

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-007

PHARMACOLOGY REVIEW(S)

INTEROFFICE MEMO

TO: NDA 22007
FROM: C. Joseph Sun, Ph. D., Supervisory Pharmacologist
Division of Pulmonary and Allergy Products
DATE: March 6, 2007

I concur with pharmacologist's recommendation that pharmacology and toxicology of formoterol fumarate inhalation solution have been adequately studied and the drug product is approvable from a preclinical standpoint.

Preclinical Pharmacology and toxicology assessment of formoterol fumarate is primarily based on a prior FDA finding of safety and effectiveness for Foradil Aerolizer (NDAs 21831 and 21279). The applicant conducted a 14-day inhalation study to compare product performance or toxicity of the inhalation solution with the approved dry powder formulation.

Pharmacology: Formoterol fumarate is a selective long-acting beta-2 agonist that possesses bronchospasmolytic activity. It has been demonstrated to bind to beta-2 receptors in several in vitro and in vivo studies models.

General toxicity: One-month inhalation study in dogs and chronic inhalation toxicity study (one year) in rats and oral toxicity study (one year) in dogs were conducted. Cardiac toxicity, common to beta-adrenergic receptor agonist, was observed in general toxicity studies in rats and dogs. The cardiotoxicity appeared to be consequence of pharmacological activity that increased heart rate. The effects were determined to be clinically monitorable and would not be an issue. Other target organs of toxicity identified in rats were testes, spleen, salivary gland, nasal cavity and lungs. Given the extensive nonclinical and clinical experience, safety margin of these findings observed in rats is considered adequate. There were no apparent differences in toxic effect between the inhalation solution and the approved dry powder formulation in a 14-day inhalation study with rats, indicating no differences in product performance of these two formulations.

Reproductive toxicity: No impairment of fertility was observed in rats following oral administration. It was not teratogenic in rats and rabbits treated with oral doses. Delayed ossification and/or decreased fetal weight were observed in rats. Formoterol has been found to be teratogenic in rats and rabbits in other laboratories and therefore such effect should be mentioned in the labeling. It has shown to cause stillbirth and neonatal mortality when given to rats during the late stage of pregnancy. Thus, pregnancy category C is appropriate.

Genotoxicity: Formoterol was negative in Ames test, chromosome aberration assay in human lymphocytes, mouse lymphoma assay and rat micronucleus assay.

Carcinogenicity: Carcinogenicity studies were conducted with mice and rats. In mice, adrenal subcapsular adenomas and carcinomas, hepatocarcinomas and uterine leiomyomas and leiomyosarcomas were observed. In rats, ovarian leiomyomas and theca cell tumors were observed.

Labeling: Carcinogenesis, mutagenesis and impairment of fertility and pregnancy category C sections have been incorporated with the above-mentioned preclinical findings.

There are no outstanding preclinical issues.

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/s/

Joseph Sun
3/6/2007 05:16:47 PM
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-007
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	06/29/06
PRODUCT:	Formoterol fumarate inhalation solution
INTENDED CLINICAL POPULATION:	Chronic obstructive pulmonary disease
SPONSOR:	Dey, L.P.
DOCUMENTS REVIEWED:	Electronic Submission
REVIEW DIVISION:	Division of Pulmonary and Allergy Products
PHARM/TOX REVIEWER:	Timothy W. Robison, Ph.D.
PHARM/TOX SUPERVISOR:	C. Joseph Sun, Ph.D.
DIVISION DIRECTOR:	Badrul Chowdhury, M.D., Ph.D.
PROJECT MANAGER:	Akilah Green

Date of review submission to Division File System (DFS): February 20, 2007

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval.

B. Recommendation for nonclinical studies

None

C. Recommendations on labeling

Recommendations for revisions of the applicant's proposed labeling are attached at the end of the review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

From a nonclinical perspective, safety of formoterol fumarate is primarily based upon a prior FDA finding of safety and effectiveness for Foradil[®] Aerolizer[™] (NDA 20-831 and NDA 21-279), as described in the drug's approved labeling. The applicant conducted a 14-day inhalation toxicology study with rats to compare product performance of formoterol fumarate inhalation solution with the approved dry powder formulation of formoterol fumarate.

A safety concern with formoterol fumarate, common to β -adrenergic receptor agonists, is the potential for cardiac toxicity that was observed in general toxicology studies with rats and dogs. Myocardial fibrosis was evident in male rats following inhalation exposure to formoterol at 400 $\mu\text{g}/\text{kg}/\text{day}$ (deposited dose of 40 $\mu\text{g}/\text{kg}/\text{day}$) for 1 year. In contrast, myocardial fibrosis was evident in dogs following inhalation exposure to formoterol at a dose as low as 3 $\mu\text{g}/\text{kg}/\text{day}$ (deposited dose of 0.6 $\mu\text{g}/\text{kg}/\text{day}$) for 1 month. The cardiotoxicity of β agonists appeared to be a consequence of pharmacological activity that increased heart rate. These effects were judged to be monitorable in a clinical setting and would not be an issue.

In a chronic inhalation toxicology study with rats, additional target organs of toxicity beside the heart were the testes, spleen, salivary gland, nasal cavity, and lungs. The NOAEL was identified as the lowest deposited dose of 3 $\mu\text{g}/\text{kg}/\text{day}$ based upon a lack of histopathology findings. The safety margin for the clinical dose of formoterol fumarate inhalation solution based upon the chronic inhalation toxicology study with rats is considered adequate given the extensive nonclinical and clinical experience with formoterol fumarate.

Formoterol fumarate administered throughout organogenesis did not cause malformations in rats or rabbits following oral administration. When given to rats throughout organogenesis, oral doses of 0.2 mg/kg and above delayed ossification of the fetus, and doses of 6 mg/kg and above decreased fetal weight. Formoterol fumarate has been shown to cause stillbirth and neonatal mortality at oral doses of 6 mg/kg and above in rats receiving the drug during the late stage of pregnancy; however, these effects were not produced at a dose of 0.2 mg/kg.

Formoterol fumarate was negative in a battery of genotoxicity tests to assess potential mutagenic and clastogenic activity. A total of four carcinogenicity studies were conducted with formoterol fumarate in mice and rats. In mice, adrenal subcapsular adenomas and carcinomas, hepatocarcinomas, and uterine leiomyomas and leiomyosarcomas were observed. In rats, ovarian leiomyomas and theca cell tumors were observed. There were no safety concerns for uterine and ovarian tumor findings in mice and rats given that β_2 -adrenergic agonists, such as formoterol, are known to produce tumors of the female rodent genital tract that have been judged to be class effects. There were no safety concerns for other tumor findings in mice given that there was an adequate safety margin between the dose without these tumor findings in mice and the proposed clinical dose.

In a 14-day inhalation toxicology study to compare product performance, there were no apparent differences in toxic effects between the applicant's formoterol fumarate inhalation solution and the approved formoterol fumarate dry powder.

B. Pharmacologic activity

Formoterol fumarate is a selective long-acting beta₂-adrenergic receptor agonist (beta₂-agonist). Formoterol has been extensively characterized in standard *in vivo* and *in vitro* models and has been shown to preferentially bind to beta₂-adrenergic receptors. The pharmacologic effects of beta₂-adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenylate cyclase. Increased intracellular cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially mast cells.

C. Nonclinical safety issues relevant to clinical use

None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-007

Review number: #01

Sequence number/date/type of submission: #000/June 29, 2006/Initial Submission

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Dey, L.P.

2751 Napa Valley Corporate Drive
Napa, CA 94558

Manufacturer for drug substance:

~~_____~~
b(4)

Merck Development Centre Private Limited,
Plot No. 1A/2, M.I.D.C. Industrial Estate,
Taloja, Panvel, Dist: Raigad, Maharashtra – 410208
DMF 19202

Reviewer name: Timothy W. Robison,
Division name: Pulmonary and Allergy Products
HFD #: 570

Review completion date: February XX, 2007

Drug:

Trade name: Formoterol fumarate inhalation solution

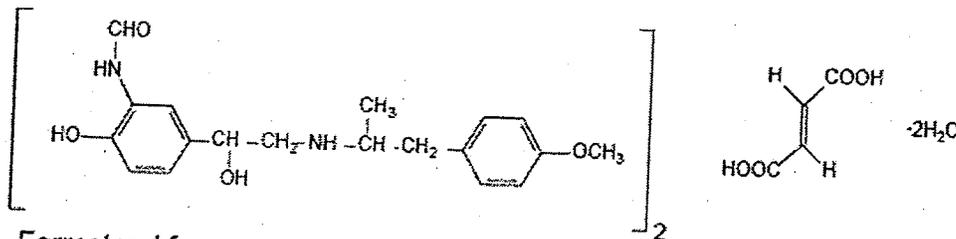
Generic name: Formoterol fumarate dihydrate

Chemical name: \pm 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl] formanilide fumarate dihydrate

CAS registry number:

Molecular formula/molecular weight: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$ / 840.92

Structure:



Relevant INDs/NDAs/DMFs:

b(4)

- IND _____
- IND 68,782 (Dey, Formoterol fumarate inhalation solution)
- NDA 20-831 (Foradil® Aerolizer™, Novartis Pharmaceuticals Corporation)
- NDA 21-279 (Foradil® Aerolizer™, Novartis Pharmaceuticals Corporation)

Drug class: β_2 -Adrenergic Agonist

Intended clinical population: Chronic Obstructive Pulmonary Disease (COPD)

Clinical formulation: Formoterol Fumarate Inhalation Solution 20 mcg/2 mL is a sterile, clear, colorless, _____ solution of the dihydrate form of formoterol fumarate, a racemic mixture of (R,R)- and (S,S)-enantiomers. Formoterol Fumarate Inhalation Solution, 20 mcg/2 mL is provided in a 2 mL single-use low-density polyethylene (LDPE) vial manufactured by _____ technology. Each vial contains 2 mL of a clear, colorless solution composed of 20 mcg of formoterol (as formoterol fumarate) in an isotonic, sterile aqueous solution containing citric acid, sodium citrate and sodium chloride. Each unit dose vial is overwrapped in a pre-printed foil laminate pouch and is packaged in cartons of _____ and 60 pouches.

b(4)

Table 3.2.P.1.1 Formulation Composition of Formoterol Fumarate Inhalation Solution 20 mcg/2 mL

INGREDIENT	FUNCTION	CONCENTRATION IN DRUG PRODUCT	AMOUNT PER VIAL (2 mL)
Formoterol Fumarate Dihydrate	Active	0.010 mg/mL*	0.020 mg*
Sodium Chloride, USP	_____	_____	_____
Sodium Citrate _____ USP			
Citric Acid _____ USP			

b(4)

* On an anhydrous basis

Route of administration: Inhalation (Nebulizer)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-007 are owned by Novartis or are data for which Dey has obtained a written right of reference. Any information or data necessary for approval of NDA 22-007 that Dey, L.P. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Dey, L.P. does not own (or from FDA reviews or

summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-007.

Studies reviewed within this submission: None

The following studies were provided in the NDA submission. They were reviewed under IND 68,782 (see attached reviews).

1. Maximum Tolerated Dose-Range-Finding Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate in Rats (Final Report).
2. 14-Day Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate Dihydrate in Rats (Final Report).

Studies not reviewed within this submission:

b(4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

See attached review of NDA 20-831.

2.6.2.2 Primary pharmacodynamics

See attached review of NDA 20-831.

2.6.2.3 Secondary pharmacodynamics

See attached review of NDA 20-831.

2.6.2.4 Safety pharmacology

See attached review of NDA 20-831.

2.6.2.5 Pharmacodynamic drug interactions

See attached review of NDA 20-831.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not provided by the sponsor.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

See attached review of NDA 20-831.

2.6.4.2 Methods of Analysis
See attached review of NDA 20-831.

2.6.4.3 Absorption
See attached review of NDA 20-831.

2.6.4.4 Distribution
See attached review of NDA 20-831.

2.6.4.5 Metabolism
See attached review of NDA 20-831.

2.6.4.6 Excretion
See attached review of NDA 20-831.

2.6.4.7 Pharmacokinetic drug interactions
See attached review of NDA 20-831.

2.6.4.8 Other Pharmacokinetic Studies
See attached review of NDA 20-831.

2.6.4.9 Discussion and Conclusions
See attached review of NDA 20-831.

2.6.4.10 Tables and figures to include comparative TK summary
See attached review of NDA 20-831.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY
Not provided by the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

See attached reviews of NDA 20-831 for the general toxicology of formoterol and IND _____ and IND 68,782 for bridging studies.

In a 4-day inhalation toxicology study, male rats were exposed to formoterol fumarate inhalation solution at total doses of 166, 499, and 1673 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder at total doses of 522 and 1692 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of formoterol fumarate inhalation solution were 11.6, 34.9, and 117.1 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Deposited doses of formoterol fumarate dry powder were 36.5 and 118.4 $\mu\text{g}/\text{kg}/\text{day}$, respectively. There were no treatment-related clinical signs, effects on body weight gain, serum Tropinin T levels, or target organs of toxicity. There were no apparent differences in toxic effects between formoterol fumarate inhalation solution

and formoterol fumarate dry powder. Dose selections for formoterol fumarate inhalation solution and formoterol fumarate dry powder were considered adequate. The high dose of formoterol fumarate inhalation solution was limited by the aqueous solubility of formoterol fumarate. The high dose of formoterol fumarate dry powder was approximately equivalent to the high dose used with innovator product.

In a 14-day inhalation toxicology study, 5 rats/sex/group were exposed to formoterol fumarate inhalation solution (FFIS) at total doses of 195, 584, and 1966 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder (FFD) at a total dose of 1966 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of FFIS were 13.7, 40.9, and 137.7 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The deposited dose of FFD was 121.5 $\mu\text{g}/\text{kg}/\text{day}$. There were no treatment-related effects on clinical signs, hematology, or clinical chemistry parameters. Body weight gain and food consumption were increased for formoterol treatment groups, which is an expected effect of B_2 agonists. Absolute and relative heart weights were increased for males in the mid dose FFIS, high dose FFIS, and high dose FFD groups and females in the high dose FFIS and high dose FFD groups. Histopathological evaluation of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates. These organs and tissues were previously identified as target organs in inhalation toxicology studies conducted with formoterol in rats. For the heart, cardiomyopathy was observed for 1 of 5 male rats in the high dose FFIS group. Myocardial fibrosis was previously reported in inhalation toxicology studies conducted with formoterol in rats. There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and formoterol fumarate dry powder.

Genetic toxicology:

See attached review of NDA 20-831.

Carcinogenicity:

See attached review of NDA 20-831.

Reproductive toxicology:

See attached review of NDA 20-831.

Special toxicology:

See attached review of NDA 20-831.

2.6.6.2 Single-dose toxicity.

See attached review of NDA 20-831.

2.6.6.3 Repeat-dose toxicity

See attached reviews of NDA 20-831, IND _____, and IND 68,782.

2.6.6.4 Genetic toxicology

See attached review of NDA 20-831.

2.6.6.5 Carcinogenicity

See attached review of NDA 20-831.

2.6.6.6 Reproductive and developmental toxicology

See attached review of NDA 20-831.

2.6.6.7 Local tolerance

See attached review of NDA 20-831.

2.6.6.8 Special toxicology studies

See attached review of NDA 20-831.

2.6.6.9 Discussion and Conclusions

See attached review of NDA 20-831.

2.6.6.10 Tables and Figures

See attached review of NDA 20-831.

2.6.7 TOXICOLOGY TABULATED SUMMARY

See attached review of NDA 20-831.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Formoterol Fumarate Inhalation Solution 20 µg/2 mL (FFIS) in the present application is indicated for long-term, twice daily administration in the maintenance treatment of bronchoconstriction in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and emphysema. Formoterol fumarate as Foradil® Aerolizer™ from Novartis was approved for the treatment of asthma (NDA 20-831) and chronic obstructive pulmonary disease (NDA 21-279).

From the product labeling, formoterol fumarate is a long-acting selective beta₂-adrenergic receptor agonist. Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. *In vitro* studies have shown that formoterol has more than 200-fold greater agonist activity at beta₂-receptors than at beta₁-receptors. Although beta₂-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta₁-receptors are the predominant receptors in the heart, there are also beta₂-receptors in the human heart comprising 10%-50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta₂-agonists may have cardiac effects. The pharmacologic effects of beta₂-adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from

cells, especially from mast cells. In vitro tests show that formoterol is an inhibitor of the release of mast cell mediators, such as histamine and leukotrienes, from the human lung. Formoterol also inhibits histamine-induced plasma albumin extravasation in anesthetized guinea pigs and inhibits allergen-induced eosinophil influx in dogs with airway hyper-responsiveness. The relevance of these in vitro and animal findings to humans is unknown.

From a nonclinical perspective, safety of formoterol fumarate is primarily based upon a prior FDA finding of safety and effectiveness for Foradil® Aerolizer™ (NDA 20-831 and NDA 21-279), as described in the drug's approved labeling. The applicant conducted a 14-day inhalation toxicology study with rats to compare product performance of formoterol fumarate inhalation solution with the approved dry powder formulation of formoterol fumarate.

Preclinical pharmacology and toxicology studies conducted with formoterol fumarate to support approval of Foradil® Aerolizer™ were reviewed under NDA 20-831 (see reviews dated June 1, 1998, March 13, 2000, April 25, 2000, April 26, 2000, April 27, 2000, and May 10, 2000; and a communication between Dr. Luqi Pei and Dr. Joseph DeGeorge, Chair of the Carcinogenicity Assessment Committee).

A safety concern with formoterol fumarate, common to β -adrenergic receptor agonists, is the potential for cardiac toxicity that was observed in general toxicology studies with rats and dogs. Increased heart rates as well as clinical signs that included reddening of the mouth and ventral surface were evident in animals treated with formoterol by the oral or inhalation routes. Myocardial fibrosis was evident in male rats following inhalation exposure to formoterol at 400 $\mu\text{g}/\text{kg}/\text{day}$ (deposited dose of 40 $\mu\text{g}/\text{kg}/\text{day}$) for 1 year. In contrast, myocardial fibrosis was evident in dogs following inhalation exposure to formoterol at a dose as low as 3 $\mu\text{g}/\text{kg}/\text{day}$ (deposited dose of 0.6 $\mu\text{g}/\text{kg}/\text{day}$) for 1 month. The cardiotoxicity of β agonists appeared to be a consequence of pharmacological activity that increased heart rate. Increased heart rate could be a consequence of direct interaction with β receptors in the heart, although, it was most likely a result of reflex tachycardia due to β_2 -mediated vasodilation and hypotension. Ischemic changes (i.e., focal necrosis and fibrosis) occur in oxygen deprived regions of the heart. The papillary muscle in the left ventricle appears to be extremely sensitive to oxygen deprivation. If tachycardia were required for cardiotoxicity, no effect would be expected with doses that do not increase heart rate. Clinical experience suggests that approved inhaled doses of the dry powder formulation as well as the proposed dose of formoterol fumarate inhalation solution only modestly increase heart rate. The large sustained increases of heart rate observed in dogs treated with formoterol do not appear to occur in a clinical setting. Further, these effects were considered to be monitorable in a clinical setting. Thus, development of formoterol was allowed to proceed despite the lack of a NOAEL for cardiac effects in dogs.

In a 6/12-month inhalation toxicology study with rats, additional target organs of toxicity beside the heart were the testes, spleen, salivary gland, nasal cavity, and lungs. The only histopathological finding at the 6-month interim sacrifice was degeneration of

seminiferous tubules in the testes for male rats in the high dose group. At 12-months, these changes in the testes were evident for male rats in mid and high groups. Testicular changes were still evident in mid and high dose groups following the 8-week recovery period. These histopathological changes at 6 and 12 months and at the end of the recovery period correlated with necropsy findings of an increased incidence of small or flaccid testes. For the lungs of female rats in the high dose group at 12 months, there was an increased severity of large foamy macrophages around the bronchioles. For the nasal cavity of male and female rats in the high dose group at 12 months, there was an increased incidence and severity of goblet cell proliferation in the anterior region. For the spleen of male and female rats in mid and high dose groups at 12 months, there was an increased incidence and/or severity of extramedullary hematopoiesis. For the salivary gland of male and female rats in the high dose group at 12 months, there was an increased incidence of very mild hypertrophy of serous acini. The NOAEL was identified as the lowest deposited dose of 3 µg/kg/day based upon a lack of histopathology findings. The safety margin for the clinical dose of formoterol fumarate inhalation solution based upon the chronic inhalation toxicology study with rats is smaller than normally desired (i.e., 10-fold), although it is considered acceptable based upon the extensive nonclinical and clinical experience with formoterol fumarate.

