

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-393 & 21-394

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-393 (Liqui-Gels), 21-394 (Caplets)

Review number: 001

Sequence number/date/type of submission: 000/October 16, 2001/Original NDA

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Wyeth Consumer Healthcare

Five Giralda Farms

Madison, NJ 07940-0871

Reviewer name: Maria I. Rivera

Division name: Anti-inflammatory, Analgesic and Ophthalmic Drug Products

HFD #: 550

Review completion date: April 11, 2002

Drug:

Trade name: Advil PM Liqui-Gels; Advil PM Caplets

Components

Advil PM Liqui-Gels: Ibuprofen/Diphenhydramine hydrochloride

Advil PM Caplets: Ibuprofen/Diphenhydramine citrate

Generic name: Diphenhydramine Citrate

Chemical name: 2 - (diphenylmethoxy) - N, N - dimethylethanamine monocitrate or ethanamine, 2-(diphenylmethoxy)-N,N-dimethyl-,2-hydroxy-1,2,3-propanetricarboxylate (1:1)

CAS registry number: 88637-37-0

Molecular formula/molecular weight: $C_{17}H_{21}NO \cdot C_6H_8O_7/447.49$

Manufacturer: _____

Generic name: Diphenhydramine Hydrochloride

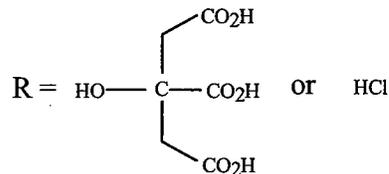
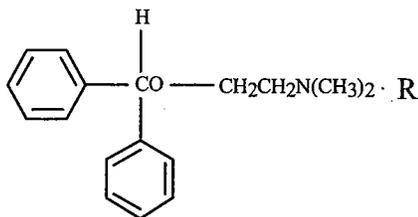
Chemical name: 2-(diphenylmethoxy)-N,N-dimethylethanamine hydrochloride or β -dimethylaminoethyl benzhydryl ether hydrochloride

CAS registry number: 147-24-0

Molecular formula/molecular weight: $C_{17}H_{21}NO \cdot HCl/291.82$

Manufacturer: _____

Structure:



Generic name: Ibuprofen

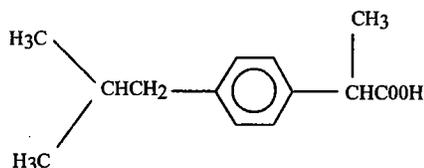
Chemical name: (±)-2-(p-isobutylphenyl)propionic acid or (R,S)-2-(4-isobutylphenyl)-propionic acid

CAS registry number: 15687-27-1

Molecular formula/molecular weight: C₁₃H₁₈O₂/206.28

Manufacturer: _____

Structure:



Relevant INDs/NDAs/DMFs:

NDA 18-989 Advil[®]

IND 56,521 Advil PM Liqui-Gels

IND 44,767 Advil PM Caplets

IND _____

DMF _____

DMF ' _____

DMF _____

Drug class: NSAID and antihistamine

Indication: Night-time analgesic/sleep aid

Clinical formulation: Advil PM Liqui-Gels contain 200 mg ibuprofen and 25 mg diphenhydramine hydrochloride. Advil PM Caplets contain 200 mg ibuprofen and 38 mg diphenhydramine citrate. Refer to the Tables in the following 2 pages for each product composition.

Advil PM Liqui-Gels

Component	mg/liqui-gel	
Gelatin Shell Gelatin,		
FD & C Blue No. 1 D & C Red No. 33 Fractionated Coconut Oil, EP ^B Lecithin NF		
Total Shell Weight		
Fill Material		mg/liqui-gel
Ibuprofen, USP Diphenhydramine HCl, USP Polyethylene Glycol Potassium Hydroxide, NF Purified Water, USP		
Total Fill Weight		
Total Liqui-gel Weight	887	

1 Page(s) Withheld

 ✓ § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 § 552(b)(4) Draft Labeling

Route of administration: oral

Proposed use: Relief of occasional sleeplessness associated with — minor aches and pains in persons \geq 12 years of age. The directions are to take two capsules at bedtime not to exceed 2 capsules in 24 hr.

Disclaimer: Tabular and graphical information is from Sponsor's submission unless stated otherwise. Parts of the text material were also copied from the Sponsor's submission.

**APPEARS THIS WAY
ON ORIGINAL**

Executive Summary

I. Recommendations

- A. **Recommendation on Approvability:** Approval is recommended.
- B. **Recommendation for Nonclinical Studies:** No additional clinical studies are recommended. Both ibuprofen and diphenhydramine are currently approved OTC drugs and the safety profile of each active ingredient is well established.
- C. **Recommendations on Labeling:** No recommendations based on the preclinical data.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

Ibuprofen and diphenhydramine hydrochloride individually and in various dosage combinations were assessed for safety in a series of toxicology studies. These studies included acute oral studies in rats, 2- and 13-week oral toxicity studies in rats and dogs, and oral teratology studies in rats and rabbits.

Ibuprofen alone, diphenhydramine alone, and three combinations of both drugs at ratios of 2:1, 4:1, and 8:1 (ibuprofen:diphenhydramine) were studied for acute oral toxicity in male and female rats. Rats given ibuprofen alone showed evidence of classical non-steroidal anti-inflammatory drugs (NSAIDs) associated toxicity. The gastrointestinal (GI) tract and principal abdominal viscera showed reddening and darkening. The LD₅₀ was calculated to be 1225 mg/kg. The LD₅₀ for rats given diphenhydramine alone was 275 mg/kg. These animals also showed darkening or reddening of the major abdominal viscera plus the lungs and thymus. The rats that died early after treatment with the combination generally presented with darkening or reddening of the major organs in the thorax and abdomen; animals that died later presented toxic signs similar to those treated with ibuprofen alone. The LD₅₀ values for rats given the drug combinations at 2:1, 4:1, and 8:1 ratios were 700, 840, and 880 mg/kg, respectively. The Sponsor concluded that the acute oral toxicity in rats after administration of ibuprofen alone or in combination with diphenhydramine was moderately toxic; whereas, that after administration of diphenhydramine alone was very toxic. No significant acute toxicological interactions occurred between the two drugs.

In the 2- and 13-week repeat-dose toxicity studies, rats given ibuprofen alone or in combination with diphenhydramine showed no definite difference in the findings for the drug combinations given at ibuprofen: diphenhydramine ratios of 4:1 or 8:1. In the 2-week study, nonulcerative inflammation involving one or more of the following tissues: duodenum, jejunum, and ileum in rats at the three highest drug combinations (60:15.0, 150:18.75 and 150:37.5 mg/kg/day). At the two highest combinations, hepatocellular hypertrophy and extramedullary hematopoiesis in liver and spleen were seen. The effects were noted similarly in rats of both sexes. Secondary effects included decreased hemograms suggesting GI bleeding. The toxicity was characteristic of that expected from propionic acid NSAIDs. Male and

female rats given 37.5 and 100 mg/kg/day diphenhydramine alone presented with excessive salivation and papillary dilatation (100 mg/kg/day only). Microscopically, males given 100 mg/kg/day of diphenhydramine alone had mild vacuolation of the hepatocytes.

In the 13-week study, rats given ibuprofen alone (16 mg/kg/day) or in combination with diphenhydramine (50:12.5 and 100:25 mg/kg/day) showed similar findings as those described above for the 2-week study. In addition, renal papillary necrosis and/or edema was observed. The NOEL was 25:6.25 mg/kg/day for the drug combination. The Sponsor concluded that ibuprofen toxic effects were not potentiated by the addition of diphenhydramine. However, in both the 2- and 13-week repeat-dose toxicity studies, the incidence and/or severity of intestinal inflammation and hepatocellular hypertrophy (2-week study) and severity of renal microscopic changes (13-week study) were slightly higher in the animals receiving the high-dose combination of ibuprofen + diphenhydramine compared to those receiving ibuprofen (13-week study) or diphenhydramine alone (both studies). Therefore, the combination may have slightly potentiated ibuprofen toxicity in this species.

Dogs were given ibuprofen alone at 16 mg/kg/day; diphenhydramine alone at 4 or 20 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 4:1 mg/kg/day, 8:2 mg/kg/day, or 16:4 mg/kg/day; or ibuprofen and diphenhydramine at an 8:1 ratio of 16:2 mg/kg/day. The results from both the 2- and 13-week studies revealed no findings that could be attributed to ibuprofen or diphenhydramine alone or in combination. Therefore, no maximum tolerated dose was identified at doses of the combination up to 16:4 mg/kg/day for 13-weeks. Dogs were given considerably lower doses of ibuprofen and diphenhydramine, alone and in combination, compared to those used to dose rats. The Sponsor lowered the doses because of the fact that dogs are more sensitive to the adverse effects of NSAIDs, especially ibuprofen, compared to rats. However, based on the results of the 2-week study, the Sponsor should have selected doses higher than 16 mg ibuprofen + 4 mg diphenhydramine/kg/day for the 13-week study.

The combination of ibuprofen and diphenhydramine was not teratogenic in rats or rabbits when given at oral doses up to 100:19 mg/kg/day and 60:15 mg/kg/day, respectively.

B. Pharmacologic Activity

Ibuprofen, a propionic acid derivative, is an NSAID with analgesic and antipyretic properties that has been available over-the-counter since 1984. Currently, the indications for this product include relief of minor aches and pains associated with the common cold, headache, toothache, muscular aches, and backache, for the minor pain of arthritis, for the pain of menstrual cramps, and for the reduction of fever. The mode of action appears to be the inhibition of prostaglandin synthesis, specifically, the cyclooxygenase enzymes COX1 and COX2.

Diphenhydramine is an H1 receptor antagonist of the ethanolamine class that is available over-the-counter for use as a sedative, hypnotic, antihistaminic, antitussive, and antiemetic agent. By virtue of their sedative effect, antihistamines are the most logical choice for the sleep component of the combination proposed in this IND and diphenhydramine is considered one of the most sedating of the group.

C. Nonclinical Safety Issues Relevant to Clinical Use

In the rat 13-week study, a slight increase in the incidence and/or severity of renal, GI and liver effects was observed with the combination at doses of 100 mg ibuprofen + 25 mg diphenhydramine/kg/day compared to 100 mg/kg/day ibuprofen alone. A similar effect was also observed in the 2-week study. No PK data was provided to determine any drug-drug interactions. In Study AE-97-09, the Sponsor reported that Advil PM Liqui-Gels were shown to have a slower rate, but equivalent extent of absorption relative to currently marketed, single ingredient diphenhydramine liqui-gel and ibuprofen liqui-gel formulations. Relative to a currently marketed single ingredient ibuprofen tablet formulation, Advil PM Liqui-Gels had an equivalent extent of absorption and an earlier time to reach peak plasma concentrations, but had a lower maximum plasma concentration. Therefore, drug-drug interactions were not observed in the clinical trial.

III. Administrative

A. Reviewer signature: _____
Maria I. Rivera, Ph.D.

B. Supervisor signature (Josie Yang, Ph.D.):

Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

NDA 21-393/Original NDA

NDA 21-394/ Original NDA

HFD-550/Division File

/Pharm/Tox TL/Yang

/MO/Lim

/SMO/Chambers

/PM/Dean

/Pharm/Tox/Rivera

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:..... 1

II. SAFETY PHARMACOLOGY: 1

III. PHARMACOKINETICS/TOXICOKINETICS: 1

IV. GENERAL TOXICOLOGY:..... 2

V. GENETIC TOXICOLOGY: 27

VI. CARCINOGENICITY: 27

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY: 27

VIII. SPECIAL TOXICOLOGY STUDIES:..... 40

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:..... 40

X. APPENDIX/ATTACHMENTS:..... 41

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

No preclinical studies were submitted with the ibuprofen/diphenhydramine combination. Ibuprofen and diphenhydramine have been studied extensively and the basic pharmacologic properties of the drugs are well known. Ibuprofen, a propionic acid derivative, is an NSAID with analgesic and antipyretic properties and has been available over-the-counter since 1984. Currently, the indications for this product include relief of minor aches and pains associated with the common cold, headache, toothache, muscular aches, and backache, for the minor pain of arthritis, for the pain of menstrual cramps, and for the reduction of fever. The mode of action appears to be the inhibition of prostaglandin synthesis, specifically, the cyclooxygenase enzymes COX1 and COX2.

Diphenhydramine is an H1 receptor antagonist of the ethanolamine class and is available over-the-counter for use as a sedative, hypnotic, antihistaminic, antitussive, and antiemetic agent. By virtue of their sedative effect, antihistamines are a logical choice for the sleep-aid component of the combination proposed in this IND and diphenhydramine is considered one of the most sedating of the group.

