CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020607

PHARMACOLOGY REVIEW(S)

Strongin

M E M O R A N D U M

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

DATE: August 13, 1997 AUG 19 1997

FROM: Pharmacology Team Leader
Division of Gastrointestinal and
Coagulation Drug Products, HFD-180

SUBJECT: NDA 20,607 (Arthrotec) -
Amendment Dated June 18, 1997 -
Revised Draft Labeling

TO: NDA 20,607

In amendment dated June 18, 1997, the sponsor submitted a revised draft labeling. The portions of the draft labeling which relate to preclinical pharmacology and toxicology studies are evaluated and the appropriate recommendations are provided below.

1. PRECAUTIONS

a. Animal Toxicology

Evaluation: The display "ANIMAL TOXICOLOGY" gives the impression of a separate section from "PRECAUTIONS" section. The information under this caption pertains entirely to misoprostol. In the presently approved labeling for cytotec, it is shown as a subsection of "Animal Toxicology" under "PRECAUTIONS" section. The revised draft labeling should also use a similar format.

b. Carcinogenesis, Mutagenesis, Impairment of Fertility

Evaluation: The display "CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY" gives the impression of a separate section from "PRECAUTIONS". The revised draft labeling should adopt the standard caption for the subsection as "Carcinogenesis, Mutagenesis, Impairment of Fertility". There are several deficiencies in the contents of this subsection. They are:

(1) Information on ARTHROTEC is included as the last paragraph and it is also incomplete. This subsection should start with this information.

(2) Information from a teratology study in rabbits is misplaced in this subsection.

(3) The contents of the labeling are not in line with the currently used CDER wording.

(4) The quoted ratios between animal doses and recommended maximum human doses for diclofenac sodium and misoprostol are either inaccurate or not computed on a body surface area basis.

(5) Information for misoprostol mutagenic potential is incomplete.

(6) Information from teratological testing of misoprostol is misplaced in this subsection. All the above cited deficiencies should be corrected.

c. Pregnancy. Teratogenic Effects. Pregnancy Category-

Evaluation: This subsection is incomplete and inadequate. Animal teratology study results for arthrotec, diclofenac sodium and misoprostol are missing and they should be included.

Teratogenic Effects:

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/S/ 8/19/97
Jasti B. Choudary, B.V.Sc., Ph.D.

Attachment: Marked Up Revised Draft Labeling

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Talarico

JBC/hw/8/15/97

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31 Page(s) Redacted

DRAFT LABELING

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AUG - 9 1996

Reviewer: Gerald A. Young, Ph.D. Review # 1
Pharmacologist, HFD-180

Sponsor & Address: G.D. Searle & Co.
Skokie, IL 60077

Date of Submission: Original Submission - December 22, 1995
Amendment - February 5, 1996

Date of Receipt by HFD-180: Original Submission - December 28, 1995
Amendment - February 6, 1996

Date of Review: August 9, 1996

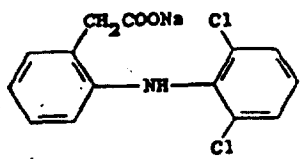
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUGS: ARTHROTEC® 50/ARTHROTEC® 75 tablets

Fixed combinations of diclofenac sodium and misoprostol

Drug (1): Diclofenac sodium (GP 45840)

2-[(2,6-dichlorophenyl)amino]benzeneacetic acid monosodium salt/
[o-(2,6-dichloroanilino)phenyl] acetic acid sodium salt

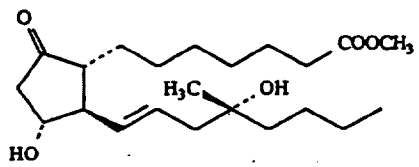


$C_{14}H_{10}Cl_2NNaO_2$ MW 318.13

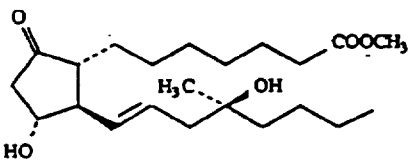
CATEGORY: Anti-inflammatory

Drug (2): Misoprostol (SC-29333)

(11α,13E)-(±)-11,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester



(±)-S-form



(±)-R-form

$C_{22}H_{38}O_5$ MW 382.54

CATEGORY: Anti-ulcerative

FORMULATION: Enteric-coated core containing 50 mg (ARTHROTEC® 50) or 75 mg (ARTHROTEC® 75) of diclofenac sodium surrounded by an outer mantle containing 200 mcg of misoprostol. Formulation includes the inactive ingredients lactose, microcrystalline cellulose, starch (corn), povidone (polyvidone) K-30, magnesium stearate, methacrylic acid copolymer

sodium hydroxide, talc, triethyl citrate, crospovidone, colloidal silicon dioxide and hydrogenated castor oil.

PROPOSED MARKETING INDICATION: For acute and chronic treatment of the signs and symptoms of osteoarthritis and rheumatoid arthritis in patients at risk of developing NSAID-induced gastrointestinal ulcers.

DOSE: The highest recommended daily dose of ARTHROTEC® 50 would deliver 150 mg/day of diclofenac sodium and 600 mcg/day of misoprostol. The highest recommended daily dose of ARTHROTEC® 75 would deliver 150 mg/day of diclofenac and 400 mcg/day of misoprostol.

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of study	Study/Report #	Drug Batch #	Page #
Pharmacology	----		4
Acute Oral Toxicity in the Mouse	PSA-87F-0331/0332	Misoprostol (M): 02800 Diclofenac (D): 57-B	6
Acute Oral Toxicity in the Rat	PSA-87F-0331/0332	M: 02800 D: 57-B	6
4-Week Oral Toxicity in the Rat	PSA-87F-0333	M: 01600 D: C0771	8
6-Month Oral Toxicity in the Rat	PSA-89F-0335	M: 02800 D: 57-B	10
4-Week Oral Toxicity in the Dog	PSA-87F-0334	M: 02800 D: 57-B	20
6-Month Oral Toxicity in the Monkey	PSA-98F-0336	M: 02800 D: 57-61A	24
MUTAGENICITY			
Ames Assay	PSA-89S-3556	M: 3D07510 D: 8935025	33
Forward Mutations in Chinese Hamster Ovary Cells	PSA-89S-3549	M: 3D07510 D: 8935025	34
Chromosomal Aberration Assay in Rat Lymphocytes	PSA-89S-3560	M: 3D07510 D: 8935025	35
Mouse Micronucleus Assay	PSA-89S-3558	M: 3D07510 D: 8935025	37
REPRODUCTIVE TOXICITY			
Segment II Oral Teratogenic Study in Rabbits	PSA-87S-3110	M: 01300 D: C0785	38

PHARMACOLOGY:

Primary Pharmacology

1. *In vivo* effects

Effects of diclofenac, misoprostol, and diclofenac:misoprostol combinations on phenylbenzoquinone-induced abdominal writhing in the mouse

Phenylbenzoquinone-induced (0.1 ml/10 g body weight, i.p.) abdominal writhing in mice was dose-dependently inhibited by either diclofenac (ED_{50} = 0.4 mg/kg; 1.0-10.0 mg/kg, p.o.) or misoprostol (ED_{50} = 2.0 mg/kg; 0.1-10.0 mg/kg, p.o.). The inhibitory effects of diclofenac:misoprostol combinations (0.1:0.1, 0.5:0.1, 0.1:0.3, and 0.1:1.0 mg/kg, p.o.) suggested synergism.

Effects of diclofenac, misoprostol, and diclofenac:misoprostol combinations on formalin-induced behavioral scores in the rat

Formalin-induced (intradermal administration of 0.1 ml of 2.5% formalin into the dorsal surface of one hindpaw) behavioral reactions were dose-dependently inhibited by diclofenac (-22.4%-66.2%, % of difference from control; 3-30 mg/kg, p.o.), but misoprostol (0.3 and 1.0 mg/kg, p.o.) had little or no inhibitory effect. In the case of the diclofenac:misoprostol combinations, inhibitory effects of lower doses of diclofenac (3 and 10 mg/kg, p.o.) seemed to be enhanced by misoprostol (1.0 mg/kg, p.o.), while inhibitory effects of a higher dose of diclofenac (30 mg/kg, p.o.) seemed to be decreased by misoprostol (0.3 and 1.0 mg/kg, p.o.). The reason for these interactions is not clear.

Effects of misoprostol on diclofenac-induced gastric ulcers in rats

Diclofenac (10 and 30 mg/kg, p.o.) produced dose-dependent increases in mean ulcer length/rat (2.0 and 32.2 mm) and mean number of lesions/rat (1.7 and 18.6). Co-administration of misoprostol (0.01-1.0 mg/kg) significantly inhibited diclofenac-induced (30 mg/kg, p.o.) mean ulcer length/rat and mean number of lesions/rat .

Effect of diclofenac, misoprostol, and diclofenac:misoprostol combinations on carrageenan-induced paw edema in the rat

Carrageenan-induced (subcutaneous administration of 0.25 mg in 0.1 ml of saline) rat paw edema in rats was inhibited by either diclofenac (ED_{40} = 1.84 mg/kg; 0.01-10.0 mg/kg, p.o.) or misoprostol (ED_{40} = 0.18 mg/kg; 0.003-0.3 mg/kg, p.o.). In the

case of diclofenac:misoprostol combinations, 0.01 and 0.03 mg/kg oral doses of misoprostol decreased the ED_{40}s of diclofenac (0.01-10.0, p.o.) to 0.04 and 0.36 mg/kg, respectively. When diclofenac (0.3-10 mg/kg, p.o.) was coadministered with oral misoprostol at diclofenac:misoprostol ratios of 100:1 and 10:1, there were no effects on the ED_{40}s of diclofenac. Thus, misoprostol either increased the efficacy of diclofenac-induced inhibition of carrageenan-induced paw edema or had no effect.

Prophylactic effect of diclofenac and diclofenac:misoprostol combinations in the rat adjuvant-induced arthritis model

Male rats were inoculated in the base of the tail with 2.0 mg of *M. butyricum* suspended in 0.05 ml of white paraffin oil. Beginning on the day of inoculation, rats were orally administered diclofenac (0.03, 0.1, 0.3 or 1.0 mg/kg/day), misoprostol (0.01, 0.03, 0.1 or 0.3 mg/kg/day) or diclofenac:misoprostol combinations of diclofenac (0.01, 0.03 or 0.3 mg/kg/day) with a fixed misoprostol dose of 0.3 mg/kg/day for 18 days. On Day 19, rats were sacrificed by CO_2 asphyxiation. In Experiment 1A, paw volume/body weight (PV/BW) ratios in the hydroxypropylmethylcellulose (HPMC) vehicle, distilled water and normal groups were 2.35, 2.82 and 1.5, respectively. Diclofenac (0.1-1.0 mg/kg, p.o.) dose-dependently reduced the mean PV/BW ratio (1.59-1.86) and increased the % reduction of PV/BW ratio (72.7-93.2%), while misoprostol (0.01-0.10 mg/kg, p.o.) had no significant effect on the mean PV/BW ratio and the % reduction of PV/BW ratio. In Experiment 1B, the PV/BW ratios in the HPMC vehicle, distilled water and normal groups were 2.30, 2.53 and 1.59, respectively. Diclofenac (0.03-0.3 mg/kg, p.o.) dose-dependently reduced the mean PV/BW ratio (1.64-1.87) and increased the % reduction of PV/BW ratio (70.2-94.7%), while misoprostol (0.03-0.3 mg/kg, p.o.) had no significant effect on the mean PV/BW ratio and the % reduction of PV/BW ratio. In Experiments 1A and 1B, there were no treatment-related effects on histopathology of ankle joints. In Experiment 2, the PV/BW ratios in the HPMC vehicle and normal groups were 2.26 and 1.49, respectively. Higher doses of diclofenac alone (0.1 and 0.3 mg/kg, p.o.) and diclofenac:misoprostol combination (0.3:0.3 mg/kg, p.o.) reduced the mean PV/BW ratio (1.62-1.99 and 1.80, respectively) and increased the % reduction of the PV/BW ratio (35.1-83.1% and 72.7%, respectively). There were no differences between diclofenac alone and diclofenac:misoprostol combination treatments. In Experiment 2, histopathology of the ankle joints was reduced by the 0.03 mg/kg dose of diclofenac alone, but not the 0.01 mg/kg dose. The histopathological effects of the diclofenac:misoprostol combination (0.01:0.3 mg/kg, p.o.) were not different from those produced by diclofenac alone (0.1 mg/kg, p.o.).

In summary, misoprostol inhibited diclofenac-induced gastric ulcers in rats; this directly supports the proposed marketing indication for ARTHROTEC® 50 and ARTHROTEC® 75. Moreover, diclofenac reduced the severity of adjuvant-induced arthritis in a rat model; diclofenac:misoprostol combinations produced similar reductions in severity. Thus, misoprostol did not alter the anti-inflammatory effects of diclofenac in this rat model. Furthermore, 100:1 and 10:1 diclofenac:misoprostol combination doses did not alter inhibition of carrageenan-induced paw edema in rats, compared to diclofenac alone.

Finally, either diclofenac or misoprostol dose-dependently increased the number of mice in which phenylbenzoquinone-induced abdominal writhing was inhibited; inhibitory effects of diclofenac:misoprostol combinations suggested additivity. On the other hand, diclofenac dose-dependently inhibited formalin-induced behavioral scores in rats, while misoprostol had little or no effect; there was an unexplainable interaction between diclofenac and misoprostol on formalin-induced behavioral scores. Thus, interactive analgesic effects of diclofenac and misoprostol appear to be complex.

