# CENTER FOR DRUG EVALUATION AND RESEARCH

**Application Number** 20-610

## PHARMACOLOGY REVIEW(S)

M E M O R A N D U M

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

DATE: June 5, 2000

FROM: Supervisory Pharmacologist
Division of Gastrointestinal and Coagulation Drug Products, HFD-180

SUBJECT: NDA 20-610 (Colazide/Balsalazide disodium/ —— /Colazal Capsules)-
Amendments Dated April 28, 2000 and May 23, 2000- Sponsor's Labeling
Submissions in Response to Approvable Letter dated March 24, 2000.

TO: NDA 20-610

In the approvable letter dated March 24, 2000, the sponsor was asked to submit a final printed labeling (FPL) for the drug. Sponsor in response submitted a draft labeling designated as "Salix, Inc. Proposed Labeling" in amendment dated April 28, 2000 and a so-called "labeling mock-ups for the package insert" in amendment dated May 23, 2000. Both of these versions are at variance with the Division's version dated June 10, 1998 prepared in response to comments by the Acting CDER Associate Director of Pharmacology and also the version attached to the approvable letter dated March 24, 2000. The preclinical portions of the labeling provided by the sponsor in the above submissions contain some unnecessary inappropriate changes, some incorrect and irrelevant information and some needless repetitions of wordings. The preclinical portions (SECTION: PRECAUTIONS, SUBSECTIONS (1) Carcinogenesis, Mutagenesis, Impairment and of Fertility (2) Nursing Mothers) of present "Salix labeling" designated hereafter as "Sponsors version" are reviewed under "Evaluation" followed by the recommended version designated as "Recommended FDA Final Draft".

PRECAUTIONS:

1) Carcinogenesis, Mutagenesis, Impairment of Fertility:

Sponsor's version:

"**Carcinogenesis, Mutagenesis, Impairment of Fertility:** In a 24 month rat (Spraque Dawley) carcinogenicity study, oral (dietary) balsalazide disodium at doses up to 2 grams/kg/day was not tumorigenic. For a 50 kg person of average height this dose represents 2.4 times the recommended human dose on a body surface area basis.

Balsalazide disodium was not genotoxic in an in vitro bacterial cell mutation assay (Ames test), an in vitro human lymphocyte chromosomal aberration test, an in vitro mouse lymphoma (L5178Y/TK+/-) forward mutation test, or an in vitro mouse micronucleus test. However, balsalazide disodium exhibited equivocal genotoxic activity when tested in vitro in the Chinese hamster lung cell (CH V79/HGPRT) forward mutation test.

4-aminobenzoyl-β-alanine, a metabolite of balsalazide disodium, was not genotoxic when tested in vitro in the Ames and mouse lymphoma (L5178Y/TK+/-) mutation tests but was positive in the in vitro human lymphocyte chromosomal aberration test. N-acetyl-4-aminobenzoyl-β-alanine, a conjugated metabolite of balsalazide disodium, was not genotoxic in the in vitro Ames or mouse lymphoma (L5178Y/TK+/-) mutation tests, or an in vitro human lymphocyte chromosomal aberration test. Balsalazide disodium at oral doses up to 2 grams/kg/day, 2.4 times the recommended human dose based on body surface area, was found to have no effect on fertility and reproductive performance in rats. The administration of balsalazide disodium during late gestation and lactation, at doses up to 2 grams/kg/day to the pregnant rat, had no demonstrable adverse effects on the development or reproductive function of the male or female offspring."

Evaluation:

The term "in vitro bacterial cell mutation assay" should be replaced with "AMES test". The term "in vitro bacterial cell mutation assay" is generic in nature. Given that there are several different types of in vitro bacterial cell mutation assays, the use of this term would appear to require the identification of bacterial tester strains used in assays. The "AMES test" is a term that is easily recognized by clinicians and known to refer to reverse mutation assays with Salmonella typhimurium and Escherichia coli tester strains. The word "in vitro" preceding each test is repeated several times. All the tests with the exception of the mouse micronucleus test are in vitro tests, i.e. Ames test, human lymphocyte chromosomal aberration test, mouse lymphoma cell (L5178Y/TK+/-) forward mutation test and Chinese hamster lung cell (CH V79/HGPRT) forward mutation test. The result of some of these tests with two metabolites are included in the last paragraph and the word "in vitro" preceded each of these tests repeatedly. To avoid these needless repetitions, the "in vitro" word should be used for all those tests collectively when they are discussed the first time. The mouse micronucleus test which is an in vivo test is listed incorrectly as an in vitro test. That needs to be corrected. The last sentence in the second paragraph of sponsor's version, describes the positive results with balsalazide in the Chinese hamster lung cell (CH V79/HGPRT) forward mutation test as "equivocal genotoxic activity." The test results were considered positive by the agency and it should be reflected in the labeling. The results of testing for effects on fertility and reproductive performance should be described as a separate paragraph. In this subsection, it is normal to include the results of testing in adult male and females for effects on fertility and reproductive performance. Any adverse test results of interest concerning development

and reproductive performance of the offspring belong to another subsection "pregnancy". Therefore the last sentence in this subsection which describes lack of effect on development or reproductive function of the male and female offspring should be deleted.

Recommended FDA Final Draft:

"Carcinogenesis, Mutagenesis, Impairment of Fertility:

In a 24-month rat (Sprague-Dawley) carcinogenicity study, oral (dietary) balsalazide sodium at doses up to 2 g/kg/day was not tumorigenic. For a 50 kg person of average height this dose represents 2.4 times the recommended human dose on a body surface area basis.

Balsalazide disodium was not genotoxic in the following in vitro tests: Ames test, human lymphocyte chromosomal aberration test and mouse lymphoma cell (L5178Y/TK+/-) forward mutation test or in the in vivo mouse micronucleus test. However, it was genotoxic in the in vitro Chinese hamster lung cell (CH V79/HGPRT) forward mutation test. 4-aminobenzoyl-β-alanine, a metabolite of balsalazide disodium, was not genotoxic in the Ames test and the mouse lymphoma cell (L5178Y/TK+/-) forward mutation test but was positive in the human lymphocyte chromosomal aberration test. N-acetyl-4-aminobenzoyl-β-alanine, a conjugated metabolite of balsalazide disodium, was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y/TK+/-) forward mutation test or the human lymphocyte chromosomal aberration test.

Sponsor's version:

Evaluation:

data should be deleted and the contents should be rewritten to comply with the aforecited format.

Recommended FDA Final Draft:

"Nursing Mothers:

It is not known whether balsalazide disodium is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Colazal is administered to a nursing woman."

/S/

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

cc:
NDA
HFD-180
HFD-181/CSO/Ms. McNeil
HFD-180/Dr. Choudary
R/D typed by deg: 6/6/00
F/t deg: 6/6/00
MN20610.doc

APPEARS THIS WAY
ON ORIGINAL

MEMORANDUM

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

DATE: June 10, 1998

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

SUBJECT: NDA 20,610 (Balsalazide Disodium) -
Preclinical Portion of Labeling

TO: NDA 20,610

The following version of the labeling (subsection "Carcinogenesis, Mutagenesis, Impairment of Fertility") addresses the suggestions/comments of Dr. P. Andrews (acting for Dr. DeGeorge). This should replace the previous version.

1. "Carcinogenesis, Mutagenesis, Impairment of Fertility:

In a 24-month rat (Sprague-Dawley) carcinogenicity study, oral (dietary) balsalazide disodium at doses up to 2 g/kg/day (12 g/$m^2$/day) was not tumorigenic. For a 50 kg person of average height (1.46 $m^2$ body surface area), this dose represents 2.4 times the recommended human dose (~5 g/$m^2$/day) on a body surface area basis. A short-term assay of carcinogenic potential of balsalazide disodium in p53 (+/-) transgenic mice is ongoing. Balsalazide disodium was not genotoxic in the Ames test, the human lymphocyte chromosomal aberration test, the mouse lymphoma cell (L5178Y/TK+/-) forward mutation test or the mouse micronucleus test. However, it was genotoxic in Chinese hamster lung cell (CH V79/HGPRT) forward mutation test. 4-aminobenzoyl-ß-alanine, a metabolite of balsalazide disodium, was not genotoxic in the Ames test and the mouse lymphoma cell (L5178Y/TK+/-) forward mutation test but was positive in the human lymphocyte chromosomal aberration test. N-acetyl-4-aminobenzoyl-ß-alanine, a conjugated metabolite of balsalazide disodium, was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y/TK+/-) forward mutation test or the human lymphocyte

chromosomal aberration test. Balsalazide disodium at oral doses up to 2 g/kg/day (12 g/$m^2$/day, 2.4 times the recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance in rats."

6/10/98.

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

cc:
NDA 20,610
HFD-180
HFD-181/CSO, Ms. McNeil
HFD-180/Dr. Choudary

JBC/hw/6/10/98
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APPEARS THIS WAY
ON ORIGINAL

NDA 20,610 Review #1

Sponsor & Address: Salix Pharmaceuticals, Inc.
Palo Alto, CA

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

NOV - 4 1997

Date of Submission: June 23, 1997

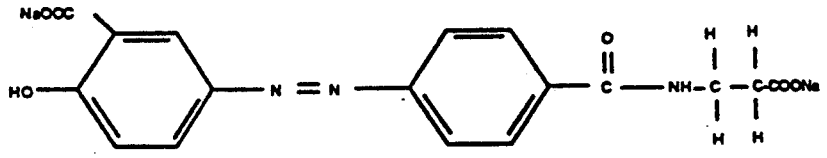
Date of HFD-180 Receipt: June 24, 1997

Date of Review: November 3, 1997

**REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA**
**Original Summary**

DRUG: Balsalazide disodium / Colazide, Capsules

(E)-5-[[4-[[(2-carboxyethyl)amino]carbonyl]phenyl]azo]-2-hydroxybenzoic acid, disodium salt, dihydrate




Molecular Formula: $C_{17}H_{13}N_3O_6Na_2 \cdot 2H_2O$ MW: 437.32

CATEGORY: Anti-ulcerative colitis

Related INDs: ——

Marketing Indications and Dose: Colazide is used for treatment of active ulcerative colitis. Patients will be given three 750 mg capsules three times a day for 8 weeks. This represents a total daily dose of 6.75 grams or 0.135 g/kg/day if 50 kg body weight assumed.

