CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20535

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY OVERALL SUMMARY AND EVALUATION:

The pre-clinical section of NDA 20535 was reviewed by Drs. W.C. Josie Yang (Pharmacology and Toxicology) and Conrad H. Chen (Pharmacokinetics, Reproduction, and Mutagenicity).

Bromfenac sodium, AHR-10282B, is a cyclooxygenase inhibitor possessing analgesic, anti-inflammatory, and antipyretic activities in various animal experimental models. It belongs to a nonsteroidal anti-inflammatory drug class (NSAID) without any narcotic-like activity. Bromfenac did not possess any significant effects on the central nervous system and cardiovascular function.

The pharmacokinetic/pharmacodynamic correlation was studied in the rat and mouse models. In the anti-inflammatory model (carrageenan-induced foot edema), the effective dose of Bromfenac at 0.032 and 0.01 mg/kg for fed and fasted rats, respectively, produced corresponding plasma levels of 0.06 and 0.30 μg/ml. In the mice analgesic model (acetylcholine or phenylbenzoquinone induced writhing), the estimated EC_{50} for Bromfenac were 0.37 and 0.47 μg/ml for fed and fasted mice, respectively.

The absorption and excretion of Bromfenac were rapid in all animal species studied. Food ingestion reduced the bioavailability of Bromfenac in animals and man. Tissue distribution widely occurred, but mainly in organs of elimination. No significant accumulation of the drug was observed. Bromfenac was highly bound to plasma protein (99%). The metabolism and excretion patterns were similar in monkeys and humans. Elimination of Bromfenac and its metabolites occurred mainly via renal pathway in monkey and man and via feces through biliary excretion in rat. In the pregnant rat, Bromfenac entered the fetuses but at a lower level than in the dam. Bromfenac was also found in rat milk at a lower concentration than that in the plasma of lactating dam.

In the acute toxicity study, the LD_{50} for female rats was 39.6 mg/kg po and 15.0 mg/kg iv, and for male rats was 46.0 mg/kg iv. The predominant toxicity observed in these studies was GI related. Hemorrhagic spots in the GI tract, thickened intestinal walls, and adhesions of intestine to peritoneal walls were major characteristics of GI lesions. Kidney toxicity was also observed, which included hematuria and pale kidneys at necropsy. The maximum nontoxic doses were $\leq$10 mg/kg po for the rat, rabbit and dog, and $\leq$1.0 mg/kg iv for the rat. It appeared that female rats were more susceptible to Bromfenac-caused toxicity than male rats.

Long-term toxicity/carcinogenicity studies were conducted in mice and rats. In a two-year study in mice, drug-induced toxicities were observed in the liver and the stomach at doses of 5.0/7.5 mg/kg/day. These lesions were identified as ulcers and/or subacute inflammation in the glandular mucosa of the stomach, and cytological alterations in the hepatocytes. There was no treatment-related increases in tumor incidences in all animals. The report showed that an eight-week dose range finding study was conducted in mice prior to the two-year study. However, the study result was not submitted in the NDA. In a 13-week toxicity study in rats, no treatment-related toxicities were found

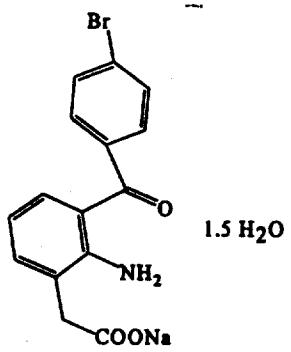
in animals at ≤0.5 mg/kg/day. At 2.5 mg/kg/day, intestinal ulcers/necrosis was observed. In a 24-month study in rats, dose-dependent hepatic (vacuolar alterations, cytoplasmic changes, inflammation, and necrosis) and gastrointestinal (inflammation, and necrosis) toxicities were identified at 12- and 24-month postmortem macro- and/or micro-scopic examinations. Nephrotoxicity (papillary necrosis) was also revealed at terminal necropsy. However, there were no treatment-associated increases in the tumor incidences in animals. No drug-related macro- and microscopic changes were observed during the six-month interim sacrifices in all doses (0.05, 0.3, and 0.6 mg/kg/day). It appeared that female rats were more sensitive to intestinal toxicity than male rats in this study. In a 13-week study in rhesus monkeys, no toxicity was found at 15 mg/kg/day. Emesis was found at 45 and 135 mg/kg/day and GI lesions were found at 135 mg/kg/day. A 12-month study was conducted in cynomolgus monkeys. Treatment-related death and enteric toxicity (ulcers) occurred in animals receiving 10 and 30 mg/kg/day in this study.

Bromfenac at doses up to 0.9 mg/kg/day in male and female rats did not cause any adverse effects in the fertility. However, increased postimplantation embryonic loss, increased stillborn pups, decreased live pups at birth, prolonged gestation period, and dystocia were observed in the rat reproduction studies. No fetal malformations were found in rats at doses up to 0.9 mg/kg/day and in rabbits at doses up to 7.5 mg/kg/day. The postnatal growth of pups from lactating dams receiving 0.9 mg/kg/day of Bromfenac was decreased.

Bromfenac was evaluated for mutagenicity potential in the Ames test, the Chinese hamster ovary cell chromosomal aberration test, the mouse lymphoma forward mutation assay, in vivo mouse micronucleus assay, and *in vivo/vitro* rat unscheduled DNA synthesis assay. Bromfenac was not mutagenic in these tests.

DIVISION OF ANTI-INFLAMMATORY, ANALGESIC AND DENTAL PRODUCTS
PHARMACOLOGY AND TOXICOLOGY REVIEW

NDA	**20-535**
DRUG:	**Bromfenac Sodium (AHR-10282B)**
SPONSOR:	Wyeth-Ayerst Laboratories 145 King of Prussia Road Radnor, PA 19087
SUBMISSION DATE:	December 30, 1994
RECEIVED BY REVIEWER:	January 6, 1995
TYPE OF SUBMISSION:	Original
DATE COMPLETED:	December 18, 1995
REVIEWER:	W. C. Josie Yang, Ph.D.& Conrad Chen, Ph.D.
DRUG CATEGORY:	NSAID
FORMULA:	2-amino-3-(4-bromobenzoyl)benzeneacetic acid, sodium salt ($C_{15}H_{11}BrNNaO_3 \cdot 1.5H_2O$); MW=383.17; pKa 4.3
RELATED DRUGS:	IND
INDICATION:	Management of acute & chronic pain, including pain of osteoarthritis and primary dysmenorrhea
DOSAGE FORM:	25 mg and 50 mg capsules
RECOMMENDED DOSE:	25 to 50 mg every 6 to 8 hours as necessary, not to exceed 150 mg/day



PRECLINICAL/LABORATORY STUDIES:

Pharmacology Studies

A. PRIMARY ACTIVITY-EFFECTS RELATED TO THE THERAPEUTIC INDICATION STUDIES

I. *IN VIVO* STUDIES

[1.] ANALGESIC ACTIVITY

a. Mouse Studies

(1.) Potency of Orally Administered AHR-10282 Relative to Zomepirac (AHR-9801) Acetylcholine-Induced Abdominal Constriction Analgesic Assay in Mice (GTR 830363) (Vol 1.18, p 1)

Report Nº: 83-0363
Study Aim: To determine the potency of orally administered AHR-10282 relative to Zomepirac (AHR-9801) at pretreatment times of 10, 20, and 300 min in preventing abdominal constriction induced by the intraperitoneal injection of acetylcholine (ACH).
Compound: AHR-10282 & Zomepirac in 0.5% Tween 80 in dist. H_2O
Control Vehicle: 0.5% Tween 80 in dist. H_2O
Dose & Route: AHR-10282 & Zomepirac: 0.316, 0.1, and 0.0316 mg/kg, 10 ml/kg, po; ACH: 6 mg/kg, 10 ml/kg, i.p.
Animal: Adult ♀ mice, ICR-Swiss Webster, weighing 20 - 30 g, 10/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: No
Study Design: Mice, 10/group, orally received various doses of AHR-10282, Zomepirac (AHR-9801) or control vehicle 10, 20 or 300 min prior to the i.p. injection of ACH (6 mg/kg). The animals were observed for the presence of abdominal constriction.

Results: AHR-10282 and Zomepirac caused a dose-dependent protection on ACH-induced abdominal constriction. The ED_{50} for AHR-10282 and Zomepirac at pretreatment time 10, 20, and 300 min were 0.20 and 0.73, 0.09 and 0.56, and 0.21 and 0.59 mg/kg, respectively. The data showed that AHR-10282 was more potent than Zomepirac in preventing ACH-induced abdominal contraction.

(2.) Pharmacologic Studies of AHR-10282 in Mice (GTR 840025) (Vol 1.18, p 12)

Report Nº: 84-0025

Results:

- The ability to block ACH-induced abdominal constriction was not modified by the repeated oral doses (0.316 mg/kg, t.i.d. for 4 days and once on day 5) of AHR-10282 suggesting that AHR-10282 did not induce tolerance in contrast to Meperidine HCl.
- AHR-10282 (3.16 mg/kg) did not prevent the abdominal constriction induced by PGE_2 (31.6 µg/kg) indicating lack of direct blocking effects on PGE_2 by AHR-10282.
- Naloxone (5 mg/kg) given s.c. immediately after oral administration of AHR-10282 (0.316 mg/kg) did not antagonize the inhibitory effect of AHR-10282 on ACH induced abdominal constriction, but it did significantly reduce the activity of 31.6 mg/kg of meperidine. These results suggested that AHR-10282 was not a narcotic-like compound.
- Hydrocortisone but not indomethacin significantly enhanced the antieffusive activity of AHR-10282.

- AHR-10282 (3.16 mg/kg) was inactive in blocking the nociceptive response in the artery clip assay. This evidence further demonstrated that AHR-10282 had no narcotic properties.
- AHR-10282 was 11.5 times more potent than indomethacin in suppressing the activity of cyclooxygenase from bovine seminal vesicles.
- AHR-10282 did not alter gastric secretion in the rat.
- AHR-10282 (up to 10^{-4} M) did not antagonize the contraction induced by histamine, serotonin or acetylcholine in isolated guinea pig ileum.
- AHR-10282 (10^{-5} M) did not reduce contractions (spontaneous and PGF2α induced) of the isolated rat uterus.

(3.) Comparison of the Analgesic activity of Orally Administered AHR-10282B in Fasted Mice with its Activity in Fed Mice (Acetylcholine-Induced Abdominal Constriction Assay) and the Determination of Concentrations of AHR-10282 in the Plasma (GTR860066)(Vol 1.18, p 25)

Report Nº: 86-0066
Study Aim: To compare the analgesic activity of orally administered AHR-10282B in fasted mice, with its activity in fed mice, and to determine the concentrations of AHR-10282 in the plasma following administration of specific doses.
Compound: AHR-10282 in 0.5% Tween 80 in dist. H_2O, ACH
Control Vehicle: 0.5% Tween 80 in dist. H_2O
Dose & Route: AHR-10282: 0.032, 0.1, 0.178 and 0.316 mg/kg, 10 ml/kg po; ACH: 6 mg/kg, 10 ml/kg, i.p.
Animal: ♀ mice, ICR-Swiss Webster, age 3-5 wk, weighing 20 - 24 g, 10/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: No
Study Design: Mice (fasted overnight & fed), 10/group, orally received (by gavage) various doses of AHR-10282 or control vehicle 20 min prior to the i.p. injection of ACH (6 mg/kg). The animals were observed for the presence of abdominal constriction. The animals were sacrificed right after the constriction test, and blood samples were obtained for plasma AHR-10282 level analysis.

Results: The ED_{50} of AHR-10282 for fasted and fed mice were 0.12 and 0.15 mg/kg, respectively. The AHR-10282 plasma concentrations at the ED_{50} were 0.47 µg/ml for fasted mice and 0.38 µg/ml for fed mice.

(4.) Determination of the Duration of Analgesic Activity of Orally Administered AHR-10282B (Acetylcholine-Induced Abdominal Constriction Assay) and the Determination of Concentrations of AHR-10282 in the Plasma (GTR860067)(Vol 1.18, p 52)

Report Nº: 86-0067
Study Aim: To determine the duration of analgesic activity of orally administered AHR-

10282B in mice and to determine the concentrations of AHR-10282 in the plasma following administration of specific doses.

Compound: AHR-10282 in 0.5% Tween 80 in dist. H_2O & ACH

Control Vehicle: 0.5% Tween 80 in dist. H_2O

Dose & Route: AHR-10282: 0.316 mg/kg, 10 ml/kg po; ACH: 6 mg/kg, 10 ml/kg, i.p.

Animal: ♀ mice, ICR-Swiss Webster, age 2-4 wk, weighing 20 - 24 g, 10/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: No

Study Design: Groups of 10 mice were given 0.316 mg/kg AHR-10282B or control vehicle. At 20 min, 1 hr, 4 hr, 24 hr, 48 hr, 72 hr, and 96 hr post dosing, ACH was administered i.p. at 6 mg/kg. The abdominal responses were observed, and blood samples were collected for the determination of AHR-10282 plasma levels immediately after observation period.

Results: AHR-10282 was able to protect animals from ACH challenge at 20 min, 1 hr and 4 hr post administration. It did not show any protective activity by 24 hr post administration.

(5.) Determination of the Potency of AHR-10282B Relative to Suprofen (AHR-9956) in the Acetylcholine (ACH)-Induced Abdominal Constriction Test in Mice (GTR 860122)(Vol 1.18, p 74)

Report Nº: 86-0122

Study Aim: To determine the potency of analgesic activity of orally administered AHR-10282B relative to orally administrated Suprofen (AHR-9956) in the ACH-induced abdominal constriction test in mice.

Compound: AHR-10282 & Suprofen (AHR-9956) in 0.5% Tween 80 in dist. H_2O; ACH

Control Vehicle: 0.5% Tween 80 in dist. H_2O

Dose & Route: AHR-10282 : 0.316 mg/kg, 10 ml/kg po; Suprofen (AHR-9956) : 3.16 mg/kg; ACH: 6 mg/kg, 10 ml/kg, i.p.

Animal: ♀ mice, ICR-Swiss Webster, weighing 18 - 30 g, 10/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: No

Study Design: Groups of 10 mice were given 0.316 mg/kg AHR-10282B, 3.16 mg/kg Suprofen, or control vehicle. At 20 min post dosing, ACH was administered i.p. at 6 mg/kg. The abdominal responses were observed, and blood samples were collected for the determination of AHR-10282 plasma levels immediately after observation period.

Results: The ED_{50} for AHR-10282 & Suprofen were 0.07 and 0.47 mg/kg. The protective potency of AHR-10282 against ACH challenge was 6.71 times stronger than that of Suprofen.

(6.) Determination of the Analgesic Activity of AHR-10282B Relative to that of Suprofen (AHR-9956): Potency in the Acetylcholine (ACH)-Induced Abdominal Constriction Test in Mice

(20 min prechallenge Time) (GTR 860263)(Vol 1.18, p 81)

Report Nº: 86-0263

Results: Same as report Nº 86-0122.

(7.) The Analgesic Activity of Orally Administered AHR-10282B, Piroxicam, and Suprofen in the Acetylcholine (ACH)-Induced Abdominal Constriction Test in Mice (GTR 860273)(Vol 1.18, p 88)

Report Nº: 86-0273
Study Aim: To determine the analgesic activity of orally administered AHR-10282B, piroxicam and Suprofen at various intervals prior to ACH challenge in mice.
Compound: AHR-10282, Piroxicam & Suprofen (AHR-9956) in 0.5% Tween 80 in dist. H_2O; ACH
Control Vehicle: 0.5% Tween 80 in dist. H_2O
Dose & Route: AHR-10282: 0.032-1.0 mg/kg, 10 ml/kg po; Piroxicam: 0.032-100 mg/kg; Suprofen (AHR-9956): 0.01-100 mg/kg; ACH: 6 mg/kg, 10 ml/kg, i.p.
Animal: 790 ♀ mice, ICR-Swiss Webster, weighing 16 - 35 g, 10/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: No
Study Design: Groups of 10 mice were given various doses of AHR-10282B, piroxicam, Suprofen, or control vehicle. At 20, 60, 180 and 300 min post dosing, ACH was administered i.p. at 6 mg/kg.

Results: The ED_{50} for AHR-10282, piroxicam & Suprofen were listed as followings.

Prechallenge Administration Time (min)	ED_{50} (mg/kg) p.o.		
	AHR-10282B	Piroxicam	Suprofen
10.00	0.20	ND*	0.68
20.00	0.08	0.25	ND
60.00	0.08	0.73	2.75
180.00	0.13	2.33	ND
300.00	0.31	ND	13.90

* ND: Not Done

It appeared that AHR-10282 was more potent than piroxicam and suprofen in blocking ACH-induced abdominal constriction. In addition, its protection effect lasted longer.

(8.) The Analgesic Activity of Orally Administered AHR-10282B Relative to that of Piroxicam (AHR-9800), Flurbiprofen (AHR-4458), Ketoprofen (AHR-3130), Ketorolac Tromethamine

(AHR-4952), and Pemedolac (AHR-7475) in Mice (GTR 900014)(Vol 1.18, p 100)

Report Nº: 90-0014
Study Aim: To determine the activity of orally administered AHR-10282B relative to that of a variety of clinically used NSAIDs in the assay of ACH-induced writhing in mice.
Compound: AHR-10282, Piroxicam, Flurbiprofen, Ketoprofen, Ketorolac & Pemedolac in 0.5% Tween 80 in dist. H_2O; ACH
Control Vehicle: 0.5% Tween 80 in dist. H_2O
Dose & Route: AHR-10282, Piroxicam, , Flurbiprofen (AHR-4458), Ketoprofen (AHR-3130), Ketorolac Tromethamine (AHR-4952), and Pemedolac (AHR-7475): 0.032-100 mg/kg, 10 ml/kg p.o.; ACH: 6 mg/kg, 10 ml/kg, i.p.
Animal: 790 ♀ mice, ICR-Swiss Webster, weighing 18 - 32 g, 10/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: No
Study Design: AHR-10282B, piroxicam, flurbiprofen, ketoprofen, ketorolac, pemedolac, or control vehicle were given to groups of 10 mice at various times before ACH challenge. The mice were observed for the presence of abdominal constriction immediately post ACH injection.

Results: The analgesic activity of orally administered AHR-10282 relative to piroxicam, ketoprofen, flurbiprofen, ketorolac, and pemedolac was illustrated in the following table. The data showed that AHR-10282 was more potent than reference compounds in inhibiting abdominal constriction induced by ACH.

Pretreatment Time (hr)	ED_{50} (mg/kg)					
	AHR-10282B	Piroxicam	Flurbiprofen	Ketoprofen	Ketorolac	Pemedolac
0.6	0.10	0.47				
1	0.06 - 0.16		7.70	0.50	0.25	0.42
3	0.09		22.92			
5	0.22 - 0.39	2.17 - 1.78		6.83		
8	0.87	3.63				
12	4.25	18.32				

(9.) Evaluation of the Possible Antibradykinin effect of Bromfenac (GTR 16319)(Vol 1.18, p 113)

Report Nº: 16319
Study Aim: To determine the potential anti-bradykinin effect of bromfenac assess by the competitive binding of bradykinin in guinea pig ileum homogenate and on bradykinin-induced writhing in mice.

Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in dist. H_2O, 1-30 mg/kg; PGE_2: 1 mg/kg, 0.1 ml/10 g i.p.; Bradykinin, 0.5 mg/kg i.p.

Animal: Adult ♂ Hartley Guinea pigs, weighing 350 - 500 g; ♂ CD-1 mice, weighing 14-19 g, 10/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: No

Results:

- Bromfenac and reference NSAIDs (indomethacin, aspirin, etodolac, naproxen, sulindac, diflunisal, and acetaminophen) did not affect the binding of ^{3}H-bradykinin (80 pM) in the guinea pig ileum.
- Bromfenac, 1-30 mg/kg p.o., did not exhibit inhibitory effects on bradykinin-induced writhing in mice.
- **These results implied that bromfenac did not bind to bradykinin receptors.**

(10.) Central Effects of Bromfenac Sodium, Dexpemedolac and Etodolac Sodium in Mice (GTR 24837)(Vol 1.18, p 121A)

Report Nº: 24837

Study Aim: To determine whether the potential antinociceptive effects of bromfenac had a central component.

