

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21-374**

**PHARMACOLOGY REVIEW**

**PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST**

NDA Number: 21374

Applicant: Whitehall-Robins

Stamp Date: July 31, 2001

Drug Name: Advil Cold & Sinus Liqui-Gel

IS THE PHARM/TOX SECTION OF THE APPLICATION FILABLE? Yes [ x ] No [ ]

The following parameters are necessary in order to initiate a full review, i.e., complete enough to review but may have deficiencies.

	Parameters	Yes	No	Comment
1	On its face, is the Pharmacology/Toxicology section of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the Pharmacology/Toxicology section of the NDA indexed and paginated in a manner to allow substantive review begin?	X		
3	On its face, is the Pharmacology/Toxicology section of the NDA legible so that substantive review can begin?	X		
4	Are ALL required* and requested IND studies completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility*, juvenile studies, ocular toxicity studies*, acute adult studies*, chronic adult studies*, maximum tolerated dosage determination, dermal irritancy, ocular irritancy, photocarcinogenicity, animal pharmacokinetic studies, etc)?	X		Toxicology studies were conducted to qualify the _____ . Reference was made to NDA 19771 and NDA 20402 for all other required non-clinical pharm/tox* studies.  NDA 19771: CoAdvil®/Advil® Cold & Sinus Tablets and Caplets NDA 20402: Advil (Provel®) Liqui-Gels
5	If the formulation to be marketed is different from that used in the toxicology studies, has the sponsor made an appropriate effort to either repeat the studies with the to be marketed product or to explain why such repetition should not be required?	X		The liquid formulation differs in the formation of an _____ . However, the _____ has been qualified.
6	Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in mg/m <sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57?	X		
7	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions?	X		
8	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	X		
9	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
10	Has the sponsor submitted a statement(s) that the pharm/tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns?	X		The reviewer did not find animal <del>welfare</del> welfare concern statement for the micronucleus assay.
11	From a pharmacology perspective, is this NDA fileable?	X		

Note:

Reviewing Pharmacologist:

\_\_\_\_\_  
Maria I. Rivera, Ph.D.

\_\_\_\_\_  
Date:

Acting Team Leader:

\_\_\_\_\_  
Robert Osterberg, Ph.D.

\_\_\_\_\_  
Date

cc:

Original NDA

HFD-550/Division File

HFD-550/Pharm-Tox/M. Rivera

HFD-550/Pharm-ToxTL/R. Osterberg

HFD-550/MO

HFD-550/PM

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this page is the manifestation of the electronic signature.**

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/s/

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Maria I. Rivera  
9/13/01 02:41:29 PM  
PHARMACIST

This is the fileability checkist for Advil Liqui-Gel. Please sign.

Robert Osterberg  
9/14/01 02:02:58 PM  
PHARMACOLOGIST

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## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

**NDA number:** 21374

**Review number:** One (Safety Review)

**Serial number/date/type of submission:** N-000/Jul. 31, 2001/Original NDA

**Information to sponsor:** Yes (x) No ( )

**Sponsor and/or agent:** Whitehall-Robins Healthcare, Five Giralda Farms, Madison, NJ 07940

Contacts: David S. Smith Phone: (973)660-8698

Sharon Heddish Phone: (973)660-5753

**Manufacturer for drug substance:** R.P. Scherer, 2725-Scherer Drive, St. Petersburg, FL

**Reviewer name:** Maria I. Rivera

**Division name:** Analgesic, Anti-Inflammatory and Ophthalmic Drug Products

**HFD #:** 550

**Review completion date:** September 28, 2001

**Drug:**

**Trade name:** Advil Cold & Sinus Liqui-Gels

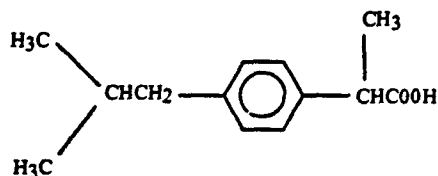
**Generic name:** Ibuprofen

**Chemical name:** ( $\pm$ )-2-(p-isobutylphenyl)propionic acid or (R,S)-2-(4-isobutylphenyl)propionic acid

**CAS registry number:** 15687-27-1

**Molecular formula/molecular weight:** C<sub>13</sub>H<sub>18</sub>O<sub>2</sub>/206.27

**Structure:**



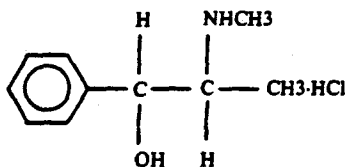
**Generic name:** Pseudoephedrine Hydrochloride

**Chemical name:** (+)- $\alpha$ -[1-(methylamino)ethyl]benzenemethanol hydrochloride

**CAS registry number:** 345-78-8

**Molecular formula/molecular weight:** C<sub>10</sub>H<sub>15</sub>NO.HCL/201.69

**Structure:**



**Relevant INDs/NDAs:**

Advil Cold & Sinus Liqui-Gels

Advil Capsules

NDA 19771 Advil Cold & Sinus Tablets/Caplets

NDA 20402 Advil (Provel) Liqui-Gels

**Drug class:** NSAID and decongestant (sympathomimetic)

**Indication:** Temporary relief of symptoms associated with the common cold, sinusitis or flu, including nasal congestion, headache, fever, body aches, and pain in adults and children 12 years of age or older.

**Clinical formulation:**

<u>Fill Material</u>	<u>mg/liqui-gel</u>
Ibuprofen, USP	200
Pseudoephedrine HCl, USP	30.0
Potassium Hydroxide, NF	
Polyethylene Glycol	
Purified Water, USP	
<u>Gelatin Shell</u>	
Gelatin, NF	
Purified Water, USP <sup>b</sup>	
FD & C Red No. 40	
D & C Yellow No. 10	

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**Route of administration:** Oral

**Proposed use:** One capsule every 4 – 6 hr while symptoms persist. If symptoms do not respond to 1 capsule, 2 capsules may be used. Not to exceed 6 capsules in any 24-hr period unless directed by a doctor.

**Disclaimer:** Tabular and graphical information is from sponsor's submission unless stated otherwise.

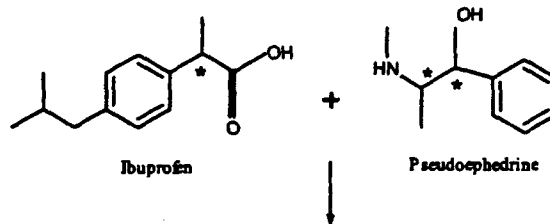
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## OVERALL SUMMARY AND EVALUATION:

### Introduction:

Whitehall-Robins Healthcare is sponsoring a liqui-gel formulation for the combination of ibuprofen and pseudoephedrine. The product is intended as a nonprescription drug for the temporary relief of symptoms associated with the common cold, sinusitis or flu including nasal congestion, headache, fever, body aches and pain in adults and children 12 years of age or older. The liqui-gel formulation contains 200 mg ibuprofen and 30 mg pseudoephedrine as in the Advil Cold & Sinus tablet formulation. The latest was approved for OTC marketing in 1989 (NDA 19-771). The rationale for developing the new product was that a liqui-gel formulation would provide for a faster rate but equivalent extent of absorption compared to the tablet formulation.

The safety and efficacy of the proposed combination as well as of the individual components have been established through extensive clinical evaluation and marketing experience. However, the combination of ibuprofen and pseudoephedrine hydrochloride in a liqui-gel formulation leads to the formation of \_\_\_\_\_ (henceforth refer to as \_\_\_\_\_) as shown in the diagram below:



Formation of the : \_\_\_\_\_

During the Sponsor meeting conducted on July 21, 1999, an agreement was reached with the FDA to conduct a qualification program to confirm the product's safety and fulfill FDA requirements for the \_\_\_\_\_. The agreement included the following:

- The battery of nonclinical safety studies will include a 14-day oral toxicity study with a 4-day ulcerogenicity component in mice, an *in vivo* micronucleus study in mice, and an *in vitro* bacterial mutagenicity study (Ames test).
- The oral toxicity and genotoxicity studies will be conducted with the finished product containing \_\_\_\_\_ at the level to be qualified. The Sponsor used concentrations of 2%, 4% and 8% relative to pseudoephedrine hydrochloride.



- After review of product stability data, a finished product specification of \_\_\_\_\_ relative to pseudoephedrine at expiration date was considered appropriate.

The nonclinical qualification studies have already been reviewed by Dr. Hamid Amouzadeh under \_\_\_\_\_ and will be summarized in the current review.

**Safety evaluation:** The 14-day mice toxicity study showed no ibuprofen/pseudoephedrine-related toxic effects at \_\_\_\_\_. Therefore, an MTD was not reached in this study. The 4-day ulcerogenicity component of the study showed no ulceration in the formulation control or formulation with \_\_\_\_\_. The positive control (indomethacin) showed ulceration of the intestine and peritonitis in some animals. However, the experimental design may have not been appropriate because the highest dose of the \_\_\_\_\_ was a NOAEL and only a single dose was administered. The ibuprofen/pseudoephedrine formulation with the \_\_\_\_\_ showed no evidence of being mutagenic in the micronucleus assay or the Ames test.

The anticipated human exposure of the \_\_\_\_\_ (Supplement N-006, p. 85), is 0.105 mg/kg/day (60 kg person). In the 14-day continuous exposure study, mice were treated up to 1.1 mg/kg/day without serious adverse effects. Therefore, the levels of exposure of the \_\_\_\_\_ in the Advil Cold & Sinus Liqui-Gels may be safe based on the lack of toxicity observed in mice at a level 10 fold higher than the human exposure on a mg/kg comparison. The HED, however, will be = \_\_\_\_\_ specification limit.