APPEARS THIS WAY
ON ORIGINAL

## PRECLINICAL STUDIES AND TESTING LABORATORIES:

| Type of Study | Study # | Lot # | Lab | Page # |
|---|---|---|---|---|
| Pharmacology | | | | 4-7 |
| Absorption, Distribution, Metabolism and Excretion | | | | 7-17 |
| Mice | 1067/43-1010 | | | |
| Rats | 1067/8-1011<br>1067/9-1011 | | | |
| Rabbits | 1067/42-1010 | | | |
| Dogs | 1067/10-11 | | | |
| Acute Toxicity | | | | |
| Mice & Rats: p.o., i.p., i.v. | 1067/45-1050 | C6832.7-10 | 1 | 17-18 |
| Oral & I.V. with 4-ABA in mice & rats | 1067/46-1050 | V87-12.3.3-03 | 1 | 18 |
| Subacute/Subchronic/Chronic Toxicity | | | | |
| 28-day oral (diet) in mice and rats | 661/TX/M-RT/1/87 | ----- | 2 | 19 |
| 7-day oral (diet) in rats | 661/Tx/Rt-M/0d/7d/87 | 261001 | | 18 |
| 9-day oral (diet) in rats | 661/Tx/Rt/0d/9d/81 | ---- | 2 | 19 |
| 14-day oral in rats | Not given | 261001 | 2 | 20 |
| 14-day oral in rats | 7405-1067/29 | C6832.7-10 | 1 | 21 |
| 14-day oral (diet) in rats | 7402-1067/5 | C6832.7-10 | 1 | 22 |
| 26-week oral in rats | 1067/4-1050 | C8632.7-13<br>BN E6832.7D-03 | 1 | 23-25 |
| 96-week oral (diet) in rats | 661/LS/RT/81 | 261001<br>261004 | 2 | 26-28 |
| 10-day oral in ferrets | 661/Tx/F/0d/10d/84 | 261006 | 2 | 28 |
| 28-day oral in ferrets | 661/Tx/F/0i/28d/88 | ---- | 2 | 28-29 |
| 26-week oral (diet) in ferrets | 661/TX/F/6/84 | 261006 | 2 | 29-30 |
| 14-day oral in dogs | 7399-1067/1 | C6832.7-10 | 1 | 30-31 |
| 28-day oral in dogs | 7477-1067/2 | C6832.7-10 | 1 | 31-33 |
| 52-week oral in dogs | 1067/3-1050 | C6832.7-13<br>BN 1754H<br>BN E6832.7D-01<br>BN E6832.7D-03 | 1 | 33-35 |

| Type of Study | Study # | Lot # | Lab | Page # |
|---|---|---|---|---|
| Special Toxicity | | | | |
| Chemoprevention of Intestinal Tumor Formation in B6-Min/+ Mice | | | | 35 |
| Carcinogenicity | | | | |
| 104-week oral diet) in rats | 1067/6-1050 | BN 1754H<br>3G2901 | 1 | 42-50 |
| Reproductive Toxicity | | | | |
| Segment I oral fertility and reproductive toxicity study in rats | 93/GYS007/0862 | 261R29 | 3 | 51-53 |
| Segment II oral teratology study in rats | 93/GYS006/054 | 261R29 | 3 | 54-57 |
| Segment II oral teratology study in rabbits | 93/GYS009/0802 | 261R29 | 3 | 57-60 |
| Segment III oral pre- and postnatal reproductive toxicity study in rats | 93/GYS008/0618 | 261R29 | 3 | 60-61 |

| Type of Study | Study # | Lot # | Lab | Page # |
|---|---|---|---|---|
| Mutagenicity | | | | |
| Tests for BSZ | | | | |
| Ames test | 1067/40 | BN 1754H | 1 | 62-63 |
| Ames test | ---- | ---- | 4 | 63-64 |
| In vitro chromosome aberration tests | 89/BEB001/0468<br>1076/44 | 261015 | 3 | 64-65 |
| CHO/HGPRT mutation assay | 89/BEB002/0739 | 161015 | 3 | 66-67 |
| Mouse lymphoma cell assay at tk locus | 1067/59-1052 | 3G2901 | 1 | 69-70 |
| Mouse micronucleus test | 89/BEB003/0434 | 261015 | 3 | 68-69 |
| Tests for 4-ABA | | | | |
| Ames test | 4L198 | 00207AJ | 5 | 73 |
| Mouse lymphoma cell assay at tk locus | 1067/54-1052 | SCS 939 | 1 | 70 |
| In vitro chromosome aberration test | 1067/57 | BN 1754H | 1 | 71-72 |
| Tests for NABA | | | | |
| Ames test | 1067/056-1052 | ---- | 1 | 73-74 |
| Mouse lymphoma cell assay at tk locus | 1067/55-1052 | SCS 936 | | 74-75 |
| In vitro chromosome aberration test | 1067/058-1052 | SCS 936 | 1 | 75-76 |

2. Biorex Laboratories Ltd, London, England

**PHARMACOLOGY:**

Balsalazide (BSZ) contains 5-aminosalicylic acid (5-ASA) and 4-aminobenzoyl-β-alanine (4-ABA). 5-ASA, the active moiety, is attached to 4-ABA via azo-bond. 4-ABA is a carrier moiety. BSZ is cleaved by bacterial azoreduction in the colon to release equimolar quantities of 5-ASA and 4-ABA. The anti-inflammatory effects of BSZ were demonstrated in a number of animal models *in vivo*.

## Primary Activity

### Anti-Inflammatory Activity

The following pharmacology studies were reviewed previously under [redacted] on June 10, 1994 and this review is attached below.

1. **Effects on Carrageenin Induced Ulcerative Colitis**: BSZ (200 mg/kg/day for 8 days) significantly reduced (57%) the severity of the lesions in colon and rectum of rats when ulcerative colitis was induced by carrageenin.

2. **Effects on Carrageenin Induced Edema in Rats**: BSZ (100 mg/kg, P.O.) and indomethacin (10 mg/kg, P.O.) both significantly reduced carrageenin induced edema in rat paw. The potency of BSZ was one-tenth of that seen with indomethacin.

3. **Cell Membrane Stabilizing Activity**: Compound 48/80 (0.3 mcg/ml) induced histamine release of rat peritoneal mast cells was inhibited by 25% and from 42% at 2 X $10^{-5}$M and 1 X $10^{-4}$M of BSZ respectively. Additionally, BSZ and SASP (sulphasalazine: comparator) both dose dependently decreased melittin-induced hemolysis (25% with 5 X $10^{-4}$M). Data indicated that BSZ has some cell membrane stabilizing activity.

Following new studies were not previously reviewed. These studies are reviewed below.

4. Effects on Ethanol-Induced Gastric Necrosis In Rats:

Treatment with a single oral dose of BSZ at 70 and 100 mg/kg markedly inhibited ethanol-induced gastric necrosis (1 ml of absolute ethanol orally) and the inhibition is comparable to that of carbenoxolone sodium (oral dose of 70 mg/kg). This inhibitory effect was antagonized by indomethacin at subcutaneous dose of 10 mg/kg.

5. Effects On Ethanol/TNBS-Induced Rectocolonic Lesions In Rats:

To induce rectocolonic lesions in rats, 0.25 ml 30% ethanol containing 20 mg 2,4,6 trinitrobenzene-sulphonic acid (TNBS) was given to rats by enema administration. Seven days after dosing with ethanol BSZ was given to these rats orally at 100 mg/kg/day for 14 consecutive days. The colonic lesion score and the biochemical markers of reactive oxygen in response to inflammatory reactions, glutathione (GSH) concentration and myeloperoxidase (MPO) activity were then measured on day 21 (termination day). Sponsor did not specify how the colonic lesion was scored. BSZ reduced rectocolonic damage by ~36% as compared to the control and completely inhibited the increase in mucosal MPO activity in this model. GSH content was not significantly altered.

Analgesic Effects:

The potential analgesic effects of SBZ was tested in mice using acetic acid-induced writhing test. The results indicated that pretreatment with oral dose of BSZ (144 mg/kg) and 5-ASA (63 mg/kg) significantly inhibited the number of writhes induced by acetic acid as compared to the control, suggesting that BSZ and 5-ASA have analgesic activity. However, the analgesic activity of SBZ was not found at 100 mg/kg in another writhing test in mice. The analgesic activity was also demonstrated in a carrageenin-induced inflamed paw model in rats. In this study, BSZ (144 mg/kg), 5-ASA (63 mg/kg) and 4-ABA (69 mg/kg) were given orally 2 hours prior to the injection of the carrageenin suspension into one hind paw. BSZ, 5-ASA and 4-ABA significantly increased the pain threshold and the analgesic activity was similar to ibuprofen.