II. SAFETY PHARMACOLOGY:

No preclinical studies were submitted with the ibuprofen/diphenhydramine combination. The Sponsor made reference to Whitehall-Robins Healthcare NDA 18-989 for preclinical data supporting use of ibuprofen over-the-counter with dosing to 1200 mg/day. Diphenhydramine HCl is Generally Recognized as Safe and Effective (GRASE) for over-the-counter use as an antihistamine, a nighttime sleep aid, an antitussive and an anti-emetic, with dosing to 300 mg/day (21 CFR 336, 338 & 341). Thus, preclinical safety has been established for the individual ingredients of the new combination proposed in this application. The Sponsor conducted an evaluation of the literature published from 1966 through July 2000. The following databases were searched: Medline, Embase, Biosis, Toxline, Derwent Drug File, and SciSearch. The Sponsor stated that no information was found to question the safety of the proposed combination.

III. PHARMACOKINETICS/TOXICOKINETICS:

No preclinical studies were submitted with the ibuprofen/diphenhydramine combination. The human PK profiles for ibuprofen and diphenhydramine are well established and are summarized in the table below.

Pharmacokinetic and Pharmacodynamic Characteristics of Ibuprofen and Diphenhydramine

PK Characteristic	Ibuprofen 400 mg	Diphenhydramine 50 mg
C _{max}	35-40 mcg/mL	65-75 ng/mL
T _{max}	0.75-2 hrs	3-4 hrs
Half-Life	2 hrs	9-10 hrs
PD Characteristic	Ibuprofen 400 mg	Diphenhydramine 50 mg
Time to Onset of Effect	15-30 min post dose	1-2 hrs post dose
Duration of Effect	up to 6 hrs post dose	up to 8 hrs post dose
Min. Effective Plasma Conc.	6-10 mcg/mL	30-50 ng/mL

IV. GENERAL TOXICOLOGY:

The Sponsor submitted several studies evaluating potential unique toxicities for the combination of ibuprofen and diphenhydramine. Some of these studies (BRT#s 84-24, 84-32, 85-09, 84-33, 85-12, 84-35, 85-07, 84-36 and 85-08) were commissioned by

An access letter providing permission for reference by Whitehall-Robins is provided in the submission (Vol. 451, pg. 1). These studies were previously submitted to the Agency under IND — but were not reviewed. The studies presented under IND — evaluated numerous doses and ratios of the ibuprofen and diphenhydramine combinations. These studies include acute oral studies in rats, 2- and 13-week oral toxicity studies in rats and dogs, and teratology studies in rats and rabbits. The studies reveal no unexpected toxicity for the combination of these two ingredients. Study #93-4058 was commissioned by Whitehall-Robins but not previously submitted to FDA.

Study title: An Acute Oral Toxicity in albino Rats Administered Test Article MV#1405-34, MV#1518-112, MV#1913-157, MV#1913-43 or MV#1913-44

Key study findings: No significant acute toxicological interactions occurred between ibuprofen and diphenhydramine in rats treated with combinations of both drugs at ratios of 2:1, 4:1, and 8:1 (ibuprofen:diphenhydramine).

Study no: BRT#84-24

Volume #, and page #: 32, 1

This study was previously submitted under IND — and was reviewed by Conrad Chen, Ph.D. The review is included in the APPENDIX/ATTACHMENTS section. A summary of the study is provided below.

Ibuprofen alone, diphenhydramine alone, and three combinations of both drugs at ratios of 2:1, 4:1, and 8:1 (ibuprofen:diphenhydramine) were studied for acute oral toxicity in male and female — Sprague-Dawley derived rats. Rats given ibuprofen alone showed evidence of classical

effects from toxicity with a non-steroidal anti-inflammatory drug (NSAID). The gastrointestinal (GI) tract and principal abdominal viscera showed reddening and darkening. All deaths occurred between days 3 and 7. The LD₅₀ was calculated to be 1225 mg/kg. Rats given diphenhydramine alone or in combination with ibuprofen had mortality on Day 1 with a few deaths delayed throughout the first 8 days after dosing. The rats that died early generally presented with darkening or reddening of the major organs in the thorax and abdomen; the later deaths showed signs similar to those rats given ibuprofen alone (ascites and/or hydrothorax, reddening and/or distention of the GI tract, black mucoid material in the GI tract and multiple adhesions among the abdominal viscera). The LD₅₀ for rats given diphenhydramine alone was 275 mg/kg. The LD₅₀ values for rats given the drug combinations were 700, 840, and 880 mg/kg for the 2:1, 4:1, and 8:1 ratios, respectively. The Sponsor concluded that the acute oral toxicity in rats after administration of ibuprofen alone or in combination with diphenhydramine was moderately toxic; whereas, that after administration of diphenhydramine alone was very toxic. No significant acute toxicological interactions occurred between the two drugs.

Study title: Fourteen-Day Oral Toxicity Study in Rats

Key study findings:

- No unexpected toxicological effects occurred in rats treated with combinations of ibuprofen and diphenhydramine at ratios of 4:1 and 8:1 (ibuprofen:diphenhydramine).
- The incidence and/or severity of intestinal inflammation and hepatocellular hypertrophy was slightly more in rats treated with 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day compared to rats treated with 60 mg ibuprofen + 15.0 mg diphenhydramine/kg/day or 150 mg ibuprofen + 18.75 mg diphenhydramine/kg/day, and were not present in animals treated with 100 mg/kg/day diphenhydramine alone.

Study no: BRT#84-32

Volume #, and page #: 33, 2

Conducting laboratory and location: —

Date of study initiation: February 4, 1985

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch #: 1934-48

Ibuprofen + diphenhydramine (4:1): Batch # 1934-46

Ibuprofen + diphenhydramine (8:1): Batch # 1938-033

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods (unique aspects): No unique aspects

Dosing:

Species/strain: Rats/ — CD®

#/sex/group or time point (main study): 5 rats/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: 8 – 9 weeks

Weight: 190 – 218 g for males, 137 – 158 g for females

Doses in administered units:

Group	Treatment	Dosage level (mg/kg/day)
1	Vehicle	
2	Ibuprofen + Diphenhydramine (4:1)	24 + 6.0
3	Ibuprofen + Diphenhydramine (4:1)	60 + 15.0
4	Ibuprofen + Diphenhydramine (4:1)	150 + 37.5
5	Ibuprofen + Diphenhydramine (8:1)	150 + 18.75
6	Diphenhydramine	37.5
7	Diphenhydramine	100

Route, form, volume, and infusion rate: gavage, 10 ml/kg

Observations and times:

Clinical signs: Observed twice daily for moribundity, mortality, and signs of overt toxicity

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Prior to study initiation and on day 12

EKG: Not determined

Hematology: Erythrocyte counts (RBC), hematocrit, hemoglobin concentration, MCH (calculated), MCV (calculated), MCHC (calculated), total leukocyte count, differential leukocyte count, platelet count.

Clinical chemistry: Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glucose, urea nitrogen, total bilirubin, total cholesterol, albumin, calculated globulin, total protein, creatine, sodium, potassium, chloride and calcium.

Urinalysis: Volume, color and appearance, pH, specific gravity, protein, glucose, ketones, urobilinogen, nitrites, bilirubin, occult blood, microscopy of spun deposit.

Gross pathology: Adrenal (2), bone (femur), bone marrow (femur), brain (fore, mid, hindbrain), eye with optic nerve, esophagus, stomach (glandular and nonglandular), duodenum, jejunum, ileum, cecum, colon, rectum, ovary (2), testis with epididymis (2), heart, kidney (2), liver (3 sections), lung with mainstem bronchi (2), lymph nodes (thoracic, abdominal), mammary region (female only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular with submandibular lymph node), sciatic

nerve, skeletal muscle, skin, spinal cord (cervical, midthoracic and lumbar), spleen, thymic region, thyroid/parathyroid complex, trachea, urinary bladder, uterus, all gross lesions

Organs weighed: Absolute and relative (to body and to brain weight) weights of liver, kidney (2), heart, adrenal (2), ovary (2), testes (2), and brain.

Histopathology: Refer to Histopathology Inventory Table for organ examined. All organs listed in the Table were examined from the control and the following groups: ibuprofen + diphenhydramine 150 + 37.5 and 150 + 18.75 mg/kg/day; diphenhydramine 37.5 and 100 mg/kg/day. In addition, duodenum, ileum, jejunum, liver and spleen from groups ibuprofen + diphenhydramine 24 + 6.0 and 60 + 15.0 mg/kg/day.

Toxicokinetics: Not performed

Results:

Mortality: None

Clinical signs: Excessive salivation was observed in all animals dosed with 37.5 and 100 mg/kg/day diphenhydramine alone. Pupillary dilatation was observed in 3 males and all 5 females in the 100 mg/kg/day diphenhydramine alone. Two males dosed with the 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day showed labored breathing and firm areas in the abdomen. The Sponsor considered these effects as incidental.

Body weights: Statistically significant decreases, as compared to control group, were noted in males in the 150 + 37.5 mg/kg/day combination group at Week 1 (-12%) and Week 2 (-18%), and in the 100 mg/kg/day diphenhydramine group at Week 2 (-6%). In females, a statistically significant decrease as compared to control was noted in the 100 mg/kg/day diphenhydramine group at Week 1 (-10%) and Week 2 (-12%).

Food consumption: Statistically significant decreases, as compared to control values, were noted at Week 1 in the 150 + 37.5 mg/kg/day (-17%) and 150 + 18.75 mg/kg/day (-6%) combination male groups and at Week 1 in the 100 mg/kg/day diphenhydramine female group (-16%).

Ophthalmoscopy: No test-article related effects

Electrocardiography: Not determined

Hematology: Test article related changes were noted in the animals receiving 150 mg/kg/day ibuprofen with 37.5 or 18.75 mg/kg/day of diphenhydramine. Similar effects were observed with the two combinations and the Sponsor attributed these findings to the ibuprofen component. Affected parameters (with statistical significance at either $p < 0.05$ or $p < 0.01$) are listed in the table below:

Parameter	I + D: 150 + 37 mg/kg/day		I + D: 150 + 18.75 mg/kg/day	
	M	F	M	F
Erythrocytes	-30%	-16%	-20%	-20%
Hemoglobin	-33%	-15%	-17%	-16%
Hematocrit	-33%	-15%	-18%	-18%
Platelets	+88%	+27%	+21%	+29%

Note: Values represent % change relative to control group; I = Ibuprofen; D = Diphenhydramine

In males, there was also a statistically significant increase in leucocytes (+35%) and in segmented neutrophils (+140%) in the 150 + 18.5 mg/kg/day combination group and in the low-dose (37.5 mg/kg/day) diphenhydramine group (+48%). These last two findings were not observed in the higher-dose combination groups and therefore, considered to be spurious changes (more indicative of animal variation and sample size than effects due to test article related toxicity).

Clinical chemistry: Statistically significant decreases (and probably related to ibuprofen) were observed in albumin and total protein in the two combination groups receiving 150 mg/kg/day ibuprofen. Males showed decreases in albumin of 15% and 6%, and females showed decreases of 8% and 11% in the combination groups treated with 150 mg ibuprofen + 37.5 mg or 18.75 mg diphenhydramine/kg/day, respectively. Females treated with 160 mg ibuprofen + 18.75 mg diphenhydramine/kg/day showed a 15% decrease in total protein. Other statistically significant effects were: decreased calcium levels in males in the 150 mg ibuprofen + 37.5 mg (-6.4%) or 18.75 mg diphenhydramine/kg/day (-3.7%) combination groups, and 25 – 50% increases in blood urea nitrogen (BUN) values in males in all treatment groups (except the 150 + 37.5 mg/kg/day combination group). In the 150 + 37.5 mg/kg/day combination group, there was a 100% increase in BUN but it was not statistically significant. The increase in BUN was not considered to be indicative of toxicity as there were no signs of kidney toxicity by histopathology. The Sponsor stated that these values were also within the range of normal variation for rats of this strain and age (data not submitted). The reviewer also noted elevated AST values (16 – 30%) in all treated male groups except in males dosed with 150 mg ibuprofen + 37.5 mg diphenhydramin/kg/day combination (no difference from control). These values did not reach statistical significance.

Urinalysis: No test-article related effects.

Organ weights: Statistically significant increases were observed in the relative weight of liver (relative to body weight) in the following groups: 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day (25%) and 100 mg/kg/day diphenhydramine (15%) males; 150 mg ibuprofen + 18.75 mg diphenhydramine/kg/day (16%) and 100 mg/kg/day diphenhydramine (17%) females. Microscopic observations showed trace to mild hepatocellular hypertrophy in both male and female combination groups listed above. However, liver weight was not significantly increased and the relative liver weight increase was considered to be a reflection of the low body weights in these groups. Females showed a

significant decrease in absolute and relative (to brain) ovaries weight in the groups treated with 60 mg ibuprofen + 15 mg diphenhydramine/kg/day (13%), 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day (11%) and 37.5 mg/kg/day diphenhydramine (16%). These findings were not accompanied by microscopic changes. Therefore, these findings were considered as physiological differences.