Safety margin for the clinical dose of formoterol fumarate inhalation solution in adult subjects

Species	Study Duration	Doses (Deposited Dose) µg/kg/day	NOAEL (Deposited Dose) µg/kg/day	Safety margin for the clinical dose of formoterol fumarate inhalation solution
				40µg/50kg = 0.8
Rat	6/12-months Dry powder	3, 12, & 40	3	3.8
Dog	4-Weeks Dry powder	0.6, 2.8, & 11	None ^a	

a. A NOAEL was not established in the 4-week inhalation toxicology study with formoterol in dogs based upon findings of increased heart rate and myocardial fibrosis at all doses. The cardiotoxicity of β agonists appeared to be a consequence of pharmacological activity related to increased heart rate. These effects were considered to be monitorable in a clinical setting.

Formoterol fumarate administered throughout organogenesis did not cause malformations in rats or rabbits following oral administration. When given to rats throughout organogenesis, oral doses of 0.2 mg/kg and above delayed ossification of the fetus, and doses of 6 mg/kg and above decreased fetal weight. Formoterol fumarate has been shown to cause stillbirth and neonatal mortality at oral doses of 6 mg/kg and above in rats receiving the drug during the late stage of pregnancy. These effects, however, were not produced at a dose of 0.2 mg/kg.

Formoterol fumarate was negative in a battery of genotoxicity tests to assess potential mutagenic and clastogenic activity. Four carcinogenicity studies were conducted with formoterol fumarate, drinking water and dietary studies in both mice and rats. In mice,

adrenal subcapsular adenomas and carcinomas, hepatocarcinomas, and uterine leiomyomas and leiomyosarcomas were observed. In rats, ovarian leiomyomas and theca cell tumors were observed. There were no safety concerns for uterine and ovarian tumor findings in mice and rats given that β_2 -adrenergic agonists, such as formoterol, are known to produce tumors of the female rodent genital tract that have been judged to be class effects. There were no safety concerns for other tumor findings in mice given that there was an adequate safety margin between the dose without these tumor findings in mice and the proposed clinical dose.

In a 14-day inhalation toxicology study to compare product performance, rats were exposed to formoterol fumarate inhalation solution (FFIS) at total doses of 195, 584, and 1966 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder (FFD) at a total dose of 1966 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of FFIS were 13.7, 40.9, and 137.7 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Histopathological evaluation of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates. These organs and tissues were previously identified as target organs in inhalation toxicology studies conducted with formoterol in rats. For the heart, cardiomyopathy was observed in 1 of 5 male rats in the high dose FFIS group. Myocardial fibrosis was previously reported in inhalation toxicology studies conducted with formoterol in rats. There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and the approved formoterol fumarate dry powder.

Conclusions: Based primarily upon a prior FDA finding of safety and effectiveness for Foradil[®] Aerolizer[™] (NDA 20-831 and NDA 21-279), there is a complete nonclinical pharmacology and toxicology program for the applicant's formoterol fumarate inhalation solution, which supports the safety of the proposed clinical dose of 20 μg BID.

Unresolved toxicology issues (if any): None.

Recommendations: From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval.

Evaluation of labeling:

The product labeling was adjusted to conform to labeling used for the recently approved Foradil[®] Certihaler[™].

The $\text{AUC}_{0-24 \text{ hr}}$ for a clinical dose of Formoterol fumarate inhalation solution at 20 μg BID was estimated to be 73 $\text{pg}\cdot\text{hr}/\text{mL}$ or 0.212 $\text{pmol}\cdot\text{hr}/\text{mL}$ (assuming linear pharmacokinetics from an $\text{AUC}_{0-24 \text{ hr}}$ of 445.1 $\text{pg}\cdot\text{hr}/\text{mL}$ obtained with a dose of 244 μg). A molecular weight of 344.45 for formoterol base was used to convert 73 $\text{pg}\cdot\text{hr}/\text{mL}$ to 0.212 $\text{pmol}\cdot\text{hr}/\text{mL}$.

From Review #03 of NDA 20-831 dated March 13, 2000, it was reported that a dose of 20 $\text{mg}/\text{kg}/\text{day}$ administered to male and female rats in the dietary carcinogenicity study resulted in AUC values of 547 and 418 $\text{nmol}\cdot\text{hr}/\text{L}$, respectively. The mean exposure for males and females combined was 483 $\text{nmol}\cdot\text{hr}/\text{L}$. Assuming linear pharmacokinetics,

exposures for dietary doses of 5 and 0.5 mg/kg/day were estimated as shown in the table below. Further, in Review #03, it was determined that pharmacokinetic data from an oral gavage study (Study B4/1991) could provide reasonable estimates of systemic exposure in the drinking water carcinogenicity study.

Systemic exposures in the rat dietary carcinogenicity study.

Dose, mg/kg/day	AUC, nmol/hr/L			Rat to human exposure ratios
	Male	Female	Mean	
20	547	418	483	2279
5 (Estimate)			120.75	570
0.5 (Estimate)			12.08	57

From Review #03 of NDA 20-831 dated March 13, 2000, it was reported that a dietary dose of 50 mg/kg/day administered to male and female mice resulted in AUC values of 113 and 202 nmole/hr/L, respectively. The mean exposure for males and females combined was 158 nmole/hr/L. Assuming linear pharmacokinetics, AUC values for dietary doses of 5 and 2 mg/kg were estimated as shown in the table below.

Systemic exposures in the mouse dietary carcinogenicity study.

Dose, mg/kg/day	AUC, nmol/hr/L			Mouse to human exposure ratios
	Male	Female	Mean	
50	113	202	158	746
20 (Estimate)			63	297
5 (Estimate)			15.8	74.6
2 (Estimate)			6.3	29.7

For a dose of 69 mg/kg/day in the 2-year drinking water study with mice, the AUC was estimated to be 218 nmol/hr/L based upon linear extrapolation from the dose of 50 mg/kg/day in the 2-year dietary carcinogenicity study with mice.

From other sections of the labeling, exposure margins between nonclinical doses and the clinical dose (20 µg BID) were determined by expressing doses on a mg/m² basis.

Drug: Formoterol Fumarate Inhalation Solution

		# daily							
age	mg/dose	doses	mg/day	kg	mg/kg	factor	mg/m ²		
Adult	COPD	0.02	2	0.04	50	0.0008	37	0.03	
		conv.			Dose Ratio				
route	mg/kg/d	factor	mg/m ²	Adults	Dose Ratio		Adults-Rounded		
Carcinogenicity:									
mouse	Oral	2	3	6	202.70			200	
mouse	Oral	5	3	15	506.76			500	
mouse	Oral	20	3	60	2027.03			2000	
mouse	Oral	50	3	150	5067.57			5100	
mouse	Oral	69	3	207	6993.24			7000	
rat	Oral	0.5	6	3	101.35			100	
rat	Oral	5	6	30	1013.51			1000	
rat	Oral	15	6	90	3040.54			3000	
rat	Oral	20	6	120	4054.05			4100	
Reproduction and Fertility:									
rat	Oral	3	6	18	608.11			600	
Teratogenicity:									
rat	Oral	0.2	6	1.2	40.54			41	
rat	Oral	6	6	36	1216.22			1200	
Overdosage:									
rat	inhalation	156	6	936	31621.62			32000	

Conversion, Correction, and Rounding Factors:

Human Age (yr)	Weight (kg)	Factor (kg/m ²)	Species	Factor (kg/m ²)	Exposure greater than x-times human	Round to nearest
0	3	25	dog	20	1	1
1	10	25	guinea pig	8	10	5
2	12	25	hamster	4	100	10
4	16	25	monkey	12	1000	100
6	20	25	mouse	3	10000	1000
12	50	37	rabbit	12		
			rat	6		

2 Page(s) Withheld

 Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

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Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
C. Joseph Sun, Ph.D.,

- cc: list:
NDA 22-007, HFD-570
GreenA, HFD-570
KaiserJ, HFD-570
SunC, HFD-570
RobisonT, HFD-570

APPENDIX/ATTACHMENTS

- Appendix 1 Review #01 of NDA 20-831 dated June 26, 1997
- Appendix 2 Review #01 of IND dated May 7, 2002
- Appendix 3 Review #01 of IND 68,782 dated January 2, 2004
- Appendix 4 Review #02 of IND 68,782 dated May 13, 2004
- Appendix 5 Review #03 of IND 68,782 dated January 25, 2005

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Appendix 1

Review #01 of NDA 20-831 dated June 26, 1997

**APPEARS THIS WAY
ON ORIGINAL**

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA
DIVISION OF PULMONARY DRUG PRODUCTS

BACKGROUND INFORMATION:

Reviewer Name: Tracey Zoetis, M.S.
Division Name: Division of Pulmonary Drug Products
HFD No. HFD-570
Review Completion Date: June 1, 1998
NDA No. 20-831 b(4)
Related INDs: -----
Serial Number: 000
Submission Date: June 26, 1997
Information to Communicate to Sponsor: See Recommendations
Sponsor or Agent: Novartis Pharmaceuticals Corporation
59 Route 10
Hanover, NJ 07936

Manufacturer: _____

DMF # _____

DMF# _____

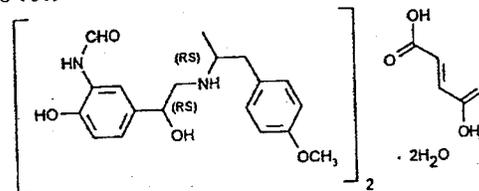
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DRUG

Code Name: Formoterol fumarate
Generic Name: Not applicable
Trade Name: Foradil®
Chemical Name: \pm 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]formanilide fumarate dihydrate
CAS Registry No.: 45229-80-7
Molecular Formula: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$
Molecular Weight: 840.9

Chemical Structural Formula:



Drug Class:

beta-2-adrenoceptor agonist

Indication: Prevention and maintenance treatment of bronchoconstriction in patients 6 years of age and older with reversible obstructive airways disease, including patients with symptoms of nocturnal asthma, and for the prevention of exercise-induced bronchospasm.

Clinical formulation (and components): Micronized Formoterol fumarate (_____ mg/capsule) _____ with lactose (to 25 mg/capsule), placed in hard gelatin capsules. b(4)

Route of Administration: Inhalation

Proposed clinical protocol or use: Formoterol fumarate will be administered twice daily via an _____^m oral inhalation device. The maximum daily dose is _____ mcg. b(4)

Previous clinical experience: This drug is marketed in two forms in several countries. The earliest approval for the aerosol solution was in September 1990 in Switzerland. The earliest approval for the Dry Powder Capsules was January 1993 in New Zealand. The aerosol solution form is currently marketed in Austria, Denmark, Dubai, Greece, Holland, Hong Kong, Israel, Italy, South Africa, Spain, Switzerland and Turkey. The Dry Powder Inhalation form is marketed in Austria, Denmark, Egypt, Finland, Holland, Ireland, Israel, Sweden, Switzerland, and the United Kingdom. It is available free of charge in New Zealand, in the absence of reimbursement.

Disclaimer: Some of the information contained in this review may have been obtained directly from the Sponsor's submission.

INTRODUCTION AND DRUG HISTORY

Information regarding this drug was originally submitted to the FDA when it was being developed in the form of an _____ by Ciba-Geigy i _____ (IND _____). Development of the drug shifted to a dry powder inhalation form and this application was submitted to the agency by Novartis Pharmaceutical Corporation in February 1995 (IND / _____). Several preclinical studies were reviewed during the course of drug development and those reviews were considered along with the current NDA submission. b(4)

STUDIES REVIEWED WITHIN THIS SUBMISSION

A comprehensive battery of studies were submitted to support the safety of Formoterol fumarate. The types of studies reviewed within this submission include:

- Pharmacokinetics/Toxicokinetics
- Toxicology
- Carcinogenicity
- Reproductive Toxicology
- Genetic Toxicology
- Special Toxicology (e.g., dermal sensitization and irritation studies).

In addition, safety pharmacology studies were considered and an overall summary is provided in this review.

Individual studies included in this submission and reviewed include the following.

Pharmacokinetics and Toxicokinetics

INDEX OF ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES INCLUDED IN THIS REVIEW

No.	Study Title	Study No.
1.	(Fomoterol fumarate): Disposition and metabolism of Fomoterol fumarate, a new bronchodilator, in rats and dogs	Sasaki et al. 1982
2.	Absorption, distribution, metabolism and excretion of formoterol fumarate (BD 40A): 1. Pharmacokinetics in the rat and dog	Kamimura et al. 1984
3.	Absorption and excretion of BD 40A in dogs	Hasegawa et al. 1983
4.	(Fomoterol fumarate): Pharmacokinetics in male and female rats; determination of formoterol in plasma of rats after a single peroral dose of Fomoterol fumarate, 12.5, 25, and 50 mg/kg body weight	Ackermann et al. 1991
5.	(Fomoterol fumarate): 2-week inhalation toxicity study in rats; determination of Fomoterol in urine of rats after pulsed inhalation exposure to Fomoterol fumarate.	Ackermann et al. 1986
6.	(Fomoterol fumarate): 3-month inhalation toxicity study in dogs; determination of Fomoterol in urine of dogs after daily inhalation of Fomoterol fumarate using metered dose aerosol packs	Ackermann et al. 1987
7.	(Fomoterol fumarate): 3 month inhalation toxicity study in dogs	— R 6/1991 supplement to 806161
8.	(Fomoterol fumarate): Absorption and disposition studies in mice	DM 1/1991
9.	Absorption and distribution of Fomoterol fumarate in rats	— F-1-4-1 906174
10.	Determination of Fomoterol in plasma of rats during administration of Fomoterol fumarate via drinking water for 3 months and after a single peroral dose (Dose levels 12.5, 25, and 50 mg/kg body weight at the end of the treatment)	
11.	Absorption and disposition studies in male and female rats after doses of 5 to 50 mg/kg [¹⁴ C]CGP 25827A	DM 2/1991
12.	Pharmacokinetics of the enantiomers of Fomoterol in plasma of male and female rats after single 10 mg/kg peroral administration of the enantiomers	— 1994/015

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No.	Study Title	Study No.
13.	3-month oral (via drinking water); pharmacokinetic study in rats; Determination of Fomoterol in plasma of rats after a single peroral dose (dose level: 12.5, 25, and 50 mg/kg body weight), following administration via drinking water for 3 months	916052
14.	Plasma concentrations of the unchanged drug following intravenous administration of BD 40B and BD 40A in dogs	F-1-4-16
15.	Absorption, distribution, metabolism and excretion of Fomoterol fumarate (BD40A): 3. Plasma concentrations and urinary excretion of unchanged drug after continuous administration in dogs	F-1-4-7
16.	Pharmacokinetics of the enantiomers of fomoterol in male dogs after single 0.3 mg/kg peroral administration of the enantiomers	1994/063
17.	Absorption, distribution, metabolism and excretion of Fomoterol fumarate (BD 40A): 4. Plasma Fomoterol and cAMP concentrations	F-1-4-8
18.	Fomoterol fumarate: metabolic fate after repeated oral administration of Fomoterol fumarate in rats	Sasaki et al 1983
19.	Absorption, distribution, metabolism and excretion of Fomoterol fumarate (BD 40A): 2. Binding of unchanged drug to plasma protein	F-1-4-6
20.	(Fomoterol fumarate): In vitro binding of fomoterol to serum proteins	R 41/1991
21.	The transpulmonary transport and metabolism of ³ H-CGP 25827A (Fomoterol fumarate) following intra-tracheal instillation to the isolated rat perfused lung	#93/02/C/2R
22.	Biotransformation of Fomoterol fumarate: Isolation and structure elucidation of metabolites formed in vitro in hepatocytes of rat, guinea pig, marmoset and dog identification of metabolites in human ex vivo biological samples	DM(EU)28/1996
23.	Evaluation of a new chemical entity, CGP 25827, as an inhibitor of human P450 enzymes	XT-0424496-25827, 1996-7804 supplement to 907172
24.	Plasma concentration of Fomoterol in mice from a 24-month carcinogenicity study with oral administration of CGP 25827A in the diet	43/1993 supplement to 907172
25.	Three-month oral (via drinking water) toxicity study in mice; determination of Fomoterol in plasma of mice during 3-month administration of Fomoterol fumarate via drinking water at concentrations of 0.25, 0.5 and 1 mg/ml water	B23/1992 supplement to 906303
26.	Three-month range finding toxicity study in mice; determination of fomoterol in plasma of mice after 1- and 3-month administration of Fomoterol fumarate in the diet at nominal dose levels of 0, 40, 140, 400 and 1400 ppm	B106/1990 supplement to 906171
27.	28-day palatability study in rats; determination of Fomoterol in plasma of rats after 28 day administration of Fomoterol fumarate in the diet at daily doses of 0, 0.4, 1.8, 4.5, and 18 mg/kg body weight	B 84/1990 supplement to 896027
28.	3-month range finding study in rats (administration in food). Determination of Fomoterol in urine and plasma of rats during 3 month administration of CGP 25827A in the diet at nominal daily doses of 0, 0.5, 2, 5, and 20 mg/kg body weight	B 68/1991 supplement to 906205
29.	24-month carcinogenicity study in rats; determination of Fomoterol in plasma and urine of rats during 24 month administration of CGP 25827A in the diet at nominal daily doses of 0, 0.5, 2, 5, and 20 mg/kg body-weight	B14/1992 supplement to 886178
30.	Determination of Fomoterol in urine, plasma and the respiratory tract of rats form an acute pulsed inhalation toxicity study with CGP 25827A dry powder formulation (1/69)	49/1993 supplement to 926108

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No.	Study Title	Study No.	
31.	Plasma concentrations and urinary excretion of Fomoterol in rats from a 28-day, repeated dose, pulsed inhalation toxicity study with CGP 25827A dry powder formulation	20/1993 supplement to 926111	b(4)
32.	Determination of Fomoterol in urine of rats during an 90 day repeated dose inhalation toxicity study with Fomoterol fumarate dry powder formulation	R 49/1991 supplement to 906154	b(4)
33.	Plasma concentrations and urinary excretion of Fomoterol in rats from a 6/12 month inhalation toxicity study with a lactose powder formulation of CGP 25827A (MDPI formulation, 1/73)	1996/061 supplement to 936115	
34.	3-month inhalation toxicity study in rats; determination of formoterol in urine of rats after daily inhalation of Fomoterol fumarate using metered dose aerosol packs	R 33/1992 supplement to 906274	b(4)
35.	Determination of Fomoterol in urine of dogs after twice daily inhalation of inhalable nebulized solution Fomoterol fumarate for 28 consecutive days	R31/1991 supplement to 906249	
36.	1 month comparative inhalation toxicity in dogs; determination of Fomoterol in urine of beagle dogs during 28 day administration of CGP 25827A by the inhalation route. Comparison of tow different batches of the solution aerosol formulation of CGP 25827A	B 57/1991 supplement to 90-6158	
37.	Urinary excretion and plasma concentrations of Fomoterol in dogs from a 4-week inhalation toxicity study with CGP 25827A dry powder formulation (1/69)	32/1993 supplement to 926074	b(4)
38.	13 week inhalation toxicity study in dogs; determination of formoterol in urine of beagle dogs during daily inhalation of CGP 25827A, dry powder formulation	B 65/1991 supplement to 906155	
39.	Plasma concentrations and urinary excretion of Fomoterol in dogs from a 6/12 month inhalation toxicity study with CGP 25827A lactose powder (MDPI formulation suspension 1/73)	(F) 1995/004 supplement to 936116	b(4)
40.	Plasma concentrations and urinary excretion of Fomoterol in dogs from a 13-week inhalation toxicity study with CGP 25827A as a suspension (HFA reformulation)	(F) 1995/016	
41.	Urinary excretion and plasma concentrations of the (S,S) enantiomer of formoterol in dogs from a 4-week inhalation toxicity study with CGP 29502A dry powder formulation (1/73)	1996/044 supplement to 936225	b(4)
42.	Urinary excretion and plasma concentrations of the (R,R) enantiomer of Fomoterol in dogs from a 4-week inhalation toxicity study with CGP 29503A powder formulation (1/73)	1996/045 supplement to 936226	b(4)
43.	Fomoterol fumarate. Absorption, distribution, metabolism and excretion of Fomoterol fumarate (BD40A): 7. Plasma concentrations and rate of urinary excretion in man	F-1-4-13	
44.	Fomoterol fumarate: Pharmacokinetics study with formoterol dry powder inhalation capsule via Aerolizer™	984008	