Compound: Bromfenac (AHR-10282), Dexpemedolac, Etodolac, Ketorolac, Morphine and PBQ(2-phenyl-1,4-benzoquinone), Substance P (SP)

Dose & Route:

Injection Volume: Intracerebroventricularly (i.c.v.): 5 μl; Intrathecally (i.t.): 4 μl

Bromfenac: 5, 10, 20 μg/mouse i.c.v., and 1, 10, 100 μg/mouse i.t.

Dexpemedolac: 10, 25, 50 μg/mouse i.c.v., and 5, 10, 20 μg/mouse i.t.

Etodolac: 10, 30, 100 μg/mouse i.c.v. or i.t.

Ketorolac: 10, 30, 100 μg/mouse i.c.v., and 2, 20, 50 μg/mouse i.t.

Morphine: 0.01, 0.1, 1.0 μg/mouse i.c.v., and 0.001, 0.01, 0.1 μg/mouse i.t.

PBQ (2-phenyl-1,4-benzoquinone): 0.02%, 0.15 ml/10 g i.p.

Substance P (SP): 10 pmol/ 4 μl i.t.

Control Vehicle: H_2O, Saline or 2% benzyl alcohol in H_2O

Animal: ♂CD-1 mice; weighing 17-25 g, 8/group for PBQ-writhing assay; weighing 15-24 g, 8-10/group for SP induced nociceptive response test

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: Analgesic activity was quantitated as the inhibition of the writhing response induced by i.p. injection of PBQ or as the inhibition of biting or scratching induced by i.t. injection of SP. Mice, group of 8, were fasted overnight and were given bromfenac, dexpemedolac, etodolac, ketorolac, morphine, or the vehicle by i.c.v. or i.t. route of administration 10 or 20 min prior to PCB challenge. Sixty min after oral or 20 min after i.t.

administration of test compounds listed above, the mice received an i.t. injection of SP. The mice were observed for the presence of writhing response or behavior change immediately post PCB or SP injection.

Results:
The ED_{50} values of i.c.v. or i.t. administered bromfenac, dexpemedolac, etodolac, ketorolac, and morphine in PBQ-induced writhing and SP-induced behavior change tests are shown in the following table.

Test Compound	ED_{50} (μg/mouse)			
	PBQ-Induced Writhing Assay		SP-Induced Behavior Change Test	
	i.c.v. Route	i.t. Route	Oral Route	i.t. Route
Dexpemedolac	22	11	Inactive	29
Ketorolac	37	17	Inactive	>>200
Bromfenac	6.9	21	Inactive	31
Etodolac	28	45	Inactive	63
Morphine	0.05	≈0.002	32	0.38

These data demonstrated that a high dose of bromfenac administered i.c.v. was required to generate effect in the writhing assay, indicating lack of a central effect. In addition, i.t. administration of bromfenac and reference NSAIDs did not block the biting behavior induced by SP.

(11.) Duration of Analgesic Action and Plasma Levels of Bromfenac in Fasted and Fed Mice (GTR 19708)(Vol 1.18, p 122)

Report Nº: 19708
Study Aim: To determine the duration of analgesic action of AHR-10282 and its relationship with plasma levels in fasted and fed mice using PBQ writhing model.
Compound: Bromfenac (AHR-10282), PBQ (2-phenyl-1,4-benzoquinone)
Dose & Route: Bromfenac, 0.1-2.0 mg/kg, 0.1 ml/10 g p.o.; PBQ: 0.02%, 0.15 ml/15 g i.p.
Control Vehicle: 0.5% Tween 80
Animal: ♂ CD-1 mice, weighing 20-28 g,
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Results: In fasted mice, Bromfenac (1 mg/kg) produced a maximum analgesic effect within 30 min and this effect declined to 50% within 4 to 8 hr post treatment. In fed mice, Bromfenac caused a significant effect within 30 min and peaked at 60 min post dosing. It declined to a nonsignificant level after 8 hr. The pharmacokinetic parameters in fasted and

fed mice receiving single oral dose of Bromfenac (1 or 2 mg/kg) were exhibited in the following table (Vol 1.18, p 136, Table 6). The results indicated that dose normalized AUC and C_{max} values were 22.6 and 1.8 times higher in the fasted animals, respectively. The elimination of Bromfenac in plasma was more rapid in fed animals than in fasted animals (1.5 vs 2.4 hr). There was a positive correlation between PBQ-writhing and plasma Bromfenac concentrations in fasted and fed animals.

PK Parameters	Fasted (1 mg/kg)	Fed (2 mg/kg)	Fed Normalized (Calculated for 1 mg/kg)
C_{max} (ng/ml)	2390	2822	1411
T_{max} (ng/ml)	0.5	0.5	0.5
AUC_{0-24} (ng·hr/ml)	8898	6964	3482
$T_{½}(\lambda_z)$ (hr)	2.4	1.5	1.5
MRT (hr)	3.6	2.4	2.4

The ED_{50} values of bromfenac in the fasted and fed mice administered 60 min prior to PBQ challenge were 0.26 (0.10-0.65) mg/kg p.o. and 0.63 (0.23-1.7) mg/kg p.o., respectively. For the time course study, the estimated ED_{80} doses (1 mg/kg p.o. in fasted mice and 2 mg/kg p.o. in fed mice) were used, and the kinetic effects of PBQ-induced abdominal constriction are listed as follows (Vol 1.8, p 133, Table 3):

Pretreatment time (hr)	% Inhibition of Writhing	
	Fasted Mice (1 mg/kg p.o.)	Fed Mice (2 mg/kg p.o.)
0.5	93***	62**
1.0	83***	89***
2.0	94***	53*
4.0	48**	55*
8.0	55*	17
16.0	25	-33[a]
24.0	28	18

*: p≤0.050; **: p≤0.010; ***: p≤0.001

[a]: Mean number of writhes increased by 33% vs respective control.

(12.) Effects of Intraperitoneally administered Bromfenac and Its Metabolites in Mouse PBQ Writhing Assay (GTR 20421)(Vol 1.18, p 142)

Report Nº: 20421

Study Aim: To determine the duration of analgesic action of AHR-10282 and its relationship with plasma levels in fasted and fed mice using the PBQ writhing model.

Compound: Bromfenac (AHR-10282), and Bromfenac metabolites (AHR-11665-B-1, AHR-11652-3, WAY-127039-A-1); reference NSAID, indomethacin; PBQ

(2-phenyl-1,4-benzoquinone)

Dose & Route: Bromfenac and its metabolites (AHR-10240-3, AHR-11665-B-1, AHR-11652-3, WAY-127039-A-1), 1.0-50 mg/kg, 0.1 ml/10 g p.o.; PBQ: 0.02%, 0.15 ml/15 g i.p.; Indomethacin: 6 mg/kg p.o.

Control Vehicle: 0.5% Tween 80

Animal: ♂ CD-1 mice, weighing 20-28 g, 8/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: Mice were fasted overnight and received i.p. injection of Bromfenac and its metabolites, AHR-11665-B-1, AHR-11652-3, WAY-127039-A-1, 10 min prior to PBQ challenge. The number of writhes was counted for 15 min immediately post i.p. injection of PBQ.

Results: The ED_{50} of Bromfenac administered by the i.p. route measured as the inhibition of writhing response induced by PBQ was 0.057 mg/kg. The metabolites of Bromfenac, AHR-10240-3, AHR-11665-B-1, AHR-11652-3, and WAY-127039-A-1 were inactive at 10 mg/kg (i.p.). The metabolites AHR-11665-B-1, AHR-11652-3, WAY-127039-A-1 but not AHR-10240-3, at 50 mg/kg i.p. exhibited significant protective effects against PBQ-induced writhing with ED_{50} values of 37.5, 26.3 for AHR-11665-B-1 and AHR-11652-3, respectively. WAY-127039-A-1, at doses of 30 and 100 mg/kg, caused writhing by it self. All metabolites caused toxic effects at 30-100 mg/kg and some were lethal at the highest level of 100 mg/kg. Therefore, the inhibitory effects against the PBQ-induced response by metabolites of Bromfenac might be due to their toxicity rather than their analgesic properties.

b. Dog Studies

(1.) The Potency of AHR-10282B Relative to Zomepirac (AHR-9801) in Blocking the Bradykinin-Induced Response in Dogs (GTR 830526)(Vol 1.18, p 150)

Report Nº: 83-0526

Study Aim: To determine the potency of AHR-10282B relative to Zomepirac in blocking the bradykinin-induced response in dogs.

Compound: Bromfenac (AHR-10282), Zomepirac (AHR-9801) in 0.5% Tween 80 in H_2O

Dose & Route: Bromfenac and Zomepirac: 0.316, 1.0, and 3.16 mg/kg p.o.; Bradykinin: 0.002 μg i.p.

Control Vehicle: 0.5% Tween 80 in H_2O

Animal: 18 ♀ mongrel dogs, weighing 8.6-14.3 kg, 3/dose/compound

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: This was a crossover study. Two times, 4x, or 8x threshold doses of bradykinin was given i.p. 30, 60, or 120 min post administration of AHR-10282 (0.032, 0.1, 0.316 mg/kg p.o.), or Zomepirac (0.1, 0.316, 1.0 or 3.16 mg/kg), or control vehicle (0.5%

Tween 80 in H_2O) to groups of 6 dogs, respectively.

Results: Both AHR-10282 and Zomepirac caused a dose-dependent block of the response to bradykinin with ED_{50} values of 0.08 and 0.46 mg/kg, respectively. AHR-10282 was 5.75 times more potent than Zomepirac in blocking the response to bradykinin.

[2.] ANTIINFLAMMATORY ACTIVITY

(a.) Comparison of the Anti-inflammatory Activity of AHR-10282 with Indomethacin in the 5-Hr Evans Blue-Carrageenan Pleural Effusion Assay in Rats (GTR 830347)(Vol 1.18, p 165)

Report Nº: 83-0347
Study Aim: To determine the potency of AHR-10282B relative to indomethacin in blocking the 5 hr effusive response to pleural irritation.
Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.03, 0.16, 0.8 and 4.0 mg/kg p.o.; Indomethacin: 0.16, 0.8, and 4.0 mg/kg, 10 ml/kg p.o.; 0.075% Evans Blue-0.5% Carrageenan suspension, intrapleural injection
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 66 fasted ♂ Sprague-Dawley rats, weighing 291 - 433 g, 7/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: One hour following the administration of AHR-10282, indomethacin or control vehicle (0.5% Tween 80 in H_2O), the animals were anesthetized and administered intrapleurally with 0.075% Evans Blue-0.5% Carrageenan suspension. The animals were sacrificed 5 hr post Carrageenan injection, then the pleural fluids were removed from the first animals in each group and measured.

Results: The potency of AHR-10282 and indomethacin in the 5 hr Evans Blue-Carrageenan pleural effusion is presented in the following table. Based on the data presented, AHR-10282 was 7.5 times more potent than indomethacin in this model.

Compound	Dose (mg/kg)	Pleural Fluid (ml)	% Decrease
Indomethacin	0.16	6.7 ± 0.8	2.3
	0.8	5.9 ± 0.8	9.0
	4.0	4.7 ± 1.2[a]	27.4
AHR-10282	0.03	6.2 ± 0.4	4.9
	0.16	5.6 ± 0.6	14.3
	0.8	4.9 ± 0.6[a]	25.1
	4.0	4.3 ± 0.3[a]	34.0
Control (0.5% Tween 80)	10 ml/kg	6.5 ± 0.6	-

[a] $P \leq 0.05$ as compared with the control group

(b.) Comparison of the Anti-inflammatory Activity of AHR-10282 with Indomethacin in Normal and Adrenalectomized Rats by Using the Carrageenan-Induced Foot Edema Assay (GTR 830349)(Vol 1.18, p 176)

Report №: 83-0349
Study Aim: To determine whether the anti-inflammatory activity of AHR-10282 was mediated by stimulation of the adrenal gland in rats.
Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.8 mg/kg p.o.; Indomethacin: 4.0 mg/kg, 10 ml/kg p.o.; 0.1% Carrageenan suspension in 0.1 ml, injection
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: Fasted ♀ Sprague-Dawley rats, weighing 140 - 200 g, 6/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: The adrenalectomy was performed 72 hr prior to the beginning of the experiment. Prior to dosing, the limb volume of the right foot of each animal was determined. One hr post administration of AHR-10282 or indomethacin or control vehicle, 0.1 ml of a 1% Carrageenan was injected into the right hind paw, and three hr later the limb volume of injected foot was measured.

Results: AHR-10282 (0.8 mg/kg) and indomethacin (4.0 mg/kg) caused a reduction in Carrageenan induced edema by 63.2% and 49.1% in normal rats and by 63.8% and 42.5% in adrenalectomized rats, respectively. Adrenalectomy did not intervene the anti-inflammatory activity of AHR-10282 and indomethacin. **Therefore, the anti-inflammatory properties of Bromfenac was independent of the adrenal glands.**

(c.) Comparison of the Antiinflammatory Activity of AHR-10282 with Indomethacin in the 17-Hr Evans Blue-Carrageenan Pleural Effusion Assay in Rats (GTR 830361)(Vol 1.18, p 187)

Report №: 83-0361
Study Aim: To determine the potency of AHR-10282B relative to indomethacin in blocking the 17 hr effusive response to pleural irritation.
Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.03, 0.16, 0.8 and 4.0 mg/kg p.o.; indomethacin: 0.16, 0.8, and 4.0 mg/kg, 10 ml/kg p.o.; 0.075% Evans Blue-0.5% Carrageenan suspension, intrapleural injection
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 56 fasted ♂ Sprague-Dawley rats, weighing 311 - 458 g, 7/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: The study procedures were the same as those described in the Report № 83-0347, except the animals were sacrificed 17 hr post Carrageenan injection.

Results: The potency of AHR-10282 and indomethacin in the 17 hr Evans Blue-Carrageenan pleural effusion was presented in the following table:

Compound	Dose (mg/kg)	Pleural Fluid (ml)	% Decrease
Indomethacin	0.16	6.8 ± 1.4	3.1
	0.8	6.3 ± 1.9	9.1
	4.0	4.5 ± 0.6	35.9[a]
AHR-10282	0.03	6.0 ± 1.2	14.1
	0.16	4.8 ± 0.8	31.3[a]
	0.8	4.3 ± 0.7	39.0[a]
	4.0	4.1 ± 0.4	40.7[a]
Control (0.5% Tween 80)	10 ml/kg	7.0 ± 0.4	-

[a] $P \leq 0.05$ as compared with the control group

The Data indicated that anti-inflammatory activity of AHR-10282 was 20 times more potent than indomethacin in the reduction effusive response to pleural irritation induced by Carrageenan.

(d.) Antiinflammatory Potency of AHR-10282 Relative to Indomethacin in Rats with Adjuvant-Induced Arthritis (GTR 830364)(Vol 1.18, p 213)

Report Nº: 83-0364

Study Aim: To determine the anti-inflammatory activity of AHR-10282 relative to indomethacin in adjuvant-induced arthritis.

Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O

Dose & Route: Bromfenac: 0.0032-0.316 mg/kg p.o.; indomethacin 0.0032-3.16 mg/kg, 10 ml/kg p.o.; Adjuvant (15 mg of dead *Mycobacterium butyricum* in 1 ml): 0.05 ml, subplantar surface injection

Control Vehicle: 0.5% Tween 80 in H_2O

Animal: Mature ♀ Lewis-Wistar rats, weighing 150 - 250 g, 6-7/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: On day 0 the lower part of both hind legs were shaved and tattooed at the point where the Achilles tendon enters the gastrocnemius muscle. Adjuvant was given by injection into the subplantar surface of left hind foot on day 1. Eighteen days following the injection of the adjuvant, the animals were weighed and the limb volumes (ml) of injected and un-injected feet were determined by a Statham Pressure Transducer. AHR-10282, indomethacin, or the vehicle was given orally daily 5 days a week from day 18 to day 28. On day 29 the animals were weighed and the limb volumes of both hind feet were determined.

Results: One animal in the group receiving 3.16 mg/kg indomethacin died and postmortem

examination showed peritoneal adhesion and peritonitis indicative of G.I. toxicity. Both AHR-10282 (0.316 mg/kg) and indomethacin (3.16 mg/kg) induced a significant increase in the 11- day body weight gain and a significant decrease in the secondary lesion score. AHR-10282 was more potent than indomethacin in decreasing the edema induced by adjuvant.

(e.) Interaction Between AHR-10282 and Hydrocortisone in the 5-Hr Evans Blue-Carrageenan Pleural Effusion Assay in Rats (GTR 830362)(Vol 1.18, p 200)

Report Nº: 83-0362
Study Aim: To determine the potency of AHR-10282B relative to indomethacin in blocking the 5 hr effusive response to pleural irritation.
Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.8 mg/kg p.o.; Indomethacin: 4.0 mg/kg, 10 ml/kg p.o.; 0.075% Evans Blue-0.5% Carrageenan suspension, intrapleural injection; Hydrocortisone, 75 mg/kg p.o.
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 66 fasted ♂ Sprague-Dawley rats, weighing 266 - 400 g, 7/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: Same as "Report Nº 83-0347".

Results: The results of the interaction of AHR-10282 and indomethacin with hydrocortisone in the 5 hr Evans Blue-Carrageenan pleural effusion are presented in the following table:

Compound	Dose (mg/kg)	Pleural Fluid (ml)	Decrease (%)
AHR-10282 (A)	0.8	4.2 ± 0.2[a]	35.0
Indomethacin (B)	4.0	4.3 ± 0.3[a]	30.2
Hydrocortisone (C)	75	4.4 ± 0.4[a]	28.8
A+C		2.8 ± 0.2[a]	54.1
B+C		3.2 ± 0.5[a]	48.0
Control	10 ml/kg	6.2 ± 0.6	

[a] P≤0.05 (Dunnett's test) as compared with control

It appeared the anti-inflammatory effect of AHR-10282 was potentiated by the concomitant administration of hydrocortisone.

(f.) Effect of AHR-10282B Rectal Temperature of Normothermic and Hyperthermic Rats (GTR 830432)(Vol 1.18, p 230)

Report Nº: 83-0432

Study Aim: To determine whether AHR-10282B possessed antipyretic properties.
Compound: Bromfenac (AHR-10282), Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.8 mg/kg p.o.; Indomethacin: 4.0 mg/kg, 10 ml/kg p.o.; 15% Brewer's yeast, 10 ml/kg s.c.
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 45 ♂ Sprague-Dawley rats, weighing 150 - 250 g, 6/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282, indomethacin, or vehicle were orally administered to groups of 6 normothermic and yeast-induced hyperthermic rats. Rectal temperatures were measured 0.5, 1, and 3 hr post dosing.

Results: AHR-10282 significantly reduced the rectal temperature of hyperthermic rats at 0.5, 1, and 3 hr post dosing; in contrast, indomethacin did not significantly altered temperature until 1 hr post administration. Neither AHR-10282 nor indomethacin affected rectal temperature in normothermic rats.

(g.) Comparison of the Antiinflammatory Activity of Orally Administered AHR-10282B in Fasted Rats, with Its Activity in Fed Rats, and Determination of the Concentration of AHR-10282 in the Plasma (GTR 860121)(Vol 1.18, p 243)

Report Nº: 86-0121
Study Aim: To determine the anti-inflammatory action of AHR-10282 and its relationship with plasma levels in fasted and fed rats in the Carrageenan-induced foot edema model.
Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.01, 0.032, 0.316, 1.0, and 3.16 mg/kg p.o.; 1.0% Carrageenan suspension in 0.1 ml, injection
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 126 ♂ Sprague-Dawley rats, weighing 150 - 180 g, age 4-5 wk, 9/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282 or vehicle were orally administered to groups of 9 fasted and fed rats 1 hr prior to Carrageenan injection into the left hind paw. The limb volumes and blood samples were obtained prior to dosing and 3 hr post Carrageenan challenge.

Results: AHR-10282 caused a dose-dependent suppression of paw edema-induced by Carrageenan in both fed and fasted rats. Plasma concentrations of AHR-10282 were significantly higher in fasted rats.

(h.) The Effect of Blocking Gastric Acid Secretion on the Antiinflammatory Activity of AHR-10282B (Carrageenan foot Edema Assay in Rats) (GTR 860141)(Vol 1.18, p 275)

Report Nº: 86-0141

Study Aim: To evaluate the effect of blocking gastric acid secretion, on the anti-inflammatory action of AHR-10282, and its relationship with plasma levels in fasted and fed rats in the Carrageenan-induced foot edema model.

Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O and Omeprazol in ETOH

Dose & Route: Bromfenac: 0.01, 0.032, 0.10 and 0.316 mg/kg p.o.; Omeprazol : 0.1 and 0.2 mg/kg i.v.; 1.0% Carrageenan suspension in 0.1 ml, injection

Control Vehicle: 0.5% Tween 80 in H_2O and 10% ETOH in saline

Animal: 126 ♂ Sprague-Dawley rats, weighing 160 - 225 g, age 4-5 wk, 6/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: Omeprazole, 0.1 or 0.2 mg/kg, was given i.v. to the rats 3 to 4 hr prior to the oral administration of AHR-10282. All rats were injected with Carrageenan into the left hind paw one hr post dosing with AHR-10282. Then hind foot volumes were measured and recorded 3 hr post Carrageenan challenge.

Results: Intravenous injection of Omeprazole (a blocker of gastric acid) at either 0.1 or 0.2 mg/kg did not enhance anti-inflammatory potency of AHR-10282. On the contrary, the anti-inflammatory potency of AHR-10282 was reduced by the presence of 0.1 or 0.2 mg/kg Omeprazole in one out of two experiments to 0.55 and 0.37, respectively.

(I.) Determination of the Duration of the Antiinflammatory Activity of Orally Administered AHR-10282B (Carrageenan foot Edema Assay in Rats) and of the Concentration of AHR-10282 in the Plasma at Various Times After Drug Administration (GTR 860183)(Vol 1.18, p 283)

Report Nº: 86-0183

Study Aim: To evaluate the duration of the anti-inflammatory activity of orally administered AHR-10282 and to determine the correlation between the anti-inflammatory activity and the level of AHR-10282 in the plasma in fed rats.

Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O

Dose & Route: Bromfenac: 0.316 mg/kg p.o.; 1.0% Carrageenan suspension in 0.1 ml, injection

Control Vehicle: 0.5% Tween 80 in H_2O

Animal: 85 ♂ Sprague-Dawley rats, weighing 155 - 215 g, age 4-5 wk, 9/group

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Study Design: AHR-10282 was given orally to fed ♂ rats at 1, 4, 24, 48, and 72 hr prior to Carrageenan challenge.

Results: Significant reductions (42% and 49%) in edema caused by Carrageenan were

observed only in animals receiving 0.316 mg/kg AHR-10282 1 or 4 hr prior to challenge. Plasma levels of AHR-10282 were detectable only at 1-7 hr post dosing, with a range of 0.25-0.56 μg/ml. No significant anti-inflammatory effects were observed when AHR-10282 was given 24, 48 or 72 hr prior to Carrageenan challenge.

(j.) Determination of the Duration of the Antiinflammatory Activity of Orally Administered AHR-10282B (Carrageenan foot Edema Assay in Rats) and of the Concentration of AHR-10282 in the Plasma- Pretreatment Time 0.5 - 48 Hr (GTR 870468)(Vol 1.18, p 304)

Report №: 87-0468
Study Aim: To evaluate the duration of the anti-inflammatory activity of orally administered AHR-10282 in **fasted rats,** and to determine the levels of AHR-10282 in the plasma.
Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.316 mg/kg p.o.; 1.0% Carrageenan suspension in 0.1 ml, injection
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 204 fasted ♂ Sprague-Dawley rats, weighing 150 - 195 g, age 4-5 wk, 6-9/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282 was given orally to fasted ♂ rats at 0.5-48 hr prior to Carrageenan challenge.

Results: Significant reductions (30-70%) in edema caused by Carrageenan were observed only in animals receiving 0.316 mg/kg AHR-10282 0.5 to 48 hr prior to challenge. Plasma levels of AHR-10282 were detectable 0.5-30 hr post dosing with range of 0.02-1.61 μg/ml.

(k.) The Effects of Bromfenac on Fluid Accumulation and Inflammatory Cell Infiltration in a Rat Model of Reverse Passive Arthus Pleurisy (GTR 21603)(Vol 1.18, p 336)

Report №: 21603
Study Aim: To determine the anti-inflammatory effects of Bromfenac on fluid accumulation and inflammatory cell infiltration in a rat model of reverse passive Arthus pleurisy.
Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.1, 0.3 and 1.0 mg/kg p.o.; Indomethacin: 8 mg/kg p.o.; Bovine Serum albumin (BSA): 5 mg/animal in 0.2 ml
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: Fasted ♂ Lewis rats, weighing 170 - 200 g
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: A reverse passive Arthus reaction pleurisy was induced by the i.v. injection

of BSA (5 mg/animal in 0.2 ml saline), followed by an intrapleural injection of rabbit anti-BSA (1 mg/animal in 0.2 ml saline). AHR-10282, indomethacin, or vehicle control was given orally to fasted ♂ rats at 1 hr prior to anti-BSA injection. Animals were sacrificed at the time of peak neutrophil infiltration (≈4 hr post anti-BSA), the fluid exudate in the pleural cavity was collected and measured. For the determination of cellular infiltration, the pleural exudate was aspirated and the plural cavity was then rinsed with 3 ml of 0.1% EDTA in 0.9% saline. The wash was pooled with the exudate and cell number was determined by Coulter ZBI counter.

Results: Orally administered Bromfenac generated a dose-related inhibition (22.1-35.8%) of cellular infiltration with an ED_{30} of 0.24 mg/kg. All tested doses gave significant inhibition (20-22%) in pleural fluid accumulation, and this suppressive effect was not dose-dependent. The reference compound, indomethacin, also caused a significant decrease in cellular infiltration and fluid accumulation (25.8% and 38.2%, respectively).

(l.) In Vivo Inhibition of Prostaglandin Production by Dexpemedolac, Bromfenac and Other NSAIDs (GTR 25295)(Vol 1.18, p 342A)

Report Nº: 25295
Study Aim: To determine the effects of orally administered AHR-10282 (Bromfenac) on PG production in the rat brain .

Results: Bromfenac had inhibitory effects on PGI_2 production in the rat brain with an ED_{50} of 3.2 mg/kg p.o. but it had no effects on PGE_2 production.

II. *IN VITRO* STUDIES

(1.) Comparison of the activity of AHR-10282 with Indomethacin (AHR-9587) on the Resting Tone of Guinea Pig Tracheal Strips (GTR 830278) (Vol 1.18, p 343)

Report Nº: 83-0278

Results: Both AHR-10282 & Indomethacin induced dose-dependent relaxation of the resting tone of guinea pig tracheal strips, and AHR-10282 was 18.7 and 35.6 times more potent than Indomethacin in two independent experiments via regression analysis.

(2.) The effect of AHR-10282 on Prostaglandin Synthesis in Various tissues (GTR 84130) (Vol 1.18, p 360)

Report Nº: 84-0130
Study Aim: To determine the effect of AHR-10282 on prostaglandin synthesis by microsomal preparation from bovine seminal vesicles, rabbit kidney medulla, and rabbit uterus, and to compare its effect with those of Indomethacin and Ibuprofen.

Results: AHR-10282 and reference NSAIDs, Indomethacin & Ibuprofen, inhibited PGE_2 and $PGF_{2\alpha}$ synthesis. The IC_{50} values of AHR-10282, Indomethacin, and Ibuprofen on PGE_2 and $PGF_{2\alpha}$ formation in microsomes of various tissues are presented in the following table, and the relative order of potency was AHR-10282>Indomethacin>Ibuprofen.

Drug		IC_{50} (μM)/Tissue Microsome		
		Bovine Seminal Vesicle	Rabbit Kidney Medulla	Rabbit Uterus
AHR-10282	PGE_2	0.777	0.136	0.667
	$PGF_{2\alpha}$	0.792	0.155	0.308
Indomethacin	PGE_2	9.38	0.894	22.11
	$PGF_{2\alpha}$	9.55	0.984	5.60
Ibuprofen	PGE_2	6.47	13.08	106.12
	$PGF_{2\alpha}$	224.38	12.55	-

(3.) Biomedical Studies on The Effect of AHR-10282 on Prostaglandin H Synthase Activity (GTR 870281) (Vol 1.19, p 1)

Report №: 87-0281

Study Aim: To determine the *in vitro* effects of AHR-10282 on the activities of sheep vesicular gland prostaglandin H (PGH) synthase.

Compound: AHR-10282 and its ethyl ester (AHR-11779); indomethacin (AHR-9578) and its ethyl ester (AHR-14661); acetamidophenol (AHR-3148), flubiprophen (AHR-4448), AHR- 5850D and its ethyl ester (AHR-6133); aspirin (AHR-9503). All compounds, except AHR-10282 and AHR-5850D which were dissolved in reaction buffer, were dissolved in absolute ethanol.

Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220

Compliance with GLP/QAU: N/A

Results: Time- and concentration-dependent inactivation of oxygenase activity was observed when PGH synthase was preincubated with AHR-10282 and the reference compounds. The results indicated that AHR-10282 was a more efficient, irreversible, time-dependent inhibitor than flurbiprofen or indomethacin (Vol 1.19, p 011, Figure 2). The relative potency of SC-58635 and various reference drugs on PGH synthase activity was presented in the following table. The relative potency was expressed as the estimated kapp/[I] and the unit is $\mu M^{-1} \cdot min^{-1}$.

Compounds	Lipid-Depleted Microsomes	Purified PGH Synthase
Acetamidophenol	0.0	ND[a]
Aspirin	0.00049 ± 0.00005	ND
Indomethacin	1.97 ± 0.32	1.64 ± 0.56
Indomethacin Ethyl Ester	0.0	ND
Flurbiprofen	2.82 ± 0.61	2.88 ± 0.70
AHR-5850D	2.77 ± 0.23	ND
AHR-6133	0.0	ND
AHR-10282	12.0 ± 0.10	12.3 ± 3.7
AHR-11779	0.0	ND

[a] ND = not determined

(4.) Inhibition of *In Vitro* Platelet Aggregation by AHR-10282B (GTR 870357) (Vol 1.19, p 42)

Report Nº: 87-0357
Study Aim: To determine the *in vitro* effects of AHR-10282 on the activities of sheep vesicular gland prostaglandin H (PGH) synthase.
Compound: AHR-10282 and indomethacin (AHR-9578) were dissolved in 0.9% saline.
Animals: ♂ New Zealand white rabbits, weighing 2.7 - 3.7 kg
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Results: AHR-10282 and reference article Indomethacin inhibited arachidonic acid-induced platelet aggregation with IC_{50} of 0.39 μM and 1.63 μM respectively. AHR-10282 was more potent (2.57-6.73 times) than Indomethacin in this test.

(5.) *In Vitro* Effect of Bromfenac on Calcium Ionophore-Induced Eicosanoid Release Using Human Whole Blood (GTR-19707) (Vol 1.19, p 54)

Report Nº: 19707

Results: Bromfenac inhibited thromboxan B_2 biosynthesis from human blood platelets in a dose-dependent manner with an IC_{50} of 0.11 μM. At a concentration of 10 μM, Bromfenac and reference NSAIDs, Indomethacin, and Ketoprofen did not affect LTB_4 biosynthesis by neutrophils. The IC_{50} values for Indomethacin, Piroxicam and Ketoprofen in ionophore-induced TXB_2 production were 0.23, 4.49, and 0.64 μM, respectively. Therefore, Bromfenac was 6-40 times more potent than reference NSAIDs in reduction of TXB_2 production by blood platelets *in vitro*.

(6.) Evaluation of Bromfenac in Panlabs Biomedical and Selected Functional Assays (GTR 20021)(Vol 1.19, p 61)

Report Nº: 20021

Results: Bromfenac was evaluated for its interaction at various sites and for its effect on different enzymes. Bromfenac, at the level of 10 μM, did not show any affinity for 41 of 42 receptor site tested, but it displaced the binding of neuropeptide Y by 41%. Bromfenac inhibited ram seminal vesicle cyclooxygenase activity but not any other tested enzymes with an IC_{50} of 11 μM.

B. Ancillary Pharmacology-Effects Related to Possible Adverse Reactions

[1.] Central Nervous System (CNS) Pharmacology

(a.) Effects of AHR-10282 on Behavior and Spontaneous Cerebral Cortical Activity in Cats with Chronically Implanted Electrodes (GTR 820171) (Vol 1.19, p 132)

Report Nº: 82-0171
Study Aim: To determine the effects of AHR-10282 on behavior and on spontaneous cerebral cortical activities in cats.
Compound: AHR-10282 in gelatin capsule, 2.5 mg/kg/day for 5 days p.o.
Animals: 3 adult mongrel cats, 1♂ and 2 ♀, weighing 2.5-3.15 kg
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Results: Emesis was observed in each animal. AHR-10282 did not affect behavior and normal spontaneous cerebral cortical activities in all cats.

[2.] Cardiovascular Pharmacology

(a.) Effect of Intravenously Administered AHR-10282B on the Cardiovascular Responses to Autonomic Agents in Anesthetized Dogs (GTR 830424) (Vol 1.19, p 143)

Report Nº: 82-0171
Study Aim: To assess the possible effects of AHR-10282 on the autonomic nervous system function in dogs.
Compound: AHR-10282 in dist. H_2O at the concentrations of 25 & 50 mg/ml
Dose & Route: AHR-10282B, 1-30 mg/kg i.v.
Animals: 5 adult ♀ mongrel dogs, weighing 2.5-3.15 kg
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Results: AHR-10282B, dose range from 1-30 mg/kg, did not significantly change the magnitude of blood pressure induced by epinephrine, acetylcholine or histamine. At doses of 10 and 30 mg, AHR-10282B potentiated the response to isoproterenol; at the dose of 30 mg/kg, it enhanced the response to DMPP (1-1-dimethyl-4-phenyl-piperazinium iodide). AHR-10282B did not modify the heart rate in response to autonomic nervous system agonists, epinephrine, norepinephrine and isoproterenol. These results implied that AHR-10282B had no distinguishable anti-adrenergic, anticholinergic, antihistaminic properties.

(b.) Cardiohemodynamic Effects of AHR-10282B Administered Intravenously to Anesthetized Dogs (GTR 830438) (Vol 1.19, p 160)

Report №: 83-0438
Study Aim: To assess the possible effects of AHR-10282 on cardiovascular function in anesthetized dogs.
Compound: AHR-10282 in dist. H_2O at the concentrations of 25 & 50 mg/ml
Dose & Route: AHR-10282B: 1, 3, 10 & 30 mg/kg i.v.
Animals: 5 adult, 2♂ & 3♀ mongrel dogs, weighing 10.1-13.7 kg
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Results: Intravenous injection of AHR-10282 at dosages of 3, 10, and 30 mg/kg increased mean arterial blood pressure significantly ($p \leq 0.05$), but it did not alter femoral arterial flow or peripheral resistance. There were no significant changes in heart rate, cardiac output, right ventricular pressure, and rate of change of left ventricular pressure at any dose tested. At the highest dose 30 mg/kg, AHR-10282 increased left ventricular pressure significantly ($p \leq 0.05$). Based on results obtained from these observation, i.v. injection of AHR-10282 had little or limited effects on cardiovascular function.

[3.] GASTROINTESTINAL PHARMACOLOGY

(a.) Gastric Toxicity of Orally Administered AHR-10282 Relative to Indomethacin in Rats (GTR 830416) (Vol 1.19, p 176)

Report №: 83-0416
Study Aim: To determine the gastric toxicity of Bromfenac relative to Indomethacin in rats.
Compound: Bromfenac (AHR-10282) & Indomethacin in 0.5% Tween 80 in H_2O
Dose & Route: AHR-10282 & Indomethacin: 1, 3, 9 mg/kg p.o.
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: Fasted ♂ Sprague-Dawley rats, weighing 180 - 238 g, 7/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A

Study Design: Six hr after dosing with AHR-10282, Indomethacin or vehicle, the animals were kill by CO_2 inhalation, and the stomachs were removed for examination.

Results: Both AHR-10282 and Indomethacin induced a dose-related increase in the gastric irritation. There was no difference in potency to induce gastric damage in rats between AHR-10282 and Indomethacin.

(b.) The Potency and Intestinal Toxicity of Orally Administered AHR-10282 Relative to Indomethacin in Rats (GTR 830417) (Vol 1.19, p 187)

Report Nº: 83-0417
Study Aim: To determine the intestinal toxicity of AHR-10282 relative to Indomethacin by using the same dosing regimen.
Compound: Bromfenac (AHR-10282) in 0.5% Tween 80 in H_2O
Dose & Route: Bromfenac: 0.75, 1.5, 3.0 or 6.0 mg/kg p.o.; Indomethacin: 1, 2, 4, or 6 mg/kg p.o.
Control Vehicle: 0.5% Tween 80 in H_2O
Animal: 63 Fasted ♂ Sprague-Dawley rats, weighing 174 - 226 g, 7/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: Group of 7 rats were orally dosed with AHR-10282 (0.75, 1.5, 3.0 or 6.0 mg/kg) or Indomethacin (1, 2, 4, or 6 mg/kg) on days 1-5 and 8-11. On day 12, all rats were sacrificed and the extent of GI damages was scored.

Results: AHR-10282 and Indomethacin caused a dose-related increase in intestinal toxicity scores. AHR-10282 at 0.75 mg/kg and Indomethacin at 1 or 2 mg/kg did not generate any intestinal damages. Death was observed in 6 animals receiving 6.0 mg/kg AHR-10282 and 3 animals receiving 6.0 mg/kg Indomethacin. AHR-10282 was 1.78 times more potent than Indomethacin in inducing intestinal damages. Weight loss was observed in animals at 6 mg/kg of AHR-10282 or Indomethacin.

C. *OTHER STUDIES*

[1.] IMMUNOPHARMACOLOGY ACTIVITY

(a.) Evaluation of AHR Compounds for Immunomodulatory Activity Part II: Influence of AHR-10282 on Mitogen-Induced Lymphocyte Blastogenesis in Mice (GTR 840265) (Vol 1.19, p 199)

Report Nº: 84-0265
Study Aim: To determine the effects of AHR-10282 on ConA and LPS-induced blastogenesis in mice.
Compound: AHR-10282 and Cyclophosphamide (Cytoxan®) in PBS, pH 7.4

Dosage & Route: AHR-10282: 0.1, 1.0, 10.0, and 20 mg/kg for 7 days, po; Cyclophosphamide: 10 and 50 mg/kg
Animals: 130 ♂ BALB/c mice, 3 - 4 wk old and weighing 15 - 20 g
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: The mice received either AHR-10282 (0.1, 1.0, 10.0, and 20 mg/kg), vehicle or cyclophosphamide (10 and 50 mg/kg) for 7 days. Animals were sacrificed on the last dosing day and spleens were removed aseptically. Single cell suspensions were prepared and cells were cultured with mitogens, ConA & LPS for 72 hr.

Results:

- AHR-10282, at any tested dosages, did not modify spleen lymphocyte response to ConA stimulation.
- Little but not consistent increases in the responses of cells obtained from mice receiving 0.1 or 10 mg/kg AHR-10282 to LPS-induced stimulations were noted.
- Cyclophosphamide at the dose of 50 mg/kg caused a suppression in lymphocyte response to Con A or LPS stimulations.

(b.) **Evaluation of AHR Compounds for Immunomodulatory Activity Part IV: Influence of AHR-10282 on Macrophage Activation (GTR840282) (Vol 1.19, p 211)**

Report Nº: 84-0282
Study Aim: To determine if AHR-10282 modulates *Corynebacterium parvum*-induced macrophage activity in mice.
Compound: AHR-10282 in PBS, pH 7.4
Dosage & Route: AHR-10282: 0.1, 1.0, 10.0, and 50 mg/kg for 7 days, po; Cyclophosphamide: 10 and 50 mg/kg
Animals: 20 ♂ BALB/c mice, 4 - 8 wk old and weighing 18 - 22 g, 5/group/dose
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: Mice were given (P.O.) AHR-10282 or vehicle for 7 days. On day 5, macrophages were activated by the i.p. injection of *C. parvum* vaccine, and animals were killed on day 10. Peritoneal exudate cells (PEC) were then collected and macrophage cytotoxic activities were measured by incubation of PEC with various ratios of Lewis Lung carcinoma cells for 2 hr.

Results: No significant changes in the macrophage cytotoxic activities against Lewis Lung carcinoma cells were attributable to AHR-10282 treatment.