TOXICOLOGY:

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ACUTE TOXICITY:

1. Acute oral toxicity in the rat and mouse (Study No. PSA-87F-0331/0332)

This study was previously submitted as an Amendment to NDA 19-268 (3/11/88) and was previously reviewed (8/24/88). A copy of the review follows:

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The acute toxicity of diclofenac/misoprostol combination was conducted by Searle at France facility from Nov. 4-Dec. 5, 1986. Results of the studies is summarized below:

cies/strain	No./Sex	Body weight /age	Route	Dose range (mg/kg)	M : D	LD 50	Time to death(days)	Maximum non-lethal dose(mg/kg)	Toxic signs
se/CD-1	10		P.O.	Misoprostol(M)	20 :	> 20	-	> 20	(1)
				Diclofenac (D)	50	150(M)	10	100(M)	(2)
					100	150(F)	8	< 50(F)	
					150				
					225				
					300				
				"Low" Association	0.2 : 50	150(M)	5	50(M)	(3)
				Misoprostol/	0.4 : 100				
				Diclofenac	0.6 : 150	190(F)	6	100(F)	
				(ratio: 2/500)	0.9 : 225				
					1.2 : 300				
				"High" Association	0.27 : 50	150(M)	6	50(M)	(4)
				Misoprostol/	0.53 : 100				
				Diclofenac	0.8 : 150	190(F)	9	100(F)	
				(ratio: 2/375)	1.200 : 225				
					1.600 : 300				
t/CD	10		P.O.	Misoprostol (M)	20,000 :	> 20	-	> 20	(5)
				Diclofenac (D)	50	230(M)	8	100(M)	(6)
					100				
					150	100(F)	16	50(F)	
					300				
					600				
				"Low" Association	0.2 : 50	280(M)	28	150(M)	(7)
				Misoprostol/	0.4 : 100				
				Diclofenac	0.8 : 200	150(F)	7	50(F)	
				(ratio: 2/500)	1.2 : 300				
					2.4 : 600				
				"High" Association	0.26 : 50	240(M)	14	150(M)	(8)
				Misoprostol/	0.53 : 100				
				Diclofenac	1.07 : 200	110(F)	9	50(F)	
				(ratio: 2/375)	1.6 : 300				
					3.2 : 600				

1): Protration, reduced motor activity.
2), (3), (4): Reduced motor activity, prostration, convulsion.
5): loses stool, prostration, reduced motor activity.
6), (7), (8): loose stool, prostration, reduced motor activity, arch back/hair standing.

There were no significant differences between the LD50 values of the two combinations and the diclofenac alone except in female rats, where the LD50 values of the low combination was significantly higher than the LD 50 for diclofenac alone. In general, it was more toxic in female rats. In mice, no significant differences in the LD50 appeared, either among the two combinations and diclofenac, or between sexes.

Addendum to the Review:

The minimum oral lethal dose of misoprostol in male and female mice was not determined (>20 mg/kg). The minimum oral lethal dose of diclofenac was 50 mg/kg in males and females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:500 ratio) was 0.4:100 mg/kg in males and 0.8:200 mg/kg in females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:375 ratio) was 0.53:100 mg/kg in males and 1.07:200 mg/kg in females.

The minimum oral lethal dose of misoprostol in male and female rats was not determined (>20 mg/kg). The minimum oral lethal dose of diclofenac was 150 mg/kg in males and 100 mg/kg in females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:500 ratio) was 0.9:225 mg/kg in males and 0.4:50 mg/kg in females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:375 ratio) was 1.2:225 mg/kg in males and 0.27:50 mg/kg in females.

SUBACUTE/CHRONIC TOXICITY:

Rats

1. 4-Week Oral Toxicity of Diclofenac Sodium/Misoprostol (Study No. PSA-87F-0333)

This study was previously submitted as an Amendment to NDA 19-268 (3/11/88) and was previously reviewed (8/24/88). A copy of the review follows:

Four-Week Oral Toxicity Study of Misoprostol/Diclofenac in Rats

Testing laboratory: Searle at France facility

Date of the study: Jan-Feb., 1987

Animals: Charles River CD rats weighing 132-303 g and 40 days of age were used.

Methods: Six groups of rats each consisting of 10 males and 10 females were given orally vehicle (0.5% methylcellulose (w/v) and 0.1% polysorbate 80 in distilled water), low combination (misoprostol:diclofenac of 0.002:0.5 mg/kg), medium combination (0.008:2.0), high combination (0.024:6.0), low diclofenac (0.5 mg/kg) and high diclofenac (6.0 mg/kg) per day for 4 weeks. Lot numbers for misoprostol and diclofenac are 2800 and 578, respectively. Five other groups each consisting of 15 males and 15 females were tested for pharmacokinetics.

Results: There were no mortalities and toxic signs in the study.

Feces examiniation: Fecal samples were examined for the presence of blood (HEMOCCULT II test). There was a significant increase in the positive finding in animals treated with diclofenac alone, when compared to controls or to animals treated with the combination.

Body weight and food consumption: normal.

Clinical chemistry: A decrease in albumin concentration was observed in all treated females groups. This may be attributable to the higher plasma(3.5-6 times) concentration of diclofenac in females than in males.

Hematology and urinalysis: normal

Pharmacokinetics: Diclofenac concentrations were higher in females than in males. Dose proportionality was seen. There was evidence of dose accumulation. There was no pharmacokinetic difference between diclofenac alone and the combination except in males on day 24 where Cmax was three times higher in the declofenac alone group.

Gross pathology and organ weights: Normal.

Histopathology: Gastric lesions were observed in 9 of the 20 rats in the high dosage group of diclofenac alone. Atrophy/inflammation/fibrosis was seen in the pancrease of 4 animals in the high dosage groups of both diclofenac and the combination. Focal granulomas were noted in the livers of 3 of 10 females in the high dosage group of disclofenac alone.

In conclusion, comparison of diclofenac with diclofenac/misoprostol combination treatment did not reveal increased toxicity or any change in pharmacokinetics of diclofenac due to the addition of misoprostol. Dose selection was inadequate since high, medium, and low dosages only represents 1.5, 1/2 and 1/8 respectively, of the maximum human dose.

Addendum to the Review:

As shown in the following table for male rats, there were dose-related increases in C_{max} and AUC values for diclofenac and diclofenac:misoprostol combinations. T_{max} values were predominantly between 0.5 and 2.0 h. There were no significant differences in toxicokinetic parameters between diclofenac and diclofenac:misoprostol combinations.

Toxicokinetic parameters for orally administered diclofenac and diclofenac:misoprostol combinations in the male rat

Diclofenac and Diclofenac:Misoprostol Combinations (mg/kg, p.o.)	Day 1			Day 24		
	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)
0.5	0.13	1.0	0.28	0.36	0.50	0.54
6.0	0.85	0.50	2.92	3.33	0.50	5.78
0.002:0.5	0.17	1.00	0.49	0.29	2.00	0.86
0.008:2.0	0.49	0.50	1.05	0.28	1.00	0.90
0.024:6.0	0.81	2.00	3.42	0.61	7.00	3.32

As shown in the following table for female rats, there were dose-related increases in C_{max} and AUC values; there was no accumulation over 24 days. Plasma levels of diclofenac and diclofenac: misoprostol combinations were higher in females than males. The predominant T_{max} value was 0.50 h. There were no significant differences in toxicokinetic parameters between diclofenac and diclofenac:misoprostol combinations.

Toxicokinetic parameters for orally administered diclofenac and diclofenac:misoprostol combinations in the female rat

Diclofenac and Diclofenac:Misoprostol Combinations (mg/kg, p.o.)	Day 1			Day 24		
	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)
0.5	0.19	2.0	0.55	0.25	0.50	0.62
6.0	1.14	0.50	5.54	2.94	0.50	7.90
0.002:0.5	0.09	0.50	0.34	0.21	0.50	0.59
0.008:2.0	0.61	0.50	1.81	1.18	0.50	2.54
0.024:6.0	2.84	0.50	5.22	3.49	0.50	10.81

2. 6-Month Oral Toxicity of Diclofenac Sodium/Misoprostol (Study No. PSA-89F-0335)

This study was submitted as

Six-Month Oral Toxicity Study of the Combination of Misoprostol/Diclofenac in the Rat. (Report No. PSA-89F-0335)

Testing Laboratories: Searle Recherche et Développement, Valbonne, France

Study Started: March 31, 1987

Study Completed: February 9, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Crl:CD(SD)Br; Males and females; Approximately 7 weeks of age; Weight; Males: 191-295 g, Females: 133-193 g

Drug Batch No.: Misoprostol: Lot No. 02800
Diclofenac: Lot No. 57B

Methods: The possible toxicological interaction of the combination of diclofenac and misoprostol versus diclofenac alone was evaluated in 26 week oral toxicity study in rats. Briefly, rats (20/sex/group) were orally (by gavage) administered Diclofenac alone at doses of 1, 2.5, and 6 mg/kg or in combination with misoprostol at doses of 4, 10 and 24 ug/kg, for 26 consecutive weeks. The dosage ratio of the combination (1 part misoprostol to 250 parts diclofenac) was chosen to mimic the likely clinical ratio. Misoprostol was administered as a dispersion which consisted of 1 part misoprostol and 100 parts hydroxypropyl methylcellulose, whereas, Diclofenac was dissolved in distilled water containing 0.5% methylcellulose 400 (w/v)and 0.1% polysorbate 80 (w/v). Diclofenac alone and the combination were administered in a total volume of 0.4 ml/kg. Animals in the control group (20/sex) received vehicles alone. An additional pharmacokinetic group (35 animals/sex/group) was used to determine the bioavailability of diclofenac and misoprostol. After 26 weeks of treatment 5 animals/group underwent a 4 week reversal period. Animals were observed at least 2X daily for signs of toxicity and/or mortality, with physical examinations conducted once at pretreatment, and weekly during the treatment period and reversal phase. Ophthalmologic examinations were performed at pretreatment and during weeks 13, 26, and 30 (recovery animals only). Body weights were recorded twice at pretreatment twice during the first 4 weeks and once weekly thereafter. Food consumption was determined once at pretreatment and weekly thereafter. Clinical chemistry and hematology was determined in plasma samples collected via the retro-orbital sinus from 10 animals/sex/group during weeks 8 and 17 and from all animals during weeks 25 and 31, with urinalysis conducted on samples from the same animals at the same time periods. Blood samples (approximately 1 ml) were also collected from 3 rats per

sampling time per sex per dosage group at 0, 0.25, 0.5, 1, and 6 hours after dosing on day 1, 95 and 179 and on days 1, 80, and 165 for determination of absorption and pharmacokinetics of diclofenac and misoprostol, respectively (each animal was bled only once for a given day). Following sacrifice, all animals in the main study group underwent gross pathologic examinations with determination of organ weights. Complete histological examinations were conducted on tissues from animals in the control, high dose combination and high dose diclofenac groups, and from all animals which did not survive to the scheduled sacrifice. Histological examinations of stomach, duodenum, jejunum, ileum, cecum, colon, and rectum were also conducted on all animals in remaining groups, along with any tissue which had gross lesions.

Results:

1. **Observed Effects:** Diclofenac-related clinical signs were observed in animals of the high dosage groups (diclofenac and combination) which were found dead or killed in extremis. These signs included: thin appearance, reduced motor activity, soiled urogenital area, pale extremities, labored breathing, and coldness to the touch.

2. **Mortality:** Treatment-related mortality included 3 females each in the high dose diclofenac and high dose combination groups. In addition, 1 male in the high dose diclofenac group and 1 female each in the high dose diclofenac and combination groups were sacrificed in extremis during weeks 22, 8, and 18. Other mortalities which resulted from apparent intubation errors included: 1 female control rat, 1 low dose combination male, 2 mid dose combination males, 1 high dose combination rat/sex.

3. **Body Weight/Food Consumption:** Diclofenac alone and in combination with misoprostol at the mid and high doses produced small reductions in body weight gains over the 26 week treatment period (Day 1 through Day 179). Mean body weight gains were reduced in mid and high dose diclofenac groups by 7.43 and 14.18% in males and by 6.5 and 12.34% in females, respectively. Similar reductions in mean body weight gains of 24.89 and 10.69% in males and of 7.78 and 2.57% in females were observed in rats in the mid and high dose combination groups. There were no apparent treatment-related changes in food consumption in any groups tested.

4. **Hematology:** At week 25, females exhibited dose-dependent increased neutrophils (95 and 507%, and 49 and 549% in mid and high dose diclofenac and mid and high dose combination groups, respectively; from a mean control value of 0.88 x 10^9 cells/L). Increased neutrophils resulted in significant increases in white blood cells (WBC) in the high dose diclofenac (77%) and high dose combination groups (91% from a control value of 8.2 x 10^9 cells/L). High dose females (diclofenac and combination) also

exhibited significant increases absolute monocytes (206 and 223%) and eosinophils (300 and 154%) compared to control values of 0.31 x 10^9/L and 0.11 x 10^9/L, respectively. Small, but significant decreases in red blood cells (RBCs, 11%), hemoglobin (Hb, 15%) and hematocrit (Hct, 12%) were observed in high dose combination females, while high dose diclofenac females only showed decreased hemoglobin (6.38%). Females in the high dose diclofenac and combination groups also exhibited increased platelets (24 and 37% over a mean value for control females of 901 x 10^9 platelets/L) which resulted in a slight decrease in prothrombin times (8.9%) compared to a control value of 10.1 seconds. In general hematological effects were not evident in rats by the end of the reversal period, with the exception of WBCs and neutrophils which remained elevated (117% and 319%) in high dose combination females at the end of the reversal period (reversal week 5) compared to mean control values of 7.6 x 10^9 WBCs/L and 1.35 x 10^9 neutrophils/L, respectively.