Toxicology

INDEX OF ACUTE TOXICOLOGY STUDIES INCLUDED IN THIS REVIEW

No.	Study Title	Study No.	
1.	Acute Oral Toxicity Study in Juvenile Rats	D-1-3	
2.	Acute Oral Toxicity Study in Chinese Hamster	825339	b(4)

No.	Study Title	Study No.
3.	Acute Oral Toxicity Study in Dogs	
4.	(Active Ingredient in Air/CFC Propellant): Acute Toxicity Study in Rats	846402
5.	(Active Ingredient in Air/CFC Propellant): Acute Inhalation Toxicity Study in Rats	841012
6.	(Active Ingredient in Air/CFC Propellant): Acute Inhalation Toxicity Study in Rats	841011
7.	(Active Ingredient in Air/CFC Propellant): Acute Inhalation Toxicity Study in Dogs	906238
8.	(Solution Aerosol): Acute Inhalation Toxicity Study in Dogs	855190
9.	(Solution Aerosol): Acute Comparative Inhalation Toxicity Study in Dogs	886174
10.	(Suspension Aerosol): Acute Inhalation Toxicity Study in Dogs	896160
11.	(Dry Powder Formulation 1:1000): Acute Pulsed Inhalation Toxicity Study in Rats	896183
12.	(Dry Powder Formulation 1:69): Acute Pulsed Inhalation Toxicity Study in Rats	926108
13.	Acute Toxicity Study in Mice and Rats (iv, ip, sc)	D-1-1
14.	Acute Intraperitoneal Study in Mice	D-1-1
15.	Acute Subcutaneous Study in Mice	D-1-1
16.	Acute Intravenous Toxicity Study in Rats	D-1-1
17.	Acute Intraperitoneal Study in Rats	D-1-1
18.	Acute Subcutaneous Toxicity Study in Rats	D-1-1
19.	Acute Intravenous Toxicity Study of Optical Isomers in Mice	D-1-4
20.	Acute Intravenous Toxicity Study of Decomposition Products in Mice	D-1-5

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INDEX OF REPEATED DOSE TOXICITY STUDIES

No.	Study Title	Study No.
1.	28-Day Palatability Study in Rats	896027
2.	13-Week (Feeding) Rangefinding Study in Rats	906205
3.	13-Week Oral (Drinking Water) Toxicity Study in Rats	906174
4.	3-Month Oral (Drinking Water) Toxicity Study in Rats	916052
5.	(Suspension Aerosol): Preliminary Inhalation Tolerance Study in Rats	906223
6.	(Dry Powder Formulation 1:1000): 13-Week Inhalation Toxicity Study in Dogs	906155
7.	(Dry Powder Formulation 1:69): 4-Week Inhalation Toxicity Study in Rats	926111
8.	(Dry Powder Formulation 1:1000): 13-Week Inhalation Toxicity Study in Rats	906154
9.	13-Week Inhalation Toxicity Study in Rats	906224
10.	(1:73 Powder Formulation): 26/52-Week Inhalation Toxicity Study in Rats	936115
11.	13-Week Feeding Toxicity Study in Mice	906171
12.	13-Week Drinking Water Toxicity Study in Mice	D-8-3
13.	13-Week Drinking Water Toxicity Study in Mice	906303
14.	52-Week Oral (Capsule) Toxicity Study in Dogs	850751
15.	(Dry Powder Formulation 1:69): Preliminary Inhalation Toxicity Study in Dogs	926109
16.	Inhalation Feasibility Study in Dogs	936077
17.	(Dry Powder Formulation 1:69) 4-Week Inhalation Toxicity Study in Dogs	926074
18.	(Solution Aerosol): 4-Week Comparative Inhalation Toxicity Study in Dogs	906158
19.	(1:73 Powder Formulation): 52-Week Inhalation Toxicity Study in Dogs	936116

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*Carcinogenicity***INDEX OF CARCINOGENICITY STUDIES INCLUDED IN THIS REVIEW**

No.	Study Title	Study No.
20.	104-Week Oral Carcinogenicity Study in Mice	906172
21.	24-Month Oral Carcinogenicity Study in Rats	886178

*Reproductive Toxicology***INDEX OF REPRODUCTIVE TOXICOLOGY STUDIES INCLUDED IN THIS REVIEW**

No.	Study Title	Study No.
22.	Oral Segment I Study in Rats	D-4-1
23.	Oral Segment I Study in Rats	820741
24.	Oral Segment II Study in Rats	D-4-2
25.	Oral Segment II Study in Rabbits	D-4-3
26.	Oral Segment III Study in Rats	D-4-4
27.	Oral Segment III (Foster Nursing) Study in Rats	D-4-5

*Genetic Toxicology***INDEX OF MUTAGENICITY AND GENOTOXICITY STUDIES INCLUDED IN THIS REVIEW**

No.	Study Title	Study No.
28.	Mutagenicity Tests in Microorganisms	D-7-1
29.	Reversion Test in Bacteria	D-7-2
30.	Salmonella/Mammalian-Microsome Mutagenicity Test	841042
31.	V79 Chinese Hamster Cells Point Mutation Test	841043
32.	Unscheduled DNA Synthesis in Rat Hepatocytes	841039
33.	Unscheduled DNA Synthesis in Human Fibroblasts	841041
34.	Test for Transformation Inducing Properties in Mammalian Fibroblasts	841044
35.	Chromosome Analysis on Chinese Hamster Somatic Cells	841040
36.	Micronucleus Test in Mice	D-7-3
37.	Micronucleus Test in Rats	8962251
38.	Chromosome Studies on Chinese Hamster Ovary Cell Line CCL 61 <i>In Vitro</i>	896209

*Special Toxicology***INDEX OF SPECIAL TOXICOLOGY STUDIES INCLUDED IN THIS REVIEW**

No.	Study Title	Study No.
1.	Antigenicity Test in Mice	D-6-2
2.	Skin Sensitization: Optimization: Study in Guinea Pigs	840421
3.	5-Day Intravenous Local Tolerability Study in Rabbits	855125
4.	Skin Irritation (Local Tolerability) Study in Rabbits	835278

STUDIES NOT REVIEWED WITHIN THIS SUBMISSION

Several studies had been submitted and reviewed throughout the course of the IND phase of study. These studies were not reviewed with the current submission. A list of the studies reviewed prior to this submission is presented below. Virgil Whitehurst, Ph.D. was the reviewer for the IND submissions.

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PHARMACOLOGY

Formoterol fumarate provides therapeutic benefit by relieving and preventing bronchoconstriction by relaxing airway smooth muscle via specific interaction with beta-2-adrenoceptors. The efficacy of formoterol at beta-2-adrenoceptors has been measured in both functional airway smooth muscle relaxation and biochemical second messenger assays where cAMP were determined. High levels of efficacy and potency agonism were demonstrated using conditions of induced tone or the presence of high levels of cholinergic agonists. Treatment with formoterol or other beta-2-adrenoceptor agonists is associated with reassertion relaxation, suggesting that it is functionally retained in or near the beta-2-adrenoceptor despite extensive washing of *in vitro* airway smooth muscle preparations.

The onset of action of formoterol is comparable to that of albuterol, yet the duration of action was consistently greater than that of isoproterenol or albuterol. The onset of action was reported to be 1.7 ± 0.3 minutes for formoterol, 0.8 ± 0.2 minutes for albuterol, and 17.6 ± 5.0 minutes for salmeterol when administered to guinea pig isolated trachea. (Jeppson et al., 1989). The duration of action of formoterol was in excess of 6 hours in isolated human bronchus (Advenier et al, 1991).

The Sponsor also addressed binding, potency, and selectivity of the RR and SS enantiomers of Formoterol, but the data are inconclusive since complete separation of the constituent enantiomers was not demonstrated.

The pharmacodynamic effects of Formoterol fumarate were consistent with those that would be expected of a highly selective beta-2-adrenoceptor agonist.

SAFETY PHARMACOLOGY

Information included in this section was summarized from reports provided by the Sponsor and published literature.

Cardiovascular system effects

Formoterol fumarate is associated with increased heart rate, maximum dp/dt, pulmonary artery and capillary pressure, coronary blood flow, cardiac output and myocardial oxygen consumption (Sponsor Study No. 10/91, January 29, 1991). Decreased peripheral, pulmonary and coronary resistance were also observed in this same study. The selectivity of beta-adrenoceptor agonists for the beta-2-adrenoceptor has been demonstrated for Formoterol fumarate, although there is also a cardiovascular response to the drug. Generally, beta-2-adrenoceptor mediated effects include relaxation of airway smooth muscle, although some cardiovascular activity has also been noted. Beta-1-adrenoceptor mediated effects include changes in contractility or beating rate of myocardial preparations *in vitro*.

Formoterol has been shown to have high selectivity for the beta-2-adrenoceptor subtype. Studies have demonstrated that formoterol displaces labeled beta-1-adrenoceptors selectively labeled with a beta-adrenoceptor antagonist (³H-CGP 12177) (Lemoine, et al. 1992a; Lemoine, et al. 1992b; Kaumann, et al. 1985; and Lemoine et al., 1985). Homologous down regulation of beta-2 but not beta-1-adrenoceptors has been demonstrated after 14 days of treatment with high doses of formoterol (Kompta et al., 1995 and Kompta et al. 1994).

Although bronchoselectivity has been demonstrated in studies with Formoterol fumarate, there exists a subdominant population of functionally coupled beta-2-adrenoceptors in the myocardium of animals and humans. Based on curve fitting of concentration-response data obtained in guinea pig studies, Formoterol fumarate appears to act principally on beta-2-adrenoceptors in the right atrium to induce increased contraction rate while acting with much lower affinity at beta-1-adrenoceptors in the left atrium to mediate some degree of increased contraction force. This effect coupled with an apparent reflex tachycardia secondary to a beta-2-adrenoceptor mediated vasodilatation and hypotension are likely responsible for the changes observed in the cardiovascular system after treatment with Formoterol fumarate.

Immune system effects

Fomoterol has inhibitory effects on a number of inflammatory cells and processes because beta-2-adrenoceptors are widely distributed in effector cells and responding tissues. It is a potent inhibitor of anaphylactic degranulation of mast cells and basophils, resulting in suppression of mediator release from immunologically sensitized tissues. Fomoterol inhibits both active and passive anaphylaxis *in vivo*.

Formoterol reduces eosinophil adhesion to venules and the Sponsor postulates that this may be the mechanism by which formoterol attenuates eosinophilic lung inflammation in experimental animals. Formoterol attenuates eosinophilic activation directly, however little effect was demonstrated in human alveolar macrophage superoxide production.

Gastrointestinal effects

Formoterol decreases gastric acid secretion and decreases gastric, duodenal and ileal motility, but without significant effect on gastric transit time.

PHARMACOKINETICS AND TOXICOKINETICS:

The absorption, distribution, metabolism, and elimination properties of Formoterol fumarate were studied in rats, mice, and dogs using radiolabeled and non-radiolabeled analytical methods. Analytical methods evolved over time to quantify unchanged Formoterol fumarate in plasma and urine collected from animals. Results were obtained using

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Pharmacokinetic Parameters

Mean pharmacokinetic values were obtained from a variety of species and time points. At this writing, some of the data are unclear and the Sponsor is requested to clarify the data. The AUC data play a critical role in the evaluation of the safety of Formoterol fumarate. The Sponsor noted in their submission that there were difficulties with the method(s) used to evaluate plasma levels in animals studies. The values provided are much higher than would reasonably be expected in a drug of this type and class. In addition, we note that the C_{max} identified in the mouse dietary carcinogenicity study is 6.3 nmol/l for a 50 mg/kg/day dose, far below the number provided in the mouse drinking water study (AUC = 4300 nmol/ml) and used for comparison to humans.

Absorption

In the rat, oral absorption was measured using radio-labeled Formoterol fumarate after a single dose of 50 µg/kg (—-F-1-4-1). ³H-Formoterol fumarate was primarily absorbed from the small intestine. Enterohepatic circulation was also demonstrated in this study by

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administering bile collected from animals dosed with ^3H -Formoterol fumarate to naïve animals. In this case, 69% of the administered dose was absorbed.

Distribution

The distribution of radio-labeled Formoterol fumarate was studied using both _____ in mice (DM 1/1991) and rats (_____-F-1-4-1). These studies indicate the accumulation of Formoterol fumarate does not occur at a level or a rate that would pose a safety concern. b(4)

Following a *single oral dose* of 0.5 or 5 mg ^3H -Formoterol fumarate/kg in 3 rats/group, concentrations of the test material were found in various tissues and organs within 30 minutes of dosing (_____-F-1-4-1). Concentrations were highest in the kidney, liver, lung, plasma, and whole blood. Concentrations of ^3H -Formoterol fumarate increased in various tissues for the first 6 hours and decreased dramatically by the sample collection at 24 hours. b(4)

Following *repeated oral administration* of 0.05 mg ^3H -Formoterol fumarate/kg/day for 21 days in rats, the concentrations of radioactivity in tissues gradually increased until Day 14 and remained constant thereafter (Sasaki, et al. 1983).

Protein Binding

The binding of Formoterol fumarate to plasma protein was assessed *in vitro* using rat, dog, and human plasma (_____-F-1-1-6), and *in vivo* in dogs after oral dosing with 0.1 mg Formoterol fumarate/kg. *In vitro* binding was 50 to 65% for all species and independent of the concentration tested (0.1 - 100 ng/ml). *In vivo* binding in the dog ranged from 44 to 60% and was constant over the 10-hour post-dose sample collection period. Formoterol fumarate primarily bound to albumin with only negligible amounts bound to α -1-acid glycoprotein or γ -globulin (_____, R 41/1991). b(4)

Metabolism

Data from rat and dog studies demonstrate that Formoterol fumarate is metabolized extensively after oral administration before reaching systemic circulation (Sasaki et al. 1983). The primary metabolite is a direct phenolic O-glucuronide (1a) and can be found in mice, rats, dogs, and humans. Other metabolites also occur via glucoronidation. The following diagram illustrates the biotransformation pathway of Formoterol fumarate. A table comparing the metabolic profiles of several species is also presented.

b(4)

Species Comparison of the Metabolic Profiles of Formoterol fumarate			
Test Model	Major Metabolite (% of dose)	Other Metabolites	Reference
<i>In vitro Hepatocytes</i>			
Rat	Ia ¹	IIa and IIb ²	DM(EU) 28/1996
Guinea Pig	Ia		DM(EU) 28/1996
Dog	Ia		DM(EU) 28/1996
Marmoset	Ia	Ib ³	DM(EU) 28/1996
<i>In vivo Models</i>			
Mouse (oral)	Ia (~70% in urine)		DM 1/1991
Rat (oral)	Ia (95% in bile)		Sasaki et al. 1982
	I ⁴ (5% in urine)		
Dog (oral)	Ia (~80% in bile)		Sasaki et al. 1982
	I (~20% in urine)		

¹ Direct phenolic O-glucuronide.² Phenolic glucuronides of the O-desmethyl metabolite.³ Direct aliphatic O-glucuronide.⁴ Unchanged Formoterol fumarate.

Human (oral)	I	(6% in urine)	B 46/87
	Ia	(24% in urine)	
	Ib	(5% in urine)	
	II	(<1% in urine)	
	IIa + IIb	(15% in urine)	

Transpulmonary transport and metabolism

In vitro models using perfused rat lung and radio-labeled Formoterol fumarate indicate that the drug is rapidly transported to the blood from the airway and is not metabolized by the lung (93/02/C/2R).

Elimination

Elimination of Formoterol fumarate and its metabolites is rapid and complete. It occurs to a great extent within the first 24 hours following dosing and is complete within 4 to 5 days (Sasaki et al. 1982, —, F-1-4-5, — F-1-4-3, DM 1/1991, DM 2/1991). In rats and dogs, excretion occurs through hepatic metabolism and most of the radio-labeled Formoterol fumarate is recovered from the bile and/or feces (DM 2/1991, —, F-1-4-3). In mice, renal excretion is evidenced by a relatively high percentage of radio-labeled Formoterol fumarate recovered in urine (DM 1/1991). With repeated dosing in dogs, plasma levels of Formoterol fumarate and urinary excretion concentrations increase during the first 2 days after the initiation of dosing and remain constant thereafter (—, F-1-4-7). Results of excretion studies in rats, dogs, and mice are summarized in the following table.

Mean Cumulative Excretion of Radio-labeled Formoterol fumarate					
Dose	% in Bile	% in Urine	% in Feces	% Total	Reference
Rats					
5 mg/kg	72.11	21.12	6.37	99.60	DM 2/1991
20 mg/kg	75.22	21.08	3.57	99.87	
50 mg/kg	70.76	19.96	5.00	94.45	
Dogs					
10 µg/kg	-	36.8	52.1	88.9	— F-1-4-3
100 µg/kg	-	36.9	52.0	88.9	
Mice					
6 mg/kg	-	64.22	33.6	97.82	DM 1/1991
60 mg/kg	-	75.88	23.80	99.68	

Conclusions Regarding Pharmacokinetics and Toxicokinetics

Formoterol fumarate is absorbed after first pass glucuronidation. Oral absorption and bioavailability vary between species as a result of the extent of first pass metabolism and enterohepatic circulation. In rats, glucuronide metabolites are excreted in bile and

Formoterol fumarate is then re-absorbed in the small intestine. Plasma protein binding was similar between rat, dog, and human plasma and ranged from 50 to 65%. Biotransformation products occur as a result of O-glucoronidation, with direct phenolic glucuronide as a primary metabolite in mice, rats, dogs, and humans. Elimination of Formoterol fumarate appears to be rapid and complete within 4 to 5 days of dosing. In rats, most radio-labeled Formoterol fumarate is recovered in the bile and feces. In mice, renal excretion is evidenced by relatively high levels of radio-labeled Formoterol fumarate recovered in urine.

TOXICOLOGY

A comprehensive data base of single dose and repeat dose toxicology studies were submitted and reviewed to support the safety of Formoterol fumarate. Studies by the oral route were performed in rats, mice, and dogs. Studies by the inhalation route using the dry powder formulation were performed in rats and dogs for up to one year.

The studies were, for the most part, adequately designed and performed in accordance with Good Laboratory Practice standards, except where otherwise noted in the individual reviews of each study. Findings of toxicity were consistent with the pharmacologic action of beta-2-adrenoceptor agonists.

Individual review of the studies in presented followed by an overall summary of the studies.

Acute Toxicity

The acute toxicity of Formoterol fumarate was studied in mice, rats and dogs by oral, intravenous (i.v.), intraperitoneal (i.p.), subcutaneous (sc.) and inhalation routes of administration. These studies are summarized in the following table.