(c.) Evaluation of AHR Compounds for Immunomodulatory Activity Part V: Influence of AHR-10282 on Serum Immunoglobulin G (IgG) Levels (GTR 850078) (Vol 1.19, p 222)

Report Nº: 85-0087
Study Aim: To determine the influence of orally administered AHR-10282 on the alteration of total serum IgG levels in mice.
Compound: AHR-10282 in PBS, pH 7.4
Dosage & Route: AHR-10282: 0.1, 1.0, 10.0, and 20 mg/kg for 7 days, po; Cyclophosphamide: 10 and 50 mg/kg
Animals: 165 ♂ BALB/c mice, 3 - 6 wk old and weighing 15 - 20 g
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: Mice were given (p.o.) AHR-10282 or vehicle for 7 days. On day 6, macrophages were activated by the i.p. injection of Sheep Red Blood Cells (SRBC), and on day 10 the mice were sacrificed. Serum samples were collected for serum IgG determination. The mean serum IgG concentrations of treated mice were compared with those obtained from a group of 125 control mice.

Results: Treatment of mice with AHR-10282 did not affect serum total IgG levels.

Conclusion: AHR-10282 did not possess immunomodulatory properties.

[2.] EFFECTS ON GLUTATHIONE LEVELS IN LIVER AND KIDNEY

(a.) The Effect of AHR-10282 on Reduced Glutathione (GSH) Levels in Liver and Kidney of Rats Pretreated with Saline or Phenobarbital (GTR 18427) (Vol 1.19, p 228)

Report Nº: 86-0186
Study Aim: To determine the effect of a single dose of AHR-10282 on the reduced glutathione levels in liver and kidney of rats.
Compound: AHR-10282, phenobarbital and Na salicylate were dissolved in saline.
Dosage & Route: Animal assignment and dosage schedule are shown in the following table (Vol 1.19, p 232).

Group	Nº Animals	Pretreatment[a] (i.p.)		Treatment[b] (i.p.)	
		Compound	Dose	Compound	Dose
A	45	Saline	1 ml/kg/day	Saline	1 ml/kg
B	45	Saline	1 ml/kg/day	Na Salicylate	800 mg/kg
C	45	Saline	1 ml/kg/day	AHR-10282	40 mg/kg
D	45	Phenobarbital	75 mg/kg/day	Saline	1 ml/kg
E	45	Phenobarbital	75 mg/kg/day	AHR-10282	40 mg/kg

[a] Compound were injected i.p. on days 1, 2, and 3.
[b] Animals received a single i.p. bolus dose on day 4.

Animals: ♂ Sprague Dawley rats, weighing 140 - 160 g
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: The same number of animals from each group were killed at 20,40, and 70 min, and 2, 3.5, 5.5, 10, 14, and 25 hr after dosing. Livers and kidneys were removed immediately and the levels of GSH were measured.

Results: There was an apparent diurnal difference in the normal physiological levels of GSH in the liver. AHR-10282 (40 mg/kg) caused a significant decrease (70% and 80% respectively) in liver and kidney GSH levels in the saline pretreated animals at 14 hr post dosing. Similar observation was noted in the animals pretreated with phenobarbital. The drop in the levels of GSH was a transient response, and the levels returned to physiological levels by 25 hr post treatment.

D. SUMMARY AND EVALUATION OF PHARMACOLOGY STUDIES

(1.) Analgesic Activity

AHR-10282 was shown to be more potent than Zomepirac, Suprofen, Piroxicam, Ketoprofen, Ketorolac, Flurbiprofen, and Pemedolac in inhibiting acetylcholine (ACH)-induced abdominal constriction in mice, with ED_{50} of 0.20, 0.09, and 0.21 mg/kg at pretreatment time 10, 20 and 300 min, respectively. The ED_{50} values of Bromfenac for the prevention of ACH-induced abdominal constriction in fasted mice or fed mice were similar (0.12 vs 0.15 mg/kg), and the maximum analgesic effect was peaked at ≈30 min postdose in fasted mice and at 60 min postdose in the fed mice. The duration of analgesic action was longer and the values of AUC and C_{max} were higher in the fasted mice. But in the PBQ-induced writhing test, the ED_{50} values for Bromfenac (administered 60 min prior to challenge) were 0.26 and 0.63 mg/kg p.o. in fasted mice and fed mice, respectively. Bromfenac was not a narcotic-like compound by the evidence that (1) Naloxone did not block the suppressive effect of Bromfenac against ACH-induced abdominal constriction; (2) the nociceptive response in artery clip assay was not blocked by Bromfenac; (3) high dose of Bromfenac administered i.c.v. was required to prevent PBQ-induced writhing; (4) Bromfenac did not block the biting behavior caused by substance P (SP).

AHR-10282 was 5.75 times more potent than Zomepirac in blocking the response to bradykinin, with an ED_{50} of 0.08 mg/kg in dogs.

(2.) Anti-inflammatory Activity

The anti-inflammatory action of Bromfenac was demonstrated in several preclinical animal models. The effective therapeutic doses (mg/kg) obtained from various models were listed as following:

Experimental Model		Animal Species	Effective Dose (mg/kg)
Evans Blue-Carrageenan Pleural Effusion	5 hr	Rat	0.8 mg/kg; ↓25.1-35% pleural fluid
	17 hr		0.16 mg/kg; ↓31.3% pleural fluid
Carrageenan-Induced Foot Pad Edema		Rat	0.8 mg/kg; ↓63.8% foot edema
Adjuvant (*Mycobacterium butyricum*) -Induced		Rat	0.316 mg/kg; ↓ 57.1% secondary lesion
Reverse Passive Arthus Pleurisy		Rat	ED_{50}=0.24 mg/kg

It was shown to **reduce the rectal temperature of yeast-induced hyperthermic rats** but not in normothermic rats. Bromfenac had suppressive effect on PGI_2 but not PGE_2 production in the rat brain with an ED_{50} of 3.2 mg/kg.

In the *in vitro* studies, the pharmacological actions of Bromfenac were also elucidated including:

i. AHR-10282 was shown to be more potent than Indomethacin in relaxation of the resting tone of guinea pig tracheal strips;

ii. The syntheses of PGE_2 and $PGE_{2\alpha}$ by bovine seminal vesicle, rabbit kidney medulla or rabbit uterus, PGH synthase activity, calcium ionophore-induced eicosanoid released by human whole blood, and arachidonic acid-induced platelet aggregation were also inhibited by Bromfenac in a dose-dependent manner. This in vitro suppressive activity was much more potent than reference NSAIDs, Indomethacin, and/or Ibuprofen.

(3.) Ancillary Pharmacology-Effects Related to Possible Adverse Reactions

Studies in cats revealed that Bromfenac did not have effects on central nervous system (behavior and normal spontaneous cerebral activities). Results obtained from the studies in dogs indicated that Bromfenac did not have anti-adrenergic, anticholinergic and anti-histaminic properties, but it had little or limited effects on cardiovascular function.

Experiments in rats demonstrated that Bromfenac, like reference NSAID Indomethacin, caused a dose-related increase in the gastric irritation and intestinal toxicity. The potency to induce gastric damages in rats were similar between Bromfenac and Indomethacin; however, Bromfenac was 1.78 times more potent than Indomethacin in causing intestinal damages in rats.

(4.) Other Effects

AHR-10282 did not possess immunomodulatory properties by the evidence that it did not modify spleen cells response ConA or LPS stimulation, and did not affect serum total IgG response to SRBC. Bromfenac induced a significant decrease in the GSH levels in the kidneys or livers of either saline or phenobarbital pretreated rats.

Pharmacokinetics

Animal Studies: The pharmacokinetics of Bromfenac was studied in various animal species, including mouse, rat, rabbit, dog, rhesus monkey, cynomolgus monkey, and man. These studies were

contained in Vol 1.45-1.48 of the NDA submission. Only the summary of these studies will be reported here.

A. Absorption.

In a series of intravenous studies of Bromfenac in animals, the following pharmacokinetic parameters were obtained.

Following a single IV administration:

Species	Dose (mg/kg)	$T_{½}$ (hr)	Clp (ml/kg·hr)	Vss (ml/kg)
Mouse	1.0	1.5	90.8	171
Rat	1.0	4.1	17.8	123
Dog	1.0	2.6 ± 0.5	32.8 ± 16.4	87.7 ± 25.7
Cynomolgus Monkey	1.0	1.2 ± 0.2	125 ± 20.0	117 ± 21.0

The results showed that the terminal half-life was short (except rat), the plasma clearance (Clp) was high, and the steady state volume of distribution was small.

The pharmacokinetic parameters of Bromfenac after a single oral dose are summarized in the following table:

Species	Dose (mg/kg)	Cmax (µg/ml)	Tmax (hr)	$T_{½}$ (hr)	F[a]
Mouse	1.0	2.59	0.25	1.3	41
Rat	1.0	5.03	0.50	4.3	44
Rabbit	7.5	7.62 ± 7.62	0.60 ± 0.30	1.7 ± 0.6	ND[b]
Dog	1.0	7.75 ± 4.85	0.80 ± 0.30	1.4 ± 0.4	76
Cynomolgus Monkey	1.0	2.96 ± 1.94	0.50 ± 0.00	1.2 ± 0.3	45
Human	50 mg[c]	4.61 ± 1.27	0.58 ± 0.2	1.1 ± 0.1	ND[b]

[a] F=absolute bioavailability.
[b] ND=not determined.
[c] Pharmacokinetic values from 50 mg dose have been selected for comparison to 1 mg/kg dose in all species except rabbit (7.5 mg/kg).

It appeared that Bromfenac was rapidly absorbed after oral dosing, with maximal plasma concentrations reached within 0.25 hr to 0.8 hr after dosing.

The terminal elimination half-life was less than 2 hr in all species, including man (except in rat which was 4.3 hr). Absolute bioavailability was higher in dog (76%) and about 40% in mouse, rat and monkey.

The pharmacokinetic parameters of Bromfenac at different doses by iv or oral

administrations are shown below:

Species	Dose (mg/kg)	Route	Cmax (μg/ml)	Tmax (hr)	$AUC_{0-\infty}$ (μg·hr/ml)	$T_{½}$ (hr)
Mouse	1.0	ig[b]	2.6	0.25	4.5	1.3
	5.0	ig	16.9	0.25	34.5	1.3
	7.5	ig	24.8	0.25	58.8	1.2
Rat	0.6	ig	2.8	0.25	18.0	3.5
	6.0	ig	23.5	0.25	168	4.8
	0.3	iv	NA[a]	NA	13.1	4.4
	3.0	iv	NA	NA	169	4.7
Rhesus monkey	15	ig	33.3	0.33	58.7	4.3
	45	ig	150	0.67	247	2.6
	15	iv	NA	NA	79.2	4.9
	45	iv	NA	NA	382	6.4
Cynomolgus monkey	3.0	ig	5.34	0.92	10.5	1.42
	30	ig	36.8	2.78	133	2.69
	3.0	iv	NA	NA	21.4	1.40
	30	iv	NA	NA	478	3.65

[a] NA=Not applicable.
[b] intragastric

In mouse and rat, increasing dose did not produce notable effects on the elimination half-life of Bromfenac. In mouse, Cmax increased proportionally with dose whereas the AUC increased slightly greater than proportionally with increasing dose. In rat, AUC and Cmax increased proportionally with increasing dose. In monkeys, increasing dose produced increases in the elimination half-life of Bromfenac in most cases. Both Cmax and AUC increased in a greater than proportional manner, with increasing dose suggesting a possibility of saturable elimination at high dose.

In a series of animal studies, the pharmacokinetic parameters of Bromfenac after single dose administration was compared with that of after multiple dose administrations. It was found that 14 days of multiple dosing in mouse, rat, rabbit, and monkey did not affect the pharmacokinetic parameters of Bromfenac. The lack of increase in Cmax and AUC were consistent with the short half-life of Bromfenac and indicated lack of accumulation of Bromfenac in these animals.

The bioavailability of Bromfenac was affected by food ingestion. An average reduction of more than 50% was found in fed condition in the mouse, rat, dog and cynomolgus monkey. Cmax

and AUC were reduced in the fed condition but Tmax and $T_{½}$ were not affected.

Species	Dose, mg/kg	Cmax, µg/ml	Tmax, hr	$T_{½}$, hr	$AUC_{0-\infty}$ µg·hr/ml
Mouse	1.0 (fasting)	2.4	0.5	2.4	8.9
	2.0 (fed)	2.8	0.5	1.5	7.0
Rat	1.0 (fasting)	5.03	0.5	4.3	24.5
	1.0 (fed)	2.14	1.0	5.1	14.8
Dog	1.0 (fasting)	7.75	0.8	1.4	26.5[b]
	1.0 (fed)	4.13	0.9	1.4	8.7[b]
Cynomolgus Monkey	15.0 (fasting)	85.1	0.42	ND[a]	101[b]
	15.0 (fed)	29.0	0.21	ND[a]	53.7[b]

[a] ND=not determined
[b] AUC_{0-24}

In rat and dog, the bioavailability of Bromfenac was also affected by food, even after intravenous administration.

Species	Dose (mg/kg)	$AUC_{0-\infty}$ (µg·hr/ml)	$T_{½}$ (hr)	Clp (ml/kg·hr)	Vd_{ss} (ml/ kg)
Rat	1 (fasted)	56.1	4.1	17.8	123
	1 (fed)	42.3	3.8	23.7	141
Dog	1 (fasted)	35.1 ± 12.6	2.6 ± 0.5	32.8 ± 16.4	87.7 ± 25.7
	1 (fed)	12.7 ± 4.9	1.9 ± 0.8	89.0 ± 35.5	135 ± 35.7

Systemic clearance and apparent volume of distribution were higher in fed rats and fed dogs. It appeared that the decrease in the bioavailability of Bromfenac with food was mediated in part by changes in clearance.

B. Protein Binding.

The in vitro binding of Bromfenac to the plasma proteins of six animal species was assessed by equilibrium dialysis. At a Bromfenac concentration of 1.4 µg/ml the percentages of unbound drug in the plasma of mice, rats, dogs, cynomolgus monkeys, rhesus monkeys, and humans were 2.51, 0.48, 0.19, 0.41, 0.33, and 0.16%, respectively. The binding of Bromfenac to plasma proteins was independent of concentration between 1.4 to 10 µg/ml. At a concentration of 53 µg/ml, however, there was a significant increase in the percentage of unbound drug detected in rat, cynomolgus monkey and human plasma. The high extent of binding to plasma proteins is consistent with the small volume of distribution.

C. Distribution.

The distribution of radioactivity from ^{14}C-Bromfenac administered orally or intravenously were determined in rats, rhesus monkeys, and cynomolgus monkeys. The radioactivities were measured by autoradiography or by analysis of isolated organs/tissues. Distribution after oral and intravenous doses was rapid and widespread and generally similar in males and females. Much of the radioactivity was associated with the GI tract, liver, and kidney. The lower radioactivity was found in the brain, testes, eyes, muscle, thymus, bone marrow and spleen.

At a dose of 0.9 mg/kg, radioactivity was found to cross the placenta. However, the exposure of fetus to ^{14}C-Bromfenac was less than the exposure in the dam. It was found that the concentration of radioactivity in the milk of lactating rats receiving ^{14}C-Bromfenac sodium was lower than those in the plasma.

D. Enzyme Induction.

The concentration of cytochrome P-450, a mediator of drug metabolism, and the activity of ethoxy and pentoxyresorufin O-dealkylase, two cytochrome P-450 dependent enzyme activities, were assessed in liver microsomes from mice receiving Bromfenac sodium, at 0.2, 1.0, 5.0, and 7.5 mg/kg/day for seven consecutive days. Bromfenac did not show any effects on liver weight, liver microsomal protein content, cytochrome P-450 content or epoxy or pentoxyresorufin O-dealkylase activity when compared with control group at the doses studied.

Rats were pretreated with phenobarbital (75 mg/kg/day) or saline intraperitoneally once daily for 3 days. Animals were then treated with Bromfenac (40 mg/kg) on 4th day. Livers and kidneys from animals were collected at several time points and determined for the reduced glutathione (GSH) contents. It was found that Bromfenac produced a small but statistically significant decrease in GSH levels, both in liver and kidney of both phenobarbital and saline pretreated animals.

E. Metabolism.

The metabolism of orally administered ^{14}C-Bromfenac was studied in bile-duct cannulated rats. Free and conjugated Bromfenac and AHR-11665 (benzoic acid metabolite) accounted for the main biliary radioactivity at 0 to 4 hours, whereas AHR-11665 was the major metabolite at 4 to 24 hours. AHR-10240 (cyclic amide metabolite) was also present in bile. These metabolites constituted approximately 65 to 90% of the biliary ^{14}C. Alkaline and enzymatic hydrolysis indicated that conjugates were glucuronides of Bromfenac and AHR-11665.

The plasma and urinary metabolite profiles of Bromfenac in cynomolgus monkey and human were similar. Analysis of plasma showed the presence of mainly Bromfenac. Urinary metabolite profiles were characterized by two peak pairs and AHR-10240 in HPLC system. No free or conjugated Bromfenac was detected in urine, and none of the urinary metabolites were found in plasma.

F. Excretion.

The following table lists the extent of elimination of radioactivity in urine and feces after oral administration of ^{14}C-Bromfenac.

Species	Dose, mg/kg	Urine, % of dose	Feces, % of dose
Rat[a]	0.6	27	50
Cynomolgus Monkey[b]	1.0	70	22
Human[a]	50 mg	82	13

[a] 0-4 days
[b] 0-7 days

The data indicated that cynomolgus monkey and human are similar in the excretion pattern. The Bromfenac-derived material was primarily excreted by the renal route. In rat, the drug-derived material was excreted mainly in the feces.

G. *SUMMARY AND EVALUATION OF ADME STUDIES*

The pharmacokinetic/pharmacodynamic correlation was studied in rat and mouse models. In the anti-inflammatory model (carrageenan-induced foot edema), the effective dose of Bromfenac at 0.032 and 0.01 mg/kg for fed and fasted rats produced corresponding plasma levels of 0.06 and 0.30 µg/ml. In the mice analgesic model (acetylcholine or phenylbenzoquinone induced writhing), the estimated EC_{50} for Bromfenac were 0.37 to 0.47 µg/ml for fed and fasted mice, respectively.

The absorption and excretion of Bromfenac were rapid in all animal species studied. Food ingestion reduced the bioavailability of Bromfenac in animals and man. Tissue distribution widely occurred, but mainly in organs of elimination. No significant accumulation of the drug was observed. Bromfenac was highly bound to plasma protein (99%). The metabolism and excretion patterns were similar in monkeys and humans. Elimination of Bromfenac and its metabolites occurred mainly via the renal pathway in monkey and man and via the feces through biliary excretion in rat. In the pregnant rats, Bromfenac entered the fetuses but at a lower level than the dam. Bromfenac was also found in rat milk at a lower concentration than that in the plasma of lactating dam.

Toxicology & Carcinogenicity Studies

A. *ACUTE TOXICITY STUDIES*

a. ORAL STUDIES

[1.] Rat Studies
AHR-10282B: Dose-Range Study to Determine the Pharmacologic and Toxicologic Effects After Acute Oral Administration to Mature Female Rats (GTR 83-0412)(Vol 1.20, p 1-29)

Report Nº: 83-0412

Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B in mature female rats.
Compound: AHR-10282B in dist. H_2O
Dose & Route: AHR-10282B: 0, 6.81, 10, 14.7, 21.5, 31.6, 46.4 mg/kg, 1 ml/100 g body weight p.o.
Control Vehicle: Dist. H_2O, 1 ml/100 g body weight p.o.
Animal: Mature ♀ Sprague-Dawley rats, weighing 140 - 175 g, 5/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (0, 1, 3.16, 6.81, 10, 14.7, 21.5, 31.6, 46.4, 100, 316, and 1000 mg/kg) was orally administered to each rat on day 0. The animals were observed daily for 7 days and post mortem examination was conducted. Body weights of each animal were determined daily.

Results: The LD_{50} was 39.6 mg/kg. The following observations were present in most of treatment groups: piloerection; hypothermia; blanched ears, feet and eye; hypotonia; dark fecal pellets; bloated abdomen; toe walking; ptosis; weak grip strength. Bloody fluid accumulation in the peritoneal cavity, adhesions of the intestines, hard nodules in the intestinal wall, and thickened intestine were seen at necropsy in animals received doses ≥10 mg/kg; hard nodules in intestinal wall, and hemorrhagic area in the duodenum were observed in animal receiving <10 mg/kg of AHR-10282. The incidence of these observations was dose-related. Therefore, the predominant toxicity observed in the present study was gastrointestinal associated.