5. Blood Chemistry: Treatment-related changes in blood chemistry included decreased total protein in females of both the high dose diclofenac and the high dose combination groups , which was due mainly to decreased albumin concentrations in these groups and in the high dose diclofenac and high dose combination groups, respectively).. The decreased albumin concentrations in females also resulted in reduced A/G ratios during week 24 in these same groups (11% and 22%) in the high dose diclofenac and high dose combination groups, respectively. The aforementioned reductions in total protein, albumin and A/G ratios persisted through the recovery period. Other significant changes in biochemical parameters included: 1) increased aspartate aminotransferase levels in: mid dose diclofenac males (60.78 and 35.4%; weeks 17 and 24) and in high dose combination group (328.84%, recovery week 5); 2) increased alanine aminotransferase in mid dose diclofenac males (90 and 42%; weeks 17 and 24) and in high dose diclofenac females (275% during recovery week 5); 3) increased alkaline phosphatase in high dose diclofenac and combination females (11 and 22% , respectively, during week 24) and in all treatment groups, except low and mid dose combination females, during week 5 of the recovery period (%; not dose related).

6. Urinalysis: No treatment-related changes were observed in urinalysis.

7. Ophthalmologic Examinations: Ophthalmoscopic observations included the following: hyper-reflectivity of the retina (one female in the high dose combination group); 5 cases of retinal hemorrhage (animals of both sexes across all treatment groups), sporadic incidence of corneal opacity, superficial keratitis (associated in 2 cases with edema or dystrophy) and absence of identifiable ocular structures (a few animals in all dose groups) and finally, three cases of capsular or incipient cataracts of the posterior part of the lens were observed across the control and low dose combination groups. In all cases ophthalmologic observations appeared sporadically or possibly related to trauma or infection (presumably due to blood collection) and did not appear treatment-related.

8. Organ Weights: Treatment-related changes in organ weights included: increased absolute and relative weights for liver in females from the high dose combination group (increases of 17,19 and 17% for absolute liver weights and liver/body and liver/brain weight ratios, respectively) and increased absolute and relative weights for spleen in females from both the high dose diclofenac group (increases of 30.4, 38.5, and 25.2% for absolute spleen weights and spleen/body and spleen/brain weight ratios, respectively) and the high dose combination group (increases of 64, 69 and 64% for absolute spleen weights and spleen/body and spleen/brain weight ratios, respectively). The aforementioned changes in liver and spleen weights persisted to a similar degree at the end of the recovery period (recovery week 5) with no evidence of reversibility.

9. Gross Pathology: Four females from each high dosage group (High dose Combination [HC] and High dose Diclofenac [HD]) and one male from the HD group which died or were killed in extremis exhibited gross lesions in the abdominal and/or thoracic cavities which included: abdominal cavity peritonitis (3 females each in the HC and HD groups); adhesion of various organs including heart (1 HD female), lungs (1 HC male), intestines (2 HC females) and liver (1 of 5 HC females); edema of the viscera (1 HC female); ulcerations of the jejunum (2 HD females) and small intestines (1 female each in the HC and HD groups); enlarged spleen (1 HD female) and mucosal hyperemia of the stomach (1 HD female) red discoloration of the pancreas (1 HD female) and discoloration of the thymus (1 male each in the mid dose combination group and in the HC). Treatment-related gross pathological findings in animals which survived to the scheduled sacrifice are presented in Table 1 (succeeding page).

Table 1. Gross Pathological Findings in Rats Following Six months of Oral Administration of Vehicle, Diclofenac or the Combination of Diclofenac/Misoprostol.

	Males							Females						
Group	CT	LC	MC	HC	LD	MD	HD	CT	LC	MC	HC	LD	MD	HD
No. of Animals	15	14	13	14	15	15	14	14	15	15	11	15	15	12
Gross Observation:														
Jejunum														
Dilated/thickened...	0	0	0	0	0	0	0	0	0	0	3	0	0	1
Thickening.....	0	0	0	0	0	0	1	0	0	0	1	0	0	4
Hyperemia.....	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Nodule.....	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Mesenteric lymph node														
Enlarged.....	0	0	0	0	0	0	0	0	0	0	3	0	0	1
Submaxillary lymph node, Enlarged.....	0	2	0	1	1	0	1	0	0	0	0	0	0	0
Enlarged Spleen.....	0	0	0	0	0	0	0	0	0	1	1	0	0	1
Stomach														
Mucosal hyperemia...	0	0	0	1	0	1	1	0	0	0	0	0	0	0
Thickened wall.....	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Dark spot.....	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Thymus														
Enlarged.....	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Small petechial Hemorrhages.....	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Uterus														
Dilated/thickened...	----------							0	2	1	2	1	1	0

CT = Vehicle control;
LC = Low dose Diclofenac/misoprostol [4 ug/kg: 1 mg/kg]
MC = Mid dose Diclofenac/misoprostol [10 ug/kg:2.5 mg/kg]
HC = High dose diclofenac/misoprostol [24 ug/kg:6 mg/kg]
LD = Low dose Diclofenac [1 mg/kg]
MD = Mid dose Diclofenac [2.5 mg/kg]
HD = High dose Diclofenac [6 mg/kg]

Briefly, the data in Table 1 show that High dose Combination (HC, Misoprostol/Diclofenac) and High dose Diclofenac (HD) treatments produced nodules, dilation and/or thickening in the jejunum (5

females and 1 male from the HD group and 7 females from the HC group). Other gross lesions included: enlarged spleens (1 female each, in the HC and HD groups and 1 female in the Mid dose Combination group [MC]); enlarged mesenteric lymph nodes (3 HC females and 1 HD female); hyperemia of the jejunum (1 HD male and 1 Mid dose Diclofenac [MD] female); mucosal hyperemia of the stomach (1 male each, in the HC, HD and MD groups); thickened stomach wall (1 HC male); dark spot in the stomach (1 HD male and 1 Low dose Combination [LC] female); enlarged thymus (1 HC male) and petechial hemorrhages in the thymus (1 HD male). Females in both treatment groups (diclofenac and combination) also exhibited an increased incidence of dilated/thickened uterus. However, this latter finding was not dose dependent and its relationship to treatment is unknown. Gross observations in rats at the end of the recovery period included: jejunal dilation/thickening (2 of the 4 HC females); jejunal hyperemia 1 of 4 HC females); abdominal cavity adherence (1 of 4 HC females); enlarged spleen (1 of 4 HC females), liver nodule (1 of 5 HD males) and enlarged submaxillary lymph nodes (1 male each in the low and high dose combination and low and high dose diclofenac groups). Uterine dilation was also observed in 1 of 5 and 2 of 5 females in the LC and MC groups. Finally, stomach mucosal hyperemia was seen in 1 of 5 Low dose Diclofenac (LD) females the at the end of the reversal period.

10. Histopathology: The incidence of treatment-related histological observations in rats which survived to the scheduled sacrifice is presented in Table 2 (succeeding pages). Briefly, these data show that diclofenac and the combination of diclofenac/misoprostol produced ulcerations in: the glandular mucosa of the stomach (1 MD and 3 HD males and in 1 female each in the LC and HC groups); the mucosa of the jejunum, mostly accompanied by a parietal granulation (7 HC females and 1 male and 6 females from the HD groups) and the mucosa of the cecum (1 female each in the HD and HC groups). Additional treatment-related findings included: extramedullary hematopoiesis of the spleen (5 HC females and 1 HD female); peritonitis of the jejunum (1 HC female); increased incidence of adenitis of the sub-maxillary and mesenteric lymph nodes (females in both high dose groups), with plasma cell infiltration of the sub-maxillary lymph node (3 HD males) and sinusal edema/cystic spaces in the mesenteric lymph nodes (5 HC females and 4 HD females). Finally, acute hemorrhage of the thymus and loss of thymic cortical lymphocytes was also observed in 1 HD male and in 1 HC male, respectively. Other possible treatment-related effects include a slight increased incidence of hepatic vacuolation (HD males and females), necrosis (1 male and 1 female in the HD and HC groups, respectively), and non-suppurative hepatitis (males in both the HC and HD groups). Finally, a low incidence of uterine luminal distension was observed in 1 to 3 females in all treatment groups (combination and diclofenac) versus none in the control group.

Table 2. Histopathological Findings in Rats Following Six months of Oral Administration of Vehicle, the Combination of Diclofenac/Misoprostol or Diclofenac alone.

	Males							Females						
Group	CT	LC	MC	HC	LD	MD	HD	CT	LC	MC	HC	LD	MD	HD
No. examined*	15	14	13	14	15	15	14	14	15	15	11	15	15	12
Microscopic Findings:														
Stomach														
Glandular Mucosa ulceration.....	0	0	0	0	0	1	3	0	1	0	1	0	1	1
Spleen														
Extramedullary hematopoiesis.....	0	-	-	0	-	-	0	0	-	-	5	-	-	1
Jejunum														
Mucosal ulceration...	0	0	0	0	0	0	1	0	0	0	7	0	0	6
Parietal granulation.	0	0	0	0	0	0	0	0	0	0	7	0	0	6
Mucosal hyperemia....	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Peritonitis.....	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cecum														
Mucosal ulceration...	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Colon														
Lymphoid hyperplasia.	0	2	3	3	4	1	0	1	4	1	0	3	3	1
Heart														
Chronic myocarditis..	2	-	-	4	-	-	3	1	-	-	0	-	-	0
Myocardial necrosis..	0	-	-	1	-	-	2	0	-	-	0	-	-	0
Liver														
Hepatocytic vacuolation.....	4	-	-	2	-	-	7	4	-	-	5	-	-	6
Necrosis.....	0	-	-	1	-	-	0	0	-	-	0	-	-	1
Non-suppurative hepatitis.....	3	-	-	8	-	-	8	9	-	-	10	-	-	4
Submaxillary LN														
Adenitis.....	4	2	-	2	1	-	2	3	-	-	6	-	-	6
Plasma cell infiltration.....	0	-	-	0	-	-	3	1	-	-	0	-	-	0

- = No animals examined
CT = Vehicle control
LC = Low dose Diclofenac/misoprostol [1 mg/kg:4 ug/kg]
MC = Mid dose Diclofenac/misoprostol [2.5 mg/kg:10 ug/kg]
HC = High dose diclofenac/misoprostol [6 mg/kg:24 ug/kg]
LD = Low dose Diclofenac [1 mg/kg]
MD = Mid dose Diclofenac [2.5 mg/kg]
HD = High dose Diclofenac [6 mg/kg]

Table 2. (Cont...)

Cont...	Males							Females						
Group No.	CT	LC	MC	HC	LD	MD	HD	CT	LC	MC	HC	LD	MD	HD
No. examined	15	0	0	14	0	0	14	14	2	1	11	0	1	12
Microscopic Findings:														
Mesenteric LN														
Adenitis.....	0	-	-	0	-	-	1	0	-	-	4	-	-	6
Sinusal edema/cystic spaces.....	0	-	-	0	-	-	0	0	-	-	5	-	-	4
Thymus														
Acute congestion/ hemorrhage.....	0	-	-	0	-	-	1	0	-	-	0	-	-	0
Loss of Cortical lymphocytes.....	0	-	-	1	-	-	0	0	-	-	0	-	-	0
Uterus														
Luminal distension..	-	-	-	-	-	-	-	0	2	1	3	-	1	3

- = No animals examined
CT = Vehicle control
LC = Low dose Diclofenac/misoprostol [1 mg/kg:4 ug/kg]
MC = Mid dose Diclofenac/misoprostol [2.5 mg/kg:10 ug/kg]
HC = High dose diclofenac/misoprostol [6 mg/kg:24 ug/kg]
LD = Low dose Diclofenac [1 mg/kg]
MD = Mid dose Diclofenac [2.5 mg/kg]
HD = High dose Diclofenac [6 mg/kg]

Histological findings in animals which were sacrificed at the end of the recovery period were limited ulcerations with parietal granulations (2 of 4 HC females). The four females from each of the high dose groups and the one male from the high dose diclofenac group which died or were killed in extremis exhibited similar, but more severe histological lesions including: erosions/ulcers of the forestomach, glandular mucosa, jejunum, ileum, Cecum and rectum; diffuse peritonitis in multiple organs including, stomach, spleen, pancreas, duodenum, jejunum, ileum, cecum, colon, liver, ovaries, urinary bladder and mesenteric lymph node; pericarditis (1 HD female); acute gastritis (1 HD female); extramedullary hematopoiesis in the spleen; cortical hypertrophy of the adrenal glands (2 females each in the HD and HC groups); adenitis and plasma cell infiltration of the submaxillary lymph node, adenitis and sinusoidal edema/cystic spaces of the mesenteric lymph node and acute congestion/ hemorrhage of the thymus with loss of cortical lymphocytes.

11. Plasma Concentrations: Diclofenac and the combination of diclofenac and misoprostol were rapidly absorbed following oral dosing with maximal plasma levels of diclofenac attained between 0.25 and 1.0 hr in all dose groups. A summary of the Cmax and AUC values for diclofenac on days 1, 95 (week 14) and 179 (week 26) is presented in Table 3, (below).

Table 3. Cmax and AUC values for Diclofenac in Rats Following Oral Administration of Diclofenac Alone or in combination with misoprostol on Days 1, 95, and 179.