**Safety issues relevant to clinical use:** none

**Other clinically relevant issues:** none

**Conclusions:** The nonclinical safety studies did not reveal any contraindication to the use of the liqui-gel formulation by humans for up to 7 days for cold symptoms as specified on the product label (3 days for fever). The studies accomplished the goal of qualification of the \_\_\_\_\_ to a maximum level of \_\_\_\_\_ at product expiration date.

**Communication review:**

**Labeling review:** The product label is very similar to that of Advil Cold & Sinus Tablets and does not contain any Pharm/Tox data to be reviewed.

**RECOMMENDATIONS:**

**Internal comments:** In the fax dated June 28, 2000, the Sponsor was requested to provide the results of the chemical analysis of the dosing solutions for mutagenicity studies. The Sponsor provided tables with the doses for each experiment. However, the following issues need further clarification.

- The Sponsor did not specify whether these are theoretical values or actual levels measured analytically.

- It is not clear based on which active ingredient the \_\_\_\_\_ is obtained from. If it is based on pseudoephedrine concentration, then the numbers given in the table do not agree with the respective percentage.
- The protocol for the Ames test states that the ibuprofen/pseudoephedrine/ \_\_\_\_\_ was received by the performing laboratory (Bacteria Reverse Mutation Assay, page 8). It is then assumed that this stock was used to prepare the different dilutions with final concentrations of \_\_\_\_\_ ug/plate. The table, therefore, is not adequate because ibuprofen/pseudoephedrine containing \_\_\_\_\_ may have not been used in the assay and the Sponsor was asked to specify the actual dose of \_\_\_\_\_ (µg/plate) used.

**External recommendations (to sponsor):** The Sponsor should clarify on which active ingredient the determination of the \_\_\_\_\_ dose was based on. If it was based on pseudoephedrine concentration (as specified on \_\_\_\_\_, Supplement N-006, p. 4-1-5), then the numbers in the dose tables for all three studies do not correspond with the percentage. The dose table reported for the Ames Test is not consistent with the protocol. The Sponsor should also clarify whether the doses in the tables are theoretical or analytical values.

**Draft letter content for sponsor (dated Sept-25-01):**

In the fax dated June 28, 2000, Whitehall-Robins Healthcare was requested to provide the results of the chemical analysis of the dosing solutions for mutagenicity studies. The Sponsor provided tables with the doses for each experiment. However, the Sponsor needs to clarify the following issues:

- Please, specify whether the values in the dose tables are theoretical values or actual levels measured analytically.
- Please, clarify based on which active ingredient the \_\_\_\_\_ was obtained. If it was based on the pseudoephedrine concentration (as specified on \_\_\_\_\_, Supplement N-006, p. 4-1-5), then the numbers given in the table do not agree with the respective percentage.
- The protocol for the Ames test states that the ibuprofen/pseudoephedrine/ \_\_\_\_\_ was received by the performing laboratory (Bacterial Reverse Mutation Assay, page 8). It is then assumed that this stock was used to prepare the different dilutions with final concentrations of \_\_\_\_\_, µg/plate. The table, therefore, is not adequate because ibuprofen/pseudoephedrine containing \_\_\_\_\_ may have not been used in the assay. Please, specify the actual dose of \_\_\_\_\_ (µg/plate) used and how they were obtained.

Please call if you have any questions or concerns.

**NDA issues: N/A**



**Studies reviewed within this submission:**

- A 14-Day Oral (Gavage) Toxicity Study in CD-1 Mice with a 4-Day Ulcerogenicity Component
- Bacterial Reverse Mutation Assay
- Mammalian Erythrocyte Micronucleus Test

**Studies not reviewed within this submission: none**

**Introduction and drug history:**

The combination of ibuprofen and pseudoephedrine hydrochloride has been available OTC from Whitehall-Robins Healthcare since receiving FDA approval on September 19, 1989 (NDA 19-771). Originally marketed under the name CoAdvil<sup>®</sup>, its name was changed to Advil<sup>®</sup> Cold and Sinus in 1991. In the current NDA, Whitehall-Robins Healthcare is proposing a liqui-gel formulation for the combination of ibuprofen and pseudoephedrine. The liqui-gel dosage form (200 mg ibuprofen and 30 mg pseudoephedrine hydrochloride), indication and use are the same as for Advil<sup>®</sup> Cold and Sinus.

Both ibuprofen and pseudoephedrine hydrochloride have extensive histories of safe and effective use, both as individual ingredients as well as in combination. Ibuprofen as an OTC analgesic/fever reducer has been available for use in adults since 1984 (Whitehall-Robins Healthcare, NDA 18-989). Pseudoephedrine hydrochloride is generally recognized as safe and effective (Category I) as an OTC nasal decongestant, and is indicated for use in adults and in children down to 2 years of age. Pseudoephedrine hydrochloride has been available in OTC nasal decongestant products since the early 1960s; in 1976 it was granted monograph status.

A solubilized 200 mg ibuprofen formulation was originally approved by FDA on April 20, 1994 (NDA 20-402) for Sandoz Pharmaceutical Corporation under the tradename Provel<sup>™</sup> (solubilized potassium ibuprofen liqui-gels; licensed from R. P. Scherer). On April 22, 1996, NDA 20-402 was transferred to Whitehall-Robins Healthcare. In 1998, Whitehall-Robins Healthcare launched an ibuprofen 200 mg liqui-gel formulation under the brand name Advil Liqui-Gels. The liqui-gel formulation of ibuprofen has a faster rate but equivalent extent of absorption compared to the reference standard tablet (Nuprin<sup>®</sup> 200 mg). Therefore, it was expected that a liqui-gel formulation of the ibuprofen/pseudoephedrine hydrochloride combination product would provide for a faster rate but equivalent extent of absorption compared to the tablet Advil Cold & Sinus formulation.

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**NONCLINICAL PHARMACOLOGY/TOXICOLOGY:  
IBUPROFEN/PSEUDOEPHEDRINE HYDROCHLORIDE**

Reference is made to the following application sponsored by Whitehall-Robins Healthcare for nonclinical pharmacology and toxicology information on ibuprofen/pseudoephedrine hydrochloride:

CoAdvil® /Advil® Cold & Sinus Tablets and Caplets NDA 19-771 for ibuprofen/pseudoephedrine data:

- Original NDA Submission (19-771) dated September 1, 1987 – Volume 1.4 of 1.15, Non-Clinical Pharmacology and Toxicology; Overview of Non-Clinical Pharmacology and Toxicology for Ibuprofen, Pseudoephedrine, and Ibuprofen/Pseudoephedrine.

Summaries of the following are provided in NDA 19-771: for ibuprofen: mode of action, anti inflammatory activity, analgesic and antipyretic activity, general pharmacology, pharmacokinetics and metabolism, and toxicology; for pseudoephedrine hydrochloride: mode of action, cardiovascular effects, bronchodilatory activity, central nervous system effects, general pharmacology, pharmacokinetics and metabolism, and toxicology; for the combination of ibuprofen/pseudoephedrine hydrochloride: an oral toxicity study in mice and rats.

- Amendment to pending NDA 19-771 dated September 16, 1988 – Study Report for Teratology Study in Rats WH 441-22; Report #3199.1.

This amendment to pending NDA 19-771 provided the report of a teratology study in rats to evaluate the potential toxic and teratogenic effects of ibuprofen/pseudoephedrine hydrochloride when administered orally to pregnant rats during the period of major organogenesis.

- Submission of Post-Approval/Phase IV Commitment dated (NDA 19-771) March 1, 1996 – Study Report for Oral Teratology Study in Mice with WH-441- 22; Report #3199.2.

This submission of a post-approval Phase IV commitment provided a report of a teratology study performed to detect and evaluate the potential embryotoxic or teratogenic effects of ibuprofen/pseudoephedrine hydrochloride when administered orally by gavage to pregnant mice during the period of major organogenesis.

Reference is also made to the following application for nonclinical pharmacology and toxicology information on the liqui-gel:

Advil (Provel®) Liqui-Gels NDA 20-402 for ibuprofen softgel 200 mg:

- Original NDA submission (20-402) for Advil (Provel) Liqui-Gels dated September 24, 1993, Volume 1.5 out of 1.10 Nonclinical Pharmacology and Toxicology.

This section of NDA 20-402 provides a summary of ibuprofen liqui-gel ester formation and hydrolysis, an assessment of *in vivo* safety and *in vitro* mutagenicity for the ibuprofen-PEG ester, reports of subchronic, ulcerogenicity, and mutagenicity studies.

**TOXICOLOGY:** \_\_\_\_\_

**Study title: A 14-Day Oral (Gavage) Toxicity Study in CD-1 Mice with a 4-Day Ulcerogenicity Component**

**Key study findings:**

- The combination of ibuprofen/pseudoephedrine with \_\_\_\_\_ was well tolerated for 14 consecutive days in mice.
- No ulcerogenicity was observed five days after administration of a single oral dose.

**Study no:** 3513.4

**Volume #, and page #:** 5, 3-1

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** January 31, 2000

**GLP compliance:** yes

**QA report:** yes

**Drug, lot #, % purity:** \_\_\_\_\_ at in a formulation of 3.5 mg/ml ibuprofen and 0.5 mg/ml pseudoephedrine hydrochloride, lot # and % purity: not provided

**Methods:** Animals in the toxicity phase were administered a single oral dose of the appropriate material for 14 consecutive days and were euthanized on day 15. Animals in the ulcerogenicity phase received a single oral dose on day 1 and euthanized on day 5.