## Secondary Activity

Central Nervous System: A single oral dose of BSZ and 4-ABA at 300, 1000 and 3000 mg/kg did not produce any effects in clinical signs and general behavior, locomotor activity and pentylenetetrazol-induced tonic convulsion in mice. SBZ did not alter the barbiturate-induced sleeping time at a single oral dose of 100 mg/kg. A single oral dose of SBZ at 200 mg/kg had no effects on leptazol-induced convulsion in mice. A single oral dose of SBZ (144 mg/kg), 5-ASA (63 mg/kg) and 4-ABS (69 mg/kg) had no effects on spontaneous motor activity in mice.

Respiratory System: A single i.v. dose of BSZ and B769A, an impurity of BSZ, at 10 mg/kg did not have any effects on respiratory rate in anesthetized rats. A single i.v. dose (10 mg/kg) or oral dose (1 g/kg) of BSZ had no effects on respiratory rate in anesthetized ferrets. A single i.v. dose (10 mg/kg) of BSZ, 5-ASA (10 mg/kg), 4-ABA (10 mg/kg), Nac-5-ASA

(10 mg/kg) and BX769A (5 mg/kg) did not alter the respiratory rate in anesthetized cats. A single intraduodenal dose of BSZ or 4-ABA at 300, 1,000 and 3,000 mg/kg had no effects on respiratory rate in beagle dogs.

Cardiovascular System: A single i.v. dose of BSZ and BX769A at 10 mg/kg did not have any effects on blood pressure and heart rate in anesthetized rats. A single i.v. dose (10 mg/kg) or oral dose (1 g/kg) of BSZ did not alter the blood pressure and heart rate in anesthetized ferrets. A single i.v. dose (10 mg/kg) of BSZ, 5-ASA (10 mg/kg), 4-ABA (10 mg/kg), Nac-5-ASA (10 mg/kg) and BX769A (5 mg/kg) did not have any effects on blood pressure and heart rate in anesthetized cats. A single intraduodenal dose of BSZ or 4-ABA at 300, 1,000 and 3,000 mg/kg had no effects on heart rate, blood pressure and EKG in beagle dogs.

Gastrointestinal System: A single oral dose of BSZ (144 or 200 mg/kg) and 4-ABA (69 mg/kg) did not have any effects on gastrointestinal motility (passage of charcoal) in rats. Slight inhibition (24% as compared to control) of gastrointestinal motility was noted after treatment orally with 5-ASA (63 mg/kg) in rats. The inhibitory effects (27-30%) were also noted following a single oral dose of sulphasalize (131 mg/kg) or sulphapyridine (81 mg/kg). Morphine (s.c. 10 mg/kg), however, produced maximum inhibition (70%) in this study. A single oral dose of BSZ (300 and 1,000 mg/kg) or 4-ABA at 300, 1,000 and 3,000 mg/kg had no effects on gastrointestinal motility (charcoal transport) in mice. A single oral dose of BSZ at 3,000 mg/kg increased the charcoal transport (~36%) in mice. BSZ and 4-ABA at concentrations of 0.1, 1 and 10 mg/ml had no effects on spontaneous movement and contraction induced by acetylcholine, histamine and barium in isolated guinea pig ileum. SBZ and 4-ABA slightly inhibited the spontaneous activity of rabbit jejunum at $10^{-3}$ M.

Renal System: A single oral dose of BSZ (10 and 100 mg/kg), 5-ASA (63 mg/kg) and 4-ABA (69 mg/kg) did not produce any effects on urinary volume and electrolyte concentrations in rats. Sulphasalazine (SASP) and its carrier molecule, sulphapyridine (SP), were also included in this study. A single oral dose of SASP (131 mg/kg) and SP (81 mg/kg) did not alter the urinary volume and electrolyte concentrations. BSZ significantly decreased the urinary volume (-61%) and increased in the urinary concentrations of sodium (+191%), potassium (+119%) and chloride (+123%) at 3,000 mg/kg in rats. These changes were also seen at lower doses (300 and 1,000 mg/kg) but less significant. Decrease in the urinary volume (40%) and increase in the sodium, potassium and chloride were also found following a single oral dose of 4-ABA at 3,000 mg/kg in rats.

Estrogenic Activity: Estrogenic effects of BSZ and its metabolites were studied in ovariectomized mice or sexually immature female rats. In the ovariectomized mice, a single oral dose of BSZ at 100 mg/kg or 5-ASA at 50 mg/kg or 4-ABA at 50 mg/kg produced some estrogenic effect (up to 30-50% of the positive control, estradiol at 5 μg/kg, s.c.). This change was not seen at lower dose of BSZ (5 mg/kg). In the sexually immature female rats, repeated oral dose of BSZ at 100 mg/kg/day for 3 days significantly increased the uterine weight. This change was not seen at lower doses (1 and 5 mg/kg/day). No anti-estrogenic effects were seen in both studies.

**ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):**

**ABSORPTION:**

MOUSE:

Plasma Concentrations and Urinary Excretion of BSZ and its Metabolites Following Dietary Administration of BSZ in Mice
(1067/43-1010)

Methods: To determine the plasma concentration and urinary recovery of BSZ and its metabolites, BSZ was given to mice in diet at 0, 120, 500, and 1,000 mg/kg for 14 days. Urine samples were collected from 3 mice/sex/group over 24 hours on days 5 and 14. Plasma samples were collected on day 14. The plasma and urinary concentrations of BSZ and its metabolites were determined using

Results: Plasma concentrations of BSZ, 5-ASA and 4-ABA were generally proportional to the dose administered. The median maximum plasma concentrations of BSZ were 0.0243, 0.0742-0.164 and 0.0714-0.213 nmol/ml (0.01, 0.033-0.07 and 0.03-0.093 mg/ml) in the low, mid and high dose groups, respectively. The median maximum plasma concentrations of total 5-ASA were 1.39-1.82, 7.65-9.94 and 12.34-19.97 nmol/ml (0.21-0.28, 1.17-1.52 and 1.89-3.1 mg/ml) in the low, mid and high dose groups, respectively. The median plasma concentrations of 4-ABA were 1.43-1.6 and 2.57-4.15 nmol/ml (0.3-0.33 and 0.54-0.86 ng/ml) in the mid and high dose groups, respectively (urinary data represented at Excretion Section).

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RAT:

Absorption, Distribution, Metabolism And Excretion Following Oral And Intravenous Administrations of $^{14}$C-BSZ in Rats (1067/8-1011)

Methods: To study pharmacokinetics of BSZ, BSZ was labeled with $^{14}$C with specific radioactivity of —— . A single dose of $^{14}$C-BSZ was given to rats orally at 120 and 2,000 mg/kg or intravenously at 120 mg/kg. The blood samples were collected at 1, 3, 6, 9, 12, 18, and 24 hours after dosing. The urine and fecal samples were collected from 0-6, 6-12, 12-24, 24-48, 48-72, and 72-96 hours after dosing. Tissue samples from a variety of organs were also collected for the radioactivity distribution study. BSZ and its metabolites in plasma, urine and fecal samples were determined using HPLC. Radioactivity was determined using liquid scintillation counter. In this study, plasma protein binding (in vitro) assays for $^{14}$C-BSZ and $^{14}$C-ABA were also conducted in human, dog, and rat plasma.

Results: Following a single oral dose, the maximum plasma concentration of BSZ was reached within 1 hour and the plasma level of BSZ was very low. The oral bioavailability of BSZ was very low (0.52-0.62%). The pharmacokinetic parameters measured after a single oral or i.v. dose were summarized in the table 7.5 on page 097 in volume 1.009. This table is attached below.

**TABLE 7.5**

**Summary of mean plasma radioactivity, balsalazide and metabolites pharmacokinetic parameters following a single oral or intravenous dose of ($^{14}$C)-balsalazide to the rat**

| Compound | Dose of balsalazide (mmol/kg) | Dose route/group | $C_{max}$ (nmol/mL) | $T_{max}$ (h) | $AUC_{(0-\infty)}$ (nmol.h/mL) | Urine (nmole/24h) | Clr (mL/min) |
|---|---|---|---|---|---|---|---|
| Radioactivity | 0.274 | Oral (A1) | 3.574 | 9.00 | 40.72 | NA | NA |
| Radioactivity | 4.573 | Oral (B1) | 26.78 | 12.00 | 373.8 | NA | NA |
| Radioactivity | 0.274 | IV (C1) | NA | NA | 269.0 | NA | NA |
| Balsalazide | 0.274 | Oral (A) | 1.091 | 1.00 | 3.072 | 273.5 | 1.484 |
| Balsalazide | 4.573 | Oral (B) | 8.505 | 1.00 | 42.56 | 3470 | 1.359 |
| Balsalazide | 0.274 | IV (C) | NA | NA | 491.9 | 18452 | 0.625 |
| NABA | 0.274 | Oral (A) | 1.838 | 9.00 | 15.73 | 1897 | 2.009 |
| NABA | 4.573 | Oral (B) | 10.71 | 12.00 | 137.0 | 10382 | 1.263 |
| NABA | 0.274 | IV (C) | NA | NA | ID | 1303 | ID |
| NASA | 0.274 | Oral (A) | 7.585 | 9.00 | 50.75 | 6867 | 2.255 |
| NASA | 4.573 | Oral (B) | 51.28 | 9.00 | 622.0 | 75690 | 2.028 |
| NASA | 0.274 | IV (C) | NA | NA | 29.16 | 5580 | 3.189 |
| ABA | 0.274 | Oral (A) | 0.310 | 9.00 | ID | ID | ID |
| ABA | 4.573 | Oral (B) | 3.974 | 12.00 | 34.30 | 6238 | 3.040 |
| ABA | 0.274 | IV (C) | ID | ID | ID | ID | ID |
| ASA | 0.274 | Oral (A) | 1.946 | 9.00 | ID | ID | ID |
| ASA | 4.573 | Oral (B) | 73.05 | 12.00 | 522.2 | 17700 | 0.565 |
| ASA | 0.274 | IV (C) | NA | NA | ID | ID | ID |

BSZ = Balsalazide
NABA = N-acetyl-4-aminobenzoyl-β-alanine
NASA = N-acetyl-5-amino-salicylic acid
ABA = 4-aminobenzoyl-β-alanine
ASA = 5-amino-salicylic acid
NA = Not applicable
ID = Insufficient data to derive parameter

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In plasma, the major radiolabeled compound was NABA, the metabolite of 4-ABA since the radiolabel was associated with the 4-ABA moiety of the BSZ molecule.