Gross pathology: The following changes were evident in males dosed with the combination of 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day: mucosal thickening with serosal adhesions in the small intestine in one animal, enlarged spleen and enlarged abdominal lymph nodes in two animals. The Sponsor considered all other changes to be agonal or spontaneous changes.

Histopathology: Treatment related microscopic changes were seen in males and females in the 60 + 15 mg/kg/day combination group (Group 3), 150 + 37.5 mg/kg/day combination group (Group 4) and 150 + 18.75 mg/kg/day combination group (Group 5); and in males in the 100 mg/kg/day diphenhydramine group (Group 7). There were no treatment related microscopic changes in the 24 + 6 mg/kg/day combination group (Group 2) and the 37.5 mg/kg/day diphenhydramine group (Group 6). The changes in Groups 3, 4 and 5 were inflammation of the small intestine. In addition, Groups 4 and 5 also had hepatocellular hypertrophy, and extramedullary hematopoiesis in the spleen and liver. Two males in Group 4 also had reactive hyperplasia in the abdominal lymph node. The change seen in males of Group 7 was mild vacuolation of hepatocytes. These changes are presented in the following table:

Sex	Males							Females							
	Group Number	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Ibuprofen mg/kg/day	0	24	60	150		0	0	0	24	60	150	150	0	0	
Diphenhydramine	0	+	15	37.5		37.5	100	0	6	15	37.5	18.75	37.5	100	
SMALL INTESTINE	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(3)
Inflammation of duodenum jejunum and/or ileum															
- trace				2							1				
- mild				2							2				
- moderate				1	3							1	1		
- severe				1											
LIVER	(5)	(5)	(5)	(5)	(5)	(7)	(8)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Hypertrophy															
- trace				1								3			
- mild				3	1										
Hematopoiesis, mild				3	1			1				1	2		
Vacuolar change, mild							4								
SPLEEN	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Increased hematopoiesis,															
- mild				1	1	2	1					1	2		
- moderate				4	2							1	1		
LYMPH NODE	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Reactive hyperplasia															
- mild				2											

() = number examined

Nonulcerative or ulcerative inflammation involving the duodenum, jejunum and/or ileum was seen in males and females of Groups 3, 4 and 5. Trace to mild hepatocellular hypertrophy was also seen in males and females of Group 4 and in males of Group 5. Hepatocellular hypertrophy was not

evident in females of Group 5. The incidence and/or severity of intestinal inflammation and hepatocellular hypertrophy was slightly more in Group 4 compared to Groups 3 and 5. Intestinal inflammation or hepatocellular hypertrophy was not evident in Groups 6 or 7 but males in Group 7 had mild vacuolation of periportal or centrilobular hepatocytes. The vacuoles were suggestive of fatty change. Vacuolation of hepatocytes was not evident in females of Group 7. In males and females of Groups 4 and 5, in addition to intestinal inflammation and hepatocellular hypertrophy, extramedullary hematopoiesis in liver and spleen was also seen. Mild hematopoiesis in the liver of one control female and mildly increased hematopoiesis in the spleen of one Group 6 male were considered to be incidental findings.

In this study, the abdominal lymph node was not one of the tissues to be histopathologically evaluated, however, it was examined in two males of Group 4 due to macroscopic enlargement. Microscopic examination of these nodes revealed a mild reactive hyperplasia which suggested an inflammatory process in the intestinal tract. Microscopic changes other than the ones discussed were all considered to be agonal or spontaneous changes unrelated in occurrence to oral administration of the test articles.

Toxicokinetics: Not performed

Summary of individual study findings:

Groups of rats were administered orally diphenhydramine alone at 37.5 or 100 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 24:6 mg/kg/day, 60:15 mg/kg/day, or 150:37.5 mg/kg/day or at an 8:1 ratio of 150:18.75 mg/kg/day. Decrease body weight gain and decrease food consumption were noted in the groups receiving the two highest-dose combinations and in the group receiving diphenhydramine alone at 100 mg/kg/day. A few rats at the highest drug combination (ibuprofen, 150 mg/kg/day and diphenhydramine, 37.5 mg/kg/day) showed evidence of GI irritation. In addition, a few rats given ibuprofen at 150 mg/kg/day and diphenhydramine at 18.75 or 37.5 mg/kg/day showed low hemograms and increased platelet concentrations as well as decreased total protein or albumin concentrations, or both. Microscopically, nonulcerative inflammation involving one or more of the following tissues: duodenum, jejunum, and ileum in rats at the three highest drug combinations. At the two highest combinations, hepatocellular hypertrophy and extramedullary hematopoiesis in liver and spleen were seen. The effects were noted similarly in rats of both sexes. Male and female rats given both doses of diphenhydramine alone presented with excessive salivation and papillary dilatation at 100 mg/kg/day. Microscopically, males given 100 mg/kg/day of diphenhydramine alone had mild vacuolation of the hepatocytes. The no observable effect level (NOEL) for the drug combination of ibuprofen and diphenhydramine was determined to be 24 mg/kg/day and 6 mg/kg/day, respectively. The Sponsor concluded that the observed adverse effects were a result of treatments with each agent independently, and were not a result of the combination, per se. However, the incidence and/or severity of intestinal inflammation and of hepatocellular hypertrophy was slightly more in animals treated with 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day than in animals treated with 150 mg ibuprofen + 18.75 mg diphenhydramine/kg/day and these adverse effects were not observed in the 37.5 mg/kg/day or 100 mg/kg/day diphenhydramine groups. This suggests a

possible potentiation effect of ibuprofen toxicity when administered in combination with diphenhydramine to rats.

Study title: Fourteen-Day Oral Toxicity in Dogs

Key study findings: At the concentrations used in this study, administration of ibuprofen, diphenhydramine or combination of ibuprofen + diphenhydramine was not toxic to beagle dogs.

Study no: BRT #84-33

Volume #, and page #: 34, 2

Conducting laboratory and location: _____

Date of study initiation: March 1, 1985

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below:

Ibuprofen: Batch # 1938-21

Diphenhydramine: Batch # 1938-20

Ibuprofen + diphenhydramine (4:1): Batch # 1938-15

Ibuprofen + diphenhydramine(8:1): Batch # 1938-19

Formulation/vehicle: All test materials were received diluted 1:14 with starch.

Methods (unique aspects): No unique aspects

Dosing:

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 2 dogs/sex/group

Satellite groups used for toxicokinetics or recovery: none

Age: ~ 6 months

Weight: 7.6 - 11.4 kg males, 7.2 -9.2 kg females

Doses in administered units:

Daily Dosage (mg/kg/day)	
Ibuprofen	Diphenhydramine
0*	0*
4	1
8	2
16	4
16	2
0	4
16	0
0	20

*; Capsules filled with starch at 280 mg/kg/day.

Route, form, volume, and infusion rate: oral, gelatin capsules, twice daily (at least 6 hours apart)

Observations and times:

Clinical signs: Twice daily for moribundity, mortality and signs of overt toxicity, diarrhea, emesis, and inappetence. Detailed observations of appearance and condition, behavior and activity, excretory functions, respiration, orifices, eyes and palpable masses on Days 8 and 14.

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Once prior to study initiation and once prior to study termination

EKG: Once prior to study initiation and once prior to study termination

Hematology: Once prior to study initiation and once prior to study termination. The following parameters were measured: total leukocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count, differential leukocyte count, prothrombin time and activated partial thromboplastin time. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were automatically calculated by the analyzer.

Clinical chemistry: Once prior to study initiation and once prior to study termination. The following parameters were measured: sodium, potassium, chloride, calcium, alkaline phosphatase, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatinine, total protein, albumin, A/G ratio (calculated), cholesterol and glucose.

Urinalysis: Once prior to study initiation and once prior to study termination. The following parameters were measured: color, appearance, microscopic examination of sediment, specific gravity, pH, protein, glucose, occult blood, nitrites, bilirubin, ketones and urobilinogen.

Gross pathology: The following organs were examined in all animals: adrenal (2), all gross lesions, aorta, bone (rib), bone marrow (rib), bone marrow smears, brain (fore, mid and hind), eye including optic nerve (2), gallbladder, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon and rectum), gonads (ovary (2) and testis with epididymis (2)), heart, kidney (2), liver, lung with mainstem bronchi (2), lymph nodes (thoracic: mediastinal and abdominal: mesenteric), mammary gland (female only), pancreas, pituitary, prostate, salivary gland, mandibular with submandibular lymph node, sciatic nerve, skeletal muscle (thigh), skin, spinal cord (cervical, midthoracic and lumbar), spleen, thymus, thyroid/parathyroid (2), trachea, urinary bladder, uterus

Organs weighed: Refer to Histopathology Inventory Table. In addition the pituitary and parathyroid were weighed.

Histopathology: Refer to Histopathology Inventory Table

Toxicokinetics: Not determined

Other:

Fecal analysis or occult blood: Collected and analyzed on days 1, 4, 13 and 14

Physical Examination: Once prior to initiation and at study termination. Physical examination included an inspection for general condition that consisted of an examination of the head and neck, thorax, abdomen, external reproductive organs, skin and extremities. Heart and lung sounds were evaluated by percussion and auscultation (using a stethoscope).

Results:

Mortality: None

Clinical signs: No test-article related effects

Body weights: No test-article related effects

Food consumption: It was difficult to determine a test-article related effect because only 2 animals/sex/group were used. The Sponsor's summary was based on the differences relative to control values. Due to differences observed between control and treatment groups at Week 0, the reviewer decided it was more appropriate to compare each group with its own Week 0 values. Using this convention, the following changes were observed at Week 1: a ~ 19% decrease in g/animal/day and g/kg/day in males treated with 16 mg ibuprofen + 2 mg diphenhydramine/kg/day, a ~30% decrease in g/animal/day and g/kg/day in males treated with 20 mg/kg/day diphenhydramine, a ~ 28% decrease in g/animal/day and g/kg/day female controls, ~ 24% decrease in g/animal/day and g/kg/day in female treated with 16 mg/kg/day ibuprofen, and a ~ 28% decrease in females treated with 20 mg/kg/day diphenhydramine. The reviewer did not consider the decreases in food consumption of toxicological significance for the following reasons: the number of animals used was not enough to determine a test-article related effect, a dose-related effect was not observed in the combination groups, and a decrease was also observed in the control group at Week 1 compared to Week 0.

Ophthalmoscopy: No test-article related effects

Electrocardiography: No test-article related effects

Hematology: No test-article related effects

Clinical chemistry: No test-article related effects

Urinalysis: No test-article related effects

Organ weights: No test-article related macroscopic changes

Gross pathology: No test-article related macroscopic changes

Histopathology: No test-article related microscopic changes

Toxicokinetics: Not performed

Fecal analysis or occult blood: Positive results occurred in some animals at various intervals, but there was not an apparent trend to suggest a test-article related effect.

Physical Examination: No test-article related abnormalities were reported. Pupils partially constricted were noted in one or more animals/group. The Sponsor also mentioned that slight irregular heart rhythm was noted at the terminal physical in 1 female in the 4 + 1 mg/kg/day and 16 + 4 mg/kg/day ibuprofen + diphenhydramine dose groups.

Summary of individual study findings:

Groups of beagle dogs were orally given drug-filled gelatin capsules consisting of ibuprofen alone at 16 mg/kg/day; diphenhydramine alone at 4 or 20 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 4:1mg/kg/day, 8:2 mg/kg/day, or 16:4 mg/kg/day; or ibuprofen and diphenhydramine at an 8:1 ratio of 16:2 mg/kg/day. The only effect observed was changes in food consumption. However, only 2 male and 2 female beagle dogs were used on each group which makes it difficult to determine a test-article related effect. The doses used in this study were not high enough to induce toxicity after treatment with ibuprofen or diphenhydramine alone or in combination.

Study title: Thirteen-Week Oral Toxicity Study in Rats

Key study findings:

- GI and renal toxicity was noted in rats; however, all adverse findings could be associated with the ibuprofen component of the drug combination. Rats treated with diphenhydramine alone showed no toxicity.
- The incidence and severity of renal microscopic changes (kidney papillary necrosis and papillary edema) was **slightly** higher in the group of rats that received a combination of 100 mg of ibuprofen + 25 mg of diphenhydramine/kg/day compared to the rats that received 100 mg/kg/day ibuprofen alone.
- The combination of 25:6.25 mg/kg/day (ibuprofen:diphenhydramine) was considered a NOEL.