**Dosing:**

**Species/strain:** Mouse/Crl:CD-1<sup>®</sup>(ICR)BR

**#/sex/group or time point:** 10/sex/group as shown in the table below.

Group	No. of Mice				Treatment	Dose
	Ulcerogenicity		Toxicity			
	M	F	M	F		
1	10	10	10	10	Vehicle <sup>a</sup>	0 mg/kg
2	5 <sup>b</sup>	10	10	10	Formulation Control <sup>c</sup>	0 mg/kg
3	10	10	—	—	Indomethacin	15 mg/kg
4	10	10	10	10		
5	—	—	10	10		
6	10	10	10	10		

<sup>a</sup>PEG600/water (86.5%/13.5%); <sup>b</sup>Ten males were originally dosed with the undiluted formulation control material resulting in their deaths. An additional five males from the same shipment were subsequently dosed with the correct formulation; <sup>c</sup>3.5 mg/ml ibuprofen and 0.5 mg/ml pseudoephedrine hydrochloride in vehicle

**Age:** 9-11 weeks

**Weight:** 22 – 25 g

**Doses in administered units:**

	<b>Ibuprofen (mg/kg)</b>	<b>Pseudoephedrine Hydrochloride (mg/kg)</b>	
	12.9	1.9	
	26.1	4.0	
	52.0	8.1	

**Route, form, volume, and infusion rate:** oral (gavage)

**Observations and times:**

**Clinical Signs:** Twice daily.

**Body Weight:** Days -2, -3, or -4, 1, 4, 8 and 14 (days 1 and 4 only for ulcerogenicity phase), and prior to euthanasia.

**Food Consumption:** Days -3 to -1, 1, 4, 8 and 14 (only for toxicity group).

**Hematology:** Day of euthanasia (only for toxicity group).

**Clinical Chemistry:** Day of euthanasia (only for toxicity group).

**Gross Necropsy:** Day of euthanasia or time of unscheduled death, see Appendix for organs examined in the toxicity phase. In the ulcerogenicity phase, the following GI tract organs were examined: esophagus, stomach, small and large intestines.

**Histopathology:** All tissues and organs collected at necropsy from all animals in the control and high-dose groups and from any low-dose or mid-dose animals that died in the toxicity phase; the esophagus, large and small intestines, and stomach from all animals in the ulcerogenicity phase.

The following information was copied from the review written by the previous reviewer, Hamid Amouzadeh, for \_\_\_\_\_, (Review completed December 13, 2000, pp. 3-6). Additional comments by the current reviewer are in bold italics.

**Results:****A. Toxicity Phase**

**Clinical Observations and Mortality:** One formulation control female, one low-dose male and one high-dose male died during the study. The formulation control female died due to a gavage error on day 2, while the low- and high-dose males died on days 3 and 4, respectively. Clinical signs in these animals included decreased activity, cool to touch, urine/fecal staining, labored/shallow breathing and abdomen appeared distended/purple (high-dose male only). There were no significant differences in clinical observations noted for the males in the toxicity study. There was a slight increase in the incidence of open lesions in the anogenital region for the low-dose females and localized anal swelling in the low- and high-dose females. A dose response was not apparent. No other significant differences were noted during the toxicity phase.



**Body Weight and Food Consumption:** There were no toxicologically meaningful differences among the groups in body weight and food consumption in the toxicity phase.

**Clinical Pathology:** Total leukocyte count was significantly decreased for the mid- and high dose males on day 15. This was not considered toxicologically significant since the results were within the SLI historical control range. No other toxicologically significant differences in hematology were noted. A significant decrease in triglycerides was noted in the low-, mid- and high-dose males and the high-dose females on day 15. The decrease was dose-related. Although not statistically significant, triglycerides were also decreased for the formulation control, low- and mid-dose females. No other toxicologically meaningful differences were found among the groups.

**Gross Pathology:** During the toxicity phase, perforation of the esophagus, dark red lungs and fluid content in the abdominal cavity were noted in the formulation control female that died on day 2, indicating a gavage error as the cause of death. There were no observations noted at gross necropsy for the vehicle control male that died during blood collection. In the low-dose (2%) male that died on day 3, wet matting of the hair coat and dark red stomach were noted. In the high-dose (8%) male that died on day 4, abnormal contents of the cranial cavity and wet matting/matted material in the hair coat were noted at gross necropsy. No significant gross necropsy findings were noted for the males or females at scheduled euthanasia on day 15. Several incidences of periovarian cysts were noted at necropsy for females in the toxicity phase. However, this finding can be expected in mice of this strain and age.

**Organ Weight:** Absolute and relative kidney weights were significantly decreased for the low dose males. This was not considered toxicologically significant since a dose-response was not observed. In the females, a significant decrease in absolute and relative thymus weights was noted in the high-dose females. All other female groups in the toxicity phase had thymus weights comparable to the vehicle control.

**Histopathology:** No test article-related microscopic changes were observed in any of the tissues and organs examined from animals in the toxicity phase of the study.

## **B. Ulcerogenicity Phase**

**Clinical Observations and Mortality:** One low-dose female was euthanized moribund on day 1 due to error. In addition, one low-dose moribund female was euthanized on day 2 due to adverse clinical signs, including decreased activity, wobbly gait, tremors, shallow breathing, cool to touch, dehydration, urine stain, eyes dark in color and eyelids partially closed. No significant differences in clinical observations were noted in the surviving animals during the ulcerogenicity phase.

**Body Weight:** No toxicologically significant differences were noted in body weights or weight gain during the ulcerogenicity phase.

**Gross Pathology:** Perforation of the esophagus, dark red lungs and fluid content in the abdominal cavity were noted in the low-dose female that died on day 1, indicating a gavage error was the cause of death. In the low-dose female that was euthanized on day 2, abnormal contents in the cecum and colon and wet matting of the hair coat were noted at gross necropsy. At scheduled necropsy on day 5, no significant observations were noted for the vehicle control, formulation control, low-dose or high-dose males and females. Lesions in the small intestine and jejunum were noted in males and females in the

positive control group. In addition, lesions in the stomach and ileum, distended stomach and diverticulum in the jejunum were noted in the females in the positive control group. During examination of the stomach and intestine under low-powered magnification, lesions were noted in the positive control males and females only. The total percent ulcerogenicity was 42 for males and 61 for females in the positive control group. The total percent ulcerogenicity was zero in the vehicle control, formulation control, low- and high-dose males and females.

**Histopathology:** Ulceration of the intestine (moderate ulceration of the jejunum or perforation of the colon by transmural ulceration) was observed in three positive control females. These changes were interpreted as treatment-related. In three males and one other female in the positive control group, minimal to mild chronic active peritonitis was present. This suggested that bowel perforation was probably present in these animals also. Minimal inflammation was noted in other segments of the gastrointestinal tract of the positive control animals, however, it cannot be determined whether these lesions were background or test article induced. No ulceration or peritonitis was observed in any of the vehicle control, formulation control, and low-dose or high dose animals.

**Sponsor's Conclusions:** Based on the results of the main toxicity study, a dosage level of \_\_\_\_\_ in an ibuprofen/pseudoephedrine hydrochloride formulation would be considered the NOAEL in mice *following 14 consecutive days of oral (gavage) administration.* In the ulcerogenicity study, digestive tract lesions were observed in the indomethacin control group, indicating that the test regimen utilized could detect gastrointestinal lesions. No digestive tract lesions were observed in the control or test article groups.

**Previous Reviewer's Comments:** The reviewer concurs with the findings with the following exceptions. In the toxicity part of this study, the MTD of the \_\_\_\_\_ was not reached. In fact, the highest dose was the NOAEL dose. It is indicated that the maximum amount of \_\_\_\_\_ is expected to reach about \_\_\_\_\_ based on the earlier prototypes \_\_\_\_\_ (Vol., 33, section II, p. 31). However, the maximum amount of \_\_\_\_\_ in this drug product after 2 years has not been determined analytically. Chemical stability study under accelerated condition (3 months) indicated an \_\_\_\_\_ Both of these levels are well below the NOAEL for \_\_\_\_\_). The drug product might be safe provided the actual \_\_\_\_\_ does not exceed the suggested value of 1 % to a great extent. Because proper chemical analysis was not done, the actual value is not known. However, the level of \_\_\_\_\_ is set at no more than 2% in the product specification \_\_\_\_\_ (Vol. 1, p. 3). *The Sponsor provided a chart under Supplement N-006 (p. 6-1-82) which includes the percentage \_\_\_\_\_ in the liqui-gels under several experimental conditions. The \_\_\_\_\_ mentioned above was analytically measured in liqui-gels stored for 2 years at 25°C, 60% relative humidity.*

It is indicated that the highest dose of \_\_\_\_\_ was chosen to produce toxicity. Because the highest dose was the NOAEL, higher doses of the \_\_\_\_\_ should have been tested to accomplish the stated objective of the study and to establish an MTD. Male and female mice were treated on different days. Animals of both sexes should have been treated on each day to eliminate any possible variability.

The actual mg/kg dose of the \_\_\_\_\_ given to animals should have been included instead of the percentage. *The Sponsor submitted the information under Supplement N-006, p. 4-1-6 (see doses in table above).*

Four animals died during the toxicity part of the study. One female from formulation control group died on day 2 because of gavage error, which was verified during necropsy. One male in vehicle control group died during blood collection, perhaps because of trauma. No gross pathology was noted at necropsy for this animal. Two males, one each at \_\_\_\_\_ died on days 3 and 4, respectively. The male given \_\_\_\_\_ had wet matting of the hair coat and dark red stomach at necropsy. The male given \_\_\_\_\_ had abnormal contents of the cranial cavity and wet matting/matted material in the hair coat at necropsy. Although histological changes were observed in the stomach and the cranium, the cause of death was not identified in these animals. The significance of changes in the weight of kidney and thymus and slight increase in the lesions in the anogenital regions in the females could not be determined.