Absorption, Metabolism and Excretion Following Oral and I.V. Administration of $^{14}$C-ABA in Rats
(1067/9-1011)

Methods: To study pharmacokinetics of 4-ABA, 4-ABA was labeled with $^{14}$C with specific radioactivity of —— A single oral or i.v. dose of $^{14}$C-4-ABA was given to rats at 64 mg/kg. The blood samples were collected at 0.25 (15 minutes), 2, and 6 hours after dosing. The urine and fecal samples were collected from 0-6, 6-12, and 12-24 hours after dosing. The plasma and urine levels of 4-ABA and 4-NABA was determined using HPLC. The radioactivity was determined using liquid scintillation counter.

Results: Following a single oral dose of $^{14}$C-4-ABA, maximum plasma levels of 4-ABA, NABA and radioactivity were 0.0034, 0.0116 and 0.0163 μmol/ml, respectively, and these were reached 15 minutes after dosing. Following i.v. dose, the plasma concentrations of 4-ABA, NABA and radioactivity measured at 15 minutes after dosing were —— , respectively. The plasma radioactivity declined quickly and was below the limit of detection by 6 hours (i.v.) Or 24 hours (oral) after dosing. AUC values were not provided in this report. The oral absorption was ~14% as calculated using the radioactivity found in the urine following oral and i.v. administrations.

RABBITS:

Pharmacokinetics Of BSZ and Its Metabolites in Pregnant Rabbits
(1067/42-1010)

Methods: Plasma concentrations of BSZ were determined in pregnant New Zealand white rabbits following oral dose of BSZ at 600 mg/kg/day (1.37 mmol/kg/day) from gestation days 6 to 12. Blood samples were collected at 1, 2, 4, 8, 12, 24 hours after dosing on gestation days 6 and 12. The plasma level of BSZ and its metabolites were analyzed using HPLC.

Results: Maximum plasma level of BSZ was reached one hour after dosing. The plasma half life for BSZ was 1.32 hours on day 6 and 3.27 hours on day 12 calculated in one rabbit. There was no apparent accumulation over time. The plasma concentrations of BSZ and its metabolites are summarized in the following table.

Median Plasma Levels of BSZ and Its Metabolites

| Plasma Levels | BSZ | 5-ASA | NASA | 4-ABA | NABA |
|---|---|---|---|---|---|
| $C_{max}$<br>nmol/ml<br>µg/ml | 6.14-7.06<br>2.69-3.09 | 87.2-93.5<br>13.4-14.3 | 193.3-232<br>37.7-45.3 | 31-56.7<br>6.46-11.8 | 55.5-69.7<br>13.9-17.4 |
| AUC<br>nmol•hr/ml<br>µg•hr/ml | 16.4-17.3<br>7.17-7.57 | 715.3-801.3<br>109.5-122.7 | 2039-2185<br>398-427 | 432-700<br>90-146 | 968-1001<br>242-251 |

DOGS:

Absorption, Metabolism and Excretion Following Oral and I.V. Administrations of $^{14}$C-BSZ in Dogs
(1067/10-1011)

Methods: To study pharmacokinetics of BSZ, BSZ was labeled with $^{14}$C with specific radioactivity of 266 µCi/mg. A single dose of $^{14}$C-BSZ was given to dogs orally at 120 and 2,000 mg/kg or intravenously at 120 mg/kg. The radiolabeling is associated with the 4-ABA moiety of the molecule. Following oral dosing, blood samples were collected before and 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 72, and 96 hours after dosing. Following i.v. dosing, blood samples were collected before and 2, 5, 15, and 30 minutes and 1, 2, 3, 4, 6, 12, 24, 48, 72, and 96 hours after dosing. Urine samples were collected before and 0-3, 3-6, 6-12, 12-24, 24-48, 48-72, and 72-96 hours after dosing. The fecal samples were collected before and 0-6, 6-24, 24-48, 48-72, and 72-96 hours after dosing. The plasma, urine and feces levels of 4-ABA and 4-NABA were determined using
The radioactivity was determined using

Results: Following a single oral dose, the maximum plasma concentration of BSZ was reached within 1 hour and the plasma level of BSZ was very low. The oral bioavailability of BSZ was less than 0.5%. The pharmacokinetic parameters measured after a single oral or i.v. dose were summarized in the tables 7.2, 7.3, 7.4 and 7.5 on pages 195, 196, 197 and 198 in volume 1.013. These tables are attached below.

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## TABLE 7.2

**Pharmacokinetic parameters of balsalazide and its metabolites following a single oral administration of ($^{14}$C)-balsalazide to male and female dogs at a nominal dose of 0.274 mmole/kg body weight**

| Animal No | Dose (mmole) | Component | $T_{max}$ (h) | $C_{max}$ (mmole/ml) | $AUC_{0-t}$ (mmole.h ml$^{-1}$) | Time range (h) for AUC estimate | Urine (μmole) | Renal Clearance (mL/min) | Bioavailability (%) |
|---|---|---|---|---|---|---|---|---|---|
| 121M | 2.726 | BSZ | 1.00 | 0.384 | 0.908 | 0 - 6 | 1.716 | 31.50 | 0.471 |
| | | ASA | 8.00 | 16.34 | 270.2 | 0 - 48 | 911.6 | 52.80 | |
| | | ABA | 10.00 | 1.194 | 29.34 | 0 - 48 | 564.6 | 252.7 | |
| | | NASA | 48.00 | 1.128 | 42.21 | 0 - 72 | 74.69 | 29.49 | |
| 123F | 2.241 | BSZ | 1.00 | 0.281 | 0.936 | 0 - 6 | 1.633 | 29.08 | 0.414 |
| | | ASA | 8.00 | 11.75 | 137.7 | 0 - 24 | 334.2 | 40.45 | |
| | | ABA | 16.00 | 3.044 | 27.54 | 0 - 24 | 195.2 | 118.1 | |
| | | NASA | 16.00 | 1.245 | 11.47 | 0 - 24 | 19.06 | 27.70 | |

Note: NABA was not detected

## TABLE 7.3

**Pharmacokinetic parameters of balsalazide and its metabolites following a single oral administration of ($^{14}$C)-balsalazide to male and female dogs at a nominal dose of 4.573 mmole/kg body weight**

| Animal No | Dose (mmole) | Component | $T_{max}$ (h) | $C_{max}$ (mmole/ml) | $AUC_{0-t}$ (mmoles h ml$^{-1}$) | Time range (h) for AUC estimate | Urine (μmole) | Renal Clearance (mL/min) | Bioavailability (%) |
|---|---|---|---|---|---|---|---|---|---|
| 221M | 44.82 | BSZ | 1.00 | 2.042 | 5.646 | 0 - 12 | 173.4 | 188.9 | 0.178 |
| | | ASA | 8.00 | 43.83 | 380.2 | 0 - 72 | 774.9 | 33.97 | |
| | | ABA | 8.00 | 3.326 | 43.29 | 0 - 48 | 320.0 | 113.9 | |
| | | NASA | 16.00 | 0.688 | 21.60 | 0 - 48 | 24.33 | 18.77 | |
| 223F | 38.25 | BSZ | 2.00 | 2.475 | 11.31 | 0 - 16 | 1220 | 1783 | 0.293 |
| | | ASA | 12.00 | 62.92 | 607.8 | 0 - 36 | 914.1 | 25.07 | |
| | | ABA | 12.00 | 6.568 | 48.98 | 0 - 36 | 553.3 | 188.3 | |
| | | NASA | 24.00 | 0.852 | 12.97 | 0 - 36 | 21.59 | -27.75 | |

Note: NABA was not detected

TABLE 7.4

**Pharmacokinetic parameters of balsalazide and its metabolites following a single intravenous administration of ($^{14}$C)-balsalazide to male and female dogs at a nominal dose of 0.274 mmole/kg body weight**

| Animal No | Dose (mmole) | Component | $T_{max}$ (h) | $C_{max}$ (nmole/ml) | $AUC_{0-t}$ (nmole.h $ml^{-1}$) | Time range (h) for AUC estimate | Urine (μmole) | Renal Clearance (mL/min) | Plasma Clearance (mL/min) |
|---|---|---|---|---|---|---|---|---|---|
| 321M | 2.756 | BSZ | (0.833) | (426.2) | 195.0 | 0 - 12 | 243.2 | 20.01 | 235.5 |
| | | ASA | 12.00 | 34.81 | 343.6 | 0 - 24 | 531.9 | 22.65 | NC |
| | | ABA | 12.00 | 4.528 | 46.00 | 0 - 24 | 317.2 | 100.5 | NC |
| | | NASA | 12.00 | 0.564 | 7.224 | 0 - 24 | 11.79 | 27.20 | NC |
| 323F | 2.429 | BSZ | (0.833) | (830.1) | 244.9 | 0 - 12 | 294.8 | 19.54 | 165.2 |
| | | ASA | 6.00 | 5.971 | 54.15 | 0 - 24 | 120.5 | 37.10 | NC |
| | | ABA | 0.50 | 0.310 | 2.364 | 0 - 12 | 47.96 | 223.4 | NC |
| | | NASA | 24.00 | 0.177 | ND | NR | NR | NR | NC |