Acute Toxicity of Formoterol fumarate				
Species	Route	Dose (mg/kg - oral, i.v., i.p., sc.) (mg/l - inhalation)	Findings	Reference
Mouse	Oral	0, 3850, 5000, 6500, 8450, 11000, 143000(♀)	LD ₅₀ :6696 -♂ and 8308 -♀	D-1-1
Rat	Oral	0, 593 (♂), 889 (♂), 1330 (♂), 2000, 3000, 4500, 6750, 10100 (♀)	LD ₅₀ :3125 -♂ and 5583 -♀	D-1-1
Juvenile Rat	Oral	7-day old 0, 680, 810, 970 1170, 1400, 1680, 2020 22-day old 5000 (♀), 6000, 7200, 8600, 10400, 12400	7-day old LD ₅₀ :1120 -♂ and 1260 -♀ 22-day old LD ₅₀ :7990 -♂ and 7480 -♀	D-1-3
Chinese Hamster	Oral	10, 100, 300, 600, 1000	LD ₅₀ :322 (♂/♀)	825339

b(4)

Species	Route	Dose (mg/kg - oral, i.v., i.p., sc.) (mg/l - inhalation)	Findings	Reference
Dog	Oral	Escalating doses in 2 dogs/sex: .0001, .001, .01, .1, 1, 3, 10, 30, 100, 3000	.001 - ↑ heart rate .1 - ventricular extrasystol; ↑ SGOT 1 - ↑ ALK PHOS; salivation; crawling; runny nose 10 - ↑ SGPT; emesis 3000 - death (1/2 ♂) <u>Necropsy:</u> myocardial necrosis and hemorrhage; lung congestion and bronchopneumonia; yellow liver with fatty degeneration.	YBD D-1-2
Rat	Inhalation	.3, .81, .94, 1.82, 5.7	LC ₅₀ : 1.35	846402
Rat	Inhalation	.05, 1, 5	LC ₅₀ : 4.8	841012
Rat	Inhalation	.5, 1, 5	LC ₅₀ : 6.07	841011
Dog	Inhalation	0, 6.7, 14.3, 37.3, 101.5	No mortality; ↑ heart rate	906238
Dog	Inhalation	12 µg/dog (aerosol metered dose)	↑ heart rate; ↑ contraction force	855190
Dog	Inhalation	12 µg/dog fresh and outdated batches	No difference between batches. ↑ heart rate; ↑ contraction force; focal necrosis and mineralization in papillary heart muscle	886174
Dog	Inhalation	2.4 mg/dog (metered dose)	None remarkable.	896160
Rat	Inhalation	3.5	LC ₅₀ > 3.5 (no mortality)	896183
Rat	Inhalation	3.91, 3.78, 4.52	LC ₅₀ > 4.52 (no mortality)	926108
Mouse	Intravenous	0, 53, 61, 70.1, 80.6, 92.7, 106.6	LD ₅₀ : 71.8 (♂), 70.9 (♀)	D-1-1
Mouse	Intraperitoneal	141, 169, 203, 244, 293, 351	LD ₅₀ : 238 (♂), 206 (♀)	D-1-1
Mouse	Subcutaneous	424 (♂), 508, 610, 732, 878, 1054	LD ₅₀ : 642 (♂), 6665 (♀)	D-1-1
Rat	Intravenous	70.1, 80.6, 92.7, 106.6, 122.6 141	LD ₅₀ : 97.8 (♂), 100.8(♀)	D-1-1
Rat	Intraperitoneal	118 (♂), 141, 169, 203, 244, 293, 351 (♀)	LD ₅₀ : 172 (♂), 207 (♀)	D-1-1
Rat	Subcutaneous	555, 722, 938, 1220, 1586, 2062 (♀)	LD ₅₀ : 997 (♂), 1097 (♀)	D-1-1

Repeat Dose Oral Toxicity Studies

Repeat dose toxicity studies were performed in rats, mice and dogs. These studies are reviewed below.

Rats

The subchronic toxicity of Formoterol fumarate in rats was studied by the oral route via dietary and drinking water administration and by the inhalation route. The oral studies were conducted up to a duration of 3 months and were considered in selecting doses for the carcinogenicity studies (presented later in this review). The inhalation studies were conducted up to a duration of 1 year.

Findings were consistent between routes of administration and included effects on the heart and reproductive organ systems. In rats dosed up to 3 months in the drinking water at levels of 10 mg/kg/day and above, myocardial fibrosis, increased uterine weights, and

b(4)

decreased testes, epididymides, seminal vesicles, and prostate weights were observed. Body weight and food consumption were higher for all treated groups when compared to controls, also without regard to route of administration.

After 3 months of inhalation treatment at levels of 0.34 mg/kg/day and above, increases were observed in heart weight, red cell parameters, and body weight and food consumption. The kidney and liver weights were higher than control for rats treated at 0.442 mg/kg/day. One year of inhalation treatment at a level of 120 µg/kg/day and higher was associated with degeneration of seminiferous tubules that did not recover after an 8-week period without treatment. All other findings recovered after the 8-week period and are described in the individual review summaries presented below.

Individual reviews of the oral and inhalation repeat dose toxicity studies are presented below. Each study review is organized by background information, methods, results, and conclusions.

1. 28-Day Palatability Study in Rats

BACKGROUND INFORMATION

Study Title:	28-Day Palatability Study in Rats
Sponsor Study No.:	896027
Study Dates:	April 4 - May 2, 1989
Report Date:	September 22, 1989
Test Facility:	CIBA-GEIGY Limited Experimental Toxicology 4332 Stein Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	19:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No:	810187
Purity:	Not stated
Control Article:	Diet

Test System

Species/Strain:	Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route:	Diet
Housing:	Individual
Duration of Exposure:	28 Days

Dosing Information

Five rats/sex were assigned to treatment groups receiving 0, 5, 20, 50, and 200 ppm of CGP 25827A in the diet. These doses correspond to 0, 0.4, 1.7, 4, and 18 mg/kg/day. The purpose of this study was to evaluate the palatability of CGP 25827A in diet and to provide information regarding drug levels in plasma and microscopic changes in the heart.

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Plasma Level Determinations	At termination (reported separately)
Gross pathology	At termination
Histopathology	At termination (heart only)

RESULTS

Antemortem Observations

There were no remarkable observations in mortality, clinical signs, body weight, or food consumption data.

Gross Pathology

There were no remarkable gross observations.

Histopathology

There was no microscopic evidence of cardiotoxicity, including myocardial fibrosis in rats treated with CGP 25827A in the diet at levels up to 18 mg/kg/day for 28 days.

CONCLUSION

No signs of toxicity were noted in rats treated with CGP 25827A in the diet at levels up to 18 mg/kg/day for 28 days. Additionally, CGP 25827A appeared to be palatable to rats in the diet at the tested levels.

2. 3-Month Range Finding Study in Rats (Administration in Food)**BACKGROUND INFORMATION**

Study Title: 3-Month Range Finding Study in Rats (Administration in Food)
Sponsor Study No.: 906205 (Supplement to 886178, 24-month study)
Study Dates: June 26 - September 25, 1990
Report Date: January 8, 1992
Test Facility: CIBA-GEIGY Limited
 Short/Long-term Toxicology
 4332 Stein
 Switzerland
GLP Status: Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page 22:1

METHODS***Test Material***

Test Article: CGP 25827A
Batch No: 810589
Purity: 100.3%
Control Article: Diet

Test System

Species/Strain: Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route: Diet
Housing: 5/cage
Duration of Exposure: 3 months

Dosing Information

Ten rats/sex were assigned to treatment groups receiving CGP 25827A at levels of 0, 0.5, 2, 5 and 20 mg/kg/day in the diet. The purpose of this study was to supplement the dietary carcinogenicity study in rats with pharmacokinetic data generated from the same species, strain and test material used in the previous study. Five rats/sex/group were used for each sample collection interval. Pharmacokinetic data were presented in a separate report (B68/1991).

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly

Parameter	Frequency of Measurement
Food consumption	Weekly
Drug Level Determinations	Reported separately
• Plasma -	• Weeks 5 and 14
• Urine -	• Weeks 3 and 11
Gross pathology	Weeks 5 and 14

RESULTS

Mortality

There were no remarkable observations in mortality.

Clinical Signs

There were no remarkable observations in clinical signs.

Body Weight

Mean absolute body weight and body weight gain values were higher in treated groups when compared to controls throughout the study.

Food Consumption

Food consumption values were consistently higher for all treated groups when compared to controls. Food consumption ratios, calculated as g food consumed/body weight/day, were not different between control and treated groups.

Gross Pathology

There were no remarkable gross observations.

CONCLUSION

Minimal toxicity information was collected in this study as the purpose was to evaluate plasma levels of CGP 25827A, when administered to rats in the diet at levels of 0, 0.5, 2, 5 and 20 mg/kg/day. This study was conducted as a supplement to a carcinogenicity study in rats (No. 886178). Pharmacokinetic data were presented in a separate report (No. _____ R 6/1992).

b(4)

Body weight values were higher for all treated groups when compared to controls.

3. 3-Month Oral (via drinking water) Toxicity Study in Rats

BACKGROUND INFORMATION

Study Title: 3-Month Oral (via drinking water) Toxicity Study in Rats
Sponsor Study No.: 90-6174
Study Dates: August 24 - December 3, 1990
Report Date: September 22, 1989
Test Facility: CIBA-GEIGY Limited
Preclinical Safety, Section of Experimental Toxicology
Basel,
Switzerland
GLP Status: Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page 20:193 and 21:1

METHODS

Test Material

Test Article: CGP 25827A **b(4)**
Batch No: 400190
Purity: _____
Stability: 7 days under study conditions
Control Article: Water

Test System

Species/Strain: Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route: Drinking water
Housing: Individual
Duration of Exposure: 3 months

Dosing Information

Ten rats/sex were assigned to treatment groups receiving 0, 0.125, 0.25, and 0.5 mg/ml of CGP 25827A in the drinking water. Overall mean test article intake calculated from water consumption averaged 0, 10, 19, and 43 mg/kg/day for Group 1 - 4 males, respectively, and 0, 13, 27 and 40 mg/kg/day for Group 1 - 4 females, respectively. Five rats/sex/group were designated as satellite animals and received a single gavage dose of CGP 25827A at levels of 0, 12.5, 25, and 50 mg/kg for Groups 2 - 5, respectively.

Observations

Toxicity was assessed by evaluating the following parameters:

Water Consumption

No remarkable findings were noted for water consumption.

Hearing Test

No remarkable findings were noted for hearing tests.

Ophthalmology

No remarkable findings were noted for ophthalmology.

Hematology

Thrombocyte counts were generally lower for animals in all treated groups when compared to control groups. However coagulation factors were not affected.

Serum Chemistry

No consistent differences from control or dose-response relationships were revealed in analysis of serum chemistry parameters.

Urinalysis

No remarkable findings were noted in urinalysis.

Organ Weights

Epididymides, seminal vesicle, and prostate weights of males in the high dose group were slightly less than those in control group. Mean uterine weights were slightly higher for all females in treated groups when compared to controls. No microscopic correlates were observed for the noted organ weight changes.

Gross Pathology

An increase in skeletal muscle mass was noted for animals in all treated groups. Although not stated, this finding appears to refer to an increase in *volume* as it correlates with higher-than-control body weight values for treated animals.

Histopathology

Microscopic findings were revealed in the heart and thigh muscle.

In the heart, the incidence of granulation tissue and fibrosis was 0/10, 1/10, 2/10, and 0/10 for Groups 1 - 4 males, respectively and 0/10, 0/10, 0/10 and 1/10 for Groups 1 - 4 females, respectively.

Hypertrophy was observed in the thigh muscle of all treated rats and no control rats. Mononuclear focal infiltrates and single cell necrosis were also observed in most of the treated animals and none of the controls.

No increase in mitotic activity was revealed in the analysis of thyroid C cells.

CONCLUSION

CGP 25827A, when administered to rats in the drinking water at levels averaging 0, 10, 19, and 43 mg/kg/day for Group 1 - 4 males, respectively, and 0, 13, 27 and 40 mg/kg/day for Group 1 - 4 females, respectively, resulted in microscopic changes in the heart (all dose groups) and thigh musculature (all dose groups). Weights of male reproductive organs (epididymides, seminal vesicles, and prostate) were lower in treated than control. Uterine weights were slightly increased for all treated groups when compared to controls. Body weight and food consumption values were higher for all treated groups when compared to controls. A no observable effect level (NOEL) was not identified for these findings.

No increase in mitotic activity was revealed in thyroid C cells.

4. 3-Month Oral (via drinking water) Pharmacokinetic Study in Rats

BACKGROUND INFORMATION

Study Title:	3-Month Oral (via drinking water) Pharmacokinetic Study in Rats
Sponsor Study No.:	90-6052
Study Dates:	June 10 - September 16, 1991
Report Date:	May 11, 1992
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division Basel, Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page	21:349

METHODS

Test Material	
Test Article:	CGP 25827A
Batch No:	400190 b(4)
Purity:	_____

Stability: 7 days under study conditions
Control Article: Solvent to CGP 25827A and acidified tap water
Test System
Species/Strain: Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route: Drinking water followed by a gavage dose after 90 days
Housing: Individual
Duration of Exposure: 3 months

Dosing Information

Five rats/sex were assigned to treatment groups receiving 0, 0.125, 0.25, and 0.5 mg/ml of CGP 25827A in the drinking water for three months. Overall mean test article intake calculated from water consumption averaged 0, 9, 21, and 44 mg/kg/day for Group 1 - 4 males, respectively, and 0, 11, 25 and 47 mg/kg/day for Group 1 - 4 females, respectively. All animals and received a single gavage dose of CGP 25827A at levels of 0, 12.5, 25, and 50 mg/kg for Groups 1 - 4, respectively, following the 3-month treatment period.

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Body weight	Pretest and three times per week
Water consumption	Daily

Blood samples were taken for analysis of CGP 25827A levels in plasma and were presented in a separate report (← R 16/1992). **b(4)**

RESULTS

Mortality

No mortality was noted.

Body Weight

Mean absolute body weight and body weight gain values were higher in treated groups when compared to controls during the first 2 weeks of study. After Week 2, the rate of body weight gain was similar between treated and control groups but mean body weight values remained consistently higher for treated groups when compared to controls throughout the study.

Water Consumption

No remarkable findings were noted for water consumption.

CONCLUSION

Minimal toxicity information was collected in this study as the purpose was to evaluate plasma levels of CGP 25827A, when administered to rats in the drinking water at levels of 0, 12.5, 25 and mg/kg/day. This study was conducted in an attempt to clarify inconsistencies in pharmacokinetic data from a previous 3-month study in rats (90-6174). Pharmacokinetic data were presented in a separate report (— R 6/1992). **b(4)**

Body weight values were higher for all treated groups when compared to controls.

5. Preliminary Inhalation Tolerance Study in Rats

Study Title: Preliminary Inhalation Tolerance Study in Rats
Sponsor Study No.: 90-6223
Laboratory Study No.: 650361
Study Dates: October 9 - 19, 1990
Report Date: March 7, 1991
Test Facility: _____

GLP Status: Compliant
NDA Volume:Page 33:275

METHODS

Test Article: CGP 25827A
Batch No: 14/909/1
Purity: — %
Control Article: Aerosol fluorocarbon propellants
Purity: Not stated
Species/Strain: Sprague-Dawley Rats
Route: Nose Only Inhalation
Exposure Conditions: Aluminum cylindrical chamber with apparatus to actuate aerosol cans to achieve target concentrations.
Duration of Exposure: Group 1-- single dose; Groups 2, 3, 4 -- 7 days
Dose Levels: Group 3 - 0.85 mg/kg/day; Group 4 - 5.52 mg/kg/day

Dosing Information					
Group	No. Animals per sex	No. of Cans x Actuations/min.	Chamber Conc. (mg/l)	Nominal Conc. (mg/l)	Mass Mean % of Particles with <6 µm Diameter
1 (High) ^a	5	6 x 6	0.13	0.043	96.4
2 (Control) ^b	5	6 x 6	.012	0	93.8
3 (Low)	5	3 x 2	.002	0.007	96.6
4 (High)	5	6 x 6	0.13	0.043	96.0

^a Single dose group.

^b Values reported represent placebo excipients only.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Daily
Food consumption	Weekly
Clinical Pathology	Day 8
Gross pathology (including organ weights)	Study termination: (after Day 1 for Group 1 and Day 7 for Groups 2 - 4)

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	Two accidental deaths were attributed to blood sampling: one Group 2 male and one Group 4 female.
Clinical signs	There were no remarkable clinical signs.
Body weight	Body weight gain values were higher in both groups of treated animals when compared to controls.
Food consumption	There were no effects on food consumption.
Clinical Pathology	The following hematology and clinical chemistry parameters were significantly different from control: <ul style="list-style-type: none"> • Hemoglobin concentration - ↓ Group 3 ♂♀ and Group 4 ♀; • White blood cell count - ↓ Group 4 ♀; • Blood urea nitrogen - ↓ Group 3 - 4 ♂♀; • Creatinine - ↓ Group 4 ♀ • Total bilirubin - ↓ Group 4 ♀ • Lactic dehydrogenase ↑ Group 3 ♂ and Group 4 ♂♀.
Gross pathology (including organ weights)	Lung weights were higher for Group 4 females when compared to control. There was no gross correlate for this finding or any other remarkable gross observation.

CONCLUSION

In absence of histopathology, the interpretation of the increased lung weight in high dose females and changes in clinical pathology of treated animals is uncertain. The results in this study were used to select doses for a 3 month study in rats (90-6224).

6. Preliminary Inhalation Feasibility Study in Rats (MDPI Formulation)**BACKGROUND INFORMATION**

Study Title: Preliminary Inhalation Feasibility Study in Rats (— Formulation) **b(4)**
Sponsor Study No.: 93-6078
Laboratory Study No.: 653006
Study Dates: April 14, 1993 - May 22, 1993
Report Date: June 24, 1994
Test Facility: _____
GLP Status: Compliant
NDA Volume:Page 35:1

METHODS

Test Article: CGP 25827A Dry Powder Formulation (Foradil)
Batch No: 1066/1
Purity: 99.9%
Vehicle: Lactose at a ratio of 1:69 (CGP 25827A:lactose)
Purity: NA
Species/Strain: Sprague Dawley Rat
Route: Nose Only Inhalation
Exposure Conditions: Rotating brush generator with monitored air flow, temperature, and humidity.
Duration of Exposure: 5 days

Dosing Information					
Group	No. Animals per sex	Dose (mg/kg/day)	Gravimetric Conc. (µg/l air)	Exposure	Mean % of Particles <3 µm Diameter
1	5	3.19	158	10 min. on 20 min. off for 250 min.	50.7
2	5	-	-	1 h on 1 day	-
3	5	0.52	95	1 h on 1 day	56.1
4	5	0.69	51	1 h/day for 5 days	57.2

The purpose of this study was to demonstrate that the described dosing apparatus would yield similar systemic exposure to a different system. Doses were selected based on

previous studies with CGP 25827A. Exposure could not be demonstrated in Group 2 animals so the experiment was repeated in Group 3 animals and dosing was extended to a 5-day interval in Group 4 animals.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Clinical signs	During dosing and 1 hour post dose during treatment and daily for 14 days after the last treatment.
Body weight	Prior to treatment and at least weekly intervals thereafter.
Urine for Proof of Absorption	After the single exposure (Groups 1 and 3) and on Day 5 (Group 4)
Blood for Proof of Absorption	After the single exposure (Groups 1 and 3)

RESULTS

There were no remarkable findings in the parameters measured. Urine and blood levels of CGP 25827A were not included in this report.

CONCLUSION

This study was to examine the feasibility of the dosing apparatus with the dry powder formulation of CGP 25827A. Target concentrations as measured by _____ concentration and particle size were achieved for Groups 1, 3, and 4. Definitive evidence of exposure (i.e., blood and urine levels of CGP 25827A) was not included in the final report. b(4)

7. 28-Day Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation (1/69) in Rats

BACKGROUND INFORMATION

Study Title: 28-Day Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation (1/69) in Rats
Sponsor Study No.: 926111
Laboratory Study No.: 323605
Study Dates: July 28, 1992 - August 28, 1992
Report Date: April 7, 1993
Test Facility: _____ b(4)

GLP Status: Compliant
NDA Volume:Page 38:1

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/69)
Batch No: 1066/1 and 1066/2

Purity: _____
Control Article: Lactose DMV 100 Mesh
Purity: U.S.P.
Species/Strain: Wistar rats, Han-Ibm., outbred, SPF-quality
Route: Nose Only Pulsed Inhalation
Exposure Conditions: _____ aerosol generator _____
 _____, discharged through a _____ . This
 method was selected to achieve the required test article
 concentrations with a mass median aerodynamic diameter
 of 3 μm or less.
Duration of Exposure: 29 days

b(4)

b(4)

Dosing Information

Group	No. Animals per sex	Exposure Duration (min.)	Dose Levels (mg/kg/day)	Target Conc. (mg/l air)	Gravimetric Conc. ($\mu\text{g/l}$ air)	Mean % of Particles <3 μm Diameter
1 (Air Control)	15	250	-	0	-	-
2 (Lactose)	15	90	-	1.7	1.64	-
3 (Low)	15	10	0.156	1.7	1.73	1.197
4 (Mid)	15	30	0.504	1.7	1.81	1.290
5 (High)	15	90	1.153	1.7	1.69	0.970

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Water Consumption	Weekly
Ophthalmoscopy	Prior to treatment and at the end of the study
Urine for Proof of Absorption	During the first and last weeks of exposure
Plasma for Proof of Absorption	At the end of the third week of treatment
Clinical Pathology	At the end of Week 4
Gross pathology (including organ weights)	At the end of Week 4
Histopathology (adrenal, heart, lungs, liver, 4 levels of the nasal cavity, spleen, testes, trachea, ovaries, and mandibular lymph nodes)	At study termination for control and high dose groups; gross lesions and mandibular lymph nodes were examined from all groups.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	A statistically significant increase in body weight gain was noted in all groups of treated males when compared to controls. Mean body weights and body weight gain values were significantly higher for all groups of treated females when compared to controls.
Food consumption	Treated males from Group 5 and females from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	Glucose levels for all treated groups were significantly lower than control values. The response was dose-related in females. Statistically significant differences from control values were reported for various other parameters in hematology and clinical chemistry determination. These changes could not be definitively attributed to treatment with CGP 25827A because they did not occur in a dose related response, were inconsistent between air- and lactose- control comparisons, and were within historical control ranges.
Gross pathology	
Organ Weights	Heart weights (and/or corresponding ratios) were significantly higher for males and females in all dose groups when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (1.15 mg/kg/day). Notably, there were no adverse effects on the respiratory system.