Study no: BRT #85-09

Volume #, and page #: 35, 2

Conducting laboratory and location: —

Date of study initiation: May 23, 1985

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1979-69

Ibuprofen: Batch # 1979-58

Ibuprofen + diphenhydramine (4:1): Batch # 1979-70

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods (unique aspects): No unique aspects

Dosing:

Species/strain: Rats/ — CD®
 #/sex/group or time point (main study): 15 rats/sex/group
 Satellite groups used for toxicokinetics or recovery: none
 Age: 6 weeks
 Weight: 181 – 210g males, 132 – 156g females
 Doses in administered units:

Treatment	Dosage level (mg/kg/day)
Vehicle	
Ibuprofen + Diphenhydramine (4:1)	25 + 6.25
Ibuprofen + Diphenhydramine (4:1)	50 + 12.5
Ibuprofen + Diphenhydramine (4:1)	100 + 25.0
Ibuprofen	100
Diphenhydramine	25

Route, form, volume, and infusion rate: oral, gavage, once daily at a volume of 10 ml/kg

Observations and times:

Clinical signs: Twice daily for signs of moribundity, mortality and overt toxicity. Detailed observations were recorded weekly.

Body weights: Weekly beginning with the pretest period

Food consumption: Weekly

Ophthalmoscopy: Once during the pretest period and once during the last week of study

EKG: Not performed

Hematology*: Determined on whole blood and included the following parameters: total leukocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count, reticulocyte count, and differential leukocyte count. The hematological indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were automatically calculated by the analyzer.

Clinical chemistry*: Determined on serum and included the following parameters: sodium, potassium, chloride, calcium, alkaline phosphatase, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatine, total protein, albumin, globulin (calculated), cholesterol and glucose.

Urinalysis*: Color, appearance, microscopic examination of sediment, specific gravity, volume, pH, protein, glucose, occult blood, nitrite, bilirubin, ketones and urobilinogen

*Conducted on 10 randomly selected animals/sex/group at 13 weeks of study.

Gross pathology: All animals received a complete postmortem examination. The Sponsor did not listed in details the organs examined but stated that the contents of the abdominal, thoracic and cranial cavities were examined both *in situ* and after removal and dissection.

Organs weighed: Refer to Histopathology Inventory Table

Histopathology: Refer to Histopathology Inventory Table. A full microscopic examination of tissues was done in the control and high-dose ibuprofen + diphenhydramine groups. Rats in the other groups had limited histopathology on selected tissues.

Toxicokinetics: Not performed

Results:

Mortality: A summary of mortality is presented in the following table:

Dosage level (mg/kg/day)	Sex	Number of deaths	Week(s) of Death
Control	M	1	14
	F	0	-
Ibuprofen + Diphenhydramine, 100 + 25	M	1	2
	F	3	9, 12, 12
Ibuprofen, 100	M	0	-
	F	1	8
Diphenhydramine, 25	M	0	-
	F	1	13

No deaths occurred in the animals treated with the mid- and low-dose combination of ibuprofen + diphenhydramine. The death of the female in the 25 mg/kg/day diphenhydramine group was due to bleeding during blood collection. The ibuprofen treated female had hypothermia, labored breathing, firm areas in the abdomen and excessive lacrimation. There were no visible abnormalities prior to death in the other animals.

Clinical signs: The following findings were observed in a few control or treated animals and were considered incidental and not treatment-related: hair loss, excessive salivation, abnormal contour and impaired function of the hindlimb, red and swollen hindlimb and upper incisors malaligned.

Body weights: No treatment-related effects

Food consumption: No treatment-related effects were reported. A few statistically significant increases or decreases were observed in the high-dose ibuprofen + diphenhydramine combination, the 100 mg/kg/day diphenhydramine and the 100 mg/kg/day ibuprofen groups. However, these differences were less than 10% and not considered toxicologically significant.

Ophthalmoscopy: No treatment-related effects

Electrocardiography: Not performed

Hematology: There were some changes in hematograms in both males and females from the ibuprofen + diphenhydramine high-dose and 100 mg/kg/day ibuprofen groups. Statistically significant decreases were seen in erythrocyte (10 - 15%), hemoglobin (~10 - 16%) and hematocrit values (~10 - 15%). Other statistically significant findings were: increased reticulocyte counts in males in the ibuprofen + diphenhydramine high-dose group (207%) and 100 mg/kg/day ibuprofen group (78%); increased platelet counts in the ibuprofen + diphenhydramine high-dose group in males (45%) and in the 100 mg/kg/day ibuprofen group in females (10.8%).

Clinical chemistry: In the ibuprofen + diphenhydramine high-dose combination and ibuprofen 100 mg/kg/day groups, total protein was decreased in both males (15% and 12%, respectively) and females (12% on each group). The decrease was primarily due to decreased albumin and/or globulin. Males in both groups had a slight (6 - 7%) but statistically significant decrease in calcium levels. Other statistically significant changes were decrease glucose (13%) in males and increase BUN (28%) in females in the ibuprofen + diphenhydramine high-dose combination group, and increase in aspartate aminotransferase values (20%) in the 100 mg/kg/day ibuprofen group in males.

Urinalysis: No treatment-related effects

Organ weights: Statistically significant increases in mean absolute and mean relative weights (relative to brain and/or body weight) of kidney and liver were seen in females that received a combination of ibuprofen and diphenhydramine in doses of 50 + 12.50 (~10 - 13%) and 100 + 25 mg/kg/day (~20%). Females that received 100 mg/kg/day of ibuprofen alone (~12%) and 25 mg/kg/day of diphenhydramine alone (~13%) had statistically significant increases in mean absolute and mean relative weights (to brain or body weight) of liver. Treatment related microscopic changes were seen in the kidney but no treatment related microscopic changes were evident in the livers of treated females. The biological significance of these increases in liver weight seen in treated females is unknown.

Females that received 25 mg of ibuprofen plus 6.25 mg of diphenhydramine/kg/day had a statistically significant increase in mean absolute and mean relative weights of the adrenals (~14%). Adrenal weight differences were not evident in higher dose groups, nor were any treatment related macroscopic or microscopic changes seen in those adrenals. Therefore, these adrenal weight differences were considered a biological variation. Males that received 100 mg of ibuprofen plus 25 mg of

diphenhydramine had a statistically significant increase (15%) in the group mean relative liver weight (relative to body weight only). Two males from this group had mild hepatocellular hypertrophy but there was no absolute liver weight increase. Also, the males of this dose group had a lower body weight compared to other groups. The relative liver weight increase was therefore considered a reflection of the low body weight.

Gross pathology: Test article related macroscopic changes were seen in 2/15 males and 2/15 females that received a combination of 100 mg of ibuprofen and 25 mg of diphenhydramine/kg/day. The changes seen were ulcers, diverticuli or mucosal necrosis in the jejunum and/or erosions in the glandular mucosa of the stomach. A focus in the renal papilla was observed in one male from this dose group. However, no corresponding microscopic changes were observed in the renal papilla of this male. Enlarged abdominal lymph nodes were seen in a few rats that received 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day (1 male) or 100 mg of ibuprofen/kg/day alone (1 male and 3 females). Abdominal lymph node enlargement was considered a response to gastrointestinal inflammation seen microscopically. Other macroscopic changes observed were considered to be agonal, spontaneous or due to gavage injuries.

Histopathology: Treatment related microscopic changes were seen in all groups of rats that received a combination of ibuprofen and diphenhydramine or ibuprofen alone. In general, the incidence or severity of these changes was slightly higher in the group of rats that received a combination of 100 mg of ibuprofen and 25 mg of diphenhydramine/kg/day compared to the group that received ibuprofen alone at a dose of 100 mg/kg/day. No treatment related changes were evident in rats that received diphenhydramine alone at a dose of 25 mg/kg/day. Treatment related changes are presented in the following table:

The salient lesions of test article related significance in the kidneys were papillary necrosis and papillary edema. The incidence of these lesions was slightly increased in the group that received 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day compared to the group that received ibuprofen alone at a dose of 100 mg/kg/day. Trace renal papillary necrosis was also seen in one female that received 50 mg of ibuprofen plus 12.50 mg of diphenhydramine/kg/day. The presence of hyaline casts in renal tubules, commonly seen at the corticomedullary junction, was considered secondary to the papillary lesions. Hyaline casts were seen in

Group	1		2		3		4		5		6	
Ibuprofen (mg/kg/day)	0		25		50		100		100		0	
+	+		+		+		+		+		+	
Diphenhydramine (mg/kg/day)	0		6.25		12.50		25		0		25	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
<u>KIDNEY</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Papillary necrosis												
- trace						1		2	1	1		
- mild							4	6	1			
Edema, papilla, mild							1	2				
Hyaline cast, trace/mild					2	2	10	7	5	5		
Chronic progressive nephropathy												
- trace	1				1	2	3	8	3	1		1
- mild	1				4		7	2	9	2		3
- moderate							1					
<u>STOMACH, GLANDULAR</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation,												
- mild							1					
- severe								1				
<u>DUODENUM</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation												
- mild							1					
- moderate								1				
<u>JEJUNUM</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation ulcerative												
- moderate								1				
- severe								2				
<u>CECUM</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation												
- mild				1		1	2	1	1	4		
- moderate							1		1	1		
<u>LIVER</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Hypertrophy, mild							2					
<u>SPLEEN</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Increased hematopoiesis												
- mild		4		4	1	6	4	5	10	4	2	4
<u>LYMPH NODE, ABDOMINAL</u>	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(0)	(3)	(0)	(0)
Edema, mild/moderate								2	1	3		
Reactive hyperplasia												
- mild							1	1	1	1		

groups that received a combination of ibuprofen and diphenhydramine in doses of 50 + 12.50 mg/kg/day or 100 + 25 mg/kg/day and in the group that received 100

mg/kg/day of ibuprofen alone. The incidence of hyaline casts was slightly higher in the group that received 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day. There were other microscopic renal changes such as papillitis, papillary hemorrhage and mineralization. These were considered secondary changes due to papillary necrosis.

Lesions suggestive of chronic progressive nephropathy, a spontaneous disease of rats, were seen in some control and treated groups of rats. The incidence of this nephropathy was slightly higher in the groups that received 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day or 100 mg/kg/day of ibuprofen alone. This lesion was characterized by tubular regeneration, interstitial fibrosis, glomerulosclerosis and/or interstitial lymphocytic infiltration. All of the preceding features were not present in every animal. The increased incidence might be due to additional tubular injury caused by the test articles.

Test-article related gastrointestinal lesions were ulcerative or nonulcerative inflammation in the glandular stomach, duodenum, jejunum and/or cecum. Inflammatory lesions in the stomach, duodenum and jejunum were evident only in the group that received a combination of 100 mg of Ibuprofen and 25 mg of diphenhydramine/kg/day. The incidence of cecal inflammation was very low. It was seen in females at all dose groups except those that received 25 mg/kg/day of diphenhydramine alone. Inflammation of the cecum was not evident in males that received a combination of ibuprofen and diphenhydramine in doses of 25 + 6.25 mg/kg/day or 50 + 12.50 mg/kg/day.

Mild hepatocellular hypertrophy was seen in two males that received 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day. However, there was no corresponding absolute liver weight increase in this group. Neither hepatocellular hypertrophy nor any other significant treatment related changes in the liver were seen in treated groups of females.

In the spleen, a slightly increased incidence of hematopoiesis was seen in groups of males that received either 100 mg of ibuprofen + 25 mg of diphenhydramine/kg/day or 100 mg/kg/day of ibuprofen alone. There was no corresponding evidence of hemolysis or injury to bone marrow. This increased incidence of hematopoiesis was considered to be a secondary response. It was probably caused by chronic blood loss *via* the gastrointestinal route. This assumption was based on microscopic findings. Edema or reactive hyperplasia in abdominal lymph nodes was seen in a few rats that received either 100 mg of ibuprofen plus 25 mg of diphenhydramine/kg/day or 100 mg/kg/day of ibuprofen alone. These lymph node reactions were considered a secondary response to gastrointestinal inflammation.

All microscopic changes other than those previously discussed were considered to be spontaneous, agonal or iatrogenic in nature.

Histopathology on animals that died during the course of study: Mortalities occurred in one male in the control group, one male and three females in the 100 mg ibuprofen + 25 mg diphenhydramine/kg/day group, 1 female in the ibuprofen 100 mg/kg/day group, and 1 female in the diphenhydramine 25 mg/kg/day group. One female that received 100 mg of ibuprofen + 25 mg of diphenhydramine/kg/day died of test article related ulcerative jejunitis. The cause of death could not be determined for another female that died from the same dose group. In all other rats that died, the cause of death was either gavage injury or spontaneous urinary tract infection.