NC = Not calculated, as the "dose" (i.e. the amount of balsalazide metabolised to this product) is unknown
NR = No Result
ND = None detected
Note: NABA was not detected
( ) = Values from the first post-dose sample

TABLE 7.5

**Plasma radioactivity pharmacokinetic parameters following a single intravenous administration of ($^{14}$C)-balsalazide male and female dogs at a nominal dose of 0.274 mmole/kg body weight**

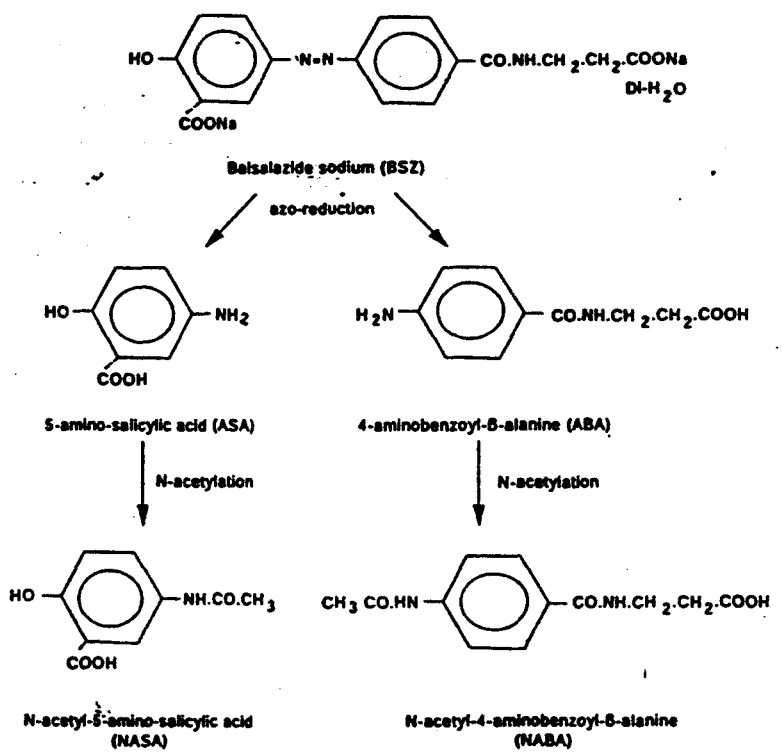
| Animal No | $T_{max}$ (h) | $C_{max}$ (nmole/ml) | $AUC_{0-t}$ (nmole.h $ml^{-1}$) | Time range (h) for AUC estimate |
|---|---|---|---|---|
| 321M | (0.033) | (2009) | 902.3 | 0 - 6 |
| 322M | (0.033) | (2415) | 976.5 | 0 - 4 |
| 323F | (0.033) | (2532) | 940.2 | 0 - 6 |
| 324F | (0.033) | (1900) | 989.6 | 0 - 4 |
| Mean | | | 952.2 | |
| SD | | | 39.26 | |

NA = Not applicable
( ) = Values from the first post-dose sample

N-acetylation. Metabolites formed after i.v. dose are similar to those after oral dose. In plasma, 5-ASA is the major metabolite. The metabolic pathway was depicted in Figure 1 on page 107 in volume 1.008. This figure is attached below.

FIGURE 1

METABOLISM OF BALSALAZIDE SODIUM (COLAZIDE®)




**EXCRETION:**

MOUSE:

Absorption Concentrations and Urinary Excretion of BSZ and Its Metabolites Following Dietary Administration of BSZ in Mice (1067/43-1010)

Methods: The methods were described above on page 7.

Results: The results indicated that BSZ in the urine was less than 1% of the daily dose in all groups and close to the limit of detection. The total recoveries of 5-ASA and its metabolite, NASA, in the urine were comparable among all dose groups ranging from 8-21% of the dose administered. The total recoveries of 4-ABA and its metabolite, NABA, in the urine were ~2.5-6.41% of the dose administered.

RAT:

Absorption, Distribution Metabolism and Excretion Following Oral and Intravenous Administrations of $^{14}$C-BSZ in Rats
(1067/8-1011)

Methods: The methods were described above on page 8.

Results: Following a single oral dose, 4-ABA was abundant in feces and was only detected in small amounts in plasma. The majority of radioactivity was recovered from the feces (~92%) with only a small amount in the urine (4.5-8%) for both doses. In the urine, low amount of BSZ was recovered with the major metabolite being NABA. In the feces, large amounts of 4-ABA were recovered. Following a single i.v. dose, the radioactivity was recovered in both feces (~58%) and urine (39%). Majority of these in the feces and the urine were 4-ABA and intact BSZ, respectively.

Absorption, Metabolism and Excretion Following Oral and I.V. Administration of $^{14}$C-ABA in Rats
(1067/9-1011)

Methods: The methods were described above on page 9.

Results: The results indicated that most of the radioactivity was associated with NABA (11.5% of the dose) in the urine following oral dose. The majority of radioactivity was associated with 4-ABA (80% of the dose) in the urine after i.v. dose. Following a single i.v. dose of $^{14}$C-BSZ, the radioactivity was recovered in the feces (57.8%, mainly 4-ABA) and in the urine (38.9%, mainly BSZ).

Following information were obtained from the pharmacology review of [redacted] dated June 10, 1994.

Methods: Rats were given a single oral dose of 50 mg/kg of BSZ. Urine and feces samples were collected daily for 4 days. The levels of Balsalazide, 5-ASA (5-aminosalicylic acid: active moiety) and ABA (4-aminobezoyl-beta alanine: carrier molecule) were measured calorimetrically.

Results: In rats, about 22% and 14% of the administered dose were eliminated in feces and urine as 5-ASA (in man after a single oral dose of 2 g BSZ, about 35% in urine and about 46% in feces were excreted as 5-ASA, while about 19% in urine and 72% in feces were excreted as ABA. The serum concentrations of BSZ, ABA and 5-ASA were below detection limit [1-2 mcg/ml]).

DOG:

In a study in dogs reviewed above (study 1067/10-1011), fecal elimination was the major route of excretion following both oral and intravenous doses. The radioactivity recovered in feces was 32-65% and 13-25% after oral doses at 120 and 2000 mg/kg, respectively. Following i.v. administration, the radioactivity was recovered in both feces (64-75%) and urine (16-29%).

The plasma and urinary concentrations of BSZ and its metabolites were also determined in some toxicity studies. These data are presented in each corresponding study.

To compare the similarities and differences between mice, rats, rabbits and dogs, the pharmacokinetic parameters of BSZ are summarized in the following table.

Pharmacokinetics of BSZ in mice, rats, rabbits and dogs

| Species & Dose | $C_{max}$, nmol/ml | AUC, nmol•h/ml | Cl, ml/min | F (%) |
|---|---|---|---|---|
| Mouse: | | | | |
| Oral | | | | |
| 120 mg/kg | 0.0243 | ---- | ---- | ---- |
| 500 mg/kg | 0.074-0.164 | ---- | ---- | ---- |
| 1000 mg/kg | 0.0714-0.213 | ---- | ---- | ---- |
| Rat: | | | | |
| Oral | | | | |
| 120 mg/kg | 1.091 | 3.072 | 1.484 | 0.52-0.62 |
| 2000 mg/kg | 8.505 | 42.56 | 1.359 | |
| I.V. | | | | |
| 120 mg/kg | ---- | 491.9 | 0.625 | ---- |
| Dog: | | | | |
| Oral: | | | | |
| 120 mg/kg | 0.281-0.384 | 0.91-0.94 | 29-32 | <0.5% |
| 2000 mg/kg | 2.475-2.842 | 5.65-11.3 | 189-1783 | |
| I.V. | | | | |
| 120 mg/kg | ---- | 952.2 | 20 | ---- |

In general, the plasma concentration of BSZ was very low after oral administration. Its oral bioavailability was ~0.5-0.6% in rats and dogs. In rabbits, BSZ was quickly eliminated with a half life of ~1.3 hours. BSZ was metabolized to 5-ASA, an active moiety and 4-ABA, the carrier molecule. 5-ASA and 4-ABA were then metabolized via N-acetylation into N-acetyl-5-amino-salicylic acid (NASA) and N-acetyl-4-aminobenzoyl-b-alanine (NABA), respectively.

In rats, radioactivity was detected in the gut (oral) and duodenum and kidney (i.v.). BSZ was highly bound to plasma protein (~96-99%) in rats, dogs and humans. The major route of excretion was by urine and feces. Following oral administration, BSZ was mainly found in the feces with little amount in the urine. Following i.v. administration, urine and fecal excretions were the major route in both rats and dogs.

**TOXICITY:**

**ACUTE TOXICITY:**

The acute toxicity studies were previously submitted to [redacted] and reviewed on June 10, 1994. The review is reproduced below.

**Methods:** The acute oral, I.P. and I.V. toxicity of Balsalazide was studied in mice and rats of both sexes. The vehicle for oral dose was 1% methylcellulose or 0.9% NaCl, for I.P. and I.V. administration the vehicles were 0.9% NaCl and 5% dextrose respectively. The volume of administration was fixed at 10 ml/kg for gavage dose, 0.2 ml/animals for I.P. dose and 5 ml/kg for I.V. dose. All animals were observed for clinical signs and mortality for 7 days (rats which received I.V. infusion dose [0.5 ml/min] were observed for only 48 hr). At the end of observation period, all animals were sacrificed and subjected to complete necropsy.