Group #	Sex	Cmax (ug/ml)			AUC (ug·hr/ml)		
		Day 1	Day 95	Day 179	Day 1	Day 95	Day 179
Control	M	0.00	0.00	0.00	0.00	0.00	0.00
LD	M	0.18	0.31	0.44	0.62	1.68	0.62
MD	M	0.52	1.14	1.01	2.13	3.57	3.29
HD	M	1.32	3.60	2.90	4.16	6.50	10.96
LC	M	0.23	0.30	0.33	0.20	0.77	1.07
MC	M	0.56	0.70	1.50	2.04	1.63	2.05
HC	M	1.24	1.95	1.67	4.31	6.66	3.65
Control	F	0.00	0.00	0.00	0.00	0.00	0.00
LD	F	0.28	0.85	0.86	1.01	1.99	2.03
MD	F	0.85	2.01	3.82	1.48	2.62	5.05
HD	F	5.24	3.13	4.13	8.25	8.83	14.52
LC	F	0.29	0.78	1.32	0.85	2.34	2.98
MC	F	1.54	1.09	1.71	2.05	4.42	5.07
HC	F	1.89	5.02	2.73	5.34	8.60	8.89

Control = Vehicle controls ;
LD = Low dose Diclofenac [1 mg/kg]
MD = Mid dose Diclofenac [2.5 mg/kg];
HD = High dose Diclofenac [6 mg/kg];
LC = Low dose Diclofenac/misoprostol [1 mg/kg:4 ug/kg]
MC = Mid dose Diclofenac/misoprostol [2.5 mg/kg:10 ug/kg]
HC = High dose diclofenac/misoprostol [6 mg/kg:24 ug/kg]

Briefly,these data show that increasing doses of Diclofenac (alone or in combination with misoprostol) produced variable, but linear increases in Cmax and AUC values which were generally dose proportional in both sexes. Overall, Cmax and AUC values were increased in females compared to males and were generally greater on Day 179 compared to Day 1. However, misoprostol did not interfere with the pharmacokinetics of diclofenac at the doses tested herein, with the possible exception of male rats on day 179, where mean AUC values were higher after administration of diclofenac alone (AUC = 10.96 $\pm$ 2.85 μg.hr/ml) compared to the combination (AUC = 3.7 $\pm$ 0.29 μg.hr/ml). However, since such

differences were not observed in any other dose groups or time points, this difference could be due to intergroup variability.

Plasma concentration data on SC-30695, the main metabolite of misoprostol (day 80 [week 12] and day 165 [week 24], data not shown), only demonstrated that misoprostol was well absorbed on days 80 and 165 in the high dose combination group, as analytical problems and instability of SC-30695 precluded further assessments.

In conclusion, 6-month oral administration of misoprostol at doses of 4, 10, and 24 ug/kg had no apparent effect on the toxicity profile of diclofenac (coadministered orally, at respective doses of 1, 2.5 and 6 mg/day) in rats, compared to the toxicity profile of diclofenac alone. Diclofenac alone or in combination with misoprostol produced clinical signs of thin appearance, reduced motor activity, soiled urogenital area, pale extremities labored breathing, coldness to touch and death in high dose rats. Mild reductions in body weight gains, alterations in hematology, clinical chemistry and increased organ weights for spleen and/or liver compared to control values were also observed in mid and high dose rats of both treatment groups. Target organs of toxicity included: 1) organs of the GI tract (stomach jejunum, cecum, and colon); 2) spleen (enlarged with extramedullary hematopoiesis); 3) Lymph nodes (enlarged with adenitis, sinusoidal edema and plasma cell infiltration; and 4) thymus, with increased incidence of congestion/hemorrhage and loss of cortical lymphocytes. In general, gross and histological signs were most prevalent in both high dose groups, and were more severe in females compared to males. Females also had higher diclofenac plasma concentrations compared to their male counterparts. However, coadministration of misoprostol had minimal effects on the absorption or pharmacokinetics of orally administered diclofenac in rats. The no effect oral dose for the combination of misoprostol and diclofenac in rats (at a dose ratio of 1:250) was 4 µg/kg and 1 mg/kg, respectively.

Dogs

1. 4-Week Oral Toxicity of Diclofenac Sodium/Misoprostol (Study No. PSA-87F-0334)

This study was previously submitted as an Amendment to NDA 19-268 (3/11/88) and was previously reviewed (8/24/88). A copy of the review follows:

Four Week Oral Toxicity Study of Diclofenac/Misoprostol in Dogs

Testing Laboratory: Searle in France

Date of the study: Dec 16, 1986 to Jan 16, 1987.

GLP requirement: A statement of compliance with GLP regulation was included.

Animals: Beagle dogs weighing and about 6-8 months of age were used.

Methods: Six groups of animals each consisting of 4 males and 4 females were given control (empty capsules), low diclofenac (0.5 mg/kg/day), high diclofenac (2 mg/kg/day), low combination (misoprostol:diclofenac of 0.002:0.5 mg/kg/day), medium combination (0.004:1) and high combination (0.008:2 mg/kg/day) by capsule for 4 weeks.

Results:

Mortality: One male the high dosage diclofenac group was found dead on day 17. Two males and one female of the high dosage combination were found dead between days 20 to 25. All remaining surviving dogs in these two groups were sacrificed on day 25 due to the mortality level and the general poor condition.

Clinical signs: Dark feces, red tinged in feces, bloody feces, loose stools and emesis, reduced motor activity, salivation, pallor of mucosa, and thinness of body were observed in all drug-treated groups.

Body weight: There were marked body weight decreases in the high diclofenac and high combination groups in the study. However, there were no differences in body weight changes from study day 1 among all dosage groups.

Food consumption: Food consumption was reduced more frequently in animals of the two high dosage groups.

EKG: None of the changes were considered to be drug-related.

Clinical chemistry: Dose-related decreases of protein , and albumin (20-50%) concentrations were observed in all treated groups except in females of the low combination group. Calcium concentration was decreased A decrease of alanine aminotransferase activity was observed. There was a decrease in alkaline phosphatase. Decreases of sodium concentration (7%) was observed in high diclofenac group. Choride concentration was normal.Triglyceride concentration was increased in the high dosage groups and the medium dosage group.Creatinine concentration was increased (8%) in high diclofenac group. There were no significant differences in the above-mentioned parameters between diclofenac alone and combination groups.

Hematology: There was a dose dependent decrease of red blood cell count), hematocrit ', and hemoglobin in the high dosage groups and medium dosage group. Mean corpuscular hemoglobin and volume was decreased (10%) in the high dosage groups. White blood cell count were markedly increased in all dosage groups. There was an increase in percentage of neutrophils with a concomitant decrease in percentage of lymphocytes in all treated groups. In general, the above-mentioned parameters were not enhanced by addition of misoprostol.

Urinalysis: Normal except some contamination of urine by blood from feces was observed.

Bioavilability: Plasma diclofenac concentrations were lower in males. No pharmacokinetic difference was observed after the administration of diclofenac alone or in combination with misoprostol.

Organ weights: Spleen weights were dose-dependently increased at all doses (50-170%) and more pronounced in males than in females. Thymus weights were decreased (67%) in animals at the high dosage of diclofenac alone and in combination with misoprostol. Kidney weight increased dose dependently in all treated groups, but they were not statistically significant. Lower prostate , testis, and epididymis weights were observed in a few animals treated with the combination of misoprostol and diclofenac (2 out of 4 at the low, 1 out of 4 at the medium and 2 out of 4 at the high dosage group). Again, the differences were not statistically significant.

Gross pathology: Ulcerations in GI tracts were found in animals of the high dosage groups and medium combination group. In the kidney, white or red discoloration of the papilla was observed in animals of high dosage groups. The prostate was reduced in size in 2 (sponsor stated 3, instead) out of 4 animals of high dosage combination group.

Histopathology: Ulceration or erosions were observed in GI tracts in animals of all treatment groups except in males of both the low dosage of diclofenac alone and the combination. Other major histopathological findings are summarized below:

	Male						Female					
Groups *	1	2	3	4	5	6	1	2	3	4	5	6
No. examined	4	4	4	4	4	4	4	4	4	4	4	4
Kidney												
papilary edema	0	0	1	1	3	4	0	1	2	0	2	2
papillary necrosis	0	0	2	0	0	0	0	0	1	0	0	1
Spleen												
Extramedullary hematopoisis	0	1	3	0	0	2	0	1	3	0	3	2
Thymus atrophy	0	0	2	0	1	2	0	0	2	0	0	2
Prostate hypoplasia	0	0	0	2	1	3						
Testis												
Oligospermia	0	0	0	1	1	3						
Epididymis												
no spermatozoa	0	1	0	1	1	2						

* 1: control, 2: low diclofenac, 3: high diclofenac
4: low combination, 5: medium combination, 6: high combination

The data indicated that prostate hypoplasia, oligospermia in testis, no spermatozoa in epididymis, and papillary edema in kidneys were observed dose-dependently in the males of all combination groups. Thus, renal toxicity and lethality due to diclofenac were somewhat enhanced by addition of misoprostol at dose equavalent to 1/2 of the recommended maximum human dose and combination of two compounds might induce toxicities in male reproductive organs. Applicant mentioned that the reproductive toxicity may be attributable to vriable matuiry of the organs due to animal's age (6-8 months). Again, this study did not provide safety margin of the combination. Dose selection was inadequate since high dose only represents 1/2 of the maximum human dose. However, there appears to be limiting toxicity i.e., mortality with high dose.

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Addendum to the Review:

As shown in the following table for male dogs, there were dose-related increases in AUC values; C_{max} values were more variable. T_{max} values varied between 1 to 4 h. There were no significant differences in toxicokinetic parameters between diclofenac and diclofenac:misoprostol combinations.

Toxicokinetic parameters for orally administered diclofenac and diclofenac:misoprostol combinations in the male dog

Diclofenac and Diclofenac:Misoprostol Combinations (mg/kg, p.o.)	Day 1			Week 4		
	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)
0.5	1.58	1.13	4.94	1.80	0.63	3.91
2.0	6.32	1.00	15.69	1.79	2.33	6.44
0.002:0.5	0.92	3.25	3.25	1.68	2.50	4.42
0.004:1.0	2.94	1.63	6.67	2.08	1.88	6.10
0.008:2.0	4.46	1.75	14.72	1.23	4.00	6.23

As shown in the following table for female dogs, there were dose-related increases in AUC values; C_{max} values were more variable. T_{max} values varied between 0.25 to 3.5 h. There were no differences between males and females. There were no significant differences in toxicokinetic parameters between diclofenac and diclofenac:misoprostol combinations.

Toxicokinetic parameters for orally administered diclofenac and diclofenac:misoprostol combinations in the female dog

Diclofenac and Diclofenac:Misoprostol Combinations (mg/kg, p.o.)	Day 1			Week 4		
	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC (µg*h/ml)
0.5	1.94	0.25	5.28	1.69	2.13	4.11
2.0	5.48	2.38	14.78	4.83	1.38	16.16
0.002:0.5	1.46	0.88	3.78	0.91	3.38	3.00
0.004:1.0	3.75	0.63	9.39	3.03	1.88	6.23
0.008:2.0	5.65	2.75	16.22	6.31	0.63	16.63

Monkeys

1. 6-Month oral toxicity of Diclofenac Sodium/Misoprostol (Study No. PSA-89F-0336)

This study was previously submitted as A copy of the review follows:

Six-Month Oral Toxicity Study of the Combination of Misoprostol/Diclofenac in the Cynomolgus Monkey. (Report No. PSA-89F-0335)

Testing Laboratories: Searle Recherche et Développement, Valbonne, France

Study Started: June 9, 1987

Study Completed: March 13, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Cynomolgus Monkeys; Age not indicated; Weight; Males: 2.5-3.8 kg, Females: 2.2-3.5 kg

Drug Batch Nos.: Misoprostol: Lot No. 02800
Diclofenac: Lot No. 57-61A

Methods: The possible toxicological interaction of the combination of diclofenac and misoprostol versus diclofenac alone was evaluated in 26 week oral toxicity study in monkeys. Briefly, monkeys (6/sex/group) were orally administered (by nasogastric route) diclofenac alone at doses of 6, and 50 mg/kg or at doses of 6, 17, and 50 mg/kg in combination with misoprostol at respective doses of 24, 68, and 200 ug/kg for a period of 6 months. The basis of dose selection was not indicated. Diclofenac was dissolved in distilled water and misoprostol was dispersed in hydroxypropyl methylcellulose, HPMC (1 part misoprostol:100 parts HPMC) and administered in a volume of 3 ml each. The diclofenac alone group received an additional 3 ml/kg distilled water. Control animals (6/sex) received 6 ml/kg distilled water. After 26 weeks of treatment, 2 animals/group (except for high dose diclofenac females, where only one animal was selected) underwent a 4 week reversal period. Animals were observed prior to and approximately 2-3 hours post dosing for signs of toxicity and/or mortality, with physical examinations conducted once at pretreatment, during weeks 7, 14, and 24 of the treatment phase and during and during week 4 of the reversal period. Ophthalmologic examinations were performed on anesthetized animals once at pretreatment, during week 24 of treatment and during week 3 of the reversal phase. Body weights were recorded once at pretreatment, once one week before the first day of treatment for selected animals, on day 4 of the study and every 2 weeks thereafter. Measurements of food and water consumption were not indicated. Serial venous blood samples (8 ml total) were collected from all animals including controls on days 1, 85 (week 13) and 176 (week 26) for determinations of diclofenac and SC-30695 (primary metabolite of misoprostol) concentrations. Additional venous blood samples (2.5 ml) were collected at pretreatment, and before dosing during weeks 4, 8, 14, 18, and 25 and at the end of the reversal period for analysis of clinical chemistry and hematology. Urinalysis was not indicated. Electrocardiographic examinations were performed on anesthetized animals at pretreatment, after treatment during weeks 14 and 24 and during week 4 of the recovery period. Complete gross and histological examinations were performed on all animals which died or were killed in extremis and at the scheduled sacrifice.

Results:

1. **Observed Effects:** Monkeys found dead or killed in extremis exhibited clinical signs of toxicity which included reduced motor activity, prostration, hypothermia, paleness of mucosae, edema and redness of eyelids swelling and wounds of the skin. Treatment- related clinical signs in surviving animals included: loose stools (males in the mid and high dose combination groups and females mainly in the high dose combination group); salivation before treatment (mid and high dose combination males and high dose combination females); and a dose-related increased incidence of small exudative wounds, primarily located on the

fingers, legs and tail, (males and females of the mid and high dose combination and high dose diclofenac groups). Finally, the Sponsor stated that instances of convulsions and prostration were observed in a few animals during drug administration. However, individual animal data regarding these latter observations were not provided. The onset and duration of clinical signs were not reported, but were stated not to be present during the reversal phase.