8. Subchronic (90-Day) Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation

BACKGROUND INFORMATION

Study Title: Subchronic (90-Day) Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation

Sponsor Study No.: 90-6145

Laboratory Study No.: 248580

Study Dates: April 2 - July 4, 1990

Report Date: June 3, 1991

Test Facility: _____

b(4)

GLP Status: Compliant
 NDA Volume:Page 35:1

METHODS

Test Article: CGP 25827A Dry Powder Formulation
 Batch No: 14/704/80
 Purity: 99.3%
 Control Article: Lactose 150 MESH BB
 Purity: Not stated
 Species/Strain: Albino Rat, Tif:RAIf (SPF)
 Route: Nose Only Pulsed Inhalation
 Exposure Conditions: _____ aerosol generator _____
 _____, discharged through a _____ . This method was selected to minimize particle size-related selection within the formulation between the excipient and the active ingredient.
 Duration of Exposure: 91 - 94 days

b(4)

Dosing Information

Group	No. Animals per sex	Dose (µg/kg/day)		Target Conc. (mg/l air)	Active Ingredient Conc. (µg/l air)	Mean % of Particles <3 µm Diameter
		Males	Females			
1 (Air Control)	15	-	-	0	-	-
2 (Lactose)	15	-	-	1.0	-	66.2
3 (Low)	15	2.5	3.7	0.1	0.11	84.7
4 (Mid)	15	8.1	12.2	0.3	0.36	78.9
5 (High)	15	26.1	39.2	1.0	1.16	58.8

Doses were selected based on previous studies with CGP 25827A. The high dose with the highest technically feasible dose using the dosing apparatus described.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Prior to treatment and at the end of the study
Urine for Proof of Absorption	After the first exposure and 1 and 3 months
Clinical Pathology	Weeks 6 and 13
Gross pathology (including organ weights)	After 91 days of treatment
Histopathology (complete tissue list including 4 levels of the nasal cavity)	At study termination for control and high dose groups; gross lesions and mandibular lymph nodes were examined from all groups.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	A dose related increase in body weight gain was noted in all groups of treated males, with statistical significance for Group 5. Mean body weights for Group 4 females and Groups 4 and 5 males were consistently higher than control values.
Food consumption	Treated animals from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	The following parameters were significantly different from control: <ul style="list-style-type: none"> • Glucose - Weeks 6 and 13, ↓ Group 3 - 5 ♂; • Aspartate aminotransferase - Week 6 and 13, ↑ Group 5 ♂; • Alanine aminotransferase - Week 6, ↑ Group 5 ♀; Week 13, ↑ Groups 5 ♂♀.
Gross pathology	
Organ Weights	Heart weights were significantly higher for males and females in Group 5 when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (26.1 and 39.2 µg/kg/day for males and females, respectively). Notably, there were no adverse effects on the respiratory system.

9. 3 Month Inhalation Toxicity Study in Rats**BACKGROUND INFORMATION**

Study Title: 3 Month Inhalation Toxicity Study in Rats
Sponsor Study No.: 90-6224
Laboratory Study No.: 650361
Study Dates: October 12, 1990 - February 20, 1991
Report Date: January 30, 1992
Test Facility: _____

b(4)

GLP Status: Compliant
NDA Volume:Page 34:1

METHODS

Test Article: CGP 25827A
Batch No: 14/909/1 **b(4)**
Purity: _____
Control Article: Aerosol fluorocarbon propellants
Purity: Not stated
Species/Strain: Sprague-Dawley Rats
Route: Nose Only Inhalation
Exposure Conditions: Aluminum cylindrical chamber with apparatus to actuate aerosol cans to achieve target concentrations.
Duration of Exposure: 3 months

Dosing Information

Group	No. Animals per sex	Dose mg/kg/day	No. of Cans x Actuations/min x h/day	Chamber Conc. (µg/l)	Nominal Conc. (µg/l)	Mass Mean % of Particles with <6 µm Diameter
1 (Control) ^a	10	0	6 x 6 x 4 h	0	0	94.3
2 (Low)	10	0.034	3 x 2 x 1 h	2.0	7.2	92.1
3 (Mid)	10	0.136	3 x 4 x 2 h	4.0	14.4	94.2
4 (High)	10	0.442	6 x 6 x 4 h	6.7	43.2	94.2

^a Values reported represent placebo excipients only.

The low dose was selected as a small multiple of the anticipated human dose. The high dose was the highest dose that could be achieved using the dosing apparatus for this study.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Prior to treatment and During Weeks 6 and 13
Clinical Pathology	Weeks 7 and 13
Gross pathology (including organ weights)	After 91 days of treatment
Histopathology (complete tissue list including anterior and posterior nasal cavities, larynx, anterior and posterior trachea, and lung sections through bronchioles)	At study termination for control and high dose groups.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	Body weights for treated animals were 25 - 35% higher than control values for all treated groups of males and females.
Food consumption	Treated animals from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	The following parameters were significantly different from control: <ul style="list-style-type: none"> • Hemoglobin concentration - Week 7, ↑ Group 3 - 4 ♂; Week 13, ↑ Group 3 ♂ • Hematocrit - Week 7, ↑ Group 3 - 4 ♂; • Mean cell hemoglobin - Week 7, ↑ Group 2 and 3 ♂; Week 13, ↑ Group 2,3 ♂; • Mean cell volume - Week 7, ↑ Groups 2 - 4 ♂; • Glucose - Week 7, ↓ Group 3 - 4 ♂; Week 13, ↓ Groups 3 - 4 ♂.
Gross pathology	
Organ Weights	Heart weights were significantly higher for all treated groups of animals when compared to controls. Kidney and liver weights for high dose males were higher than controls. Thyroid weights were higher than controls for mid dose males. Lung weights were higher for Group 2 females when compared to controls. Adrenal weights were higher for Group 2 and 3 females when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (0.442 mg/kg/day). Notably, there were no adverse effects on the respiratory system.

10. 6/12 Month Inhalation Study in Rats (— Formulation 1/73)**b(4)****BACKGROUND INFORMATION**

Study Title: 6/12 Month Inhalation Study in Rats (— Formulation 1/73)

Sponsor Study No.: 936115

Laboratory Study No.: 653179

Study Dates: June 10, 1993 - August 5, 1994

Report Date: March 8, 1996

Test Facility: _____

GLP Status: Compliant

b(4)**b(4)**

NDA Volume:Page 39:1

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/73)
Batch No: 1103/1 and 1173/1
Purity: _____
Control Article: Lactose
Purity: Not stated
Species/Strain: Sprague-Dawley derived albino (Tif:RAIf) rats
Route: Nose Only Pulsed Inhalation
Exposure Conditions: Rotating brush generator with monitored air flow, temperature, and humidity.
Duration of Exposure: 1 year with and 8-week recovery

b(4)

Dosing Information

Group	No.			Dose Levels ($\mu\text{g}/\text{kg}/\text{day}$)	Mean % of Particles <3.5 μm Diameter
	Animals/sex: Main Study	Animals/sex: 6-Mo. Interim	Animals/sex: Recovery		
1 (Lactose)	20	10	5	0	47.5
2 (Low)	20	10	5	30	50.4
3 (Mid)	20	10	5	120	46.7
4 (High)	20	10	5	400	44.0

The results of previous toxicity studies and a feasibility study using the exposure conditions described for the current study were considered in dose selection. In a 28-day inhalation study in rats, doses were 15.6, 50.4, and 115.3 $\mu\text{g}/\text{kg}/\text{day}$ and findings included significantly increased heart weights at all doses without microscopic correlate. In a 90-day study, doses were 2.5, 8.1, and 26.1 $\mu\text{g}/\text{kg}/\text{day}$ and findings included increased heart weights for high dose females only and there was no microscopic correlate.

The low dose was a small multiple of what was thought to be the intended human therapeutic dose (48 $\mu\text{g}/\text{day}$). The high dose was described by the Sponsor as the "maximum tolerated dose that can be administered to the test model of the 52 week duration of the study without causing unnecessary distress or suffering." The basis for this claim was not stated.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Rectal temperature	Week 25
Ophthalmoscopy	Prior to treatment and at Weeks 6, 12, 25, 39, and 51
Urine for Proof of Absorption	Weeks 26 and 51

Parameter	Frequency of Measurement
Plasma for Proof of Absorption	Weeks 26 and 51 (predose, immediately postdose, and 0.5, 1, 2, 4, and 8 hours postdose)
Clinical pathology	Prior to treatment and at Weeks 7, 13, 36, 39, and 52; and after the 8-week recovery period
Gross pathology (including organ weights)	After 6 months and 12 months of treatment and 8 weeks of recovery
Histopathology	After 6 months and 12 months of treatment and 8 weeks of recovery. Complete tissue list for all control and high dose animals and salivary glands, heart, spleen, lungs, nasal cavity and testes were examined from animals in all groups.

RESULTS

Mortality

No remarkable observations.

Clinical signs

No remarkable observations.

Body weight

Body weight gain was higher for treated males when compared to controls throughout the growth phase of the study (Weeks 1 - 15). Thereafter, body weight gain values for treated males approached that of controls and was less than controls for the latter part of the study. Body weight gain values for females were consistently greater than control values throughout the study.

Food consumption

No remarkable findings.

Rectal temperature

No remarkable findings.

Ophthalmoscopy

No remarkable findings.

Urine for Proof of Absorption

Not included in this report.

Plasma for Proof of Absorption

Not included in this report.

Clinical Pathology

Glucose levels for all treated groups of animals were consistently decreased compared to control values. Statistically significant differences from control values were reported for various other parameters in hematology and clinical chemistry determinations, however the changes could not be definitively attributed to treatment with CGP 25827A because they did not occur in a dose related response, were inconsistent over time, or were not biologically meaningful.

Gross pathology

Organ Weights

Treatment related changes in organ weights were observed in the heart (all dose groups), lung (all dose groups), liver (mid and high dose groups), and testes (mid and high dose groups). The changes in the liver and testes weights persisted through the recovery period.

Increased heart weights were noted for all groups of treated animals when compared to controls as early as 6 months, persisted through the 12-month observation, but were not present after the 8-week recovery period. Lung weights for all treated groups were higher than control values at the 6 (males and females) and 12 (females) month intervals, but were not noted at the 8-week recovery interval. Thyroid weights were not measured at the 6 month interval but were significantly higher than control for high dose females at the 12 month interval.

Testes weights for the mid and high dose males were lower than control values at the 6- and 12-month intervals and at the 8-week recovery interval. The other organ weight change that persisted through the recover period was lower-than-control liver weights for males at 6 months (high dose) and at the recovery interval (mid and high dose).

Necropsy Observations

An increased incidence of small or flaccid testes was observed in mid and high dose group males at the 6 and 12 month intervals and after recovery when compared to controls.

Histopathology

Remarkable microscopic findings were observed in the testes (Group 3 and 4 males); spleen (Group 3 and 4 females); salivary gland (Group 4 males and females); nasal cavity (Group 4 males and females); lungs (Group 4 females); and heart (Group 4 males). All findings except degeneration of germinal epithelium of the seminiferous tubules resolved during the 8-week recovery period.

The only remarkable finding at the 6 month interval was degeneration of seminiferous tubules. Only high dose and control animals were examined at this interval, thus the finding was only identified in the high dose males. At the 12 month interval, this finding was revealed in mid dose rats as well, and did not resolve for the mid or high dose males after the 8 week recovery period.

The findings in the lung and nasal cavity were confined to mid and/or high dose animals at the 12 month interval and were not present after 8 weeks of recovery. Findings in the lung consisted of an increased severity of "large 'foamy' macrophages" around the terminal bronchioles of the high dose females. In the nasal cavity, an increased incidence and severity of goblet cell proliferation was observed in the anterior region of the cavity in high dose males and females when compared to controls.

Findings in the spleen consisted of increased incidence and/or severity of extramedullary hematopoiesis in mid and high dose animals at the 12 month interval.

In the salivary gland, very mild hypertrophy of serous acini was noted in 1/20, 0/20, 0/18 and 10/20 males in Groups 1 - 4, respectively and in 2/20, 2/20, 2/20 and 9/20 females in Groups 1 - 4, respectively, at the 12 month interval.

The notable finding in the heart was an increased incidence of myocardial fibrosis in high dose males when compared to controls.

CONCLUSION

After one year of treatment with CGP 25827A via dry powder inhalation, rats receiving 120 and 400 $\mu\text{g}/\text{kg}/\text{day}$ had degeneration of seminiferous tubules that did not recover after an 8-week period without treatment. The no observable adverse effect level (NOAEL) for this irreversible finding was 30 $\mu\text{g}/\text{kg}/\text{day}$ in rats. — times the expected human dose daily inhaled dose of $\approx \mu\text{g}$.

Findings in the heart (myocardial fibrosis), lung (presence of foam cells) were consistent with those observed in the rat dietary carcinogenicity study with CGP 25827A.

b(4)

Mice

Three month dose range finding studies were conducted in mice: 1 via dietary administration and 2 via drinking water administration.

After 3 months of dietary administration, mice treated at levels of 40 mg/kg/day and above had increased heart weights without microscopic correlate, and decreased glucose levels and increased urea levels. Red cell parameters were elevated in groups treated at levels of 400 mg/kg/day and above. Kidney weights were higher than control in animals treated at levels of 140 and above, but histopathologic evaluation was not performed on this organ.

After 3 months of treatment with the test material in the drinking water, no remarkable changes were observed in animals dosed as high as 450 mg/kg/day.

A second 3 month study was conducted to supplement information obtained in the carcinogenicity study (reported later in this review). Reviews of this and the other 3 month studies are presented below.

11. 3-Month Range Finding Study in Mice (Administration in Food)

BACKGROUND INFORMATION

Study Title:	3-Month Range Finding Study in Mice (Administration in Food)
Sponsor Study No.:	906171
Study Dates:	May 22 - August 22, 1990
Report Date:	February 28, 1992
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division 4002 Basle Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986, Section 4, 2.2(e)
NDA Volume:Page	18:1

METHODS

Test Article:	CGP 25827A
Batch No.:	810187
Purity:	_____ b(4)
Control Article:	Diet
Species/Strain:	Mouse/Tif:MAGf (SPF), hybrids of NIH x MAG
Route:	Oral in the diet
Duration of Exposure:	3 months

Animals were assigned to groups and administered CGP 25827A in the diet. Target doses and actual doses measured in the diet are presented in the following table. In the last

column, plasma levels associated with each group are presented; mean values represent a sample size of 2 for each group at the end of the study and ND stands for not detected.

Group	No. Animals per sex	Target Dose (mg/kg/day)	Actual Dietary Levels		Mean Plasma Levels after 3 months (pmol/g)	
			(mg/kg/day)			
			Males	Females	Males	Females
1	9	0	-	-	ND	ND
2	9	40	5.73	8.97	ND	ND
3	9	140	22.3	33.5	3.4	2.5
4	9	400	61.8	82.3	7.0	6.9
5	9	1400	246	335	32.9	35.8

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Water consumption	Weekly
Clinical pathology	1 and 3 months
Blood level determinations	1 and 3 months (2/sex/group)
Organ weight	3 months
Gross pathology (tissues were saved)	3 months
Histopathology	3 months (heart only)

RESULTS

In- life Observations

There were no remarkable differences from control in mean body weight values. Treated mice ate and drank more than control mice, but not in a consistent dose-response pattern.

Clinical Pathology

Slightly higher than control values for erythrocyte, hemoglobin, and hematocrit were observed in the females treated at the 400 (Group 4) and 1400 (Group 5) mg/kg/day level. Mean values reported for these parameters are reported in the following table with statistically significant findings noted with an asterisk.

Group	Mean Hematology Values of Parameters Affected by CGP 25827					
	RBC (T/l)		Hb (mmol/l)		Hct (l)	
	Males	Females	Males	Females	Males	Females
1	11.4	11.1	10.6	10.7	0.503	0.509
2	12.0	11.6	10.7	10.9	0.503	0.523

Group	RBC (T/l)		Hb (mmol/l)		Hct (l)	
3	12.1	11.8	11.4	11.2	0.527	0.532
4	12.4	12.5*	11.2	11.5*	0.532	0.551
5	12.1	12.6*	10.9*	11.9*	0.518*	0.568*

Serum chemistry parameters that differed from control included decreased glucose levels (mean values were 7.0, 4.4, 4.6, 4.8, and 4.0 for groups 1 - 5, respectively) and increased urea levels (mean values were 8.3, 11.8, 12.0, 11.2, and 14.3 for groups 1 - 5, respectively) for all treated groups of males. Liver enzyme levels of high dose males were elevated above control.

Gross and Microscopic Pathology

Effects on the heart and kidney were evidenced by changes in organ weight. Mean kidney weights, kidney-to-body and kidney-to-brain weight ratios were higher than control, with statistical significance often achieved in male groups treated at 140, 400, and/or 1400 mg/kg/day levels. Mean heart weight, heart-to-body and heart-to-brain weight ratios were consistently higher than control values for all treated groups of males and females.

There were no remarkable gross findings in tissues examined.

No treatment-related findings were revealed by microscopic evaluation of the heart in animals from any dose group.

CONCLUSION

After 3 months of dosing with CGP 25827A at levels up to 1400 mg/kg/day mice exhibited signs of effects on red cell parameters, heart, kidney, and perhaps liver. Erythrocyte, hemoglobin, and hematocrit values were elevated above control in the 400 and 1400 mg/kg/day female groups. Decreased glucose levels and increased urea levels relative to control were noted for all dose groups. Effects on the liver were evidenced by increases in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in males in the 1400 mg/kg/day dose group. Effects on the heart and kidney were evidenced by increases in weight in groups 2 (heart only), 3, 4, and 5. Only the heart was examined microscopically and no correlate to the increased weight or any other findings were revealed.

12. Thirteen Week Drinking Water Study in Mice

BACKGROUND INFORMATION

Study Title: Thirteen Week Drinking Water Study in Mice
 Sponsor Study No.: -D-8-3
 Laboratory Study No.: 2069-102
 Study Dates: May 17, 1979 - August 22, 1979

b(4)

Report Date: March 6, 1980
 Test Facility: _____

b(4)

GLP Status: Compliant
 NDA Volume:Page 17:1

METHODS

Test Article: CGP 25827A (BD40A)
 Batch No.: L6
 Control Article: Acidified water
 Species/Strain: Mouse/B6C3F1
 Route: Drinking water
 Duration of Exposure: 13-weeks

Fifteen mice per sex were randomly assigned to four groups receiving CGP 25827A at concentrations of 0, 0.25, 0.50, and 1.00 mg/ml. Overall mean compound consumption values were 96, 185, and 397 mg/kg/day for males in Groups 2 - 4, respectively, and 101, 194, and 450 mg/kg/day, respectively for females in Groups 2 - 4, respectively. Mice received test material or control water via *ad libitum* exposure in the drinking water. Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Weekly
Body weight	Weekly
Food consumption	Weekly
Water consumption	Twice weekly
Ophthalmoscopic Examinations	Pretest, Weeks 6 and 13
Organ weight data	Week 13
Gross pathology	Week 13
Histopathology	Week 13

RESULTS

No treatment-related findings were noted for any of the parameters evaluated.

CONCLUSION

The no observable effect level (NOEL) for CGP 25827A is 1.0 mg/ml (~ 400 mg/kg/day) in mice treated for 13-weeks via oral administration in the drinking water.