**Results:**

Acute Toxicity Study of Balsalazide

| Species (strain) | Route | No./Sex/ Group | Dose (g/kg) | LD 50 (g/kg) | Highest Non-Lethal Dose (g/kg) |
|---|---|---|---|---|---|
| Mice (Albino) | P.O. | 10 | 2, 4 | ND | 4 |
| Mice (Albino) | I.P. | 10 | 2 | ND | 2 |
| Mice (Crl:CD(ICR)BR) | P.O. | 3 | 5 | ND | 5 |
| Mice (Crl:CD-1(ICR)BR) | I.V. | 3 | 0.6 | ND | 0.6 |
| Rats (Wistar) | P.O. | 10 | 2 | ND | 2 |
| Rats (Wistar) | I.P. | 6 | 0.5, 1 | ND | 1 |
| Rat (Crl:CD(SD)BR) | P.O. | 3 | 5 | ND | 5 |
| Rat (Crl:CD(SD)BR) | I.V. | 3 | 0.6 | ND | 0.6 |
| Rat (Crl:CD(SD)BR) | I.V. (0.5 ml/min) | 1 | 0.12, 0.30, 0.6 | ND | 0.6 |

ND = not determined

**The highest tested dose in mice (4 g/kg oral, 2 g/kg I.P. and 0.6 g/kg I.V.) and rats (2 g/kg oral, 1 g/kg I.P. and 0.6 g/kg I.V.) were the highest non-lethal dose. No clinical signs were seen in mice and rats at the respective highest non-lethal dose.**

**ACUTE TOXICITY OF 4-ABA:**

**Methods:** The acute oral and I.V. toxicity of 4-ABA was studied in Crl:CD-1(ICR)BR mice (3/sex/group) and Crl:CD(SD)BR rats (3/sex/group) of both sexes. The vehicle for oral dose was 1% methylcellulose, for I.V. administration the vehicles was 1 N HCl (pH was adjusted to 4). The volume of administration was fixed at 20 ml/kg for gavage dose and 5 ml/kg for I.V. dose. All animals were observed for clinical signs and mortality for 7 days. At the end of observation period, all animals were sacrificed and subjected to complete necropsy.

**Results:** In both species the highest tested dose (1000 mg/kg) was non-lethal dose. No clinical signs were seen in mice and rats at the highest tested dose.

Addendum: In the acute toxicity study with 4-ABA (report 1067/46-1050), the oral dose was 1000 mg/kg and i.v. dose was 150 mg/kg for both mice and rats.

**SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY:**

A 7-Day Acceptance Study in Rats
(661/Tx/Rt-M/Od/7d/87)

Methods: This is not a GLP study. To determine acceptability and tolerance of BSZ in rats, rats (5/sex/group) were given BSZ at 2, 4 and 8 g/kg in diet for 7 days. Following parameters were monitored: clinical signs of toxicity (daily), body weights (on days 1, 3, 7), food (daily) and water consumptions (twice weekly), liver and kidney weights and gross pathological examination on the liver, kidneys and GI tract.

Results: Diarrhea was seen in all treated animals and the severity was dose dependent. Body weight gain was reduced by ~6, 6 and 15% in the low, mid and high dose males and ~15% in the low and high dose females as compared to the control. The body weight gain in the mid dose females, however, was increased ~15% as compared to the control. Slight changes in food and water consumption were not clearly dose dependent. Macroscopic examination revealed that the incidence of distended caecum was slightly increased in the low and mid dose groups (not in the high dose group) and the incidence of the distended caecum was 1, 2, 3 and 0 in the control, low, mid and high dose females.

A 9-Day Acceptance Study in Rats
(661/Tx/Rt/Od/9d/81)

Methods: This is not a GLP study. To determine acceptability and tolerance of BSZ in rats, rats (5/sex/group) were given BSZ at 1 g/kg in diet for 9 days. Following parameters were monitored: clinical signs of toxicity, body weights and food consumption, hematology, blood chemistry, urinalysis, organ weights and gross and histopathological examinations.

Results: There were no treatment related changes in clinical signs of toxicity, body weight, food consumption, organ weight and histopathological examination. Changes in hematocrit, serum bicarbonate and urinary specific gravity and protein were minor and not considered to be biologically significant.

The following repeated dose toxicity studies were previously submitted to ——— and reviewed on June 10, 1994 and June 28, 1995. The review is reproduced below.

**28-Day Acceptance Study of Balsalazide in Mice and Rats**
**(# 661/TX/M-RT/1/87)**

**Methods: This is not a GLP study. Experimental details were not written clearly. Groups of Albino mice (5/sex/group) and Wistar rats (5/sex/group) were given 8 and 12 g/kg of Balsalazide via diet ("two parts of drug solution at the appropriate dose were mixed with 3 parts of crushed diet") for 28 days. The control group animals received unmedicated diet. It should be noted that at highest dose about 17-21% and 10-12% of the diet represented drug in mice and rats respectively. All animals were observed for clinical signs and mortality daily. Body weights were recorded on days 0, 5, 9, 15, 20 and 28 of the study and food and water intakes were recorded daily. Blood samples were collected prior to terminal sacrifice to measure Balsalazide and its metabolite levels in plasma and hematological tests. At the end of study period all animals were sacrificed and subjected to complete necropsy. Only liver, kidneys and gastrointestinal tract were examined microscopically.**

Results: Dose related diarrhea (with fecal occult blood) was seen in treated rats of both sexes. There was no diarrhea in treated mice. Dose-related decrease in body weight gain was evident in treated mice of both sexes (males: low dose = 16% and high dose = 10%; females: low dose = 23% and high dose = 52%) and treated male rats (low dose = 7% and high dose = 22%) and significant reduction in body weight gain was also seen in high dose treated female rats (30%). Food and water intake data were not reported properly (graphs are not labelled). According to sponsor "all mice and rats finished the food given, and water consumption increased in dose dependent fashion in treated rats of both sexes". Dose related leucocytosis (low dose: 76% and high dose: 111%) was seen in treated male rats and dose related increase in platelet counts (low dose: 36% and high dose: 50%) was seen in treated female rats. Hematological test results in mice were not reported. Histopathological changes in cecum (chronic inflammation/erosions), mid colon (chronic inflammation), rectum and preanal (chronic inflammation/ ulceration) were seen in treated rats (severity increased at high dose).

This a non-GLP study. Not all toxicological parameters were evaluated, hence study as such is not very informative. Furthermore, at highest dose about 17-21% and 10-12% of the diet represented drug in mice and rats respectively, which is not acceptable (according to NCI Guideline drug level should not exceed more than 5% of the diet). Significant reduction in body weight gains in treated mice (both sexes) and high dose treated female rats were seen. Dose related leucocytosis (in male rats) and increase in platelet counts (in female rats) were seen. Histopathological examination revealed chronic inflammation/ erosions/ulceration in cecum, colon, rectum and preanal of treated rats. A maximum tolerated dose was not identified in this study.

## 14-Day Oral Toxicity Study in Rats
**(Study # not given)**

Methods: Groups of Wistar rats (10/sex/group) were given 2 g/kg of Balsalazide orally (gavage) for 14 consecutive days. The volume of administration was 2 ml/rat. The control animals received vehicle (water) in similar fashion. All animals were observed for clinical signs and mortality daily, body weights were recorded twice weekly. Food intakes were recorded weekly and water intakes were recorded daily. Blood samples were collected from abdominal aorta, just before sacrifice, for hematological and serum chemistry tests. Urine samples during the last week of treatment were also collected for urinalysis. At the end of study period all surviving rats were sacrificed and subjected to complete necropsy and histological examinations.

**Results**: Body weight gain was significantly increased (46%) in treated males, while in females body weight gain was reduced by 10% when compared to control values. In male rats food intake was increased by 19% compared to control. Water intakes were increased by 139% and 22% in treated males and females respectively. No biologically significant effect on hematological and serum chemistry tests were seen. No treatment related histological abnormality was evident in treated rats. Thus 2 g/kg (25 times the maximum proposed clinical dose [4 g/day; 0.08 g/kg; 50 kg body wt. assumed) of Balsalazide for 14 days did not produce any toxicity in rats (both sexes).

**14-Day Oral (gavage) Dose Range Finding Study in Rats**
**(Report # 7405-1067/29)**

Groups of Crl:CD(SD)BR rats (5/sex/group) were given 0 (1% methylcellulose), 120, 500, 2000 and 3000 mg/kg/day of BSZ orally (gavage) for 14 consecutive days. Clinical signs such as salivation, padding motions and cold to touch were seen in high dose treated rats. One female from high dose group died on day 4 of the study. Cause of death was not determined. In this study only body weight and food consumption were monitored, and treatment had no effect on these parameters. Hence, 3000 mg/kg/day (37.5 times the maximum proposed clinical dose [4 g/day = 0.08 g/kg; 50 kg body wt. assumed) did not produce any toxicity in rats. According to sponsor 3000 mg/kg/day is the "maximum practicable" high dose in future studies due to solubility (300 mg/ml) and the diameter of the dosing catheter. Sponsor also indicated that proof of absorption will be reported separately in report # 1067/29-1010.

Addendum: The toxicokinetic data were not previously submitted. The serum levels of BSZ and its metabolites were increased with increased dose. Very small amount of SBZ was detected in the serum. The serum levels of BSZ, 5-ASA and 4-ABA and their metabolites are summarized in the following table.