2. Mortality: Treatment-related mortality was seen in 1 high dose combination (HC) male (No. 87-6879) which died in week 14. In addition, 1 high dose diclofenac (HD) male (No. 87-6904) and 2 HD females (Nos.87-6907 and 87-6908) were killed in extremis at weeks 4 and 14, and 2, respectively. Finally, one high dose combination female (No. 87-6885) died in week 18, with death attributed to an intubation error.

4. Body Weight/Food Consumption: Mean body weight loss of 6%, (compared to day -4 body weights) were observed in high dose diclofenac males and in the high dose diclofenac. In comparison, control males and males in the high dose combination treatment groups exhibited mean body weight gains of 16 and 19%, respectively. Body weight loss was also seen in 1 female per each high dose group, while, complete suppression of body weight gain was also seen in 1 female each in the mid and high dose combination groups. In comparison, control females exhibited mean body weight gains of 9.68% compared to day -4 body weights. One high dose diclofenac male and 2 high dose diclofenac females which died or were killed in extremis prior to completion of the treatment also showed body weight loss of or complete suppression of body weight gain. Data on the effects of treatment on food and water consumption were not provided.

5. Hematology: Treatment-related hematological alterations which were evident at the end of the treatment period (week 25) included: decreased red blood cells (12.2% and 13.2%); hemoglobin (24 and 8%) and hematocrit (14.8 and 4.9%)in males in the high dose diclofenac and combination groups, respectively. High dose diclofenac males also exhibited reduced mean corpuscular volumes (13.7%) and reduced mean corpuscular hemoglobin concentrations (13.8%). Other treatment-related findings included: increased reticulocytes (0.8% and 1.1%) in high dose diclofenac males and females compared to values of 0.5% and 0.2% in control males and females, respectively. Monkeys in both high dose treatment groups also had increased neutrophils; (% and absolute; 41-80%) and decreased lymphocytes (% and absolute; . Finally, increased platelet counts were seen high dose diclofenac males (126.6% from control values of 387 x 10^9/L) and females (31.8%) and in high dose combination males (26%). Partial reversal of the aforementioned changes were observed in animals which underwent recovery, with the exception of reticulocytes which remained elevated in the high dose diclofenac group (males and females 100 and 233%, respectively).

6. Blood Chemistry: Treatment-related effects on blood chemistry included: decreased albumen (in the mid and high dose combination groups and in the high dose diclofenac groups, increased globulin in the high dose diclofenac group, and decreased albumin to globulin ratios ( ) in the high dose diclofenac group and in the mid and high dose combination groups. Additional treatment-related effects included: decreased calcium both sexes in the high dose diclofenac group and males in the high dose combination group); increased chloride (1.8 to 4.5%) in low and high dose diclofenac groups and in the mid and high dose combination groups; increased triglycerides (30 and 100% in low and high dose diclofenac males and 50% and 79% in high dose diclofenac and combination females, respectively); decreased aspartate aminotransferase (, both sexes in both high dose groups); decreased alanine aminotransferase (all treatment groups except females in both low dose groups); decreased alkaline phosphatase (, in individual males and females in the mid and high dose combination groups and in the high dose diclofenac groups), except for one high dose combination male which had increased alkaline phosphatase activity (236% compared to a mean control values of 1460 IU/l); increased urea (, males in both high dose groups and 27-54% in females in all treated groups); and increased creatinine levels (21%, in high dose diclofenac males and (13%, 28% and 38% in females in the low and high dose diclofenac groups and in the high dose combination groups, respectively. The high dose diclofenac female, (No. 87-6908), which was killed in extremis during week 2 also showed dramatic changes in blood biochemistry (i.e. large increases in plasma urea, creatinine, triglycerides, potassium, aspartate aminotransferase, and globulin and decreased albumin, total protein, calcium and chloride) indicative of acute renal failure. With the exception of chloride levels, which remained elevated in low and high dose diclofenac animals (2.7%), all other changes in blood chemistry were not observed following the recovery period.

7. Urinalysis: Urinalysis was not indicated.

8. Physical/Electrocardiographic/Ophthalmologic Examinations: No treatment-related changes in rectal temperature were observed. Other observations made during physical examinations (i.e. loose stools, salivation, wounds) are reported under Observed Effects (above). Various electrocardiographic differences including: reductions in P wave amplitude (low dose combination group versus low dose diclofenac group); decreased heart rate and/or increased QT intervals (mid dose combination group and low dose diclofenac group versus controls); slight decrease in PQ and QT (mid dose combination group compared to controls) and persistent sinus arrhythmia with a low heart rate (96 bpm) at week 24 (1 low dose diclofenac male) were observed. However, ECG changes were not dose-related and did not occur in high dose groups. Therefore their relationship to treatment is unknown. Several ophthalmoscopic observations were noted, however, these appeared incidental in nature, since they occurred sporadically and were not dose related.

9. **Organ Weights:** Treatment-related changes in absolute and/or relative weights for organs are summarized in Table 4, (succeeding page). Briefly, treatment-related changes in organ weights occurred mainly in males of both high dose groups and included: 1) increased relative weights (organ to body weight ratios) for liver and kidney; 2) decreased absolute and relative weights for testes, epididymis, and prostate (HD only); and 3) decreased absolute and relative weights for thymus in HD). In comparison, high dose females (HD and HC) only showed decreased absolute and relative weights for uterus. At the end of the reversal period kidney weights (absolute and relative) remained mildly elevated) but only in the HC group. Treated males which underwent the recovery period showed a dramatic rebound in absolute and relative weights for reproductive organs (testes, epididymis and prostate).

TABLE 4. Treatment-related changes in absolute and/or relative (organ/final body weight [ORG/FBW]; and organ/brain weight [ORG/BRN]) organ weights for male and female monkeys (expressed as a percent change from respective control values).

SEX	Organ	Dose	Absolute	Relative Weights	
			Δ% in Wt	Δ% ORG/FBW	Δ% ORG/BRN
M	Liver	LD	----	↑ 11*	----
		HD	----	↑ 19*	----
		HC	----	↑ 18*	----
M	Kidney	HD	----	↑ 19*	----
		HC	----	↑ 19*	----
M	Testes	LD	↓ 28	↓ 35	↓ 29
		HD	↓ 67*	↓ 62	↓ 64
		HC	↓ 70*	↓ 69	↓ 71
M	Epididymis	LD	↓ 16	----	----
		HD	↓ 47*	↓ 33	↓ 33
		HC	↓ 37*	↓ 33	↓ 33
M	Prostate	HD	↓ 27	----	↓ 50
M	Pituitary	MC	↑ 28*	↑ 21	↑ 33
		HC	↑ 35*	↑ 41	↑ 33
M	Thymus	HD	↓ 66	↓ 57	↓ 57
F	Uterus	HD	↓ 49	↓ 42	↓ 50
		HC	↓ 29	↓ 20	↓ 25

* = Statistically significant change from control values.
---- = Comparable to control values
↑ or ↓ = increased or decreased relative to mean control weights
LD = Low Dose Diclofenac (6 mg/kg)
HD = High Dose Diclofenac (50 mg/kg)
LC = Low Dose Combination Misoprostol:Diclofenac (24 ug:6 mg/kg)
MC = Mid Dose Combination Misoprostol:Diclofenac (68 ug:17 mg/kg)
HC = High Dose Combination Misoprostol:Diclofenac (200 ug:50 mg/kg)

Increased absolute and relative organ weights for recovery males were as follows: 1) for testes (increases 140-220% in Low Dose Diclofenac group [LD], in HD, in low dose combination [LC] groups, in mid dose combination [MC] groups, and in HC); epididymis in HD, 33-60% in LC, in MC and in HC); and prostate (50-100% in LD, in HD, in LC, ; in MC and in HC) Finally, recovery males exhibited increased absolute and relative weights for adrenal glands (in HD) and dose-dependent decreases in absolute and relative weights for thymus (in HD, in MC and in HC). HD recovery females also showed increased absolute and relative weights for ovaries ( ) and thymus ( ) and decreased absolute and relative weights for uterus (. In comparison, HC recovery females only showed increased absolute and relative pituitary weights (%).

10. **Gross Pathology:** Gross pathological findings in animals which died or were sacrificed in extremis (1 male and 2 females in the high dose diclofenac groups and 1 male in the high dose combination group) included one or more of the following: ulcers/erosions of the skin, hemorrhage in the stomach, ulcer(s) in the duodenum, discoloration of the jejunum, colon, heart, kidney and/or lung, enlarged lymph nodes, and fibrous adhesions in the lung, liver, heart (pericardial adhesion) and brain (meningeal adherence).

Gross findings in animals sacrificed at the end of the treatment included: 1) hyperemia and or hemorrhage of the cecum and duodenum (2 of 3 males and 2 of 3 females in the high dose diclofenac [HD] groups, and 1 of 4 females in the mid dose combination [MC] group), 2) hyperemia of the jejunum was also seen in 1 of the 2 aforementioned HD females; 3) enlarged lymph nodes (2 of 3 HD males); 4) fibrous adhesions/plaques around the stomach and/or liver were also seen in 1 of 3 HD males and 1 of 4 LD males. In addition, treated males exhibited an overall increased incidence of nodules (brown or hyperemic) in the cecum, colon and/or jejunum (2 of 6 MC males, 3 of 6 HC males,3 of 6 LD males and in 1 of 6 HD males versus 0 of 6 males each in the control and low dose combination groups). In contrast, the incidence of these types of nodules in treated females was comparable to that seen in control females. Gross pathological changes observed in recovery animals included: hyperemia of the cecum (1 of monkey/sex in the HD group and 1 of 2 males in the HC group. Other gross pathological findings, such as the presence of parasites (Oesophagostomum spp.), and a limited incidence of enlarged ovaries, spleen, thymus and thyroid occurred either at comparable incidence in control animals or sporadically.

11. Histopathology: Treatment-related histological changes in animals which died or were sacrificed in extremis (1 male and 2 females in the high dose diclofenac groups and 1 male in the high dose combination group) included one or more of the following: peritonitis of the stomach, duodenum or liver; congestion of the duodenum, jejunum and colon; mucosal ulcerations in the cecum and or duodenum and ulcerations of the skin. One or both of the aforementioned high dose diclofenac females also exhibited cortical tubular dilation and glomerulonephritis of the kidney, acute sinusoidal congestion of the spleen, reactive hyperplasia/ nonspecific lymphadenitis in the mesenteric lymph node, fibrous adhesions of the lungs and acute myocarditis and pericarditis. Whereas the aforementioned HD male which died showed portal subacute inflammation in the liver, sinusal edema in the mesenteric lymph node, acute myocarditis, acute testicular inflammation and degeneration, and epididymitis.

Treatment-related histological findings observed in monkeys sacrificed at the end of the treatment period included: mucosal ulceration/peritonitis of the stomach (1 of 4 HD males), duodenum (1 of 4 HD males), and cecum (3 of 4 MC females, 2 of 3 males and 3 of 3 females each, in the HC group and HD groups); mucosal hemorrhage in the cecum (1 of 3 HD males); cortical tubular dilation of the kidney (1 of 4 MC females, 2 of 3 HC females and 1 monkey/sex in the HD group); glomerulonephritis 1 of 3 females each at the HC and HD groups); peritonitis in the liver (1 of 3 HD males); portal subacute inflammation of the liver (1 of 4 LD males and 1 of 3 HD females); lymphoid hyperplasia of the spleen (1 of 4 LC males, 1 of 3 males and 2 of 3 females in the HC group, 1 of 4 LD females and 2 of 3 monkeys/sex in the HD group); increased incidence of reactive hyperplasia/nonspecific lymphadenitis in the mesenteric lymph node (2 of 3 HC males, 3 of 3 HD males and 2 of 2 HD females versus none of 4 males and only 1 of 4 female control animals) and sinusal edema in the mesenteric lymph node (1 of 3 HC males).

Histological observations in recovery monkeys included: mucosal hemorrhage of the cecum (2 of 2 HC males and 1 of 2 males each in the LD and HD groups; and lymphoid hyperplasia of spleen (1 monkey/sex in the LD group); myeloid hyperplasia of the spleen (both HC recovery males); reactive hyperplasia/nonspecific lymphadenitis and sinusal edema of the mesenteric lymph nodes (1 of 3 MC males and 1 of 2 HC females, respectively). Intralobular hemorrhage of the thymus (both HC recovery females) and myositis of the skeletal muscle (one of 2 recovery females each in the HC and HD groups) were also seen in recovery animals. However, neither of these findings were observed in animals at the end of the treatment period.

12. **Plasma Levels of the Drug:** Data on the absorption of orally administered diclofenac, alone or in combination with misoprostol on days 1, 85 (week 13), and 176 (week 26) in monkeys is presented in Table 5, (succeeding page). Briefly, diclofenac and the combination of diclofenac and misoprostol were rapidly absorbed following oral dosing with maximal plasma levels of diclofenac attained between 0.25 and 1.21 hr in all dose groups. The addition of misoprostol did not affect absorption or pharmacokinetics of diclofenac, with the possible exception of females in the high dose combination group on day 176, where Cmax and AUC values were approximately double those seen in the high dose diclofenac group. Increases in plasma concentrations and AUC values on days 1, 85 (week 13) and 176 (week 26) appeared linear but disproportional to dose (greater than expected) in both groups, with no sex-related differences observed. Finally, there was no evidence of accumulation with repeated dosing since, plasma concentrations and AUC values tended to be some what lower (both sexes) on day 176 compared to day 1 of dosing.

Table 5. Mean Cmax and AUC values for Diclofenac in Plasma in Cynomolgus Monkeys Following Oral Administration of Diclofenac Alone or in combination with misoprostol on Days 1, 85, and 176.

