      |                      |                      |
| 22          |                    |                      |                      |                      |
| 23          |                    |                      |                      |                      |
| 24          |                    |                      |                      |                      |
| Mean        | 66.4               | 73.3                 | 65.8                 | 70.0                 |
| S.D.        | 12.2               | 10.3                 | 9.7                  | 9.0                  |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-10

Ratios of HCTZ Urinary Recovery Following Administration of the  
Combination Dosage Forms to Recovery of the Separate Entity  
Administered Concurrently with Lisinopril as a Reference

| Subject No.    | $\left( \frac{B/A}{C/A} \right) \left( \frac{\% \text{ Dose in Urine (Combination)}}{\% \text{ Dose in Urine (Separate Entity)}} \right)$ |               |               |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------|---------------|---------------|
|                | 20/12.5                                                                                                                                   | (C/A) 20/6.25 | (D/A) 20/25.0 |
| 1              |                                                                                                                                           |               |               |
| 2              |                                                                                                                                           |               |               |
| 3              |                                                                                                                                           |               |               |
| 4              |                                                                                                                                           |               |               |
| 5              |                                                                                                                                           |               |               |
| 6              |                                                                                                                                           |               |               |
| 7              |                                                                                                                                           |               |               |
| 8              |                                                                                                                                           |               |               |
| 9              |                                                                                                                                           |               |               |
| 10             |                                                                                                                                           |               |               |
| 11             |                                                                                                                                           |               |               |
| 12             |                                                                                                                                           | *             | *             |
| 13             |                                                                                                                                           |               |               |
| 99             |                                                                                                                                           |               |               |
| 15             |                                                                                                                                           |               |               |
| 16             |                                                                                                                                           |               |               |
| 17             |                                                                                                                                           |               |               |
| 18             |                                                                                                                                           |               |               |
| 19             |                                                                                                                                           |               |               |
| 20             |                                                                                                                                           |               |               |
| 21             |                                                                                                                                           |               |               |
| 22             |                                                                                                                                           |               |               |
| 23             |                                                                                                                                           |               |               |
| 24             |                                                                                                                                           |               |               |
| Geometric Mean | 1.09                                                                                                                                      | .993          | 1.07          |
| S.D.           | .23                                                                                                                                       | .185          | .21           |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-9

Total Urinary Recovery of HCTZ Following Dosing  
Concomitantly (A) and in Combination (B,C,D) with 20 mg Lisinopril

| Subject No. | % of Dose in Urine |                      |                      |                      |
|-------------|--------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>12.5 mg  | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                    |                      |                      |                      |
| 2           |                    |                      |                      |                      |
| 3           |                    |                      |                      |                      |
| 4           |                    |                      |                      |                      |
| 5           |                    |                      |                      |                      |
| 6           |                    |                      |                      |                      |
| 7           |                    |                      |                      |                      |
| 8           |                    |                      |                      |                      |
| 9           |                    |                      |                      |                      |
| 10          |                    |                      |                      |                      |
| 11          |                    |                      |                      |                      |
| 12          |                    | *                    |                      |                      |
| 13          |                    |                      |                      |                      |
| 99          |                    |                      |                      |                      |
| 15          |                    |                      |                      |                      |
| 16          |                    |                      |                      |                      |
| 17          |                    |                      |                      |                      |
| 18          |                    |                      |                      |                      |
| 19          |                    |                      |                      |                      |
| 20          |                    |                      |                      |                      |
| 21          |                    |                      |                      |                      |
| 22          |                    |                      |                      |                      |
| 23          |                    |                      |                      |                      |
| 24          |                    |                      |                      |                      |
| Mean        | 66.4               | 73.3                 | 65.8                 | 70.0                 |
| S.D.        | 12.2               | 10.3                 | 9.7                  | 9.0                  |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-10

Ratios of HCTZ Urinary Recovery Following Administration of the  
Combination Dosage Forms to Recovery of the Separate Entity  
Administered Concurrently with Lisinopril as a Reference

| Subject No.    | $\left( \frac{\% \text{ Dose in Urine (Combination)}}{\% \text{ Dose in Urine (Separate Entity)}} \right)$ |                  |                  |
|----------------|------------------------------------------------------------------------------------------------------------|------------------|------------------|
|                | (B/A)<br>20/12.5                                                                                           | (C/A)<br>20/6.25 | (D/A)<br>20/25.0 |
| 1              |                                                                                                            |                  |                  |
| 2              |                                                                                                            |                  |                  |
| 3              |                                                                                                            |                  |                  |
| 4              |                                                                                                            |                  |                  |
| 5              |                                                                                                            |                  |                  |
| 6              |                                                                                                            |                  |                  |
| 7              |                                                                                                            |                  |                  |
| 8              |                                                                                                            |                  |                  |
| 9              |                                                                                                            |                  |                  |
| 10             |                                                                                                            |                  |                  |
| 11             |                                                                                                            |                  |                  |
| 12             |                                                                                                            | *                | *                |
| 13             |                                                                                                            |                  |                  |
| 99             |                                                                                                            |                  |                  |
| 15             |                                                                                                            |                  |                  |
| 16             |                                                                                                            |                  |                  |
| 17             |                                                                                                            |                  |                  |
| 18             |                                                                                                            |                  |                  |
| 19             |                                                                                                            |                  |                  |
| 20             |                                                                                                            |                  |                  |
| 21             |                                                                                                            |                  |                  |
| 22             |                                                                                                            |                  |                  |
| 23             |                                                                                                            |                  |                  |
| 24             |                                                                                                            |                  |                  |
| Geometric Mean | 1.09                                                                                                       | .993             | 1.07             |
| S.D.           | .23                                                                                                        | .185             | .21              |

-- incomplete urine collection

\* Excluded from mean (differs by more than 3 S.D.)

Table 9-11

Renal Clearance of HCTZ Following Dosing  
Concomitantly (A) and in Combination (B,C,D) with 20 mg Lisinopril

| Subject No. | Cl <sub>r</sub> (ml/min) |                      |                      |                      |
|-------------|--------------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>12.5 mg        | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                          |                      |                      |                      |
| 2           |                          |                      |                      |                      |
| 3           |                          |                      |                      |                      |
| 4           |                          |                      |                      |                      |
| 5           |                          |                      |                      |                      |
| 6           |                          |                      |                      |                      |
| 7           |                          |                      |                      |                      |
| 8           |                          |                      |                      |                      |
| 9           |                          |                      |                      |                      |
| 10          |                          |                      |                      |                      |
| 11          |                          |                      |                      |                      |
| 12          |                          |                      |                      |                      |
| 13          |                          |                      |                      |                      |
| 99          |                          |                      |                      |                      |
| 15          |                          |                      |                      |                      |
| 16          |                          |                      |                      |                      |
| 17          |                          |                      |                      | *                    |
| 18          |                          |                      |                      |                      |
| 19          |                          |                      |                      |                      |
| 20          |                          |                      |                      |                      |
| 21          |                          |                      |                      |                      |
| 22          |                          |                      |                      |                      |
| 23          |                          |                      |                      |                      |
| 24          |                          |                      |                      |                      |
| Mean        | 322                      | 308                  | 356                  | 294                  |
| S.D.        | 75                       | 87                   | 73                   | 55                   |

-- incomplete urine collection

\* Excluded from mean (differs by more than 4 S.D.)

Table 9-12

Area Under the HCTZ Plasma Concentration versus Time Curve (AUC)  
Following Dosing Concomitantly (A) and in Combination (B,C,D)  
With 20 mg Lisinopril

| Subject No. | AUC (mg·hr/l)     |                      |                      |                      |
|-------------|-------------------|----------------------|----------------------|----------------------|
|             | Dose A<br>12.5 mg | Dose B<br>20/12.5 mg | Dose C<br>20/6.25 mg | Dose D<br>20/25.0 mg |
| 1           |                   |                      |                      |                      |
| 2           |                   |                      |                      |                      |
| 3           |                   |                      |                      |                      |
| 4           |                   | *                    | *                    | *                    |
| 5           |                   |                      | *                    |                      |
| 6           |                   |                      |                      |                      |
| 7           |                   |                      |                      |                      |
| 8           |                   |                      | *                    |                      |
| 9           |                   |                      |                      |                      |
| 10          |                   |                      |                      |                      |
| 11          |                   |                      |                      |                      |
| 12          |                   |                      |                      |                      |
| 13          |                   |                      |                      |                      |
| 99          |                   |                      |                      |                      |
| 15          |                   |                      |                      |                      |
| 16          |                   |                      |                      |                      |
| 17          |                   |                      |                      |                      |
| 18          |                   |                      |                      |                      |
| 19          |                   |                      |                      |                      |
| 20          |                   |                      |                      |                      |
| 21          |                   |                      |                      |                      |
| 22          |                   |                      |                      |                      |
| 23          |                   |                      |                      |                      |
| 24          |                   |                      |                      |                      |
| Mean        | .465              | .493                 | .191                 | .990                 |
| S.D.        | .116              | .092                 | .024                 | .172                 |

\* Excluded from mean (differs by more than 3 S.D.)

Representative standard curve for lisinopril RIA.

TABLE: 9-13

a).

| Nominal Conc (ng/ml) | % Control Binding | Read-Back Value (ng/ml)* | Calc./Nominal |           |
|----------------------|-------------------|--------------------------|---------------|-----------|
| 0.4                  | 87.57             | 0.409                    | 1.02          |           |
|                      | 89.10             | 0.353                    | 0.88          |           |
|                      | 86.28             | 0.460                    | 1.15          | Mean 1.02 |
| 1.0                  | 75.38             | 1.014                    | 1.01          |           |
|                      | 76.26             | 0.960                    | 0.96          |           |
|                      | 75.68             | 0.996                    | 1.00          | Mean 0.99 |
| 2.0                  | 61.95             | 2.158                    | 1.08          |           |
|                      | 63.67             | 1.972                    | 0.99          |           |
|                      | 62.60             | 2.087                    | 1.04          | Mean 1.04 |
| 4.0                  | 50.01             | 3.985                    | 1.00          |           |
|                      | 51.00             | 3.788                    | 0.95          |           |
|                      | 51.12             | 3.765                    | 0.94          | Mean 0.96 |
| 10                   | 33.10             | 10.00                    | 1.00          |           |
|                      | 33.89             | 9.543                    | 0.95          |           |
|                      | 33.82             | 9.581                    | 0.96          | Mean 0.97 |
| 20                   | 22.29             | 20.75                    | 1.04          |           |
|                      | 22.31             | 20.73                    | 1.04          |           |
|                      | 22.29             | 20.75                    | 1.04          | Mean 1.04 |
| 40                   | 14.62             | 41.67                    | 1.04          |           |
|                      | 15.17             | 39.30                    | 0.98          |           |
|                      | 15.30             | 38.75                    | 0.97          | Mean 1.00 |
| 100                  | 7.91              | 105.4                    | 1.05          |           |
|                      | 8.18              | 100.4                    | 1.00          |           |
|                      | 8.15              | 100.9                    | 1.01          | Mean 1.02 |
| 160                  | 5.77              | 165.3                    | 1.03          |           |
|                      | 5.99              | 157.1                    | 0.98          |           |
|                      | 6.27              | 147.2                    | 0.92          | Mean 0.98 |

Conc. for 50% inhibition 3.99 ng/ml  
 B-zero 41.67%  
 NSB 0.96%

\*Using a 3rd degree polynomial

(Lab book/page: 8735/307 File MK-521 XII)

b).

Representative cumulative QC sample data through 9/86.

| Serum Code | Nominal (ng/ml) | Mean (ng/ml) | Inter-Assay CV% | No. of Assays |
|------------|-----------------|--------------|-----------------|---------------|
| 11         | 2               | 1.88         | 6.58            | 47            |
| 21         | 10              | 9.75         | 4.70            | 47            |
| 31         | 100             | 95.96        | 5.23            | 47            |

(Lab book 8915/211 File MK-521 XI, XII)

| Urine Code | Nominal (ug/ml) | Mean (ug/ml) | Inter-Assay CV% | No. of Assays |
|------------|-----------------|--------------|-----------------|---------------|
| 15         | 0.20            | 0.20         | 6.06            | 33            |
| 25         | 1.00            | 1.00         | 3.56            | 33            |
| 35         | 10.0            | 10.07        | 5.91            | 33            |

(Lab book 8735/307 File MK-521 XI, XII)

Representative standard curve for lisinopril RIA.

TABLE: 9-13

a).

| Nominal Conc<br>(ng/ml) | % Control<br>Binding | Read-Back Value<br>(ng/ml)* | Calc./Nominal |
|-------------------------|----------------------|-----------------------------|---------------|
| 0.2                     | 87.57                | 0.409                       | 1.02          |
|                         | 89.10                | 0.353                       | 0.88          |
|                         | 86.28                | 0.460                       | 1.15          |
|                         |                      |                             | Mean 1.02     |
| 1.0                     | 75.38                | 1.014                       | 1.01          |
|                         | 76.26                | 0.960                       | 0.96          |
|                         | 75.68                | 0.996                       | 1.00          |
|                         |                      |                             | Mean 0.99     |
| 2.0                     | 61.95                | 2.158                       | 1.08          |
|                         | 63.67                | 1.972                       | 0.99          |
|                         | 62.60                | 2.087                       | 1.04          |
|                         |                      |                             | Mean 1.04     |
| 4.0                     | 50.01                | 3.985                       | 1.00          |
|                         | 51.00                | 3.788                       | 0.95          |
|                         | 51.12                | 3.765                       | 0.94          |
|                         |                      |                             | Mean 0.96     |
| 10                      | 33.10                | 10.00                       | 1.00          |
|                         | 33.89                | 9.543                       | 0.95          |
|                         | 33.82                | 9.581                       | 0.96          |
|                         |                      |                             | Mean 0.97     |
| 20                      | 22.29                | 20.75                       | 1.04          |
|                         | 22.31                | 20.73                       | 1.04          |
|                         | 22.29                | 20.75                       | 1.04          |
|                         |                      |                             | Mean 1.04     |
| 40                      | 14.62                | 41.67                       | 1.04          |
|                         | 15.17                | 39.30                       | 0.98          |
|                         | 15.30                | 38.75                       | 0.97          |
|                         |                      |                             | Mean 1.00     |
| 100                     | 7.91                 | 105.4                       | 1.05          |
|                         | 8.18                 | 100.4                       | 1.00          |
|                         | 8.15                 | 100.9                       | 1.01          |
|                         |                      |                             | Mean 1.02     |
| 160                     | 5.77                 | 165.3                       | 1.03          |
|                         | 5.99                 | 157.1                       | 0.98          |
|                         | 6.27                 | 147.2                       | 0.92          |
|                         |                      |                             | Mean 0.98     |

Conc. for 50% inhibition 3.99 ng/ml  
 S-zero 41.67%  
 MSB 0.96%

\*Using a 3rd degree polynomial

(Lab book/page: 8735/307 File MK-521 XII)

b).

Representative cumulative QC sample data through 9/86.

| Serum<br>Code | Nominal<br>(ng/ml) | Mean<br>(ng/ml) | Inter-<br>Assay<br>CV% | No. of<br>Assays |
|---------------|--------------------|-----------------|------------------------|------------------|
| 11            | 2                  | 1.88            | 6.58                   | 47               |
| 21            | 10                 | 9.75            | 4.70                   | 47               |
| 31            | 100                | 95.96           | 5.23                   | 47               |

(Lab book 8915/211 File MK-521 XI, XII)

| Urine<br>Code | Nominal<br>(ug/ml) | Mean<br>(ug/ml) | Inter-<br>Assay<br>CV% | No. of<br>Assays |
|---------------|--------------------|-----------------|------------------------|------------------|
| 15            | 0.20               | 0.20            | 6.06                   | 33               |
| 25            | 1.00               | 1.00            | 3.56                   | 33               |
| 35            | 10.0               | 10.07           | 5.91                   | 33               |

(Lab book 8735/307 File MK-521 XI, XII)

Table: 9-14 a)

## INTERDAY PRECISION OF PLASMA HYDROCHLOROTHIAZIDE

| SAMPLE NUMBER                             |   |   |   |   |   | MEAN | S.D. | %CV  |
|-------------------------------------------|---|---|---|---|---|------|------|------|
| 1                                         | 2 | 3 | 4 | 5 | 6 |      |      |      |
| HIGH CONCENTRATION (SPIKED: 200 ng/ml)    |   |   |   |   |   | 193  | 5.44 | 2.82 |
| MEDIUM CONCENTRATION (SPIKED: 80.0 ng/ml) |   |   |   |   |   | 72.9 | 1.83 | 2.51 |
| LOW CONCENTRATION (SPIKED: 10.0 ng/ml)    |   |   |   |   |   | 10.1 | 0.87 | 8.61 |

Table: 9-14 b)

## INTRADAY PRECISION OF PLASMA HYDROCHLOROTHIAZIDE

| SAMPLE NUMBER                             |   |   |   |   |   | MEAN | S.D. | %CV  |
|-------------------------------------------|---|---|---|---|---|------|------|------|
| 1                                         | 2 | 3 | 4 | 5 | 6 |      |      |      |
| HIGH CONCENTRATION (SPIKED: 200 ng/ml)    |   |   |   |   |   | 199  | 12.7 | 6.38 |
| MEDIUM CONCENTRATION (SPIKED: 80.0 ng/ml) |   |   |   |   |   | 79.3 | 3.12 | 3.93 |
| LOW CONCENTRATION (SPIKED: 10.0 ng/ml)    |   |   |   |   |   | 9.56 | 0.38 | 3.94 |

Table: 9-15 a)

## INTERDAY PRECISION OF URINE HYDROCHLOROTHIAZIDE

| SAMPLE NUMBER                             |   |   |   |   |   | MEAN | S.D. | %CV  |
|-------------------------------------------|---|---|---|---|---|------|------|------|
| 1                                         | 2 | 3 | 4 | 5 | 6 |      |      |      |
| HIGH CONCENTRATION (SPIKED: 32.0 ug/ml)   |   |   |   |   |   | 32.3 | 1.21 | 3.75 |
| MEDIUM CONCENTRATION (SPIKED: 16.0 ug/ml) |   |   |   |   |   | 16.4 | 0.18 | 1.16 |
| LOW CONCENTRATION (SPIKED: 2.00 ug/ml)    |   |   |   |   |   | 1.82 | 0.09 | 4.89 |

Table: 9-15 b)

## INTRADAY PRECISION OF URINE HYDROCHLOROTHIAZIDE

| SAMPLE NUMBER                             |   |   |   |   |   | MEAN | S.D. | %CV  |
|-------------------------------------------|---|---|---|---|---|------|------|------|
| 1                                         | 2 | 3 | 4 | 5 | 6 |      |      |      |
| HIGH CONCENTRATION (SPIKED: 32.0 ug/ml)   |   |   |   |   |   | 29.8 | 0.26 | 0.84 |
| MEDIUM CONCENTRATION (SPIKED: 16.0 ug/ml) |   |   |   |   |   | 16.5 | 0.16 | 1.03 |
| LOW CONCENTRATION (SPIKED: 2.00 ug/ml)    |   |   |   |   |   | 1.85 | 0.01 | 0.51 |

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N-19085-1 OF 5

NDA

19-085-

NDA

19-085

AP/Ltr

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NDA 19-035

Boehringer Ingelheim Pharmaceuticals, Inc.  
90 East Ridge  
P.O. Box 363  
Ridgefield, CT 06377

Attention: Ruth Wasserman  
Associate Director, Regulatory Affairs

Gentlemen:

Please refer to your new drug application dated July 29, 1983, submitted pursuant to section 505(b) of the Federal Food, Drug and Cosmetic Act for the preparation Atrovent (ipratropium bromide) Inhalation Aerosol.

We also refer to our letter dated December 19, 1986 which declared this application approvable and to your communications dated December 19, 22 and 23, 1986.

Additional reference is made to a December 23, 1986 verbal commitment by Dr. Wilson of Boehringer Ingelheim to change the Product Title on the carton and immediate container labels to "Atrovent (ipratropium bromide) Inhalation Aerosol" within 6 months or at the next printing, whichever occurs first.

We have completed the review of this application including the draft labeling submitted in your last amendment and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in that draft labeling. Accordingly, the application is approved, effective as of the date of this letter.

The final printed labeling (FPL) must be identical to the draft labeling. Marketing the product with FPL that is not identical to the draft labeling may render the product misbranded and an unapproved new drug. Please submit twelve copies of the FPL to FDA as soon as available. For administrative purposes this submission should be designated "FPL Supplement" to the approved NDA 19-035. Approval of this supplement by FDA is not required before the labeling is used. Should additional information relating to the safety and effectiveness of this drug product become available prior to our receipt of the final printed labeling, revision of that labeling may be required.

NDA 19-085  
Page 2

Please submit one market package of the drug product when it is available.

We remind you that you must comply with the requirements set forth under 21 CFR 314.80 and 314.81 for an approved NDA.

Sincerely yours,

*Botstein*

Paula Botstein, M.D.  
Acting Deputy Director (Medical Affairs)  
Office of Drug Research and Review  
Center for Drugs and Biologics

NDA

19-085

Clinical Data

NDA 19-085 Atrovent Inhaler  
ipratropium bromide  
Bronchodilator

Boehringer Ingelheim  
Submitted: 12/1/86  
Received: 12/3/86  
Reviewed: 12/10/86

#### REVIEW AND EVALUATION OF CLINICAL DATA

This amendment to the NDA consists of:

1. Safety update, foreign data from clinical trials and spontaneous postmarketing reports (foreign) as well as a summary of literature since the July 2, 1985 update.
2. Foreign marketing status
3. Revised adverse reaction section of the package insert incorporating adverse experience data from foreign experience, the compassionate use program and additional clinical trials conducted since the NDA as well as the 90-day studies.
4. Two adverse experience summary tables as requested; one containing all adverse experiences reported from clinical trials and one containing reports of all adverse events except for the 90-day studies. Separate tabulations of the controlled and uncontrolled trials from which the summary tables were derived are also included.

Also included is the Sixth Annual Emergency Program Progress Report, all previous progress reports and a summary of patients withdrawn from controlled clinical trials conducted since the NDA was filed.

There is also a commitment to performing the following Phase IV Studies:

1. A 90-day controlled study designed to show efficacy and safety of Atrovent in asthma.
2. Shorter term studies to evaluate the interaction, including efficacy and safety of Atrovent with other drugs used in the treatment of patients with asthma and with chronic obstructive pulmonary disease.

The firm understands the design of those studies will be finalized in discussions with this Agency after approval for marketing of Atrovent.

These data are submitted as a result of communications dated November 13, 1986 (telephone) and November 25, 1986 telephone (Mr. Wardell).

1. Safety update - Atrovent MDI

Summary of Foreign Data - Atrovent MDI

Sources

1. Unpublished data (foreign clinical trials and foreign marketing experience)
2. Published literature

DEC 17 1986

Unpublished Data

January 1985 to November 15, 1986 16 adverse reactions were reported in 10 patients regarding use of Atrovent MDI - Table 1.

TABLE 1

Foreign Unpublished Adverse Events  
Associated with the use of Atrovent® MDI

| <u>Body System/Event</u>                     | <u>Atrovent® MDI</u> |
|----------------------------------------------|----------------------|
| <u>Cardiovascular</u>                        |                      |
| Blood pressure decrease                      | 1                    |
| Fainting                                     | 1                    |
| <u>Central and Peripheral Nervous System</u> |                      |
| Dizziness                                    | 1                    |
| Grand mal                                    | 1                    |
| <u>Dermatological</u>                        |                      |
| Flushing                                     | 1                    |
| Giant urticaria                              | 1                    |
| <u>Psychiatric</u>                           |                      |
| Psychosis                                    | 1                    |
| <u>Respiratory</u>                           |                      |
| Bronchoconstriction                          | 1                    |
| Bronchospasm                                 | 4                    |
| Cyanosis                                     | 1                    |
| Dysnoea                                      | 1                    |
| Increased breathlessness                     | 1                    |
| Respiratory arrest                           | 1/16                 |

Unpublished Data

January 1985 to November 15, 1986 16 adverse reactions were reported in 10 patients regarding use of Atrovent® MDI - Table 1.

TABLE 1

Foreign Unpublished Adverse Events  
Associated with the use of Atrovent® MDI

| <u>Body System/Event</u>                     | <u>Atrovent® MDI</u> |
|----------------------------------------------|----------------------|
| <u>Cardiovascular</u>                        |                      |
| Blood pressure decrease                      | 1                    |
| Fainting                                     | 1                    |
| <u>Central and Peripheral Nervous System</u> |                      |
| Dizziness                                    | 1                    |
| Grand mal                                    | 1                    |
| <u>Dermatological</u>                        |                      |
| Flushing                                     | 1                    |
| Giant urticaria                              | 1                    |
| <u>Psychiatric</u>                           |                      |
| Psychosis                                    | 1                    |
| <u>Respiratory</u>                           |                      |
| Bronchoconstriction                          | 1                    |
| Bronchospasm                                 | 4                    |
| Cyanosis                                     | 1                    |
| Dysnoea                                      | 1                    |
| Increased breathlessness                     | 1                    |
| Respiratory arrest                           | 1                    |

1/16

TABLE 2

Summary of Unpublished Adverse Reactions from Foreign Trials and Marketing Experience with Atrovent® MDI from January 1985 - November 1986

| <u>Age</u> | <u>Sex</u> | <u>Reporting Country</u> | <u>Indication</u>                  | <u>Adverse Event</u>                                          | <u>Concomitant Medication</u>                          | <u>Outcome</u>                                      | <u>Comment</u>         |
|------------|------------|--------------------------|------------------------------------|---------------------------------------------------------------|--------------------------------------------------------|-----------------------------------------------------|------------------------|
| 76         | M          | G.B.                     | Chronic obstructive airway disease | Grand mal seizure                                             | Ventolin<br>Phyllocontin                               | Reaction occurred again after drug was discontinued |                        |
| Unknown    | F          | F                        | Chronic bronchitis                 | Giant urticaria                                               | Vibromycine<br>Becotide<br>Ventoline<br>Trentadil      |                                                     | Positive rechallenge   |
| 68         | M          | F                        | Chronic bronchitis                 | Bronchospasm                                                  | Ventolin                                               | Death ✓                                             | No further information |
| Unknown    | Unknown    | G.B.                     |                                    | Psychosis                                                     | Ventolin<br>Becotide<br>Intal                          | Unknown                                             | No further information |
| 61         | M          | Aus                      | Unknown                            | Dysnoea<br>Bronchospasm<br>Cyanosis                           | Unknown                                                | Drug discontinued                                   | No further information |
| Unknown    | Unknown    | G.B.                     | Unknown                            | Bronchospasm                                                  | Unknown                                                | Unknown                                             | No further information |
| Unknown    | F          | G.B.                     | Ca lung                            | Increased breathlessness                                      | Prednisolone                                           | Drug discontinued                                   | No further information |
| Unknown    | Unknown    | F                        | Unknown                            | Bronchospasm                                                  | Bricanyl<br>Ventoline                                  | Drug continued                                      | No further information |
| 58         | F          | G.B.                     | Unknown                            | Blood pressure decreased<br>Fainting<br>Dizziness<br>Flushing | Salbutamol<br>Thyroxine<br>Becloforte<br>Glibenclamide | Unknown                                             | No further information |
| Unknown    | Unknown    | G.B.                     | Unknown                            | Broncho-constriction                                          | Unknown                                                | Unknown                                             | No further information |

Aus = Australia    F = France    G.B. = Great Britain

Summary data for each patient is presented in Table 2. One death occurred in a 68 year old male patient from France who developed bronchospasm in association with Atrovent MDI; no additional information is available on this patient. One patient developed giant urticaria with Atrovent which recurred on rechallenge. A 76 year old male in the U.K. had a grand mal seizure on Atrovent MDI and another after discontinuation of Atrovent. One patient became psychotic while on Atrovent plus Intal, Becotide and Ventolin. No further information was available on that patient. All other adverse reactions are well known and previously reported in patients administered Atrovent MDI.