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Median serum levels of BSZ and its metabolites (nmol/ml)

| Dose (mg/kg/day) | BSZ | 5-ASA | NASA | 4-ABA | NABA |
|---|---|---|---|---|---|
| 120, males | 0.1 | 0.411 | 3.94 | <0.24 | 0.747 |
| females | <0.0229 | 0.334 | 3.01 | <0.24 | 0.454 |
| 500, males | 0.585 | 3.03 | 9.82 | <0.24 | 2.14 |
| females | 0.0805 | 4.38 | 18.67 | <0.24 | 1.48 |
| 2000, males | 3.1 | 21.25 | 31.21 | 0.544 | 7.24 |
| females | 0.395 | 33.13 | 46.9 | 1.72 | 6.54 |
| 3000, males | 6.71 | 44.48 | 55.74 | 1.45 | 11.75 |
| females | 0.608 | 53.12 | 60.78 | 1.87 | 4.64 |

The urinary excretion of BSZ was very low (<1% of dose) as compared to total 5-ASA ranging from ~7.33-11.3% of the dose. The urinary excretion of 4-ABA was ~0.7-4.41% of the dose.

**14-Day Palatability Oral (dietary administration) Study in Rats (Report # 7402-1067/5)**

**Groups of Crl:CD(SD)BR rats (10/sex/group) were given 120, 500, 1000 and 2000 mg/kg/day of BSZ via diet for 14 days. The control rats were given unmedicated diet. Drug intakes were generally in good agreement with intended levels (low dose: 117.6-133.2 mg/kg/day, mid dose: 497.1-563.5 mg/kg/day, high dose: 979.6-1052.9 mg/kg/day and highest dose: 1977.2-2231.6 mg/kg/day). The highest tested dose was the no effect dose, and 2000 mg/kg/day was palatable. Sponsor suggested that 2000 mg/kg/day should be the highest dose level for the carcinogenicity study. Sponsor indicated that proof of absorption will be reported separately in report # 1067/5-1010.**

Addendum: The toxicokinetic data were not previously submitted. The serum levels of BSZ and its metabolites were increased with increased dose. Very small amount of SBZ was detected in the serum. The serum levels of the metabolites (5-ASA, NASA, 4-ABA and NABA) appeared to be higher in females than those in males. The serum levels of BSZ, 5-ASA and 4-ABA and their metabolites are summarized in the following table.

Median serum levels of BSZ and its metabolites (nmol/ml)

| Dose (mg/kg/day) | BSZ | 5-ASA | NASA | 4-ABA | NABA |
|---|---|---|---|---|---|
| 120, males | 0.1 | 0.411 | 3.94 | <0.24 | 0.747 |
| females | <0.0229 | 0.334 | 3.01 | <0.24 | 0.454 |
| 500, males | 0.585 | 3.03 | 9.82 | <0.24 | 2.14 |
| females | 0.0805 | 4.38 | 18.67 | <0.24 | 1.48 |
| 2000, males | 3.1 | 21.25 | 31.21 | 0.544 | 7.24 |
| females | 0.395 | 33.13 | 46.9 | 1.72 | 6.54 |
| 3000, males | 6.71 | 44.48 | 55.74 | 1.45 | 11.75 |
| females | 0.608 | 53.12 | 60.78 | 1.87 | 4.64 |

The urinary excretion of BSZ was very low (<1% of dose) as compared to total 5-ASA ranging from ~7.33-11.3% of the dose. The urinary excretion of 4-ABA was ~0.7-4.41% of the dose.

**14-Day Palatability Oral (dietary administration) Study in Rats (Report # 7402-1067/5)**

**Groups of Crl:CD(SD)BR rats (10/sex/group) were given 120, 500, 1000 and 2000 mg/kg/day of BSZ via diet for 14 days. The control rats were given unmedicated diet. Drug intakes were generally in good agreement with intended levels (low dose: 117.6-133.2 mg/kg/day, mid dose: 497.1-563.5 mg/kg/day, high dose: 979.6-1052.9 mg/kg/day and highest dose: 1977.2-2231.6 mg/kg/day). The highest tested dose was the no effect dose, and 2000 mg/kg/day was palatable. Sponsor suggested that 2000 mg/kg/day should be the highest dose level for the carcinogenicity study. Sponsor indicated that proof of absorption will be reported separately in report # 1067/5-1010.**

Addendum: The toxicokinetic data were not previously submitted. The serum levels of BSZ and its metabolites were increased with increased dose. Very small amount of SBZ was detected in the serum. The serum levels of the metabolites (5-ASA, NASA, 4-ABA and NABA) appeared to be higher in females than those in males. The serum levels of BSZ, 5-ASA and 4-ABA and their metabolites are summarized in the following table.

Median serum levels of BSZ and its metabolites (nmol/ml)

| Dose (mg/kg/day) | BSZ | 5-ASA | NASA | 4-ABA | NABA |
|---|---|---|---|---|---|
| 120, males | 0.0306 | <0.327 | 1.13 | <0.24 | 0.64 |
| females | 0.0619 | 0.332 | 2.7 | 0.244 | 1.15 |
| 500, males | 0.112 | <0.327 | 3.33 | 0.363 | 2.2 |
| females | 0.33 | 4.63 | 15.82 | 1.43 | 3.29 |
| 1000, males | 0.238 | 3.22 | 15.55 | 1.06 | 4.96 |
| females | 0.162 | 32.13 | 28.46 | 16.51 | 7.81 |
| 2000, males | 0.152 | 11.66 | 17.8 | 11.52 | 12.36 |
| females | 0.347 | 41.03 | 51.04 | 5.46 | 7.2 |

The urinary excretion of BSZ was very low (<1% of dose) as compared to total 5-ASA ranging from ~7-17% of the dose. The urinary excretion of 4-ABA was ~2-6% of the dose.

## 26-Week Oral (gavage) Toxicity Study in Rats (Report # 1067/4-1050)

**Testing Laboratories:** [redacted]

**Study Started:** August 24, 1992

**Study Completed:** November 1, 1993

**GLP Requirements:** A Statement of Compliance with the GLP regulations (U.K.) quality assurance unit was included.

**Animals:** Crl:CD(SD)BR rats (39 days old; males: 190.8-224.1 g and females: 137.5-183.4 g).

**Drug Batch No.:** C 6832.7-13 and BNE 6832.7D-03

**Methods:** Groups of rats (20/sex/group) were given 0 (1% methylcellulose), 120, 600 and 3000 mg/kg/day of BSZ orally via gavage for 26 weeks. The volume of administration was 10 ml/kg. Additionally 10 rats/sex/group were included in each group to assess drug absorption ((proof of absorption i.e. levels in blood and urine will be reported separately in report #-1067/4-1010). All animals were observed daily for clinical signs and mortality. Body weight and food and water consumptions were recorded weekly.

Ophthalmoscopic examinations were performed on all animals at pre-dose and on all animals in control and high dose group in week 25 of the study. Blood samples were collected from orbital sinus at weeks 4, 13 and 26 of the study for hematological and serum chemistry tests. Eighteen hr. urine samples were also collected at weeks 4, 12 and 25 of the study for urinalysis, during which time animals were fasted. At the end of treatment period all animals were sacrificed and subjected to complete necropsy. Histopathological examinations were conducted on all tissues of control and high dose groups, all gross lesions and kidney, stomach and bladder from low and mid dose groups.

Results:

1. Observed Effects: Clinical signs such as salivation and paddling motions were seen in all high dose treated rats.

2. Mortality: A total of 6 animals (3 from control group, 1 from low dose group and 2 from high dose group) died or sacrificed during the study period. These deaths were considered not to be treatment related.

3. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, only high dose treated males body weight gains were reduced by 9% when compared to the control values. Food intakes were not affected by the treatment. Water intakes were increased by 32-35% and 45-60% in high dose treated males and females respectively compared to their respective control values.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry and Urinalysis: In high dose treated rats (both sexes) serum cholesterol and serum phospholipid levels were decreased by 25-30% and 20-26% respectively when compared to their control values. In high dose treated males, increases in urinary volume (107%), sodium (725%), potassium (78%) and chloride (100%) excretion were seen. Increased electrolyte excretion was also seen in high dose treated females but magnitude of increase was lower than that seen in high dose males. Increased incidences of blood and protein were seen in the urine of high dose treated rats (both sexes).

6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.

7. Organ Weights: Only kidney weights were increased by 12% in high dose treated rats of both sexes.

8. Gross Pathology: Cecal distention (males: 2/20 in mid dose and 16/20 in high dose; females: 13/20 in high dose) and thickening of the fundic region of the stomach (males: 3/20 in high dose and females: 2/20 in high dose) were seen in treated rats.

9. Histopathology: Histopathological examination revealed gastritis in the stomach, corticomedullary mineralization and papillary urothelial hyperplasia/mineralization in the kidneys and urothelial hyperplasia in the bladder of mid and high dose treated rats. The cecal distention noted macroscopically was not associated with any histopathological changes. The incidences of these findings were as follows:

| Incidences of Histopathological Findings in Rats | | | | | |
|---|---|---|---|---|---|
| Organs | Sex | Control | Low Dose | Mid Dose | High Dose |
| # Examined | M/F | 20 | 20 | 20 | 20 |
| Stomach | | | | | |
| Gastritis | M | 0 | 0 | 3 | 20 |
| | F | 0 | 0 | 5 | 20 |
| | | | | | |
| Kidney | | | | | |
| Corticomedullary mineralization | M | 0 | 0 | 0 | 1 |
| | F | 5 | 6 | 9 | 14 |
| Papillary urothelial hyperplasia/mineralization | M | 0 | 0 | 1 | 8 |
| | F | 0 | 2 | 0 | 0 |
| | | | | | |
| Bladder | | | | | |
| Urothelial hyperplasia | M | 0 | 0 | 0 | 10 |
| | F | 0 | 0 | 0 | 9 |

**The data indicated that stomach, kidney and bladder are the target organs of toxicity and the lowest tested dose (120 mg/kg/day) is the no effect dose in this study.**

Addendum: The toxicokinetic data were not previously submitted. The serum samples were not analyzed. The urinary excretion of BSZ was very low from non-measurable to 1.71% of dose) as compared to total 5-ASA ranging from ~3.62% to 25.03% of the dose. The urinary excretion of 4-ABA was ~0.865-8.84% of the dose.