The objective of the ulcerogenicity study is not clear. It was not stated whether the ulcerogenicity of the ibuprofen or the \_\_\_\_\_ was tested. If the aim of study was to determine the ulcerogenicity of ibuprofen, the dose and the duration of the treatment was not adequate. Because of the low dose, short duration of administration, and most important, the difference in the potencies of indomethacin and ibuprofen, comparison with indomethacin positive control at the tested dose does not prove the lack of ulcerogenicity of the drug. Indomethacin is one of the most potent NSAIDs with regard to ulcerogenicity and the ulcerogenicity of ibuprofen is less than that of indomethacin (Mitchell, *et. al.*, 1994 and McCarthy, 1999)<sup>1</sup>. If the ulcerogenicity of the \_\_\_\_\_ was being tested, the experiment should have been designed appropriately, i.e. the \_\_\_\_\_ alone should have been administered at increasing doses for a longer period.

**Current Reviewer's Comments:** The reviewer concurs with the Sponsor conclusion and the previous reviewer's comments. An \_\_\_\_\_ the ibuprofen/pseudoephedrine formulation was a NOAEL in mice under the conditions of the study. It is not clear based on which active ingredient the % \_\_\_\_\_ is obtained from. If it is based on pseudoephedrine concentration, then the numbers given in the dose table do not agree with the respective percentage. It was not specified whether these are theoretical values or actual levels measured analytically

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<sup>1</sup> McCarthy DM (1999), Comparative toxicity of non-steroidal anti-inflammatory drugs, *Am J Med*, 107(6A):37S-46S; Mitchell JA, Akarasereenont P, Thiemermann C., Flower RJ, and Vane JR (1994). Selectivity of non-steroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci*, 90(24): 11693-7.

**Histopathology Inventory for NDA # 21374**

Study	3513.4
Species	Mouse
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X*
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	
Liver	X*
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X*
Pancreas	X
Parathyroid	X*
Peripheral nerve	X
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X
Sciatic nerve	
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X*
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X
Zymbal gland	
Standard List	

X, histopathology performed  
 \*, organ weight obtained

**GENETIC TOXICOLOGY:**

**Study title:** Bacterial Reverse Mutation Assay

**Key findings:** The test article, combination of ibuprofen and pseudoephedrine, was not mutagenic in any of the bacterial strains tested.

**Study no:** AA25CF.510.BTL

**Study type:** Ames Test

**Volume #, and page #:** Vol. 5, page not labeled

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** December 28, 1999

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** Combination of ibuprofen and pseudoephedrine with \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Si

**Previous Reviewer's Comments:** The assay was performed according to an established method and was adequately described. The reviewer concurs with the findings. The results indicate that the \_\_\_\_\_ in a combination of ibuprofen and pseudoephedrine hydrochloride was not mutagenic in this assay. It is not clear which substance of the combination the concentration of test article cited in Tables 11-20 (\_\_\_\_\_ Vol. 3, pp. 246 - 270) refers to. The actual concentration (µg/plate) of each test substance should have been presented. It was not stated whether the genotype of the bacteria was verified prior to experiments.

**Current Reviewer's Comments:** The reviewer concurs with the Sponsor's conclusion that under the conditions of the study, combination of ibuprofen and pseudoephedrine hydrochloride plus \_\_\_\_\_ did not cause a positive response in any of the bacterial strains tested. The Sponsor has addressed the underlined comments from the previous reviewer. Regarding the actual µg/plate of each test substance, the Sponsor submitted the following table:

Actual concentration of each substance	
Ibuprofen* (mg/plate)	Pseudoephedrine Hydrochloride* (mg/plate)
4.4	0.7
8.8	1.4
17.6	2.7

\*Amounts represent maximum dosing that could be evaluated for strain TA102. All other strains received approximately triple the dosing amounts listed.

The assay protocol states that the performing laboratory received the combination of ibuprofen/pseudoephedrine with \_\_\_\_\_ (Current study, page 8). It is then assumed that this stock was used to prepare the different dilutions with final concentrations of 0.05 – 150 µg/plate. The table, therefore, is not consistent because ibuprofen/pseudoephedrine combination containing \_\_\_\_\_ may have not been used in the assay. It is also not clear whether the Sponsor performed chemical analysis of the dosing solution as requested by the previous reviewer \_\_\_\_\_, External Recommendations).

The verification of each bacterial strain was stated on page 12 of the current NDA under Section: **Criteria for a valid test.**

**Study title: Mammalian Erythrocyte Micronucleus Test**

**Key findings:** The combination of ibuprofen and pseudoephedrine containing up to \_\_\_\_\_ was negative in the micronucleus assay.

**Study no:** AA25CF.123.BTL

**Volume #, and page #:** Vol. 5, page not labeled

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** December 28, 1999

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** Combination of ibuprofen and pseudoephedrine with \_\_\_\_\_ lot # 91358-78, % purity was not specified but purity was reported by the Sponsor to be 6.58 mg/g or 7.24 mg/ml

**Formulation:** 3.5 mg/ml ibuprofen and 0.5 mg/ml pseudoephedrine hydrochloride

**Methods:**

**Strains/species/cell line:** Mice/ICR

**Dose selection criteria:** Two pilot studies were conducted. For the initial pilot study, formulation with \_\_\_\_\_ and formulation control were administered by oral gavage to three male and three female mice at a dose volume of 10 ml/kg body weight. Mortality rates were as follows: 3/3 male mice and 2/3 female mice treated with formulation control and 3/3 male and 3/3 female treated with formulation with \_\_\_\_\_. Due to excessive mortality, the second pilot study was performed. Formulation with \_\_\_\_\_ and formulation control were administered by oral gavage to three male and

three female mice at dose volumes of 2.5 and 5 ml/kg. Mortality rates were as follows: 1/3 male mice treated with formulation control at dose volumes of 2.5 and 5 ml/kg, 1/3 male and 1/3 female mice treated with formulation with \_\_\_\_\_ at dose volume of 2.5 ml/kg, and 1/3 male mice treated with formulation with \_\_\_\_\_ dose volume of 5 ml/kg. Therefore, a dose volume of 5 ml/kg was selected for the micronucleus assay to administer the formulation with \_\_\_\_\_

**Test agent stability:** Not determined.

**Controls:**

**Vehicle:** \_\_\_\_\_

**Negative controls:** \_\_\_\_\_ e

**Positive controls:** \_\_\_\_\_

**Exposure conditions:**

**Incubation and sampling times:** Femur bone marrow was sampled 24 and 48 hr after dosing, except for the \_\_\_\_\_ and the positive control groups (24 hr only).

**Doses used in definitive study:** Formulation with \_\_\_\_\_ administered at a dose volume of 5 ml/kg.

**Study design:** The test-article-vehicle mixture, the vehicle alone, and the positive control were given as a single oral gavage administration. The animal grouping was as follows:

Treatment	No. mice/sex dosed	No. mice/sex used for bone marrow collection	
		24 hr	48 hr
Negative control (saline)	10	5	5
Vehicle control (PEG/H <sub>2</sub> O)	10	5	5
Formulation control	10	5	5
_____	5	5	
_____	5	5	
_____	10	5	5*
Positive control	5	5	

\*Only four male mice were available for bone marrow collection at 48 hr

**Analysis:**

**No. of replicates:** Two slides of femur bone marrow from each mouse was prepared.

**Counting method:** Using \_\_\_\_\_, 2000 polychromatic erythrocytes were scored for the presence of micronuclei. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated. The proportion of polychromatic erythrocytes to total erythrocytes was also recorded per 1000 erythrocytes.

**Criteria for positive results:** The test article was considered to induce a positive response if a dose-responsive increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically elevated relative to the negative control (physiological saline,  $p \leq 0.05$ , Kastenbaum-Bowman Tables) at any sampling time. If a single treatment group was significantly elevated at one sacrifice time with no evidence of a dose-response, the assay was considered a suspect or unconfirmed positive and a repeat assay recommended.



**Summary of individual study findings:**

**Study validity:** The study was conducted adequately. The mean incidence of micronucleated polychromatic erythrocytes did not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative control (physiological saline). The incidence of micronucleated polychromatic erythrocytes in the positive control group was significantly increased relative to the negative control group ( $p \leq 0.05$ , Kastenbaum-Bowman Tables).

The previous reviewer and the Sponsor had a discussion regarding the evidence for systemic exposure to the \_\_\_\_\_ (see Previous Reviewer's Comments below). The Sponsor response was: *"The report indicated that there was a reduction in the ratio of polychromatic erythrocytes to total erythrocytes. This is considered adequate proof of exposure as recommended by the ICH Guideline (S2A). Therefore, the study is acceptable as conducted."*

**Study outcome:** The number of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes in the formulation with degradant, in the formulation control and vehicle control treated groups was not statistically increased relative to the negative controls in either male and female mice at either 24 or 48 hr after dosing. Slight to moderate reductions of 5% to 27% in the ratio of polychromatic erythrocytes to total erythrocytes were observed in the formulation control and the formulation with degradant-treated groups relative to the negative control.

**Sponsor's Conclusions:** All criteria for a valid test were met. Under the conditions of the assay described in this report, combination of ibuprofen and pseudoephedrine did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow and was concluded to be negative in the micronucleus test using male and female ICR mice.

**Previous Reviewer's Comments:** The assay was performed according to an established method and was adequately described. However, it is difficult to assess the results of this study because no evidence of exposure of the animals to the \_\_\_\_\_ was provided. It is stated that *"the reduction in the ratio of polychromatic erythrocyte to total erythrocyte suggest that there was bioavailability of the test article to the bone marrow target tissue"* (Vol. 3, pp. 199). This is just a speculation. The only proof of exposure is the measurement of the drug in the system. It was stated that *"in total, mortality was observed in 4/14 male and 2/13 female mice treated with formulation control and in 6/15 male and 3/15 female mice treated with formulation with \_\_\_\_\_"* (Vol. 3, pp. 199). The mortality rates are summarized below.