Published data

January 1, 1985 to November 1986 a total of 44 articles were published on Atrovent MDI (Appendix A) of which three contained safety data (Appendix B). Data reported in these articles originated in two countries, Great Britain and Canada (Table 3).

TABLE 3

Country of Origin of Published Literature Reporting  
Adverse Events Associated with the use of Atrovent® MDI

| <u>Country</u> | <u>Number of Articles</u> | <u>Senior Author</u> |
|----------------|---------------------------|----------------------|
| Great Britian  | 2                         | Hockley<br>Spencer   |
| Canada         | 1                         | Vakil                |

The adverse reactions are classified according to body system (Table 4).

TABLE 4  
Published Literature Events Reported  
Among 32 Patients Receiving Atrovent® MDI

| <u>Body System/Adverse Event</u>             | <u>Number of pts. experiencing adverse event</u> | <u>Senior Author</u> |
|----------------------------------------------|--------------------------------------------------|----------------------|
| <u>Cardiovascular</u>                        |                                                  |                      |
| Fall in blood pressure                       | 9* + 1                                           | Vakil & Hockley      |
| Increase in systolic blood pressure          | 1                                                | Vakil                |
| <u>Central Nervous System</u>                |                                                  |                      |
| Drowsiness                                   | 1                                                | Vakil                |
| <u>Central and Peripheral Nervous System</u> |                                                  |                      |
| Tremor                                       | 2                                                | Vakil & Hockley      |
| Dizziness                                    | 1                                                | Vakil                |
| Vertigo                                      | 1                                                | Vakil                |
| <u>Dermatological</u>                        |                                                  |                      |
| Rash                                         | 1                                                | Hockley              |
| <u>Heart Rate and Rhythm</u>                 |                                                  |                      |
| Tachycardia                                  | 3                                                | Vakil                |
| Fall in pulse rate                           | 9                                                | Hockley              |
| <u>Musco-skeletal</u>                        |                                                  |                      |
| Myalgia                                      | 1                                                | Vakil                |
| <u>Oro-otolaryngeal</u>                      |                                                  |                      |
| Bitter taste                                 | 6                                                | Hockley              |
| Sore throat                                  | 2                                                | Hockley & Spencer    |
| Stomatitis                                   | 1                                                | Spencer              |
| Ulcerative stomatitis                        | 2                                                | Spencer              |
| Throat irritation                            | 1                                                | Vakil                |
| Swollen lips and tongue                      | 1                                                | Spencer              |
| <u>Respiratory</u>                           |                                                  |                      |
| Cough                                        | 2                                                | Vakil & Hockley      |
| Bronchopneumonia                             | 1                                                | Vakil                |
| Asthmatic attack                             | 1                                                | Vakil                |
| <u>Vision Disorders</u>                      |                                                  |                      |
| Eye irritation                               | 1                                                | Hockley              |

48

\*In a cross-over study in 9 patients diastolic B.P. was significantly lower than base line at 8 hr at 200 ug, and with 80 ug the systolic pressure was significantly lower at 2 hr than base line.

There are a total of 48 events reported in a total of 32 patients treated. No unusual reactions not previously reported in association with use of Atrovent MDI are reported in this published literature. Two patients withdrew from a

study because of side effects, one tremor and the other throat irritation, myalgia and coughing. Another two withdrew according to the author due to reasons not connected with the study, one developed pneumonia on the first day of the study and the other due to Meiner's disease detected 2 months into the study. Another developed inflamed buccal mucosa with ulceration after 4 puffs (76 ugs) of Atrovent MDI and his lips and tongue became swollen. After 48 hours of cessation of use of the inhaler, he was normal and he declined a rechallenge.

Events of clinical interest occurring with other Atrovent preparations from foreign sources

There is one report of increased SGOT and hepatomegaly from Canada in a 35 year old male who used Atrovent solution and the only other medication he used was Triamcinolone. The cause of the liver problem was not determined. Chorea athetoid movements were reported in one patient on Atrovent solution for severe asthma. This patient also had hypoglobulinemia and epilepsy and was receiving salbutamol, ventolin, carbamazepine, codeine and prednisolone. A 74 year old male in Australia reported enlargement of the left breast due to a diffuse firm and non-tender swelling after 180 days on Atrovent solution. There were two reports of chest pain. There was one report of respiratory and one circulatory arrest - no further information is known on these cases. All known information obtainable is shown on Table 5. Sparsity of information in these cases makes it impossible to establish a causal relationship.

Summary (by the firm) - confirmed

The unpublished and published adverse reactions associated with Atrovent MDI from foreign clinical sources is consistent with that seen in the clinical trials and the Atrovent compassionate program in the U.S. The analysis of those data does not suggest any changes in the proposed package insert. [Table 5 is on the following page.]

TABLE 5  
 Events of Clinical Interest from Foreign Sources (Unpublished) using  
 Formulations of Atrovent® other than MDI from January 1985 - November 1986

| <u>Age</u>                       | <u>Sex</u> | <u>Reporting Country</u> | <u>Indication</u>                      | <u>Adverse Event</u>                                                         | <u>Concomitant Medication</u>                                         | <u>Outcome</u>         | <u>Comment</u>                                                                                                        |
|----------------------------------|------------|--------------------------|----------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------|------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <u>Atrovent® Solution 0.025%</u> |            |                          |                                        |                                                                              |                                                                       |                        |                                                                                                                       |
| 35                               | M          | Can                      | Unknown                                | SGOT increased<br>Hepatomegaly                                               | Triamcinolone                                                         | Drug continued         | No further information                                                                                                |
| 62                               | M          | Unknown                  | Emphysema                              | 1)Bronchospasm-mild<br>2)Bronchospasm-severe<br>plus circulatory<br>collapse | Terbutaline<br>Prednisolone<br>Hydrocortisone                         | Drug dis-<br>continued | No further information                                                                                                |
| 74                               |            | Aus                      | Chronic<br>obstructive<br>lung disease | Enlarged left breast,<br>diffuse firm<br>non-tender swelling                 | Intal<br>Ventolin<br>Theodur<br>Aspirin                               | Swelling<br>persists   | Occured after 180 days<br>on drug                                                                                     |
| 65                               | F          | S.A.                     | Emphysema                              | Chest pain                                                                   |                                                                       |                        | This reaction occurred<br>only with one spray<br>can after years of<br>using drug.                                    |
| Unknown                          | F          | G.B.                     | Severe asthma                          | Chorea athetoid<br>movements                                                 | Salbutamol<br>Ventolin<br>Corbamazepine<br><br>Codein<br>Prednisolone | Drug<br>continued      | Other co-existing<br>conditions Hypogamma<br>globulinema and<br>epilepsy.                                             |
| 48                               | M          | Sw                       | Unknown                                | Chest pain                                                                   | None                                                                  |                        | No further information                                                                                                |
| Unknown                          | F          | G.B.                     | Unknown                                | Respiratory arrest                                                           | Unknown                                                               | Patient<br>recovered   | Vague report given to<br>sales representative<br>by junior physician<br>who did not respond<br>to follow-up requests. |

Aus = Australia, Can = Canada, Sw = Sweden, G.B. = Great Britain

## 2. Foreign Marketing Status

FOREIGN MARKETING STATUS

On July 2, 1985 we submitted the attached list of the countries where ATROVENT® Inhaler is currently marketed. In addition to the attached list, the following countries have introduced ATROVENT® into the market:

France  
Venezuela  
Mexico  
Ireland  
Singapore  
Taiwan

ATROVENT® has also been approved in the following countries where marketing has not begun:

Costa Rica  
Panama  
Honduras  
El Salvador  
Guatemala  
Jamaica  
Trinidad  
Curacao  
Tanzania  
Poland

There are registrations pending in Ecuador and Surinam.

6/21/85 Introduction Status ATROVENT Metered Aerosol  
 .....

C o u n t r i e s

|                        |       |                    |
|------------------------|-------|--------------------|
| Germany                | 03/75 |                    |
| <u>R_e_g_i_o_n_I</u>   |       |                    |
| Austria                | 10/74 |                    |
| Belgium                | 09/77 |                    |
| Denmark                | 03/78 |                    |
| Finland                | 09/78 |                    |
| Netherlands            | 09/77 |                    |
| Norway                 | 05/79 |                    |
| Sweden                 | 02/79 |                    |
| Switzerland            | 03/76 |                    |
| Tunisia                | 02/80 |                    |
| U K                    | 08/77 |                    |
| <u>R_e_g_i_o_n_II</u>  |       |                    |
| Canada                 | 01/83 |                    |
| Japan                  | 04/81 | marketed by Teijin |
| <u>R_e_g_i_o_n_III</u> |       |                    |
| Argentina              | 03/82 |                    |
| Brasilia               | 03/84 |                    |
| Chile                  | 06/82 |                    |
| Columbia               | 10/83 |                    |
| Peru                   | 07/84 |                    |
| Portugal               | 01/81 |                    |
| Spain                  | 10/79 |                    |
| Uruguay                | 04/82 |                    |
| <u>R_e_g_i_o_n_IV</u>  |       |                    |
| Australia              | 03/81 |                    |
| New Zealand            | 03/80 |                    |
| R S A                  | 03/78 |                    |
| Thailand               | 11/84 |                    |

3. Package Insert Adverse ReactionsADVERSE REACTIONS

Adverse reaction information concerning Atrovent® is derived from 90 day controlled clinical trials (N=254), other controlled clinical trials using recommended doses of Atrovent® (N=377) and a compassionate use program (N=1924). Additional information is derived from foreign postmarketing experience and the published literature.

Adverse reactions reported in greater than one percent of patients in the 90 day controlled clinical trials appear in the following table:

| Reaction                      | Percent of Patients                 |                                        |
|-------------------------------|-------------------------------------|----------------------------------------|
|                               | <u>Ipratropium Bromide</u><br>N=254 | <u>Metaproterenol Sulfate</u><br>N=249 |
| <u>Cardiovascular</u>         |                                     |                                        |
| Palpitations                  | 1.8                                 | 1.6                                    |
| <u>Central Nervous System</u> |                                     |                                        |
| Nervousness                   | 3.1                                 | 6.8                                    |
| Dizziness                     | 2.4                                 | 2.9                                    |
| Headache                      | 0.4                                 | 2.0                                    |
| <u>Dermatological</u>         |                                     |                                        |
| Rash                          | 1.2                                 | 0.4                                    |
| <u>Gastrointestinal</u>       |                                     |                                        |
| Nausea                        | 2.8                                 | 1.2                                    |
| Gastrointestinal distress     | 2.4                                 | 2.8                                    |
| Vomiting                      | 0                                   | 1.2                                    |
| <u>Musculoskeletal</u>        |                                     |                                        |
| Tremor                        | 0                                   | 2.4                                    |
| <u>Ophthalmological</u>       |                                     |                                        |
| Blurred vision                | 1.2                                 | 0.8                                    |
| <u>Oro-Otolaryngeal</u>       |                                     |                                        |
| Dry mouth                     | 2.6                                 | 0.8                                    |
| Irritation from aerosol       | 1.6                                 | 1.6                                    |
| <u>Respiratory</u>            |                                     |                                        |
| Cough                         | 5.9                                 | 1.2                                    |
| Exacerbation of symptoms      | 2.4                                 | 3.6                                    |

The Adverse Reactions Table for package insert lists for those greater than 1% for both Atrovent and metaproterenol sulfate. By body systems there were 7 greater in the metaproterenol group and there were 6 systems greater in the Atrovent (ipratropium) group, and in one system oro-otolaryngeal (irritation from aerosol) the percentage incidence was the same in both treatment groups.

In this tabulation, the highest percentage, 5.9% in the Atrovent group was cough and the highest for metaproterenol was nervousness, 6.8%.

Under Ophthalmological only blurred vision is reported, 1.2% in Atrovent patients and 0.8% in metaproterenol-treated patients.

Additional adverse reactions reported in less than one percent of the patients, considered possibly due to Atrovent® include urinary difficulty, fatigue, insomnia and hoarseness.

The compassionate use program was an uncontrolled, open-label study that included seriously ill patients taking many concomitant medications. Many had severe underlying cardiorespiratory disease. About 7% of patients treated in the compassionate use program discontinued the program because of adverse events. Of patients currently in the program, about 57% have been treated for two or more years with Atrovent®.

Of the 2301 patients treated in the compassionate use program and in clinical trials other than the 90 day studies, the most common adverse reactions reported were: dryness of the oropharynx, about 5 in 100; cough, exacerbation of symptoms and irritation from aerosol, each about 3 in 100; headache, about 2 in 100; nausea, dizziness, blurred vision/difficulty in accommodation, and drying of secretions, each about 1 in 100. Less frequently reported adverse reactions that were possibly due to Atrovent® include:

Cardiovascular: Palpitations, about 7 in 1000; tachycardia about 6 in 1000.

Central Nervous System: Fatigue, insomnia, each about 6 in 1000; paresthesia, drowsiness, each about 2 in 1000; coordination difficulty, about 1 in 1000.

Dermatological: Rash, about 4 in 1000; itching, about 2 in 1000; hives, flushing, alopecia, each less than 1 in 1000.

Gastrointestinal: Gastrointestinal distress, about 8 in 1000; diarrhea, constipation, vomiting, each about 3 in 1000.

Genitourinary: Urinary difficulty, about 6 in 1000.

Musculoskeletal: Tremor, about 4 in 1000.

Oro-Otolaryngeal: Hoarseness about 7 in 1000; mucosal ulcers, less than 1 in 1000.

A single case of giant urticaria with positive rechallenge has been reported from the foreign marketing experience (see Contraindications).

In this tabulation, the highest percentage, 5.9% in the Atrovent group was cough and the highest for metaproterenol was nervousness, 6.8%.

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Central Nervous System: Fatigue, insomnia, each about 6 in 1000; paresthesia, drowsiness, each about 2 in 1000; coordination difficulty, about 1 in 1000.

Dermatological: Rash, about 4 in 1000; itching, about 2 in 1000; hives, flushing, alopecia, each less than 1 in 1000.

Gastrointestinal: Gastrointestinal distress, about 8 in 1000; diarrhea, constipation, vomiting, each about 3 in 1000.

Genitourinary: Urinary difficulty, about 6 in 1000.

Musculoskeletal: Tremor, about 4 in 1000.

Oro-Otolaryngeal: Hoarseness about 7 in 1000; mucosal ulcers, less than 1 in 1000.

A single case of giant urticaria with positive rechallenge has been reported from the foreign marketing experience (see Contraindications).

The foregoing is information on a revised package insert relative to the Adverse Reaction Section incorporating adverse experience data from foreign experience, the compassionate use program and additional clinical trials conducted since the NDA, as well as the 90-day studies.

4. Adverse Reaction Summary Tables:

These consist of two summary tables requested of the firm. One contains all adverse experiences reported from clinical trials and the other containing reports of all adverse experiences except for the 90-day studies. Separate tabulations of the controlled and uncontrolled trials from which the summary tables were derived are also included.

Table one shows totals of data from 2,555 patients

| <u>Adverse experience</u> |                                     |
|---------------------------|-------------------------------------|
| Cardiovascular            | 65                                  |
| CNS                       | 156                                 |
| Dermatologic              | 29                                  |
| Endocrine                 | 1                                   |
| G. I.                     | 88                                  |
| G. U.                     | 23                                  |
| Metabolic/nutrition       | 4                                   |
| Musculo/skeletal          | 25                                  |
| Ophthalmologic            | 45                                  |
| Oro-otolaryngeal          | 215                                 |
| Respiratory               | 195                                 |
| Vascular extracardiac     | 1                                   |
| Other                     | 11                                  |
| TOTAL                     | 758 of 2,555 patients<br>evaluated. |

Table 2 shows - Clinical Trials  
except for 90-day studies

| <u>Adverse experience system</u> | <u>Number of Reactions</u> | <u>Number of patients treated</u> |
|----------------------------------|----------------------------|-----------------------------------|
|                                  |                            | 2,301                             |
| Cardiacvascular                  | 61                         |                                   |
| CNS                              | 132                        |                                   |
| Dermatologic                     | 26                         |                                   |
| Endocrine                        | 1                          |                                   |
| G. I.                            | 73                         |                                   |
| G. U.                            | 22                         |                                   |
| Metabolic/nutrition              | 4                          |                                   |
| Musculo/skeletal                 | 25                         |                                   |
| Ophthalmological                 | 41                         |                                   |
| Oro-otolaryngeal                 | 206                        |                                   |
| Respiratory                      | 173                        |                                   |
| Vascular extracardiac            | 1                          |                                   |
| Other                            | 11                         |                                   |
| TOTAL                            | 603                        | in 2,301 patients<br>studied      |

Highlights of Table one

|                          | <u>Adverse reactions</u> |                |
|--------------------------|--------------------------|----------------|
|                          | <u>Numbers</u>           | <u>Percent</u> |
| <u>Cardiovascular</u>    |                          |                |
| Palpitations             | 22                       | 0.9            |
| Tachycardia              | 14                       | 0.5            |
| <u>CNS</u>               |                          |                |
| Dizziness                | 29                       | 1.1            |
| Fatigue                  | 13                       | 0.5            |
| Headache                 | 46                       | 1.8            |
| Insomnia                 | 13                       | 0.5            |
| Nervousness              | 26                       | 1.0            |
| <u>Dermatological</u>    |                          |                |
| Rash                     | 13                       | 0.5            |
| <u>Gastrointestinal</u>  |                          |                |
| G. I. Distress           | 24                       | 0.9            |
| Nausea                   | 35                       | 1.4            |
| <u>G. U.</u>             |                          |                |
| Urinary difficulty       | 13                       | 0.5            |
| <u>Musculo/skeletal</u>  |                          |                |
| Tremor                   | 10                       | 0.4            |
| <u>Ophthalmological</u>  |                          |                |
| Blurred vision           | 22                       | 0.9            |
| Other ophthalmological   | 15                       | 0.6            |
| <u>Oro-otolaryngeal</u>  |                          |                |
| Dryness                  | 126                      | 4.9            |
| Hoarsness                | 17                       | 0.7            |
| Irritation from aerosol  | 63                       | 2.5            |
| <u>Respiratory</u>       |                          |                |
| Cough                    | 86                       | 3.5            |
| Dryness of Secretions    | 25                       | 1.0            |
| Exacerbation of symptoms | 66                       | 2.6            |

Highlights of Table one

|                          | <u>Adverse reactions</u> |                |
|--------------------------|--------------------------|----------------|
|                          | <u>Numbers</u>           | <u>Percent</u> |
| <u>Cardiovascular</u>    |                          |                |
| Palpitations             | 22                       | 0.9            |
| Tachycardia              | 14                       | 0.5            |
| <u>CNS</u>               |                          |                |
| Dizziness                | 29                       | 1.1            |
| Fatigue                  | 13                       | 0.5            |
| Headache                 | 46                       | 1.8            |
| Insomnia                 | 13                       | 0.5            |
| Nervousness              | 26                       | 1.0            |
| <u>Dermatological</u>    |                          |                |
| Rash                     | 13                       | 0.5            |
| <u>Gastrointestinal</u>  |                          |                |
| G. I. Distress           | 24                       | 0.9            |
| Nausea                   | 35                       | 1.4            |
| <u>G. U.</u>             |                          |                |
| Urinary difficulty       | 13                       | 0.5            |
| <u>Musculo/skeletal</u>  |                          |                |
| Tremor                   | 10                       | 0.4            |
| <u>Ophthalmological</u>  |                          |                |
| Blurred vision           | 22                       | 0.9            |
| Other ophthalmological   | 15                       | 0.6            |
| <u>Oro-otolaryngeal</u>  |                          |                |
| Dryness                  | 126                      | 4.9            |
| Hoarsness                | 17                       | 0.7            |
| Irritation from aerosol  | 63                       | 2.5            |
| <u>Respiratory</u>       |                          |                |
| Cough                    | 86                       | 3.5            |
| Dryness of Secretions    | 25                       | 1.0            |
| Exacerbation of symptoms | 66                       | 2.6            |

TABLE 1  
All Adverse Experiences Reported in Clinical  
Trials with ATROVENT® Inhaler<sup>a</sup>

|                                  | <u>Number of Patients (%)</u> |
|----------------------------------|-------------------------------|
| Total number of patients treated | 2,555                         |
| <u>Adverse Experience</u>        |                               |
| <b>CARDIOVASCULAR</b>            |                               |
| Arrhythmias                      | 9 (0.4)                       |
| Cardiomyopathy                   | 1 (0.04)                      |
| Cerebrovascular accident         | 2 (0.1)                       |
| Chest pain                       | 3 (0.1)                       |
| Congestive heart failure         | 1 (0.04)                      |
| Lightheaded (not dizzy)          | 1 (0.04)                      |
| Myocardial infarction            | 4 (0.2)                       |
| Palpitations                     | 22 (0.9) ✓                    |
| Syncope                          | 6 (0.2)                       |
| Tachycardia                      | 14 (0.5) ✓                    |
| Thrombosis                       | 1 (0.04)                      |
| Increased blood pressure         | 1 (0.04)                      |
|                                  | <u>65</u>                     |
| <b>CENTRAL NERVOUS SYSTEM</b>    |                               |
| Decreased mental alertness       | 6 (0.2)                       |
| Dizziness                        | 29 (1.1) ✓                    |
| Drowsiness                       | 3 (0.1)                       |
| Fatigue                          | 13 (0.5) ✓                    |
| Headache                         | 46 (1.8) ✓                    |
| Insomnia                         | 13 (0.5) ✓                    |
| Myasthenia gravis                | 1 (0.04)                      |
| Nervousness                      | 26 (1.0) ✓                    |
| Paresthesia                      | 5 (0.2)                       |
| Psychiatric reaction             | 1 (0.04)                      |
| Seizures                         | 1 (0.04)                      |
| Tinnitus                         | 3 (0.1)                       |
| Weakness                         | 5 (0.2)                       |
| Coordination difficulties        | 2 (0.1)                       |
| Somnolence                       | 1 (0.04)                      |
|                                  | <u>156</u>                    |

NOTE ✓  
Reactions

<sup>a</sup>Source Data: ATROVENT® Emergency Program as of 9/14/86  
ATROVENT® NDA 7/29/83  
NDA updates 3/14/85  
Studies performed since NDA as of 11/13/86

**TABLE 1**  
**All Adverse Experiences Reported in Clinical**  
**Trials with ATROVENT® Inhaler<sup>a</sup>**

|                             | <u>Number of Patients (%)</u> |
|-----------------------------|-------------------------------|
| <b>DERMATOLOGICAL</b>       |                               |
| Alopecia                    | 2 (0.1)                       |
| Diaphoresis                 | 2 (0.1)                       |
| Dry skin                    | 2 (0.1)                       |
| Flushing                    | 2 (0.1)                       |
| Hives                       | 2 (0.1)                       |
| Itching                     | 5 (0.2)                       |
| Purpura                     | 1 (0.04)                      |
| Rash                        | 13 (0.5) ✓                    |
|                             | <u>27</u>                     |
| <b>ENDOCRINE DISORDERS</b>  |                               |
| Adrenal insufficiency       | 1 (0.04)                      |
|                             | <u>1</u>                      |
| <b>GASTROINTESTINAL</b>     |                               |
| Appetite changes            | 4 (0.2)                       |
| Constipation                | 8 (0.3)                       |
| Diarrhea                    | 7 (0.3)                       |
| Gastronenteritis            | 1 (0.1)                       |
| Gastrointestinal distress   | 24 (0.9) ✓                    |
| Gastrointestinal hemorrhage | 1 (0.1)                       |
| Nausea                      | 35 (1.4) ✓                    |
| Pancreatitis                | 1 (0.1)                       |
| Peptic ulcer disease        | 1 (0.04)                      |
| Vomiting                    | 6 (0.2)                       |
|                             | <u>88</u>                     |
| <b>GENITOURINARY</b>        |                               |
| Impotence                   | 2 (0.1)                       |
| Loss of sex drive           | 1 (0.04)                      |
| Prispism                    | 2 (0.1)                       |
| Renal Calculus              | 1 (0.04)                      |
| Urinary difficulty          | 13 (0.5) ✓                    |
| Urinary frequency           | 4 (0.2)                       |
|                             | <u>23</u>                     |

<sup>a</sup>Source Data: ATROVENT® Emergency Program as of 9/14/86  
 ATROVENT® NDA 7/29/83  
 NDA updates 3/14/85  
 Studies performed since NDA as of 11/13/86