		Cmax (ug/ml)			AUC (ug·hr/ml)		
Group #	Sex	Day 1	Day 85	Day 176	Day 1	Day 85	Day 176
Control	M	0.0	0.0	0.0	0.0	0.00	0.0
LD	M	5.0	3.89	4.5	5.9	5.97	5.1
HD	M	88.1	50.19	44.5	225.0	125.48	132.3
LC	M	5.0	5.22	7.0	8.0	6.64	8.6
MC	M	28.7	14.30	12.1	40.8	28.53	22.7
HC	M	111.4	70.38	61.7	219.9	169.11	150.7
Control	F	0.0	0.00	0.0	0.0	0.00	0.0
LD	F	5.0	6.24	3.6	6.6	6.24	4.5
HD	F	100.5	35.40	25.3	200.2	76.35	53.2
LC	F	7.4	2.46	4.8	9.9	2.79	5.6
MC	F	25.4	12.88	15.7	36.5	20.71	21.8
HC	F	93.9	37.42	48.6	202.2	101.16	104.4

Control = Vehicle controls
LD = Low dose Diclofenac [6 mg/kg]
HD = High dose Diclofenac [50 mg/kg]
LC = Low dose Diclofenac/misoprostol [6 mg/kg: 24 ug/kg]
MC = Mid dose Diclofenac/misoprostol [17 mg/kg:68 ug/kg]
HC = High dose diclofenac/misoprostol [50 mg/kg:200 ug/kg]

The instability of SC-30695 (the main metabolite of misoprostol) and other analytical problems prevented obtaining full results for absorption of misoprostol. However, SC-30695 metabolite data from day 176 suggested that misoprostol was well absorbed with average plasma concentrations of 439 and 684 pg/ml, 1299 and 1183 pg/ml, and 2400 and 2120 pg/ml in male and female monkeys in the low, mid, and high dose combination groups, respectively.

In conclusion, misoprostol had no adverse toxicological interaction on the toxicity profile of diclofenac following 6-month oral administration in monkeys. Diclofenac alone or in combination with misoprostol produced treatment-related clinical signs including: reduced motor activity, prostration, convulsions, mild losses in body weights and death in both high dose groups. Alterations in hematology (males in both high dose groups and females in the high dose diclofenac group) and blood chemistry (both diclofenac groups and mid and high dose combination groups) were also observed. Urinalysis was not indicated. Target organs of toxicity included: 1) organs of the GI tract (i.e. stomach, duodenum, jejunum, cecum and colon), 2) kidney; and 3) the liver. Ulcerations of the skin, along with increased numbers neutrophils and decreased lymphocytes in high dose animals also suggested possible treatment-related effects on immune function. Toxic effects occurred in animals of the mid and high dose combination groups and the low and high dose diclofenac groups (no mid dose diclofenac group included) and were more prevalent and severe in males versus females and in the diclofenac versus combination groups. Pharmacokinetic analysis showed that in general, misoprostol did not affect the absorption or pharmacokinetics of diclofenac in the monkey. The low dose combination dosage 24 µg/kg misoprostol plus 6 mg/kg diclofenac could be considered the no effect dose in the monkey.

MUTAGENICITY:

1. Ames Assay of Diclofenac Sodium/Misoprostol in *Salmonella typhimurium* (Study No. PSA-89S-3556)

This study

A copy of the review follows:

Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) (Study No. S.A. 3556)

Testing Laboratories:

Study Started: July 18, 1989

Study Completed: September 28, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch No.: Diclofenac, Lot No. 8935025
Misoprostol, Lot No. 3D07510

Methods: The mutagenic activity of Misoprostol/diclofenac (1:250 ratio) at concentrations of 0.04/10, 0.2/50, 0.4/100, 2/500, 4/1000 and 20/5000 ug/plate, was tested in two independent studies using the AMES test (standard method); tester strains Salmonella typhimurium TA97, TA98, TA100, TA1535 and TA1538 in both the presence and absence of an S-9 mix (metabolic activation system). The basis of dose selection was not provided, however, a maximum concentration of 5000 ug/plate of test agent is standard for the assay system. Positive controls in tests without S-9 metabolic activation were: sodium azide (1 ug/plate) for test strains TA1535 and TA100, 2-nitrofluorene (2.5 ug/plate) for strains TA1538 and TA98, and IRC-191 acridine (0.5 ug/plate) for Strain TA97, whereas, 2-aminoanthracene (1 ug/plate) was used for all 5 strains in the presence of the S-9 metabolic activation system. Criteria for a positive mutagenic effect in the above assays was a dose-related increase in the mean number of revertants/plate of at least 2 times greater than the vehicle control at two or more successive doses.

Results: At the highest concentration of 20/5000 ug/plate, Misoprostol/diclofenac was cytotoxic, as evidenced by reduced or eliminated growth of background lawn. However, Misoprostol/ diclofenac, at concentrations ranging from 0.04/10 to 20/5000 ug/plate produced no treatment-related increases in the numbers of revertant colonies in any of the bacterial strains tested in the presence or absence of a S-9 metabolic activation system. In comparison, the positive controls produced the expected increases in histidine revertant colonies, supporting the validity of the study. Therefore, the combination of Misoprostol/diclofenac, tested negative for mutagenic activity (induction of revertant strains) in the Ames assay.

2. Mutagenic Potential of Diclofenac Sodium/Misoprostol in the CHO/HGPRT Assay (Study No. PSA-89S-3549)

This study

A copy of the review follows:

CHO/HGPRT In Vitro Mammalian Cell Mutation Assay with Misoprostol/Diclofenac (Report No. S.A. 3549)

Testing Laboratories: G.D. Searle & Co., Skokie IL

Study Started: July 26, 1989

Study Completed: September 28, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch No.: Diclofenac, Lot No. 8935025
Misoprostol, Lot No. 3D07510

Methods: The ability of misoprostol/diclofenac to induce a mutation at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus was tested in cultured Chinese hamster ovary (CHO) cells. Briefly, CHO cells were exposed to misoprostol/diclofenac at a ratio of 1:250, and concentrations of 0.05/12.5, 0.40/100, 0.80/200, and 1/250 ug/ml misoprostol/diclofenac for approximately 20-24 hours in the absence of the S-9 metabolic activation system and approximately 4 hours in the presence of the S-9 metabolic activation system. Dose selection was based on a Range finding cytoxicity tests, where cytoxicity was observed beginning at concentrations of 0.06/15.6 ug/ml, with complete cytoxicity observed at 1.00/250 ug/ml. Exposed cells were then subcultured at 1 x 10^6 cells/100 mm dish (1 dishes/treatment culture) for the mutant expression cultures. Parallel Day 1 cytotoxicity tests were also conducted on subcultured cells (200 cells/dish; 4 dishes/treatment culture) which were fixed, stained, and scored after 7 days of incubation. Mutant expression cultures were incubated for 1 day in normal medium and for 6 days in a low serum medium. Cells were then changed to a complete medium and after 1 additional day subcultured into the mutant selection medium (Complete F12 medium plus 10 uM 6-thioguanine) at 0.2 x 10^6 cells/100 mm dish and in the complete F12 medium at 200 cells/60 mm plate for the day 9 cytoxicity test. Subcultured cells were allowed to grow for an additional 7 days and then fixed and stained. Colonies of 50 cells or more in the mutation and parallel cytoxicity dishes were then scored for calculation of the mutation frequency and % cell survival, respectively. IRC-91 acridine (IRC; 1 ug/ml) and 3-methylcholanthrene (MCA; 5 ug/ml) were used as positive

controls in the absence and presence of the S-9 metabolic activation system, respectively. A test was considered positive if mutation frequencies were ≥ 15 per 1 x 10^6 cloned cells for at least 2 successive test article concentrations and significantly higher than solvent controls.

Results: Results from the mutation experiment showed that Misoprostol/diclofenac produced a dose-dependent decrease in relative survival compared to vehicle control values from an average of 107% at 0.0.05/12.5 ug/ml to 26% at 0.40/100 ug/ml in the absence of metabolic activation and from 105% at 0.20/50 ug/ml to 22% at 1.00/250 ug/ml in the presence of the metabolic activation system. Misoprostol/diclofenac produced no evidence of increased mutagenesis in terms of an increased frequency of mutant cloned cells at any dose tested with or without activation (i.e. none of the mutant frequencies were greater mutation frequencies were ≥ 15 per 1 x 10^6 cloned cells and significantly different from controls). Both positive controls showed the expected increases in mutant frequencies (IRC = 186 mutants/10^6 cloned cells and MCA = 162 mutants /10^6 cloned cells). Therefore, Misoprostol/diclofenac tested negative for mutagenicity in the CHO/HGPRT assay system.

3. Clasogenic Potential of Diclofenac Sodium/Misoprostol in the Rat Lymphocyte Chromosomal Aberration Assay (Study No. PSA-89S-3560)

This study A copy of the review follows:

Chromosomal Aberrations in Rat Peripheral Blood Lymphocytes
(Study No. S.A. 3560)

Testing Laboratories: G.D. Searle & Co., Skokie IL

Study Started: August 15, 1989

Study Completed: September 28, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Drug Batch No.: Diclofenac, Lot No. 8935025
Misoprostol, Lot No. 3D07510

Methods: A solution of misoprostol and diclofenac, at a ratio of 1:250, misoprostol/diclofenac, and was tested for clastogenic activity in cultured rat peripheral blood lymphocytes. Briefly, cultured rat lymphocytes were arrested at metaphase and examined for chromosomal aberrations including, chromosome and chromatid gaps, deletion/breaks, and exchanges, following exposure to the combination of misoprostol/diclofenac at concentrations of 12.5, 25, 37.5, 50 and 62.5 ug/ml (expressed as the concentration of diclofenac), for 17 hours in the absence of an Aroclor-induced S-9 activation system and for 2 hours in the presence of an Aroclor-induced S-9 activation system. The high dose level, 62.5 ug/ml was selected based on cytotoxicity in a range finding study, where the lowest concentration of misoprostol/ diclofenac tested, 0.40/10 ug/ml decreased relative survival of the cells by 25 and 56% in the presence and absence of a metabolic activation system, respectively. Negative, vehicle (dimethylsulfoxide) and positive controls, triethylenemelamine (0.20 ug/ml), in the absence of the S-9 fraction and cyclophosphamide (7.5 ug/ml), in the presence of the S-9 fraction, were also included for validation of the studies. A separate study was conducted to determine the effects of misoprostol 0.001 /0.25 to 0.2/50 ug/ml (misoprostol/ diclofenac) with or without metabolic activation on cell cycle kinetics (mitotic index). A response was considered positive if the percent aberrant cells for at least two successive test article concentrations was significant or one dose level was significant with an indication of a dose response relationship compared to negative control values.

Results: Results from the cell cycle kinetics study showed that the presence and absence of the S-9 fraction, the high dose (0.2/50) misoprostol/diclofenac reduced the mitotic index by 38 and 64%, respectively, whereas lower doses tested (0.075/18.75 and 0.1/25 ug/ml) had minimal to no effects on cell cycle kinetics with or without the metabolic activation system. Mitotic indices in the clastogenicity study ranged from 1 to 11% in the misoprostol/diclofenac treated groups, compared to 17% in the negative vehicle controls indicative of severe to moderate cytoxicity at the doses tested. However, each culture had at least 50 metaphase cells which were evaluated for clastogenicity. Evaluation of the three highest doses tested 0.15/37.5, 0.21/50 and 0.25/62.5 showed that in either the presence or absence of the metabolic activation system, none of the concentrations evaluated produced treatment-related increases in numbers of aberrant cells, relative to the DMSO control values. In contrast, the positive controls, triethylenemelamine and cyclophosphamide produced average incidence of 68% and 59% aberrant cells in the in the absence and presence of the S-9 fraction metabolic activation system, respectively. Therefore, misoprostol/diclofenac tested negative for clastogenic activity in the rat peripheral blood lymphocyte chromosome aberration assay.

4. Potential of Diclofenac Sodium/Misoprostol to Induce Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells (Study No. PSA-89S-3558)

This study

A copy of the review follows:

Misoprostol/Diclofenac: Micronucleus Test in Mice Following Oral Administration (Study No. S.A. 3558)

Testing Laboratories: G.D. Searle & Co., Skokie IL

Study Started: July 27, 1989

Study Completed: September 28, 1989

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female Cr:CD_1 Mice, approximately 6 weeks of age; weighing between 23 and 35 g.

Drug Batch No.: Diclofenac, Lot No. 8935025
Misoprostol, Lot No. 3D07510

Methods: Misoprostol and Diclofenac were dissolved in a 25% polyethylene glycol 400 solution at a ratio of 1:250 and administered to groups of 12 mice (6/sex) twice by oral gavage, 24 hours apart, at doses of 0.12/30, 0.24/60 and 0.48/120 mg/kg/day of misoprostol/diclofenac. Dose selection was based on lethality of (misoprostol/diclofenac) at doses $\geq$ 0.8/200 mg/kg (misoprostol/diclofenac) in a previous dose range finding study in mice (Report No. PSA-89S-3557). The high dose level 0.48/120 mg/kg/day (misoprostol/ diclofenac) was selected as a dose level expected to produce some clinical signs of toxicity without excessive mortality. The lower doses were selected at half fold intervals from the high dose. Both negative (vehicle) and positive (cyclophosphamide, 20 mg/kg/day) controls were concurrently tested. Mice at each dose level were killed at approximately 24 hours after the second dose, followed by harvest and examination of the bone marrow for chromosomal damage in polychromatic erythrocytes (PCEs, approximately 1000/animal), as indicated by the presence of micronuclei. A test result was considered positive if a statistically significant increase in the frequency of micronucleated PCEs, relative to the concurrent vehicle controls. A test was considered negative if the criteria for a positive response were not met.