Treatment	Male	Female
Negative control (saline)	0	0
Vehicle control (PEG600/water)	0	0
Formulation control	29%	15%
	0	0
	0	0
	40%	20%

Although there is no dose-dependency in the mortality rate, the results are interesting in that mortality was only observed in formulation control and \_\_\_\_\_ groups. Because mortality was not observed in \_\_\_\_\_ groups, no definitive conclusion could be made with the exception that mortality was about 50% higher in males than that in females. This study is considered inadequate mainly due to the lack of proof of exposure.

**Current Reviewer's Comments:** The reviewer concurs with the Sponsor's conclusion. Although systemic exposure to the \_\_\_\_\_ was not measured, the reduction observed in the ratio of polychromatic erythrocytes to total erythrocytes is an acceptable proof of exposure according to specifications in the ICH S2A document. The previous reviewer also requested the actual concentration for each substance in the test-article. The Sponsor complied with the request and the values are given in the table below.

Actual concentration of each substance

Ibuprofen (mg/kg)	Pseudoephedrine Hydrochloride (mg/kg)
435	63
880	133
1755	276

However, it was not specified whether these are theoretical values or actual levels measured analytically. Also, it is not clear based on which active ingredient the % \_\_\_\_\_ is obtained from. If it is based on pseudoephedrine concentration, then the numbers given in the table do not agree with the respective percentage.

**Genetic toxicology summary:**

In accordance with the qualification program for the \_\_\_\_\_ agreed with FDA, the Sponsor conducted an *in vitro* bacteria mutagenicity study (Ames test) and an *in vivo* micronucleus study in mice. In the Ames test, the combination of ibuprofen/pseudoephedrine/ \_\_\_\_\_ at concentrations ranging from \_\_\_\_\_ (TA102) or \_\_\_\_\_ (all other strains) was not mutagenic. In the micronucleus study in mice, the combination of ibuprofen/pseudoephedrine with \_\_\_\_\_ did not increase the incidence of polychromatic erythrocytes in bone marrow.

**Genetic toxicology conclusions:**

The combination of ibuprofen/pseudoephedrine/ \_\_\_\_\_ it did not show genetic toxicity potential in the Ames test or the *in vivo* micronucleus assay.

**Labeling recommendations:** None

**ADDENDUM TO REVIEW: N/A**

**APPENDIX/ATTACHMENTS: N/A**

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/s/

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Maria I. Rivera  
10/31/01 12:00:31 PM  
PHARMACIST

Robert Osterberg  
11/2/01 11:15:07 AM  
PHARMACOLOGIST

**APPEARS THIS WAY  
ON ORIGINAL**

**Review of Minor Toxicological Amendment:  
Response to Comments on NDA Review**

**NDA number:** 21-374

**Date/type of submission:** 12-14-01/BP

**Drug:** Advil Cold and Sinus Liquigel (Ibuprofen 200 mg/Pseudoephedrine HCl 30 mg)

**Sponsor:** White-Hall Robins Healthcare

**Reviewer name:** Maria I. Rivera

**Division:** HFD-550

**Date:** 1-7-02

White-Hall Robins submitted the answers to the fax dated September 25, 2001, in which FDA requested clarification on three issues regarding calculations made in the Toxicology section of the NDA. A summary of the answers is given below.

1. The values presented in the dose tables for ibuprofen, pseudoephedrine and \_\_\_\_\_ are actual analytical values for each of the chemical species in the stock solution.
2. The percent \_\_\_\_\_ is weight/volume percentage of the finished product, not percentage of pseudoephedrine as incorrectly specified on \_\_\_\_\_ (GC N-006). The test material percent ( \_\_\_\_\_ ) is a target value for the \_\_\_\_\_ present in the stock test solution.
3. In the Ames test, only the ibuprofen/pseudoephedrine/ \_\_\_\_\_ formulation containing the target \_\_\_\_\_ was used to prepare the various dilutions. The actual concentration of the \_\_\_\_\_ . The Sponsor included a table presenting the amount of each component contained in the \_\_\_\_\_ µg/plate test material concentrations used in both the Ames preliminary toxicity assay and in the definitive mutagenicity assay. The amount of ibuprofen, pseudoephedrine and \_\_\_\_\_ µg/plate, respectively. These values were analytically determined from the stock formulation and the test article dilution at each exposure level.

The reviewer considers the answers are acceptable and do not change the toxicological assessment done in the initial safety review. A difference exists in the calculation of the \_\_\_\_\_ in the preclinical and clinical studies. The \_\_\_\_\_ specification limit was calculated relative to pseudoephedrine for the clinical formulation, whereas it was calculated relative to the finished product on a weight/volume basis for the preclinical studies.

**Reviewer signature:** \_\_\_\_\_  
Maria I. Rivera, Ph.D.

**Date:** \_\_\_\_\_

**Supervisor concurrence:** \_\_\_\_\_  
Robert E. Osterberg, R.Ph., Ph.D.

**Date:** \_\_\_\_\_

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/s/

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Maria I. Rivera  
1/17/02 08:49:53 AM  
PHARMACOLOGIST

Robert Osterberg  
1/18/02 09:29:06 AM  
PHARMACOLOGIST

**APPEARS THIS WAY  
ON ORIGINAL**

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

**IND Number:** \_\_\_\_\_  
**Review Number:** One (Safety Review)  
**Sequence Number:** N-000  
**Date and Type of Submission:** October 11, 2000, Initial IND  
**Number of Volumes:** Three  
**Sponsor:** Whitehall-Robins Healthcare  
Five Giralda Farms  
Madison, NJ 07904  
Phone: 973 660 5753  
Fax: 973 660 7178  
Contacts: Sharon C. Heddish and Joanne Robinett

**Reviewer's Name:** Hamid R. Amouzadeh, Ph.D.  
**Division Name:** Division of Anti-Inflammatory, Analgesic and Ophthalmic Drug Products  
**HFD:** 550  
**Review completion Date:** December 13, 2000

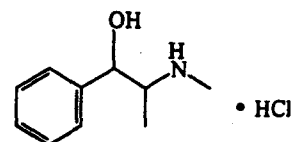
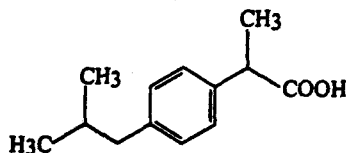
### Drug Product:

**Trade Name:** Advil Cold and Sinus Liqui-Gel  
**Components:** Ibuprofen  
**Class:** NSAID  
**Chemical Name:** (±)2-(p-isobutylphenyl)-propionic acid

**CAS Registry Number:** 15687-27-1  
**Structural Formula:** C<sub>13</sub>H<sub>18</sub>O<sub>2</sub>  
**Molecular Weight:** 206.27  
**Manufacturer:** \_\_\_\_\_

**Pseudoephedrine HCl**  
Sympathomimetic  
α-[1-(methylamino)ethyl]  
benzenemethanol  
345-78-8  
C<sub>10</sub>H<sub>15</sub>NO · HCl  
201.69

### Chemical Structures:



### Relevant DMFs/INDs/NDAs:

\_\_\_\_\_ Ibuprofen  
Pseudoephedrine hydrochloride  
NDA 18-989 Advil Tablets, Caplets, Gelcaps  
NDA 20-402 Provel™ (Advil) Liquigels  
NDA 20-589 Children's Advil Suspension  
NDA 20-267 Junior Strength Advil Tablets  
NDA 20-812 Pediatric Advil Drops  
NDA 20-944 Children's and Jr. Strength Advil Chewable Tablets

The combination product ibuprofen (200 mg) and pseudoephedrine hydrochloride (30 mg) in tablet form was the subject of the following Whitehall-Robins Healthcare applications:

NDA 19-771 Advil Cold & Sinus Tablets/Caplets  
 NDA 25,532 Ibuprofen 200 mg/Pseudoephedrine 30 mg

**Drug Class:** NSAID and Decongestant  
**Indication:** Pain relief / fever reduction / nasal decongestion  
**Clinical Formulation:**

Ingredient	Grade	mg in each Liqui-gel
Ibuprofen	USP	200
Pseudoephedrine hydrochloride	USP	30
Potassium hydroxide	NF	
Polyethylene glycol	NF	
Water	USP	

**Route of Administration:** Oral  
**Proposed Clinical Protocols:**

Protocol No.	Title	Dose	Route	n
AB-00-04	Advil® cold and sinus liqui-gel® relative bioavailability study: Single-center, randomized, open-label, single-dose, 4-way crossover	Ibuprofen (400 mg) + Pseudoephedrine hydrochloride (60 mg)*	Oral	28
AB-00-05	Advil® cold and sinus liqui-gel® food effect bioavailability study: Single-center, randomized, open-label, single-dose, 2-way crossover	Ibuprofen (400 mg) + Pseudoephedrine hydrochloride (60 mg)	Oral	28

\* Active comparator groups are: ibuprofen (200 mg), liquid pseudoephedrine hydrochloride (60 mg), and tablet form of ibuprofen (400 mg) + pseudoephedrine hydrochloride (60 mg).

**Previous Clinical Experiences:** Ibuprofen and pseudoephedrine hydrochloride alone or in combination has been used extensively in both adults and children for pain relief, analgesia and nasal decongestion for many years. The pharmacology and toxicology of these drugs are well described. Pseudoephedrine hydrochloride was included in the final nasal decongestant monograph in 1994.

**Key Words:** Anti-inflammatory, analgesia, pain, ibuprofen, fever reduction pseudoephedrine hydrochloride, nasal decongestant

**Disclaimer:** The Sponsor's materials were used in this review.



## OVERALL SUMMARY AND EVALUATION:

The subject of this IND is a liqui-gel form of the combination of ibuprofen (200 mg) and pseudoephedrine hydrochloride (30 mg). Ibuprofen and pseudoephedrine hydrochloride in combination or alone are marketed over-the-counter (OTC) in many forms for adults and children. Recently, a liqui-gel formulation of ibuprofen has been approved for OTC market (NDA 20-402). The combination of ibuprofen and pseudoephedrine hydrochloride in a liqui-gel form is unique in that a reaction between ibuprofen and pseudoephedrine hydrochloride results in the formation of a new chemical moiety or a \_\_\_\_\_ . Because this \_\_\_\_\_ was not qualified, FDA recommended a number of studies in a meeting with the sponsor on July 21, 1999.