**TABLE 1**  
**All Adverse Experiences Reported in Clinical**  
**Trials with ATROVENT® Inhaler\***

|                                            | <u>Number of Patients (%)</u> |
|--------------------------------------------|-------------------------------|
| <b>METABOLIC AND NUTRITIONAL DISORDERS</b> |                               |
| Dehydration                                | 1 (0.04)                      |
| Weight gain                                | 1 (0.04)                      |
| Weight loss                                | 2 (0.1)                       |
|                                            | <u>4</u>                      |
| <b>MUSCULO-SKELETAL</b>                    |                               |
| Pain in fingers                            | 1 (0.04)                      |
| Bone disorder                              | 1 (0.04)                      |
| Gout                                       | 2 (0.1)                       |
| Muscle pain                                | 5 (0.2)                       |
| Muscle spasm                               | 5 (0.2)                       |
| Tremor                                     | 10 (0.4) ✓                    |
| Arthralgia                                 | 1 (0.04)                      |
|                                            | <u>25</u>                     |
| <b>OPHTHALMOLOGICAL</b>                    |                               |
| Blurred vision                             | 22 (0.9) ✓                    |
| Conjunctivitis                             | 2 (0.1)                       |
| Difficulty of accommodation                | 3 (0.1)                       |
| Other - Ophthalmological                   | 15 (0.6) ✓                    |
| Decreased visual acuity                    | 3 (0.1)                       |
|                                            | <u>45</u>                     |
| <b>OEO-OTOLARYNGEAL</b>                    |                               |
| Change of voice                            | 2 (0.1)                       |
| Discoloration of teeth                     | 1 (0.04)                      |
| Dryness                                    | 126 (4.9) ✓                   |
| Epistaxis                                  | 2 (0.1)                       |
| Halitosis                                  | 1 (0.04)                      |
| Hoarseness                                 | 17 (0.7) ✓                    |
| Irritation from aerosol                    | 63 (2.5) ✓                    |
| Loss of taste                              | 1 (0.04)                      |
| Nasal mucosal ulcers                       | 2 (0.1)                       |
| Sinusitis                                  | 1 (0.04)                      |
| Swelling in posterior pharynx              | 1 (0.04)                      |
|                                            | <u>217</u>                    |

\*Source Data: ATROVENT® Emergency Program as of 9/14/86  
 ATROVENT® NDA 7/29/83  
 NDA updates 3/14/85  
 Studies performed since NDA as of 11/13/86

TABLE 1  
All Adverse Experience Reported in Clinical  
Trials with ATROVENT® Inhaler<sup>a</sup>

|                                          | <u>Number of Patients (%)</u> |
|------------------------------------------|-------------------------------|
| <b>RESPIRATORY</b>                       |                               |
| Acute Bronchitis                         | 1 (0.04)                      |
| Congestion                               | 1 (0.04)                      |
| Cough                                    | 86 (3.4) ✓✓                   |
| Drying of secretions                     | 25 (1.0) ✓                    |
| Exacerbation of symptoms                 | 66 (2.6) ✓                    |
| Hemoptysis                               | 4 (0.2)                       |
| Other - Respiratory                      | 1 (0.04)                      |
| Plaurisy                                 | 2 (0.1)                       |
| Pneumonia                                | 2 (0.1)                       |
| Respiratory failure                      | 5 (0.2)                       |
| Rhinitis                                 | 2 (0.1)                       |
|                                          | <u>195</u>                    |
| <b>VASCULAR (EXTRACARDIAC) DISORDERS</b> |                               |
| Gangrene                                 | 1 (0.04)                      |
| <b>OTHER</b>                             |                               |
| Burning sensation-head and face          | 1 (0.04)                      |
| Carcinoma                                | 5 (0.2)                       |
| Chest pain                               | 1 (0.04)                      |
| Cold intolerance                         | 1 (0.04)                      |
| Fever                                    | 3 (0.1)                       |
|                                          | <u>11</u>                     |

<sup>a</sup>Source Data: ATROVENT® Emergency Program as fo 9/14/86  
ATROVENT® NDA 7/29/83  
NDA updates 3/14/85  
Studies performed since NDA as of 11/13/86

TABLE 2  
All Adverse Experiences Reported in Clinical Trials with  
ATROVENT® Inhaler Except for the 90-day Studies\*

|                                  | <u>Number of Patients (%)</u> |
|----------------------------------|-------------------------------|
| Total number of patients treated | 2,301                         |
| <u>Adverse Experience</u>        |                               |
| <b>CARDIOVASCULAR</b>            |                               |
| Arrhythmias                      | 10 (0.4) ✓                    |
| Cardiomyopathy                   | 1 (0.04)                      |
| Cerebrovascular accident         | 2 (0.1)                       |
| Chest pain                       | 3 (0.1)                       |
| Congestive heart failure         | 1 (0.04)                      |
| Lightheaded (not dizzy)          | 1 (0.04)                      |
| Myocardial infarction            | 4 (0.2)                       |
| Palpitations                     | 18 (0.8) ✓                    |
| Syncope                          | 6 (0.3)                       |
| Tachycardia                      | 14 (0.6) ✓                    |
| Thrombosis                       | 1 (0.04)                      |
|                                  | <u>61</u>                     |
| <b>CENTRAL NERVOUS SYSTEM</b>    |                               |
| Decreased mental alertness       | 6 (0.3)                       |
| Dizziness                        | 23 (1.0) ✓                    |
| Drowsiness                       | 3 (0.1)                       |
| Fatigue                          | 12 (0.5) ✓                    |
| Headache                         | 40 (1.7) ✓                    |
| Insomnia                         | 12 (0.5) ✓                    |
| Myasthenia gravis                | 1 (0.04)                      |
| Nervousness                      | 18 (0.8) ✓                    |
| Paresthesia                      | 5 (0.2)                       |
| Psychiatric reaction             | 1 (0.04)                      |
| Seizures                         | 1 (0.04)                      |
| Tinnitus                         | 3 (0.1)                       |
| Weakness                         | 5 (0.2)                       |
| Coordination difficulties        | 1 (0.04)                      |
| Somnolence                       | 1 (0.04)                      |
|                                  | <u>132</u>                    |

\*Source Data: ATROVENT® NDA 7/29/83 (with the exception of 90-day studies)  
Emergency ATROVENT® Program as of 9/14/86  
NDA updates 3/14/85 (excluding 90-day studies)  
Studies performed since NDA as of 11/13/86

TABLE 2  
All Adverse Experiences Reported in Clinical Trials with  
ATROVENT® Inhaler Except for the 90-day Studies\*

|                             | <u>Number of Patients (%)</u> |
|-----------------------------|-------------------------------|
| <b>DERMATOLOGICAL</b>       |                               |
| Alopecia                    | 2 (0.1)                       |
| Diaphoresis                 | 2 (0.1)                       |
| Dry skin                    | 2 (0.1)                       |
| Flushing                    | 2 (0.1)                       |
| Hives                       | 2 (0.1)                       |
| Itching                     | 5 (0.2)                       |
| Purpura                     | 1 (0.04)                      |
| Rash                        | 10 (0.4) ✓                    |
|                             | <u>26</u>                     |
| <b>ENDOCRINE DISORDERS</b>  |                               |
| Adrenal insufficiency       | 1 (0.04)                      |
|                             | <u>1</u>                      |
| <b>GASTROINTESTINAL</b>     |                               |
| Appetite changes            | 4 (0.2)                       |
| Constipation                | 7 (0.3)                       |
| Diarrhea                    | 6 (0.3)                       |
| Gastroenteritis             | 1 (0.04)                      |
| Gastrointestinal distress   | 18 (0.8) ✓                    |
| Gastrointestinal hemorrhage | 1 (0.04)                      |
| Nausea                      | 28 (1.2) ✓                    |
| Pancreatitis                | 1 (0.04)                      |
| Peptic ulcer disease        | 1 (0.04)                      |
| Vomiting                    | 6 (0.3)                       |
|                             | <u>73</u>                     |
| <b>GENITOURINARY</b>        |                               |
| Impotence                   | 2 (0.1)                       |
| Loss of sex drive           | 1 (0.04)                      |
| Priapism                    | 2 (0.1)                       |
| Renal Calculus              | 1 (0.04)                      |
| Urinary difficulty          | 12 (0.5) ✓                    |
| Urinary frequency           | 4 (0.2)                       |
|                             | <u>22</u>                     |

\*Source Data: ATROVENT® NDA 7/29/83 (with the exception of 90-day studies)  
Emergency ATROVENT® Program as of 9/14/86  
NDA updates 3/14/85 (excluding 90-day studies)  
Studies performed since NDA as of 11/13/86

TABLE 2  
All Adverse Experiences Reported in Clinical Trials with  
ATROVENT® Inhaler Except for the 90-day Studies<sup>a</sup>

|                                            | <u>Number of Patients (%)</u> |
|--------------------------------------------|-------------------------------|
| <b>METABOLIC AND NUTRITIONAL DISORDERS</b> |                               |
| Dehydration                                | 1 (0.04)                      |
| Weight gain                                | 1 (0.04)                      |
| Weight loss                                | 2 (0.1)                       |
|                                            | <u>4</u>                      |
| <b>MUSCULO-SKELETAL</b>                    |                               |
| Pain in fingers                            | 1 (0.04)                      |
| Bone disorder                              | 1 (0.04)                      |
| Gout                                       | 2 (0.1)                       |
| Muscle pain                                | 5 (0.2)                       |
| Muscle spasm                               | 5 (0.2)                       |
| Tremor                                     | 10 (0.4) ✓                    |
| Arthralgia                                 | 1 (0.04)                      |
|                                            | <u>25</u>                     |
| <b>OPHTHALMOLOGICAL</b>                    |                               |
| Blurred vision                             | 22 (1.0) ✓                    |
| Conjunctivitis                             | 2 (0.1)                       |
| Difficulty of accommodation                | 3 (0.1)                       |
| Other - Ophthalmological                   | 14 (0.6)                      |
|                                            | <u>41</u>                     |
| <b>ORO-OTOLARYNGEAL</b>                    |                               |
| Change of voice                            | 2 (0.1)                       |
| Discoloration of teeth                     | 1 (0.04)                      |
| Dryness                                    | 120 (5.2) ✓                   |
| Epistaxis                                  | 2 (0.1)                       |
| Halitosis                                  | 1 (0.04)                      |
| Hoarseness                                 | 16 (0.7) ✓                    |
| Irritation from aerosol                    | 59 (2.6) ✓                    |
| Loss of taste                              | 1 (0.04)                      |
| Nasal mucosal ulcers                       | 2 (0.1)                       |
| Sinusitis                                  | 1 (0.04)                      |
| Swelling in posterior pharynx              | 1 (0.04)                      |
|                                            | <u>206</u>                    |

<sup>a</sup>Source Data: ATROVENT® NDA 7/29/83 (with the exception of 90-day studies)  
Emergency ATROVENT® Program as of 9/14/86  
NDA updates 3/14/85 (excluding 90-day studies)  
Studies performed since NDA as of 11/13/86

**TABLE 2**  
**All Adverse Experiences Reported in Clinical Trials with**  
**ATROVENT® Inhaler Except for the 90-day Studies\***

|                                          | <u>Number of Patients (%)</u> |
|------------------------------------------|-------------------------------|
| <b>RESPIRATORY</b>                       |                               |
| Acute Bronchitis                         | 1 (0.04)                      |
| Congestion                               | 1 (0.04)                      |
| Cough                                    | 71 (3.1) ✓                    |
| Drying of secretions                     | 25 (1.1) ✓                    |
| Exacerbation of symptoms                 | 60 (2.6) ✓                    |
| Hemoptysis                               | 4 (0.2)                       |
| Other - Respiratory                      | 1 (0.04)                      |
| Pleurisy                                 | 1 (0.04)                      |
| Pneumonia                                | 2 (0.1)                       |
| Respiratory failure                      | 5 (0.2)                       |
| Rhinitis                                 | 2 (0.1)                       |
|                                          | <u>173</u>                    |
| <b>VASCULAR (EXTRACARDIAC) DISORDERS</b> |                               |
| Gangrene                                 | 1 (0.04)                      |
| <b>OTHER</b>                             |                               |
| Burning sensation-head and face          | 1 (0.04)                      |
| Carcinoma                                | 5 (0.2)                       |
| Chest pain                               | 1 (0.04)                      |
| Cold intolerance                         | 1 (0.04)                      |
| Fever                                    | 3 (0.1)                       |
|                                          | <u>11</u>                     |

\*Source Data: ATROVENT® NDA 7/29/83 (with the exception of 90-day studies)  
 Emergency ATROVENT® Program as of 9/14/86  
 NDA updates 3/14/85 (excluding 90-day studies)  
 Studies performed since NDA as of 11/13/86

Appendix I  
Adverse Experiences During Emergency Treatment with Atrovent MDI  
Highlights

Cut off date September 15, 1986; 1,924 patients

|                          | <u>Adverse reactions</u> |                |
|--------------------------|--------------------------|----------------|
|                          | <u>Numbers</u>           | <u>Percent</u> |
| <u>Cardiovascular</u>    |                          |                |
| Palpitations             | 18                       | 0.9            |
| Tachycardia              | 11                       | 0.6            |
| <u>CNS</u>               |                          |                |
| Dizziness                | 20                       | 1.0            |
| Fatigue                  | 10                       | 0.5            |
| Headache                 | 30                       | 1.6            |
| Insomnia                 | 12                       | 0.6            |
| Nervousness              | 18                       | 0.9            |
| <u>Dermatological</u>    |                          |                |
| Rash                     | 9                        | 0.5            |
| <u>Gastrointestinal</u>  |                          |                |
| G. I. Distress           | 16                       | 0.8            |
| Nausea                   | 24                       | 1.2            |
| <u>G. U.</u>             |                          |                |
| Urinary difficulty       | 12                       | 0.6            |
| <u>Ophthalmological</u>  |                          |                |
| Blurred vision           | 22                       | 1.1            |
| Other eye                | 11                       | 0.6            |
| <u>Otolaryngeal/oro</u>  |                          |                |
| Dryness                  | 113                      | 5.9            |
| Hoarsness                | 16                       | 0.8            |
| Irritation from aerosol  | 56                       | 2.9            |
| <u>Respiratory</u>       |                          |                |
| Cough                    | 68                       | 3.5            |
| Dryness of Secretions    | 25                       | 1.3            |
| Exacerbation of symptoms | 52                       | 2.7            |

EMERGENCY ATROVENTO  
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APPENDIX 1  
Adverse Events During Emergency Treatment  
With ATROVENTO MDI

|                                  | Cutoff Date:<br>September 15, 1986 | Cutoff Date:<br>December 20, 1985 |
|----------------------------------|------------------------------------|-----------------------------------|
|                                  | Number of Patients %               | Number of Patients %              |
| Total number of patients treated | 1,924                              | 1,686                             |
| <b>CARDIOVASCULAR</b>            |                                    |                                   |
| Arrhythmias                      | 8 (0.4)                            | 4 (0.2)                           |
| Cardiomyopathy                   | 1 (0.05)                           | 1 (0.06)                          |
| Cerebrovascular accident         | 2 (0.1)                            | 2 (0.1)                           |
| Chest pain                       | 3 (0.2)                            | 3 (0.2)                           |
| Congestive heart failure         | 1 (0.05)                           | 0 (0.0)                           |
| Lightheaded (not dizzy)          | 1 (0.05)                           | 1 (0.06)                          |
| Myocardial infarction            | 4 (0.2)                            | 2 (0.1)                           |
| Palpitations                     | 18 (0.9)                           | 18 (1.1)                          |
| Syncope                          | 6 (0.2)                            | 5 (0.3)                           |
| Tachycardia                      | 11 (0.6)                           | 7 (0.5)                           |
| Thrombosis                       | 1 (0.05)                           | 1 (0.06)                          |
|                                  | <u>46</u>                          | <u>46</u>                         |
| <b>CENTRAL NERVOUS SYSTEM</b>    |                                    |                                   |
| Decreased mental alertness       | 6 (0.3)                            | 4 (0.2)                           |
| Dizziness                        | 20 (1.0)                           | 18 (1.1)                          |
| Drowsiness                       | 3 (0.2)                            | 2 (0.1)                           |
| Fatigue                          | 10 (0.5)                           | 8 (0.5)                           |
| Headache                         | 30 (1.6)                           | 27 (1.6)                          |
| Insomnia                         | 12 (0.6)                           | 9 (0.5)                           |
| Myasthenia gravis                | 1 (0.05)                           | 0 (0.0)                           |
| Nervousness                      | 18 (0.9)                           | 16 (0.9)                          |
| Paresthesia                      | 5 (0.2)                            | 5 (0.3)                           |
| Psychiatric reaction             | 1 (0.05)                           | 0 (0.0)                           |
| Seizures                         | 1 (0.05)                           | 1 (0.06)                          |
| Tinnitus                         | 3 (0.2)                            | 3 (0.2)                           |
| Weakness                         | 5 (0.2)                            | 3 (0.2)                           |
|                                  | <u>115</u>                         | <u>97</u>                         |

**APPENDIX 1**  
Adverse Events During Emergency Treatment  
With ATROVENTO® MDI

|                             | Cutoff Date:<br>September 15, 1986 | Cutoff Date:<br>December 20, 1985 |
|-----------------------------|------------------------------------|-----------------------------------|
|                             | Number of Patients %               | Number of Patients %              |
| <b>DERMATOLOGICAL</b>       |                                    |                                   |
| Alopecia                    | 2 (0.1)                            | 2 (0.1)                           |
| Diaphoresis                 | 2 (0.1)                            | 2 (0.1)                           |
| Dry skin                    | 2 (0.1)                            | 2 (0.1)                           |
| Flushing                    | 2 (0.1)                            | 2 (0.1)                           |
| Hives                       | 1 (0.05)                           | 1 (0.06)                          |
| Itching                     | 4 (0.2)                            | 4 (0.2)                           |
| Purpura                     | 1 (0.05)                           | 1 (0.06)                          |
| Rash                        | 9 (0.5) ✓                          | 7 (0.4)                           |
|                             | <u>25</u>                          | <u>21</u>                         |
| <b>ENDOCRINE DISORDERS</b>  |                                    |                                   |
| Adrenal insufficiency       | 1 (0.05)                           |                                   |
| <b>GASTROINTESTINAL</b>     |                                    |                                   |
| Appetite changes            | 4 (0.2)                            | 4 (0.2)                           |
| Constipation                | 5 (0.2)                            | 5 (0.3)                           |
| Diarrhea                    | 5 (0.2)                            | 5 (0.3)                           |
| Gastroenteritis             | 1 (0.05)                           | 0 (0.0)                           |
| Gastrointestinal distress   | 16 (0.8) ✓                         | 12 (0.7)                          |
| Gastrointestinal hemorrhage | 1 (0.05)                           | 0 (0.0)                           |
| Nausea                      | 24 (1.2) ✓                         | 23 (1.4)                          |
| Pancreatitis                | 1 (0.05)                           | 1 (0.06)                          |
| Peptic ulcer disease        | 1 (0.05)                           | 0 (0.0)                           |
| Vomiting                    | 5 (0.3)                            | 5 (0.3)                           |
|                             | <u>63</u>                          | <u>55</u>                         |
| <b>GENITOURINARY</b>        |                                    |                                   |
| Impotence                   | 2 (0.1)                            | 2 (0.1)                           |
| Loss of sex drive           | 1 (0.05)                           | 1 (0.06)                          |
| Priapism                    | 2 (0.1)                            | 2 (0.1)                           |
| Renal Calculus              | 1 (0.05)                           | 0 (0.0)                           |
| Urinary difficulty          | 12 (0.6) ✓                         | 8 (0.5)                           |
| Urinary frequency           | 4 (0.2)                            | 3 (0.2)                           |
|                             | <u>27</u>                          | <u>16</u>                         |

APPENDIX 1  
Adverse Events During Emergency Treatment  
With ATROVENTO MDI

|                                            | Cutoff Date:<br>September 15, 1986 | Cutoff Date:<br>December 20, 1985 |
|--------------------------------------------|------------------------------------|-----------------------------------|
|                                            | Number of Patients %               | Number of Patients %              |
| <b>METABOLIC AND NUTRITIONAL DISORDERS</b> |                                    |                                   |
| Dehydration                                | 1 (0.05)                           | 1 (0.06)                          |
| Weight gain                                | 1 (0.05)                           | 1 (0.06)                          |
| Weight loss                                | 2 (0.1)                            | 1 (0.06)                          |
|                                            | <u>4</u>                           | <u>3</u>                          |
| <b>MUSCULO-SKELETAL</b>                    |                                    |                                   |
| Cout                                       | 2 (0.1)                            | 2 (0.1)                           |
| Muscle pain                                | 4 (0.2)                            | 3 (0.2)                           |
| Muscle spasm                               | 5 (0.2)                            | 4 (0.2)                           |
| Tremor                                     | 6 (0.3)                            | 5 (0.3)                           |
|                                            | <u>17</u>                          | <u>14</u>                         |
| <b>OPHTHALMOLOGICAL</b>                    |                                    |                                   |
| Blurred vision                             | 22 (1.1) ✓                         | 20 (1.2)                          |
| Conjunctivitis                             | 2 (0.05)                           | 2 (0.1)                           |
| Difficulty of accomodation                 | 2 (0.05)                           | 2 (0.1)                           |
| Other - Ophthalmological                   | 11 (0.6) ✓                         | 10 (0.6)                          |
|                                            | <u>47</u>                          | <u>34</u>                         |
| <b>ORO-OTOLARYNGEAL</b>                    |                                    |                                   |
| Change of voice                            | 2 (0.1)                            | 2 (0.1)                           |
| Discoloration of teeth                     | 1 (0.05)                           | 1 (0.06)                          |
| Dryness                                    | 113 (5.9) ✓                        | 101 (6.0)                         |
| Epistaxis                                  | 2 (0.1)                            | 2 (0.1)                           |
| Halitosis                                  | 1 (0.05)                           | 1 (0.06)                          |
| Hoarseness                                 | 16 (0.8) ✓                         | 16 (0.9)                          |
| Irritation from Aerosol                    | 56 (2.9) ✓                         | 47 (2.8)                          |
| Loss of taste                              | 1 (0.05)                           | 1 (0.06)                          |
| Nasal mucosal ulcers                       | 2 (0.1)                            | 1 (0.06)                          |
| Swelling in posterior pharynx              | 1 (0.05)                           | 1 (0.06)                          |
|                                            | <u>195</u>                         | <u>123</u>                        |

APPENDIX 1  
Adverse Events During Emergency Treatment  
With ATROVENTO MDI

|                                          | Cutoff Date:<br><u>September 15, 1986</u> | Cutoff Date:<br><u>December 20, 1985</u> |
|------------------------------------------|-------------------------------------------|------------------------------------------|
|                                          | Number of Patients    %                   | Number of Patients    %                  |
| <b>RESPIRATORY</b>                       |                                           |                                          |
| Cough                                    | 68 (3.5) //                               | 60 (3.6)                                 |
| Drying of secretions                     | 25 (1.3) //                               | 22 (1.3)                                 |
| Exacerbation of symptoms                 | 52 (2.7)                                  | 46 (2.7)                                 |
| Hemoptysis                               | 4 (0.2)                                   | 4 (0.2)                                  |
| Other - Respiratory                      | 1 (0.05)                                  | 1 (0.06)                                 |
| Pleurisy                                 | 1 (0.05)                                  | 1 (0.06)                                 |
| Pneumonia                                | 2 (0.1)                                   | 1 (0.06)                                 |
| Respiratory failure                      | 5 (0.2)                                   | 3 (0.02)                                 |
| Rhinitis                                 | 2 (0.1)                                   | 2 (0.1)                                  |
|                                          | <u>160</u>                                | <u>150</u>                               |
| <b>VASCULAR (EXTRACARDIAC) DISORDERS</b> |                                           |                                          |
| Gangrene                                 | 1 (0.05)                                  | 0 (0.0)                                  |
|                                          | <u>1</u>                                  | <u>0</u>                                 |
| <b>OTHER</b>                             |                                           |                                          |
| Burning sensation-head and face          | 1 (0.05)                                  | 1 (0.06)                                 |
| Carcinoma                                | 5 (0.2)                                   | 2 (0.1)                                  |
| Chest pain                               | 1 (0.05)                                  | 0 (0.0)                                  |
| Cold intolerance                         | 1 (0.05)                                  | 1 (0.06)                                 |
| Fever                                    | 3 (0.2)                                   | 2 (0.1)                                  |
|                                          | <u>11</u>                                 | <u>6</u>                                 |

**APPENDIX 2**  
**Overall Incidence of Adverse Experiences Among 631 Patients Treated**  
**With Atrovent® in Double-blind, Controlled Clinical Trials**  
**Within the Recommended Dose<sup>a</sup>**

|                                  | <u>NUMBER OF PATIENTS (%)</u> |
|----------------------------------|-------------------------------|
| Total number of patients treated | 631                           |
| <u>Adverse Experience</u>        |                               |
| <u>Cardiovascular</u>            |                               |
| Palpitations                     | 4 (0.6) ✓                     |
| Tachycardia                      | 3 (0.5)                       |
| Arrhythmia                       | 2 (0.3)                       |
| Increased blood pressure         | 1 (0.2)                       |
|                                  | 10                            |
| <u>Central Nervous System</u>    |                               |
| Headache                         | 16 (2.5) ✓                    |
| Dizziness                        | 9 (1.4) ✓                     |
| Nervousness                      | 8 (1.3) ✓                     |
| Fatigue                          | 3 (0.5)                       |
| Coordination difficulties        | 2 (0.3)                       |
| Weakness                         | 1 (0.2)                       |
| Insomnia                         | 1 (0.2)                       |
| Somnolence                       | 1 (0.2)                       |
|                                  | 41                            |
| <u>Dermatological</u>            |                               |
| Rash                             | 4 (0.6) ✓                     |
| Itching                          | 1 (0.2)                       |
| Hives                            | 1 (0.2)                       |
|                                  | 6                             |
| <u>Gastrointestinal</u>          |                               |
| Nausea                           | 11 (1.7) ✓                    |
| Gastrointestinal distress        | 8 (1.3) ✓                     |
| Constipation                     | 3 (0.5)                       |
| Diarrhea                         | 2 (0.3)                       |
| Vomiting                         | 1 (0.2)                       |
|                                  | 25                            |
| <u>Genitourinary</u>             |                               |
| Urinary difficulty               | 1 (0.2)                       |

<sup>a</sup>Source Data: Atrovent NDA 7-29-83  
 Update of 90-day studies 3-14-85  
 Update of continuing studies at time of NDA other  
 than 90-day 3-14-85  
 Clinical trials conducted since NDA as of 11-13-86

**APPENDIX 2**  
**Overall Incidence of Adverse Experiences Among 631 Patients Treated**  
**With Atrovent® in Double-blind, Controlled Clinical Trials**  
**Within the Recommended Dose\***

Musculoskeletal

|                 |         |
|-----------------|---------|
| Tremor          | 4 (0.6) |
| Arthralgia      | 1 (0.2) |
| Bone disorder   | 1 (0.2) |
| Muscle pain     | 1 (0.2) |
| Pain in fingers | 1 (0.2) |
|                 | <hr/>   |

6

Ophthalmological

|                               |         |
|-------------------------------|---------|
| Decreased visual acuity       | 3 (0.5) |
| Other ophthalmological        | 4 (0.6) |
| Difficulties of accommodation | 1 (0.2) |
|                               | <hr/>   |