Toxicokinetics: Not performed

Summary of individual study findings:

Groups of rats were administered orally ibuprofen alone at 100 mg/kg/day; diphenhydramine alone at 25 mg/kg/day; or combinations of ibuprofen and diphenhydramine at 4:1 ratios of 25:6.25 mg/kg/day, 50:12.5 mg/kg/day, or 100:25 mg/kg/day. Toxicity was noted in rats; however, all adverse findings could be associated with treatment from the ibuprofen component of the drug combination; rats treated with diphenhydramine alone showed no toxicity. The dose effects seen in the 13-week study were similar to those observed in the 2-week study, previously described, but in addition, rats treated for the longer duration showed renal involvement. Renal papillary necrosis or edema, or both, were seen in rats at the middle and high dose combination groups as well as in rats given ibuprofen alone. In this study, the combination of 25:6.25 mg/kg/day (ibuprofen:diphenhydramine) was considered a NOEL.

The Sponsor stated that there was no indication that a 4:1 combination of ibuprofen and diphenhydramine at the doses stated above potentiated the toxic effects of ibuprofen. However, the incidence and severity of renal microscopic changes (kidney papillary necrosis and papillary edema) were slightly higher in the group of rats that received a combination of 100 mg of ibuprofen + 25 mg of diphenhydramine/kg/day compared to the rats that received 100 mg/kg/day ibuprofen alone (refer to Table above). In the 2-week study, a slightly higher incidence and severity in GI and hepatic effects were noted in the group administered 150 mg ibuprofen + 37.5 mg diphenhydramine/kg/day than in the group administered 150 mg ibuprofen + 18.75 mg diphenhydramine/kg/day or diphenhydramine alone at 37 or 100 mg/kg/day. Therefore, the combination may increase some of the ibuprofen adverse effects in rats.

Study title: Thirteen Week Oral Toxicity in Dogs

Key study findings:

- No treatment-related toxic effects were observed in dogs treated with ibuprofen or diphenhydramine alone or in combination.
- A maximum tolerated dose (MTD) was not achieved.

Study no: BRT #85-12

Volume #, and page #: 36, 2

Conducting laboratory and location:**Date of study initiation:** May 28, 1985**GLP compliance:** Yes**QA report:** yes (x) no ()**Drug, lot #, radiolabel, and % purity:** Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1979-076

Ibuprofen: Batch # 1979-77

Ibuprofen + diphenhydramine (4:1): Batch # 1979-78

Formulation/vehicle: All test materials were received diluted 1:14 with starch.**Methods (unique aspects):** No unique aspects**Dosing:**

Species/strain: Dogs/Beagle

#/sex/group or time point (main study): 4 dogs/sex/group

Satellite groups used for toxicokinetics or recovery: None

Age: ~ 5 months

Weight: 9.1 to 11.9 kg males, 6.8 to 10.8 kg females

Doses in administered units:

Daily Dosage (mg/kg/day)	
Ibuprofen	Diphenhydramine
0*	0*
4	1
8	2
16	4
16	0
0	4

*, Capsules filled with starch at 280 mg/kg/day.

Route, form, volume, and infusion rate: oral, gelatin capsules, twice daily (at least 6 hours apart)

Observations and times:

Clinical signs: Twice daily for moribundity, mortality and signs of overt toxicity, diarrhea, emesis, and inappetence. Detailed observations of appearance and condition, behavior and activity, excretory functions, respiration, orifices, eyes and palpable masses on were conducted at least once a week.

Body weights: weekly, beginning one week before study initiation

Food consumption: weekly, beginning one week before study initiation

Ophthalmoscopy: Once prior to study initiation and once prior to study termination

EKG: Once prior to study initiation, at 1 month and once prior to study termination

Hematology: Once prior to study initiation and at 1, 2, and 3 months of study. The following parameters were measured: total leucocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count, reticulocyte count, differential leucocyte count, prothrombin time and activated partial thromboplastin. Mean corpuscular volume (MVC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were automatically calculated by the analyzer.

Clinical chemistry: Once prior to study initiation and at 1, 2, and 3 months of study. The following parameters were measured: sodium, potassium, chloride, calcium, alkaline phosphatase, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatine, total protein, albumin, globulin (calculated), albumin/globulin (A/G) ratio (calculated), cholesterol and glucose.

Urinalysis: Once prior to study initiation and at 1, 2, and 3 months of study. The following parameters were measured: color, appearance, microscopic examination of sediment, specific gravity, volume, pH, protein, glucose, occult blood, nitrite, bilirubin, ketones and urobilinogen.

Gross pathology: All animals received a complete postmortem examination. The Sponsor did not list in details the organs examined but stated that the contents of the abdominal, thoracic and cranial cavities were examined both *in situ* and after removal and dissection. A full tissue complement was collected from all animals.

Organs weighed: Refer to Table. In addition, the pituitary and parathyroid were weighted.

Histopathology: Refer to Table

Toxicokinetics: Not determined

Other:

Fecal analysis or occult blood: Once prior to study initiation and at 1, 2, and 3 months of study.

Physical Examination: Once prior to study initiation and at 1, 2, and 3 months of study. Physical examination included an inspection for general condition that consisted of examination of the head and neck, thorax, abdomen, external reproductive organs, skin and extremities. Heart and lung sounds were evaluated by percussion and auscultation (using a stethoscope).

Results:

Mortality: All dogs survived to study termination.

Clinical signs: There were no dose-related differences although the incidence of emesis tended to be higher in the treated animals. Incidental findings present in a few control and/or treated dogs

included soft stool, diarrhea, red vaginal discharge, focal alopecia in the shoulder region, injection of sclera and ptialism.

Body weights: No test-article related effects

Food consumption: In general, the trend towards decreased food consumption (g/animal/day or g/kg/day) was observed in all groups compared to controls. This trend was probably related to the lower food consumption observed at Week 0 (prior to test-article administration). In males, a statistically significance decrease was observed at Week 1 in mean g/kg/day food consumption in the 4 mg/kg/day diphenhydramine group compared to control (-32%). In females, statistically significant decreases in mean g/animal/day food consumption occurred at Week 2 and Week 5 in all combination treated groups (-30% to 35%), at Week 5 in the 4 mg/kg/day diphenhydramine group (-37%), at Week 3 in the 8 + 2 mg/kg/day combination group (-39%), and in the 16 mg/kg/day ibuprofen group (-31%). At Week 13 (study termination), food consumption was comparable to that of the control group for both males and females.

Ophthalmoscopy: No test-article related effects

Electrocardiography: No test-article related effects

Hematology: In males, hemoglobin values in the 4 + 1, 8 + 2, 16 + 4 mg/kg/day combination groups, and in ibuprofen 16 mg/kg/day group were lower than the control mean value at 3 months. These mean values were also lower at pretest, and compared to the pretest value, there was not a decrease in hemoglobin. The Sponsor also stated that hemoglobin values in these four groups were within historical control values. Reticulocytes counts in control and all treated groups tended to show a decrease when compared to pretest values in males as well as females. Therefore, both hemoglobin and reticulocyte counts changes were not considered test-article related.

Clinical chemistry: In males, total protein values were decreased compared to control values in males at the 2 or 3 months interval in the 8 + 2 (-13%) and 16 + 4 mg/kg/day combination groups (-10%), ibuprofen 16 mg/kg/day group (-13%) and diphenhydramine 4 mg/kg/day group (-10%). In addition, increased glucose values were noted in females in the same groups at 2 months (+12 – 16%), and in the 16 + 4 mg/kg/day combination group at 1 month (+12%). Cholesterol levels were elevated in females only in the 8 + 2 (+24%), 16 + 4 mg/kg/day combination groups (+30%), and in the ibuprofen 16 mg/kg/day group (+30%) at the 1 and/or 2 month interval. Whether these findings are test-article related or due to normal animal variation is doubtful because of the following reasons: values from animals of the opposite sex were not similarly affected, the differences were slight, values were within historical ranges (as stated by the Sponsor, data not shown). In addition, the decrease in total protein values was not apparent when compared to pretest values for each group.

Urinalysis: No test-article related effects

Organ weights: In males, mean relative (to body weight) liver weights were statistically significantly increased in 8 + 2 and 16 + 4 mg/kg/day combination groups (15% increase in each

group). This finding was not accompanied by any histological abnormalities and therefore, considered of no toxicological significance.

Gross pathology: No treatment-related effects

Histopathology: No treatment-related effects

Toxicokinetics: Not performed

Other:

Fecal analysis or occult blood: No test-article related effects

Physical Examination: No test-article related effects

Summary of individual study findings:

Groups of beagle dogs were orally given drug-filled gelatin capsules consisting of ibuprofen alone at 16 mg/kg/day; diphenhydramine alone at 4 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 4:1mg/kg/day, 8:2 mg/kg/day, or 16:4 mg/kg/day. The results of the physical, ophthalmologic, and electrocardiographic examinations, clinical pathology studies, and anatomic pathology examinations showed that there were no adverse effects following treatment with ibuprofen or diphenhydramine, alone or in combination. Some aberrant laboratory values occurred in control and drug-treated dogs, which often were statistically significant at $p < 0.05$, but showed no relationship to treatment. In this study, the maximum tolerated dose (MTD) was not achieved.

Toxicology summary:

Ibuprofen and diphenhydramine hydrochloride individually and in various dosage combinations were assessed for safety in a series of toxicology studies. These studies included acute oral studies in rats, 2- and 13-week oral toxicity studies in rats and dogs, and oral teratology studies in rats and rabbits.

Ibuprofen alone, diphenhydramine alone, and three combinations of both drugs at ratios of 2:1, 4:1, and 8:1 (ibuprofen:diphenhydramine) were studied for acute oral toxicity in male and female rats. Rats given ibuprofen alone showed evidence of classical NSAID-related toxicity. The gastrointestinal (GI) tract and principal abdominal viscera showed reddening and darkening. The LD_{50} was calculated to be 1225 mg/kg. The LD_{50} for rats given diphenhydramine alone was 275 mg/kg. These animals also showed darkening or reddening of the major abdominal viscera plus the lungs and thymus. Rats that died early after treatment with the combination generally presented with darkening or reddening of the major organs in the thorax and abdomen; animals that lived longer presented toxic signs more similar to treatment with ibuprofen alone (ascites and/or hydrothorax, reddening and/or distention of the GI tract, black mucoid material in the GI tract and multiple adhesions among the abdominal viscera). The LD_{50} values for rats given the drug combinations were 700, 840, and 880 mg/kg for the 2:1, 4:1, and 8:1 ratios, respectively. The Sponsor concluded that the acute oral toxicity in rats after administration of ibuprofen alone or in combination with diphenhydramine was moderately toxic; whereas, that after administration of diphenhydramine alone was very toxic. No significant acute toxicological interactions occurred between the two drugs.

In the 2- and 13-week repeat-dose oral toxicity studies, similar findings were observed in rats given ibuprofen alone or in combination with diphenhydramine at 4:1 or 8:1 (ibuprofen:diphenhydramine) ratios. In the 2-week study, nonulcerative inflammation was observed in one or more of the following tissues: duodenum, jejunum, and ileum in rats at the three highest drug combinations (60:15.0, 150:18.75 and 150:37.5 mg/kg/day). At the two highest combinations, hepatocellular hypertrophy and extramedullary hematopoiesis in liver and spleen were seen. The effects were noted similarly in rats of both sexes. Secondary effects included decreased hemograms suggesting GI bleeding. The toxicity was characteristic of that expected from propionic acid non-steroidal antiinflammatory drugs (NSAIDs). Diphenhydramine effects observed in male and female rats were excessive salivation at 37.5 and 100 mg/kg/day and papillary dilatation at 100 mg/kg/day. Microscopically, males given 100 mg/kg/day of diphenhydramine alone had mild vacuolation of the hepatocytes.

In the 13-week study, rats given ibuprofen alone (16 mg/kg/day) or in combination with diphenhydramine (50:12.5 and 100:25 mg/kg/day) had similar findings as those described above plus renal papillary necrosis and/or edema. The NOEL for the drug combination was 25:6.25 mg/kg/day. The Sponsor concluded that ibuprofen-related toxicity was not potentiated by the addition of diphenhydramine. However, in both the 2- and 13-week repeat-dose toxicity studies, the incidence and/or severity of intestinal inflammation and hepatocellular hypertrophy (2- and 13-week studies) and severity of renal microscopic changes (13-week study) was slightly higher in the animals receiving the high-dose combination of ibuprofen + diphenhydramine compared to those receiving ibuprofen or diphenhydramine alone. Therefore, the combination may have slightly potentiated ibuprofen toxicity in this species.