13. 3-Month Oral Toxicity Study in Mice

BACKGROUND INFORMATION

Study Title: 3-Month Oral Toxicity Study in Mice
Sponsor Study No.: 906303
Study Dates: January 28 - May 7, 1991
Report Date: August 6, 1992
Test Facility: CIBA-GEIGY Limited
 Pharmaceutical Division
 Preclinical Safety
 Basel, Switzerland
GLP Status: Compliant with GLP Switzerland, Procedures and Principles, March 1986, Section 4, 2.2(e)
NDA Volume:Page 17:55

METHODS

Test Article: CGP 25827A
Batch No.: 400190
Control Article: Acidified tap water
Species/Strain: Mouse/Tif:MAGf(SPF)
Route: Drinking water
Duration of Exposure: 3 months

This study was designed to supplement a carcinogenicity study⁵ with information regarding:

- actual water consumption and test article intake
- plasma levels of CGP 25827A
- serum electrolyte levels (to assess adrenal gland function)
- _____ analysis of the mitotic index of the non-glandular stomach mucosa and adrenal gland.

b(4)

Twenty-two mice per sex were randomly assigned to three groups receiving CGP 25827A at concentrations of 0.25, 0.50, and 1.00 mg/ml. Within treatment groups, animals were divided into groups of 6 for clinical chemistry, 6 for hematology, and 10 for satellite study. A group of 12 control mice per sex received acidified water and was also divided into 2 groups of 6 for clinical chemistry and hematology, respectively. Thus, the total number of animals used for in-life, gross and microscopic evaluations were 12 controls and 22 treated mice/sex/group. Mice received test material or control water via *ad libitum* exposure in the drinking water. Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Daily and then 3 times per week beginning with Week 5

⁵ 104-Week Drinking Water Toxicity Study in Mice, _____, Project No. 2069-105, September, 16, 1983.

b(4)

Parameter	Frequency of Measurement
Food consumption	Twice per week
Water consumption	Daily with correction for spillage
Hearing test	Pretest, Weeks 6 and 13
Ophthalmology	Pretest, Weeks 6 and 13
Clinical biochemistry	Pretest, Weeks, 5 and 15
Hematology	Pretest, Weeks 5, 9, and 14
Organ weight	Weeks 13 - 15
Gross pathology	Weeks 13 - 15
Histopathology	Weeks 13 - 15

RESULTS

The Sponsor calculated individual water consumption and test article intake after recovery of spilled water. Water consumption for the high dose males was low and yielded lower than target values for test article intake. The test article intake values for high dose males was similar to that of mid-dose females. Test article intake values for high dose females were about 60% higher than that for high dose males. Using a graph provided the Sponsor, the overall mean test article intake values can be estimated as 65, 100, and 165 mg/kg/day for males and 80, 180, and 270 mg/kg/day for females. Results of plasma analysis were not reported in this study.

There were no remarkable findings in clinical chemistry parameters.

Microscopic evaluation of the adrenal gland revealed minimal focal hypertrophy or hyperplasia in several male mice in the mid and high dose groups. No increase in mitotic activity of adrenal cortical cells was revealed in this study. There was no mention of findings or increases in mitotic indices in the non-glandular stomach.

Other microscopic findings included congestion and increased hematopoiesis in the spleen in all dose groups and hypertrophy of the mandibular salivary gland in females from all dose groups and from males from the high dose group.

CONCLUSION

The Sponsor attempted to replicate the conditions of the referenced carcinogenicity study, with the exception of data collection techniques for water consumption, plasma sampling, and evaluation of mitotic indices in two tissues.

Findings unique to only one of the two studies are summarized in the following table.

Carcinogenicity Study Only	Current Study Only
<ul style="list-style-type: none"> adrenal spindle cell hyperplasia 	<ul style="list-style-type: none"> water consumption and thus test article intake calculations were higher for females when compared to males in all dose groups splenic congestion and hematopoiesis with

- concomitant changes in red blood cell parameters
- hypertrophy of the mandibular salivary gland

While not previously reported for mice, the findings in the mandibular salivary gland had been seen in rats and were considered by the Sponsor to be characteristic of the response to β -agonists.

Dogs

The toxicity of CGP 25827A was tested in dogs via both the oral and inhalation routes of administration for up to 1 year. Effects revealed in these studies are attributed to an exaggeration of pharmacologic effects of beta agonists. Dogs treated for one year by the inhalation route exhibited increased heart rate and force of contraction at daily doses of $\geq 2 \mu\text{g}/\text{kg}/\text{day}$ (.002 mg/kg/day) via inhalation and $\geq 0.01 \text{ mg}/\text{kg}/\text{day}$ via oral administration. Myocardial fibrosis was noted in the dogs treated orally and did not fully resolve during the one-month recovery phase. This finding was not noted in the inhalation study.

There were no signs of overt toxicity in dogs treated with CGP 25827A at a level of 15.16 $\mu\text{g}/\text{kg}/\text{day}$ via inhalation for one year.

Reviews of studies conducted in dogs is presented below.

14. 12 Month Oral Toxicity Study in Beagle Dogs (CGP 25827A)

BACKGROUND INFORMATION

Study Title:	12 Month Oral Toxicity Study in Beagle Dogs (CGP 25827A)
Sponsor Study No.:	85-751
Laboratory Study No.:	Not applicable
Study Dates:	March 10, 1986 – April 8, 1987
Report Date:	April 18, 1988
Test Facility:	CIBA GEIGY Limited Experimental Toxicology 4332 Stein, Switzerland
GLP Status:	Compliant
NDA Volume:Page	26:1

METHODS

Test Article:	CGP 25827A
Batch No (analytical content of active [mg]):	11/806/1 (1.9); 11/805/1 (0.9); 11/804/1 (0.05); 11/803/1 (0.1); 11/802/1 (0.5); 11/801/1 (1.0)
Control Article:	Gelatin capsule with "Placebo EE, batch 09/797/1"

Species/Strain: Beagle dog
Route: Oral capsule
Duration of Exposure: 12 months

Animals were randomly assigned to study groups and the test material was administered in gelatin capsules illustrated in the table below. Doses were selected based on observed toxicity in previous studies with CGP 25827A. The high dose was expected to cause myocardial lesions and was estimated to be the maximum tolerated dose for beagle dogs subjected to a 1-year study. Slight pharmacodynamic effects (increased heart rates) were expected at the low dose. Four dogs/sex/group were treated for 1 year and terminated. An additional 2 dogs/sex/group remained on study for a 1-month recovery period.

Group	No. Animals/Sex	Dose ($\mu\text{g}/\text{kg}/\text{day}$)
1	6	0
2	6	0.01
3	6	0.1
4	6	1.0

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Prior to treatment and at Weeks 14, 28, and 51, and at the end of the 4-week recovery period. Measurements were taken immediately before the daily dose in all animals and at 1, 3, and 5 hours after dosing in 2 dogs/sex/group.
Ophthalmology	Prior to treatment, during Week 52, and at the end of the 4-week recovery period
Hearing test	Prior to treatment, during Week 52, and at the end of the 4-week recovery period
Hematology	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Serum chemistry	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Urinalysis	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Gross pathology	4 dogs/sex/group after 52 Weeks and 2 dogs/sex/group after the 4-week recovery period
Organ weights	Same schedule as gross pathology. Organs weighed included: adrenals, brain, cervical lymph nodes, epididymides, heart, kidneys, liver, lung, ovaries, parathyroid, pituitary, prostate, spleen, submandibular salivary glands, testes, thymus, thyroid, and uterus.
Histopathology	Same schedule as gross pathology

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Reddening of the skin (ears and abdomen) and mucous membranes (gingival and conjunctiva) were reported for Groups 3 and 4 animals. This observation as first noted shortly following daily dosing and resolved. This observation was not noted during the 4-week recovery period.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Ophthalmology	Alterations in the appearance of the fundus were noted in Groups 3 and 4 animals. The incidence of remarkable ophthalmoscopic findings in treated groups is summarized in the following table. Microscopic examination of ocular tissues revealed atrophy of the tapetum lucidum. These findings did not resolve during the 4-week recovery period

Incidence of Treatment-Related Ophthalmoscopic Findings

Finding	0.01 mg/kg/day		0.1 mg/kg/day		1.0 mg/kg/day	
	Males	Females	Males	Females	Males	Females
Expansion of pigmentation in the fundus	0/6	0/6	0/6	1/6	4/6	3/6
Orange spots in the tapetal area	0/6	0/6	1/6	4/6	5/6	4/6
Hyperreflective foci in the tapetal area	0/6	0/6	0/6	0/6	1/6	0/6

Parameter	Remarkable Observations
Hearing test	No remarkable observations.
Electrocardiograph	Bradycardia was noted in all treated groups. Heart rates were slightly increased when compared to controls in all treated groups 1, 3 and 5 hours after dosing and decreased thereafter, reaching markedly lower heart rates than controls by 22 hours after dosing.
Hematology	Decreased hemoglobin in group 3 males, hypochromasia and microcytosis in group 4 animals, increased platelet counts in group 2 males and group 3 females.
Serum chemistry	Decreased glucose in all treated groups; decreased cholesterol and HDL in group 4 males; decreased triglycerides in group 3 males and group 4 females; decreased phospholipid in group 4 animals; increased potassium in all treated groups; and increased phosphate in group 4 females.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weight	Decreased heart weights in all treated groups of males.
Histopathology	Focal myocardial fibrosis in all groups of treated animals. This effect did not resolve during the one-month treatment-free period.

CONCLUSION

Observations noted in this study were consistent with those reported in previous studies and are attributed to an exaggeration of the pharmacologic activity of this β agonist. All treated groups exhibited pharmacologic signs of exposure to CGP 25827A (Foradil).

Clinical signs (reddening of the skin and mucous membranes) and increased heart rates are likely vasodilatory effects and were not noted during the course of the recovery phase.

All signs of toxicity resolved within the 1-month recovery period with the exception of focal fibrosis of the myocardium and atrophy of the ocular tapetum lucidum. Myocardial fibrosis is consistent with findings in other studies with β agonists. The tapetum lucidum atrophy is considered to be of negligible clinical significance since humans are an atepetal species. The NOAEL was not identified in this effect.

15. Preliminary Inhalation Toxicity Study in the Dog

BACKGROUND INFORMATION

Study Title: Preliminary Inhalation Toxicity Study in the Dog
Sponsor Study No.: 926109
Laboratory Study No.: 321906
Study Dates: Not Stated
Report Date: June 8, 1993
Test Facility: _____

b(4)

GLP Status: Non-compliant
NDA Volume:Page 40:294

METHODS

Test Article: CGP 25827A (Foradil)
Batch No: Not stated
Purity: Not stated
Control Article: None
Purity: Not stated
Species/Strain: Dog (strain not stated)
Route: Inhalation
No. of Animals: 2
Dose levels: 1.5 – 2.4 mg/kg/day
Duration of Exposure: 5 days

Toxicity was assessed by evaluating gross and microscopic pathology.

RESULTS

Myocardial necrosis was observed in both animals and was considered to be a result of treatment with CGP 25827A.

CONCLUSION

This study is of limited value in the overall evaluation of the toxicity of the drug due to several reasons including: the lack of proper controls, low sample size, unspecified

conditions of exposure, lack of the full battery of parameters needed to assess toxicity, and the lack of a no observable effect level.

16. Inhalation Feasibility Study in Dogs (CGP 25827A (Foradil) - Formulation b(4)

BACKGROUND INFORMATION

Study Title: Inhalation Feasibility Study in Dogs (CGP 25827A (Foradil) - Formulation b(4)
Sponsor Study No.: 93-6077
Laboratory Study No.: 653011
Study Dates: April 2 - 16, 1993
Report Date: June 24, 1994
Test Facility: _____ b(4)
GLP Status: Compliant
NDA Volume:Page 41:1

METHODS

Test Article: CGP 25827A
Batch No: 1066/1
Purity: _____ b(4)
Control Article: Not Applicable
Purity: Not Applicable
Species/Strain: Beagle dog
Route: Oral Inhalation
Exposure Conditions: Rotating brush generator with compressed air dispersion.
Duration of Exposure and Dose Levels: Single doses of 20 and 50 µg/kg were given to one male and one female on separate occasions. The time between doses was not specified in the report. Animals were sacrificed on Day 4.

Particle Size Distribution: _____ b(4)
 µg/kg exposures, respectively.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	During and after dosing
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Predose and 5, 10, 30, 60, 120, and 240 minutes after dosing
Respiratory function	3 times over the course of the study
Blood for proof of absorption	Predose and at 30 minutes after each dose

Parameter	Frequency of Measurement
Urine for proof of absorption	Urine was collected for 24 h immediately after dosing.
Gross pathology of the heart	Day 4
Histopathology of the heart	Day 4

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Increased heart force and reddening of the mouth, gums, and sometimes ventral surface of the abdomen were reported for one or both dogs immediately following exposure.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Electrocardiograph	Heart rates were markedly increased with a maximum effect at 30 minutes to 1 hour post dose. Heart rates remained increased for up to 4 hours post dose.
Respiratory function	No remarkable observations.
Gross pathology of the heart	Pale foci were observed in the left ventricular papillary muscle in both animals. Valvular thickening was also observed.
Histopathology of the heart	Acute myocardial degeneration was observed in both animals.

CONCLUSION

This study demonstrated that the CGP 25827A dry powder formulation could be administered to dogs in a direct passive inhalation dosing setting.

The heart was affected by treatment with CGP 25827A at levels of 20 and 50 µg. Clinical signs (reddening of the mouth and ventral surface) and increased heart rates are likely vasodilatory effects. The Sponsor reported that the myocardial degeneration was consistent with anoxic damage indirectly associated with vasodilatation.

17. 4-Week Inhalation Toxicity Study in Dogs with CGP 25827A(Foradil)Dry Powder Formulation (1/69) in the Dog

BACKGROUND INFORMATION

Study Title: 4-Week Inhalation Toxicity Study in Dogs with CGP 25827A(Foradil)Dry Powder Formulation (1/69) in the Dog

Sponsor Study No.: 926074

Laboratory Study No.: 321917

Study Dates: July 9 - august 6, 1992

Report Date: January 22, 1993

Test Facility: _____

b(4)

GLP Status: Compliant
 NDA Volume:Page 41:52

b(4)

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/69)
 Batch No: 1066/1
 Purity: _____ b(4)
 Control Article: Lactose 100 Mesh
 Purity: Not stated
 Species/Strain: Beagle dog
 Route: Oral Inhalation
 Exposure Conditions: Rotating brush generator with a _____ b(4)

Duration of Exposure: 4 Weeks

Animals were randomized into study groups and dosed as follows:

Dosing Information			
Group	No. Animals/sex	Dose Levels of Lactose or Test Article as Supplied (mg/kg/day)	Active Dose Calculated from Analysis of Filter Samples (µg/kg/day)
1 (Lactose)	3	2	0
2 (Low)	3	0.1	3
3 (Mid)	3	0.5	14
4 (High)	3	2	55

The mean percentage \pm S.D. of particles found at an aerodynamic diameter of 4.6 µm or below was 76.0 \pm 3.1% for CGP 25827A and 48.4 \pm 7.6 for lactose.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Weekly
Food consumption	Daily
Ophthalmoscopic exams	Predose and after 4 weeks
Electrocardiograph	Predose Day 1 – 30 minutes postdose (all groups) and 2 hours postdose (Groups 2 and 4) Week 1 – Predose; and at 10 minutes and then 2 hours postdose Week 2 – Predose and 2 hours postdose Week 4 – Predose and 10 minutes and then 2 hours postdose

Parameter	Frequency of Measurement
Respiratory function	Predose, on Day 2 and after 4 weeks
Hematology	Predose and after 4 weeks
Serum Chemistry	Predose and after 4 weeks
Urinalysis	Predose and after 4 weeks
Blood for proof of absorption	After 1 week: Prior to the daily dose and 30 minutes after dosing in Group 4 animals only
Urine for proof of absorption	Urine was collected from all animals for 24 h immediately after dosing at the end of Weeks 1 and 4.
Gross pathology	After 4 weeks.
Organ weights	Weighed organs include: adrenal glands, brain, heart, kidneys, liver, lungs, pituitary gland, prostate gland, spleen, testes, epididymides, thyroid gland with parathyroids.
Histopathology	After 4 weeks.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Erythema of the mucous membranes of the oral cavity and abdominal skin was observed in all dogs treated with CGP 25827A. This observation was noted daily shortly following dose administration and persisted for approximately 1 to 5 hours.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Electrocardiograph	Heart rates from all treated groups were increased at 30 minutes and 2 hours post dose on Day 1 when compared to control and pre-test values. This change was accompanied by increased P-wave amplitude and decreased QT-intervals. Frank tachycardia was observed in dogs from all dose groups. These changes were also observed at subsequent intervals.
Respiratory function	No remarkable observations.
Hematology	No remarkable observations.
Serum chemistry	Serum electrolyte levels were slightly off balance in treated animals when compared to controls and pretest values. Potassium levels increased slightly with increasing dose while sodium levels were significantly lower than controls for Groups 3 and 4 females.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weights	No remarkable observations.
Histopathology	Myocardial fibrosis was observed in 0/3, 1/3, 0/3, and 2/3 males from Groups 1 - 4, respectively and in 0/3, 0/3, 1/3, and 1/3 females from Groups 1 - 4, respectively. Severity increased with increasing dose. A no effect level for this observation was not identified in this study.

Dosing Information		
Group	No. Animals per sex	Target Dose (µg/day)
1	2	0
2	4	1200 (outdated)
3	4	1200 (fresh)

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Clinical signs	Daily
Body weight	Twice weekly
Food consumption	Daily
Ophthalmoscopic Examinations	Pretest and Week 4
Electrocardiograms	Pretest and Week 4
Respiratory Function	Pretest and Week 4
Clinical Pathology	Pretest and Day 27
Gross pathology (including organ weights)	Study termination
Histopathology	Study termination

RESULTS

Parameter	Findings
Clinical signs	No remarkable findings.
Body weight	No remarkable findings.
Food consumption	No remarkable findings.
Ophthalmoscopic Examinations	No remarkable findings.
Electrocardiograms	No remarkable findings.
Respiratory Function	No remarkable findings.
Clinical Pathology	A slight reduction in hemoglobin and red blood cell counts were noted in dogs treated with CGP 25827A when compared to controls. No differences were noted between groups treated with fresh and outdated CGP 25827A.
Gross pathology (including organ weights)	Spleen weights were lower for dogs treated with CGP 25827A when compared to controls. No differences were noted between groups treated with fresh and outdated CGP 25827A.
Histopathology	There were no remarkable gross findings. No remarkable findings.

CONCLUSION

There were no differences between groups treated with fresh CGP 25827A or with CGP 25827A that was outdated.

19. 6/12 Month Inhalation Toxicity Study in Dogs (CGP 25827A (Foradil))**BACKGROUND INFORMATION**

Study Title: 6/12 Month Inhalation Toxicity Study in Dogs (CGP 25827A (Foradil))
Sponsor Study No.: 93-6116
Laboratory Study No.: 653184
Study Dates: May 21, 1993 - August 24, 1994
Report Date: March 15, 1996
Test Facility: _____ **b(4)**

GLP Status: Compliant
NDA Volume:Page 42:1

METHODS

Test Article: CGP 25827A (Foradil)
Batch No: 1103/1 (Weeks 1 - 42) and 1173/1 (Weeks 42 - 52) **b(4)**
Purity: _____
Control Article: Lactose 100 Mesh
Purity: Not stated
Species/Strain: Beagle dog
Route: Oral Inhalation
Exposure Conditions: Rotating brush generator with compressed air dispersion.
Duration of Exposure: 12 months

Animals were randomly assigned to study groups and the test material was administered as follows:

Group	No. Animals/Sex	Target Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Achieved Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Particle Size (%<6 μm via I)
1	6	0	0	-
2	4	2	2.01	87
3	4	6	5.44	83
4	6	18	15.16	89

b(4)

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Predose, immediately following dosing and 1 hour after dosing on Day 1 and during Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Ophthalmology	Prior to treatment and during Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Respiratory function	Prior to treatment and at Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Hematology	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period
Serum chemistry	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period
Urinalysis	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period
Blood for proof of absorption	Serial samples were collected on Day 1 and during Weeks 28 and 52
Urine for proof of absorption	Urine was collected for 24 h on Day 1 and during Weeks 28 and 52
Gross pathology	4 dogs/sex/group after 52 Weeks and 2 dogs/control and high dose groups after an 8 week recovery period
Organ weights	Same schedule as gross pathology. Organs weighed included: adrenals, brain, heart, kidneys, liver, lung, ovaries, pancreas, parathyroid, pituitary, prostate, spleen, testes, thymus, thyroid, and uterus.
Histopathology	Same schedule as gross pathology. Special staining _____ was used to examine mucus-secreting cells of the trachea.

b(4)

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Increased heart force and reddening of the mouth, gums, ears and ventral surface of the abdomen were reported for all dogs treated with CGP 25827A (Foradil). This observation was not noted during the 8-week recovery period.
Body weight	Body weight values in treated animals were consistently higher than control values during the treatment phase of study. The high dose animals exhibited a gradual body weight loss during the 8-week recovery period.
Food consumption	No remarkable observations.
Ophthalmology	No remarkable observations.