8

Oro-otolaryngeal

|                         |          |
|-------------------------|----------|
| Dry mouth               | 13 (2.1) |
| Irritation from aerosol | 7 (1.1)  |
| Hoarseness              | 1 (0.2)  |
|                         | <hr/>    |

21

Respiratory

|                          |          |
|--------------------------|----------|
| Cough                    | 18 (2.8) |
| Exacerbation of symptoms | 14 (2.2) |
| Bronchitis               | 1 (0.2)  |
| Pleurisy                 | 1 (0.2)  |
| Sinusitis                | 1 (0.2)  |
| Congestion               | 1 (0.2)  |
|                          | <hr/>    |

36

\*Source data: Atrovent NDA 7-29-83  
 Update of 90-day studies 3-14-85  
 Update of continuing studies at time of NDA other  
 than 90-day 3-14-85  
 Clinical trials conducted since NDA as of 11-13-86

**APPENDIX 2**  
**Overall Incidence of Adverse Experiences Among 631 Patients Treated**  
**With Atrovent® in Double-blind, Controlled Clinical Trials**  
**Within the Recommended Dose\***

Musculoskeletal

|                 |         |
|-----------------|---------|
| Tremor          | 4 (0.6) |
| Arthralgia      | 1 (0.2) |
| Bone disorder   | 1 (0.2) |
| Muscle pain     | 1 (0.2) |
| Pain in fingers | 1 (0.2) |
|                 | 8       |

Ophthalmological

|                               |         |
|-------------------------------|---------|
| Decreased visual acuity       | 3 (0.5) |
| Other ophthalmological        | 4 (0.6) |
| Difficulties of accommodation | 1 (0.2) |
|                               | 8       |

Oro-otolaryngial

|                         |          |
|-------------------------|----------|
| Dry mouth               | 13 (2.1) |
| Irritation from aerosol | 7 (1.1)  |
| Hoarseness              | 1 (0.2)  |
|                         | 21       |

Respiratory

|                          |          |
|--------------------------|----------|
| Cough                    | 18 (2.8) |
| Exacerbation of symptoms | 14 (2.2) |
| Bronchitis               | 1 (0.2)  |
| Pleurisy                 | 1 (0.2)  |
| Sinusitis                | 1 (0.2)  |
| Congestion               | 1 (0.2)  |
|                          | 36       |

\*Source data: Atrovent NDA 7-29-83  
 Update of 90-day studies 3-14-85  
 Update of continuing studies at time of NDA other  
 than 90-day 3-14-85  
 Clinical trials conducted since NDA as of 11-13-86

Appendix 2  
 Overall incidence of Adverse Reactions among 631 patients  
 Treated with Atrovent in double-blind, controlled clinical trials  
 within the recommended dose

|                         | <u>Highlights</u>                          |                |
|-------------------------|--------------------------------------------|----------------|
|                         | <u>Adverse reactions</u><br><u>Numbers</u> | <u>Percent</u> |
| <u>Cardiovascular</u>   |                                            |                |
| Palpitations            | 4                                          | 0.6            |
| <u>CNS</u>              |                                            |                |
| Headache                | 16                                         | 2.5            |
| Dizziness               | 9                                          | 1.4            |
| Nervousness             | 8                                          | 1.3            |
| <u>Dermatological</u>   |                                            |                |
| Rash                    | 4                                          | 0.6            |
| <u>Gastrointestinal</u> |                                            |                |
| Nausea                  | 11                                         | 1.7            |
| <u>Musculo/skeletal</u> |                                            |                |
| Tremor                  | 4                                          | 0.6            |
| <u>Ophthalmological</u> |                                            |                |
| Decreased visual acuity | 3                                          | 0.5            |
| Other                   | 4                                          | 0.6            |
| <u>Oro-otolaryngeal</u> |                                            |                |
| Dryness                 | 13                                         | 2.1            |
| Aerosol irritation      | 7                                          | 1.1            |
| <u>Respiratory</u>      |                                            |                |
| Cough                   | 18                                         | 2.8            |
| Exacerbation            | 14                                         | 2.2            |

**APPENDIX 3**  
**Incidence of all Adverse Experiences Reported in Double-blind  
 Controlled Clinical Trials with the Exception  
 of the 90-day Studies\***

|                               | <u>Number of Patients (%)</u> |
|-------------------------------|-------------------------------|
| <u>Total Treated</u>          | <u>377</u>                    |
| <u>Adverse Experience</u>     |                               |
| <u>Cardiovascular</u>         |                               |
| Tachycardia                   | 3 (0.8)                       |
| Arrhythmia                    | 2 (0.5)                       |
|                               | <u>5</u>                      |
| <u>Central Nervous System</u> |                               |
| Headache                      | 10 (2.6)✓                     |
| Dizziness                     | 3 (0.8)                       |
| Fatigue                       | 2 (0.5)                       |
| Coordination difficulties     | 1 (0.3)                       |
| Somnolence                    | 1 (0.3)                       |
|                               | <u>17</u>                     |
| <u>Dermatological</u>         |                               |
| Rash                          | 1 (0.3)                       |
| Itching                       | 1 (0.3)                       |
|                               | <u>2</u>                      |
| <u>Gastrointestinal</u>       |                               |
| Nausea                        | 4 (1.1)✓                      |
| Gastrointestinal distress     | 2 (0.5)                       |
| Constipation                  | 2 (0.5)                       |
| Diarrhea                      | 1 (0.3)                       |
| Vomiting                      | 1 (0.3)                       |
|                               | <u>10</u>                     |

\*Source Data: ATROVENT® NDA submitted 7-29-83 (with the exception of the 90 day studies)  
 Update of ongoing NDA studies other than 90-day 3-14-85  
 Clinical trials conducted since NDA as of 11-13-86

APPENDIX 3  
Incidence of all Adverse Experiences Reported in Double-blind  
Controlled Clinical Trials with the Exception  
of the 90-day Studies<sup>a</sup>

|                               |           |
|-------------------------------|-----------|
| <u>Musculoskeletal</u>        |           |
| Tremor                        | 4 (1.1) ✓ |
| Arthralgia                    | 1 (0.3)   |
| Bone disorder                 | 1 (0.3)   |
| Muscle pain                   | 1 (0.3)   |
| Pain in fingers               | 1 (0.3)   |
|                               | 8         |
| <u>Ophthalmological</u>       |           |
| Difficulties of accommodation | 1 (0.3)   |
| Other ophthalmological        | 3 (0.8)   |
|                               | 4         |
| <u>Oro-otolaryngial</u>       |           |
| Dry Mouth                     | 7 (1.8) ✓ |
| Irritation from aerosol       | 3 (0.8)   |
|                               | 10        |
| <u>Respiratory</u>            |           |
| Exacerbation of symptoms      | 8 (2.1) ✓ |
| Cough                         | 3 (0.8)   |
| Bronchitis                    | 1 (0.3)   |
| Sinusitis                     | 1 (0.3)   |
| Conjestion                    | 1 (0.3)   |
|                               | 14        |

<sup>a</sup>Source Data: ATROVENT<sup>®</sup> NDA submitted 7-29-83 (with the exception of the 90-day studies)  
Update of ongoing NDA studies other than 90-day 3-14-85  
Clinical trials conducted since NDA as of 11-13-86

Appendix 3  
Incidence of all adverse reactions reported in double-blind trials  
with the exception of the 90-day studies

|              | <u>Highlights</u> |                |
|--------------|-------------------|----------------|
|              | <u>Numbers</u>    | <u>Percent</u> |
| Headache     | 10                | 2.6            |
| Nausea       | 4                 | 1.1            |
| Tremor       | 4                 | 1.1            |
| Dry Mouth    | 7                 | 1.8            |
| Exacerbation | 8                 | 2.1            |

**APPENDIX 3**  
**Incidence of all Adverse Experiences Reported in Double-blind  
 Controlled Clinical Trials with the Exception  
 of the 90-day Studies<sup>a</sup>**

Musculoskeletal

|                 |           |
|-----------------|-----------|
| Tremor          | 4 (1.1) ✓ |
| Arthralgia      | 1 (0.3)   |
| Bone disorder   | 1 (0.3)   |
| Muscle pain     | 1 (0.3)   |
| Pain in fingers | 1 (0.3)   |
|                 | 8         |

Ophthalmological

|                               |         |
|-------------------------------|---------|
| Difficulties of accommodation | 1 (0.3) |
| Other ophthalmological        | 3 (0.8) |
|                               | 4       |

Oro-otolaryngial

|                         |           |
|-------------------------|-----------|
| Dry Mouth               | 7 (1.8) ✓ |
| Irritation from aerosol | 3 (0.8)   |
|                         | 10        |

Respiratory

|                          |           |
|--------------------------|-----------|
| Exacerbation of symptoms | 8 (2.1) ✓ |
| Cough                    | 3 (0.8)   |
| Bronchitis               | 1 (0.3)   |
| Sinusitis                | 1 (0.3)   |
| Conjestion               | 1 (0.3)   |
|                          | 14        |

<sup>a</sup>Source Data: ATROVENT® NDA submitted 7-29-83 (with the exception of the 90-day studies)  
 Update of ongoing NDA studies other than 90-day 3-14-85  
 Clinical trials conducted since NDA as of 11-13-86

Appendix 3  
 Incidence of all adverse reactions reported in double-blind trials  
 with the exception of the 90-day studies

Highlights

|              | Adverse reactions |         |
|--------------|-------------------|---------|
|              | Numbers           | Percent |
| Headache     | 10                | 2.6     |
| Nausea       | 4                 | 1.1     |
| Tremor       | 4                 | 1.1     |
| Dry Mouth    | 7                 | 1.8     |
| Exacerbation | 8                 | 2.1     |

APPENDIX 4  
Overall Incidence of Adverse Experiences Among  
Patients Treated with ATROVENT® For 90 Days

| <u>Adverse Experience</u>            | <u>Number of Patients (%)</u> |                        |
|--------------------------------------|-------------------------------|------------------------|
|                                      | <u>NDA Submission</u>         | <u>Completed Study</u> |
| <b>TOTAL</b>                         | 228                           | 254                    |
| <b><u>Cardiovascular</u></b>         |                               |                        |
| Increased Blood Pressure             | 0 (0.0)                       | 1 (0.4)                |
| Palpitations                         | 4 (1.8)                       | 4 (1.6)                |
|                                      | <u>4</u>                      | <u>5</u>               |
| <b><u>Central Nervous System</u></b> |                               |                        |
| Coordination Difficulties            | 1 (0.4)                       | 1 (0.4)                |
| Dizziness                            | 6 (2.6)                       | 6 (2.4)                |
| Fatigue                              | 0 (0.0)                       | 1 (0.4)                |
| Headache                             | 6 (2.6)                       | 6 (2.4)                |
| Insomnia                             | 1 (0.4)                       | 1 (0.4)                |
| Nervousness                          | 7 (3.1)                       | 8 (3.1)                |
| Weakness                             | 0 (0.0)                       | 1 (0.4)                |
|                                      | <u>21</u>                     | <u>27</u>              |
| <b><u>Dermatological</u></b>         |                               |                        |
| Hives                                | 0 (0.0)                       | 1 (0.4)                |
| Rash                                 | 3 (1.3)                       | 3 (1.2)                |
|                                      | <u>3</u>                      | <u>4</u>               |
| <b><u>Gastrointestinal</u></b>       |                               |                        |
| Constipation                         | 1 (0.4)                       | 1 (0.4)                |
| Diarrhea                             | 1 (0.4)                       | 1 (0.4)                |
| Gastrointestinal Distress            | 6 (2.6)                       | 6 (2.4)                |
| Nausea                               | 7 (3.1)                       | 7 (2.8)                |
|                                      | <u>15</u>                     | <u>15</u>              |
| <b><u>Genitourinary</u></b>          |                               |                        |
| Urinary Difficulty                   | 1 (0.4)                       | 1 (0.4)                |
| <b><u>Ophthalmological</u></b>       |                               |                        |
| Blurred Vision                       | 1 (0.4)                       | 3 (1.2)                |
| Other Ophthalmological               | 0 (0.0)                       | 1 (0.4)                |
|                                      | <u>1</u>                      | <u>4</u>               |
| <b><u>Oro-otolaryngeal</u></b>       |                               |                        |
| Dryness                              | 4 (1.8)                       | 6 (2.4)                |
| Hoarseness                           | 0 (0.0)                       | 1 (0.4)                |
| Irritation from Aerosol              | 4 (1.8)                       | 4 (1.6)                |
|                                      | <u>8</u>                      | <u>11</u>              |
| <b><u>Respiratory</u></b>            |                               |                        |
| Cough                                | 14 (6.1)                      | 15 (5.9)               |
| Exacerbation of Symptoms             | 6 (2.6)                       | 6 (2.4)                |
| Pleurisy                             | 1 (0.4)                       | 1 (0.4)                |
|                                      | <u>21</u>                     | <u>22</u>              |

Appendix 4  
Overall Incidence of Adverse Reactions  
Among Patients Treated with Atrovent 90-days

Highlights  
Completed study 254 Patients

|                         | <u>Adverse reactions</u> |                |
|-------------------------|--------------------------|----------------|
|                         | <u>Numbers</u>           | <u>Percent</u> |
| <u>Cardiovascular</u>   |                          |                |
| Palpitations            | 4                        | 1.8            |
| <u>CNS</u>              |                          |                |
| Dizziness               | 6                        | 2.4            |
| Headache                | 6                        | 2.4            |
| Nervousness             | 8                        | 3.1            |
| <u>Dermatological</u>   |                          |                |
| Rash                    | 3                        | 1.2            |
| <u>Gastrointestinal</u> |                          |                |
| G. I. Distress          | 6                        | 2.4            |
| Nausea                  | 7                        | 2.8            |
| <u>Ophthalmological</u> |                          |                |
| Blurred vision          | 3                        | 1.2            |
| <u>Oro-otolaryngeal</u> |                          |                |
| Dryness                 | 6                        | 2.4            |
| Aerosol irritation      | 4                        | 1.6            |
| <u>Respiratory</u>      |                          |                |
| Cough                   | 15                       | 5.9            |
| Exacerbation            | 6                        | 2.4            |

Atrovent Emergency Program Update  
Sixth Annual Progress Report  
September 1986

This program originated in December 1979 and through September 15, 1986, the cutoff date for this report, 2169 patients have been enrolled by 474 clinical investigators. The status as of 9/15/86 is:

|                                                                                  | <u>9/15/86</u> | <u>Number of Patients</u> |
|----------------------------------------------------------------------------------|----------------|---------------------------|
| Enrolled                                                                         |                | 2,169                     |
| Never received drug or<br>unable to obtain any informa-<br>tion regarding status |                | 245                       |
| Total Treated                                                                    |                | 1,924                     |
| Active Patients                                                                  |                | 824                       |
| Discontinued Patients                                                            |                | 1100                      |
| Lack of efficacy                                                                 |                | 403                       |
| Death                                                                            |                | 118                       |
| Adverse experience                                                               |                | 140                       |
| Drug no longer required<br>or desired                                            |                | 160                       |
| Lost to follow up                                                                |                | 162                       |
| Other                                                                            |                | 117                       |

\*Adverse experiences:

Tabulated in Table 1 (following) comparison with incidences for the 1985 report shows no significant changes in the incidence or severity of the symptoms from the 5th annual report.

\*Dropouts due to adverse experiences

140 in number from the entire program and 14 since the 5th annual report (Table 2). More detailed information is provided in Appendix I. Respiratory symptoms including exacerbation of symptoms, cough and drying of secretions have accounted for a total of 55 patients. Dryness of the mouth and throat, irritation from aerosol, hoarseness and sore throat have accounted for a total of 37 patients.

Other adverse experiences which led to discontinuation of the drug are:

|                                              | Cutoff date:<br>September 15, 1985 | Cutoff date:<br>December 20, 1985 |
|----------------------------------------------|------------------------------------|-----------------------------------|
|                                              | Number of Patients                 | Number of Patients                |
| Palpitations and tachycardia                 | 5                                  | 5                                 |
| Chest pain                                   | 3                                  | 3                                 |
| Myocardial infarction                        | 1                                  | 1                                 |
| Headache                                     | 4                                  | 4                                 |
| Nervousness                                  | 2                                  | 2                                 |
| Syncope                                      | 1                                  | 1                                 |
| Cerebrovascular Accident                     | 1                                  | 1                                 |
| Other CNS complaints                         | 6 (1)**                            | 5                                 |
| Rash                                         | 3                                  | 3                                 |
| Blurring of Vision                           | 6 (1)**                            | 5                                 |
| Gastrointestinal Complaints                  | 8 (1)**                            | 7                                 |
| Elevated SGOT and SGPT                       | 1                                  | 1                                 |
| Cardiomyopathy and acute respiratory failure | 1                                  | 1                                 |
| Loss of Sex Drive                            | 1                                  | 1                                 |
| Musculo-chondral pain                        | 1                                  | 1                                 |
| Arrhythmia                                   | 1                                  | 1                                 |
| Urinary Difficulty                           | 2                                  | 2                                 |
| Muscle spasm                                 | 1 (1)**                            | 0                                 |
|                                              | <u>48</u>                          | <u>44</u>                         |
|                                              | +4                                 |                                   |

\* Reported in the 5th Annual Report.

\*\* Details of patients who discontinued the program since the 5th Annual Report are discussed in Appendix 2.

This table shows one report of a previously unreported reaction, muscle spasm. Three reported reactions, "other CNS complaints," "blurring of vision," and "gastrointestinal complaints" showed an increase in number of one since the 5th annual report to discontinuation of the drug. This table shows nothing that is cause for action.

Severe or unusual reactions not necessarily related to treatment with Atrovent is described in 16 cases on page 3 through 6 of this report. This are:

|                                    |           |
|------------------------------------|-----------|
| Arrhythmias                        | 4         |
| Carcinoma                          | 5         |
| Cardiomyopathy/respiratory failure | 4         |
| Myocardial infarct                 | 2         |
| Syncop                             | 1         |
| TOTAL                              | <u>16</u> |

### Deaths

As of September 15, 1986 118 deaths have been reported in patients on Atrovent or within 2 weeks of stopping the drug. 38 of these deaths were reported since the 5th annual report. Among these, the age range was 12 to 86 years; of these, 23 were respiratory and the remaining unknown or cancer.

### Clinical Laboratory data

Clinically significant changes from initial lab values are shown in table 4 below. Since the beginning of this program, no consistent pattern of abnormalities has been noted which can be attributed to Atrovent therapy.

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### Clinical Laboratory data

Clinically significant changes from initial lab values are shown in table 4 below. Since the beginning of this program, no consistent pattern of abnormalities has been noted which can be attributed to Atrovent therapy.

TABLE 4  
LABORATORY TESTS IN SUPPORT OF SAFETY IN 2,169 PATIENTS TREATED WITH ATROVENT®

| <u>TEST</u>            | <u>TOTAL TESTED<sup>a</sup></u> | <u>NUMBER OF PATIENTS WITH CLINICALLY SIGNIFICANT CHANGES FROM INITIAL VALUE<sup>+</sup></u> |
|------------------------|---------------------------------|----------------------------------------------------------------------------------------------|
| <u>Hematology</u>      |                                 |                                                                                              |
| Hematocrit             | 1065                            | (4) <sup>+b</sup>                                                                            |
| Hemoglobin             | 1067                            | (3) <sup>c</sup>                                                                             |
| Red Blood Count        | 472                             |                                                                                              |
| White Blood Count      | 1071                            | (4) <sup>d</sup>                                                                             |
| Platelets              | 559                             |                                                                                              |
| <u>Blood Chemistry</u> |                                 |                                                                                              |
| Glucose                | 929                             | (9) <sup>e</sup>                                                                             |
| BUN                    | 826                             | (2) <sup>f</sup>                                                                             |
| Creatinine             | 855                             | (1) <sup>g</sup>                                                                             |
| Uric Acid              | 923                             | (2) <sup>h</sup>                                                                             |
| Total Protein          | 904                             | (2) <sup>i</sup>                                                                             |
| Albumin                | 908                             | (2) <sup>j</sup>                                                                             |
| Globulin               | 419                             |                                                                                              |
| Total Bilirubin        | 903                             |                                                                                              |
| Alkaline Phosphatase   | 941                             | (4) <sup>k</sup>                                                                             |
| SGOT                   | 936                             | (1) <sup>l</sup>                                                                             |
| SGPT                   | 389                             | (1) <sup>m</sup>                                                                             |
| LDH                    | 827                             | (4) <sup>n</sup>                                                                             |
| Gamma GT               | 180                             | (1) <sup>o</sup>                                                                             |
| Calcium                | 942                             |                                                                                              |
| Phosphorus             | 811                             | (6) <sup>p</sup>                                                                             |
| Sodium                 | 809                             | (1) <sup>q</sup>                                                                             |
| Potassium              | 838                             | (1) <sup>r</sup>                                                                             |
| Chloride               | 733                             | (1) <sup>s</sup>                                                                             |
| <u>Urinalysis</u>      | 816                             | (2) <sup>t</sup>                                                                             |

<sup>a</sup> This includes only patients who had more than one laboratory value recorded, and includes data collected to 9/15/86.

\* As determined per physician monitor.

+ The figures in parenthesis represent the total number of patients with clinically significant changes reported since the 5th Annual Report and for the period of 1/3/86-9/15/86. Refer to the following pages for the list of pertinent footnotes.

Table 4 continues with an explanation of the conditions associated with the 51 laboratory abnormalities, usually further information being requested, or concomitant medical conditions such as "malnourished." No direct causal relationship could be established to administration of Atrovent.

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| Calcium                | 942                             |                                                                                   |
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Table 4 continues with an explanation of the conditions associated with the 51 laboratory abnormalities, usually further information being requested, or concomitant medical conditions such as "malnourished." No direct causal relationship could be established to administration of Atrovent.

Discontinued patients:

This section is in tabular form listing the investigator (study number), total enrolled in NDA both as of April 5, 1984 and total number of patients enrolled as of 10/21/82 (as given in the original NDA). The total enrolled as of November 13, 1986 is given as 2627 patients, and the number in the NDA is given as 1801. The number of dropouts since the NDA of those on Atrovent is tabulated as 23 and reasons given are those hospitalized in some cases, protocol violation, lack of efficacy, lost to follow-up, patient unable to be off previous medication, lost interest in study, administrative failure. These apply only to controlled studies - COPD.

The number of adverse reactions for this same group, i.e. patients on Atrovent in controlled studies, COPD is given as 16 for adverse reactions in this group since NDA (pages 89-101 section 6 of submission).

The number of adverse reactions in the controlled studies - COPD since NDA is given for Alupent is 8.

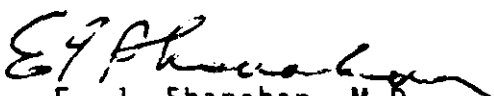
This concludes the review of the submitted data listed in the cover letter. In addition copies of the five preceding annual reports are submitted as requested by HFN 101. The sixth, reviewed report in this MOR is cumulative both qualitatively and quantitatively.

Conclusion

The sixth annual safety report on the emergency use program for Atrovent is complete with no new serious reactions nor significant increases in numbers of reactions relative to the population study numbers.

Recommendation

Continue ongoing reports on safety data for the Atrovent emergency program.

  
E. J. Shanahan, M.D.  
12/10/86

NDA 19-085

HFN-160

HFN-340

HFN-160 Dr. Winkler

R/D EJShanahan 12/10/86

R/D Init. by JWWinkler 12/10/86, PGWalters 12/11/86

FT OLA 250SN 0156N 12/15/86

Doc. Room 160

Appendix I

An explanation of the in patients who discontinued for adverse experience from December 20, 1985 to present (cumulative total: 140 patients). The following was reported:

Patient - Burning chest discomfort, worsening of dyspnea; post Atrovent FEV<sub>1</sub> improved but patient discontinued voluntarily.

Patient - Blurring of vision, Atrovent stopped, ophthalmological evaluation - early cataracts.

Patient - Patient's condition worsened due to myasthenia gravis - investigator noted possible retained secretions.

Patient - Patient discontinued due to dry mouth.

Patient - Cough aggravated on Atrovent.

Patient - Patient developed a mild irritant reaction - discontinued.

Patient - Patient experienced recurrent nausea on Atrovent, therefore discontinued.

Patient - No significant improvement; irritation and coughing - discontinued.

Patient - Chest burning on Atrovent - subsided after discontinuing treatment.

Patient - Poor patient compliance.

Patient - Patient experienced muscle spasm.

Patient - Increased shortness of breath, coughing, chest tightness on Atrovent 3 days.

Patient - Bronchospasm, no significant improvements.

Patient - Developed cough, stopped Atrovent. Status pending awaiting evaluation of cough.

Appendix 2 is an explanation of fatalities in 38 patients who died on treatment or within two weeks after discontinuation of Atrovent. This covers the period of December 20, 1985 to present, (cumulative total 118). None could be shown to be caused by administration of Atrovent.

NDA 19-085 Atrovent Inhaler  
Ipratropium Bromide  
Bronchodilator

Boehringer Ingelheim Ltd.  
Submitted: ~~7/17/85~~ 7/2/85  
Reviewed: 8/29/85

1. REVIEW AND EVALUATION OF LABELING

Draft labeling has been submitted as a result of a meeting between representatives of the firm and this Agency held on June 19, 1985. The topic of the meeting was a discussion of labeling for this new NDA.

The submitted, revised draft labeling concerns the sections titled CLINICAL PHARMACOLOGY, INDICATIONS, and USAGE, PRECAUTIONS, and a statement concerning "inclusion of the Rx legend on the insert."

Essentially, the submitted draft labeling reflects the conclusions reached at the noted meeting with one exception, concerning the statement made under the section titled INDICATIONS and USAGE reading "approximately one-half of asthmatic patients have been shown to respond to ATROVENT<sup>R</sup>. (See CLINICAL PHARMACOLOGY)."

RECOMMENDATIONS:

1. The statement, which is the final sentence of the section titled INDICATIONS and USAGE including the words in parenthesis regarding use in asthmatic patients should be deleted from that section of the labeling.
2. Providing 1) above is implemented, the submitted draft labeling should be approved.
3. Adverse experience tables for patients treated in the 90-day controlled clinical trials in support of the Package Inserts: For Atrovent: This tabulation shows that the total number of patients included in the NDA submission was 228 while the number that completed study was 254 - Table 1. This table is a breakdown by body systems showing the number and percentage of patients reporting adverse experiences under each system and, where available, the numbers and percentages for each sign/symptom for each body system. This was for both the 228 patients in the NDA submission and those under "Completed Study."