\_\_\_\_\_

Chemical structure of \_\_\_\_\_

In the cover letter dated October 11, 2000, the sponsor indicated that the FDA agreed to a specific program for the qualification of the degradant during a meeting on July 21, 1999. Because the minutes of this meeting were not provided and no FDA-generated meeting minutes was found, it is not clear who in the agency agreed to the \_\_\_\_\_ qualification program referenced by the sponsor. The only documentation is a fax to the sponsor by the project manager dated June 28, 2000 in which a list of the recommendations to the sponsor was included. Apparently, this is a portion of a review whose author is not known.

The studies in this submission were reviewed mainly in the context of the recommendation sent to the sponsor by fax on June 28, 2000. A number of problems were found with these studies.

In the toxicity study, maximum tolerated dose (MTD) of the \_\_\_\_\_ was not reached. In fact, the highest dose was the no-observable-adverse-effect level (NOAEL) dose. It is indicated that the maximum amount of \_\_\_\_\_ is expected to reach about \_\_\_\_\_ at two years based on the earlier prototypes (Vol., 33, section II, pp. 31). However, the maximum amount of \_\_\_\_\_ in this drug product after 2 years has not been determined analytically. Chemical stability study under accelerated condition (3 months) indicated an \_\_\_\_\_ level of about \_\_\_\_\_. Both of these levels are well below the NOAEL for \_\_\_\_\_ albeit in a combination form. The drug product might be safe provided the actual \_\_\_\_\_ does not exceed the expected value of \_\_\_\_\_, to a great extent. This is not known due to the lack of proper chemical analysis. However, the level of \_\_\_\_\_ is not expected to be higher than \_\_\_\_\_, because it was set at no more than \_\_\_\_\_ in the product specification (Vol. 1, pp. 3).

It is indicated that the highest dose of \_\_\_\_\_ was chosen to produce toxicity (Vol. 2, pp. 140). Because the highest dose was the NOAEL, higher doses of the degradant should have been tested to accomplish the stated objective of the study and to establish an MTD.

The objective of the ulcerogenicity part of the study is not clear. It was not stated whether the ulcerogenicity of ibuprofen or pseudoephedrine hydrochloride or the \_\_\_\_\_ was tested. If the study was done to determine the ulcerogenicity of the ibuprofen, the dose and the duration of the treatment was not adequate. Because of the low dose, short duration of administration, and most important, the difference in the potencies of indomethacin and ibuprofen, comparison with a positive control at the tested dose does not prove the lack of ulcerogenicity of the drug. If the ulcerogenicity of the \_\_\_\_\_ was being tested, the experiment should have been designed appropriately, i.e. the \_\_\_\_\_ alone should have been administered at increasing doses for a longer period.

The results of the mammalian erythrocyte micronucleus test were difficult to assess because no evidence of exposure of the animals to the \_\_\_\_\_ was provided. It is stated that the reduction in the ratio of polychromatic erythrocyte to total erythrocyte suggests that there was bioavailability of the test article to the bone marrow target tissue (Vol. 3, pp. 199). This is just a speculation. The actual blood levels of the \_\_\_\_\_ in test animals should have been provided.

The results of the bacterial reverse mutation assay were negative, indicating that the \_\_\_\_\_ in the combination of ibuprofen and pseudoephedrine hydrochloride was not mutagenic.

There were a few unexplained deaths in the toxicity and the mammalian erythrocyte micronucleus test. In the toxicity study, two males, one each at \_\_\_\_\_ died on days 3 and 4, respectively. Although histological changes were observed in the stomach and the cranium, the cause of death was not identified (Vol. 3, pp. 2) in these animals. In the mammalian erythrocyte micronucleus test, a number of deaths occurred in formulation control and in \_\_\_\_\_ groups. In the formulation control group, there were 29% and 15% death among males and females, respectively. In the \_\_\_\_\_ groups there were 40% and 20% death among males and females, respectively. Apparently males were more sensitive. Although there was no dose-dependency, these unexplained deaths might have significant implications.

For the genotoxicity study, the sponsor was advised (fax dated June 28, 2000) that "*a chemical analysis of dosing solution should be performed to assure that adequate levels of the \_\_\_\_\_ of interest are present in the dosing solutions*". The documentation for the chemical analysis of the dosing solution was not provided.

It was indicated that "*in order to confirm the product's safety, the \_\_\_\_\_ was qualified by conducting a series of non-clinical safety studies described in FDA guidelines*" (Vol. 1, pp. 120). It is not clear these studies were based on which specific FDA guidelines.

**Conclusions:** Overall, these studies accomplished the goal of qualification of \_\_\_\_\_ as recommended in the ICH Guideline (Q3B). However, deficiencies as described under "External Recommendations" should be addressed.

**Communication Review:** The investigator's brochure was reviewed and found adequate.

**RECOMMENDATIONS:**

**Internal Comments:** Based on the previous experiences with the combination of ibuprofen and pseudoephedrine hydrochloride and qualification of the \_\_\_\_\_, the doses proposed in the clinical trials appear safe and the clinical study can proceed.

**External Recommendations (to sponsor):** Please provide the followings:

1. The results of the chemical analysis of the dosing solution for mutagenicity studies as per recommendation in the fax dated June 28, 2000.
2. The actual dose of the \_\_\_\_\_ used in all studies (e.g. mg/kg,  $\mu$ g/plate).
3. Proof of exposure for mammalian erythrocyte micronucleus test (plasma concentration of \_\_\_\_\_).
4. Clarification on which specific FDA guidelines the qualification of \_\_\_\_\_ is based on (Vol. 1, pp. 120).

**Reviewer's Signature:**

\_\_\_\_\_  
Hamid R. Amouzadeh, Ph.D.

\_\_\_\_\_  
Date

**Team Leader's Signature:**

\_\_\_\_\_  
Robert Osterberg, Ph.D.

\_\_\_\_\_  
Date

**CC List:**

\_\_\_\_\_/Original IND  
\_\_\_\_\_/Division File  
HFD-550/PM/Gould  
HFD-550/MO/Stauffer  
HFD-550/Pharm/Tox/Amouzadeh

**Studies Reviewed in this Submission:**

Study No.	Study Title	GLP QA	Location of Report
3513.4	A 14-day oral (gavage) toxicity study in CD-1 mice with a 4-day ulcerogenicity component	Yes	Vol. 2, pp. 121-368 Vol. 3, pp. 1-185
AA25CF.123.BTL	Mammalian erythrocyte micronucleus test	Yes	Vol. 3, pp. 186-220
AA25CF.510.BTL	Bacterial reverse mutation assay	Yes	Vol. 3, pp. 221-270

GLP = Good Laboratory Practice, QA = quality assurance

**Studies Not Reviewed in this Submission: None**

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## INTRODUCTION AND DRUG HISTORY:

Ibuprofen and pseudoephedrine hydrochloride alone or in combination have been used extensively in both adults and children for pain relief, analgesia and nasal decongestion for many years. The pharmacology and toxicology of these drugs are well described. Pseudoephedrine hydrochloride was included in the final nasal decongestant monograph in 1994.

Advil® Cold & Sinus Liqui-Gel is an alternate dosage form of a previously approved ibuprofen (200 mg)/pseudoephedrine hydrochloride (30 mg) combination marketed by Whitehall-Robins since 1989. A solubilized ibuprofen capsule formulation (200 mg) was originally approved by FDA (Sandoz NDA 20-402) on April 20, 1994 under the trade name Provel™ (solubilized potassium ibuprofen liqui-gels; licensed from R. P. Scherer). On April 22, 1996, NDA 20-402 was transferred to Whitehall-Robins Healthcare. In 1998, Whitehall-Robins Healthcare launched an ibuprofen liqui-gel formulation (200 mg) under the brand name Advil Liqui-Gels.

Advil Cold & Sinus Liqui-Gels will be indicated for the same uses as Advil Cold & Sinus Tablets, i.e. the temporary relief of symptoms associated with the common cold, sinusitis, and flu, including nasal congestion, headache, body aches, pain and fever, in subjects 12 years of age or older.

The focus of this IND is to provide data for the *in vivo* pharmacokinetic evaluation of this new formulation. The pharmacokinetic program being undertaken to support the NDA approval of the formulation will evaluate the pharmacokinetic profile of ibuprofen and pseudoephedrine hydrochloride in the liqui-gel formulation.

According to the sponsor "*Prior to submitting this IND, Whitehall-Robins Healthcare sought input from FDA on the development program for Advil Cold & Sinus Liqui-Gels to ensure the requirements for NDA approval for the product would be met. A briefing document outlining the program was submitted to FDA on May 31, 2000 for an pre-IND teleconference scheduled for June 30, 2000. Prior to the conduct of the teleconference, FDA contacted Whitehall-Robins Healthcare and indicated if evaluation of food effects was included with the program, the program would be acceptable. As directed by FDA, a food effects evaluation is included as part of the program*".

The development program is comprised of the following two studies:

- A bioavailability study comparing the pharmacokinetic profile of Advil Cold & Sinus Liqui-Gels with Advil Cold & Sinus Tablets, Advil Liqui-Gels, and Children's Sudafed® Nasal Decongestant Liquid.
- A food effects bioavailability study to evaluate the rate and extent of absorption of ibuprofen and pseudoephedrine from the liqui-gel formulation under fed and fasted conditions.