[2.] Rabbit Studies
AHR-10282B: Dose-Range Study to Determine the Pharmacologic and Toxicologic Effects After Acute Oral Administration to Mature Female Rabbits (GTR 83-0261)(Vol 1.20, p 30-38)

Report №: 83-0261
Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B in rabbits.
Compound: AHR-10282B in 0.3% methyl cellulose in dist. H_2O
Dose & Route: AHR-10282B: 0, 10, 31.6, 100, 316, 1000, and 2150 mg/kg p.o.
Control Vehicle: 0.3% methyl cellulose in dist. H_2O p.o.
Animal: 14 mature (7♂ & 7♀) New Zealand White rabbits, weighing 1.7-2.9 kg, 2 (1♂ & 1♀)/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (0, 10, 31.6, 100, 316, 1000, and 2150 mg/kg) was orally administered to the rabbits on day 0. The animals were observed daily for 7 days and post mortem examination was conducted. Body weights of each animal were determined on days

0 and 7.

Results: Both animals in groups receiving 316 (on day 7) and 2150 mg/kg (on day 0) and one rabbit in the group receiving 1000 mg/kg (on day 7) were dead. No significant findings was revealed for control group and for group receiving 10 mg/kg treatment. Weight loss, increased respiration rate, and anorexia were seen in animals receiving 316 and 1000 mg/kg. In addition to the described findings, convulsions were noted in both animals receiving the highest dose, 2150 mg/kg. Postmortem examinations showed congestion of stomach in both rabbits dosed with 2150 mg/kg, and several small hemorrhagic spots on the stomach of the male rabbit. Kidneys were pale to light yellow-brown in appearance.

[3.] Dog Studies

AHR-10282B: Dose-Range Study to Determine the Pharmacologic and Toxicologic Effects After Acute Oral Administration to Mature Female Dogs (GTR 83-0313)(Vol 1.20, p 46-60)

Report Nº: 83-0313
Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B in dogs.
Compound: AHR-10282B in gelatin capsules
Dose & Route: AHR-10282B: 10, 21.5, 46.4, 100, and 215 mg/kg p.o.
Animal: 14 mature (7♂ & 7♀) mongrel dogs, weighing 8.5-12.5 kg, 2 (1♂ & 1♀)/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: Dogs orally received AHR-10282B, 10, 21.5, 46.4, 100, or 215 mg/kg on day 0. The animals were observed daily for 7 days and post mortem examination was conducted. Body weights of each animal were determined on days 0 and 7.

Results: No mortality was found. Reddish brown or dark spots in the stools were seen in animals at all dose levels. Emesis was noted at the doses of 46.4, 100 and 215 mg/kg. GI lesions seen at necropsy included hemorrhagic spots in duodenum, jejunum and ileum, congestion and thickening throughout the GI tract.

b. INTRAVENOUS STUDIES

[1.] Rat

(1.) AHR-10282B: Dose-Range Study to Determine the Pharmacologic and Toxicologic Effects after Acute Intravenous Administration to Mature Male Rats (GTR 83-0410)(Vol 1.20, p 61-79)

Report Nº: 83-0410
Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B in mature male rats.
Compound: AHR-10282B in dist. H_2O

Dose & Route: AHR-10282B: 0, 0.1, 0.316, 1.0, 3.16, 10, 31.6, and 100 mg/kg iv, 0.1 ml/100 g body wt.
Control Vehicle: Dist. H_2O, 1 ml/100 g body wt. p.o.
Animal: Mature ♂ Sprague-Dawley rats, weighing 140 - 175 g; 5/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (0, 0.1, 0.316, 1.0, 3.16, 10, 31.6, and 100 mg/kg) was administered iv to each rat on day 0. The animals were observed daily for 7 days and post mortem examination was conducted. Body weights of each animal were determined daily.

Results: Some animals at 31.6 mg/kg (2/5) and at 100 mg/kg (4/5) were dead between days 3-7 postdose. The LD_{50} were 46.0 mg/kg. Weight loss was seen in rats at 31.5 and 100 mg/kg. Diarrhea, dark fecal pellets, hematuria, blanched ears, feet, and tails, piloerection, hypotonia and bloated abdomen were major clinical signs. No major pathological findings at the necropsy in rats at ≤1.0 mg/kg. Pale kidneys but no GI lesions were observed in animals at 3.16 mg/kg. Thickened intestinal walls, adhesions of intestines to peritoneal walls and throughout the GI tract, hemorrhagic spots in the small intestine, pale liver, adhesions of lobes of the liver, and blood in GI tract were noted in animals at 31.6 and 100 mg/kg.

(2.) AHR-10282B: Dose-Range Study to Determine the Pharmacologic and Toxicologic Effects after Acute Intravenous Administration to Mature Female Rats (GTR 83-0411)(Vol 1.20, p 80-99)

Report Nº: 83-0411
Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B in mature ♀ rats.
Compound: AHR-10282B in dist. H_2O
Dose & Route: AHR-10282B: 0, 0.1, 0.316, 1.0, 3.16, 10, 31.6, and 100 mg/kg iv, 0.1 ml/100 g body wt.
Control Vehicle: Dist. H_2O, 1 ml/100 g body wt. p.o.
Animal: Mature ♂ Sprague-Dawley rats, weighing 170-210 g, 5/group
Study Location: A.H. Robins Company, R & D Division, 1211 Sherwood Ave., Richmond, VA 23220
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (0, 0.1, 0.316, 1.0, 3.16, 10, 31.6, and 100 mg/kg) was administered iv to each rat on day 0. The animals were observed daily for 7 days and post mortem examination was conducted. Body weights of each animal were determined daily.

Results: Mortality occurred in animals at 10 (2/5), 31.6 mg/kg (4/5) and at 100 mg/kg (5/5) between days 3-7 postdose. The LD_{50} was 15.0 mg/kg. Decreased body weight gains were seen in rats at 3.16 and weight loss was noted in animals at ≥10 mg/kg. No overt pharmaco-toxicological effects could be identified clinically or macroscopically in rats at ≤0.316 mg/kg. Dark fecal pellets, blanched ears, feet, and tails, piloerection, and cold feet

were observed in animals at ≥1.0 mg/kg. In addition to those clinical signs described, hypotonia, hematuria and bloated abdomen were the major clinical signs identified in rats at ≥3.16 mg/kg. Yellow tinted jejunum and bright yellow material mixed with food in the stomach were major findings at necropsy in animals at 3.16 mg/kg. Thickened intestinal walls, adhesions of intestines to peritoneal walls and throughout the GI tract, hemorrhagic spots in the small intestine, pale liver, and blood in GI tract were noted in animals at 10, 31.6 and 100 mg/kg.

It appeared that ♀ rats were more susceptible to the Bromfenac-caused toxicity. It would be important to know the PK profiles in ♀ rats.

B. SUBCHRONIC/CHRONIC CARCINOGENICITY STUDIES

a. ORAL STUDIES

[1.] Mouse Studies

(1.) Two-Year Carcinogenicity Bioassay of AHR-10282B in CD®-1 Mice (GTR 87-0426)(Vol 1.21-1.25)

Study Nº: 87-0426
Study Aim: To determine the carcinogenic and toxicologic potential of AHR-10282 in mice.
Compound: AHR-10282B in potassium phosphate buffer (PB), pH 8.4
Dose & Route: 0.2, and 1.0 mg/kg/day for 728 days and 5.0 mg/kg/day for 184 days then 7.5 mg/kg/day for the remaining 544 days, 10 ml/kg p.o.
Control Vehicle: PB, pH 8.4, 10 ml/kg body weight p.o.
Animal: Swiss mice [Crl:CDR-1(ICR)BR], ≈6 weeks old, weighing 23-31 g for ♂ and 20-26 g for ♀, 60/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: Mice, group of 60/sex, were dosed with vehicle, 0.2, or 1.0 mg/kg/day for 728 days, and the high dose group was given 5.0 mg/kg/day for 184 days, then 7.5 mg/kg/day for the remaining 544 days orally. The animals were observed 2x daily for the mortality and moribundity. Food consumption and body weights were recorded once a week for the first 14 weeks and once every 2 weeks for the remainder of the study. Hematological parameters were determined on 10 mice/sex/group at months 0, 6, 12, 15, 18, and 24. Necropsy was performed on each animal at moribund condition or the terminal schedule sacrifice.

Results:

- Mortality & Survival - The mortality and survival of each group at the end of 24 months of study are summarized in the following table. There was no difference in the survival distribution between controls and drug-treated groups.

	Dose Levels (mg/kg/day)									
	Control 1		Control 2		0.2		1		5.0/7.5	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N° of Animals	60	60	60	60	60	60	60	60	60	60
Non-accidental	21	29	19	30	24	32	21	34	26	31
Accidental Deaths	0	0	2	0	0	1	1	1	2	1
Terminal Sacrifice	39	31	39	30	36	27	38	25	32	28
Survival Rate (%)	65	51.7	65	50	60	45	63.3	41.7	53.3	46.7
Survival P Values	-	-	0.82	0.83	0.63	0.65	0.95	0.18	0.25	0.39

- Clinical Signs - Ruffled hair was observed randomly in all groups. No other findings were attributable to the treatment.
- Body Weights & Food Consumption - Body weights for the ♂ in low- and mid-dose groups were significantly increased as compared to vehicle control 1 & 2. Body weights for the ♂ at high dose were not statistically different from controls. In contrast, body weights in the ♀ at high dose were lower than control groups, with ≈5% and 8% less relative to the control 1 & 2, respectively. The mean body weight gains for each group and the difference (%) from the control 1 and control 2 are presented in the following table.

Dose Level (mg/kg/day)	♂				♀			
	Mean B. Wt.		Gain relative to control (%)		Mean B. Wt.		Gain relative to control (%)	
	Wk104	Wk 0	Control 1	Control 2	Wk 104	Wk 0	Control 1	Control 2
Control 1	38.4	26.3	-	0.00	36.6	22.6	-	-1.4
Control 2	38.8	26.7	0.00	-	37.1	22.9	1.42	-
0.2	39.1	27.0	0.00	0	36.7	22.9	-1.42	2.81
1.0	39.3	27.1	0.82	0.82	37.0	23.1	-0.71	2.11
5.0/7.5	39.5	27.0	3.30	3.30	34.3	23.1	-20.00	-21.12

There were no differences in mean daily food consumption between treatment and control groups.

- Clinical Pathology - Hematological evaluations were performed at 0, 6, 12, 15, 18, and 24 months during the course of the study. A significant decrease in lymphocyte counts was seen at the 6 mon analysis in the male at 1.0 mg/kg and a significant increase in the atypical lymphocytes was present in the ♀ receiving 1.0 mg/kg, at the 15 mon analysis. Statistically significant decreases in WBC, RBC, hemoglobin and hematocrit values were seen in animals at 5.0/7.5 mg/kg (at the 6 mon analysis for the ♂ and at the 12 mon analysis for the ♀). There were evident increases (more than 2x higher relative to the controls) in the lymphocytes, monocytes, segmented neutrophils, and atypical lymphocytes in high dose male at the 24 mon analysis. Elevated platelet and atypical lymphocyte counts were noted at the 12, 15, 18 and 24 mon hematology analyses in high dose females. Significant ↑ in segmented neutrophils and monocytes were also observed in high dose female at the 15 mon analysis.

- Neoplastic and Non-neoplastic Incidences - Stomach and liver were the target organs. The incidences of drug-related non-neoplastic lesions, characterized as ulcers in the glandular mucosa of the stomach, subacute inflammation of the glandular mucosa of the stomach and cytological alteration involving hepatocyte were significantly higher in the high dose group. Gastric ulcers and subacute inflammation of the glandular mucosa occurred at much higher rates in the ♀ than in the ♂. A cytological alteration (enlargement of centrolobular hepatocyte nuclei) was seen in the liver of high dose groups (both ♂ & ♀). It appeared no treatment-related increase occurred in tumor incidence. The incidences of various neoplastic and non-neoplastic events are listed in the following table.

Types of Neoplastic Abnormality		Dose Levels (mg/kg/day)				
		Control 1	Control 2	0.2	1	5.0/7.5
Interstitial Cell Adenomas in the Testicles of Males		9/60	0/60	0/60	1/60	4/60
Interstitial Cell Carcinoma in the Testicles of Males		1/60	0/60	0/60	0/60	0/60
Hemangioma/ Hemangiosarcoma in All Tissues	♂	8/60	3/60	3/60	3/60	2/60
	♀	10/60	8/60	9/60	4/60	5/60
Stromal Polyps in the Uterus	♀	4/60	1/30	0/33	0/35	1/60
Stromal Sarcoma, Uterus	♀	3/60	3/60	2/60	0/60	3/60
Adenocanthoma/Adenocarcinoma, Mammary Gland	♀	4/60	0/60	3/60	3/60	2/60
Hepatocellur Adenoma	♂	8/60	14/60	10/60	11/60	10/60
	♀	1/60	5/60	0//60	0/60	6/60
Hepatocellular Carcinoma	♂	4/60	4/60	7/60	6/60	6/60
	♀	0/60	0/60	0/60	0/60	0/60
Lymphoma Malignant, Mixed (In All Tissues)	♂	4/60	3/60	4/60	5/60	5/60
	♀	18/60	14/60	11/60	9/60	13/60
Alveolar /Bronchiolar Adenoma, Lung	♂	15/60	2/60	8/60	5/60	12/60
	♀	1/60	5/60	0/60	0/60	6/60
Alveolar/ Bronchiolar Carcinoma, Lung	♂	6/60	2/60	5/60	4/60	2/60
	♀	6/60	3/60	1/60	4/60	7/60

Types of Non-Neoplastic Abnormality		Dose Levels (mg/kg/day)				
		Control 1	Control 2	0.2	1	5.0/7.5
Stomach Ulcer	♂	0/60	0/60	0/60	0/60	4/60
	♀	0/60	0/60	0/60	1/60	35/60**
Stomach , Subacute Inflammation	♂	3/60	6/60	6/60	3/60	17/60**
	♀	6/60	8/60	6/60	11/60	53/60**
Cytological Alteration in the Liver	♂	5/60	5/60	4/60	8/60	23/60**
	♀	1/60	1/60	2/60	1/60	21/60**
Amyloidosis (Kidneys, Adrenals, Hearts, Small Intestines, Salivary Glands, Thyroids and Ovary)	♂	9/60	10/60	8/60	9/60	15/60
	♀	16/60	4/60	7/60	11/60	7/60
Sternum, Developmental Malformation	♂	30/60	7/21	10/24	8/22	13/60
	♀	4/60	2/30	0/33	1/35	2/60
Bone Marrow -Hypercellurity	♂	14/60	3/21	7/24	7/22	16/60
	♀	11/60	10/30	7/33	10/35	38/60

** P≤0.001

(2.) Eight-Week Range Finding Study in CD-1 Mice on AHR-10282B (PRS 5610-R_4)(Vol 1.25, p 233-251)

Study Nº: 5610-R_4
Study Aim: To determine the dose range of AHR-10282B in order to select doses for a 2-year chronic study to be performed on mice.
Compound: AHR-10282B in potassium phosphate buffer (PB), pH 8.4
Dose & Route: 0.05, 0.125, 0.30, 0.75, 2.0, and 30.0 mg/kg, 0.1 ml/10 g body weight p.o. for 56 days; 0, 5.0, and 12.5 mg/kg p.o. for 66 days
Control Vehicle: PB, pH 8.4, 0.1 ml/10 g body weight p.o.
Animal: CD-1 mice, ≈7 weeks old, weighing ? g, 5/sex/group
Study Location:

Compliance with GLP/QAU: No
Study Design: AHR-10282B (0, 1, 3.16, 6.81, 10, 14.7, 21.5, 31.6, 46.4, 100, 316, and 1000 mg/kg) was orally administered to each mouse once daily for 56 days.

Results: Results were not submitted in the present NDA, the reviewer had requested data from the sponsor.
One ♂ & 3♀ at 30 mg/kg (days 11-39) and 1♀ at 12.5 mg/kg (day 2) died before scheduled sacrifice. Enlarged spleens were found in animals at ≥0.3 mg/kg/day. The provided data did not indicate any GI associated lesions at necropsy or histopathological examinations.

[2.] Rat Studies

(1.) AHR-10282B: 13-Week Oral Toxicity Study in Rats (GTR 83-0518)(Vol 1.26, p 1-306)

Report Nº: 83-0518
Study Aim: To determine the toxicity of AHR-10282B in a 13-week oral study in rats.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 0.1, 0.5, and 2.5 mg/kg/day, 10 ml/kg p.o. for 13 weeks
Control Vehicle: Phosphate buffer, pH 8.0, 10 ml/kg p.o.
Animal: Charles River CD® rats, weighing 120-142 g for ♂ and 90-105 g for ♀, ≈5 weeks old, 15/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 0.1, 0.5, or 2.5 mg/kg/day) was orally administered to each rat for 13 weeks by gavage. The animals were observed 2x daily, body weights and food consumption were recorded weekly, and ophthalmology examination was performed 2x, one at pretest, and the other at 3 mon postdose. Clinical pathological parameters were measured on 10/rats/sex/group at weeks 6 and 13. Postmortem examination was conducted on all animals at moribund and terminal sacrifice .

Results:

- Mortality & Clinical Signs - One ♀ in control group and 12 ♂ and 12 ♀ at 2.5 mg/kg were found dead or sacrificed in moribund condition. Firm areas on the ventral abdomen, abdominal distended, and decreased fecal output were observed in rats at 2.5 mg/kg in moribund condition.
- Body Weights & Food Consumption - Decreased body weight gain and food consumption (≈15.2-20.8%) were seen in animals at 2.5 mg/kg, and the group mean body weights at wk 13 are as following:

Dose (mg/kg/day)	Group Mean Body Weights (% Difference from Control)	
	♂	♀
0 (Control)	491	253
0.1	491	251 (-0.8%)
0.5	487 (-0.8%)	251 (-0.8%)
2.5	419 (-14.7%)	230 (-9.1%)

- Ophthalmology - No treatment related findings could be identified.
- Clinical Laboratory Observations - Increased leukocyte counts, ↑ reticulocytes, ↓RBC, ↓ hematocrit, ↓Hb, ↑ MCV, ↓ serum protein and ↓ albumin were noted for animals at 25 mg/kg at wks 6 and 13. No special changes related to the treatment could be found in the urinalysis.
- Pathology - No treatment related changes could be seen in animals at ≤0.5 mg/kg. Macroscopic changes could be identified in small and large intestines, mesenteric lymph nodes (enlarged), and abdominal cavity in animals at 2.5 mg/kg/day. Thickened mucosa, gray, yellow or greenish necrotic areas, perforation, and adhesion were major lesions in the intestinal tract. There were significant increases in liver and spleen weights (relative and absolute) which were due to extramedullary hematopoiesis in response to blood loss. Microscopic lesions could be seen in duodenum, jejunum, ileum, cecum and colon, including necrotic ulcers, necrosis of the intestinal wall, enteritis, and peritonitis. Lymphadenitis was also identified in the mesenteric lymph nodes.

(2.) AHR-10282B: Twenty Four Month Oral Chronic Toxicity and Carcinogenicity Study in Rats (Interim Report Covering Six-Months of Compound Administration) (GTR 85-0277)(Vol 1.27, 29 & 29A)

Report Nº: 85-0277
Study Aim: To determine the toxicity and carcinogenic potential of AHR-10282B in a two-year oral study in rats: 6-month interim report.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 05, 0.3 and 0.6 (changed to 0.75 at wk 27) mg/kg/day, 10 ml/kg p.o. (by gavage)
Control Vehicle: Phosphate buffer, pH 8.0, 10 ml/kg p.o.
Animal: Charles River CD® rats, 70/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 0.05, 0.3, or 0.6 mg/kg/day) was orally administered to each rat for 26 weeks by gavage. The animals were observed 2x daily; body weights and food consumption were recorded weekly for the first 14 wks then once every 2 wks. Ophthalmological examinations were done at pretest, 3 and 6 months. Clinical pathological parameters were measured on 10/rats/sex/group at 3 and 6 months. Postmortem examination was conducted on animals at moribund and scheduled 6 month interim sacrifice.