There are no remarkable differences between the latter two groups, i.e. "NDA submission" and "completed study." The highest incidences reported were in order of frequency:

- |                |                             |
|----------------|-----------------------------|
| 1. Cough       | 4. Exacerbation of Symptoms |
| 2. Nausea      | 5. Dizziness                |
| 3. Nervousness | 6. G.I. Distress            |

NOV 22 1985

NDA 19-085 Atrovent Inhaler  
Ipratropium Bromide  
Bronchodilator

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Submitted: ~~7/1/85~~ 7/1/85  
Reviewed: 8/29/85

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| 2. Nausea      | 5. Dizziness                |
| 3. Nervousness | 6. G.I. Distress            |

NOV 22 1985

Table 2 - "Overall Incidence of Adverse Experiences Among Patients Treated with ALUPENT for 90 days:

Number of patients (%)    NDA Submission = 223  
                                         Completed Study = 249

Incidence in order of frequency was:

1. Nervousness
2. Exacerbation of Symptoms
3. G.I. Distress
4. Tremor

There was no appreciable clinical significance between the Atrovent and Alupent treatment groups regarding incidence nor severity of adverse experience reporting except for more cases of insomnia reported in the Alupent-treated patients.

4. "A safety updated section comprising a summary of foreign data from clinical trials and spontaneous post marketing reports as well as a summary of the literature available since the NDA submission." The review of unpublished and published reports of adverse reactions associated with the use of Atrovent MDI from foreign data sources is consisted with the adverse reactions seen in clinical trials and the Atrovent compassionate use program in the United States. The analysis of these data shows Atrovent MDI is safe.

5. Foreign Marketing Status

There is a list of 25 countries where Atrovent Inhaler is currently marketed as well as the dates introduced in each, 1974-1984. The amount distributed for this period of time represents approximately 3 million single doses.

Atrovent has also been approved in France and Italy where marketing has not begun. There are no pending registrations, nor have any registrations been refused.

6. Patient information and exclusivity information is given in this section. Most of the information presented in this section is in the submitted NDA. Exclusivity - date first ANDA could be approved and length of exclusivity period is given as five (5) years from date of NDA approval.

Applicable patent numbers and expiration date of each are given as

3,681,500 - August 1, 1989

4,385,048 - May 24, 2000

CONCLUSION: Sections 2 through 4 above are in compliance with the agreements reached with the meeting with the representatives of the firm held on June 19, 1985.

Addendum dated 11/8/85 to Review of Labeling

Under the section titled CLINICAL PHARMACOLOGY add the following:

In these asthmatic patients who were responders to Atrovent<sup>R</sup> there was no evidence of development of tachyphylaxis.

  
E. J. Shanahan, M.D.

NDA 19-085

HFN-160

HFN-340

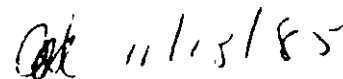
DocRm. 160

HFN-160/JWWinkler 11/7/85: NOTE: Will discuss further in-house, probably OK to leave asthma claim in "Indications" section also.

R.D. EJShanahan 11/8/85

R.D. Init by:JWWinkler 11/8/85:PHRussell 11/8/85

shg W2164K 11/14/85



NDA

19-085

Chemist Revs

DIVISION OF SURGICAL-DENTAL DRUG PRODUCTS  
Chemist's Review #8

A. 1. NDA 19-085

Date Completed: 10/23/85

APPLICANT: Boehringer Ingelheim, Ltd.  
ADDRESS: 90 East Ridge, P.O. Box 368  
Ridgefield, CT 06877

2. PRODUCT NAME(s):

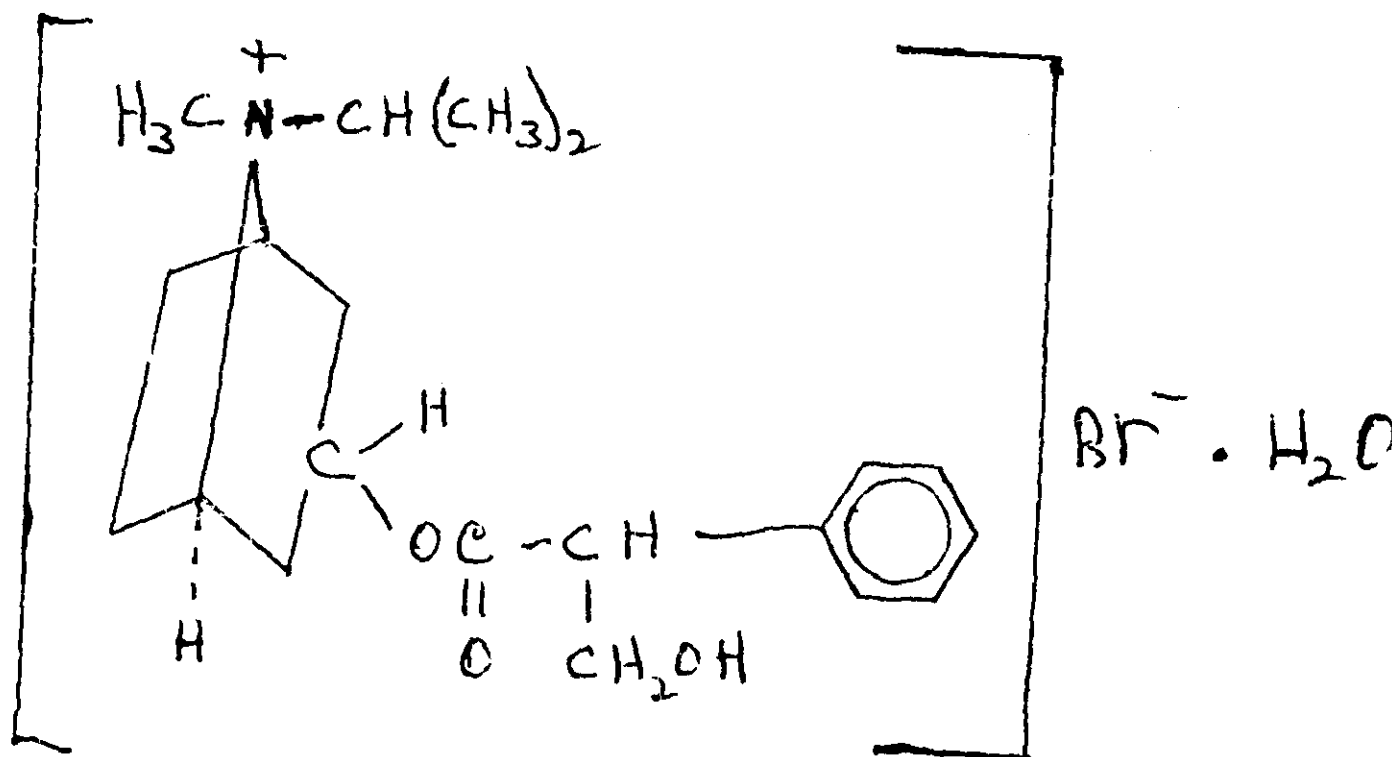
Proprietary: Atrovent Inhaler  
Non-proprietary: Ipratropium bromide  
Code Name and/or Number: Sch 1000

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Rx; Aerosol Inhalation

4. PHARMACOLOGICAL CATEGORY AND/OR PRINCIPAL INDICATION:

Anticholinergic bronchodilator used to treat bronchospasm.

5. STRUCTURAL FORMULA AND CHEMICAL NAME:



OCT 26 1985

Page 2

- B. 1. INITIAL SUBMISSION: July 29, 1983
- 2. AMENDMENT: October 11, 1985; Rec'd by Chemist on October 21, 1985.
- 4. RELATED DOCUMENTS:  
Telecon between O. H. Riggleman and Dr. C. Kumkumian (HFN-102) dated September 24, 1985.

C. REMARKS

- 1. The labeling deficiencies have been resolved. The fluorochlorohydrocarbons will be identified by their specific names where space permits (PI, container carton).
- 2. The term "Inhaler" has been permitted in the name. The term "Inhalation Aerosol" appears in the third paragraph of the description section.
- 3. The storage statement has been revised from "Store at room temperature. Avoid excessive humidity." to "Do not store above 30°C (86°F). Avoid excessive humidity."
- 4. The consultation reviews from microbiology, pharmacology, and biometrics are still out.

E.

Page 2

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4. The consultation reviews from microbiology, pharmacology, and biometrics are still out.

E.

D. CONCLUSIONS AND/OR RECOMMENDATIONS:

From a chemistry standpoint, the NDA can be approved.

The consultative reviews from biometrics, pharmacology, and microbiology should concur before approval is granted.

Draft labeling only has been reviewed. MV is in progress. EIR's are satisfactory; an update has been requested by HFN-322.

  
O. H. Riggleman

NDA 19-085  
HFN-160  
DocRm. 160  
R.D. OHRiggleman 10/24/85  
R.D. Init by:CPHoiberg 10/24/85  
shg W2147K 10/25/85

DRAFT OF CHEMIST < PART, LETTER TO APPLICANT

1. Final chemistry review is awaiting consultation reviews from pharmacology, microbiology, and biometrics.

NDA

19-085-

Pharmacology  
Reviews

N-19085-2

Date of Completion: 3/27/84

NDA 19-085

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original submission dated 7/29/83

APPLICANT: Boehringer Ingelheim Ltd.  
90 East Ridge  
P.O. Box 368  
Ridgefield, CT 06877  
(203) 438-0311

NAME OF DRUG: Descriptive: Atrovent Metered Dose Inhaler  
Sch 1000 or Sch 1000 - Br

USAN: Ipratropium bromide monohydrate

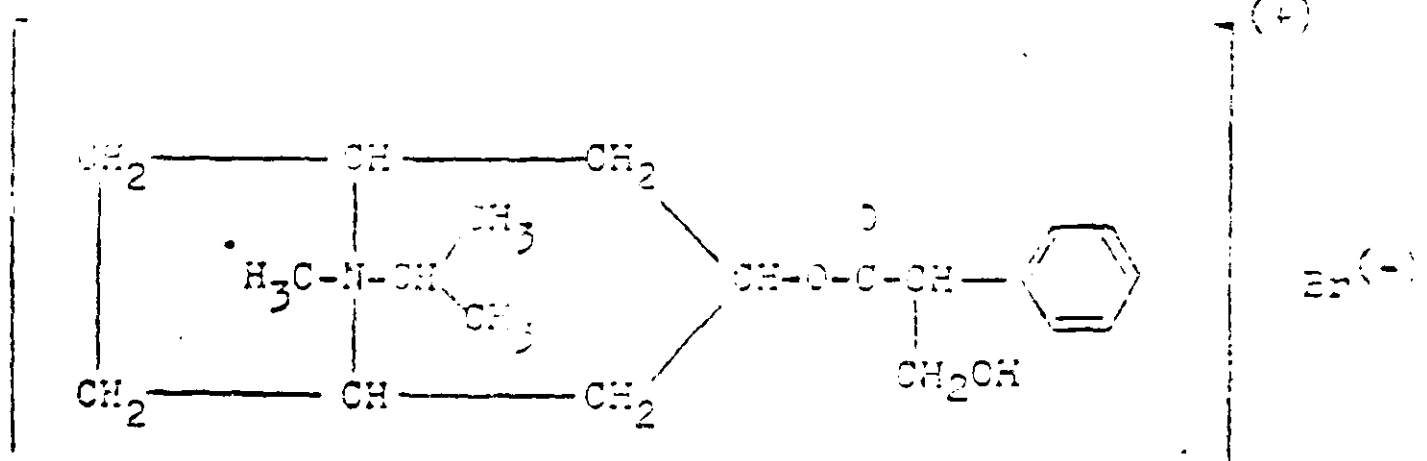
Chemical: 8-Azobicyclo[3.2.1] octane,  
3-(3-hydroxy-1-oxo-2-phenylpro-  
pxy)-8-methyl-8-(1-methyl-  
ethyl)-, bromide, monohydrate  
(endo,syn)-, (+)- or

(8r)-3a-dimethyl-2-isopropoxy-1-

3ad, 5ad-propanolamine salt

(+)-triflate monohydrate

STRUCTURE:



Empirical Formula: C<sub>20</sub>H<sub>30</sub>N<sub>03</sub>Br·H<sub>2</sub>O

Molecular Weight: 430.4

Date of Completion: 3/27/84

NDA 19-085

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original submission dated 7/29/83

APPLICANT: Boehringer Ingelheim Ltd.  
90 East Ridge  
P.O. Box 368  
Ridgefield, CT 06877  
(203) 438-0311

NAME OF DRUG: Descriptive: Atrovent Metered Dose Inhaler  
Sch 1000 or Sch 1000 - Br

USAN: Ipratropium bromide monohydrate

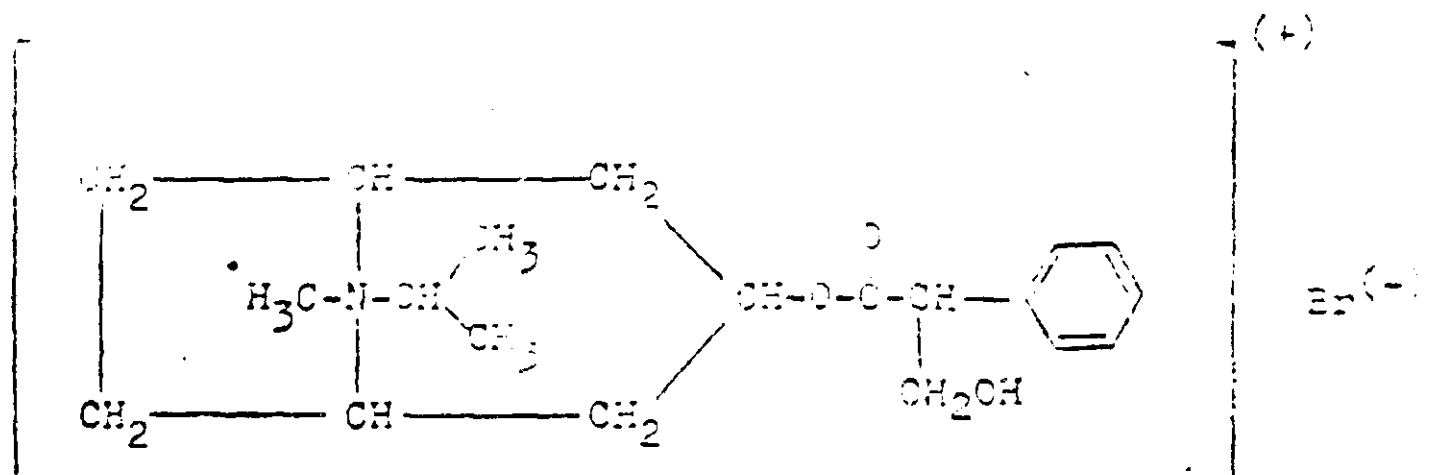
Chemical: 8-Azoniabicyclo[3.2.1] octane,  
3-(3-hydroxy-1-oxo-2-phenylpro-  
panoxy)-8-methyl-8-(1'-methyl-  
ethyl)-, bromide, monohydrate  
(endo,syn)-, (+)- or

(8r)-3-(3-hydroxy-1-oxo-2-phenylpro-

panoxy)-8-methyl-8-(1'-methyl-

ethyl)-, bromide monohydrate

STRUCTURE:



Empirical Formula: C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub>Br·H<sub>2</sub>O

Molecular Weight: 430.4

COMPOSITION OF DRUG:

| <u>Component</u>                                  | <u>% W/W</u> |
|---------------------------------------------------|--------------|
| Ipratropium bromide, micronized                   | 0.03         |
| Soya lecithin                                     | 0.10         |
| Dichlorodifluoromethane, NF<br>(Propellant 12)    | 50.33        |
| Dichlorotetrafluoroethane, NF<br>(Propellant 11*) | 20.32        |
| Trichloromonofluoromethane, NF<br>(Propellant 11) | 29.35        |
| Nitrogen                                          | 9.97         |

\* Nitrogen added in processing.

ROUTE OF ADMINISTRATION: Oral inhalation

DOSE: Usual dose is 2 inhalations 4 times a day. Patients may take additional inhalations as required; however, the total number of inhalations should not exceed 12 in 24 hours. Each actuation delivers 20 ug from the mouthpiece. Therefore, the maximum daily dose = 20 x 12 = 240 ug/day = 4.8 ug/kg/day for 50 kg subject.

NDA 19-085  
Page 3

DOSAGE FORM: Suspension aerosol with Freons for oral inhalation.

CATEGORY: Bronchodilator

CLINICAL INDICATION: Bronchodilator for the treatment of chronic obstructive pulmonary diseases.

COMPARISON DRUG: Atropine sulfate

PRECLINICAL DATA:

GLP STATEMENTS: Yes.

FOREIGN ANIMAL STUDIES: Yes, most studies have been performed at C.H. Boehringer Sohn, West Germany; and some, in Boehringer Ingelheim in Japan. Some were done at Huntingdon Research Center, England; and some, at Inveresk Research International, Scotland. A few were done at Institute for Industrial and Biological research, European subsidiary of Hazleton Labs. Inc.

REVIEW OF PRECLINICAL DATA:

PHARMACOLOGY/BIOCHEMISTRY:

Anticholinergic Effects

A. Gastrointestinal Tract

(a) Salivary Secretions (stimulated by pilocarpine)

When administered IV, SC, and intraduodenally to dogs atropine was 2, and 5 times respectively, more effective than Sch in inhibiting salivary secretions. Decreased blood pressure was slightly inhibited by Sch but not in a dose-related fashion.

Administered as an aqueous aerosol Sch was slightly less effective than atropine. Maximum inhibition occurred at 10-15 minutes. Heart rate was not consistently altered. Decreased blood pressure produced by AcCh was only slightly inhibited by Sch (max effect at 1 min).

When administered as a metered aerosol, doses of 1-8 mg/animal, Sch effectively inhibited salivary secretion but did not alter BP, heart rate or ECG consistently.

In the mouse Sch was as effective I, but 1/2 as effective PO. In the rat, Sch was 2 times as effective as atropine when given SC but only 1/50 as effective intraduodenally in inhibiting carbachol-induced salivary secretions.

(b) Gastric Secretions:

Volume of gastric secretion was only slightly affected by low doses of Sch or atropine but Sch had more effect on volume of acid secretion. Sch was about twice as potent as atropine when given SC to rats but only 1/10 as potent when given PO.

(c) Intestines:

Following IV administration of Sch to intact dogs, spasmolytic effect appeared immediately whereas with intraduodenal and oral routes it occurred more slowly. Pulse rate increased with increase in dose.

(d) Bladder:

When Sch was administered IV to anesthetized dogs spasmolytic effect was as effective as atropine and produced an immediate response with a maximum in 7 minutes. A non dose-related increase in heart rate was noted. Respiratory rate was not altered. A slight transient decrease in BP occurred. When administered intraduodenally Sch was 1/2 as effective as atropine and onset of effect was longer.

(e) Rectum:

In vitro studies on isolated guinea pig rectum indicate that Sch was at least as effective as atropine in preventing AcCh and pilocarpine-induced spasms but had no effect on BaCl<sub>2</sub> or histamine spasms.

B. Eye

In the mouse Sch was as effective as atropine when given SC but only about 1/15 as potent when given PO in producing a mydriatic effect.

C. Bronchi

In guinea pigs IV Sch was twice as effective as atropine in preventing AcCh-induced spasms.

Sch was also twice as effective when given IV to dog. With hi dose (0.3 mcg/kg and higher) spasmolytic effect was noted immediately. Sch was administered to dogs as metered aerosol (25 mcg/dog). Maximum effects occurred at 6-11 minutes. Duration of effect was longer than after IV administration.

Sch was twice as effective as atropine when given to dogs as an aqueous aerosol. After hi dose, effect was noted immediately with a maximum 6-13 minutes. Metered aerosol in effect for a slightly longer period than aqueous aerosol.

D. TS

0.3-10 mg/kg IV to rabbits did not alter areolar stimulus or prevent carbachol-induced tremors; death occurred at 20 mg/kg.

In mice centrally induced lacrimal and salivary secretions induced by exotremone were inhibited by 0.005-0.05 mg/kg Sch but not the tremors.

E. Cardiovascular

When given SC to intact dog Sch was as effective as atropine but only 1/5 as effective when given PO in producing 50% increase in heart rate. In dogs, 0.03-0.1 mg/kg IV had no effect on BP or circulatory reflexes. 0.3 mg/kg produced a slight and transitory decrease in carotid sinus pressure reflex; at 1.0 mg/kg reflex was abolished for a few minutes.

**F. Action of Sch 1000 on the Oxotremorine Test in Mice (1/30/75 review)**

Sch 1000 at low dose levels given S.C., 0.0046 and 0.05 mg/kg act as a peripheral anti-cholinergic agent and depresses markedly lacrimal and salivary secretions in mice. The mice had been given 1 mg/kg S.C. Oxotremorine, a drug which produces marked increase in lacrimal and salivary secretions. Sch 1000 had no effect on reducing tremors which are also produced by oxotremorine. Atropine used as the experimental control reduced secretions and tremors at lower than level.

**G. Mydriatic Effect of Sch 1000, administered systemically in mice: Comparison with atropine - Pharmacology Research Lab., West German**

In a test to determine the mydriatic side effect of Sch 1000, mice were given this drug S.C. and orally at a dose level of 0.1 mg/kg. The results show that the mydriatic efficacy of Sch 1000 is about 10-20 times when given S.C., however when Sch 1000 is given orally, it has 10-20 times less the mydriatic efficacy of atropine.

**H. Action of Subcutaneous and Intraduodenal Sch 1000 on Salivary Secretion in Anesthetized Rats.**

Comparison with Atropine

Pharmacology Research Lab.,  
West German

Sch 1000 given S.C. and intraduodenally at dose levels of 0.012 and 0.1 mg/kg respectively, markedly reduced salivary secretions at 0.15 mg/kg of pilocarpine in anesthetized rats, given by intraperitoneal injection of muscarinic cholinergic stimulation were used.

**I. Effect of S.C. and Enteral Sch 1000 on Gastric Secretion in the Rat**

Comparison with Atropine

Pharmacology Research Lab.,  
West German

The results of this study show that 10-30 mg/kg of Sch 1000 S.C. at a dose of 3-10 mg/kg stops the secretion of free HCl in the stomach of rats, whereas atropine must be given in dose of 30 to 100 mg/kg to achieve the same effect. Neither drug had much influence on the total gastric juice secretion in the stomach. Atropine, given by enteral administration at a dose level of 5.5 mg/kg and Sch 1000 at a dose level of 5.5 mg/kg given similarly, reduced total acidity volume in the stomach of rats by 50%.

**F. Action of Sch 1000 on the Oxotremorine Test in Mice (1/30/75 review)**

Sch 1000 at low dose levels given S.C., 0.0046 and 0.05 mg/kg acted as a peripheral anti-cholinergic agent and depresses markedly lacrimal and salivary secretions in mice. The mice had been given 1 mg/kg S.C. Oxotremorine, a drug which produces marked increase in lacrimal and salivary secretions. Sch 1000 had no effect on reducing tremors which are also produced by oxotremorine. Atropine used as the experimental control reduced secretions and tremors at lower than level.

**G. Mydriatic Effect of Sch 1000, administered systemically in mice: Comparison with atropine - Pharmacology Research Lab., West Germany**

In a test to determine the mydriatic side effect of Sch 1000, mice were given this drug S.C. and orally at a dose level of 0.1 mg/kg. The results show that the mydriatic efficacy of Sch 1000 is similar to atropine when given S.C., however when Sch 1000 is given orally, it was 10-20 times the mydriatic efficacy of atropine.

**H. (less ysc) Action of Subcutaneous and Intraduodenal Sch 1000 on Salivary Secretion in Anesthetized Rats.**

Comparison with Atropine

Pharmacology Research Lab.,  
West Germany

Sch 1000 given S.C. and intraduodenally at dose levels of 0.010 and 0.1 mg/kg respectively, reduced salivary secretions at 0.1 mg/kg. Anesthetized rats, given Sch 1000 by subcutaneous and intraduodenal stimulation were used.

**I. Effect of S.C. and Enteral Sch 1000 on Gastric Secretion in Rats**

Comparison with Atropine

Pharmacology Research Lab.,  
West Germany

The results of this study show that 10-50 mg/kg of Sch 1000 S.C. at a dose of 3-10 mg/kg stops the secretion of free HCl in the stomach of rats, whereas atropine must be given in dose of 30 to 100 mg/kg to achieve the same effect. Neither drug had much influence on the volume of gastric juice secretion in the stomach. Atropine, given by enteral administration at a dose level of 5.5 mg/kg and Sch 1000 at a dose level of 5.5 mg/kg given similarly, reduced total acidity volume in the stomach of rats by 50%.

J. Cardiovascular Effects of Oral and Subcutaneous Sch 1000 in the conscious dog: Comparison with atropine.

Pharmacology Research Labs.  
West Germany

Purpose of this study was to determine tachycardial side-effects of Sch 1000 and atropine following S.C. and oral administration in dogs. Results showed that a 50% increase in heart rate of dogs is achieved by a 0.01 mg/kg dose of Sch 1000, S.C. and a 0.040 mg/kg dose when drug is given orally. Atropine has the same effect when it is given at a dose of 0.03 mg/kg S.C. and 0.135 mg/kg orally.

K. Action of Sch 1000 administered by inhalation on respiration and Acetylcholine induced bronchospasm in Anesthetized dogs

Pharmacology Research Labs.,  
West Germany

A dose of Sch 1000 given i.v. at 0.15 mg/kg inhibited acetylcholine induced bronchospasms by 50% in dogs. An i.v. injection of atropine at 0.27 mg/kg gave similar results.

Sch 1000 given as an aqueous aerosol is twice as effective in inhibiting bronchospasms in the anesthetized dog. The effect is immediate and the maximum effect is 5-13 minutes.

When Sch 1000 is administered as a nebulated aerosol at 0.15 mg/kg it also inhibited bronchospasms by 50% in the anesthetized dog.

L. Effect on Salivary Secretion, Blood Pressure and Heart Rate in Anesthetized Dogs

Anesthetized dogs with acetylcholine produced decrease in blood pressure and tachycardia induced salivary stimulation were used in this study. Results show that 0.01 mg/kg Sch 1000 i.v. (aqueous aerosol) 100% blocked the acetylcholine decrease in blood pressure and cause an increase in heart rate. Atropine at a similar dose produces opposite results.

Sch 1000 given by aqueous aerosol in a concentration of 4% inhibited salivary flow by 50%. However, it had no effect on blood pressure and h.p. Atropine in a 3% aqueous solution inhibited salivary flow by 50% and completely blocked acetylcholine induced decrease in blood pressure.