In addition, in the cover letter dated October 11, 2000, the sponsor indicated that "*reference is made to a July 21, 1999 meeting between Whitehall-Robins and the Agency meeting during which agreement was reached regarding the program to test the \_\_\_\_\_ found in the formulation. FDA agreed that the qualification program would consist of a bacterial reverse*

*mutation assay (completed April 3, 2000), a mammalian erythrocyte micronucleus test (completed March 30, 2000), and a 14-day oral toxicity study with a 4-day ulcerogenicity component (completed May 10, 2000)".*

Because the minutes of this meeting were not provided and no FDA-generated meeting minutes was found, it is not clear who in the agency agreed to the \_\_\_\_\_ qualification program referenced by the sponsor. The only documentation is a fax to the sponsor by the project manager dated June 28, 2000 in which a list of the recommendations to the sponsor was included. Apparently, this is a portion of a review whose author is unknown.

To qualify the degradant, the sponsor provided the study reports from a 14-day oral toxicity study with a 4-day ulcerogenicity component (study no. 3513.4), a bacterial reverse mutation assay (study no. AA25CF.123.BTL) and a mammalian erythrocyte micronucleus test (study no. AA256CF.510.BTL).

#### **PHARMACOLOGY, SAFETY PHARMACOLOGY AND PHARMACOKINETICS:**

Ibuprofen is an NSAID with anti-inflammatory, analgesic and antipyretic properties. These effects are mediated through inhibition of cyclooxygenases 1 and 2. Effects of ibuprofen on the cardiovascular, respiratory and central nervous systems are minor. Ibuprofen is absorbed rapidly through the intestine with first-order kinetics, and with a peak plasma level reaching at 0.5-3 hr. The plasma elimination half-life is about 1.5-2.5 hr. The metabolism of ibuprofen is comparable among species and is thought to be mediated by cytochrome P-450 2C9. The metabolite is essentially excreted in the urine within 24 hrs. Biliary excretion is a minor route.

Pseudoephedrine is a decongestant that exerts its effect through activation of  $\alpha$ -adrenergic receptors. It has vasopressor, bronchodilatory and weak (compared to ephedrine) central nervous systems effects. In man, it is absorbed rapidly and completely through the intestine with the peak plasma level reaching at 1.4-2 hr. The plasma elimination half-life is about 1.5-2.5 hr. Pseudoephedrine is excreted essentially unchanged in the urine within 24 hrs and less than 1% of it is eliminated through *N*-demethylation to an active metabolite.

There was no interaction when ibuprofen and pseudoephedrine hydrochloride were administered together in a solid form. In liqui-gel form, the ibuprofen has a similar pharmacokinetic profile to suspension i.e. higher  $C_{max}$  and shorter  $T_{max}$  as compared to solid form. There is no difference in the safety profile of ibuprofen in either solid or liquid forms. The liqui-gel formulation has small amounts of \_\_\_\_\_, which results from reaction of ibuprofen with pseudoephedrine hydrochloride.

**TOXICOLOGY:**

**Study Title:** A 14-day Oral Toxicity Study in CD-1 Mice with a 4-day Ulcerogenicity Component  
**Study Number:** 3513.4  
**Test Site:** \_\_\_\_\_  
**GLP Compliance and Quality Assurance:** Yes  
**Location of Report:** Vol. 2, pp. 121-368, Vol. 3, pp. 1-185  
**Study Period:** January 11, 2000 – May 10, 2000  
**Species/Strain/Age/Sex/Weight:** Mouse (CrI:CD-1<sup>®</sup> (ICR) BR), ~ 9-11 weeks old, ♂ and ♀, 23-39 g

**Treatment Scheme**

Group	No. of Mice				Treatment	Dose
	A		B			
	♂	♀	♂	♀		
1	10	10	10	10	Vehicle (PEG600/water)	0 mg/kg
2	5	10	10	10	Formulation Control	0 mg/kg
3	10	10	-	-	Indomethacin	15 mg/kg
4	10	10	10	10		
5	-	-	10	!		
6	10	10	10	10		

**Notes:**

A = Ulcerogenicity group – dosed on Day 1 and observed through Day 4, euthanized on Day 5  
 B = Toxicity group – dosed for 14 consecutive days, euthanized on Day 15  
 Formulation Control = 0.5 mg/mL of pseudoephedrine hydrochloride and 3.5 mg/mL of ibuprofen in a vehicle of PEG600 and water.  
 — control was prepared in Formulation Control.

**Observation Period:** 14 days (toxicity) or 4 days (ulcerogenicity)

**Test Substance:** \_\_\_\_\_

**Parameters Observed/Measured:**

**Clinical Signs:** Twice daily.

**Body Weight:** Days -2, -3, or -4, 1, 4, 8 and 14, and prior to euthanasia (only for toxicity group).

**Food Consumption:** Days -3 to -1, 1, 4, 8 and 14 (only for toxicity group).

**Blood Collection:** Day of euthanasia (only for toxicity group).

**Clinical Pathology:** Routine parameters (only for toxicity group).

**Necropsy:** At the end of the scheduled treatment period all surviving animals in the control and high-dose group were killed and full necropsy examinations were carried out and gross findings were recorded for each animal. The following tissues were preserved for histological examination: adrenal glands, all gross lesions, aorta, bone marrow smear (femur), brain, cecum, colon, duodenum, epididymides, esophagus, extraorbital lachrymal glands, eyes, femur, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mediastinal lymph node, mesenteric lymph node, ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, rectum, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, submandibullray lymph node, submaxillary salivary gland, thymus, thyroid glands/parathyroid, tongue, trachea, and urinary bladder, uterus, and vagina. Weight of adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary (after fixation), prostate, spleen, testes, thymus, thyroid/parathyroid and uterus with cervix was measured (only for toxicity group).

**Ulcerogenicity Phase:** The entire esophagus, stomach, small and large intestine, cecum and rectum were examined after \_\_\_\_\_ The locations of lesions (focal staining by

dye, hemorrhage, or perforation of mucosal surface), if any, were recorded. The severity of the mucosal damage was assessed and scored. A percent ulceration and a mean percent ulcerogenicity were calculated. The ulcerogenic effect of the positive control was determined using the total percent ulcerogenicity, however, the  $UD_{50}$  dose (ulcerogenic dose) could not be calculated for the study since no ulcerogenicity was observed in the test-article-treated groups.

**Statistical Analysis:** Body weights, weight gain, food consumption, clinical pathology and organ weight data were analyzed by One-Way Analysis of Variance (ANOVA). When significance was observed with ANOVA, group by group comparisons was performed using the Tukey-Kramer method. All tests were two-tailed with a minimum significance level of 5% ( $p < 0.05$ ). Statistical analyses were not performed on the additional five males assigned to group 2 of the ulcerogenicity phase.

### Summary of Findings:

#### A. Toxicity Phase

**Clinical Observations and Mortality:** One formulation control female, one low-dose male and one high-dose male died during the study. The formulation control female died due to a gavage error on day 2, while the low- and high-dose males died on days 3 and 4, respectively. Clinical signs in these animals included decreased activity, cool to touch, urine/fecal staining, labored/shallow breathing and abdomen appeared distended/purple (high-dose male only). There were no significant differences in clinical observations noted for the males in the toxicity study. There was a slight increase in the incidence of open lesions in the anogenital region for the low-dose females and localized anal swelling in the low- and high-dose females. A dose response was not apparent. No other significant differences were noted during the toxicity phase.

**Body Weight and Food Consumption:** There were no toxicologically meaningful differences among the groups in body weight and food consumption in the toxicity phase.

**Clinical Pathology:** Total leukocyte count was significantly decreased for the mid- and high-dose males on day 15. This was not considered toxicologically significant since the results were within the historical control range. No other toxicologically significant differences in hematology were noted. A significant decrease in triglycerides was noted in the low-, mid- and high-dose males and the high-dose females on day 15. The decrease was dose-related. Although not statistically significant, triglycerides were also decreased for the formulation control, low- and mid-dose females. No other toxicologically meaningful differences were found among the groups.

**Gross Pathology:** During the toxicity phase, perforation of the esophagus, dark red lungs and fluid content in the abdominal cavity were noted in the formulation control female that died on day 2, indicating a gavage error as the cause of death. There were no observations noted at gross necropsy for the vehicle control male that died during blood collection. In the low-dose (2%) male that died on day 3, wet matting of the hair coat and dark red stomach were noted. In the high-dose (8%) male that died on day 4, abnormal contents of the cranial cavity and wet matting/matted material in the hair coat were noted at gross necropsy. No significant gross necropsy findings were noted for the males or females at scheduled euthanasia on day 15. Several incidences of periovarian cysts were noted at necropsy for females in the toxicity phase. However, this finding can be expected in mice of this strain and age.

**Organ Weight:** Absolute and relative kidney weights were significantly decreased for the low-dose males. This was not considered toxicologically significant since a dose-response was not observed. In the females, a significant decrease in absolute and relative thymus weights was noted in the high-dose females. All other female groups in the toxicity phase had thymus weights comparable to the vehicle control.

**Histopathology:** No test article-related microscopic changes were observed in any of the tissues and organs examined from animals in the toxicity phase of the study.



**B. Ulcerogenicity Phase**

**Clinical Observations and Mortality:** One low-dose female was euthanized moribund on day 2 due to error. In addition, one low-dose moribund female was euthanized on day 2 due to adverse clinical signs, including decreased activity, wobbly gait, tremors, shallow breathing, cool to touch, dehydration, urine stain, eyes dark in color and eyelids partially closed. No significant differences in clinical observations were noted in the surviving animals during the ulcerogenicity phase.

**Body Weight:** No toxicologically significant differences were noted in body weights or weight gain during the ulcerogenicity phase.