Results: No clinical signs were attributable to the treatment. Four rats found dead within first two weeks were replaced. The viability of each group during the first 6 months of study is shown in the following table:

	Dose Levels (mg/kg/day)									
	Control 1		Control 2		0.05		0.3		0.6	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Nº of Animals Initiated	70	70	70	70	70	70	70	70	70	70
6 Mon Interim Sacrifice	10	10	10	10	10	10	10	10	10	10
Nº of Unscheduled Death, 0-6	2	0	1	1	2	0	2	1	3	1
Nº of Surviving Animals	58	60	59	59	58	60	58	58	57	59

No drug related macro- & micro-scopic changes were found during histopathological examination. However, one ♂ at 0.6 mg/kg/day that died during study wk 25 showed perforation of the jejunum and peritonitis at necropsy and microscopically revealed changes in the intestinal tract with characteristics of inflammation in the jejunum, ileum, colon and mesenteric lymph node. The causes for some deaths as revealed by the micro- and macro-scopic findings were liver necrosis (1), malignant lymphoma (1), pyelonephritis, dosing accident (6), perforation and necrotic enteritis of jejunum (1), and chronic progressive nephropathy (1). All scheduled sacrificed animals were normal in any parameters examined (10/sex/group).

(3.) AHR-10282B: 24-Month Oral Chronic Toxicity and Carcinogenicity Study in Rats (Interim Report Covering 12-Months of Compound Administration)(GTR 86-0295)(Vol 1.28, 29B & 29C)

Report Nº: 85-0277
Study Aim: To determine the toxicity and carcinogenic potential of AHR-10282B in a two-year oral study in rats: 12-month interim report.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 0.05, 0.3 and 0.6 (changed to 0.75 at wk 27, to .90 mg/kg/day at week 36 then back to 0.60 mg/kg at week 46) mg/kg/day, 10 ml/kg p.o.
Control Vehicle: Phosphate buffer, pH 8.0, 10 ml/kg p.o.

Animal: Charles River CD® rats, 70/sex/group
Study Location:

Compliance with GLP/QAU: Yes

Study Design: AHR-10282B [0, 0.05, or 0.6 (changed to 0.75 at wk 27, to 0.90 mg/kg/day at week 36 then back to 0.60 mg/kg at week 46) mg/kg/day] was orally administered to each rat for 52 weeks by gavage. The animals were observed 2x daily; body weights and food consumption were recorded weekly for the first 14 wks then once every 2 wks. Ophthalmological examinations were done at pretest, 3, 6 and 12 months. Clinical pathological parameters (including hematology, blood chemistry and urinalysis) were measured on 10 rats/sex/group at 3, 6 and 12 months. Postmortem examination was conducted on animals at moribund and scheduled 12-month interim sacrifice (10/sex/group).

Results:

- Mortality & Survival - The mortality and survival of each group at the end of 12 months of study are summarized in the following table:

	Dose Levels (mg/kg/day)									
	Control 1		Control 2		0.05		0.3		0.6	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Nº of Animals Initiated	70	70	70	70	70	70	70	70	70	70
6/12 Mon Interim Sacrifice	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Nº of Unscheduled Death, 0-6 Mon	2	0	1	1	2	0	2	1	3	1
Nº of Unscheduled Death, 6-12 Mon	2	0	5	3	6	0	4	1	2	9
Nº of Surviving Animals	46	50	44	46	42	50	44	48	45	40

- Food Consumption & Weight Gains - There were no treatment-related significant differences in mean body weights and average food consumption as compared with both control group 1 & 2; however, there was a ≈5-6% increase in the food intake in the female rats at 0.3 mg/kg/day group. The group mean body weights, average food consumption for weeks 1-52, and the % differences from the control groups are presented in the following two tables.

Dose Level (mg/kg/day)	♂			♀		
	Mean B. Wt.	% Difference from		Mean B. Wt.	% Difference from	
		Control 1	Control 2		Control 1	Control 2
Control 1	715	-	-1.0	382	-	0.8
Control 2	722	1.0	-	379	-0.8	-
0.05	721	0.8	-0.1	382	0.0	0.8
0.30	705	-1.4	-2.4	401	5.0	5.8
0.60	713	-0.3	-1.2	371	-2.9	-2.1

Dose Level (mg/kg/day)	♂			♀		
	Average food consumption (g)	% Difference from		Average food consumption (g)	% Difference from	
		Control 1	Control 2		Control 1	Control 2
Control 1	27.0	-	-0.4	18.6	-	-0.5
Control 2	27.1	0.4	-	18.7	0.5	-
0.05	26.7	-1.1	-1.5	18.7	0.5	0.0
0.30	26.6	-1.5	-1.8	19.7	5.9	5.3
0.60	27.4	1.5	-1.1	19.0	2.2	1.6

Body weight gains for each group during week 1-52 and the difference from control groups are as follows:

Dose Level (mg/kg/day)	♂				♀			
	Mean B. Wt.		Gain relative to control (%)		Mean B. Wt.		Gain relative to control (%)	
	wk 52	wk 0	Control 1	Control 2	wk 52	wk 0	Control 1	Control 2
Control 1	715	189	-	-1.50	382	147	-	2.17
Control 2	722	188	1.52	-	379	149	-2.13	-
0.05	721	188	1.33	-0.19	382	148	-0.43	1.74
0.30	705	190	-2.09	-3.56	401	150	6.81	9.13
0.60	713	189	-0.38	-1.87	371	150	-5.96	-3.91

- Ophthalmology - Normal
- Clinical Laboratory Examinations - An increase in segmented neutrophils was noted in both ♂ & ♀ at 0.6 mg/kg/day.
- Pathology - Macroscopic examination showed tested article related lesions which were consisted of mucosal erosions, necrosis, ulcers or abscesses in small and large intestines and fibrinous and/or fibrous serosal adhesions in the abdominal cavity in both male and female rats at 0.6 mg/kg/day. No treatment associated organ weight changes could be identified in the male rats. In contrast, there were increases in the relative weights (to body weight) of the adrenal gland at 0.6 mg/kg/day and relative weights (to body and/or to brain weight) of kidneys at all dose levels in the female animals. Focal necrosis in the glandular and non-glandular stomach, mucosal ulceration in the jejunum, ileum, duodenum, and cecum, peritonitis, multifocal necrosis in the liver, and chronic progressive nephrosis were major findings in animals that died or were sacrificed at moribund during 6-12 months of the study during microscopic examinations. The non-neoplastic and neoplastic incidences of microscopic changes during this period are summarized as followings.

Neoplastic Incidence : 6-12 Mon							
Tissue	Type of Tumor	Sex	Control 1	Control 2	0.05 mg/kg	0.3 mg/kg	0.6 mg/kg
Brain	Astrocytoma	♂	1/12				
Liver	Hepatocellular Carcinoma	♂		1/5			
Kidney	Renal Cell Carcinoma	♂					1/12
Lymphoreticular System	Malignant Lymphoma, Lymphocytic	♂		2/2	1/1		
Skin	Fibrosarcoma	♀			1/1		
	Keratoacanthoma	♀				½	
Mammary Gland	Fibroadenoma/Adenoma	♀			½	1/1	
	Mixed Tumor, Malignant	♀		1/1			
Uterus	Polyp	♀	1/10		1/1		

It appeared that the liver and intestinal tract were the major target organs. **Higher frequencies in the pathological changes of the liver and intestinal tract were observed in the animals at 0.6 mg/kg/day. Tabulated results also reveal that female rats were more sensitive to the intestinal toxicity than male rats.**

Non-neoplastic Incidence of Microscopic Observation: 6 - 12 Mon												
Tissue/Organ	Lesions	Sex	Control 1		Control 2		0.05 mg/kg		0.3 mg/kg		0.6 mg/kg	
			DOS[1]	SAC[2]	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Liver	Bile Duct Hyperplasia	♂	0/2	1/10	0/3	0/2	0/3	0/2	0/0	0/1	0/2	5/10
	Inflammation/Necrosis	♂	0/2	1/10	0/3	0/2	0/3	0/2	0/0	0/1	½	6/10
		♀	0/0	0/10	0/2	0/1	0/0	0/1	0/0	0/0	2/9	7/10
	Vacuolar Changes, Trace	♂	0/2	1/10	0/3	0/2	0/3	0/2	0/0	0/1	0/2	0/10
		♀	0/0	0/10	0/2	0/1	0/0	0/1	0/0	0/0	2/9	0/10
	Peritonitis, Moderate	♂	0/2	0/10	0/3	0/2	0/3	0/2	0/0	0/1	½	0/10
		♀	0/0	0/10	0/2	0/1	0/0	0/1	0/0	0/0	3/9	0/10
Cecum	Inflammation/Necrotic, Trace & Moderate	♂	0/2	0/10	0/5	0/10	0/6	0/9	0/4	0/9	½	2/10
		♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	6/9	2/10
Ileum	Inflammation, Necrotic, Moderate	♂	0/2	0/10	0/5	0/10	0/5	0/9	0/4	0/9	0/2	1/10
		♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	6/9	1/10
Jejunum	Inflammation/ Necrotic, Mild-Severe	♂	0/2	0/10	0/5	0/10	0/6	0/9	0/4	0/9	0/2	2/10
		♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	6/9	1/10
Colon	Inflammation,	♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	5/9	0/10
Duodenum	Inflammation	♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	1/9	0/0
	Peritonitis	♀	0/0	0/10	0/3	0/10	0/0	0/10	0/1	0/10	2/9	0/10
Kidney	Chronic Progressive Nephrosis	♂	2/2	3/10	0/0	0/0	0/0	0/0	0/0	0/0	0/3	5/10
		♀	0/0	0/10	0/1	0/0	0/0	0/0	0/0	0/1	1/9	5/10

[1] Deaths & sacrifices at moribund
[2] Scheduled sacrifice

(4.) AHR-10282B: 24-Month Oral Chronic Toxicity and Carcinogenicity Study in Rats : Final Report (GTR 87-0437)(Vol 1.30-1.36)

Report №: 85-0437
Study Aim: To determine the toxicity and carcinogenic potential of AHR-10282B in a two-year oral study in rats.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 0.05, 0.3 and 0.6 (changed to 0.75 at wk 27, to 0.90 mg/kg/day at week 36 then back to 0.60 mg/kg at week 46) mg/kg/day, 10 ml/kg p.o.
Control Vehicle: Phosphate buffer, pH 8.0, 10 ml/kg p.o.
Animal: Charles River CD® rats, 70/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 0.05, 0.3 or 0.6 (changed to 0.75 at wk 27, to .90 mg/kg/day at week 36 then back to 0.60 mg/kg at week 46) mg/kg/day) was orally administered to each rat for 52 weeks by gavage. The animals were observed 2x daily; body weights and food

consumption were recorded weekly for the first 14 wks, then once every 2 wks. Ophthalmological examinations were done at pretest, 3, 6, 12, 18 and 24 months. Clinical pathological parameters (including hematology, blood chemistry and urinalysis) were measured on 10 rats/sex/group at 3, 6, 12, 18 and 24 months. Postmortem examination was conducted on animals at moribund and scheduled terminal sacrifice.

Results:

- Mortality - The mortality was higher for ♂ at 0.6 mg/kg/day. Interim sacrifice was performed at 6 and 12 months with 10 rats/sex/group. The numbers of survival animals of each group at the end of 104 week study are as follows:

	Dose Levels (mg/kg/day)									
	Control 1		Control 2		0.05		0.3		0.6	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N° of Animals Initiated	70	70	70	70	70	70	70	70	70	70
N° of Unscheduled Death, 0-6 Mon	2	0	1	1	2	0	2	1	3	1
N° of Unscheduled Death, 6-12 Mon	2	0	5	3	6	0	4	1	2	9
N° of Unscheduled Death, 12-24 Mon	29	29	20	22	19	26	26	27	32	20
6/12 Mon Scheduled Sacrifice	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Terminal (24 Mon) Sacrifice	17	21	24	24	23	24	18	21	13	20
Survival Rate (%)	24.3	30.0	34.3	34.3	32.9	34.3	25.7	30.0	18.67	28.6

- Body Weights & Food Consumption - The mean body weights of each group of rats and % difference from control groups at week 104 are listed as follows:

Dose Level (mg/kg/day)	♂			♀		
	Mean B. Wt.	% Difference from		Mean B. Wt.	% Difference from	
		Control 1	Control 2		Control 1	Control 2
Control 1	696	-	-10.3	522	-	15.2
Control 2	776	11.5	-	453	-13.2	-
0.05	752	8.0	-3.1	466	-10.7	2.9
0.30	703	1.0	-9.4	480	-8.0	6.0
0.60	705	1.3	-9.1	489	-6.3	7.9

The body weight gains for each group at week 104 as compared with control 1 or control 2 are listed in the following table.

Dose Level (mg/kg/day)	♂				♀			
	Mean B. Wt.		Gain relative to control (%)		Mean B. Wt.		Gain relative to control (%)	
	wk 104	wk 0	Control 1	Control 2	wk 104	wk 0	Control 1	Control 2
Control 1	696	189	-	-13.77	522	147	-	23.35
Control 2	776	188	15.97	-	453	149	-18.9	-
0.05	752	188	11.24	-4.08	466	148	-15.2	4.60
0.30	703	190	1.18	-12.75	480	150	-12.00	8.55
0.60	705	189	1.17	-12.24	489	150	-9.60	11.51

Group mean food consumption (g/animal/day) for weeks 1-104 and the % difference from the control groups are shown in the following table.

Dose Level (mg/kg/day)	♂			♀		
	Average food consumption (g)	% Difference from		Average food consumption (g)	% Difference from	
		Control 1	Control 2		Control 1	Control 2
Control 1	26.6	-	-1.5	19.3	-	0.5
Control 2	27.0	1.5	-	19.2	-0.5	-
0.05	26.7	0.4	-1.1	19.2	-0.5	0.0
0.30	26.8	0.8	-0.8	20.1	4.1	4.7
0.60	26.9	1.1	-0.4	20.0	3.6	4.2

- Clinical Pathology - Hematological evaluations, urinalyses and clinical chemistries were performed at 0, 6, 12, 15, 18, and 24 months during the course of the study. There was no significant difference between control and drug-treated animals. Occasionally, elevated activities in ALT, AST, AP and BUN were observed in a few animals (≈ 1-3) from each group (including control groups) at 6, 12, 15, 18, or 24 month analysis.
- Neoplastic and Non-neoplastic Incidences - **GI tract, kidney and liver were the target organs. However, the sponsor did not identify liver as the target organ in the present submission.** The incidences of drug-related non-neoplastic lesions characterized as inflammation and/or necrosis in the stomach (glandular and non-glandular), small and large bowels, papillary necrosis and/or pyelonephritis and histopathological changes involving hepatocytes, such as necrosis, vacuolar alterations, inflammation and cytoplasmic changes were significantly higher in the high dose **group. Dose-dependent GI toxicity could be identified in 6-12 months and 12-24 months histopathological examinations. Similarly, dose-related nephrotoxicity could be observed in 12-24 months histopathological reports.** There were no treatment-related increases in tumor incidences for all examined tumors. The incidences of various neoplastic and non-neoplastic events are listed in the following tables. The incidences of various neoplastic and non-neoplastic events are listed in the following tables.

Non-neoplastic Incidence of Microscopic Observation: 12 Mon - Terminal												
Tissue/Organ	Lesions	Sex	Control 1		Control 2		0.05 mg/kg		0.3 mg/kg		0.6 mg/kg	
			DOS[1]	SAC[2]	DOS	SAC	DOS	SAC	-DOS	SAC	DOS	SAC
Liver	Necrosis	♂	2/29	0/17	0/11	0/20	2/14	1/18	5/18	1/13	5/32	1/13
		♀	5/30	0/20	2/10	2/15	3/13	0/17	1/14	0/12	0/20	1/20
	Peritonitis	♂	0/29	0/17	1/11	0/20	0/14	0/18	1/18	0/13	4/32	0/13
		♀	0/30	0/20	0/10	0/15	0/13	0/17	0/14	0/12	0/20	3/20
	Vacuolar Changes	♂	7/29	0/17	5/11	3/20	5/14	3/18	4/18	0/13	7/32	0/13
		♀	3/30	2/20	5/10	3/15	3/13	2/17	4/14	3/12	2/20	2/20
	Inflammation	♂	2/29	3/17	0/11	0/20	0/14	2/18	0/18	0/13	7/32	3/13
		♀	0/30	0/20	0/10	0/15	1/13	0/17	1/14	0/12	4/20	0/20
	Cytoplasmic Alteration	♂	2/29	2/17	0/11	6/20	0/14	6/18	0/18	1/13	3/32	5/13
		♀	0/30	5/20	1/10	4/15	2/13	3/17	2/14	1/12	2/20	2/20
Jejunum	Inflammation	♂	0/29	0/17	1/20	0/24	0/20	0/23	2/27	0/18	6/32	0/13
		♀	0/30	0/20	0/22	0/24	0/26	0/24	2/27	1/21	12/20	3/20
	Peritonitis	♂	0/29	0/17	0/20	0/24	0/20	0/23	1/27	0/18	4/32	0/13
		♀	0/30	0/20	0/22	0/24	0/26	0/24	1/27	1/21	8/20	2/20
Ileum	Inflammation and/or Necrosis	♂	1/29	0/17	1/20	0/24	1/20	0/23	1/27	0/18	5/32	0/13
		♀	0/30	0/20	0/22	0/24	1/26	0/24	4/27	0/21	6/20	7/20
	Peritonitis	♂	1/29	0/17	1/20	0/24	0/20	0/23	1/27	0/18	3/32	0/13
		♀	0/30	0/20	0/22	0/24	1/26	0/24	2/27	0/21	4/20	3/20
Cecum	Inflammation and/or Necrosis	♂	2/29	0/17	1/20	1/24	2/20	3/23	8/27	3/18	18/32	0/13
		♀	2/30	0/20	3/22	1/24	3/26	2/24	11/27	9/21	13/20	5/20
Colon	Inflammation	♂	2/29	0/17	0/20	024	3/20	1/23	2/27	0/18	15/32	0/13
		♀	1/30	0/20	0/22	0/24	1/26	1/24	9/27	1/21	4/20	1/20
Duodenum	Inflammation	♂	0/29	0/17	1/20	0/24	0/20	0/23	0/27	0/18	3/32	0/13
		♀	0/30	0/20	0/22	0/24	1/26	0/24	1/27	1/21	5/20	1/20
Stomach-Glandular	Hemorrhage/Inflammation/ Necrosis	♂	4/29	0/17	1/20	0/24	8/20	0/23	4/27	0/18	15/32	0/13
		♀	6/30	0/20	4/22	0/24	4/26	1/24	10/27	1/21	7/20	3/20
Stomach-Non-Glandular	Inflammation	♂	7/29	0/17	2/20	0/24	5/20	0/23	6/27	0/18	10/32	1/13
		♀	4/30	0/20	6/22	0/24	3/26	0/24	3/27	2/21	7/20	1/20
Kidney	Chronic Progressive Nephrosis	♂	22/29	16/17	17/20	22/24	16/20	22/23	23/27	18/18	25/32	12/13
		♀	3/30	8/20	9/22	11/24	11/26	9/24	8/27	15/21	14/20	10/20
	Papillary Necrosis	♂	0/29	0/17	0/20	0/24	0/20	0/23	1/27	0/18	9/32	6/13
		♀	0/30	0/20	0/22	0/24	0/26	0/24	1/27	1/21	3/20	3/20
	Pyelonephritis	♂	1/29	1/17	0/20	0/24	0/20	0/23	0/27	0/18	1/32	1/13
		♀	0/30	0/20	0/22	0/24	0/26	0/24	2/27	0/21	2/20	1/20
Spleen	Increased Extramedullary Hematopoiesis	♂	3/29	1/17	0/1	0/1	0/1	0/0	0/1	0/1	9/32	1/13
		♀	5/30	4/20	2/3	0/0	½	0/0	0/0	1/1	8/20	3/20