A total dose of 0.6 mg of Sch 1000 given as a nebulated aerosol effectively inhibited salivary flow in dogs.

M. Effect of Metered Aerosol on ECG and Heart Rate of Anesthetized Dogs

Anesthetized dogs were given Sch 1000 at dose levels of 1, 4, and 8 mg by metered aerosol. A 7.5 mg dose of alupent in a metered aerosol was used as the reference control. Results show that Sch 1000 at these dose levels cause a transient decrease in h.r. and then an increase in h.r. for approximately 1-2 minutes. The decrease in h.r. was thought to be due to propellant gas. No pathological changes in the ECG patterns of the dogs were noted. An increase in the h.r. of the dogs receiving alupent was observed.

N. E.E.G. Studies in Rabbits

Pharmacology Research Lab.

Sch 1000 I.V. had no effect on the central nervous system of rabbits.

Other Actions demonstrated in the Pharmacology Screening Program Pharmacology Research Lab.

O. Gastrointestinal System

The effect of Sch 1000 on contractions variably induced in the isolated rectum of the guinea pig were studied in this program.

Sch 1000 was more effective than atropine in inhibiting acetylcholine induced contractions in the rectum of guinea pigs. It was more effective in inhibiting atropine induced contractions. The ED<sub>50</sub> values were 0.05 mg/kg for Sch 1000 and 0.1 mg/kg for atropine. The ED<sub>50</sub> values for Sch 1000 and atropine were 0.05 mg/kg and 0.1 mg/kg, respectively. Sch 1000 had little effect on 5-HT<sub>2</sub> or histamine contractions.

P. Expectorant Action of Sch 1000

Sch 1000 in high doses caused inhibition of bronchial secretions, however, in low doses (micrograms), Sch 1000 caused an increase in bronchial secretions.

Q. BEAGLE DOGS: Cardiotoxicity study by INHALATION: with propellant gases and under hypoxic conditions (Sept. 3, 1973): Translation (Boehinger Sohn).

- a. Under normal ventilation: Dogs(? M &?F of 7-14.5 kg) inhaled 200 whiffs (total = mg) of mixture of Sch-1000-Br and freon gases 11, 12, and 114, after pentobarbital (30-40 mg/kg I.V.) and gallamide triethiodide (4 mg/kg I.V.), and ECG were recorded from leads I and II: No pathological changes in ECG were observed. Heart rates were decreased with increased O<sub>2</sub> tension and decreased CO<sub>2</sub> tension.
- b. Under hypoventilation: Hypoxia itself produced more changes on ECG before starting infusion, and the changes became more marked as time went by, as did bradycardia.

R. BEAGLE DOGS: by I.V. infusion:

- a. Under normal ventilation: One dog at 5 mg/kg/min.: after 725 mg/kg, there was a marked bradycardia.  
  
One dog at 10 mg/kg/min: The dog tolerated up to 1550 mg/kg, but with marked bradycardia and hypotension.
- b. Under hypoventilation:  
  
10 mg/kg/min + placebo (containing freon) + atropine + benzol of 10 puffs every 5 min: Hypoventilation produced repolarization problems on dogs before the administration of infusion, and the effects were exaggerated by the infusion. Animal survived after 1000 mg/kg and 200 puffs.

It was stated that the hypotension and bradycardia produced by Sch 1000-Br is probably due to its ganglionic-blocking activity and curare like effect, but it did not produce any pathological changes on ECG.

S. IN-VITRO PROTEIN BINDING OF SCH 1000: (from 11/9/83 review)

Lab. Performing the study: C.H. Boehringer Sohn  
(Dr. Wahl, 9/24/80)

Methods: Equilibrium dialysis with/without  $^{14}\text{C}$ -Sch. 1000 (Batch B).  
Batch no of cold Sch-1000 (VIII)

Plasma 1 was a mixture of old plasma samples from 22  
persons, and was older than 1 week.

Plasma 2 was fresh from 3 male subjects and used separately.

Rat plasma was from several male rats of Chbb: THOM (SPF).  
See pages 1-3 for details.

RESULTS:

The Sch 1000 binding to human plasma was reported as an average of 18% (14-20%), to human albumin as 6% (4-10%), and to rat plasma as 8% (6-8%), and there were no significant differences between old and fresh plasma samples.

II. ADME AND PHARMACOKINETICS: (from the original review)

A. Absorption, Metabolism and Excretion Studies

RA:

After iv administration of 100 mg of RA, 20% of the dose was excreted in 24 hours in urine, 22% in feces, 18% was excreted in

bile and less than 1% was exhaled. Elimination from blood was biphasic: 1)  $t_{1/2}$  = 1.3 minutes and 2)  $t_{1/2}$  = 1.4 hours. Tissue distribution, in order of decreasing activity, was GIT, liver, kidney, bladder, heart, and brown fat.

Following PO administration of 1-25 mg of RA drug, seventy-five percent of RA was eliminated in 24 hours (5% urine, 60% feces), 3% in bile and less than 1% was exhaled. Blood levels could not be determined. Distribution studies indicate drug was found, in order of decreasing activity, in GIT, liver, kidneys, heart, lacrimal glands, and brown fat. At least three metabolites were reported but no details given as to structure.

Dog

After 5 mg/kg <sup>14</sup>C-Sch IV, elimination of RA from blood was biphasic: 1) t/2=2.5 minutes and 2) t/2=3-4 hours. About 38% of RA was eliminated in urine and 1% in feces in 24 hours. One hour after IV dosing about 73% of RA excreted in urine was due to unchanged drug; approximately 8-10 metabolites were present in small amounts. None of metabolites were further identified. Over a 7-day period, 88% was recovered (45% urine, 43% feces).

When the above dose was given PO to two dogs, maximum blood levels were noted after 3 hours. In one dog blood t/2 was 10 hours, in the other, maximum levels were maintained for 15 hours. About 23% of RA was excreted in urine in 24 hours and 54% in feces. Over a 7-day period 98% of RA was eliminated (27% urine, 71% feces). Metabolic pattern was not determined.

Man

After PO administration of 30 mg <sup>14</sup>C-Sch to 7 humans, maximum blood levels were noted 2-4 hours then slowly decreased; t/2 was about 4 hours. Twenty-nine percent of administered RA was recovered in 24 hours (8% urine, 21% feces). In 48 hours, fecal excretion accounted for 89%. Over 6-day period, the total recovery was 97% (9% urine, 89% feces).

Following IV administration of 1 mg <sup>14</sup>C-Sch to 4 humans a biphasic elimination was noted: 1) t/2=5 minutes and 2) t/2=1.2 hours. In 24 hours, 72% of RA was excreted (70% urine (52% in 4 hours) and 2% feces). Over 6-day period, 78% of RA was accounted for (72% urine, 6% feces). Based on renal excretion following IV and PO administration, absorption is approximately 17% of administered dose.

One hour after IV dosing, urine was examined for metabolites. About 71% of unchanged drug was present, one major metabolite was identified. Other metabolites were present only in small amounts, all less than 1%. With time the amount of unchanged drug decreased and major metabolite increased. Other seven metabolites remained about the same.

Four hours following PO administration, qualitative distribution of metabolites was somewhat similar to that noted after IV dosing. Amount of unchanged drug was smaller (24%). Two metabolites found following PO administration were not seen after IV administration and two found after PO administration were not observed after IV route. Metabolites were not identified structurally.

Five subjects received a total of 585 mcg. of <sup>14</sup>C-Sch in ethanol and freons via inhalation. Peak concentration occurred 3 hours after exposure. Half-life for plasma elimination was about 2 hours. Fifty-one percent of administered RA was accounted for in 24 hours (48% feces, 3% urine), as after PO administration, urinary excretion was very small, over 6 days, 72% was recovered (69% feces, 3% urine). Unchanged drug and three metabolites were found in urine but not further identified.

Dog

After 5 mg/kg <sup>14</sup>C-Sch IV, elimination of RA from blood was biphasic: 1) t/2=2.5 minutes and 2) t/2=3-4 hours. About 38% of RA was eliminated in urine and 1% in feces in 24 hours. One hour after IV dosing about 73% of RA excreted in urine was due to unchanged drug; approximately 8-10 metabolites were present in small amounts. None of metabolites were further identified. Over a 7-day period, 98% was recovered (45% urine, 43% feces).

When the above dose was given PO to two dogs, maximum blood levels were noted after 3 hours. In one dog blood t/2 was 10 hours, in the other, maximum levels were maintained for 15 hours. About 23% of RA was excreted in urine in 24 hours and 54% in feces. Over a 7-day period 98% of RA was eliminated (27% urine, 71% feces). Metabolic pattern was not determined.

Man

After PO administration of 30 mg <sup>14</sup>C-Sch to 7 humans, maximum blood levels were noted 2-4 hours then slowly decreased; t/2 was about 4 hours. Twenty-nine percent of administered RA was recovered in 24 hours (8% urine, 21% feces). In 48 hours, fecal excretion accounted for 89%. Over 6-day period, the total recovery was 97% (3% urine, 89% feces).

Following IV administration of 1 mg <sup>14</sup>C-Sch to 7 humans a biphasic elimination was noted: 1) t/2=5 minutes and 2, t/2=4.2 hours. In 24 hours, 72% of RA was excreted (70% urine (52% in 4 hours) and 2% feces). Over 6-day period, 78% of RA was accounted for (72% urine, 6% feces). Based on renal excretion following 1 mg PO administration, absorption is approximately 17% of administered dose.

One hour after IV dosing, urine was examined for metabolites. Unchanged drug was present, one metabolite was present in small amount, seven others were present only in small amount, all less than 1% of unchanged drug. One amount of unchanged drug decreased and major metabolite increased. Other seven metabolites remained about the same.

Four hours following PO administration, qualitative distribution of metabolites was somewhat similar to that noted after IV dosing. Amount of unchanged drug was smaller (2%). Two metabolites found following PO administration were not seen after IV administration and two found after PO administration were not observed after IV route. Metabolites were not identified structurally.

Five subjects received a total of 555 mcg. of <sup>14</sup>C-Ion in ethanol and freons via inhalation. Peak concentration occurred 3 hours after exposure. Half-life for plasma elimination was about 2 hours. Fifty-one percent of administered RA was accounted for in 24 hours (40% feces, 3% urine), as after PO administration, urinary excretion was very small, over 6 days, 72% was recovered (69% feces, 3% urine). Unchanged drug and three metabolites were found in urine but not further identified.

The absorption of Sch in rat, dog, and man, based on urinary excretion following IV and PO use, was 9, 60 and 11%, respectively. It would appear that absorption in rat and man were comparable but considerably higher in dog. Only small percent of RA was found in feces following IV administration to man. Apparently, drug and/or its metabolites are not secreted into intestine via the bile to a significant extent while the reverse seems to be true for the rat and dog.

Based on the quantitative distribution of unchanged drug and metabolites in the urine following IV administration, the metabolic pattern in the dog (9-11 metabolites) and man (8 metabolites) appears to be somewhat similar. However, since metabolites were not structurally identified, it is not known if they were identical. Sufficient information was not available to make a definite statement about the rat metabolism data.

**B** Pharmacokinetics of <sup>14</sup>C - Sch 1000 Metered Aerosol - Synopsis (1/24/75 rev.)

Rats Radioactive Sch 1000

# animal/dose level 39M

dose 1 mg/kg

administration and duration

I.V. and oral 15 day observation period

Results

Plasma levels could not be obtained in the rat. Excretion of drug was fairly rapid with the maximum excretion occurring within 6 hours. 5.0% of the drug was excreted via the urine and 63% was excreted via the fecal route when taken orally and 6.4% via the kidney and 39.0% in the fecal matter when the drug was given I.V.

Dog Radioactive Sch 1000

# animals/dose 2

dose level 5 mg/kg

administration and duration

I.V. and oral, 13 day observation period.

Results:

Following oral administration the maximum plasma value was found within 3 hours. 23.6% of the drug is excreted via the urine when given orally and 72% in the fecal matter while 45% is found in the urine when the drug is given I.V. and 44% in the feces. The half life of the drug when given orally is 3-4 hours.

In man, the metabolism of Sch 1000 is similar to that found in the dog. The maximum concentration of this drug in blood was observed within 3-4 hours. The drug is excreted rapidly 30-70% within the first 24 hours depending on whether the drug is given orally or I.V. when given I.V., 72% is excreted in the urine, and 6.0 in the feces. When taken orally, the drug is excreted 9.3% in the urine and 28.5% in the feces.

C. Pharmacokinetics Using Radioactive Sch 1000 Metered Aerosol

man (age 22-34)

Radioactive Sch 1000

# dose level 4M +1F

dose 469-619 mcg. average 555 mcg.

administration and duration

Inhalation; 3 puffs observations 4 hours  
Sch 1000 given as a radioactive solution in I.V. studies.

Results:

The maximum plasma levels were observed within 15 minutes of administration of the drug in plasma was 1.1 to 1.3 mcg/ml. The maximum concentration of Sch 1000 was most rapid in the first 15 minutes of administration. The average half life test was 3.7 hours. The data indicated that the maximum plasma level was 1.1 to 1.3 mcg/ml in the first 15 minutes of administration. The maximum plasma levels observed for the inhalation of Sch 1000 was 1.1 to 1.3 mcg/ml per gram of plasma.

D. The Excretion and Whole-Blood concentrations of Radioactive Sch 1000 after 1.1% administration to monkeys during a chronic inhalation toxicity study of the Non-Radioactive Drug.

Rhesus Monkeys (M. <sup>mulatta</sup> m. ~~schatta~~) Sch 1000

#Animals/Dose Level: 1F and 1M

Dose: Control and 1 mcg/kg

Administration and Duration:

Animals used were on a chronic inhalation study. Animals used were controls and the 1600 mcg/da groups. These animals were given radioactl Sch 1000 I.V., a one shot dose.

Results:

An I.V. dose of Sch 1000 at 1 mg/kg given to the monkeys before the initiation of the chronic inhalation study, and at 3 and 6 months during the inhalation study showed no significant differences in the rates of excretion. A total of 66.7% of the dose was found in the urine of the monkeys in the control group and 62.9% in the 1600 mcg/da group at the 3 months evaluations. At the same evaluations, 25.6% of the dose was found in the fecal matter of the control monkeys and 24.9% in the feces of the 1600 mcg/da monkeys. Results for pre-exposure and at 6 months were similar. Similar results were obtained when the whole blood of these monkeys was evaluated for radioactivity. The radioactivity of the control and experimental monkeys were comparable at pre-exposure, 3 and 6 months. Spon suggests that these data show there is no accumulation of drug or inhibition of drug metabolizing enzyme(s) during the administration of Sch 1000. Thin-layer chromatography analysis of the urine show that the drug is excreted unchanged by the monkeys.

E. Species Comparison of Biochemical Findings Sch 100 in Rat, Dog and Man  
(C.H. Boehringer Sohn, Germany) (3/14/77 review)

Rat

Rats, given an oral dose of 100 mg/kg Sch 1000 showed a maximum concentration in the blood within 2-4 hours, drug remained in the blood at high levels for several hours (approximately 7). Rats, given a i.v. dose of Sch 1000 (1mg/kg) had a rapid fall in blood drug concentration during the first 4.3 minutes (50% drop). During the next 3 hours, drug was lost at a slower rate. During the first 24 hours, 4.5% of drug was found in the urine and 76.9% was found in feces when Sch 1000 is given to the rat orally. When given by I.V., 57.1% was found in the urine and 39.5 was noted in the feces. A total of 5 components were found in the urine of rats given Sch 1000 orally. The largest amount of these components was unchanged drug.

Dog

Dogs, given Sch 1000 orally at a dose of 5 mg/kg showed a maximum drug concentration in the blood within 2-4 hours. Large concentrations were noted for up to 10 hours in the blood. 23.2% of oral Sch 1000 was found in the urine of the dog within 24 hours and 72.5% was found in the feces. After I.V. administration 38.4% of the dose was found in the urine and 42.6% in the feces.

Man

An oral dose of 30 mg was used in humans. As seen in the rat and dog maximum concentrations was noted in 2-4 hours. Similar results were obtained when this drug was given to man by inhalation. Dose levels in the inhalation study were 555 mcg. Excretion in man was 7.2% of the oral dose found in the urine, 85% in feces. Excretion in man was 70.1% of an I.V. dose in urine and 6.3% in feces. By inhalation, 2.8% was excreted via kidneys.

F. METABOLIC STUDY IN HUMAN URINE BY INHALATION: (11/2/78 review)

The metabolic pattern of 1 and 7 hour urine samples of a volunteer man after inhalation of 3 puffs from metered dose aerosol (489 ug Sch 1000, 68.46 uCi) showed 3 radioactive substances, one of which was identified as unchanged Sch 1000, the other as its hydrolysis product, and the third, as unknown but presumably conjugate.

The unknown metabolite was 53% in 1 hour urine and 36% in 7 hour urine, and the proportion of hydrolysis product was increased from 6.8% in 1 hour urine and 40.6% in 7 hour urine, whereas unchanged drug was 27% in 1 hour urine, and the level remained relatively constant between 10.4% and 15% of total radioactivity from 2 hour to 7 hour post dose. This may explain its long duration of effect.

II. ACUTE TOXICITY STUDIES:

A. LD<sub>50</sub> by Various Routes: See the following Table 1.

Table 1. Summary of Acute Toxicity Studies with Atrovent® by Route of Administration

| <u>Route of Administration</u> | <u>Species</u> | <u>Strain</u>    | <u>Sex</u>  | <u>Body Weight</u> | <u>No. of Animals Per Group</u> | <u>Drug Administration</u>  | <u>LD<sub>50</sub> mg/kg</u> | <u>95% Fiducial Limits</u> | <u>References</u> ★ |
|--------------------------------|----------------|------------------|-------------|--------------------|---------------------------------|-----------------------------|------------------------------|----------------------------|---------------------|
| <u>Oral</u>                    | Mice           | Chbl:<br>NMRI    | M           | 21-26 g            | 10                              | Aqueous solution<br>(5-15%) | 1240                         | (959-1425)                 | 18                  |
|                                |                |                  | F           | 17-21 g            | 10                              |                             | 1209                         |                            |                     |
|                                | Mice           |                  | M           |                    | 5                               | 0.5% Methyl-<br>cellulose   | 2300                         | (2000-2645)                | 19                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Mice           | ICR-<br>JCL      | M           | 34 g               | 10                              | Aqueous<br>solution         | 1120                         | (806-1557)<br>(1694-2481)  | 24                  |
|                                |                |                  | F           | 27 g               | 10                              |                             | 2050                         |                            |                     |
|                                | Rat            |                  | M           |                    | 5                               | Aqueous<br>solution         | 2300                         | (2054-2576)                | 19                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Rat            |                  | M           |                    | 5                               | 20% emulsion                | > 4000                       |                            | 20                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Rat            | SDL-<br>JCL      | M           | 270 g              | 10                              | Aqueous<br>solution         | 1900                         | (1545-2337)<br>(1639-2321) | 24                  |
|                                |                |                  | F           | 183 g              | 10                              |                             | 1950                         |                            |                     |
|                                | Dog            | Beagles          | M           | 12.2-16.3 kg       | 1                               | Gelatin<br>capsules         | 1300                         |                            | 21                  |
| F                              |                |                  | 9.6-13.0 kg | 1                  |                                 |                             |                              |                            |                     |
| Dog                            | Mongrels       | M                | 5.7-7.0 kg  | 1                  | Gelatin<br>capsules             | 400-<br>800                 |                              | 22                         |                     |
|                                |                | F                | 7.8-9.7 kg  | 1                  |                                 |                             |                              |                            |                     |
| Rabbit                         | Himalayan      | M                | 2.03 kg     | 5                  | Aqueous<br>solution             | 1557                        |                              | 24                         |                     |
|                                |                | F                | 1.59 kg     | 5                  |                                 | 2282                        |                              |                            |                     |
| <u>Intragastric</u>            | Rat            | FW49<br>Biberach |             |                    | 5                               |                             | 1560                         | (1380-1763)                | 20                  |
| <u>Intraduodenal</u>           | Rat            | FW49<br>Biberach |             |                    | 5                               |                             | 310                          | (274-350)                  | 20                  |

Table 1. Summary of Acute Toxicity Studies with Atrovent® by Route of Administration

| <u>Route of Administration</u> | <u>Species</u> | <u>Strain</u>    | <u>Sex</u>  | <u>Body Weight</u> | <u>No. of Animals Per Group</u> | <u>Drug Administration</u>  | <u>LD<sub>50</sub> mg/kg</u> | <u>95% Fiducial Limits</u> | <u>References</u> ★ |
|--------------------------------|----------------|------------------|-------------|--------------------|---------------------------------|-----------------------------|------------------------------|----------------------------|---------------------|
| <u>Oral</u>                    | Mice           | Chbi:<br>NMRI    | M           | 21-26 g            | 10                              | Aqueous solution<br>(5-15%) | 1240                         | (959-1425)                 | 18                  |
|                                |                |                  | F           | 17-21 g            | 10                              |                             | 1209                         |                            |                     |
|                                | Mice           |                  | M           |                    | 5                               | 0.5% Methyl-<br>cellulose   | 2300                         | (2000-2645)                | 19                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Mice           | ICR-<br>JCL      | M           | 34 g               | 10                              | Aqueous<br>solution         | 1120                         | (806-1557)                 | 24                  |
|                                |                |                  | F           | 27 g               | 10                              |                             | 2050                         | (1694-2481)                |                     |
|                                | Rat            |                  | M           |                    | 5                               | Aqueous<br>solution         | 2300                         | (2054-2576)                | 19                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Rat            |                  | M           |                    | 5                               | 20% emulsion                | > 4000                       |                            | 20                  |
|                                |                |                  | F           |                    | 5                               |                             |                              |                            |                     |
|                                | Rat            | SDL-<br>JCL      | M           | 270 g              | 10                              | Aqueous<br>solution         | 1900                         | (1545-2337)                | 24                  |
|                                |                |                  | F           | 183 g              | 10                              |                             | 1950                         | (1639-2321)                |                     |
|                                | Dog            | Beagles          | M           | 12.2-16.3 kg       | 1                               | Gelatin<br>capsules         | 1300                         |                            | 21                  |
| F                              |                |                  | 9.6-13.0 kg | 1                  |                                 |                             |                              |                            |                     |
| Dog                            | Mongrels       | M                | 5.7-7.0 kg  | 1                  | Gelatin<br>capsules             | 400-<br>800                 |                              | 22                         |                     |
|                                |                | F                | 7.8-9.7 kg  | 1                  |                                 |                             |                              |                            |                     |
| Rabbit                         | Himalayan      | M                | 2.03 kg     | 5                  | Aqueous<br>solution             | 1557                        |                              | 24                         |                     |
|                                |                | F                | 1.59 kg     | 5                  |                                 | 2282                        |                              |                            |                     |
| <u>Intragastric</u>            | Rat            | FW49<br>Biberach |             |                    | 5                               |                             | 1560                         | (1380-1763)                | 20                  |
| <u>Intraduodenal</u>           | Rat            | FW49<br>Biberach |             |                    | 5                               |                             | 310                          | (274-350)                  | 20                  |

Table 1. (continued)

| <u>Route of Administration</u> | <u>Species</u> | <u>Strain</u> | <u>Sex</u>   | <u>Body Weight</u> | <u>No. of Animals Per Group</u> | <u>Drug Administration</u> | <u>LD<sub>50</sub> mg/kg</u> | <u>95% Fiducial Limits</u> | <u>References</u> |
|--------------------------------|----------------|---------------|--------------|--------------------|---------------------------------|----------------------------|------------------------------|----------------------------|-------------------|
| <u>Intravenous</u>             | Mice           | Chb1:         | M            | 19-24 g            | 10                              | Aqueous solution           | 12.29                        | (11.02-13.43)              | 20                |
|                                |                | NMKI          | F            | 17-22 g            | 10                              |                            | 14.97                        | (14.06-15.80)              |                   |
|                                | Mice *         |               | M            | 31-36 g            |                                 | Aqueous solution           | 17.18                        | (15.9-20.3)                | 24                |
|                                |                |               | F            | 26-29 g            |                                 |                            | 20.5                         | (17.5-24.0)                |                   |
|                                | Rat            | Chbb:         | M            | 220-250 g          | 10                              | Aqueous solution           | 15.90                        | (15.04-16.70)              | 18                |
|                                |                | THOM          | F            | 192-225 g          | 10                              |                            | 15.70                        | (14.48-16.99)              |                   |
|                                | Rat            |               | M            |                    | 5                               | Aqueous solution           | 16.5                         | (13.9-19.6)                | 19                |
|                                |                |               | F            |                    | 5                               |                            |                              |                            |                   |
|                                | Rat            | SDL-          | M            | 270 g              | 10                              | Aqueous solution           | 18.                          | (15.9-20.3)                | 24                |
|                                |                | JCL           | F            | 183 g              | 10                              |                            | 20.5                         | (17.5-24.0)                |                   |
| Dog                            | Beagles        | M             | 12.7-15.5 kg | 1                  | Aqueous solution                | 17.5-20.0                  |                              | 25                         |                   |
|                                |                | F             | 10 - 12 kg   | 1                  |                                 |                            |                              |                            |                   |
| <u>Subcutaneous</u>            | Rat            | FW49          |              |                    | 5                               |                            | 1500                         | (1090-2060)                | 20                |
|                                | Rat            |               | M            |                    | 5                               | Aqueous solution           | 1960                         | (1361-2822)                | 20                |
|                                |                |               | F            |                    | 5                               |                            |                              |                            |                   |
|                                | Dog            | Mongrel       | M            | 5.4-7.4 kg         | 1                               | Aqueous solution           | .71                          |                            | 23                |
|                                |                |               | F            | 5.4-10.5 kg        | 1                               |                            |                              |                            |                   |
|                                | Rat            | SD-JCL        | M            | 270 g              | 10                              | Aqueous solution           | 1250                         | (1106-1413)                | 24                |
|                                |                |               | F            | 183 g              | 10                              |                            | 1150                         | (865-1530)                 |                   |
|                                | Mice           | ICR-JCL       | M            | 34 g               | 10                              | Aqueous solution           | 650                          | (542-780)                  | 24                |
| F                              |                |               | 27 g         | 10                 | 470                             |                            | (373-592)                    |                            |                   |
| <u>Intraperitoneal</u>         | Rat            | SD-JCL        | M            | 270 g              | 10                              | Aqueous solution           | 240                          | (205-281)                  | 24                |
|                                |                |               | F            | 183 g              | 10                              |                            | 210                          | (160-275)                  |                   |
|                                | Mice           | ICR-JCL       | M            | 34 g               | 10                              | Aqueous solution           | 94                           | (83.2-106.2)               | 24                |
|                                |                |               | F            | 27 g               | 10                              |                            | 72                           | (62.6-82.8)                |                   |

Table 1. (continued)

| <u>Route of Administration</u> | <u>Species</u> | <u>Strain</u>  | <u>Sex</u> | <u>Body Weight</u> | <u>No. of Animals Per Group</u>                                                    | <u>Drug Administration</u>                                           | <u>LC<sub>50</sub> or LD<sub>50</sub></u>                      | <u>95% Fiducial Limits</u> | <u>References</u> |        |
|--------------------------------|----------------|----------------|------------|--------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------|----------------------------|-------------------|--------|
| Inhalation                     | Rat            | CD-1           | M          | 170-197            | 6                                                                                  | Suspension in inert propellant                                       | > 11.5 ug/L <sub>i</sub> for 4 1-hr. exposures/7 days          |                            | 26                |        |
|                                |                |                | F          | 170-182            | 6                                                                                  |                                                                      |                                                                |                            |                   |        |
|                                | Rat            | FW-49 Biberach | M          | 180-200 g          | 5                                                                                  | Aerosol with 0.05 and 0.1% soy lecithin                              | > 3200 ug (20 ug/stroke for 160 strokes)                       |                            | 27                |        |
|                                |                |                | F          | 180-200 g          | 5                                                                                  |                                                                      |                                                                |                            |                   |        |
|                                | Rat            | Chbb: THOM     | M          | 200-               | 7                                                                                  | Aerosol with Atrovent <sup>R</sup> and Benzalkonium chloride, 0.025% | > 120 mg of Atrovent <sup>R</sup> and Benzalkonium for 8 hours |                            | 28                |        |
|                                |                |                | F          | 245 g              | 8                                                                                  |                                                                      |                                                                |                            |                   |        |
|                                | Guinea Pigs    |                |            | M                  | 210-380 g                                                                          | 6                                                                    | Atomized 4 & 8% solution for 1/2, 1, 2, 4 and 6 hrs.           | > 200 mg/kg for 5 hours    |                   | 30, 45 |
|                                | Guinea Pigs    | Colored        | M          | 210-380 g          | 6                                                                                  | Aerosol 4 & 8% solution for 1/2, 1, 2, 4 and 6 hrs.                  | > 18 mg/kg/6 hr. 199 mg/kg/5 hrs. Lethal                       |                            | 29                |        |
|                                | Monkey         | Rhesus         | M          | 2.1-3.0 kg         | 1                                                                                  | Metered aerosol with propellant; 100, 200 & 400 mcg/day/5 days       | > 400 mcg/day/5 days                                           |                            | 31                |        |
|                                |                |                | F          | 2.2-3.1 kg         | 1                                                                                  |                                                                      |                                                                |                            |                   |        |
| Monkey                         | Rhesus         | M              | 3.8-4.6 kg | 1                  | Aerosol 500 ug twice daily/7 days                                                  | > 500 ug/2X/day/7 days                                               |                                                                | 32                         |                   |        |
|                                |                | F              | 3.3-4.1 kg | 1                  |                                                                                    |                                                                      |                                                                |                            |                   |        |
| Monkey                         | Rhesus         | M              | 2.5        | 1                  | Aerosol 30-40 puffs/guedal airway or nasal catheter, 20 ug sodium fluorescein/puff | Fluorescent material present in tracheal bronchial tree              |                                                                | 33                         |                   |        |
|                                |                | F              | 2.60 kg    | 1                  |                                                                                    |                                                                      |                                                                |                            |                   |        |

See pages 3-5, Vol. 1, 22 of NDA for the references.