Results: The high dose of Misoprostol/Diclofenac (0.48/120 mg/kg/day) was lethal in 1 male and 2 female mice. Thus only 4 female mice were evaluated at the high dose. Since no indication of a sex related difference in micronuclei induction, values for male and female mice were pooled for statistical comparisons. Results from these tests showed no significant increases in the frequencies of micronuclei in any of the test-article treated groups compared to concurrent controls. In contrast administration of cyclophosphamide induced a significant increase in the frequency of micronucleated PCEs supporting the validity of the test. Evaluation of the relative proportions of polychromatic cells to total erythrocyte population also suggested that Misoprostol/Diclofenac had no cytotoxic or cytostatic effects at dose levels of 0.12/30, 0.24/60 and 0.48/120 mg/kg/day of misoprostol/diclofenac in mice. Therefore, under the current test conditions that the combination of Misoprostol/Diclofenac had no clastogenic, cytotoxic or cytostatic effects (measured in terms of PCE micronuclei and erythrocyte maturity) in mice at i.v. doses 0.12/30, 0.24/60 and 0.48/120 mg/kg/day (misoprostol/diclofenac).

REPRODUCTIVE TOXICOLOGY:

1. Segment II Oral Teratogenic Study of Diclofenac Sodium/Misoprostol in Rabbits (Study No. PSA-87S-3110)

This study . A copy of the review follows:

Dose-Range Finding Studies of misoprostol/diclofenac in Rabbits

Testing laboratory: Sponsor at Skokie facility.

Date of the study: Nov. 4-Dec. 3, 1986 (1st study) and March 10-April 8, 1987 (2nd study).

In the first study, misoprostol/diclofenac were administered to six groups of 6 rabbits each from days 6 through 18 of gestation period at dosage levels of 40 mcg/kg/day misoprostol/ 10 mg/kg/day diclofenac, 120 mcg/kg/day misoprostol/30 mg/kg/day diclofenac, and 400 mcg/kg/day misoprostol/100 mg/kg/day diclofenac. Three other groups each received diclofenac alone at dosage levels of 10, 30, and 100 mg/kg/day. A control group received vehicle (0.5% methylcellulose and 0.1% polysorbate 80 in distilled water). There were drug-related clinical signs of low food intake, not eating and loose stools in all drug-treated groups. There were mortalities in all treatment groups except in the low combination group. There was an increase in numbers of resorptions for all drug-treated groups.

In the second dose-range finding study, dosage levels used were: vehicle control, 4 mcg/kg/day misoprostol/1 mg/kg/day diclofenac, 12mcg misoprostol/3 mg/kg/day diclofenac, 40 mcg/kg/day/10 mg/kg/day diclofenac, 1,3, or 10 mg/kg/day of diclofenac alone. There were no deaths, clinical signs and maternal body weight changes in any drug-treated groups. There were no adverse effects on numbers of corpora lutea, implantations, resorptions and live or dead fetuses in any drug-treated groups.

Segment II Teratologic Study of Misoprostol/Diclofenac in Rabbits

Testing laboratory: Sponsor's facility at Skokie.

Date of the study: May 5 to June 11, 1987.

GLP requirement: A statement of compliance with GLP regulations was included. However, quality assurance statement was not included.

Animals: New Zealand White rabbits weighing 3.1 to 3.9 kg were used.

Methods: Four groups of animals each consisting of 15 artificially inseminated pregnant rabbits were given vehicle , 4 mcg/kg/day misoprostol/1 mg/kg/day diclofenac, 12 mcg/kg/day misoprostol/3 mg/kg/day diclofenac, and 40 mcg/kg/day misoprostol/10 mg/kg/day diclofenac orally on days 6 through 18 of gestation period. The doses of misoprostol (batch no 01300) and diclofenac (lot # C0785) were prepared as fresh daily suspensions in a volume of 4 ml/kg. All fetuses were subjected to visceral and skeletal examinations.

Results:

Mortality: One animal in the high combination died due to drug-related effect. Additional four animals, two each from the control and low combination died due to intubation errors.

Clinical signs: Low food intake was seen in the high combination group.

Body weight: In the high combination groups, there was a significant retardation (43%) in maternal body weight gain.

Dams: In the high combination group, there was a significant increase (7 times) in number of resorptions. No adverse effects on fetal weight were seen at any dosage level. There were no abnormal external, viseral and skeletal findings in the study.

In conclusion, no teratogenic effects due to combined treatment were observed in rabbits at dosage levels up to 40 mcg/kg/day misoprostol and 10 mg/kg/day diclofenac equavalent to 2.5 times the recommended maximum human dose. Significant retardation in body weight and increase in resorption numbers were reported in the females of high combination group.

4 Page(s) Redacted

DRAFT Labeling

Diclofenac Sodium

Clinical signs that may indicate diclofenac sodium overdose include GI complaints, confusion, drowsiness or general hypotonia.

Misoprostol

Clinical signs that may indicate an overdose are sedation, tremor, convulsions, dyspnea, abdominal pain, diarrhea, fever, palpitations, hypotension or bradycardia.

ARTHROTEC®

Symptoms of ARTHROTEC® overdosage should be treated with supportive therapy. In case of acute overdosage, gastric lavage is recommended. Induced diuresis may be beneficial because diclofenac sodium and misoprostol metabolites are excreted in the urine. The effect of dialysis on the elimination of diclofenac sodium (99% protein bound) and misoprostol acid remains unproven. The use of oral activated charcoal may help to reduce the absorption of diclofenac sodium and misoprostol.

SUMMARY AND EVALUATION:

Diclofenac sodium is a NSAID derivative and possesses anti-inflammatory, analgesic and antipyretic activity. The more predominant adverse effects of diclofenac sodium include the production of peptic ulceration and gastrointestinal bleeding. Diclofenac sodium is currently marketed in the U.S. and is indicated for the acute and chronic treatment of signs and symptoms of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Diclofenac sodium is available in a delayed-release formulation (Volataren®) and an immediate-release formulation (Cataflam®); Cataflam® is also indicated for the management of pain and primary dysmenorrhea.

Misoprostol is a prostaglandin E_1 derivative; it possesses antisecretory (inhibiting gastric acid secretion) and mucosal protective properties. Misoprostol is contraindicated in pregnant women because of its abortifacient property. Misoprostol (Cytotec®) is currently marketed in the U.S. and is indicated for the prevention of NSAID-induced gastric ulcers in patients at high risk of developing gastric ulceration.

Thus, it is apparent that there are some patients who receive concurrent diclofenac sodium and misoprostol medication. However, the sponsor suggests that the effectiveness of coadministration of diclofenac sodium and misoprostol is limited by (1) inconvenience to the patient of taking two sets of

medication, (2) possible mismatching of the diclofenac sodium dosing regimen and the misoprostol dosing regimen, and (3) incidence of adverse effects associated with the currently recommended 200 mcg qid dose of misoprostol (800 mcg/day).

Therefore, the sponsor is seeking approval for the marketing and use of ARTHROTEC® (fixed combinations of diclofenac sodium and misoprostol) for acute and chronic treatment of the signs and symptoms of osteoarthritis and rheumatoid arthritis in patients at risk of developing NSAID-induced gastrointestinal ulcers. The highest recommended daily dose of ARTHROTEC® 50 (50 mg of diclofenac sodium and 200 mcg of misoprostol; 3 times per day) would deliver 150 mg/day of diclofenac sodium and 600 mcg/day of misoprostol. The highest recommended daily dose of ARTHROTEC® 75 (75 mg of diclofenac sodium and 200 mcg of misoprostol; 2 times per day) would deliver 150 mg/day of diclofenac and 400 mcg/day of misoprostol.

In support of NDA 20-607, the sponsor submitted several preclinical studies of diclofenac:misoprostol combinations including pharmacology studies in mice and rats, acute oral toxicity studies in mice and rats, 4-week and 6-month oral toxicity studies in rats, 4-week oral toxicity study in dogs, 6-month oral toxicity study in monkeys, mutagenic studies (Ames test, forward mutations in Chinese hamster ovary cells, chromosomal aberrations in rat lymphocytes, mouse micronucleus assay), and a Segment II oral teratogenic study in rabbits. Pharmacology data were original; all other data had been

In preclinical pharmacological studies, it was shown that misoprostol inhibited diclofenac-induced gastric ulcers in rats; this directly supports the proposed marketing indication for ARTHROTEC® 50 and ARTHROTEC® 75. Moreover, diclofenac reduced the severity of adjuvant-induced arthritis in a rat model; diclofenac:misoprostol combinations produced similar reductions in severity. Thus, misoprostol did not alter the anti-inflammatory effects of diclofenac in this rat model. Furthermore, 100:1 and 10:1 diclofenac:misoprostol combination doses did not alter inhibition of carrageenan-induced paw edema in rats, compared to diclofenac alone.

In acute oral toxicity studies in mice; the minimum oral lethal dose of misoprostol in male and female mice was not determined (>20 mg/kg); clinical signs of toxicity were protraction and reduced motor activity. The minimum oral lethal dose of diclofenac was 50 mg/kg in males and females; clinical signs of toxicity were reduced motor activity, prostration and convulsions. The minimum oral lethal dose of the

misoprostol:diclofenac combination (2:500 ratio) was 0.4:100 mg/kg in males and 0.8:200 mg/kg in females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:375 ratio) was 0.53:100 mg/kg in males and 1.07:200 mg/kg in females. In combination studies, clinical signs of toxicity were reduced motor activity, prostration and convulsions. Thus, in mice, toxicity of misoprostol:diclofenac combinations was no greater than that of diclofenac alone; there was no significant difference between sexes.

In acute oral toxicity studies in rats; the minimum oral lethal dose of misoprostol in male and female rats was not determined (>20 mg/kg); clinical signs of toxicity were loose stools, prostration and reduced motor activity. The minimum oral lethal dose of diclofenac was 150 mg/kg in males and 100 mg/kg in females; clinical signs of toxicity were loose stools, prostration, reduced motor activity and arched back/standing hair. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:500 ratio) was 0.9:225 mg/kg in males and 0.4:50 mg/kg in females. The minimum oral lethal dose of the misoprostol:diclofenac combination (2:375 ratio) was 1.2:225 mg/kg in males and 0.27:50 mg/kg in females. In combination studies, clinical signs of toxicity were loose stools, prostration, reduced motor activity and arched back/standing hair. Thus, in rats, toxicity of misoprostol:diclofenac combinations was no greater than that of diclofenac alone; there was no significant difference between sexes.

In a 4-week oral toxicity study of diclofenac alone (0.5 and 6.0 mg/kg/day) and misoprostol:diclofenac combinations (0.002:0.5, 0.008:2.0 and 0.024:6.0 mg/kg/day) in the rat, the no effect dose of diclofenac alone was 0.5 mg/kg. The 6.0 mg/kg/day dose of diclofenac alone produced gastric lesions, atrophy/inflammation/fibrosis in the pancreas, and focal hepatic granulomas. The no effect dose of the misoprostol:diclofenac combination was 0.002:0.5 mg/kg/day. The toxicity and pharmacokinetics of orally administered diclofenac and diclofenac:misoprostol combinations did not differ in the rat.

In a 6-month oral toxicity study of diclofenac alone (1, 2.5 and 6 mg/kg/day) and misoprostol:diclofenac combinations (0.004:1, 0.01:2.5 and 0.024:6 mg/kg/day) in rats, the no effect dose of diclofenac alone was 1 mg/kg/day. The 2.5 mg/kg/day dose of diclofenac alone produced granular mucosal ulceration in the stomach, colonic lymphoid hyperplasia and uterine luminal distention; the 6 mg/kg/day dose of diclofenac alone produced granular mucosal ulceration in the stomach, extramedullary hematopoiesis in the spleen, mucosal ulceration, parietal granulation and mucosal hyperemia in the jejunum, colonic lymphoid hyperplasia, chronic myocarditis and myocardial necrosis in the heart, adenitis and sinusal edema/cystic spaces in mesenteric lymph nodes, uterine luminal distension and deaths

(3 females; 1 male and 1 female were sacrificed in extremis). The no effect dose of the misoprostol:diclofenac combination was 0.04:1 mg/kg/day). The toxicity and toxicokinetics of orally administered diclofenac and misoprostol:diclofenac combinations did not differ in rats. However, there was increased toxicity in the 6-month study compared to the 4-week study.

In a 4-week oral toxicity study of diclofenac alone (0.5 and 2.0 mg/kg/day) and misoprostol:diclofenac combinations (0.002:0.5, 0.004:1 and 0.008:2 mg/kg/day) in dogs, the minimal effect dose of diclofenac was 0.5 mg/kg/day. The 2.0 mg/kg/day dose of diclofenac alone produced renal papillary edema, renal papillary necrosis, extramedullary hematopoiesis of the spleen, thymic atrophy and deaths (1 male). The minimal effect dose of the misoprostol:diclofenac combination was 0.002:0.5 mg/kg/day. The toxicity and toxicokinetics of orally administered diclofenac and diclofenac:misoprostol combinations did not differ in the kidney, spleen and thymus. However, misoprostol:diclofenac combinations produced treatment-related prostrate hypoplasia, testicular oligospermia and absence of spermatozoa in the epididymis in males, while diclofenac alone only produced absence of spermatozoa in the epididymis. Reproductive organs of male dogs have been previously shown to be target organs of toxicity for misoprostol. Finally, there were no differences in toxicokinetics between diclofenac and diclofenac:misoprostol combinations.