Dogs were given ibuprofen alone at 16 mg/kg/day; diphenhydramine alone at 4 or 20 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 4:1mg/kg/day, 8:2 mg/kg/day, or 16:4 mg/kg/day; or ibuprofen and diphenhydramine at an 8:1 ratio of 16:2 mg/kg/day. The results from both the 2- and 13-week repeat-dose toxicity studies revealed no findings that could be attributed to ibuprofen or diphenhydramine alone or in combination. Therefore, no maximum tolerated dose was identified at doses of the combination up to 16:4 mg/kg/day for 13-weeks. Dogs were given considerably lower doses of ibuprofen and diphenhydramine, alone and in combination, compared to those used to dose rats. The Sponsor lowered the doses because of the fact that dogs are more sensitive to the adverse effects of NSAIDs, especially ibuprofen, compared to rats. However, based on the results from the 2-week study, the Sponsor should have selected doses higher than 16 mg ibuprofen + 4 mg diphenhydramine/kg/day for the 13-week study.

Toxicology conclusions: The studies in the rats showed no unexpected toxicity from treatment with a combination of ibuprofen + diphenhydramine. In the rat 13-week study, a slight increase in the incidence and/or severity of renal, GI and liver effects was observed with the combination at doses of 100 mg ibuprofen + 25 mg diphenhydramine/kg/day compared to 100 mg/kg/day ibuprofen alone. A similar effect was also observed in the 2-week study. The relevance of this finding is not clear. The NOEL for the drug combination was 25:6.25 mg/kg/day in rats. This is equivalent to a human dose of 4.05 mg/kg/day ibuprofen (243 mg/60 kg person) and 1.01 mg/kg/day diphenhydramine (60.8 mg/60 kg person). The total daily dose for each active ingredient of the proposed combination is 400 mg ibuprofen and 50 mg diphenhydramine hydrochloride (76 mg citrate salt). Therefore, the NOEL dose

in the rat is lower or similar to the intended human dose. In dogs, no toxicity was observed at doses of ibuprofen:diphenhydramine up to 16:4 mg/kg/day. This is equivalent to a human dose of 8.64 mg/kg/day ibuprofen (518 mg/60 kg person) and 2.16 mg/kg/day diphenhydramine (130 mg/60 kg person). These doses are 1.3- and 2.3- fold higher than the human total daily dose for ibuprofen and diphenhydramine, respectively. The maximum recommended OTC dose for ibuprofen is 1200 mg/day and that for diphenhydramine is 300 mg/day. Therefore, although the non-toxic doses used in the animal studies are not much different from the intended human dose, the doses proposed in the current NDA are within established OTC doses.

**APPEARS THIS WAY
ON ORIGINAL**

Histopathology Inventory for NDA # 21-393 and 21-394

Study	84-32	84-33	85-09	85-12
Species	Rat	Dogs	Rat	Dogs
Adrenals	X*	X*	X*	X*
Aorta				X
Bone Marrow smear	X	X	X	X
Bone (femur)			X (femur)	X (rib)
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X			
Colon		X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye			X	X
Fallopian tube				
Gall bladder				X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				X
Liver	X*	X*	X*	X*
Lungs	X	X	X	X
Lymph nodes, cervical				
Lymph nodes mandibular			X	X
Lymph nodes, mesenteric			X ^a	X ^a
Mammary Gland			X ^b	X
Nasal cavity				
Optic nerves				
Ovaries	X*	X*	X*	X*
Pancreas			X	X
Parathyroid	X	X*	X	X*
Peripheral nerve				
Pharynx				
Pituitary			X	X*
Prostate			X	X
Rectum	X	X	X	X
Salivary gland			X	X
Sciatic nerve			X	X
Seminal vesicles			X	
Skeletal muscle				X
Skin			X	X
Spinal cord			X	X
Spleen	X	X*	X	X*
Sternum				
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus			X ^c	X
Thyroid	X	X*	X ^d	X*
Tongue				

Trachea			X	X
Urinary bladder	X	X	X	X
Uterus			X	X
Vagina				
Zymbal gland				
Standard List				

X, histopathology performed; *, organ weight obtained; ^aAlso thoracic (mediastinal) lymph nodes; ^bMammary region; ^cThymic region; ^dParathyroids could not always be identified macroscopically. They were examined microscopically if in the plane of section and in all cases where they were noted as grossly enlarged.

V. GENETIC TOXICOLOGY:

No studies were submitted with the combination.

VI. CARCINOGENICITY:

No studies were required at this time.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: A Segment II Teratology Study in Rats with WH-555-002

Key study findings: The combination of ibuprofen and diphenhydramine was not embryotoxic, fetotoxic or teratogenic at dose levels of 12, 71 and 119 mg/kg/day.

Study no: 93-4058

Volume #, and page #: 12, 5-41-1

Conducting laboratory and location: _____

Date of study initiation: December 13, 1993

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity:

Ibuprofen (WH-1651), lot # IB1A011, _____ purity

Diphenhydramine Citrate (WH-1855), lot # DCB3C0604-P, _____ purity

Formulation/vehicle: 1% methylcellulose (w/v) in deionized water

Methods:

Species/strain: Albino rats/CD[®] (Sprague-Dawley derived) [Cr]: CD[®] (SD) BR]

Doses employed:

Group	Dosage level (mg/kg/day)			Dose volume (ml/kg)
	Combination	Ibuprofen	Diphenhydramine	
I ^a	0	0	0	10
II	12	10	2	10
III	71	60	11	10
IV	119	100	19	10

V	0	0	19	10
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*Vehicle

Route of administration: Oral, gavage

Study design: Animals were dosed daily over Days 6 –15 of gestation and sacrificed on Day 20 of gestation.

Number/sex/group: 24 female rats/group

Parameters and endpoints evaluated: Clinical signs, body weight, food consumption, gross postmortem examination, liver/body weight ratios, number of live and dead fetuses, number of early and late resorptions, number of implantation sites, presence and number of corporea lutea, fetal external malformations/variations, fetal visceral malformations/variations, and fetal skeletal malformations/variations.

Results:

In-life observations:

Mortality: No mortality occurred in any group.

Clinical signs: No treatment-related effects were observed.

Body weight: At the 71 mg/kg/day dose level, weight gain was reduced during Days 6 - 9 of gestation (58% decrease). At the 119 mg/kg/day dose level, the following effects were observed: decreased body weight at Day 9 of gestation (5.5% decrease), mean weight gain of zero over Days 6 –9 of gestation, reduced weight gain over Days 6 - 16 (21.9% decrease) and 6 - 20 (21.6% decrease) of gestation.

Food consumption: Decreased significantly in the 119 mg/kg/day dose level (-12.4% decrease) during Day 6 – 11 of gestation.

Toxicokinetics: Not performed.

Terminal and necroscopic evaluations:

Dams:

Corporea Lutea and Uterine Implantation:

No treatment-related effects in the mean number of corporea lutea, uterine implantations, viable fetuses and resorptions (early and late). One animal in the 12 mg/kg/day dose group had 15 early resorptions. However, this was considered not treatment-related because this was not observed at the higher doses.

Liver Weight: Mean maternal liver weights, absolute and relative to the corrected Day 20 gestation body weights, for the low- and mid-dose groups and the 19 mg/kg/day diphenhydramine group were comparable to controls. In the high-dose group a statistically significant increase in mean maternal liver weight (relative to body weight) was observed compared to control (7.5 % increase). This difference was not considered of toxicological significance and was attributed to the slight reduction in corrected Day 20 gestation weights seen in the high-dose group in comparison to control data.

Gross Postmortem Examinations: No treatment-related effects.

Offspring:

Body Weight: No treatment-related effects in mean fetal weights (distinguished by sex as a composite for both sexes).

External Malformations: No treatment-related effects. In the mid-dose group, one fetus was severely malformed. This fetus had the following malformations: craniorachischisis, unilateral open eye (right-sided), absence of the left eye, facial cleft (left-sided) and protruding tongue. This single occurrence was considered incidental because a similar malformation was not observed in the high-dose group.

External Variations: No treatment-related effects.

Visceral Malformations: No treatment-related effects.

Viscera Variations: No treatment-related effects.

Skeletal Malformations: No treatment-related effects in the combination treated groups. In the diphenhydramine treated group, the fetal and litter incidences of skeletal malformations were 2.2% (4/182 fetuses) and 12.5% (3/24 litters), respectively. Although these incidences were slightly higher than control values (0.5% and 4.3%, respectively), they were not statistically significant and were not considered treatment-related.

Ossification Variations: No treatment-related effects in the combination-treated groups. A slight increase was observed in the 119 mg/kg/day combination group and in the diphenhydramine-treated group compared to control (70.7%, 68.1% and 59.9%, respectively). This difference was not statistically significant. In addition, the incidences were at or just outside the low range of recent historical control data (69.0 – 93.8).

Summary of individual study findings:

Under the conditions of this study, the combination of ibuprofen and diphenhydramine citrate administered orally by gavage to pregnant CD[®] rats over the Days 6 –15 of gestation was not maternally toxic at a dose level of 12 mg/kg/day. At 71 mg/kg/day, mean weight gain was reduced during gestation Days 6 – 9, indicative of maternal toxicity. At the 119 mg/kg/day dose level, maternal toxicity was indicated from the following observations: decreased body weight on Day 9 of gestation, absence of weight change over Days 6 – 9 of gestation, reduced weight gains over Day 6 – 16 and 6 – 20 gestation intervals, and reduced food consumption over the Days 6 –11 of gestation. The combination of ibuprofen and diphenhydramine was not embryotoxic, fetotoxic or teratogenic at dose levels of 12, 71 and 119 mg/kg/day. Thus, in this rat teratology study, the NOAEL for maternal toxicity was 12 mg/kg/day and the NOAEL for developmental toxicity (embryotoxicity, fetotoxicity or teratogenicity) was 119 mg/kg/day. Diphenhydramine alone was not fetotoxic nor did it produce any discernable maternal toxicity at a dose level of 19 mg/kg/day.

The following four studies were submitted as part of the annual reports of IND — but were not previously reviewed.

Study title: Range-Finding Teratology Study in Rats

Key study findings:

- Reductions in body weight gain were observed at the high dosages of the 4:1 and 8:1 ratios of the combinations of ibuprofen and diphenhydramine as well as ibuprofen alone at 60 mg/kg/day and diphenhydramine alone at 60 mg/kg/day.
- Embryotoxic signs of equivocal biological significance included a slightly increased postimplantation loss in rats at the three 8:1 ratios as well as both doses of diphenhydramine alone, and the single dose of ibuprofen alone.
- No fetal morphological malformations/variations were observed in rats from any group.

Study no.: BRT# 84-35

Volume #, and page #: 37, 2

Conducting laboratory and location: —

Date of study initiation: Not indicated

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot # and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1934-48

Ibuprofen: Batch # 1934-49

Ibuprofen + diphenhydramine (4:1): Batch # 1934-46

Ibuprofen + diphenhydramine (8:1): Batch # 1934-47

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods:

Species/strain: Rats/ — COBS® CD®

Doses employed:

Group	Combination (mg/ml)	I:D Ratio	Dosage level (mg/kg/day)	
			Ibuprofen	Diphenhydramine
1	0*	0:0	0	0
2	0.94	4:1	7.5	1.9
3	2.5	4:1	20	5
4	7.5	4:1	60	15
5	0.845	8:1	7.5	0.95
6	2.25	8:1	20	2.5
7	6.75	8:1	60	7.5
8	1.5	0:15	0	15
9	6.0	60:0	60	0
10	6.0	0:60	0	60

*Control group was given the vehicle alone under a similar treatment regimen.

I = ibuprofen; D = diphenhydramine

Route of administration: oral, gavage

Study design: Test material was administered as a single dose from days 6 through 15 of gestation at a volume of 10 ml/kg. Cesarean section was performed on gestation day 20.

Number/sex/group: 5 females/group

Parameters and endpoints evaluated: mortality (daily), clinical signs (daily), body weights (gestation days 0, 6, 9, 12, 16 and 20), reproductive parameters (number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations and corpora lutea, number of viable fetuses, fetal sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations)

Statistical analysis: Not performed

Results:

In-life observations:

Mortality: No mortalities occurred

Clinical signs: No test-article related effects. The following findings were considered incidental: alopecia (primarily located on the forelimbs) in the control and in treatment Groups 4 and 10, hydronephrosis on one dam each in Group 8 and Group 10 and excess salivation in one dam in Group 10.

Body weight: The Sponsor did not perform a statistical analysis of the data. Slight decreases in body weight (~2 – 5%) were observed primarily in Groups 4, 9 and 10 during gestation days 6, 9 and 16, and in Group 7 during gestation day 16. Reductions in weight gain for these groups were also evident during gestation days 6 – 12. Percent differences ranged from 33 – 58 % with the greatest difference occurring in Group 10 during gestation days 6 – 9. Group 10 exhibited reduced weight gain (~10%) during the overall gestation interval (days 0 – 20).