1 Deaths & sacrifices at moribund

2 Scheduled sacrifice

Neoplastic Incidence : 0 Mon-Terminal							
Tissue	Type of Tumor	Sex	Control 1	Control 2	0.05 mg/kg	0.3 mg/kg	0.6 mg/kg
Adrenal Cortex	Carcinoma/Adenoma	♂	0/46	1/1	0/1	0/1	1/45
		♀	0/50	2/11	2/11	2/5	2/40
Adrenal Medulla	Pheochromocytoma, Benign/ Malignant	♂	7/45	2/2	1/1	0/0	2/45
		♀	0/50	0/4	0/3	1/3	1/39
Bone	Osteoma	♂	0/2	0/0	1/1	0/0	0/1
	Osteosarcoma	♀	0/1	0/1	½	0/0	0/3
Brain	Astrocytoma	♂	1/12	-	-	-	-
	Oligodendroglioma	♂	1/46	0/0	0/1	0/0	0/45
	Granular Cell Tumor, Malignant	♂	0/46	0/0	1/1	0/0	0/45
Cecum/Ileum	Leiomyoma	♀	0/50	0/46	1/50	0/46	0/40
Ileum	Leiomyoma	♂	0/46	0/44	0/43	0/45	1/44
		♀	0/50	0/46	1/49	0/48	0/40
Kidney	Lipoma	♂	1/46	0/44	0/43	0/45	0/45
	Renal Cell Carcinoma	♂	0/46	1/44	0/43	0/45	1/57
Liver	Cholangioma	♀	1/50	0/25	0/30	0/26	0/40
	Hemangioma	♂	0/46	0/31	0/32	1/31	0/45
	Adenoma/Carcinoma, Hepatocellular	♂	0/46	2/36	4/32	2/31	0/45
		♀	1/50	0/25	1/30	0/26	1/40
Lymph Nodes & Lymphoreticular System	Hemangioma	♂	1/46	0/43	0/43	0/45	0/45
		♀	0/50	0/46	1/50	0/48	0/40
	Histiocytic Sarcoma	♂	0/0	0/1	1/1	0/0	1/1
		♀	1/4	0/1	2/2	1/1	½
	Malignant Lymphoma, Lymphocytic/Mixed	♂	0/0	4/4	½	0/1	0/1
		♀	2/4	1/1	0/2	0/1	½
	Mononuclear Cell Leukemia	♀	1/4	0/1	0/2	0/1	0/2
Mammary Gland	Adenoma/Fibroma/ Fibroadenoma	♂	0/37	0/2	4/5	0/4	0/37
		♀	27/50	15/31	19/33	13/32	20/40
	Mixed Tumor, Benign/ Malignant	♂	0/37	0/2	0/5	0/4	1/37
		♀	0/50	2/32	2/31	0/31	0/40
	Adenocarcinoma	F	10/50	5/31	7/31	12/31	5/40
Oral Tissues	Papilloma	♂	0/0	0/0	1/0	1/1	0/0
Ovary	Granulosa Cell Tumor, Benign	♀	0/50	0/4	2/6	0/6	0/40
	Thecoma	♀	0/50	0/4	0/6	1/6	0/40
Pancreas	Adenoma, Acinar Cell	♂	0/46	0/2	1/4	0/1	0/45
	Adenoma/Carcinoma, Islet Cell	♂	1/46	2/2	2/4	1/1	2/45
		♀	1/49	0/0	0/1	½	2/40
Parathyroid	Adenoma	♂	0/41	0/2	0/3	1/7	0/40
Pituitary	Adenoma/Adenocarcinoma	♂	24/46	21/28	16/19	20/23	26/45
		♀	37/49	24/28	36/36	30/34	18/38
	Craniopharyngioma	♂	1/46	0/28	0/19	0/23	0/45
Preputial Gland	Adenoma	♂	0/7	0/9	0/6	0/9	1/8
Seminal Vesicle	Leiomyosarcoma	♂	0/46	0/1	0/0	1/1	0/44
Skin	Fibroma/Fibrosarcoma	♂	2/46	7/24	3/16	3/15	2/45
		♀	0/50	2/6	4/8	0/7	0/40
	Lipoma	♂	1/46	1/24	2/16	1/15	1/45
		♀	0/50	1/6	0/7	0/7	0/40
	Myxoma	♂	0/46	0/24	1/16	0/15	0/45
	Neuro-fibroma/fibrosarcoma	♂	1/46	1/24	1/16	0/15	0/45
	Keratoacanthoma/Papilloma/ Squamous Carcinoma	♂	9/46	9/24	10/16	4/16	2/45
		♀	1/50	0/6	0/7	1/9	0/40
	Sebaceous Adenoma	♂	0/46	0/24	0/16	0/15	1/45
	Trichoepithelioma	♂	0/46	2/24	0/16	1/15	2/45
		♀	0/50	0/6	0/7	1/7	0/40
	Hemangioma/Hemangiosarcoma	♂	0/46	0/24	1/16	0/15	0/45
		♀	0/50	0/6	1/7	0/7	0/40
	Undifferentiated Sarcoma	♂	1/46	0/24	0/16	0/15	0/45

Neoplastic Incidence : 0 Mon-Terminal							
Tissue	Type of Tumor	Sex	Control 1	Control 2	0.05 mg/kg	0.3 mg/kg	0.6 mg/kg
Soft Tissue, Abdomen	Fibrosarcoma	♂	0/1	½	0/16	0/0	0/3
	Leiomyosarcoma	♂	0/1	0/2	2/2	0/0	0/3
	Lipoma	♀	½	0/1	0/2	0/1	0/4
	Undifferentiated Sarcoma	♀	0/2	0/1	0/2	1/1	0/4
Soft Tissue, Tail	Fibroma	♂	1/1	0/0	0/0	0/2	0/1
Soft Tissue, Thorax	Liposarcoma	♀	1/1	0/0	0/0	0/1	0/2
Spleen	Hemangiosarcoma	♂	0/46	½	0/1	0/2	0/45
Stomach-Glandular	Adenocarcinoma	♀	1/50	0/46	0/50	0/48	0/40
Testis	Interstitial Cell Tumor, Benign	♂	2/46	2/10	1/8	1/11	3/45
Thyroid	Adenoma/Carcinoma, Follicular Cell	♂	2/46	2/4	1/1	0/3	2/45
		♀	0/50	0/0	½	½	1/40
	Parafollicular Cell Adenoma	♂	3/46	2/4	0/1	0/3	5/45
		♀	2/50	0/0	0/2	½	0/40
Uterus and Cervix	Adenoma/Adenocarcinoma	♀	1/50	1/8	0/12	0/8	1/40
	Leiomyoma/Leiomyosarcoma	♀	1/50	0/8	0/13	0/8	2/40
	Polyp	♀	4/60	2/8	4/13	0/8	1/40
	Fibroma	♀	0/50	0/1	0/1	½	0/40

The sponsor did not examine the lymphoreticular system of all animals from the control 1 and high dose groups; therefore, the incidences for malignant lymphoma (lymphocytic/mixed) and mononuclear cell leukemia listed in the report did not reflect the true frequencies for these tumors in the present study. Based on the submitted data, there were high occurrences in these types of tumors in the examined animals, as indicated in the above table; the reviewer could not draw any conclusion for this particular issue.

[3] Monkey Studies

(1.) AHR-10282: 13-Week Oral Toxicity Study in Rhesus Monkeys (GTR 83-0647)(Vol 1.37, p 1-249)

Report №: 83-0647
Study Aim: To determine the toxicity of AHR-10282B in a 13-week oral study in Rhesus monkeys.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 15, 45, and 135 mg/kg/day, 3 ml/kg p.o. for 13 weeks
Control Vehicle: Phosphate buffer, pH 8.0, 3 ml/kg p.o.
Animal: Rhesus monkeys (*Macaca mulatta*), weighing 6.5-9.2 kg for ♂ and 4.1-6.3 kg for ♀, 3/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 15, 45, or 135 mg/kg/day) was orally administered to each monkey for 13 weeks by gavage. The animals were observed 2x daily, body weights were recorded weekly, and ophthalmology examination was performed 2x, one at pretest, and the other at 3 mon postdose. EEG examinations were performed at pretest and on wk 12. Clinical pathological parameters were measured on all monkeys prior to the initiation of the study and

at 1, 2, and 3 months of study. Post mortem examination was conducted on unscheduled moribund and terminal sacrifice animals.

Results:

- Clinical Signs & Mortality - No significant drug-related findings were noted for monkeys at 15 mg/kg/day. Emesis was seen for animals at 45 and 135 mg/kg/day. One ♀ at 135 mg/kg died on day 16.
- Body Weights - Although there were no significant differences between control and treated groups, a decrease in body weights was noted in the monkeys at 135 mg/kg/day.
- Ophthalmological Examination - No significant changes related to the treatment were observed.
- EEG - Reduced heart rates (14.7-16.1%) were recorded for all treated groups as compared to those obtained during pretest.
- Clinical Laboratory Findings - Increased erythrocyte sedimentation rate (ESR) was noted for animals at 135 mg/kg/day. No other changes in hematological values could be attributable to the treatment. Decreased serum albumin levels were identified in animals at 135 mg/kg/day at one, two, and three months during the study. No detectable treatment-associated changes could be found in the urinalysis.
- Histopathology - Lesions in the GI tract were major findings (mild to moderate enteritis & gastritis) in the microscopic examination and occurred at higher incidence in the high dose (135 mg/kg/day) group. They were most serious in the ♀ that died on day 16. Gastroenteritis was the major cause of death for this monkey.

(2.) AHR-10282: One-year Oral Toxicity Study in Cynomolgus Monkeys (6-Month Interim Report)(GTR 87-0419)(Vol 1.38)

Report Nº: 87-0419
Study Aim: To determine the toxicity of AHR-10282B in a 52-week oral study in cynomolgus monkeys: 6-month interim report.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 10, and 30 mg/kg/day for 52 weeks and 90 mg/kg/day for ≈3 wks then switched to 3 mg/kg/day the remaining period of the study, 3 ml/kg p.o.
Control Vehicle: Phosphate buffer, pH 8.0, 3 ml/kg p.o.
Animal: Cynomolgus monkeys, weighing 2.2-4.0 kg for ♂ and 1.6-2.9 kg for ♀, 8/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 10, or 30 mg/kg/day or 90 mg/kg/day then switched to 3 mg/kg/day at week 6) was orally administered to each monkey for 26 weeks by gavage. The animals were observed 2x daily for mortality , moribundity and signs of overt toxicity. Body weights were measured weekly. Ophthalmological examinations were done at pretest, 13 and 25 wks. EKG was conducted pretest and at 1, 3, and 6 months of study. Clinical pathological

parameters were measured on all monkeys 2x pretest and at 1, 3 and 6 months. Postmortem examination was conducted on animals at moribund and scheduled 6 month interim sacrifice (4♂ & 3♀ at 0 and 10 mg/kg/day; 3♂ & 4♀ at 30 mg/kg and 4/sex/group at 3/90 mg/kg/day).

Results:

- Mortality & Clinical Signs - During the first two weeks of treatment, animals at 90 mg/kg/day had signs of diarrhea, emesis and inappetence; these signs disappeared after placing these animals at the dose level of 3 mg/kg/day. High incidences of emesis, soft stool and/or diarrhea as well as inappetence were also noted in the animals at 30 mg/kg during wks 1-2. One ♀ at 10 mg/kg/day died at wk 6 due to the intussusception and consequent necrosis of the jejunum; 1♂ at 30 mg/kg/day died at wk 13 due to the peritonitis; 1♀ at 0 mg/kg/ml died at wk 13 with no apparent signs.
- Body Weights - Decreased body weights were observed in animals at 90 mg/kg/day during the first few weeks of study.
- Ophthalmoscopic Examination - No treatment related findings were noted.
- Macro- & Microscopic Analysis - Ulcers in the intestines adhesions and cloudy fluid in abdominal cavity were observed at necropsy in the male at 30 mg/kg/day that died at wk 13. Microscopic examination revealed intestinal inflammation and peritonitis. No lesions related to the drug treatment were observed in animals in all groups.
- Clinical Laboratory Findings - ↑BUN, ↑AST and ALT, ↓total serum protein and ↓albumin were obtained at 3 mon for the ♂ receiving 30 mg/kg/day died at wk 13.

(3.) AHR-10282: One-Year Oral Toxicity Study in Cynomolgus Monkeys: Final Report(GTR 87-0318)(Vol 1.39-1.41)

Report №: 87-0318
Study Aim: To determine the toxicity of AHR-10282B in a 52-week oral study in cynomolgus monkeys.
Compound: AHR-10282B in phosphate buffer, pH 8.0
Dose & Route: AHR-10282B: 0, 10, and 30 mg/kg/day for 52 weeks and 90 mg/kg/day for ≈3 wks then switched to 3 mg/kg/day the remaining period of the study, 3 ml/kg p.o.
Control Vehicle: Phosphate buffer, pH 8.0, 3 ml/kg p.o.
Animal: Cynomolgus monkeys, weighing 2.2-4.0 kg for ♂ and 1.6-2.9 kg for ♀, 8/sex/group
Study Location:

Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 10, or 30 mg/kg/day or 90 mg/kg/day then switched to 3 mg/kg/day at week 6) was orally administered to each animal for 52 weeks by gavage. The animals were observed 2x daily for mortality, moribundity and signs of overt toxicity. Body weights were measured weekly. Ophthalmological examinations were done at pretest, 3, 6, 9 and 12 months. EKG was conducted pretest and at 1, 3, 6, 9 and 12 months of study. Clinical pathological parameters were measured on all monkeys 2x pretest and at 1, 3, 6, 9

and 12 months. Necropsy was done on all monkeys.

Results:

- Mortality & Clinical Signs - One ♀ in the control group died at wk 13 and the cause of death was uncertain; 2♀ at 10 mg/kg/day died at wk 6 (due to the intussusception of the small intestine) and wk 39 (unknown reason); 1♂ at 30 mg/kg/day died at wk 13 with intestinal ulceration and peritonitis. Emesis, diarrhea, weight loss and inappetence were major clinical observations in the first three weeks of study, and the severity of these findings was dose-related. Necrosis, opened lesions and bleeding in the tail-tip were seen in all treatment groups.
- Body Weights - Decreased body weights were observed in animals at 90/3 mg/kg/day during the first few weeks of study.
- Ophthalmoscopic Examination - No treatment related findings were noted.
- Macro- & Microscopic Analysis - Ulcers in the intestines, adhesions, and cloudy fluid in the abdominal cavity were observed at necropsy in the male at 30 mg/kg/day that died at wk 13. Microscopic examination revealed intestinal inflammation and peritonitis. Necrotic, mild focal dermatitis in distal tail which correspond to the gross lesions in the tail tip was noted in some of treated monkeys. No other lesions related to the drug treatment were observed in animals in all groups.
- Clinical Laboratory Findings - ↑BUN (158.9 mg/dl), ↑AST (290 IU/l) and ALT (419 IU/l), ↓total serum protein and ↓albumin were obtained at 3 mon for the ♂ receiving 30 mg/kg/day died at wk 13. One ♀ at 10 mg/kg/day had elevated ALT (107 IU/l) at 12 mon screening.

b. INTRAVENOUS STUDIES

[1] Rat Studies

(1.) Bromfenac Sodium: Two-Week Intravenous Toxicity Study in Rats (GTR 22666)(Vol 1.41A, p 1-313)

Report №: 22666
Study Aim: To determine the pharmacological and toxicologic profile of AHR-10282B administered intravenously for 2 weeks in mature rats.
Compound: AHR-10282B in Sodium Citrate, 10 mg/ml
Dose & Route: AHR-10282B: 0, 0.1, 0.25 and 0.5 mg/kg/day for 14 days iv, 1 ml/kg body wt.
Control Vehicle: Sodium citrate aqueous solution buffered with citric acid , pH 7.9
Animal: Charles River CD VAF rats, weighing 185 - 333 g for ♂ and 185-219 g for ♀, 15/sex//group
Study Location: Wyeth-Ayerst Research, Drug Safety Evaluation, Chazy, NY 12921
Compliance with GLP/QAU: Yes
Study Design: AHR-10282B (0, 0.1, 0.25 or 0.5 mg/kg/day) was administered iv to each rat once daily for 2 wks. The animals were observed 2x daily for survival and post mortem

examination was conducted. Body weights and food consumption of each animal were determined 1x pretest and weekly thereafter. Ophthalmoscopic examinations were performed on each rat 2x (wks -2 & 2). Clinical laboratory procedures were done on 10 rats/sex/group 2x (pretest & wk 2).

Results: No deaths occurred. No drug-related effects on body weights food consumption, ophthalmology, clinical chemistry profiles, organ weights and pathological (gross & microscopic) examinations. A slight increase in fibrinogen was seen in 1♂ at 0.5 mg/kg/day.

(2.) Bromfenac: Multiple Dose Intravenous Range Finding Study (GTR22575)(Vol 1.41A, p 313-461)

Report №: 22575
Study Aim: To determine the acceptable dosages and potential pharmacological and toxicologic effects of Bromfenac for a subsequent 2-week intravenously toxicity study in rats.
Compound: AHR-10282B in Sodium Citrate, 10 mg/ml
Dose & Route: AHR-10282B: 0, 0.1, 0.5, 1.0 and 2.0 mg/kg/day for 7 days iv, 1 ml/kg body wt.
Control Vehicle: Sodium citrate aqueous solution buffered with citric acid , pH 7.9
Animal: Charles River CD VAF rats, weighing 171-294 g, 5/sex//group
Study Location: Wyeth-Ayerst Research, Drug Safety Evaluation, Chazy, NY 12921
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (0, 0.1, 0.5, 1.0 or 2.0 mg/kg/day) was administered iv to each rat once daily for 7 days. The animals were observed 2x daily for survival and post mortem examination was conducted on day 8. Body weights and food consumption of each animal were determined on days -1 & 7. Clinical laboratory procedures were done on all rats 2x (pretest & wk 1).

Results: There were nine rats (1♂ & 2♀ at 1.0 mg/kg/day and 1♂ & 5♀ at 2.0 mg/kg/day) that died between days 5-8 before scheduled terminal sacrifice. Moderate to marked body weight loss was noted in rats at 2.0 mg/kg/day, and slight to moderate decreases in body weights were observed for rats at 1.0 mg/kg/day. A significant decrease in food consumption was seen in animals at ≥1.0 mg/kg/day. Decreased motor activity, ptosis, paleness, ataxia, diarrhea/loose stool, and distended abdomen were major clinical signs observed in rats at ≥1.0 mg/kg/day. Clinical laboratory examinations revealed ↓RBC, ↓Hb, ↓MCHC, ↓hematocrit, ↑MCV, ↑reticulocytes, ↑WBC, ↑platelets, ↑neutrophils and ↓albumin and total protein in samples obtained from rats at ≥1.0 mg/kg. Drug-related lesions characterized as extensive, perforating ulcers in jejunum/ileum with multiple coalescing omental adhesions, and multifocal fibrinous peritonitis were seen in animals receiving ≥1.0 mg/kg/day at postmortem gross examination. At microscopic examination, no drug-related intestinal lesions in rats at ≤0.5 mg/kg/day. Perforating ulcers in the jejunum or ileum confirming the gross findings were observed in animals at ≥1.0 mg/kg/day.

[2] Monkey Studies

(1.) Bromfenac: Multiple Dose Intravenous Range Finding Study - Monkey (GTR20931)(Vol 1.42, p 1-41)

Report Nº: 20931
Study Aim: To determine the acceptable dosages and potential pharmacological and toxicologic effects of Bromfenac for a subsequent 2-week intravenously toxicity study in monkeys.
Compound: AHR-10282B in phosphate buffer
Dose & Route: AHR-10282B: 15 and 30 mg/kg/day for 7 days iv, 0.15-0.3 ml/kg body wt.
Animal: Cynomolgus monkeys, weighing 3.7-6.3 kg, 1/sex/group
Study Location: Wyeth-Ayerst Research, Drug Safety Evaluation, Chazy, NY 12921
Compliance with GLP/QAU: N/A
Study Design: AHR-10282B (15 or 30 mg/kg/day) was administered iv to each monkey once daily for 7 days. The animals were observed 2x daily for survival and post mortem examination was conducted on day 8. Body weights and food consumption of each animal were determined on days -1 & 7. Clinical laboratory procedures were done on all animals 2x (pretest & wk 1).

Results: No deaths occurred. Slightly decreased food intake was noted in female at 30 mg/kg. Blood present in the stool (confirmed with hematest tablets) was observed on day 3 for the ♀ at 30 mg/kg and emesis was seen in the same animal on day 4. Slight to moderate ↑ in WBC, reticulocyte counts, monocyte, and fibrinogen and ↓ in RBC, Hb and hematocrit were major changes in the hematology for the treated animals. Profound elevations in AST, ALT, creatine kinase (CK) and LDH were noticed in one female at 30 mg/kg and slight to moderate increases in CK and ALD were also observed in both male and female at 15 mg/kg as well as male at 30 mg/kg on day 7. Slightly increased total bilirubin was observed in both male and female at 30 mg/kg on day 7.