OBSERVATIONS:

1. No significant differences between 24 hour or 14 day LD<sub>50</sub>s.
2. Toxic symptoms in rodents were apathy, reduced motility, varying degrees of paralysis of skeletal muscles, slight to moderate tonic convulsions and chronic type of spasms just prior to death, labored breathing, and respiratory failure preceded cardiac arrest.  
Dogs showed a pronounced increase in heart rate and a fall in rectal temperature which lasted up to 6 hours after oral and iv doses with long lasting mydriasis and drying of mucosa (anticholinergic action), sedation, and ataxia, and the death was due to cardiac or circulatory failure.
3. Toxicity was 6 times greater by intraduodenal administration than after sc administration, ~~which~~ that was similar to atropine (Ref. 20).

4. INHALATION STUDIES:

COMMENTS: The following reviews are from the previous reviews in our file with reference number if possible.

a) Acute Inhalation Toxicity in the Rat Using Sch 1000 (Ref. 25):

Rats Sch 1000 (1/24/75 review)

# animals/dose level: 6M + 6F with 12 controls

Dose: 11.5 mcg/hr (46.0 mcg day)

ADMINISTRATION AND DURATION:

Inhalation 4 times daily for 1 hour each 7 day study

RESULTS

Clinical Signs: No abnormalities were seen during exposure or post exposure.

Food Consumption - Food consumption was similar in both groups.

Bodyweight - Body weights were similar for both groups.

Freon Analysis - A freon buildup was noted in the blood 50 minutes after of exposure.

The maximum blood level was 78 mcg/ml after 50 minutes.

Terminal studies - At autopsy, no gross pathological changes were noted.

b) Metered Aerosol with Sch 1000 acute inhalation toxicity in Rats

Rats Sch 1000 (Ref. 7)

# animals/dose level: 5 M & 5 F

dose: 20-300 mcg/day

administration and duration: Inhalation administration at 5, 20, 40, 80, and 160 strokes, each stroke delivered 20 mcg of Sch 1000. Thirteen day observation period.

Results:

All animals survived this test. No incompatibility was observed. No morphological examinations were carried out.

c) The Deposition of Inhaled Fluorescein - Sodium Aerosol in Primate Lung

Rhesus Monkey, (M. Mulatta) Sch 1000

# animals/dose level: 1F & 1M.

dose: 600-800 mcg/day

administration and duration:

Sch 1000 administered by Guedal airway or by polyethylene Catheter.  
Animals given 30-40 puffs within 5-6 minutes.

Results:

Animals survived tests, and then were sacrificed and areas of trachea, lung, and bronchiolus were examined by fluorescence microscopy. All tissues examined showed fluorescein deposits. No difference was noted between the 2 methods of delivering Sch 1000.

d) The Acute Inhalation toxicity of Sch 1000 to Primates

Rhesus Monkeys (M. mulatta) Sch 1000

# animals/dose level: 2F & 2M.

dose levels: 1000 mcg/day.

administration & duration

Inhalation - Guedal airway + polyethylene catheter, 2 x daily.  
7 day test

Results:

Clinical signs

No adverse effects to the repeated inhalation were noted.

Body weight Body weights of these animals were unremarkable

Food Consumption Food consumption for all animals was comparable.

Laboratory Investigations

Hematology Hematology tests carried out pre and post exposure showed all values within normal limits.

Biochemistry Two female monkeys in the treated group had high levels of  $\text{Na}^+$ ; however 1 monkey in the control group also had a high level of  $\text{Na}^+$ .

Fluorescein Na aerosol had same "particle size distribution and aerodynamics properties" similar to those of Sch 1000 aerosol.

Sponsor suggested that condition was not thought to be drug related. One male monkey in the treated group had a high glucose value and an abnormal SAP value.

#### Urinalysis

All urine specimen were considered normal except for 1 female monkey in the treated group. The urine of this monkey contained blood pigments and red blood cells, not thought to be drug related.

#### Electrocardiograms

Pre and post electrocardiograms showed values to be within normal limits.

#### Freon levels in the blood

Relatively high levels of Freon-11 were found in the blood of the test animals after the first dosing on the third day. The half life of Freon was found to be approximately 3 minutes. No Freon-12 or 114 was detected in any of the samples. Freon 11 levels were detected to be higher in the Guedal airway group than in the nasal catheter group, 28.3 mcg/ml to 9.35mcg/ml.

#### Terminal Studies

No abnormalities were observed in these animals which were considered to be drug-related.

#### e) The Deposition of inhaled Fluorescein - Sodium aerosol in Rat Lung

|                      |                                                              |
|----------------------|--------------------------------------------------------------|
| Rats                 | Sch 1000                                                     |
| # animals/dose level | 3F                                                           |
| dose;                | 5.71 mcg/g, 11.5 $\frac{\mu\text{g}}{\text{g}}$ plus control |

#### Administration and Duration

Inhalation administration for 20 and 40 minutes, spray of 20 mcg every 15 second.

RESULTS: Fluorescent material was found in lumen of the trachea of the animals. However more material was deposited in the trachea in 20 minutes treated animals than in 40 minute treated ones. Results unexplainable. Minimal deposits of the material was found in the lung parenchyma of animal exposed for 40 minutes. Animals were evaluated using fluorescence microscopy. Fluorescein Na aerosol had same "particle size distribution and aerodynamic properties" similar to those of Sch 1000 aerosol.

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g) GUINEA PIGS: ACUTE INHALATION (Boehringer Sohn), 5/12/71: (Ref. 20):

(11/2/78 review)  
6 M/group were exposed to aerosol chambers of 4% and 8% aqueous solutions for 30 min., 1, 2, 4, and 6 hours with chamber pressure of 1.5 at. and the air mixture flow of 5-5.9 liters/min. All the animals were kept under observation for 14 days after exposure.

RESULTS:

The results showed that a 4% solution was well tolerated up to a dose of 18 mg/kg/6 hours, assuming 50% uptake. The 8% solution was well tolerated for 30 min and 1 hour, but after 2 hours breathing became troubled and rapid. After 4 hours, coughing was noted, but all animals survived.

When animals were exposed to 4 times of 8% solution, 2 out of 3 died after 4.5-5 hours (199 mg/kg/5 hours), and the third, survived animal, displayed severe respiratory distress and rapid breathing during the test. Macroscopic examination showed no pathological findings, but histopathology revealed slight emphysema and hyperemia of the lungs in two dead ones.

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COMMENTS ON ACUTE TOXICITY STUDIES:

1. IV LD<sub>50</sub>s in M and F mice were reported as 17 and 18 respectively in 3/23/83 submission, but Table 1 for Ref. 24 shows 17.13 and 20.5. Why different?
2. There was no submission in IND for 1.12 for Ref. 23...  
*etc*

6) Study of Acute Inhalant Tolerability of an Atrovent Inhalation Solution with Benzalkonium Chloride (BZ-Cl) and a BZ-Cl in Rats (Translation): (Ref. 27):

(5/4/81 review)

Lab. Performing the Study: C. H. Boehringer Sohn, Germany.

Date of the Study: 6/7/78

Study Material: Solution 1: 25 mg of Sch 1000-Br & 25 mg of BZ-Cl in 100 ml of demineralized water at pH 3.4 with HCl.

Solution 2: Same as Solution 1 except excluding Sch 1000-Br.

Solution 3: 0.9% NaCl solution.

Strain: Chbb: THOM(SPF) albino rats of 200-245 g

No of Animals/Level: 16 (3 M & 3 F ?)

Method: 16 rats were exposed, "head (nose and mouth) only" to inhalation solution (aerosol) in restraint cages in a special chamber for 8 hrs, to each solution on the same day with disinfection between 2 sessions with 1% aqueous solution of Korsolin (p. 1). and 3 rats of each group were subjected to necropsy and histological examination 16 hrs after the treatment, while the remaining 2 rats were followed up for 14 days and necropsied. Another 16 rats served as untreated controls (Group 0).

- Results:
1. The 90% of the aerosol generated in the chamber used was reported as 1 and 2 microns.
  2. The amount of inhalation solution per rat was reported as 60 ml (15 mg of Sch 1000-Br or BZ-Cl) and a total, 480 ml/8 hours.
  3. It was estimated that the amounts of Sch 1000-Br and BZ-Cl absorbed during 8 hours were about 4 mg/kg/8 hours based on body weight, respiratory volume, active ingredient concentration, and that the rat absorbed 50% of the amount of active ingredient inhaled.
  4. It was reported that observation of the rats during and after the acute inhalation study showed no intolerance to any of 3 solutions tested.
  5. Necropsy findings and histological study results will be reported later (by Dept. of Exp. Pathology and Toxicology).

COMMENT: It was only a summary type of report without any raw data.

6) Study of Acute Inhalant Tolerability of an Atrovent Inhalation Solution with Benzalkonium Chloride (BZ-Cl) and a BZ-Cl in Rats (Translation) (Ref. 2):  
(5/4/81 review)

Lab. Performing the Study: C. H. Boehringer Sohn, Germany.

Date of the Study: 6/7/78

Study Material: Solution 1: 25 mg of Sch 1000-Br & 25 mg of BZ-Cl in 100 ml of demineralized water at pH 3.4 with HCl.

Solution 2: Same as Solution 1 except excluding Sch 1000-Br.

Solution 3: 0.9% NaCl solution.

Strain: Chbb: THOM(SPF) albino rats of 200-245 g

No of Animals/Level: 16 (8 M & 8 F ?)

Method: 16 rats were exposed, "head (nose and mouth) only" to inhalation solution (aerosol) in restraint cages in a special chamber for 8 hrs, to each solution on the same day with disinfection between 2 sessions with 1% aqueous solution of Korsolin (p. 1). and 8 rats of each group were subjected to necropsy and histological examination 16 hrs after the treatment, while the remaining 8 rats were followed up for 14 days and necrosied. Another 16 rats served as untreated controls (Group 0).

- Results:
1. The 90% of the aerosol generated in the chamber used was reported as 1 and 2 microns.
  2. The amount of inhalation solution resp. inhaled was reported as 60 ml (15 mg of Sch 1000-Br or BZ-Cl) and total, 480 ml/8 hours.
  3. It was estimated that the amounts of Sch 1000-Br and BZ-Cl absorbed during 8 hours were about 4 mg/kg/8 hours based on body weight, respiratory volume, active ingredient concentration, and that the rat absorbed 50% of the amount of active ingredient inhaled.
  4. It was reported that observation of the rats during and after the acute inhalation study showed no intolerance to any of 3 solutions tested.
  5. Necropsy findings and histological study results will be reported later (by Dept. of Exp. Pathology and Toxicology).

COMMENT: It was only a summary type of report without any new data.

**II. SUBACUTE TOXICITY STUDIES:**

**2. Subacute Toxicity: (Boehringer)**

Species: Rat

Duration and Route: 3 weeks, PO (diet)

Schedule:

| <u>Dose (mg/kg)</u> | <u>= animals/group</u> |
|---------------------|------------------------|
| 0                   | 20                     |
| 10                  | 40                     |
| 100                 | 40                     |
| 500                 | 60                     |

Note: 0 dose rats were left untreated for an additional 4 weeks after the study was terminated, then sacrificed.

Mortality: 4 hi, 1 control

Body weight (weekly) was decreased in mid dose F (28%) and high dose M (20%). Food consumption (twice weekly) was correspondingly decreased in these animals. Water intake, determined on four occasions during the study, was normal. Hematological parameters, determined three times during the study, were normal. Blood glucose, SGPT, BUN, and SAP were within normal limits. Terminal qualitative and microscopic urinalysis were not unusual. Organ weights were not remarkable. No drug-induced histological alterations were noted.

**3. Subacute Toxicity: (Boehringer)**

Species: Rat

Duration and Route: Four weeks, SC, 7 days/week

Schedule:

| <u>Dose (mg/kg)</u> | <u>= animals/group</u> |
|---------------------|------------------------|
| 0                   | 20                     |
| 1                   | 40                     |
| 10                  | 40                     |
| 100                 | 60                     |

Note: 15M and 15F at 0 dose were maintained without treatment for 5 weeks after the study was completed then sacrificed

Mortality: One mid and 1 hi dose

Body weight was decreased in a dose-related manner; 23% in hi dose M and F. Food consumption was correspondingly decreased in early part of the study only. Substantial recovery occurred in non-treatment period. Water intake was not consistently altered. Variation in segmented cell values were due to inflammation and infection at site of injection. This parameter showed subsequent recovery after discontinuation of drug in hi dose animals kept for 5 additional weeks. Other hematological values were within normal limits as were usual clinical chemistries. Terminal qualitative and microscopic urinalysis of hi dose animals were normal. Organ weights were not remarkable.

The following histological changes appeared to be drug induced:

|                                                                               | % Incidence |    |     |    |
|-------------------------------------------------------------------------------|-------------|----|-----|----|
|                                                                               | Control     | Lo | Mid | Hi |
| Inflammation and necrosis at injection site *usually accompanied by hair loss | 0           | 3  | 3   | 60 |
| Depletion of birefringent** compounds in adrenals                             | 0           | 0  | 0   | 7  |
| Hemorrhagic necrosis of adrenals **                                           | 0           | 0  | 0   | 3  |

\* In hi dose rats carried 5 additional weeks, most of necrosis healed.  
\*\* Said to be due to inflammatory site reactions.

Of the two animals that died, one showed paralytic of stomach and intestine with bloody inhibition; dilated urinary bladder found in the other.

Other Comments: Distension of abdomen with gas was noted in early part of the study in hi dose M.

C. Subacute Toxicity: (Institute for Industrial and Biological Research).

Dog (Beagle): Four animals/level

Duration and Route: Eight days for low dose and 4 days for high dose; PO, (capsules), 7 days/week.

Dose: 150 or 300 mg/kg.

Mortality: None.

Body weight and food intake were decreased. No behavioral effects were noted. Mydriasis, dryness of nasal and oral mucosa and conjunctivitis occurred. Occasional vomiting was seen.

Subacute Toxicity: (Institute for Industrial and Biological Research).

Dog (Beagle): 3M, 3F/level

Duration and Route: Thirty days, PO (capsules), 7 days/week.

Dose: 0, 3, 30, 150-300 mg/kg (increased at beginning of 4th week).

Mortality: Three hi dose after dose was increased.

Body weight and food intake, decreased gradually, were decreased in M especially at hi dose. Hematological values were within normal limits. Some clinical chemistries, in addition to serum electrolytes, were also normal. Qualitative and microscopic urinalysis were unremarkable. Urine pH, determined several times during the study, was decreased in one dog that died shortly thereafter but were generally difficult to evaluate because determinations were made at different times after dosing. Many organs of hi dose dogs showed increase in relative weight.

All dogs exhibited mydriasis, dry nasal and oral mucosa while purulent conjunctivitis was noted in 2 hi dose dogs. Occasional vomiting and weakness were also seen. Histological observations such as eye and oropharyngeal inflammation due to decreased secretions were attributed to pharmacologic effects of the drug.

D. Subacute Toxicity: (Institute for Industrial and Biological Research)

Dog (Beagle): 3M, 3F/level

Duration and Route: One month, SC, injection site random, 7 days/week.

Dose: 0, 10, 20\*, 30-40 \* mg/kg (increased after 25th day).

\* On day 24 of dosing these animals received 1000 mg/kg accidentally: 6/12 died (3/6 at mid and 3/6 at hi dose). Survivors received no injection on day 25 and normal dosing was resumed from day 25 to end of study.

Body weight was decreased in all treated dogs, especially, at hi dose and food intake was correspondingly decreased in these animals. Hematological parameters, measured on several occasions during the study, were unremarkable. Heart rate, determined at various intervals, was unremarkable. Organ weight deviations were difficult to assess because of weight loss that occurred. Relative liver and kidney weights of dogs that died from overdose were increased. Histopathology: Congestion of portal spleen, pancreas and kidneys were noted in animals that died from overdose. Tissue in all animals were examined microscopically. The following changes seemed noteworthy:

|                                                | Incidence |    |     |    |
|------------------------------------------------|-----------|----|-----|----|
|                                                | Control   | Lo | Mid | Hi |
| <b>Liver</b>                                   |           |    |     |    |
| Congestion with narrowing of vascular cells    | 0         | 17 | 33  | 33 |
| Fatty Droplets                                 | 0         | 17 | 33  | 33 |
| Aggregates of histiocytes and fibroblasts      | 0         | 17 | 33  | 33 |
| <b>Thymus</b>                                  |           |    |     |    |
| Loss of distinction between cortex and medulla | 0         | 17 | 33  | 33 |
| <b>Eye</b>                                     |           |    |     |    |
| Inflammation of cornea and/or sclera           | 0         | 33 | 33  | 33 |
| <b>Adrenals</b>                                |           |    |     |    |
| Vacuolization                                  | 0         | 33 | 33  | 33 |

Other Comments:

Generally, behavior was normal. Extreme mydriasis was noted in treated dogs in addition to dry nasal and oral mucosa and irritated and inflamed conjunctiva.

E. Subacute Toxicity: (Institute for Industrial and Biological Research)

This study was performed due to mortality that occurred in SC study conducted by this same laboratory and reported above.

Dog (Beagle): 3/level

Duration and Route: 1 month SC, 7 days/week, injection site random.

Dose: Twenty, 30-40 mg/kg (increased in 4th week).

Mortality: One hi dose on day 22 of study.

Body weight, determined weekly, was markedly decreased especially at hi dose, and food intake was correspondingly reduced. Hematological parameters were normal. The usual clinical chemistries, including serum electrolytes and qualitative and microscopic urinalysis, were within normal limits. Heart rates revealed no correlation with dose or time. In the dog that died hi relative organ weights were noted (due to weight loss and agonal congestion?) and relative testicular and epididymus weights were low (underdeveloped).

Histopathology: Hypocemia was noted in the dog that died. Other alterations were: Reduction of thymus tissue 3/3 hi dose, and infiltration of cornea 1/3 hi dose.

Other Comments: Death was preceded by tremor, incoordination, ataxia, and vomiting. Pupillary dilation, conjunctival injection, and nasal mucosa, and inflammation of conjunctiva were noted.

Acute Toxicity: (Institute for Industrial and Biological Research)

Dog (Beagle): 2M, 2F/level

Duration and Route: Thirty-five days, SC (back of back, two sites), 7 days/week.

Dose: (1) Ten mg/kg from day 1-21, day 22, regular dose and 25 mg/kg 2 hours later; From day 23-35 daily dose was 40 mg/kg.

(2) Twenty-five mg/kg.

Mortality: None

Body Weight (daily): was reduced in both groups accompanied by decrease food intake.

E. Subacute Toxicity: (Institute for Industrial and Biological Research)

This study was performed due to mortality that occurred in SC study conducted by this same laboratory and reported above.

Dog (Beagle): 3/level

Duration and Route: 1 month SC, 7 days/week, injection site random.

Dose: Twenty, 30-40 mg/kg (increased in 4th week).

Mortality: One hi dose on day 22 of study.

Body weight, determined weekly, was markedly decreased especially at hi dose, and food intake was correspondingly reduced. Hematological parameters were normal. The usual clinical chemistries, including serum electrolytes and qualitative and microscopic urinalysis, were within normal limits. Heart rates revealed no correlation with dose or time. In the dog that died hi relative organ weights were noted (due to weight loss and apical congestion?) and relative testicular and epididymus weights were low (underdeveloped).

Histopathology: Hyperemia was noted in the dog that died. Other alterations were: Reduction of thymus tissue 3/3 hi dose, and infiltration of pancreas 1/3 hi dose.

Other Comments: Death was preceded by tremor, hunched posture, and vomiting. Pupillary dilation, conjunctival injection, and nasal mucosa, and inflammation of conjunctiva were noted.

Subacute Toxicity: (Institute for Industrial and Biological Research)

Dog (Beagle): 2M, 2F/level

Duration and Route: Thirty-five mg/kg SC (skin of back, two sites), 7 days/week.

Dose: (1) Ten mg/kg from day 1-21, day 22, regular dose and 25 mg/kg 2 hours later. From day 23-35 daily dose was 40 mg/kg.

(2) Twenty-five mg/kg.

Mortality: None

Body Weight (daily): was reduced in both groups accompanied by decrease food intake.

Other Comments:

At both doses, extreme pupil dilation occurred after 1st dose. Dryness of oral and nasal mucosa and conjunctivitis were noted. After dose was increased on day 29, instability, restlessness, impairment of vision, prostration, trembling, hypersensitivity, retching, and diarrhea were observed.

F. Subacute Toxicity: (Woodard)

Monkey: 1M, 1F/level.

Duration and Route:

Animals received drug by inhalation: Five minute exposures every 2 hours, 5 times/day, 5 days. Total daily dose was: Propellant mixture control, 100, 200 or 400 mcg/kg/day. Beaker placed over a monkey's head. Aerosol sprayed through hole in beaker into animals nose and mouth.

Mortality:

None.

Body weight and food intake were not altered. EKG (Lead 2), heart rate and BP were normal. Physical examination including eyes was normal. No behavioral changes were noted. Gross pathology did not reveal any abnormalities.

G. Subacute Toxicity: (Woodard)

Monkey (Rhesus): Six /level

Duration and Route: Forty-two days, inhalation

Dose: Propellant mixture control, vehicle control, 200, 400, 800, 1600 mcg/day.

Route: Two hour exposure, 5 times/day, 7 days/week - same technique as above.

Body weight and food consumption were normal. EKG (prior to dosing and after), heart rate, respiratory rate, body temperatures and neurological and ophthalmological examinations were within normal limits. Except for decreased Hgb, RBC and WBC values, in monkey that died, other treated values were unremarkable. Complete clinical chemistries and qualitative and microscopic urinalysis were also not unusual. Organ weights were also within normal range. Histological alterations were not related to drug. Two special studies were performed. One was surface tension which was found to be comparable in all monkeys. Second was determination of ciliary activity which was somewhat increased in treated animals as compared to controls but difference was short of significance.

**H. FOUR-WEEK ORAL TOXICITY STUDY IN RABBITS: E83-122:**

Lab. Performing the Study: The same as above.

Strain: Himalayan = small Russian breed.

No. of Animals/Level: 8 M + 8 F except that the high dose group had additional 6 M + 6 F recovery group with 6 weeks untreated period.

M = 1954 ± 208 g, 15 weeks old

F = 1818 ± 212 g 15 weeks old

Dosage Levels: 0, 4, 40, or 400 mg/kg (Batch No. 1/74)

COMMENTS: 4 mg/kg = about 3 times clinical dose of 1.5 mg/kg day.

400 mg/kg = 1/4 LD50.

Duration and Route: For 4 weeks (from 6/17 to 8/30/74) by stomach tube in 5 ml/kg in distilled water

Clinical Observation: All but 2 rabbits (M 25, F 36, F 37) suffered from anorexia, ataxia, and ruffling up of coat in high dose, 400 mg/kg group.

Mortality: Drug related death occurred only in high dose group: 12 M out of 13 + 9 F out of 14 died as shown in the following Table, with only two F left in the recovery group (F 36 + 37).

| sex | week | 1 | 2 | 3 | 4 | total |
|-----|------|---|---|---|---|-------|
| M   | 13   | 1 | 9 | 2 | 0 | 12    |
| F   | 14   | 0 | 4 | 4 | 1 | 9     |

Death was caused by "circulatory failure."