Food consumption: Not determined

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:

Dams: Post-implantation loss (primarily early resorptions) was elevated in Groups 5 through 10 relative to the control by 123%, 93%, 65%, 72%, 60%, and 65%, respectively. Mean pre-implantation loss in Groups 2 and 8 exceeded the control by 410% and 471%, respectively. Groups 6 and 7 also showed an 89% and 139% increase in pre-implantation loss, respectively. However, in the absence of accompanying increases in the other 4:1 ibuprofen + diphenhydramine groups (Groups 3 and 4) and in the highest-dose diphenhydramine group (Group 10), the Sponsor attributed the finding to biological variation. Variations in fetal sex distribution with respect to the concurrent and historical control were seen in Groups 5, 6, 7, and 10 (refer to Table below). However, in the absence of an effect in the groups with higher combination doses, the effects were attributed to biological variation.

Group (I + D)	Male	Female
Control	35	31
5 (7.5 + 0.5)	40	26
6 (20 + 2.5)	27	39
7 (60 + 7.5)	30	22
10 (0 + 60)	42	23

I = Ibuprofen; D= Diphenhydramine

Offspring: No treatment-related abnormalities.

Summary of individual study findings:

Mated rats were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 or 60 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day; or at 8:1 ratios of 7.5:0.95 mg/kg/day, 20:2.5, or 60:7.5 mg/kg/day during gestation days 6 - 15. Survival was 100% among rats in all groups. Effects attributable to drug-treatment were observed exclusively as noteworthy reductions in body weight gain in rats at the high dosages of the 4:1 and 8:1 ratios of the combinations of ibuprofen and diphenhydramine as well as ibuprofen alone at 60 mg/kg/day and diphenhydramine alone at 60 mg/kg/day. Embryotoxic signs of equivocal biological significance included a slightly increased postimplantation loss in rats at the three 8:1 ratios as well as both doses of diphenhydramine alone, and the single dose of ibuprofen alone. No antemortem findings, necropsy results, or fetal morphological observations were observed in rats of any group.

Study title: Teratology Study in Rats

Key study findings:

- The only notable adverse effect was a decrease in body weight gain of rats given the high dose of the combination of ibuprofen and diphenhydramine (60 + 15 mg/kg/day).
- No fetal morphological malformations/variations were observed in rats from any group.

Study no.: BRT #8507

Volume #, and page #: 39, 2

Conducting laboratory and location: _____

Date of study initiation: Not indicated

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot # and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1979-69

Ibuprofen: Batch # 1979-70

Ibuprofen + diphenhydramine (4:1): Batch # 1979-70

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods:

Species/strain: Rats/ — COBS® CD®

Doses employed:

Group	Combination (mg/ml)	I:D Ratio	Dosage level (mg/kg/day)	
			Ibuprofen	Diphenhydramine
1	0*	0:0	0	0
2	0.94	4:1	7.5	1.9
3	2.5	4:1	20	5
4	7.5	4:1	60	15
5	6.0	60:0	60	0
6	1.5	0:15	0	15

*Control group was given the vehicle alone under a similar treatment regimen.

I = Ibuprofen; D = Diphenhydramine

Route of administration: oral, gavage

Study design: Test material was administered as a single dose from days 6 through 15 of gestation at a volume of 10 ml/kg. Cesarean section was performed on gestation day 20.

Number/sex/group: 25 females/group

Parameters and endpoints evaluated: mortality (twice daily), clinical signs (daily), body weights (gestation days 0, 6, 9, 12, 16 and 20), reproductive parameters (number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations and corpora lutea, number of viable fetuses, fetal sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations)

Results:

In-life observations:

Mortality: No mortalities occurred.

Clinical signs: No test-article related effects were observed. Alopecia and hydronephrosis were observed in the control and all treatment groups and were considered incidental findings.

Body weight: Although not statistically significant, a trend towards decrease body weight was observed in Group 4 compared to control group. The trend was more manifest during gestation days 6 –9. A corresponding reduction in weight gain (-22%) was observed during the overall treatment period (gestation days 6 – 15).

Food consumption: Not measured

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:

Dams: No treatment-related abnormalities.

Offspring: Microphthalmia was the most frequently observed malformation and occurred in one fetus/litter in Groups 3, 4 and 6. The remaining malformations, anasarca, cleft palate, mandibular micrognathia, diaphragmatic hernia, vertebral anomaly with associated rib anomaly, forked rib, and bent scapula occurred as isolated incidents among the animals of the treated groups. The most frequent developmental variations were unossified sternbrae #5 and/or # 6 but occurred in comparable frequency in the control and treated rats. The Sponsor considered none of these findings as toxicologically meaningful.

Summary of individual study findings:

Mated rats were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 mg/kg/day; combinations of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day. The dosages were selected on the basis of the results from a previously conducted dose range-finding study. The only notable adverse effect was a decrease in body weight gain of rats given the high dose of the combination of ibuprofen and diphenhydramine. Survival was 100% in all groups. No positive antemortem findings, necropsy results, or fetal morphological observations were observed in rats from any group.

Study title: Range-Finding Teratology Study in Rabbits

Key study findings:

- Cesarean section revealed a slight increase in the rates of preimplantation loss and postimplantation loss in rabbits given the high dose of the 4:1 ratio and the middle and high doses of the 8:1 ratios of the combinations of ibuprofen and diphenhydramine.
- In rabbits treated with 60 mg ibuprofen + 7.5 mg/kg/day diphenhydramine, two fetuses had hydrocephaly and a third fetus had a vertebral anomaly. In rabbits treated with 60 mg/kg/day, one fetus had the following malformations: gastroschisis, ectromelia, polydactyly, syndactyly, tail anomaly and absent diaphragm. No fetal morphological malformations/variations were observed in rats from any other group.

Study no.: BRT #84-36

Volume #, and page #: 38, 2

Conducting laboratory and location:

Date of study initiation: Not indicated

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot # and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1934-48

Ibuprofen: Batch # 1934-49

Ibuprofen + diphenhydramine (4:1): Batch # 1934-46

Ibuprofen + diphenhydramine (8:1): Batch # 1934-47

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods:

Species/strain: Rabbits/New Zealand White

Doses employed:

Group	Combination (mg/ml)	I:D Ratio	Dosage level (mg/kg/day)	
			Ibuprofen	Diphenhydramine
1	0*	0:0	0	0
2	3.13	4:1	7.5	1.9

3	8.33	4:1	20	5
4	25.00	4:1	60	15
5	2.82	8:1	7.5	0.95
6	7.50	8:1	20	2.5
7	25.50	8:1	60	7.5
8	5.00	0:15	0	15
9	20.00	60:0	60	0

*Control group was given the vehicle alone under a similar treatment regimen.

I = Ibuprofen; D = Diphenhydramine

Route of administration: oral, gavage

Study design: Test material was administered as a single dose on days 6 through 18 of gestation at a volume of 3 ml/kg. Cesarean section was performed on gestation day 29.

Number/sex/group: 5 females/group

Parameters and endpoints evaluated: mortality (twice daily), clinical signs (daily), body weights (gestation days 0, 6, 9, 12, 19, 24 and 29), reproductive parameters (number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations and corpora lutea, number of viable fetuses, fetal sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations)

Results:

In-life observations:

Mortality: Two animals died; one in Group 2 on gestation Day 8 and one in Group 6 on gestation Day 28. Both rabbits had matting and/or staining of the anogenital haircoat prior to death. In addition, the Group 6 rabbit had ocular and nasal discharge and decreased defecation and the Group 2 rabbit was hypoactive. Necropsy revealed tan fibrinous material and adhesion of the lungs in the Group 2 rabbit and lung congestion with tracheal reddening in the Group 6 rabbit, but failed to delineate a cause of death in either case. One rabbit each in Groups 7 and 8 delivered on gestation Day 28 and one rabbit in Group 9 aborted in gestation days 24 - 26. The Sponsor did not consider these findings as test-article related.

Clinical signs: The only finding considered test-article related was decreased defecation in Group 8 rabbits. The other clinical observations occurred primarily as single incidents or were consistent with the normal profile of observations for these species.

Body weight: The mean maternal weight gain of all treated groups tended to be lower than control group during the gestation days 6 -18. However, the magnitude of the difference was small and given the variability of maternal weight gain, the toxicological significance of the decrease is confounded.

Food consumption: Not measured

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:

Dams: In Groups 4 and 7, when ibuprofen was administered at 60 mg/kg/day and diphenhydramine levels were 15 and 7.5 mg/kg/day, respectively, there was an increase in

the rates of preimplantation and postimplantation loss, which resulted in a decrease in the mean number of viable fetuses (see table below). Similar findings were evident in the 20 + 2.5 mg/kg/day combination group (Group 6) but to a lesser extent.

Parameter	Control	Group 4 (I + D: 60 + 15)	Group 6 (I + D: 20 + 2.5)	Group 7 (I + D: 60 + 7.5)
Viable fetuses/doe	8.8	6.0	6.4	7.0
Postimplantation loss/doe	1.0	1.6	1.0	1.8
Group mean preimplantation loss	13.3	36.7	26.0	18.6
Group mean postimplantaion loss	10.3	21.1	13.5	20.0

Not mentioned by the Sponsor was the finding that fetal sex distribution was affected in Groups 6 and 7. The male and female distribution was 51.4 vs 48.6, 39.4 vs 60.6, and 71.4 vs 28.6 in control, Group 6 and Group 7, respectively. However, this may not be of toxicological significance because the finding was not observed in the 20 + 5 mg/kg/day (Group 3) and 60 + 15 mg/kg/day (Group 4) combination groups.

Offspring: In Group 7, two fetuses had hydrocephaly and a third fetus had a vertebral anomaly. In Group 9, one fetus had the following malformations: gastroschisis, ectromelia, polydactyly, syndactyly, tail anomaly and absent diaphragm.

Summary of individual study findings:

Groups of five inseminated New Zealand White rabbits were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day; or at 8:1 ratios of 7.5:0.95 mg/kg/day, 20:2.5 mg/kg/day, or 60:7.5 mg/kg/day. Decreased defecation was observed in rabbits at 15 mg/kg/day of diphenhydramine. Cesarean section revealed a slight increase in the rates of preimplantation loss and postimplantation loss among rabbits given the high dose of the 4:1 ratio and the middle and high doses of the 8:1 ratios of the combinations of ibuprofen and diphenhydramine. These findings were treatment-related. No further fluctuations in cesarean section parameters were considered a result of treatment. In rabbits treated with 60 mg ibuprofen + 7.5 mg diphenhydramine/kg/day (Group 7), two fetuses had hydrocephaly and a third fetus had a vertebral anomaly. These anomalies were not observed in the 60 mg ibuprofen + 15 mg diphenhydramine/kg/day group (Group 4). Both anomalies have been observed in historical controls (1/57 fetuses in 1/9 litters for hydrocephaly; 4/94 fetuses in 3/15 litters for vertebral anomaly). In rabbits treated with 60 mg ibuprofen (Group 9), one fetus had the following malformations: gastroschisis, ectromelia, polydactyly, syndactyly, tail anomaly and absent diaphragm. No apparent effect on fetal morphology was observed in any other treatment groups and these anomalies may not be treatment-related. Based on the results, dosage levels of 7.5 + 1.9, 20 + 5, 60 + 15 mg/kg/day ibuprofen + diphenhydramine, 60 mg/kg/day ibuprofen and 15 mg/kg/day diphenhydramine were selected for the definitive teratology study.

Study title: Teratology Study in Rabbits

Key study findings:

- Decreased body weight gain occurred in rabbits given the high dose of the drug combination (60 mg ibuprofen + 15 mg diphenhydramine/kg/day) during the treatment period, gestation days 6 through 18.
- No fetal morphological malformations/variations were observed in rats from any group.

Study no.: BRT #85-08

Volume #, and page #: 40, 2

Conducting laboratory and location: _____

Date of study initiation: Not indicated

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot # and % purity: Lot number and % purity were not specified. Batch numbers were included and are listed below.

Diphenhydramine: Batch # 1934-48

Ibuprofen: Batch # 1934-49

Ibuprofen + diphenhydramine (4:1): Batch # 1934-46

Formulation/vehicle: 0.025% polysorbate 80/0.2% sodium carboxymethylcellulose

Methods:

Species/strain: Rabbits/New Zealand White

Doses employed:

Group	Combination (mg/ml)	I:D Ratio	Dosage level (mg/kg/day)	
			Ibuprofen	Diphenhydramine
1	0*	0:0	0	0
2	3.13	4:1	7.5	1.9
3	8.33	4:1	20	5
4	25.00	4:1	60	15
5	20.00	60:0	60	0
6	5.00	0:15	0	15

*Control group was given the vehicle alone under a similar treatment regimen.