**Gross Pathology:** Perforation of the esophagus, dark red lungs and fluid content in the abdominal cavity were noted in the low-dose female that died on day 1, indicating a gavage error was the cause of death. In the low-dose female that was euthanized on day 2, abnormal contents in the cecum and colon and wet matting of the hair coat were noted at gross necropsy. At scheduled necropsy on day 5, no significant observations were noted for the vehicle control, formulation control, low-dose or high-dose males and females. Lesions in the small intestine and jejunum were noted in males and females in the positive control group. In addition, lesions in the stomach and ileum, distended stomach and diverticulum in the jejunum were noted in the females in the positive control group. During examination of the stomach and intestine under low-powered magnification, lesions were noted in the positive control males and females only. The total percent ulcerogenicity was 42 for males and 61 for females in the positive control group. The total percent ulcerogenicity was zero in the vehicle control, formulation control, low- and high-dose males and females.

**Histopathology:** Ulceration of the intestine (moderate ulceration of the jejunum or perforation of the colon by transmural ulceration) was observed in three positive control females. These changes were interpreted as treatment-related. In three males and one other female in the positive control group, minimal to mild chronic active peritonitis was present. This suggested that bowel perforation was probably present in these animals also. Minimal inflammation was noted in other segments of the gastrointestinal tract of the positive control animals, however, it cannot be determined whether these lesions were background or test article induced. No ulceration or peritonitis was observed in any of the vehicle control, formulation control, and low-dose or high-dose animals.

**Conclusions:** Based on the results of the main toxicity study, a dosage level of \_\_\_\_\_ in an ibuprofen/pseudoephedrine hydrochloride formulation would be considered the NOAEL in mice. No digestive tract lesions were observed in the control or test article groups. Digestive tract lesions were observed in the indomethacin control group, indicating that the test regimen utilized could detect gastrointestinal lesions.

**Reviewer's Comments:** The reviewer concurs with the findings with the following exceptions. In the toxicity part of this study, the MTD of the \_\_\_\_\_ was not reached. In fact, the highest dose was the NOAEL dose. It is indicated that the maximum amount of \_\_\_\_\_ is expected to reach about 1% at two years based on the earlier prototypes (Vol., 33, section II, pp. 31). However, the maximum amount of \_\_\_\_\_ in this drug product after 2 years has not been determined analytically. Chemical stability study under accelerated condition (3 months) indicated an \_\_\_\_\_. Both of these levels are well below the NOAEL for \_\_\_\_\_. The drug product might be safe provided the actual \_\_\_\_\_ does not exceed the suggested value of 1% to a great extent. Because proper chemical analysis was not done, the actual value is not

known. However, the level of \_\_\_\_\_ in the product specification (Vol. 1, pp. 3).

It is indicated that the highest dose of \_\_\_\_\_ was chosen to produce toxicity (Vol. 2, pp. 140). Because the highest dose was the NOAEL, higher doses of the degradant should have been tested to accomplish the stated objective of the study and to establish an MTD.

Male and female mice were treated on different days (Vol. 2, pp. 141). Animals of both sexes should have been treated on each day to eliminate any possible variability.

The actual mg/kg dose of the \_\_\_\_\_ given to animals should have been included instead of the percentage.

Four animals died during the toxicity part of the study. One female from formulation control group died on day 2 because of gavage error, which was verified during necropsy (Vol. 2, pp. 151). One male in vehicle control group died during blood collection, perhaps because of trauma. No gross pathology was noted at necropsy for this animal. Two males, one each at \_\_\_\_\_ died on days 3 and 4, respectively. The male given \_\_\_\_\_ had wet matting of the hair coat and dark red stomach at necropsy. The male given \_\_\_\_\_ had abnormal contents of the cranial cavity and wet matting/matted material in the hair coat at necropsy. Although histological changes were observed in the stomach and the cranium, the cause of death was not identified (Vol. 3, pp. 2) in these animals.

The significance of changes in the weight of kidney and thymus (Vol. 2, pp. 149) and slight increase in the lesions in the anogenital regions in the females (Vol. 2, pp. 151) could not be determined.

The objective of the ulcerogenicity study is not clear. It was not stated whether the ulcerogenicity of the ibuprofen or the \_\_\_\_\_ was tested. If the aim of study was to determine the ulcerogenicity of ibuprofen, the dose and the duration of the treatment was not adequate. Because of the low dose, short duration of administration, and most important, the difference in the potencies of indomethacin and ibuprofen, comparison with indomethacin positive control at the tested dose does not prove the lack of ulcerogenicity of the drug. Indomethacin is one of the most potent NSAIDs with regard to ulcerogenicity and the ulcerogenicity of ibuprofen is less than that of indomethacin (Mitchell, et. al., 1994 and McCarthy, 1999). If the ulcerogenicity of the \_\_\_\_\_ was being tested, the experiment should have been designed appropriately, i.e. the \_\_\_\_\_ alone should have been administered at increasing doses for a longer period.

**GENETIC TOXICOLOGY:**

**Study Title:** Mammalian Erythrocyte Micronucleus Test  
**Study Number:** AA25CF.123.BTL, 3513.4  
**Test Site:** \_\_\_\_\_  
**GLP Compliance and Quality Assurance:** Yes  
**Location and Date of Report:** Vol. 3, pp. 186-220, March 30, 2000  
**Study Period:** December 28, 1999-January 30, 2000  
**Species/Strain/Age/Sex/Weight:** Mouse (ICR), 6-8 weeks old, ♂ and ♀, 24-26 g  
**Number of Animals:** 5/sex/group  
**Test Substance:** \_\_\_\_\_ in combination with ibuprofen and pseudoephedrine HCl (lot no. 91358-78)  
**Positive Controls:** \_\_\_\_\_  
**Dose/Route/Duration:** \_\_\_\_\_, oral, 24 hr and 48 hr, single dose  
**Treatment Scheme:**

Treatment	No. of Mice			
	24 hr		48 hr	
	♂	♀	♂	♀
Negative Control (saline)	5	5	5	5
Vehicle Control (PEG600/water)	5	5	5	5
Formulation Control	5	5	5	5
_____	5	5	-	-
_____	5	5	-	-
_____	5	5	5	5*
_____	5	5	-	-

Formulation Control = 0.5 mg/mL of pseudoephedrine Hydrochloride and 3.5 mg/mL of ibuprofen in a vehicle of PEG600 and water.  
 \*Only four male mice were available for bone marrow.

**Reviewer's Comments:** The assay was performed according to an established method and was adequately described. However, it is difficult to assess the results of this study because no evidence of exposure of the animals to the \_\_\_\_\_ was provided. It is stated that "the reduction in the ratio of polychromatic erythrocyte to total erythrocyte suggest that there was bioavailability of the test article to the bone marrow target tissue" (Vol. 3, pp. 199). This is just a speculation. The only proof of exposure is the measurement of the drug in the system.

It was stated that "in total, mortality was observed in 4/14 male and 2/13 female mice treated with formulation control and in 6/15 male and 3/15 female mice treated with formulation with \_\_\_\_\_" (Vol. 3, pp. 199). The mortality rates are summarized below.

Treatment	♂	♀
Negative Control (saline)	0	0
Vehicle Control (PEG600/water)	0	0
Formulation Control	29%	15%
	0	0
	0	0
	40%	20%

Although there is no dose-dependency in the mortality rate, the results are interesting in that mortality was only observed in formulation control and \_\_\_\_\_ groups. Because mortality was not observed in \_\_\_\_\_ groups, no definitive conclusion could be made with the exception that mortality was about 50% higher in males than that in females. This study is considered inadequate mainly due to the lack of proof of exposure.

**Study Title:** Microbial Mutagenesis Assay  
**Study Number:** AA256CF.510.BTL, AA25CF.123.BTL, 3513.4  
**Test Site:**  
**GLP Compliance and Quality Assurance:** Yes  
**Location and Date of Report:** Vol. 3, pp. 221-270  
**Study Period:** December 28, 1999 – February 7, 2000  
**Test Substance:** \_\_\_\_\_ in combination with ibuprofen and pseudoephedrine (lot no. 91358-78)  
**Concentration of Test Substance:** 0.15 – 150 µg/plate  
**Test System:** Salmonella typhimurium strains TA1535, TA9102, TA98 and TA100  
**Positive Controls:** \_\_\_\_\_  
**Metabolic Activation System:** Liver S9 (10%, rats treated with \_\_\_\_\_, 500 mg/kg, 5 days)  
**Replicates:** Triplicate

**Criteria for Positive Result:**

A dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA 1535 and TA 1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than three times the mean Sponsor vehicle control value. Data sets for strains TA98, TA100 and TA102 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than two times the mean sponsor vehicle control value.

**Summary of Findings:** All criteria for a valid study were met as described in the protocol. Under the conditions of this study, combination of ibuprofen and pseudoephedrine hydrochloride did not cause a positive response with any of the tester strains in the presence and absence of \_\_\_\_\_ rat liver S9. The study was concluded to be negative without conducting an independent repeat assay because no unique metabolism requirements were known about the test article and because no equivocal responses were observed in the assay that would suggest further testing is warranted.

**Reviewer's Comments:** The assay was performed according to an established method and was adequately described. The reviewer concurs with the findings. The results indicate that the \_\_\_\_\_ in a combination of ibuprofen and pseudoephedrine hydrochloride was not mutagenic in this assay. It is not clear which substance of the combination the concentration of test article cited in Tables 11-20 (Vol. 3, pp. 246 - 270) refers to. The actual concentration ( $\mu\text{g}/\text{plate}$ ) of each test substance should have been presented. It was not stated whether the genotype of the bacteria was verified prior to experiments.

**REFERENCES:**

- Kastenbaum, MA and Bowman, KO (1970) Table for determining the statistical significance of mutation frequencies. *Mut Res* 9: 527-549.
- McCarthy, DM (1999) Comparative toxicity of nonsteroidal anti-inflammatory drugs. *Am J Med*, 107(6A): 37S-46S.
- Mitchell, JA, Akarasereenont, P, Thiemermann, C, Flower, RJ and Vane, JR. (1994) Selectivity of non-steroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci*, 90(24): 11693-7.

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