Parameter	Remarkable Observations
Electrocardiograph	Heart rates were sporadically increased in all groups treated with CGP 25827A (Foradil) when compared to controls, however no consistent patterns were observed during the course of the study.
Respiratory function	No remarkable observations.
Hematology	Red cell parameters were lower in treated animals from all groups when compared to controls throughout the treatment period. This finding was present, but to a lesser degree, at the end of recovery period for high dose animals.
Serum chemistry	Triglyceride levels for treated animals were lower than control values at all serum collection intervals for males and at Weeks 7, 13 and 52 for females. Creatinine levels were consistently higher for treated groups when compared to controls. There were no remarkable observations for animals in the recovery phase of the study.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weight	No remarkable observations.
Histopathology	No remarkable observations.

CONCLUSION

Observations noted in this study were consistent with those reported in previous studies and are attributed to an exaggeration of the pharmacologic activity of this β agonist. All treated groups exhibited pharmacologic signs of exposure to CGP 25827A (Foradil). Results of the proof of absorption studies were not included in this report.

Clinical signs (reddening of the mouth and ventral surface) and increased heart rates are likely vasodilatory effects and were not noted during the course of the recovery phase. The Sponsor attributed the increased body weight effect to the anabolic effect of β agonist compounds. They further speculated that the observed increases in creatinine levels in treated animals could be either the result of an anabolic effect, with a proportional increase in muscle mass, or a consequence of lower glomerular filtration brought on by low blood pressure associated with the pharmacologic action of CGP 25827A (Foradil).

In conclusion, there were no signs of overt toxicity associated with inhalation of CGP 25827A (Foradil) at levels up to 15.16 $\mu\text{g}/\text{kg}/\text{day}$, the NOAEL, for one year in beagle dogs.

Overall Toxicology Summary

The toxicity of Formoterol fumarate was studied in rats, mice, and dogs via oral and inhalation routes of administration. Inhalation toxicology studies in rats and dogs were conducted for up to 1 year. Oral studies in mice and rats were used in selecting the doses of subsequent carcinogenicity studies and were conducted for 3 months. An oral study in dogs was conducted for up to 1 year.

Effects on the heart were consistent across species and routes of administration and were noted in both the 90-day and 1-year studies. Myocardial fibrosis was observed in rats

dosed up to 3 months in the drinking water at levels of 10 mg/kg/day and above. Heart weights were increased in rats dosed at levels of 0.34 mg/kg/day via inhalation without microscopic correlate after 3 months. One year of inhalation treatment in rats yielded increased heart weights in groups treated at .03 mg/kg/day and above, with myocardial fibrosis observed in males treated at the .4 mg/kg/day (high dose) level. After 3 months of dietary administration, mice treated at levels of 40 mg/kg/day and above had increased heart weights without microscopic correlate. Clinical signs of effects on the heart were most evident in the dog, which when dosed, turned red (gums, ears, and/or abdomen) and had increased heart rate and force of contraction. These signs were noted at levels of ≥ 2 $\mu\text{g}/\text{kg}/\text{day}$ (.002 mg/kg/day) via inhalation and ≥ 0.01 mg/kg/day via oral administration. Myocardial fibrosis was noted in the dogs treated orally (≥ 0.01 mg/kg/day) and did not fully resolve during the one-month recovery phase. This finding was not noted in one-year inhalation study up to 15.16 $\mu\text{g}/\text{kg}/\text{day}$.

Body weight and food consumption was higher than controls in all nearly all treated groups across species and routes of administration. The Sponsor attributes this to an anabolic effect of beta agonists.

One year of inhalation treatment in rats at a level of 120 $\mu\text{g}/\text{kg}/\text{day}$ and higher was associated with degeneration of seminiferous tubules that did not recover after an 8-week period without treatment. All other findings resolved after the 8-week period. This finding was not noted in other species tested.

CARCINOGENICITY

Dose range finding studies for carcinogenicity testing began in 1979. Carcinogenicity studies were conducted in rats and mice via drinking water in 1980 - 1982. Interpretation of the rat study was confounded by low survival. Additional histopathology was performed over the years to elucidate findings in the rat study, with a final amendment to the report dated 1989. Also in 1989, dietary range finding studies began in rats and mice. The rat dietary study was conducted from 1989 - 1991 and the mouse dietary study was conducted from 1990 - 1992.

The carcinogenicity studies where CGP 25827A was administered in the drinking water were reviewed prior to this submission and are not re-reviewed in this document. However the salient points from these studies necessary for the overall interpretation of the carcinogenic response to CGP 25827A have been considered during the course of this review. Individual reviews of the carcinogenicity studies where CGP 25827A was administered in the diet are presented below.

20. 24-Month Carcinogenicity Study in Rats

BACKGROUND INFORMATION

Study Title: 24-Month Carcinogenicity Study in Rats

Sponsor Study No.: 886178
Study Dates: August 28, 1989 - September 5, 1991
Report Date: March 26, 1992
Test Facility: CIBA-GEIGY Limited
Short/Long term Toxicology
4332 Stein, Switzerland (In-life testing)
4002 Basle, Switzerland (Analytical Laboratories and
Histopathology)
GLP Status: Compliant with OECD Guideline 451 and Japanese (EA 700,
MHW 1039, MITI .1014)
NDA Volume:Page 56:1 and 57 - 58:1

METHODS

Test Material

Test Article: CGP 25827A
Batch No.: 810589
Purity: _____
Control Article: Diet

Test System

Species/Strain: Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Housing: 5/cage
Age at Initiation: Approximately 5 weeks
Route: Diet
Duration of Exposure: 24 months
CAC Concurrence: No

Dosing

CGP 25827A was administered in the diet at doses of 0, 0.5, 2, 5, and 20 mg/kg/day. Doses were also selected based on the results of a previous carcinogenicity study (Sponsor Study No. 2069-104) where rats received 0, 0.125, 0.25 and 0.5 mg CGP 25827A/ml drinking water/day, yielding average daily doses of 0, 12, 25, and 51 mg/kg/day for males and 0, 18, 38, 76 mg/kg/day for females. CGP 25827A affected survival in all treated groups. A 28-day palatability study was also considered in dose selection; where animals consumed dietary levels of 0.43, 1.75, 4.46, and 18.1 mg CGP 25827A /kg/day and did not exhibit signs of systemic toxicity. A total of 75 rats/sex/group were assigned to treatment groups receiving CGP 25827A or control diet. Animals from each group were designated for "carcinogenicity study only;" "carcinogenicity and hematology;" or "carcinogenicity and drug level determinations." Dose levels and study groups of the rat dietary study are presented in the following table.

Dosing Information

Group	Carcinogenicity Only	Carcinogenicity and Hematology	Carcinogenicity and Drug Level Determinations in Blood and Urine	Target Dose
	No. Animals/sex	No. Animals/sex	No. Animals/sex	(mg/kg/day)
1	50	10	10	0
2	50	10	10	0.5
3	50	10	10	2
4	50	10	10	5
5	50	10	10	20

Evaluation Of Endpoints

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly for Months 1 - 3 and monthly thereafter
Food consumption	Weekly for Months 1 - 3 and monthly thereafter
Water consumption	Monthly
Ophthalmology	Prior to treatment and after 1 and 2 years (Control and Group 5 all intervals; Group 4 after 2 years only)
Hematology	Weeks 12, 26, 53, 78, and 105
Drug levels in Plasma	Weeks 5, 26, 53, 78, 105 (included in a separate report)
Drug levels in urine	Week 40 from individually housed rats (included in a separate report)
Prolactin levels	Months 1 and 3 (blood samples and vaginal smears) (included in a separate report)
Organ weights	Week 105 (brain, heart, liver, kidneys, adrenals, ovaries/testes, spleen, lung, pituitary)
Gross pathology	Week 105
Histopathology	Week 105

RESULTS**Mortality**

There were no treatment effects on mortality. Adjusted survival was 43/70, 47/70, 50/70, 47/70, and 44/70 for Groups 1 - 5 males, respectively, and 42/70, 40/70, 42/70, 43/70, and 33/70 in Groups 1 - 5 females, respectively.

Clinical Signs

There were no remarkable clinical signs.

Body Weight

Animals in treated groups gained weight more rapidly during the growth phase, resulting in higher than control mean body weight values for Weeks 1 - 20. As the rate of body weight gain decreased over time in all groups, mean body weight values for treated groups approached or became slightly less than controls.

Food Consumption

Animals in treated groups consistently consumed more diet than did animals in the control group. The efficiency of food utilization was lower for animals in the treated groups than in the controls.

Water Consumption

Animals in the treated groups consistently consumed slightly more water than did animals in the control group.

Ophthalmology

There were no treatment related ocular findings.

Hematology

There were no treatment related hematology findings.

Organ Weights

Organ weight data revealed effects of CGP 25827A on the liver (Group 5 males); heart (Group 2 - 5 males and Group 3 females); lung (Group 2 - 5 males and females); testes (Group 4 and 5 males); and thymus (Group 5 males). Mean data for these organs are presented in the table at the end of this section.

Liver-to-body weight ratios for Group 5 males were significantly higher than control values.

Absolute heart weights and heart-to-body weight ratios were higher for all treated groups when compared to control values. Values were similar between all treated groups within each sex. Statistically significant differences from control for heart weights included the mean absolute heart weight for Group 3 females and heart-to-body weight ratios for Groups 2 - 5 males.

Lung weights were higher for treated animals when compared to controls, with statistically significant differences occurring for absolute weights in Groups 2 - 5 males and females and for lung-to-body weight ratios in Groups 2 - 5 males and 3 - 5 females.

Mean absolute testes weights for Groups 4 and 5 males were significantly lower than control values, however this was considered to be the effect of an unusually high weight for one of the control animals (no. 30 testes weight was 18.5g). Similarly, absolute thymus weights were lower than control for all treated groups of males, however this was attributed to the unusually high weight for one of the control animals (no. 9 thymus weight was 1885 mg). There were no significant differences from control when these outliers were excluded from analysis.

Notable Mean Absolute Organ Weights and Organ-to-Body Weight Ratios

Dose Group (mg/kg/day):		Males					Females				
		0	0.5	2	5	20	0	0.5	2	5	20
Liver (g)	Absolute	21.71	23.4	22.03	20.77	23.04	16.95	18.09	17.46	16.35	16.74
	Ratio	29.46	32.32	31.21	30.35	35.87*	34.20	34.62	35.26	33.20	33.55
Kidney (g)	Absolute	5.418	5.407	4.967	4.661	4.968	3.207	3.416	3.376	3.285	3.189
	Ratio	7.576	7.678	7.047*	6.894	7.956*	6.500	6.626	6.866	6.815	6.472
Heart (g)	Absolute	2.141	2.403	2.372	2.252	2.187	1.496	1.634	1.657*	1.620	1.556
	Ratio	2.945	3.333*	3.375*	3.309*	3.476*	3.029	3.153	3.352	3.356	3.146
Lung (g)	Absolute	2.534	3.075*	3.096*	2.974*	2.931*	1.969	2.213*	2.470*	2.302*	2.264*
	Ratio	3.495	4.266*	4.452*	4.361*	4.724*	4.043	4.571	5.045*	4.806*	4.624*
Testes (g)	Absolute	4.819	4.193	4.095	3.780*	3.601*					
	Ratio	6.525	5.791	5.840	5.570	5.533					
Thymus (mg)	Absolute	250.2	173.2	158.3	146.4*	180.4	126.1	129.8	122.1	116.7	132.6
	Ratio	0.328	0.235	0.222	0.022	0.283	0.249	0.248	0.024	0.239	0.268

*Significantly different from control ($p \leq 0.05$).

Gross Pathology

Notable gross observations involved the male and female reproductive systems.

Gross findings involving the female reproductive system included ovarian changes described as "large/mass/cystic mass/nodule" at an incidence of 1/70, 0/70, 5/69, 8/70 and 8/69 in Groups 1 - 5, respectively. Ovarian cysts were observed at a higher incidence in all treated groups than controls (3/70, 15/70, 21/69, 28/70, and 26/69 in Groups 1 - 5, respectively).

Gross findings involving the male reproductive system included small seminal vesicles (6/70, 8/70, 7/69, and 1/70 in Groups 1 - 5, respectively) and small testes (7/70, 8/70, 9/70, 14/70, and 9/70 in Groups 1 - 5, respectively). Both findings were without microscopic correlate.

Histopathology

Treatment related neoplastic lesions were limited to the female reproductive system. Mesovarian leiomyoma was noted at an incidence of 0/70, 0/70, 1/69, 1/69, and 3/69 for Groups 1 - 5, respectively. The only other neoplastic finding occurred as a result of hyperplasia of ovarian granulosa/theca cells (also known as sex chord stromal cells) progressing to benign tumors at an incidence of 1/70, 5/70, 6/69, 6/69, and 8/69, for Groups 1 - 5, respectively. There were no significant differences from control in the incidence of malignant tumors. When the incidence of animals with proliferative granulosa/theca cell lesions was combined (i.e., hyperplasia, benign or malignant tumor), treatment related effects were statistically significant in all dose groups. Treatment related nonneoplastic findings in female reproductive organs consisted of an increased incidence of ovarian cysts in all treated groups and uterine polyps in Group 5. Remarkable microscopic findings in the female reproductive organs are summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Notable Microscopic Findings in Female Reproductive Organs							
		Dose (mg/kg/day):	0	.05	2	5	20
<i>Ovary</i>	N		70	70	69	69	69
Cysts	No.		7	24*	23*	34*	30*
	%		10	34	33	49	43
Granulosa/Theca cell (benign)	No.		1	5	6	6*	8
	%		1	7	9	9	12
Granulosa/Theca cell (hyperplasia)	No.		17	32	33*	31*	34*
	%		24	46	48	45	49
Granulosa/Theca cell (benign or malignant tumor)	No.		1	0	2	0	0
	%		1	0	3	0	0
Granulosa/Theca cell (hyperplasia, benign, or malignant tumor)	No.		18	36*	36*	33*	39*
	%		26	51	52	48	57
Mesovarian leiomyoma	No.		0	0	1	1	3
	%		0	0	1	1	4
<i>Uterus</i>	N		70	70	69	70	69
Polyps	No.		2	3	3	3	10*
	%		3	4	4	4	14

*Significantly different from control ($p \leq 0.05$).

The incidence of nonneoplastic lesions was increased in the thyroid of treated animals. C-cell proliferative lesions yielded significant differences from control in the incidence of hyperplasia (Groups 2 - 5 males and 4 - 5 females), but not for adenoma or carcinoma. When all C-cell proliferative lesions were combined (i.e., hyperplasia, adenoma, and carcinoma) significant differences from control were noted for Groups 3 - 5 males and 4 - 5 females. The incidence of thyroid C-cell proliferative lesions is summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence of Thyroid C-cell Proliferative Lesions

	Males	Females
--	-------	---------

	Dose Group (mg/kg/day):										
		0	0.5	2	5	20	0	0.5	2	5	20
<i>Thyroid</i>	N	67	70	68	67	69	69	67	66	67	70
C-cell hyperplasia	No.	24	35*	36*	40*	47*	17	15	17	30*	25
	%	36	50	53	60	68	25	22	26	45	36
C-cell adenoma	No.	7	3	7	6	11	4	7	14	3	9
	%	10	4	10	9	16	6	10	21	4	13
C-cell adenoma, carcinoma, or gangliocytoma	No.	8	3	8	7	11	4	8	14	3	10
	%	12	4	12	10	16	0	1	0	0	1
C-cell proliferative lesions (hyperplasia, adenoma, carcinoma, or gangliocytoma)	No.	32	37	40	42*	55*	20	22	29	31*	29
	%	48	53	59	63	80	29	33	44	46	41

*Significantly different from control ($p \leq 0.05$).

Several other nonneoplastic lesions were primarily remarkable due to increased severity when compared to control. These lesions involved the lung, heart, spleen, and Harderian gland, and are summarized in the table at the end of this section. The presence of foam cells in the lung was increased in severity in Group 5 males and Groups 2 - 5 females. The incidence of this lesion was significantly higher than control for Groups 4 and 5 females. This finding correlates with the increased lung weights noted for treated animals. Myocardial fibrosis was noted in most of the control and treated rats, but with increased severity in the Group 4 - 5 males and Group 3 females. Additional microscopic findings attributable to treatment with CGP 25827A include in increased severity of splenic hemosiderosis in all groups of treated females and increased incidence of atrophy of the Harderian gland in all groups of treated males. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence and Severity of Notable Nonneoplastic Lesions

Dose Group (mg/kg/day):		Males					Females				
		0	0.5	2	5	20	0	0.5	2	5	20
<i>Lung</i>	N	70	70	69	70	70	70	69	68	70	70
Foam Cells	Total No.	24	27	23	25	33	15	23	24	30*	29*
	%	34	39	33	36	47	21	33	35	43	41
	Mild	20	23	19	21	26	10	9	5	3	8
	Moderate	4	4	4	4	5	5	8	13	17	11
	Severe	0	0	0	0	2	0	6	6	10	10
<i>Heart</i>	N	70	70	70	70	70	70	69	68	69	70
Myocardial Fibrosis	Total No.	69	65	65	70	68	45	48	55	47	52
	%	99	93	93	100	97	64	70	81	68	74
	Mild	46	41	37	35	29	26	22	21	23	27
	Moderate	23	24	28	35	37	19	26	33	24	21
	Severe	0	0	0	0	2	0	0	1	0	4
<i>Spleen</i>	N	70	70	69	69	70	70	70	69	70	70
Hemosiderosis	Total No.	35	39	41	40	35	54	54	61	63	55
	%	50	56	59	72	50	77	77	88	90	79
	Mild	20	24	24	16	14	24	14	11	18	13
	Moderate	14	14	17	23	20	24	25	26	30	27
	Severe	1	1	0	1	1	6	14	24	15	15

Dose Group (mg/kg/day):		Males					Females				
		0	0.5	2	5	20	0	0.5	2	5	20
<i>Harderian Gland</i> Atrophy	N	69	69	69	67	70	70	70	70	68	68
	No.	30	41*	41*	50*	49*	50	58	60	52	58
	%	43	59	59	75	70	71	83	86	76	85
	Mild	23	27	30	38	35	17	22	13	13	11
	Moderate	7	14	10	12	14	31	31	39	33	39
	Severe	0	0	1	0	0	2	5	8	6	8

*Significantly different from control ($p \leq 0.05$).

CONCLUSION

Tumors observed in this study were limited to the female reproductive system and included leiomyomas in high dose females. There were no statistically significant differences in survival between treated and control groups.

Dietary treatment of rats with CGP 25827A at levels of 0, 0.5, 2, 5, and 20 mg/kg/day for two years did not yield any remarkable findings in the in-life or hematology parameters evaluated.

Evidence of a carcinogenic response was found in female reproductive tissues of animals from all treated groups. Specific types of tumors included mesovarian leiomyoma in the 20 mg/kg/day group and benign granulosa/theca cell (also known as sex chord stromal cells) in all treated groups.

Although not statistically significant, mesovarian leiomyoma is a known response to this class of drug. Other investigators have reported that early studies of several β_2 agonist failed to demonstrate mesovarian leiomyomas but later studies revealed "...that any β_2 adrenergic agonist would be expected to produce [this lesion] in rats if properly studied (adequate potency and bioavailability)..." (Sells and Gibson, 1987). The incidence of this lesion in the high dose group supports the validity of the study in that adequate doses and bioavailability of CGP 25827A were present in the rats of this study to ascertain the carcinogenic potential of the test compound for this finding (Kelly et. al., 1993; Jack et. al., 1983).

The increased incidence of benign granulosa/theca cell tumors is considered to be the endpoint of a hyperplastic response as it did not progress to malignancy. Other treatment-related effects noted in female reproductive tissues included ovarian cysts (observed grossly and microscopically) in all treated groups and uterine polyps in the 20 mg/kg/day dose group.