(2.) Bromfenac Sodium: Two-Week Intravenous Toxicity Study in Monkeys (GTR 22573)(Vol 1.42, p 42-299)

Report Nº: 22573
Study Aim: To determine the toxicological potential of intravenously administrated Bromfenac in monkeys.
Compound: AHR-10282B in sodium citrate buffer
Dose & Route: AHR-10282B: 0, 1 and 5 mg/kg/day for 14 days and 20 mg/kg/day for 9 days iv, 0.1-0.25 ml/kg body wt.
Control Vehicle: Sodium citrate aqueous solution buffered with citric acid , pH 8.8-8.9
Animal: Cynomolgus monkeys, weighing 3.7-6.3 kg, 3/sex/group
Study Location: Wyeth-Ayerst Research, Drug Safety Evaluation, Chazy, NY 12921
Compliance with GLP/QAU: N/A

Study Design: AHR-10282B (0, 1 or 5 mg/kg/day) was administered iv to each monkey once daily for 14 days or 9 days for the dose level of 20 mg/kg/day. The animals were observed 2x daily for survival and post mortem examination was conducted. Body weights and food consumption of each animal were determined on weekly basis. Ophthalmology examinations were performed on wk -2 & 2. Clinical laboratory procedures were done on all animals at wk -3, -1 & 2.

Results:

- Survival & Clinical Signs - All animals survived the entire course of treatment; due to severe local irritation at the injection sites with signs of skin discoloration, ulceration and edema, all monkeys at 20 mg/kg/day were sacrificed at day 9.
- Body Weight and Food Consumption - Normal
- Ophthalmology - Normal
- Clinical Laboratory Analysis - Hematology examination for the samples obtained at the terminal sacrifice indicated that slight ↓ in RBC and Hb, slight ↑ in WBC, neutrophils and monocytes were noted in some animals at 20 mg/kg/day. Blood Chemistry analysis at day 9 for the animals at 20 mg/kg showed slightly ↓ albumin/globulin ratio in all animals, ↑ALT (245 IU/L) in 1♂, ↑AST (IU/L) in ♂, ↑↑CK in 2 ♂ & 2♀ and ↑LDH in 2 ♂ & 2♀.
- Macro- & Microscopic Pathology - Local drug-induced skin lesions with the characteristics of swollen, firm, hemorrhage, edema and occasional ulceration of the overlaying skin around injection site were seen in monkeys at 5 & 20 mg/kg and one monkey at 1.0 mg/kg. Microscopic examination revealed that the lesions consisted of a marked to severe chronic active panniculitis and myositis and could be characterized by the mixtures of neutrophils, fibroblasts, macrophages, lymphocytes and serofibrinous exudate within subcutaneous tissues and adjacent muscle bundles. The inflammatory response in the injection site of one low dose (1.0 mg/kg) was not as severe as those observed in the 5 & 20 mg/kg dosage groups. The subcutaneous tissue of inject sites in the control monkeys and those in the 1.0 mg/kg group were slightly edematous and hemorrhagic with a small number of neutrophils adjacent to vessels in the subcutis. No other drug-related lesions.

C. *SUMMARY AND EVALUATION OF TOXICOLOGY STUDIES*

Acute Toxicity Studies:

Single dose acute toxicity of Bromfenac was examined in rats, rabbits and dogs. Based on the data from the oral and i.v. acute single dose studies, it could be concluded that the LD_{50} was 39.6 mg/kg for ♀ rats in the oral study, and 46.0 mg/kg and 15.0 mg/kg for ♂ &♀ rats, respectively, in the iv study. The predominant toxicity observed in these studies was GI related. Hemorrhagic spots in the GI tract, thickened intestinal walls, and adhesions of intestines to peritoneal walls were the major characteristics of GI lesions. Results also showed that ♀ rats were more susceptible to Bromfenac toxicity. The kidney was also the target organ, with the clinical findings of hematuria and pathological findings of pale kidneys at necropsy. The maximum nontoxic doses were ≤10 mg/kg

p.o. for the rat, rabbit and dog, and ≤1.0 mg/kg i.v. for the rat.

Subchronic and Carcinogenicity Studies:

(1) Oral Studies:

Mouse 24-Month Carcinogenicity Study - Mice, 60/sex/group, were treated with Bromfenac at either 0, 0.2, 1 or 5.0/7.5 mg/kg/day by oral gavage. There was no difference in the survival distributions. Decreased body weight gains (20 and 21% relative to control 1 and control 2, respectively) were observed in the high dose (5.0/7.5 mg/kg/day) female mice. Food consumptions were not affected by the drug treatment. Drug-induced toxicities were observed in the liver and stomach. These lesions were identified as ulcers and or subacute inflammation in the glandular mucosa of the stomach, and cytological alterations in the hepatocytes. There was no treatment-related difference in the neoplastic incidence of all examined tumors.

Rat 13-Week Subchronic Toxicity Study - Oral subchronic toxicity of Bromfenac was evaluated in the rat, 15/sex/group, at dosages of 0, 0.1, 0.5 or 2.5 mg/kg/day. High mortality (≈80% in both ♂ & ♀) was observed in the 2.5 mg/kg group due to the intestinal toxicity. Microscopic lesions consisting of necrotic ulcer, necrosis of the intestinal wall, and enteritis could be identified in duodenum, jejunum, ileum and colon. Results from this study showed that female rats were more sensitive to Bromfenac-caused toxicity.

Rat 24-Month Carcinogenicity Study - Rats, 70/sex/group, received Bromfenac at doses of 0, 0.05, 0.3, or 0.6 mg/kg/day for 24 months. Interim sacrifices, 10/group/sex, were conducted at 6 and 12 months. The GI tract, kidney and liver were the target organ. Dose-dependent hepatic (vacuolar alterations, cytoplasmic changes, inflammation, and necrosis) and gastrointestinal (inflammation, and necrosis) toxicities were identified at 12- and 24- month postmortem macro- and/or microscopic examinations. Nephrotoxicity (papillary necrosis) was not revealed at necropsy until final sacrifice. There was no treatment-associated increases in the tumor incidences for all examined tumors.

Rhesus Monkeys 13-Week Subchronic Toxicity Study - Rhesus Monkeys, 3/sex/group were dosed with Bromfenac at 15, 45, or 135 mg/kg by gavage for 13 weeks. Emesis was the major clinical signs of monkeys receiving≥45 mg/kg/day. One death (♀, 135 mg/kg/day) occurred. Increased ESR was noted in animals at 135 mg/kg. Enteritis and gastritis were major histopathological lesions seen in high dose monkeys.

Cynomolgus Monkey 12-Month Chronic Toxicity Study - The chronic toxicity of Bromfenac was investigated in monkeys (8/sex/group) at 10, 30 or 3/90 mg/kg/day by oral gavage for 52 weeks. An interim sacrifice at week 26 were performed on 3 or 4 monkeys per group per sex. Treatment related death and enteric toxicity (ulcers) occurred in animals receiving 10 and 30 mg/kg/day.

(2) Intravenous Studies:

Rat 2-Week Intravenous Toxicity Study - The intravenous subchronic toxicity was studied in groups of 15/sex rats at levels of 0, 0.1, 0.25 or 0.5 mg/kg/day for 14 days. No treatment related toxicity was observed.

Cynomolgus Monkey 2-Week intravenous Toxicity Study - Groups of 3/sex cynomolgus monkeys were intravenously given Bromfenac at levels of 1, 5, or 20 mg/kg/day for 14 days. Due to severe drug induced local irritation at the injection site, animals that received 20 mg/kg/day were terminated at day 9 post treatment. No GI toxicity was seen in this study. Marked to severe chronic active panniculitis and myositis were characterized in the animals at 5 & 20 mg/kg/day during the microscopic examination.

Reproduction Studies

A. ***Segment I-Fertility and General Reproductive Performance*** (GTR 84-0003, V.1.43, p 1)

Charles River COBS CD rats, 20/sex/group, doses: 0, 0.06, 0.3, and 1.5/0.9 mg/kg/day by gavage.
Phase I: treated male rats (beginning 60 days prior to cohabitation) were mated with untreated females.
Phase II: treated female rats (beginning 14 days prior to cohabitation until gestation day 13 or lactation day 21) were mated with untreated males.

The dosage level in the high dose group was decreased from 1.5 to 0.9 mg/kg/day on days 29 and 11 for males and females, respectively, because of the moribund condition in some animals. The drug treatment of males at dosage levels up to 0.9 mg/kg/day produced no effects on fertility and reproductive parameters. The treatment of Bromfenac at 0.9 mg/kg/day in females resulted in increased postimplantation embryonic loss. Total implantation sites were somewhat decreased in 0.3 and 0.9 mg/kg/day group. Gestation length was prolonged at 0.3 and 0.9 mg/kg/day dosage levels. Two females in 0.9 mg/kg/day group had dystocia, incomplete delivery, and died. Fertility and other reproductive parameters in treated females were not affected.

B. ***Segment II-Teratology Study in Rats*** (GTR 83-0550, V.1.43, p 189)

Charles River COBS CD rats, 25 female/group, dose: 0,0.06,0.3, and 0.9 mg/kg/day by gavage from days 6 to 15 of gestation, Cesarean sections were performed on day 20 of gestation.

There was no overt maternal toxicity. Bromfenac administration to the pregnant rats did not cause changes in in-utero survival, growth, and morphological development of fetuses at doses up to 0.9 mg/kg/day. No teratogenic effects of Bromfenac was observed in this study.

C. ***Segment II-Teratology Study in Rabbits*** (GTR 83-0551, V.1.43, p 243)

Dutch Belted rabbits, 16/group, dose: 0,1,2.5, and 7.5 mg/kg/day from days 6 to 18 of gestation by gavage, Cesarean sections were performed on day 28 of gestation.

One female in high dose group died of GI hemorrhage. One female in mid dose group aborted. Losses in mean maternal body weight were observed during the treatment period in 2.5 and 7.5 mg/kg/day groups. An increased postimplantation loss and a reduced number of viable fetuses were observed at 7.5 mg/kg/day levels. However, these findings were not statistically significant. Slight increases in the number of fetuses and litters with malformations occurred in low and mid dose groups relative to the control group. However, the incidences of malformations in high dose group were comparable to that of the control group. In addition, most malformations occurred in single fetus. No dose response relationship could be established in these fetal findings.

D. ***Segment III-Perinatal and Postnatal Study in Rats*** (GTR 83-0560, V.1.44, p 1)

Charles River COBS CD rats, 25/group, dose: 0,0.06,0.3, and 0.9 mg/kg/day from day 15 of gestation through day 20 of lactation by gavage. All dams were allowed to deliver and sacrificed on day 21 of lactation.

Maternal survivals and maternal body weights were decreased on lactation days 14 and 21 in the high dose group. Peritonitis, combined with intestinal perforation or ulceration were found in most of the deaths. An increased number of stillborn pups and a slightly decreased number of live pups per litter at birth occurred in 0.9 mg/kg/day group. Offspring growth was decreased also in this group. All pups were examined for malformation or congenital variation. The results showed that there were increased cases of undeveloped renal papillae and/or distended ureter in fetuses from the high dose group. However, distended ureter was also observed in control pups. No adverse effects of Bromfenac at 0.3 mg/kg/day was found in this study.

E. ***SUMMARY OF REPRODUCTION STUDIES***

Bromfenac at doses up to 0.9 mg/kg/day in male and female rats did not cause any adverse effects in the fertility of animals. However, increased postimplantation embryonic loss, increased stillborn pups, decreased live pups at birth, prolonged gestation period and dystocia were observed in the rat reproduction studies. No fetal malformations were found in rats at up to 0.9 mg/kg/day and in rabbits at up to 7.5 mg/kg/day dose levels. The postnatal growth of pups from lactating dams receiving 0.9 mg/kg/day of Bromfenac was decreased.

Mutagenicity Study

A. ***IN VITRO NON-MAMMALIAN CELL SYSTEM***

(1.) Ames Test (GTR 85-0307, V.1.44, p 66).

The mutagenicity of Bromfenac was examined in the Ames test using Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 in the presence and absence of rat liver S-9 metabolic activation. The results showed that Bromfenac did not exhibit mutagenic activity in these assays under the experimental conditions. The positive controls (sodium azide, 2-nitrofluorene, and 9-aminoacridine) showed positive results in these tests.

B. IN VITRO MAMMALIAN CELL SYSTEM

(1.) Chromosomal Aberration Test (GTR 89-0319, V.1.44, p 108).

The ability of Bromfenac to induce chromosomal aberrations was evaluated in Chinese hamster ovary (CHO) cells with and without rat liver S-9 metabolic activation. Mitomycin C and cyclophosphamide were used as the positive control agents in these tests. No significant increase in cells with chromosomal aberrations was observed in the Bromfenac treated groups.

(2.) Mouse Lymphoma Forward Mutation Assay (GTR 89-0354, V.1.44, p 161).

The mouse lymphoma cells were used to determine the ability of Bromfenac in inducing forward mutation at the thymidine kinase (TK) locus. In a preliminary cytotoxicity assay, Bromfenac above 0.625 mg/ml caused near total cell deaths. Therefore, dose levels below 0.6 mg/ml were used in this assay. Bromfenac did not induce notable increases in mutant frequency with and without S-9 metabolic activation at dose levels between 0.01 and 0.5 mg/ml. Only at a dose level of 0.6 mg/ml with S-9 metabolic activation was a small increase in mutant frequency observed.

C. IN VIVO MAMMALIAN SYSTEM

(1.) Micronucleus Assay (GTR 89-0320, V.1.44, p 222).

Bromfenac was administered to ICR mice as a single dose to evaluate inducement of micronuclei in bone marrow polychromatic erythrocytes. Dosages of 50, 167, and 500 mg/kg were used. Animals were sacrificed at 24, 48, and 72 hours after dosing for extraction of bone marrow. The positive control, cyclophosphamide, produced expected positive results to confirm the validity of the assay. Bromfenac did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the tested conditions.

(2.) Unscheduled DNA Synthesis (UDS) (GTR 24334, V.1.44, p 255a).

The objective of this assay was to detect DNA damage caused by test article by measuring UDS induced in rat primary hepatocytes in vivo. The existence and degree of DNA damage were inferred from an increase in net nuclear grain counts in hepatocytes obtained from treated animals. No increase in net nuclear grain count was seen at dosages of 5, 10, or 20 mg/kg of Bromfenac after the 2 to 4 hour or 15 to 16 hour sacrifice points.

D. SUMMARY AND EVALUATION OF MUTAGENICITY STUDIES

Bromfenac was evaluated for mutagenicity potentials in the Ames test, the Chinese hamster ovary cell chromosomal aberration test, the mouse lymphoma forward mutation assay, the *in vivo* mouse micronucleus assay, and *in vivo/vitro* rat unscheduled DNA synthesis assay. Bromfenac was not mutagenic in these tests.

OVERALL SUMMARY AND EVALUATION:

The pre-clinical section of NDA 20535 was reviewed by Drs. W.C. Josie Yang (Pharmacology and Toxicology) and Conrad H. Chen (Pharmacokinetics, Reproduction, and Mutagenicity).

Bromfenac sodium, AHR-10282B, is a cyclooxygenase inhibitor possessing analgesic, anti-inflammatory, and antipyretic activities in various animal experimental models. It belongs to a nonsteroidal anti-inflammatory drug class (NSAID) without any narcotic-like activity. Bromfenac did not possess any significant effects on the central nervous system and cardiovascular function.

The pharmacokinetic/pharmacodynamic correlation was studied in the rat and mouse models. In the anti-inflammatory model (carrageenan-induced foot edema), the effective dose of Bromfenac at 0.032 and 0.01 mg/kg for fed and fasted rats, respectively, produced corresponding plasma levels of 0.06 and 0.30 μg/ml. In the mice analgesic model (acetylcholine or phenylbenzoquinone induced writhing), the estimated EC_{50} for Bromfenac were 0.37 and 0.47 μg/ml for fed and fasted mice, respectively.

The absorption and excretion of Bromfenac were rapid in all animal species studied. Food ingestion reduced the bioavailability of Bromfenac in animals and man. Tissue distribution widely occurred, but mainly in organs of elimination. No significant accumulation of the drug was observed. Bromfenac was highly bound to plasma protein (99%). The metabolism and excretion patterns were similar in monkeys and humans. Elimination of Bromfenac and its metabolites occurred mainly via renal pathway in monkey and man and via feces through biliary excretion in rat. In the pregnant rat, Bromfenac entered the fetuses but at a lower level than in the dam. Bromfenac was also found in rat milk at a lower concentration than that in the plasma of lactating dam.

In the acute toxicity study, the LD_{50} for female rats was 39.6 mg/kg po and 15.0 mg/kg iv, and for male rats was 46.0 mg/kg iv. The predominant toxicity observed in these studies was GI related. Hemorrhagic spots in the GI tract, thickened intestinal walls, and adhesions of intestine to peritoneal walls were major characteristics of GI lesions. Kidney toxicity was also observed, which included hematuria and pale kidneys at necropsy. The maximum nontoxic doses were $\leq$10 mg/kg po for the rat, rabbit and dog, and $\leq$1.0 mg/kg iv for the rat. It appeared that female rats were more susceptible to Bromfenac-caused toxicity than male rats.

Long-term toxicity/carcinogenicity studies were conducted in mice and rats. In a two-year study in mice, drug-induced toxicities were observed in the liver and the stomach at doses of 5.0/7.5 mg/kg/day. These lesions were identified as ulcers and/or subacute inflammation in the glandular mucosa of the stomach, and cytological alterations in the hepatocytes. There was no treatment-

related increases in tumor incidences in all animals. The report showed that an eight-week dose range finding study was conducted in mice prior to the two-year study. However, the study result was not submitted in the NDA. In a 13-week toxicity study in rats, no treatment-related toxicities were found in animals at ≤0.5 mg/kg/day. At 2.5 mg/kg/day, intestinal ulcers/necrosis was observed. In a 24-month study in rats, dose-dependent hepatic (vacuolar alterations, cytoplasmic changes, inflammation, and necrosis) and gastrointestinal (inflammation, and necrosis) toxicities were identified at 12- and 24-month postmortem macro- and/or micro-scopic examinations. Nephrotoxicity (papillary necrosis) was also revealed at terminal necropsy. However, there were no treatment-associated increases in the tumor incidences in animals. No drug-related macro- and microscopic changes were observed during the six-month interim sacrifices in all doses (0.05, 0.3, and 0.6 mg/kg/day). It appeared that female rats were more sensitive to intestinal toxicity than male rats in this study. In a 13-week study in rhesus monkeys, no toxicity was found at 15 mg/kg/day. Emesis was found at 45 and 135 mg/kg/day and GI lesions were found at 135 mg/kg/day. A 12-month study was conducted in cynomolgus monkeys. Treatment-related death and enteric toxicity (ulcers) occurred in animals receiving 10 and 30 mg/kg/day in this study.

Bromfenac at doses up to 0.9 mg/kg/day in male and female rats did not cause any adverse effects in the fertility. However, increased postimplantation embryonic loss, increased stillborn pups, decreased live pups at birth, prolonged gestation period, and dystocia were observed in the rat reproduction studies. No fetal malformations were found in rats at doses up to 0.9 mg/kg/day and in rabbits at doses up to 7.5 mg/kg/day. The postnatal growth of pups from lactating dams receiving 0.9 mg/kg/day of Bromfenac was decreased.

Bromfenac was evaluated for mutagenicity potential in the Ames test, the Chinese hamster ovary cell chromosomal aberration test, the mouse lymphoma forward mutation assay, in vivo mouse micronucleus assay, and *in vivo/vitro* rat unscheduled DNA synthesis assay. Bromfenac was not mutagenic in these tests.

RECOMMENDATION

The approval of NDA 20535, Bromfenac, is recommended by the pharmacologists.

Josie Yang 12/19/95
W.C. Josie Yang, Ph.D.
Pharmacologist

Conrad H. Chen 12/19/95
Conrad H. Chen, Ph.D.
Pharmacologist

Concur by peer leader: Yes ☒ No ☐ Almon W Coulter 12/19/95
Almon W. Coulter, Ph.D.
Pharmacologist

cc:
NDA 20-535
HFD-550/Division File
/JYang
/CChen
/CKoerner
HFD-345
HFD-024
F/T by JYang, December 18, 1995