In a 6-month oral toxicity study of diclofenac alone (6 and 50 mg/kg/day) and misoprostol:diclofenac combinations (0.024:6, 0.068:17 and 0.2:50 mg/kg/day) in monkeys, the no effect dose of diclofenac alone was 6 mg/kg/day. The 50 mg/kg/day dose of diclofenac alone produced mucosal ulceration/peritonitis of the stomach, duodenum and cecum, mucosal hemorrhage in the cecum, cortical dilation of the kidney, glomerulonephritis, peritonitis in the liver, lymphoid hyperplasia of the spleen, reactive hyperplasia/nonspecific lymphadenitis in the mesenteric lymph node and deaths (1 male and 2 females were sacrificed in extremis). The no effect dose of the misoprostol:diclofenac combination was 0.024:6 mg/kg/day. The toxicity and toxicokinetics of orally administered diclofenac and diclofenac:misoprostol combinations did not differ in monkeys.

In a Segment II oral teratogenic study of diclofenac alone (10, 30 and 100 mg/kg/day during days 6 through 18 of gestation) and misoprostol:diclofenac combinations (0.04:10, 0.12:30 and 0.4:100 mg/kg/day during days 6 through 18 of gestation) in rabbits, there were no treatment-related teratogenic effects.

Misoprostol:diclofenac combinations were negative in mutagenic studies (Ames test, forward mutations in Chinese hamster ovary cells, chromosomal aberrations in rat lymphocytes, mouse micronucleus assay).

In the safety assessment of ARTHROTEC®, the main issue is whether the toxicity and toxicokinetics of the proposed fixed combinations of diclofenac and misoprostol in the ARTHROTEC® formulations differ from those of either diclofenac or misoprostol alone. In the 4-week and 6-month oral toxicity studies in the rat and the 6-month oral toxicity study in the monkey, there were no differences in the toxicity and toxicokinetics between diclofenac and diclofenac:misoprostol combinations. In the 4-week oral toxicity study in the dog, the misoprostol component of the diclofenac:misoprostol combination produced toxicity in the reproductive organs of male dogs. However, there were no other differences in the toxicity between diclofenac:misoprostol combinations, and there were no differences in toxicokinetic parameters between diclofenac and diclofenac:misoprostol combinations in the dog. Thus, since the sponsor would use approved daily doses of diclofenac and misoprostol in the ARTHROTEC® formulations, the preclinical toxicity data suggest that the proposed clinical use of ARTHROTEC® would be reasonably safe.

One of the inactive ingredients in ARTHROTEC® 50 and ARTHROTEC® 75 is methacrylic acid copolymer
ARTHROTEC® 50 and ARTHROTEC® 75 tablets, respectively). Methacrylic acid copolymer is listed in the INACTIVE INGREDIENT GUIDE (January 1996) and is currently used in marketed drug products at concentrations Thus, the use of methacrylic acid copolymer in ARTHROTEC® 50 and ARTHROTEC® 75 tablets is reasonably safe.

It should be noted that the sponsor submitted a Segment II oral teratogenic study for the misoprostol:diclofenac combination in only 1 species; i.e., the rabbit. It is recommended that Segment II teratogenic studies be done in 2 species. Since the misoprostol component has an abortifacient property, it seems important to assess any interactive effect of misoprostol and diclofenac in a teratogenic study using a second species.

Finally, the reviewer has suggested a revised version for the **Carcinogenesis, mutagenesis, impairment of fertility** section, the **Pregnancy** section, and the **Overdosage** section of the labeling.

RECOMMENDATIONS:

From a preclinical viewpoint, the NDA application is approvable.

APPEARS THIS WAY
ON ORIGINAL

/S/ 8/9/96

Gerald A. Young, Ph.D.
Pharmacologist, HFD-180

cc:
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Young
HFD-345/Dr. Viswanathan

/S/ 8/9/96

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APPEARS THIS WAY
ON ORIGINAL

M E M O R A N D U M

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

DATE: January 31, 1996

FROM: Pharmacology Team Leader
Division of Gastrointestinal and
Coagulation Drug Products, HFD-180

SUBJECT: NDA 20,607 (Arthrotec) - Preclinical Deficiencies

TO: NDA 20,607

The following deficiencies are noted in this application.

1. The following reports of toxicology studies of the combination of diclofenac sodium and misoprostol are not included in this application but referred to NDA 19,268. The sponsor is inconsistent on inclusion of reports of combination drug studies in this application. For example, the report of Segment II. rabbit teratology study of the combination is included in this application and as well as NDA 19,268, but the following reports are not provided in this application:

a. Acute oral toxicity study of the association misoprostol/diclofenac compared to the toxicity of each component in the rat and the mouse.
PSA-87F-0331/0332.

b. Four-week oral toxicity study of the combination of misoprostol/diclofenac in the rat.
PSA-87F-0333.

c. Four-week toxicity study of the combination of misoprostol/diclofenac in the dog.
PSA-87F-0334.

2. Report of a Segment II. teratology study in rats is not available. Sponsor was informed about this deficiency at the pre-NDA meeting. Only the report of a study in rabbits was included.

Sponsor should be asked to provide copies of the reports listed under 1.

/S/

Jasti B. Choudary, Ph.D., B.V.Sc.

cc:
NDA 20,607
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Young

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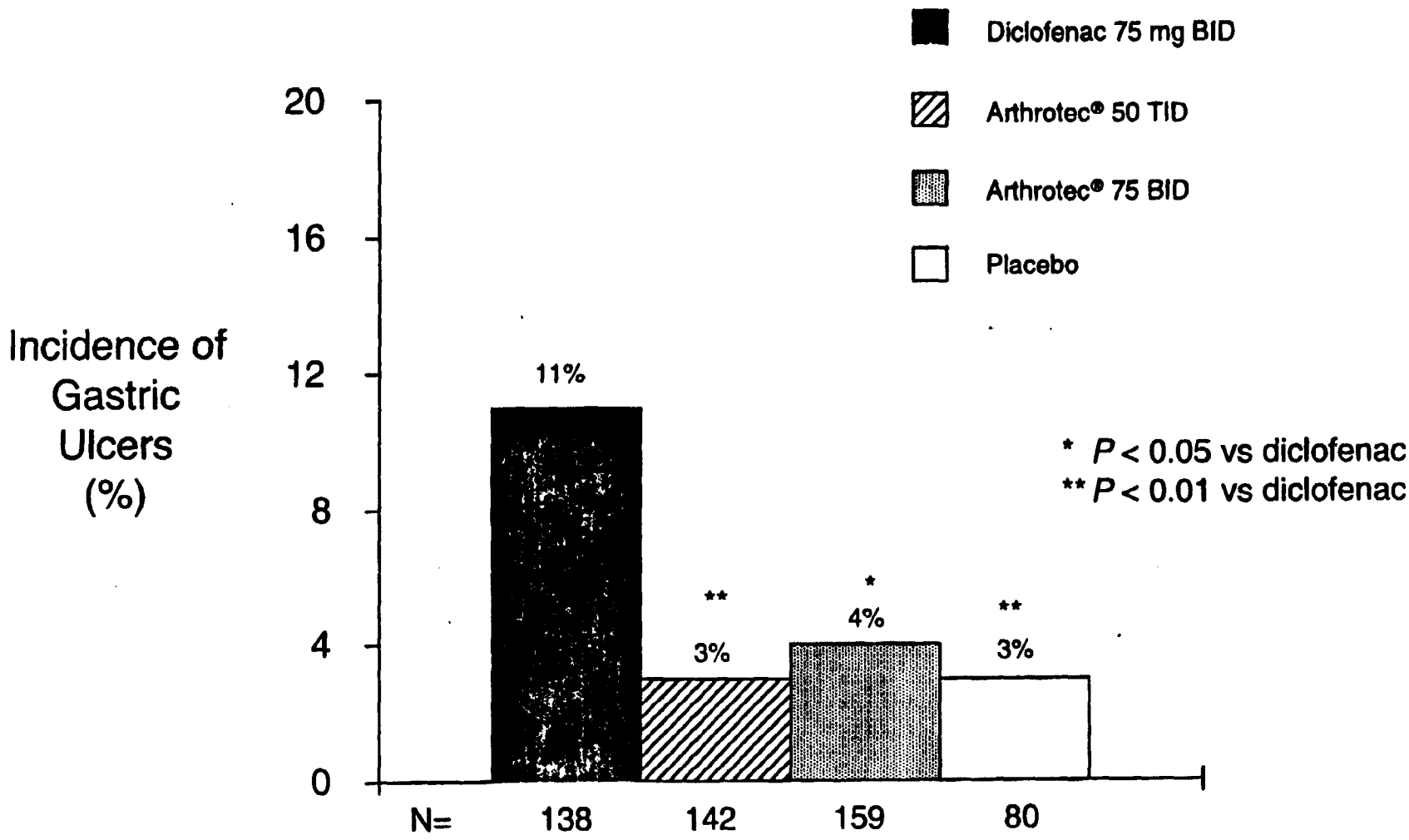
Incidence of Adverse Events Causing Withdrawal in Phase III Fixed Combination Trials

	Arthrotec®		Diclofenac		Placebo	
	%	(N)	%	(N)	%	(N)
	100.0	(2184)	100.0	(1691)	100.0	(146)
Withdrawn due to adverse event	11.2		8.2		4.1	
Abdominal Pain	4.6		3.0		2.1	
Diarrhea	3.2		1.1		0.7	
Nausea	2.1		1.6		0.7	
Dyspepsia	1.3		1.2		0.0	
Flatulence	1.1		0.3		0.0	

Arthrotec®

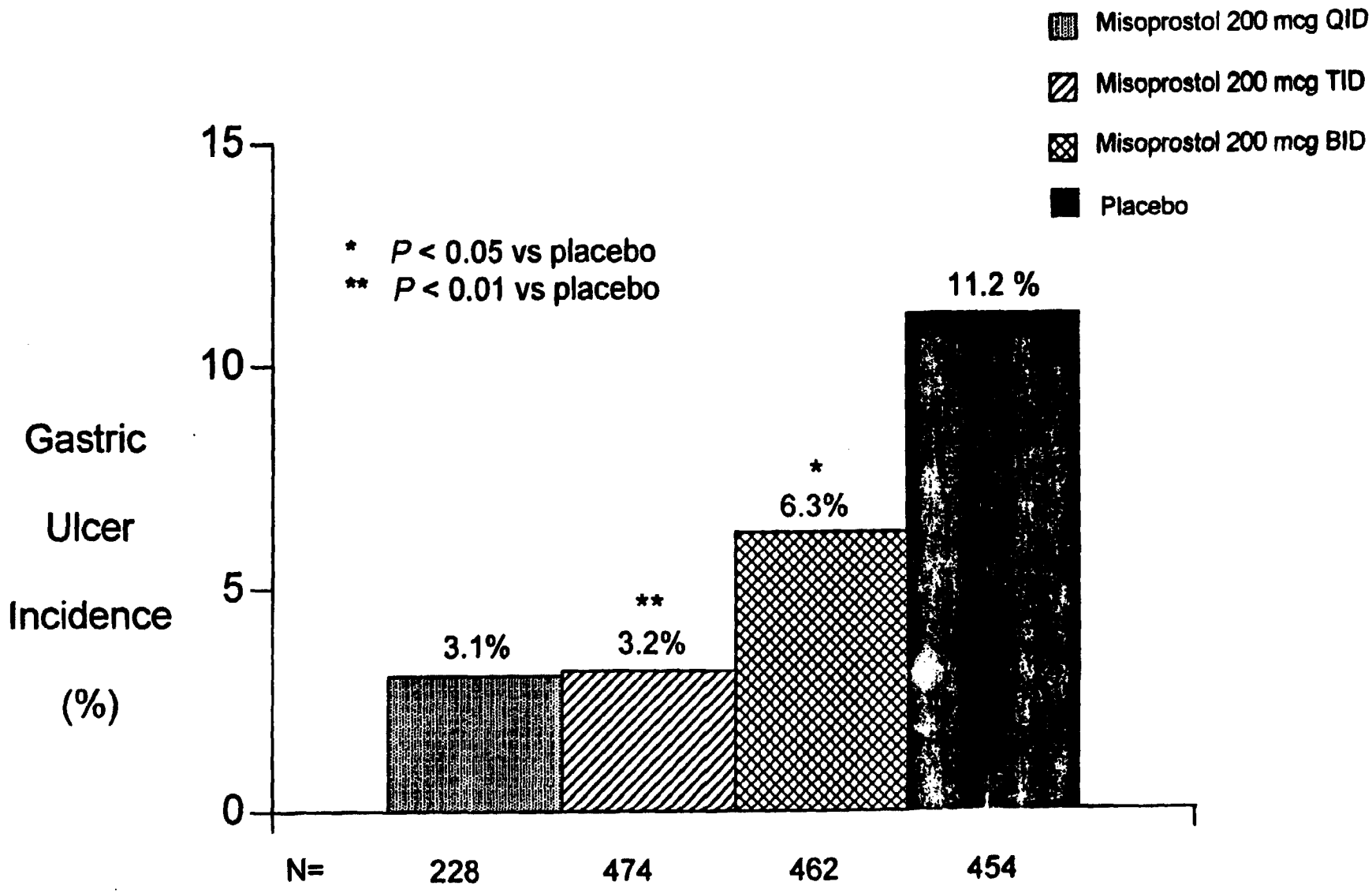
- Misoprostol protects against diclofenac-induced GU
- Two adequate and well controlled studies support the improved GU safety of Arthrotec at BID and TID dosing
  - -349 study
  - -053 study

Incidence of Gastric Ulcers (≥ 3 mm) in Patients Treated with Diclofenac and Diclofenac/Misoprostol (Study NN2-94-02-349)



2/2/96

Incidence of NSAID-Induced Gastric Ulcers (≥ 3 mm) in Patients Treated with Misoprostol 200 mcg BID-QID (12 Weeks)
(Study S81-89-02-053)



N(Various NSAIDs were allowed

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Conclusions

- Misoprostol does not interfere with the anti-arthritic properties of diclofenac in OA and RA
- Arthrotec is associated with a lower incidence of GU than diclofenac
- Safety profile of Arthrotec® is well defined