Nonneoplastic lesions were observed in the endocrine, respiratory and cardiovascular systems of animals from all treated groups. Endocrine system changes consisted of an increased incidence of thyroid C-cell hyperplasia. Respiratory system changes consisted of an increased severity of foam cells in the lungs of all treated groups of females, and

increased incidence of foam cells in the 5 and 20 mg/kg/day dose group females. Cardiovascular changes consisted of increased severity of myocardial fibrosis in the 5 and 20 mg/kg/day dose group males. Harderian gland atrophy was also increased in incidence and severity in the 5 and 20 mg/kg/day dose group males, and in incidence in the 20 mg/kg/day dose group females.

In conclusion, the carcinogenic response of rats to CGP 25827A is consistent with that reported in the literature for other β_2 -adrenoceptor stimulants. A no observable effect level of 2 mg/kg/day was identified for the benign granulosa/theca cell tumors.

21. 24-Month Carcinogenicity Study in Mice

BACKGROUND INFORMATION

Study Title:	24-Month Carcinogenicity Study in Mice
Sponsor Study No.:	906172
Study Dates:	September 24, 1990 - October 27, 1992
Report Date:	May 13, 1993
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division 4002 Basle, Switzerland
GLP Status:	Compliant with OECD Guideline 451 and Japanese (EA 700, MHW 1039, MITI .1014)
NDA Volume:Page	49:1 and 50 - 52:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No.:	000290
Purity:	_____
Control Article:	Diet

Test System

Species/Strain:	Albino mice, Tif:MAGf(SPF), MAG x NIH hybrid
Housing:	Individual
Age at Initiation:	Approximately 7 weeks
Route:	Diet
Duration of Exposure:	24 months
CAC Concurrence:	No

Dosing

The results of a 3-month study where mice were dosed at 40, 140, 400, and 1400 mg/kg/day were considered in dose selection. Findings noted at the lowest dose tested included increased heart weights, decreased glucose levels and increased urea levels when compared to controls. In addition, kidney weights were increased relative to control at the 140 mg/kg/day level and above.

A total of 85 mice/sex/group were assigned to treatment groups receiving CGP 25827A at levels of 0, 2, 5, 20 and 50 mg/kg/day. Animals from each group of this carcinogenicity study were designated for "carcinogenicity study only;" "carcinogenicity and hematology;" or "carcinogenicity and blood level determinations" as illustrated in the following table.

Dosing Information				
Group	Carcinogenicity only: No. Animals/sex	Carcinogenicity and Hematology: No. Animals/sex	Carcinogenicity and Plasma drug levels: No. Animals/sex	Target Dose (mg/kg/day)
1	50	10	25	0
2	50	10	25	2
3	50	10	25	5
4	50	10	25	20
5	50	10	25	50

Evaluation Of Endpoints

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly for Months 1 - 3 and monthly thereafter
Food consumption	Weekly for Months 1 - 3 and monthly thereafter
Hematology	Weeks 26, 53, 78, and 105
Plasma Levels	Weeks 5, 26, 53, 78, 105
Organ Weights	Week 105 (brain, heart, liver, kidneys, adrenals, ovaries/testes, spleen, lung)
Gross pathology	Week 105
Histopathology	Week 105

RESULTS

Mortality

There were no effects on survival that would preclude meaningful interpretation of tumor data or would indicate a frank treatment-related effect on mortality. For the males, survival in the treated groups was consistently higher than the control group but without a dose response relationship. Survival in Group 3 (5 mg/kg/day) males was significantly higher than in the control group. Survival in the females was similar between groups, with

slightly but not statistically significant decreased survival in Group 5 (50 mg/kg/day) compared to the control value.

The following table is a summary of overall mortality and adjusted survival. Overall mortality is indicated by the number of animals that were found dead or sacrificed *in extremis* over the total number exposed/sex/group. Adjusted survival is the number of animals alive over the total number at risk, excluding animals sacrificed according to schedule at Weeks 5, 26, 53, and 78.

Dose Group (mg/kg/day)	Overall Mortality		Adjusted Survival	
	No. (%)		No. (%)	
	Males	Females	Males	Females
0	40/85 (47)	33/85 (39)	27/67 (40)	31/66 (47)
2	32/85 (38)	36/85 (42)	33/63 (52)	31/66 (47)
5	19/85 (22)*	35/85 (41)	48/67 (71)	32/67 (48)
20	28/85 (33)	34/85 (40)	37/64 (58)	32/66 (48)
50	28/85 (33)	46/85 (54)	38/66 (58)	20/66 (30)

*Significantly different from control, $p \leq 0.05$.

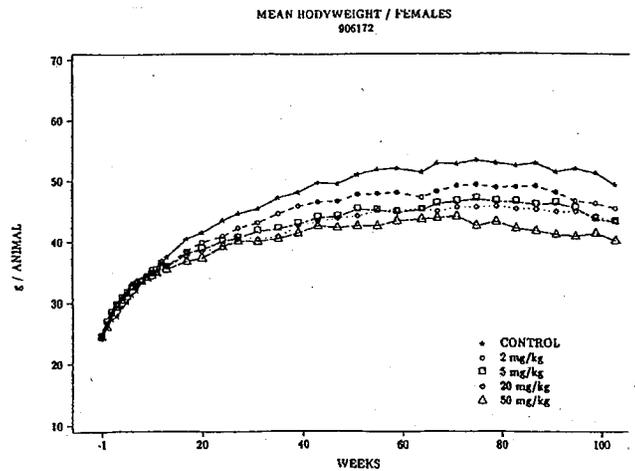
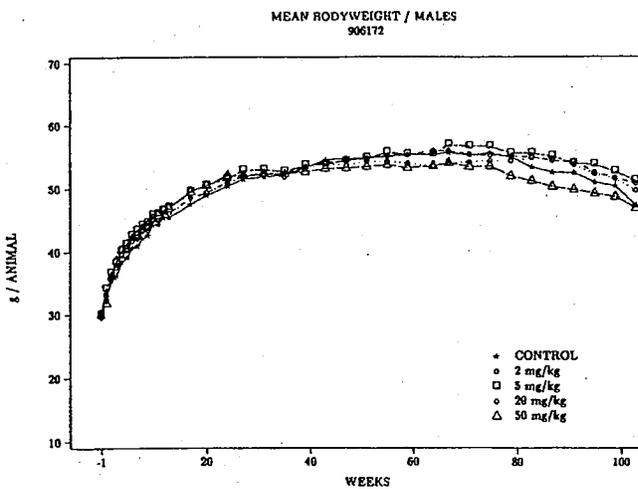
Clinical Signs

The incidence of palpable masses and swellings (females) was slightly higher in the 20 and 50 mg/kg/day dose groups when compared with controls. The number of animals exhibiting palpable masses was 1, 6, 9, 5, and 11 for Groups 1 - 5 males, respectively, and 7, 8, 13, 9, and 17 for Groups 1 - 5 females, respectively. The number of animals exhibiting swellings was 14, 11, 9, 11, and 18 for Groups 1 - 5 males, respectively, and 0, 2, 9, 15 and 13 for Groups 1 - 5 females, respectively. These findings generally correlated with enlarged livers revealed during necropsy observations. There were no other remarkable clinical signs.

Body Weight

Mean body weight and body weight gain were similar between groups during the initial growth phase of the animals (weeks 1 - 13). Thereafter, body weight values for males did not reveal a definitive compound effect, but a dose-related decrease was revealed for the females, with statistical significance achieved for Groups 2 - 5 for at least weeks 24 - 79, and for Groups 3 - 5 thereafter. The magnitude of the difference between female treated groups and controls was always less than 10% in Group 2, with percent of control values at the end of the study at 92, 87, 88, and 81, for Group 2 - 5 females, respectively. The low magnitude of the change in the Group 2 females supports a no effect level of 2 mg/kg/day for body weight effects of CGP 25827A. The lack of a difference in body weight *gain* during the growth span of the mice does not support the achievement of a maximum tolerated dose using this parameter.

Mean body weight values for the duration of the study are depicted in the following graphs.



Food Consumption

Animals in the treated groups consistently ate more than animals in the control group, achieving statistically significant differences from control in all groups of treated males and the 20 and 50 mg/kg/day group of treated females for at least 15 of the 36 measured food consumption intervals. Food consumption ratios, reported as g food/kg body weight/day, were consistently higher for treated groups of females when compared to controls, in an apparent dose response relationship throughout the study.

Hematology

There were no remarkable differences from control in hematology parameters evaluated.

Plasma Levels

Mean plasma concentrations of CGP 25827A for mice in this study were reported in a separate study (43/1993, v. 66, p. 166). Using _____

the limit of detection was estimated to be approximately 2.5 nmol/L. Plasma concentration levels of CGP 25827A were not detected in the 2 mg/kg/day group, below quantifiable limits in the 5 mg/kg/day group, and averaged 3.2 and 6.3 nmol/L in the 20 and 50 mg/kg/day dose groups respectively.

Organ Weights

Treatment related effects on organ weights were noted in the heart (Group 2, 4, and 5 females), liver (Group 5 males and 4 - 5 females), spleen (Group 4 - 5 females), and testes (Group 3 - 5 males).

Mean absolute heart weights and/or heart-to-body weight ratios were higher for treated females than control females as early as Week 5 and remained higher than controls throughout the 26, 53, 78 and 105 sacrifice intervals. Although a clear dose response relationship was not demonstrated, statistical significance was often achieved for females in Groups 2, 4, and 5. The increase in Group 3 females was statistically significant for absolute heart weight at the Week 26 interval and heart-to-body weight ratio at the week 105 interval, but was not consistently higher than control values at other sacrifice intervals. For males, the effect on heart weight and/or heart-to-body weight ratios was only evident at Week 26 in Groups 3, 4, and 5 and Week 53 in Group 3. Statistically significant differences from control were not observed for males at Weeks 78 and 105.

Liver weights were increased in group 5 males and Group 4 and 5 females at the week 105 interval. This finding was consistent with masses and swellings observed antemortem.

Spleen weights and or spleen-to-body weight ratios for females were higher than control values as early as Week 5 for Groups 3 - 5 and persisted through all other intervals for Groups 4 and 5. Statistical significance was noted in Group 3 at Week 5 and in Groups 4 and 5 at Weeks 5, 26, 53, and 78. This effect was not consistently demonstrated in male mice.

Absolute testes weights or testes-to-body weight ratios were significantly lower than control values for Groups 3, 4, and 5 at Week 105 only.

Gross Pathology

Gross findings attributable to treatment with CGP 25827A involved the liver, testes, and uterus. Liver masses and small testes in treated animals were consistent with effects observed in organ weight data. In addition, uterine masses were grossly visible in more treated than control animals. The incidence of these findings summarized in the table below includes all animals at the terminal sacrifice for the liver and testes findings. As the uterine masses were primarily observed in animals that died on test, the numbers indicated include all scheduled and unscheduled deaths for this finding.

Incidence (%) of Treatment-Related Gross Findings

mg/kg/day:	Males					Females				
	0	2	5	20	50	0	2	5	20	50
Liver - mass	64	85	61	94	100	35	37	31	53	60
Testes - small	0	6	9	21	41					
Uterus - mass						7	12	11	18	21

Histopathology

Notable microscopic findings involved the liver (Groups 2 - 5), heart (Groups 5), female genital tract/uterus (Group 2 - 5), and testes (Groups 5). The incidence of these findings is summarized in the table presented at the end of this section.

Hepatocellular hypertrophy and/or benign hepatoma was noted primarily in animals from treated groups, however these findings occurred in the absence of a dose response relationship and without statistical significance. The relatively low incidence of these lesions in Groups 4 and 5 at the terminal sacrifice can be attributed to the increased incidence of neoplastic lesions in these groups. At study termination, hepatocellular carcinoma was noted in 24, 33, 28, 36, and 57% of males in Groups 1 - 5, respectively and 3, 10, 6, 25 and 30% of females in Groups 1 - 5, respectively. The incidence of animals with liver tumors (hepatoma and carcinoma combined) was 33, 37, 47, 48 and 48 for Groups 1 - 5 males, respectively, and 13, 17, 22, 31 and 22% for Groups 1 - 5 females, respectively.

Effects on smooth muscle were evidenced by microscopic findings in the heart of Group 4 and 5 males and genital tract for all groups of treated females. The incidence of myocardial fibrosis was 8, 8, 6, 10, and 22% for Groups 1 - 5 males, respectively. The incidence of smooth muscle tumors (including leiomyoma and leiomyosarcoma) in the uterus and female genital tract was 5, 19, 19, 20 and 26% for Groups 1 - 5, respectively.

Testicular tubular atrophy was most severe in high dose males. Concomitant findings include the absence of sperm in the epididymus and impaction of seminiferous tubules in Groups 3, 4, or 5 males. The incidence and severity of testicular tubular atrophy is summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence and Severity of Testicular Tubular Atrophy				
Dose (mg/kg/day)	Mild	Moderate	Severe	Total
0	44	9	2	55
2	36	12	6	54
5	39	14	9	62
25	26	18	7	51
50	18	11	22*	51

*Significantly different from control, $p \leq 0.05$.

The following table is a summary of the incidence of the treatment-related histopathology lesions. Intervals refer to all animals sacrificed at study termination (Term) or all animals exposed to CGP 25827A (Total). Sample sizes for each of these intervals are presented at the end of the table. The number (No.) indicates the number of animals with a given lesion; multiple occurrences of a given lesion in any one animal are only counted once. The percent (%) indicates the incidence at the indicated interval. Statistically significant differences from control are based on the total number of animals exposed to CGP

25827A without regard to duration of exposure. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Summary Incidence of Treatment-Related Microscopic Findings

Dose (mg/kg/day):	Interval	Males					Females					
		0	2	5	20	50	0	2	5	20	50	
<i>Liver</i>												
Hypertrophy	Term	No.	6	17	23	20	10	3	17	14	11	2
		%	24	52	50	61	27	10	57	44	34	10
Total	No.		19	43*	38*	38*	27	12	27*	26*	18	14
		%	22	52	45	45	32	14	32	31	21	16
Benign Hepatoma	Term	No.	8	9	14	14	8	7	6	9	12	8
		%	32	27	30	42	22	23	20	28	38	40
Total	No.		24	24	31	33	25	11	12	17	19	14
		%	28	29	36	39	29	13	14	20	22	16
Carcinoma	Term	No.	6	11	13	12	21	1	3	2	8	6
		%	24	33	28	36	57	3	10	6	25	30
Total	No.		10	12	19	17	26*	2	5	3	11*	7*
		%	12	14	22	20	31	2	6	4	13	8
All Hepatocellular Tumors	Total	No.	28	31	40	40	41	11	14	19	26*	19*
		%	33	37	47	48	48	13	17	22	31	22
<i>Heart</i>												
Myocardial Fibrosis	Term	No.	1	4	3	4	13	1	0	1	0	0
		%	4	12	7	12	35	3	0	3	0	0
Total	No.		7	7	5	8	19	1	1	1	0	0
		%	8	8	6	10	22	1	1	1	0	0
<i>Testes</i>												
Tubular Atrophy	Term	No.	24	29	43	29	29					
		%	96	88	93	88	78					
Total	No.		55	54	62	51	51					
		%	65	65	73	61	60					
Absence of Sperm in Epididymus	Total	No.	2	7	12	7	24					
		%	2	8	14	8	28					
Semineferous Tubule Impaction	Total	No.	2	6	3	10	9					
		%	2	7	4	12	11					
<i>Genital Tract</i>												
Uterine Leiomyoma	Term	No.						3	4	6	7	4
		%						10	13	19	22	20
Total	No.						4	13*	13*	14*	17*	
		%					5	12	15	16	19	
Leiomyosarcoma	Total	No.					0	3	3	3	5	
		%					0	4	4	4	6	
Smooth Muscle Tumor	Total	No.					4	16*	16*	17*	22*	
		%					5	19	19	20	26	
Term n			25	33	46	33	37	31	30	32	32	20

Dose (mg/kg/day):	Interval	Males					Females				
		0	2	5	20	50	0	2	5	20	50
Total n		85	83	85	84	85	85	84	85	85	85

* Significantly different from control $p \leq 0.05$.

CONCLUSION

While there may have been a minimal effect on survival in the high dose females, the incidence of mortality is not of a magnitude that yields a statistically significant difference from control. Further, the onset of death was the same in the control and high dose group (Week 33). Although a dose response relationship in body weight was demonstrated in the females, there was no appreciable difference in body weight gain. Therefore the study failed to reach the MTD based on survival or body weight.

The Sponsor provided two separate analyses of survival and used one to support the claim that the MTD was exceeded for high dose females. The analysis used to support this conclusion would not generally be acceptable for analysis of carcinogenicity data. As stated in the methods, 85 animals/sex/group were originally assigned to the study, each subgroup was listed as part of the carcinogenicity study and was included in gross and microscopic analyses. Only the first 50 animals/sex/group were included in statistical analysis that indicated a significant effect on survival in the high dose females. Several animals dying late in the study were disregarded in this analysis as they were not included in the first 50/sex assigned to the group. In a separate analysis, all 85 animals/sex/group were included as appropriate and results indicated that the effect on survival in the high dose females was not statistically significant ($p = 0.07$). All animals at risk should be included in the denominator for survival analysis. The Sponsor reported 14/50 (28%) survival in the first 50 animals per sex assigned to the high dose group. When animals scheduled for interim sacrifice are excluded from the original 85 animals assigned to the high dose female group, the actual number at risk is 66. Thus a more accurate reflection of survival for the high dose females is 20/66 (30%) compared with 31/66 (47%) in the controls.

Body weight and body weight gain values for treated groups were similar to control values during the growth phase of the animals (weeks 1 - 13). Thereafter body weight values were lower for the 5, 20, and 50 mg/kg/day dose group females when compared to controls. The magnitude of the difference was consistently greater than 10% of the control value.

Gross and microscopic evaluation of tissues revealed treatment-related effects on the smooth muscle (heart and female genital tract), liver, spleen, and testes. The totality of gross findings, organ weight data, and microscopic findings yielded consistent interpretation of the treatment-related effects for each organ system. Neoplastic findings included hepatocarcinoma in high dose males and smooth muscle tumors (leiomyoma and/or leiomyosarcoma) in the uterus or female genital tract in all groups of treated females. The continuum of the liver lesions described as hypertrophy, benign hepatoma,

and carcinoma is consistent with that reported in the literature (Goodman et al. 1991), and increased in severity as doses increased. The smooth muscle tumors of the female reproductive organs were noted in all dose groups. Other investigators have reported identical findings with other β_2 agonists (Gibson et al. 1987, Sells and Gibson, 1987).

The incidence of myocardial fibrosis was significantly higher than control values for males in the 20 and 50 mg/kg/day dose groups. The no observable effect level (NOEL) for this finding was 5 mg/kg/day in mice.

The severity of testicular tubular atrophy was significantly greater in 50 mg/kg/day dose males when compared to controls. Concomitant findings were described as: the absence of sperm in the epididymus and impaction of seminiferous tubules in the 5, 20, and 50 mg/kg/day dose groups. Overall, the NOEL for male reproductive organs is 2 mg/kg/day.

In conclusion, this study was adequately designed and with sufficient survival to ascertain the carcinogenic potential of CGP 25827A when administered to mice at levels of 0, 2, 5, 20, and 50 for two years.

Overall Interpretation and Evaluation of Carcinogenicity Studies

The formation of leiomyomas in the female genital tract is a known response of rodents to treatment with high doses of beta agonists. The presence of mesovarian leiomyomas in both rat studies and the mouse dietary study indicates that adequate potency, bioavailability, and strain sensitivity was achieved to ascertain the carcinogenic potential of CGP 25827A (Sells and Gibson, 1987). This response has been demonstrated to be a pharmacodynamic response of beta agonists and has been prevented in mice and rats by simultaneous treatment with propranolol (a beta blocker) (Gibson et al., 1987). It was noted in rats at 15 (drinking water) and 20 (dietary) mg/kg/day.

The hyperplasia and benign granulosa/theca cell tumor formation is considered to be a pharmacodynamic effect of CGP 25827A. Beta agonists increase intracellular cAMP levels and thereby increase steroidogenesis in steroid-producing cells and could stimulate theca cells in the ovary (Aguado et al. 1982; Marsh 1975). Beta stimulation has also been shown to increase the sensitivity of theca cells to physiological gonadotropin (Dyer and Erickson, 1985).

The increased incidence of hepatic tumors observed in the mouse dietary study differs significantly from control for the 50 mg/kg/day group males and the 20 mg/kg/day group females.

Although the incidence of thyroid C-cell tumors (64 mg/kg/day males) and mammary adenomas (32 mg/kg/day females) were increased in rat drinking water study, the incidence was within historical ranges of the performing laboratory.