I = Ibuprofen; D = Diphenhydramine

Route of administration: oral, gavage

Study design: Test material was administered as a single dose from days 6 through 18 of gestation at a volume of 3 ml/kg. Cesarean section was performed on gestation day 29.

Number/sex/group: 14 females/group

Parameters and endpoints evaluated: mortality (twice daily), clinical signs (daily), body weights (gestation days 0, 6, 12, 19, 24 and 29), reproductive parameters (number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations and corpora lutea, number of viable fetuses, fetal sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations)

Results:**In-life observations:**

Mortality: No deaths occurred

Clinical signs: The most common observation was alopecia. Although this occurred more frequently in the 20 + 5 mg/kg/day and 60 + 15 mg/kg/day combination groups than in the control group (35.7%, 42.9 and 21.4%, respectively), the difference was too small and the Sponsor concluded it was of no biological significance.

Body weight: The Sponsor stated that a slight reduction in maternal body weight gain was seen during the treatment interval days 6 through 18 in the 60 + 15 mg/kg/day combination group (129 ± 166.9 vs 95 ± 179.4 , control vs treated, respectively). However, due to the high S.D. observed in this and most body weight gain values, the biological significance of this finding is questionable.

Food consumption: Not measured

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:

Dams: Rabbits treated with 60 mg/kg/day ibuprofen showed a decrease in the number of viable fetuses (-33%), and an increase in the group mean pre- (+60%) and post-implantation loss (+187%). An increase in group mean postimplantation loss was also observed in rabbits treated with 7.5 mg ibuprofen + 1.9 mg diphenhydramine/kg/day (+114%) or 15 mg/kg/day diphenhydramine (+57%). The Sponsor reported the differences were not statistically significant and were deemed not treatment-related. In addition, an increase in postimplantation loss was not observed in the higher-dose combination groups.

Offspring: There were no biologically meaningful differences in the incidence of fetal malformations/variations between control and treated groups.

Summary of individual study findings:

Inseminated New Zealand White rabbits were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 mg/kg/day; or combinations of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day. The dosages were selected on the basis of the results from a previous dose range-finding study. Survival was 100% and no abortions were noted. Decreased body weight gain occurred in rabbits given the high dose of the drug combination during the treatment period, but not during the overall gestation interval. Rabbits treated with 60 mg/kg/day ibuprofen showed a decrease in the number of viable fetuses (-33%), and an increase in the group mean pre- (+60%) and post-implantation loss (+187%). An increase in group mean postimplantation loss was also observed in rabbits treated with 7.5 mg ibuprofen + 1.9 mg diphenhydramine/kg/day (+114%) or 15 mg/kg/day diphenhydramine (+57%). There was no difference in the incidence of fetal malformations/variations observed between control and treated groups.

Reproductive and developmental toxicology summary:

A dose-range finding and two definite teratology studies were conducted in rats, and a dose-range finding and definite teratology study were conducted in rabbits.

Rats were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 or 60 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day; or at 8:1 ratios of 7.5:0.95 mg/kg/day, 20:2.5, or 60:7.5 mg/kg/day. In the dose-range finding study, survival was 100% among rats in all groups. Effects attributable to drug-treatment were reductions in body weight gain in rats at the high dosages of the 4:1 and 8:1 ratios of the combinations of ibuprofen and diphenhydramine as well as ibuprofen alone at 60 mg/kg/day and diphenhydramine alone at 60 mg/kg/day. Embryotoxic signs of equivocal biological significance included a slightly increased postimplantation loss in rats at the three 8:1 ratios as well as both doses of diphenhydramine alone, and the single dose of ibuprofen alone. No antemortem findings, necropsy results, or fetal morphological observations were observed in rats from any group. In the definite teratology study, the only notable adverse effect was a decrease in body weight gain of rats given the high dose of the combination of ibuprofen and diphenhydramine (60:15 mg/kg/day). Survival was 100% among rats in all groups. No positive antemortem findings, necropsy results, or fetal morphological observations were observed in rats from any group.

The Sponsor also submitted a second definite teratology study (commissioned by Whitehall-Robins) in which rats were given diphenhydramine alone at 19 mg/kg/day or a combination of ibuprofen and diphenhydramine at ~5:1 ratios of 10:2 mg/kg/day, 60:11 mg/kg/day, or 100:19 mg/kg/day. At the combination mid-dose, mean weight gain was reduced during gestation Days 6 – 9, indicative of maternal toxicity. At the combination high-dose, maternal toxicity was indicated from a decreased in body weight on Day 9 of gestation, an absence of weight change over Days 6 – 9 of gestation, a reduced weight gains over Day 6 – 16 and 6 –20 gestation intervals, and a reduced food consumption over the Days 6 –11 of gestation. The combination of ibuprofen and diphenhydramine was not embryotoxic, fetotoxic or teratogenic at any combination dose level. Diphenhydramine alone was not fetotoxic nor did it produce any discernable maternal toxicity at a dose level of 19 mg/kg/day.

Rabbits were given ibuprofen alone at 60 mg/kg/day; diphenhydramine alone at 15 mg/kg/day; a combination of ibuprofen and diphenhydramine at 4:1 ratios of 7.5:1.9 mg/kg/day, 20:5 mg/kg/day, or 60:15 mg/kg/day; or at 8:1 ratios of 7.5:0.95 mg/kg/day, 20:2.5 mg/kg/day, or 60:7.5 mg/kg/day. In the dose-range finding study, decreased defecation was observed in rabbits at 15 mg/kg/day of diphenhydramine. Cesarean section revealed a slight increase in the rates of preimplantation loss and postimplantation loss among rabbits given the high dose of the 4:1 ratio and the middle and high doses of the 8:1 ratios of the combinations of ibuprofen and diphenhydramine. These findings were suggestive of a treatment effect. In rabbits treated with 60 mg ibuprofen + 7.5 mg diphenhydramine/kg/day, two fetuses had hydrocephaly and a third fetus had a vertebral anomaly. Both findings have been observed in historical controls and were not observed in rabbits treated with a higher ratio of ibuprofen and diphenhydramine (60:15 mg/kg/day). Therefore, these findings may not be treatment-related. In rabbits treated with 60 mg ibuprofen, one fetus had the following malformations: gastroschisis, ectromelia, polydactyly, syndactyly, tail anomaly and absent diaphragm. No apparent effect on fetal morphology was observed in any other treatment group and these anomalies may not be treatment-related. In the definitive teratology study, decreased body weight gain occurred in rabbits given the high dose of the

ibuprofen:diphenhydramine combination (60:15 mg/kg/day) during the treatment period, but not during the overall gestation interval. Rabbits treated with 60 mg/kg/day ibuprofen showed a decrease in the number of viable fetuses (33%), and an increase in the group mean pre- (60%) and post-implantation loss (187%). An increase in group mean postimplantation loss was also observed in rabbits treated with 7.5 mg ibuprofen + 1.9 mg diphenhydramine/kg/day (114%) or 15 mg/kg/day diphenhydramine (57%). There was no difference in the incidence of fetal malformations/variations observed between control and treated groups.

Reproductive and developmental toxicology conclusions: The combination of ibuprofen and diphenhydramine was not teratogenic in rats or rabbits when given at doses up to 100:19 mg/kg/day and 60:15 mg/kg/day, respectively. These doses are equivalent to the human doses shown in the following table:

Dose (mg/kg/day)	HED	
	mg/kg/day	mg/60 kg/day
Rats		
I = 100	16	972
D = 19	3	185
Rabbits		
I = 60	19.5	1168
D = 15	4.86	292

I = Ibuprofen; D = Diphenhydramine

The total daily dose for each active ingredient of the proposed combination is 400 mg ibuprofen and 50 mg diphenhydramine hydrochloride. Therefore, the doses used in the rat studies provide a safety factor of 2.5 and ~10, and those used in the rabbit studies provides a safety factor of ~3 and ~6 for ibuprofen and diphenhydramine, respectively.

Labeling recommendations: Not applicable for OTC products.

VIII. SPECIAL TOXICOLOGY STUDIES:

None was submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: The preclinical studies did not identify any unexpected toxicity from the administration of a combination of ibuprofen and diphenhydramine to rats and dogs. In the rat studies, a slight increase of ibuprofen-induced GI, liver, and renal effects was observed in rats treated with the combination at doses of 100 mg ibuprofen + 25 mg diphenhydramine/kg/day compared to those treated with 100 mg/kg/day ibuprofen alone. The relevance of this finding is not clear. The NOEL for the drug combination was 25:6.25 mg/kg/day in rats. This is equivalent to a human dose of 4.05 mg/kg/day ibuprofen (243 mg/60 kg person) and 1.01 mg/kg/day diphenhydramine (60.8 mg/60 kg person). The total daily dose for each active ingredient of the proposed combination is 400 mg ibuprofen and 50 mg diphenhydramine hydrochloride (76 mg citrate salt). Therefore, the NOEL dose in the rat is lower or

similar to the intended human dose. In the dog study, an MTD was not reached. No toxicity was observed at doses of ibuprofen:diphenhydramine up to 16:4 mg/kg/day. This is equivalent to a human dose of 8.64 mg/kg/day ibuprofen (518 mg/60 kg person) and 2.16 mg/kg/day diphenhydramine (130 mg/60 kg person). These doses are 1.3- and 2.3- fold higher than the human total daily dose for ibuprofen and diphenhydramine, respectively. The maximum recommended OTC dose for ibuprofen is 1200 mg/day and that for diphenhydramine is 300 mg/day. Therefore, although the non-toxic doses used in the animal studies are not that different from the proposed human dose, the doses proposed in the current NDA are within established OTC doses. The combination of ibuprofen and diphenhydramine was not teratogenic in rats or rabbits when given at doses up to 100:19 mg/kg/day and 60:15 mg/kg/day, respectively.

General Toxicology Issues: In rats, a slight increase in the renal, GI and liver effects was observed with the combination at doses of 100 mg ibuprofen + 25 mg diphenhydramine/kg/day compared to those treated with 100 mg/kg/day ibuprofen alone. A similar effect was also observed in the 2-week study. No PK data was provided to determine any drug-drug interactions. In Study AE-97-09, the Sponsor reported that Advil PM Liqui-Gels were shown to have a slower rate, but equivalent extent of absorption relative to currently marketed, single ingredient diphenhydramine liquigel and ibuprofen liquigel formulations. Relative to a currently marketed single ingredient ibuprofen tablet formulation, Advil PM Liqui-Gels had an equivalent extent of absorption and an earlier time to reach peak plasma concentrations, but had a lower maximum plasma concentration. Therefore, drug-drug interactions were not observed in the clinical trial.

Recommendations: From a preclinical safety standpoint, the reviewer recommends approval.

Labeling with basis for findings: Not applicable for OTC drugs.

X. APPENDIX/ATTACHMENTS:

Addendum to review: Pharm/Tox review of IND — (Study # 84-24).

Other relevant materials (Studies not reviewed, appended consults, etc.): None

Any compliance issues: None

2 Page(s) Withheld

§ 552(b)(4) Trade Secret / Confidential

§ 552(b)(5) Deliberative Process

§ 552(b)(4) Draft Labeling

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Maria I. Rivera
4/23/02 11:17:38 AM
PHARMACOLOGIST

Josie Yang
4/23/02 06:24:59 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY NDA FILEABILITY CHECKLIST

NDA Number: 21-393

Applicant: Whitehall-Robins

Stamp Date: Oct. 18, 2001

Drug Name: Advil PM Liquigels (Ibuprofen 200 mg, Diphenhydramine HCl 25 mg)

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		The pre-clinical studies were previously submitted under IND — — One study (# 93-4058) was commissioned by Whitehall-Robins but not previously submitted.
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		For most pre-clinical studies, the active ingredients were dissolved in a mixture of 0.025% w/v Tween 80 and 0.2% w/v sodium carboxymethylcellulose. In some studies, the active ingredients were placed in gelatin capsules.
6	Are the proposed labeling sections relative to pharmacology appropriate (including human dose multiples expressed in mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?			OTC label
7	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions?	X		FDA asked the Sponsor to submit a summary of non-clinical pharmacology and toxicology from public literature or other sources for detection of possible additive, synergistic or antagonistic effects of the drug combination.
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		Studies were conducted in accordance with the standard operating procedures of the — — Study # 93-4058 complied with all aspects of the Animal Welfare Act regulations.
11	From a pharmacology perspective, is this NDA fileable?	X		

Note: NDA-21-394 simultaneously submitted with NDA 21-393.

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

**This is a representation of an electronic record that was signed electronically and
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/s/

Maria I. Rivera
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PHARMACOLOGIST

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