There were 4 accidental deaths also (1 F in control, 1 F in low, 1 M in mid, and 1 M in high dose group). See pages 13-14.

Food and Water Consumptions:

A severe, highly significant ( $p < 0.01$ ) depression of food consumption was seen in high dose group M during first 3 weeks, but the only surviving M of this group had "outstanding" consumption during the last week (=week 4). F showed dose-related and significant ( $p < 0.05$  in mid dose and  $p < 0.01$  in high dose group) depression during dosing period. See page 34. A highly significant ( $p < 0.01$ ) depression in water consumption in both M and F during the 4 week dosing period in high dose group only. See page 35.

Growth:

Both M and F high dose group lost body weight (significantly in M with  $p < 0.01$ ) during dosing period. Only M survivor in the high dose group started to regain some weight during 3rd week. F survivors (5) started gain weight during week 4 and gradually exceeded the original weight. See pages 32, 22, 23.

Comments: it was reported that due to only 1 M + 5 F including 2 F recovery group in the high dose group, the statistical analysis on hematology parameters were impossible, and there were no significant differences between groups.

Hematology:

Hb, RBC, reticulocyte and PCV of both M + F in the high dose group were significantly lower than respective control group. See pages 24-25 & 36.

Prothrombin Time:

The high dose group showed a decrease.

Death was caused by "circulatory failure."

There were 4 accidental deaths also (1 F in control, 1 F in low, 1 M in mid, and 1 M in high dose group). See pages 13-14.

Food and Water Consumptions:

A severe, highly significant ( $p < 0.01$ ) depression of food consumption was seen in high dose group M during first 3 weeks, but the only surviving M of this group had "outstanding" consumption during the last week (=week 4). F showed dose-related and significant ( $p < 0.05$  in mid dose and  $p < 0.01$  in high dose group) depression during dosing period. See page 34. A highly significant ( $p < 0.01$ ) depression in water consumption in both M and F during the 4 week dosing period in high dose group only. See page 35.

Growth:

Both M and F high dose group lost body weight (significantly in M with  $p < 0.01$ ) during dosing period. Only M survivor in the high dose group started to regain some weight during 3rd week. F survivors (5) started gain weight during week 4 and gradually exceed the original weight. See pages 32, 22, 23.

Comments: It was reported that due to only 1 M + 5 F including 2 F recovery group in the high dose group, the statistical analysis on hematology parameters were impossible, and there were no significant differences between groups.

Hematology:

Hb, RBC, reticulocyte and PCV of both M + F in the high dose group were significantly lower than respective control group. See pages 24-25 & 36.

Prothrombin Time:

The high dose group showed a decrease.

COMMENTS: Some values in hematology and coagulation time showed more changes in the control group than in other treated groups. See pages 36-47.

Clinical Chemistry:

Only M in the high dose group did not show any significant differences from the control M, so did the recovery (F) group.

Three F in the high dose group had significantly ( $p < 0.01$  or  $p < 0.05$ ) increased SGPT, cholesterol "(3 times normal value)", and SGOT, but decreased total protein (hypoalbuminemia) and  $Ca^{++}$ . See pages 49.

Organ Weight:

Significant decrease in organ weight of heart, lung, liver, kidney, ovary, thymus, (in M + F) and prostate (in M) survivor of high dose group; and heart, thymus and prostate in mid dose group M also. Adrenal gland was increased in mid and high dose group M only. See pages 55-56. No significant differences in the recovery group and control group.

Dissection Data:

Group 0 (control)

N.A.D.

Group 1 (4 mg/kg)

M 12 Agenevia testis et epididymis sinister

Group 2 (40 mg/kg)

M 21 Hypertrophy of testes and accessory  
sex glands

F 19 Focal pneumonia

F 24 Obstipation

Group 3 (400 mg/kg)

M 26 Obstipation

M 27 Enteritis catarrhalis

M 29 Enteritis catarrhalis et peritonitis  
fibrinosa

M 30 Dilatation of intestine

M 31 Obstipation, cachexia

M 32 Enteritis catarrhalis

M 33 Enteritis catarrhalis

M 34 Enteritis catarrhalis

M 35 Cachexia

M 36 Obstipation

M 37 Cachexia

M 38 Cachexia

F 15 Cachexia

F 27 Enteritis catarrhalis

F 28 Enteritis catarrhalis

F 30 Cachexia

F 33 Enteritis catarrhalis

F 34 Cachexia

F 38 Round heart, enteritis catarrhalis,  
dilatation of intestine

histopathology:

The following reports were submitted as drug related changes in the high dose group, in addition to "regular findings" on pages 15-16.

1. At 400 mg/kg dose the rabbits mostly have shown marked findings of liver ( $F_2$  and  $F_3$ ), namely
- a) a big droplet-like peripheral fatty change of lobules of the liver (M 26, 31, 36, 38, F 27, 28, 29, 33, 34); or
  - b) a severe big droplet-like diffuse fatty change including completely all lobules. In the latter cases the hepatic parenchymal cells are so loaded with fat that they appear totally cleared. Only the portal area indicates the hepatic origin in tissue sections (M 27, 28, 32, 34, F 26, 35, 38).

Mesenchymal reactions are missed in these cases.

2. On second place a diffuse fatty change ( $F_2$ ) of the myocardium was seen more often (M 27, 28, 31, 32, 35, 37, 38, F 26, 33, 34, 35, 38). The fat is dispersed within the muscle fibers in extremely fine vacuoles and degenerative damages of nuclei occur.
3. In the kidneys fatty change could be observed in the cytoplasm of some proximal convoluted tubules in several animals either sex at 400 mg/kg dosage only. The fat vacuoles surrounding the nuclei of the epithelial cells ( $F_2$ ).
4. Post portem changes may prohibit the microscopical proof of slight inflammatory lesions of intestine, as supposed by the diarrhea seen at autopsy in animals of high dosage. "

**1) FOUR WEEK ORAL TOXICITY STUDY IN RATS: E83-123:**

Lab Performing the Study: Same as above.

Strain: SD-JCL Sprague-Dawley

No. of Animals/Level: 15 M + 15 F except that the high dose group had an additional 10 M + 10 F recovery group with 6 weeks untreated period.  
M = 187 ± 17 g, F = 154 ± 10 g, both 6 weeks old.

Dosage Level: 0, 12, 120, or 1200 mg/kg/day  
Sch 1000 (Batch No. 1/74).

Duration and Route: For 4 weeks by stomach tube in 1.0 ml/100 g in distilled water.

Mortality: Only 1 M died in the mid dose group, but 9 M + 4 F in the high dose group died during the study.

Clinical Signs: Mid dose group (120 mg/kg) showed a slight weakness. Rats in high dose group were very weak with ruffling up of coat, red incrustated eyes and nose, and ataxia, especially during the first week, and they disappeared during the first week of recovery period.

Growth: Body weight gain of treated groups was generally significantly ( $p < 0.01$  in M in two high dose groups and  $p < 0.05$  in F high dose group) depressed from the first week and also dose-related especially in M.

See pages 21 and 31.

Food Consumption: Both M and F of the treated groups had generally less food consumption than respective controls and significantly ( $p < 0.05$ ) less in the high dose group during the first week. But F of the high dose group had significantly higher consumption during weeks 2-4, due to decreased consumption in the control F, and M in the same group had lesser food than control M only during week 3. See page 32.

Water Consumption:

Both M and F of the high dose group had significantly ( $p < 0.01$ ) higher water consumption during weeks 2 to 4 over control group or other 2 groups. See page 33.

COMMENTS: Page numbers for the results of each parameter were missing in the RESULTS section of this study. See pages 14-15.

Hematology:

Some values were significantly different from the control values, but they were reported as "in normal range". See pages 14 and 34-45.

Coagulation Time:

Prothrombin time showed no difference between groups.

Clinical Chemistry:

Although some values were significant, no consistent dose-related or time-related changes were seen. See pages 46-57.

Urinalysis:

No dose-related changes were reported. See pages 58-80.

Organ Weight:

It was stated that "parallel to the decrease of body weight of male and female rats at 1200 mg/kg dose all weights of the main parenchymatous organs, as heart, liver, kidneys and endocrine glands, decrease significantly too. To the contrary, the weight of the adrenals increases in both sexes."

The difference was generally at  $p < 0.01$  in M (see page 81) and  $p < 0.05$  in F (see page 82), and most of them showed a trend of dose-relationship.

Dissection Data:

Group 0 (control)

M 4 necroses of liver  
M 10 hydronephrosis  
F 9 hydrometra

Group 1 (12 mg/kg)

N.A.D.

Group 2 (120 mg/kg)

M 36 kidney infarct  
F 39 hydronephrosis

Group 3 (1200 mg/kg)

M 50 cachexia, obstipatio, edema pulmonum  
M 52 cachexia  
M 53 edema pulmonum  
M 54 meteorism  
M 56 gastric dilatatio, obstipatio of colon,  
edema pulmonum  
M 59 obstipatio, pneumonia  
M 60 cachexia, enteritis catarrhalis, pneumonia  
M 69 cachexia, enteritis catarrhalis, pneumonia  
F 53 enteritis catarrhalis, pneumonia  
F 54 enteritis catarrhalis  
F 68 cachexia, enteritis catarrhalis

Histopathology:

It was reported that "except the atrophy of male accessory sex glands following loss of body weight at high dosage, no dose-related changes occur."

Dissection Data:

Group 0 (Control)

M 4 necroses of liver  
M 10 hydronephrosis  
F 9 hydrometra

Group 1 (12 mg/kg)

N.A.D.

Group 2 (120 mg/kg)

M 36 kidney infarct  
F 39 hydronephrosis

Group 3 (1200 mg/kg)

M 50 cachexia, obstipatio, edema pulmonum  
M 52 cachexia  
M 53 edema pulmonum  
M 54 meteorism  
M 56 gastric dilatatio, obstipatio of colon,  
edema pulmonum  
M 59 obstipatio, pneumonia  
M 60 cachexia, enteritis catarrhalis, pneumonia  
M 69 cachexia, enteritis catarrhalis, pneumonia  
F 53 enteritis catarrhalis, pneumonia  
F 54 enteritis catarrhalis  
F 68 cachexia, enteritis catarrhalis

Histopathology:

It was reported that "except the atrophy of male accessory sex glands following loss of body weight at high dosage, no dose-related changes occur."

For the detailed summary report,  
see pages 16-18 and 93 to the end  
of the report.

FOUR-WEEK INHALATION STUDY IN RATS: E83-124:

Lab. Performing the Study: Name as above.

Strain: SD-JCL albino rats.

No. of Animals/Level: 12 M + 12 F for all 4 groups except  
that the high dose group had an  
additional 6 M + 6 F for "recovery  
group"

M = 178 ± 17 g, F = 144 ± 11 g, both 6  
weeks old.

Dosage Levels:

| Groups         | Dose Group               |                             |                      |
|----------------|--------------------------|-----------------------------|----------------------|
|                | number of aerosol puffs* | period of inhalation (min.) | number of rats       |
| D <sub>0</sub> | 05                       | 10                          | M 1-12<br>F 1-12     |
| D <sub>1</sub> | 10                       | 10                          | M 13-24<br>F 13-24   |
| D <sub>2</sub> | 50                       | 10                          | M 25-36<br>F 25-36   |
| D <sub>3</sub> | 300                      | 10                          | M 37-54**<br>F 37-54 |

- \* One aerosol puff contains 0.02 mg Sch 1000
- \*\* In the high dose group 6 animals of both sexes were observed untreated for 6 weeks following the end of substance administration (recovery group).

The content of Batch No. 31229 was follows:

| follows:                                                                                       | stated content<br>in g/aerosol | total content<br>aerosol incl.<br>overfill |
|------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------------------|
| Sch 1000, micronized                                                                           | 0.0060                         | 0.00690                                    |
| Soja-lecithine standardized                                                                    | 0.0105                         | 0.01210                                    |
| Monofluorotrichloromethane                                                                     | 0.4200                         | 0.48300                                    |
| <u>Propellant gas mixture:</u>                                                                 |                                |                                            |
| Monofluorotrichloromethane/<br>Difluorodichloromethane/Tetra-<br>fluorodichloroethane 23:54:23 | 20.5635                        | 23.64800                                   |

Control group received placebo (Batch No. 40224).

Duration and Route:

Inhalation for 4 weeks (9/23-10/25/74, and recovery group for additional untreated 6 weeks until 12/6/74. See pages 4 and 22 for drug administration procedure.

Clinical Signs:

High dose group had "unrest and slight ataxia" immediately after drug administration but "recovered within half a minute".

Growth:

Body weight gain of the high dose group (M + F) was significantly ( $p < 0.05$ ) less only during the first week, but the same group gained more than the respective control group in later weeks, without dose relationship. See pages 23-26.

Food and Water Consumption:

Food consumption of treated groups was significantly ( $p < 0.01$ ) higher than the control group except high dose F, but only during the first week, and it was attributed to the "comparatively low intake of the control groups." See page 27.

No differences between groups in water consumption were reported. See page 28.

Hematology:

It was reported that "hematological values throughout were in normal range." See pages 29-44.

COMMENTS: Some of the values fluctuated a lot; for example, reticulocytes of M of all 4 groups decreased to less than 25 at week 4 from 80-140 from week 0 (on page 32) although none of them were reported as significantly different.

Coagulation Time:

There were no significant differences between groups in prothrombin time.

Clinical Chemistry:

Slight but significant ( $p < 0.01$  or  $0.05$ ) decrease in total protein and cholesterol values in all three treated F were attributed to rather high control values at week 4, and there were no-dose related changes. See pages 15 and 45-49. Glucose value in F high dose group and  $Ca^{++}$  in M high dose group were slightly but significantly ( $p < 0.05$ ) higher than respective control values. See page 50. The high glucose was attributed to the "technique of blood sampling", and the high  $Ca^{++}$  value was reported as "of no biological relevance," due to the fact that some of week 0 values were also significant. See pages 14-15 and 45-60.

COMMENTS: 1.

Some of control values fluctuated so much that the data submitted may not be reliable, as was the case in Hematology above.

2.

Mistake on line 4 from the bottom of page 15: "increased" values of cholesterol should be changed to "decreased".

Urinalysis.

No significant dose-related changes were reported.

Organ Weight:

Absolute weight of pituitary gland of all treated M were significantly ( $p < 0.01$ ) smaller, than that of control M, but not dose-related, and the difference was attributed to rather high control value. See page 80.

COMMENTS: Some of the values fluctuated a lot; for example, reticulocytes of M of all 4 groups decreased to less than 25 at week 4 from 80-140 from week 0 (on page 32) although none of them were reported as significantly different.

Coagulation Time:

There were no significant differences between groups in prothrombin time.

Clinical Chemistry:

Slight but significant ( $p < 0.01$  or  $0.05$ ) decrease in total protein and cholesterol values in all three treated F were attributed to rather high control values at week 4, and there were no-dose related changes. See pages 15 and 45-49. Glucose value in F high dose group and  $Ca^{++}$  in M high dose group were slightly but significantly ( $p < 0.05$ ) higher than respective control values. See page 50. The high glucose was attributed to the "technique of blood sampling", and the high  $Ca^{++}$  value was reported as "of no biological relevance," due to the fact that some of week 0 values were also significant. See pages 14-15 and 45-60.

- COMMENTS: 1. Some of control values fluctuated so much that the data submitted may not be reliable, as was the case in Hematology above.
2. Mistake on line 4 from the bottom of page 15: "increased" values of cholesterol should be changed to "decreased".

Urinalysis:

No significant dose-related changes were reported.

Organ Weight:

Absolute weight of pituitary gland of all treated M were significantly ( $p < 0.01$ ) smaller, than that of control M, but not dose-related, and the difference was attributed to rather high control value. See page 80.

Necropsy:

Urinary calculi <sup>were</sup> ~~was~~ seen in an F(24) in low dose group and perisplenitis fibrosa in a M (25) in mid dose group.

Histopathology:

No drug induced changes were reported. For the detailed report, see page 17-18 and 92-101.



JK. Toxicity Subacute (from 1/24/75 review)

The Toxicity of Rats to Sch 1000 administered by inhalation over a period of 30 days

Rats ( Charles Rivers)

Sch 1000

# animals/dose level:

10F + 10 M

dose level: 21,600 mcg/da, 14,400 mcg/da and 7,200 mcg/da, plus control and vehicle groups. administration and duration

Inhalation - 20 mcg spray every 5 seconds for 15 minutes. Animals were exposed 15 minutes, 6,4,2 times a day in the high, medium and low dose groups respectively.

30 day study

Results:

Clinical Signs

Animal behavior appeared normal after an adjustment period.

Mortalities

Nine animals (all groups) died during this study. Animals deaths were not drug-related.

Food Consumption

Food consumption of the animals was normal following an initial drop in consumed food during the first week.

Body Weight

Animals had an initial weight loss during the first week. Animals (majority) gained weight during the final 3 weeks of the study.

Ophthalmoscopy

Examinations carried out pre-exposure and at termination recorded no abnormalities that could be attributed to treatment with Sch 1000.

Laboratory Investigations

Hematology Values obtained pre-exposure and at termination revealed no significant changes due to Sch 1000.

Biochemistry Biochemical values obtained for these animals were within normal limits.

Urinalyses

All values were within normal limits at both the pre-exposure and at termination.

Macroscopic Pathology

No morphological changes were observed which were considered to be drug induced.

Subacute Toxicity Study in Male Dogs

Dogs, beagles

Sch 1000

# animal/dose level

4 M - 6 M in control group.

Dose level: 1.5, 3.0 and 15.0 mg/kg

administration and duration: Oral administration, 7 days weekly for 13 weeks.

**Results:**

Clinical Signs

All animals survived the tests. Test animals exhibited dryness of mouth, mydriasis and inhibition of lacrimation. Vomiting and diarrhea was seen in several animals of every group.

Body Weight

Dogs in 15.0 mg/kg group showed a slight weight loss. All other dogs had comparable body weights.

Food Consumption

Food Consumption of the dogs in treatment groups was not significantly different from the control group.

Hematology

The erythrocyte sedimentation rate of 2 dogs in the 3.0 mg/kg groups were slightly accelerated during weeks 0 and 6. All other values for all dogs were within normal range.

Biochemistry

No significant differences were noted between the treated groups and the control group.

Urinalysis

Values for urine specimen were within normal limits.

Ophthalmoscopic

Examination of the eyes of these dogs showed that the animals in the 3.0 and 15.0 mg/kg groups had drug induced keratoconjunctivitis sicca. Daily rinsing with 3% boric acid with applications of aureomycin and Vitamin A ointment healed this condition in 5 of the 8 dogs in the abovementioned groups. Histological investigations showed that keratitis sicca is due to a dose-dependent inhibitory effect of Sch 1000 on lacrimation.

Autopsy Findings

Several dogs in the control group as well as the 3.0 and 15.0 mg/kg had slight lipomatosis Cordis, mainly in the right ventricle. The livers of all the dogs in this study had parasitic or bacterial granulomas. In this test, 1 dog in each of the control group and the 1.5 mg/kg group had reduction in spermatogenesis. This condition was also noted in 1 dog of the 3.0 mg/kg and 2 dogs in

the 15.0 mg/kg group. In several of these dogs the reduction was bilateral. In 2 control dogs, 6 dogs of the 1.5 and .3.0 mg/kg groups and 1 dog in the 15.0 mg/kg a partial atrophy of the glandular epithelium of the prostate was observed.

M. Subacute

1) Inhalation Study in the Rat  
(Huntingdon Research Center,  
Huntingdon, England)

In this study, 5 samples of the upper and lower trachea, bronchus and lung parenchyma were taken from each of 3 males and 3 females (rat) for electron microscopic examination. However, study can not be evaluated because dose levels, administration and duration are not included in the submission. Apparently, these particulars were submitted earlier; however, I can not locate them.

2) Inhalation Study in Monkeys

Electron Microscopy Report  
(C.H. Boehringer Solm, Germany)

This is another study in which samples were taken from trachea, bronchus bronchiolus and lung parenchyma of the monkey for electron microscopic examination again, important details of the study are missing.

N. 6-WEEK INHALATION STUDY ON RATS:

No. of Animals/group: 15 M + 15 F (average wt: M=290 g, F=225 g)  
Strain: Albino SPF rats of Chbb:THOM(SPF)  
Age at the beginning: M=64 days, F=78 days, + 2 days

| Dosage Level: | Group          | No. of whiffs/min | Actual dose (mg/ |
|---------------|----------------|-------------------|------------------|
|               | 0 (Handling)   | -                 | -                |
|               | 1 (propellant) | 36                | -                |
|               | 2              | 12                | 0.17             |
|               | 3              | 12                | 0.34             |
|               | 4              | 36                | 1.0              |

Duration in chamber: all 120 min.

Duration and Route: 6-weeks by inhalation (5/10/77-6/27/77)

|                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Growth Effect:</b>      | There was no difference. 2 rats from each group except group 2 showed decrease in body weights with unknown reasons.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Food Consumption:</b>   | There was no difference.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Mortality:</b>          | One of group 1 and 2 of group 2 died, but the death seemed to be not drug related. No deaths were seen in groups 3 and 4.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Hematology:</b>         | There were no drug induced changes.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Coagulation Test:</b>   | No test was performed as such.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>Clinical Chemistry:</b> | GPT of groups 2 and 4 females and GOT of all females were slightly increased, and glucose of females of three treated groups was decreased, but they were not dose-related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Urinalysis:</b>         | No dose related change was seen.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Organ Weights:</b>      | There was no dose related change.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Gross pathology:</b>    | There were some changes such as pleural adhesion or small brownish-red miliary foci of lung in all groups except group 2, but those changes were not dose related.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Histopathology:</b>     | <p>The examination was performed by Prof. K. Paulini of the Pathological Institute, Clinics of the Universtiy of Mainz. There was no dose related change. Common findings were bronchiectases, foam-cell-rich interstitial pneumonia, slight and various eosinophilia of heart muscle fibers, dysatelectases, PAS-positive cytoplasm of interstitial tissue and alveoli, fatty infiltration of hepatocytes, siderosis of spleen reticulum cells, and calcium-iron incrustations of distal tubules of females.</p> <p>Glandular cysts of the gastic mucosa found in 5 rats of group 1 and dilatation of the bladder found in 6 rats of group 3 were distinct. Other findings were numerous, but they were found in 1-3 rats of each group. The single finding of retinal atrophy in group 4 may be worth mentioning.</p> |

○. 13 WEEK INHALATION TOXICITY STUDY IN DOGS WITH SCH 1000-BR "POWDER  
AEROSOL" (11/9/83-review)

Lab. Performing the Study:

C.H. Boehringer Sohn

(Dr. Kollmer, 12/15/79)

Strain:

Beagle dogs

No. of Animals/Level:

3 M + 3 F

(M = 13.5 kg and 14 months, and  
F = 11.5 kg and 13 months in  
average).

Dosage Level:

| Groups | Dose in $\mu\text{g kg}^{-1}$ |                      | No. of dogs |   | Animal No.         |
|--------|-------------------------------|----------------------|-------------|---|--------------------|
|        | nominal                       | actual <sup>a)</sup> | m           | f |                    |
| C      | placebo                       |                      | 3           | 3 | 001-003<br>051-053 |
| 1      | 10                            | 7                    | 3           | 3 | 101-103<br>151-153 |
| 2      | 50                            | 36                   | 3           | 3 | 201-203<br>251-253 |
| 3      | 250                           | 179                  | 3           | 3 | 301-303<br>351-353 |

a) the nominal dose is the intended dose and the total dose administered; the actual dose was calculated by subtracting from the total amount administered the substance residue demonstrated by quantitative analysis in the inhalation apparatus.

"The experimental animals received Sch 1000-BR on inspiration in the form of a powder aerosol at the same time each day (between 8.00 and 15.00 h). Animals in groups 2 and 3 received Sch 1000-BR powder capsules containing 0.2 mg Sch 1000-BR. The adjustment of the dosage in accordance with body weight was carried out using capsules containing 0.04 mg Sch 1000-BR in animals from all the treated groups. Control animals received an aerosol with placebo capsules containing only glucose."

Justification of the dose: See pages 10-11.

Batch Nos: 88121 and 88122 for capsules containing 0.2 mg Sch-1000 and 25 mg glucose

88125 for capsules containing 0.04 mg Sch-1000 and 25 mg glucose

78119 for placebo capsule containing 25 mg glucose

Duration and Route: 13 weeks: Started on 4/17/78 for M and on 4/18/78 for F and ended on 7/18/78

The drug was introduced in the form of a powder aerosol directly into the trachea with inspiration. See inhalation apparatus on page 8.

Mortality: None

Growth: There were no significant differences between groups, although F showed a slight trend of depression.

COMMENTS: 1.

Some page numbers were missing or illegible.

2.

No group mean values were given.

Food Consumption: No significant differences between groups were reported.

Clinical Symptoms and Behavior: M and F exhibited dryness of the nose in group 2 (50 ug/kg/day) and dryness of the nose and oral mucosa in group 3 (250 ug/kg/day), and it lasted 1-2 hours in 50 ug/kg/day group and 4-5 hours in 250 ug/kg/day group.

Incidence of diarrhea and emesis were reported on pages 023 and 024, but there was no dose or time relationship.

Hematology:

There were no significant differences between groups.

COMMENTS:

Values were presented for weeks -2, 1, 6, and 13.

Myelogram (Bone marrow):

No dose or time related changes were reported for bone marrow investigation. (See page 111-114).

Coagulation Time:

No significant differences between groups were noted for partial thromboplastin time, thromboplastin time, or thrombin time.

Clinical Chemistry:

Na<sup>+</sup> of 3 treated groups was statistically significantly higher than control group, but only at week 6, and without dose-relationship. Cholesterol of 3 treated groups was slightly higher than control group at week 13, but without dose-relationship.

Urinalysis:

There were no significant differences between groups.

Organ Weights:

There were no significant differences between groups in selected organ weight, (The high dose group had larger weight of left ovary, but not significant.)

COMMENTS:

It was reported that the weight of the lung was inadvertently omitted.

Hematology:

Incidence of diarrhea and  
emesis were reported on pages  
023 and 024, but there was no  
dose- or time relationship.

There were no significant  
differences between groups.

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weeks -2, 1, 6, and 13.

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differences between groups.

Organ Weights:

There were no significant  
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high dose group had larger  
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significant.)

COMMENTS:

It was reported that the  
weight of the lung was  
inadvertently omitted.