## 96-Week Oral (diet) Toxicity Study in Rats
## (Report # 661/LS/RT/81)

Testing Laboratories: Biorex Pharmaceuticals Ltd.
Middlesex UK

Study Started: Not given.

Study Completed: Not given.

Animals: Male (mean wt.: 143 g) and female (mean wt.: 148 g) Biorex Wistar Rats.

Drug Batch No.: Not given.

Methods: Groups of rats (10-15/sex/group) were given 0, 0.1, 0.5 and 1.0 g/kg/day of Balsalazide orally (via diet) for 12 or 27 weeks. Additionally, 15 rats/sex each were included in control and high dose groups for 6 weeks recovery study. Furthermore, 15 rats/sex each were also included in control, low and mid dose groups in which treatment continued for 96 weeks. All animals were observed for clinical signs and mortality daily. Body weights, food consumption and water intakes were recorded weekly. Blood pressure and heart rate were monitored in the control and high dose groups rats after 6, 12, 27 and 96 weeks of treatment and after 6 weeks recovery period in rats treated for 27 weeks. Just before sacrifice, blood samples were collected from abdominal aorta for hematological and serum chemistry tests. Twenty-four hr. urine samples from 5 rats/group were also collected in the week prior to autopsy for urinalysis. Five male rats per group were used for fertility test after 3, 12 and 27 weeks of treatment. Fertility test was done by mating each male with 5 females of proven fertility for 4 days, then females were killed 14 days later. Number of pregnant females, corpora lutea, live/dead fetus and early/late resorptions were recorded. At the end of treatment/recovery period all surviving animals were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: No treatment related effects were seen.

2. Mortality: A total of 58 rats (21 in control group, 19 in low dose group, 17 in mid dose group and 1 in high dose group) died or were killed during the study period. All these deaths were considered not to be treatment related.

3. Body Weight/Food Consumption/Water Consumption: During the first 27 weeks of treatment, body weight gains in both sexes were not affected by the treatment. At the end of 96 weeks of treatment, body weight gains were reduced by 21% and 15% in low and mid dose treated males and 15% and 30% in corresponding females when compared to the control values (high dose was not used for 96-week treatment). Food consumptions were not affected by the treatment. Initially, in the form of "moist ball of diet" male rats were given 25 g feed with or without drug/rat/day while females received 20 g feed with or without drug/rat during the study period. Sponsor did not indicate how much food was available to rat/day at later time. Dose related increases in water consumption were seen in treated rats. Sponsor attributed this to the high sodium content in the drug.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: No treatment related effects were seen in serum chemistry tests. A one unit increase in urinary pH was evident in high dose treated rats. Urinary pH at 6 weeks after the recovery period in rats which were treated for 27 weeks were comparable in all groups.

6. Vital Signs/Physical Examination: No treatment related effects were seen.

7. Organ Weights: No treatment related effects were seen in rats treated for 27 weeks. However, at the end of 96 weeks of treatment, relative kidney weights were increased by 17% and 45% and relative testes weights were reduced by 28% and 25% in 100 and 500 mg/kg treated group respectively. Additionally, the relative weights of adrenals and kidneys were increased by 41% and 23% in 500 mg/kg/day treated females.

8. Gross Pathology: At the end of 27 weeks of treatment, no drug related effects were seen. Summary table for gross pathological examinations at week 96 reported the results of only 5-7 rats/sex/group. Hence data is not complete. In any event, at the end of 96 weeks of treatment, 2 out of 7 male rats treated with 500 mg/kg/day had pale soft kidneys with hemorrhagic foci and mottled kidneys were seen in 4/7 and 1/6 mid dose (500 mg/kg/day) treated males and females respectively.

9. Histopathology: No treatment related effects were seen. Tumors such as basophilic adenoma in pituitary (control = 0/30, low dose = 1/30 and mid dose = 1/30) was seen in treated rats. This is a common tumor and the incidence is very low, therefore can be considered as spontaneous finding.

10. Male Fertility Test: Not affected by the treatment.

**The data indicated that a dose of up to 1000 mg/kg/day for 27 weeks did not produce any drug related effect in Biorex Wistar rat. However, dose levels of 100 and 500 mg/kg/day for 96 weeks did produce decrease in body weight gain, and some gross changes in kidneys which were not evident by microscopic examinations. In this study sponsor should have used higher dose levels to elicit some toxicities. Study as such is not very informative.**

A 10-Day Acceptance Study in Ferrets
(661/Tx/F/Od/10d/84)

Methods: This was a dose ranging study for a 26-week dietary toxicity study in ferrets (not GLP study). Sponsor provided a brief summary of this study in this submission. BSZ was given to ferrets (2/sex) in diet at 600 mg/kg for 10 days. Following parameters were monitored: clinical signs of toxicity, body weight and food consumption.

Results: The major treatment related changes were decreased body weight in both males (~12%) and one female (21%) at the end of treatment.

A 28-Day Pilot Study in Ferrets
(661/Tx/F/Oi/28d/88)

Methods: This was a dose ranging study (not GLP study). Sponsor provided a brief summary of this study in this submission. Ferrets (2/sex) were given BSZ at 4000 mg/kg by oral gavage for 28 days. The dose volume was constant at 20 ml/kg body weight. Animals were under anesthesia (13.3 mg/kg ketamine and 2.7 mg/kg xylazine im) when gavage dose was given. Following parameters were monitored: clinical signs of toxicity, body weights, food and water consumptions, organ weights and histopathological examination.

Results: Recovery from anesthesia was noted ~1 hour after dosing. Following clinical signs of toxicity were seen: emesis, increased motor activity, burying their heads in the sawdust, occasional jumping, and scratching the insides of their mouths. Decreased body weight was seen in males (-26%) over the first two weeks. Water consumption was increased by ~100% and 20% in the male and female, respectively, as compared to the control. Increased liver weights (30-40%) was seen in the treated animals. Histopathological examination revealed the following: periacinar hepatocytic glycogen pallor and fatty vacuolation and occasional small clumps of perivascular chronic inflammatory cells in the liver and marked excess of mucus, a slight increase of chronic inflammatory cells in the mucosa. The central nervous system, liver and GI tract were the target organs of toxicity.

The following repeated dose toxicity studies were previously submitted to ~~~ and reviewed on June 10, 1994. The review is reproduced below.

**26 Week Oral (diet) Toxicity Study in Ferret**
**(Report # 661/TX/F/6/84)**

**Testing Laboratories**: Biorex Laboratories Ltd.
Middlesex, England

**Study Started**: Not given.

**Study Completed**: Not given.

**GLP Requirements**: Not mentioned.

**Animals**: Biorex albino ferrets (males: 2.0-2.4 kg and female: 0.9-1.2 kg).

**Drug Batch No.**: Not given.

**Methods**: Groups of ferrets (10/sex/group) were given 120, 300 and 600 mg/kg/day of BSZ via diet for 26 weeks. The control group animals were given unmedicated diet. All animals were observed daily for clinical signs and mortality. Body weights were recorded weekly and food and water intakes were recorded daily. Blood pressure and heart rate were monitored at pretest on 5 ferrets/sex/group and on 5 ferrets/sex from control and high dose groups at 0 and 6 months. Just before sacrifice, blood samples were collected from abdominal aorta for hematological and serum chemistry tests. Twenty-four hr. urine samples were also collected during the last week of study for urinalysis. At the end of study period all surviving animals were sacrificed and subjected to complete necropsy and histopathological examinations.

**Results**:

1. **Observed Effects**: No treatment related effects were seen.

2. **Mortality**: A total of 4 ferrets (2 females from control group, 1 female from low dose group and 1 female from high dose group) died or killed during the study period and cause of deaths were not treatment related.

3. **Body Weight/Food Consumption/Water Consumption**: All treated females and mid and high dose treated males lost their weights when compared to initial weights (body weight data was presented graphically). Mean weekly food consumptions were not affected by the treatment, however, water intakes were increased by 6-70% and 17-36% in treated males and females respectively.

4. **Hematology/Coagulation/Bone Marrow**: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: Only serum cholesterol levels were increased by 23% in high dose treated females when compared to the control values. Urine volume was increased by 82% in high dose treated females.

6. Blood Pressure/Heart Rates: No treatment related effects were seen.

7. Organ Weights: In high dose treated males, relative weights of brain and kidneys were increased by 11% and 18% respectively, while testes absolute as well as relative weights were decreased by 30-33% when compared to their respective control values. In high dose treated females ovaries weights were significantly reduced (absolute: 24% and relative: 16%) compared to control values. Additionally, reduced absolute (26%) and reduced relative (17%) weights of spleen were recorded in high dose treated females.

8. Gross Pathology: No summary table was provided. However, hemorrhagic lymph nodes were seen in 4/10 high dose treated males and none in any other groups.

9. Histopathology: No treatment related affects were seen.

In this study target organ of toxicity was not identified. However, drug had severe effect on body weight gain (all treated females and mid and high dose treated males lost their weights). A no effect dose in this study was not established.

**BSZ: Oral Maximum Tolerated Dose (MTD) Toxicity Study Followed by a 14-Day Fixed Oral (gavage) Dose Toxicity Study in the Dog (Report # 7399-1067/1)**

Testing Laboratories: ———

Study Started: July 6, 1992

Study Completed: September 27, 1993

GLP Requirement: A Statement of Compliance with the GLP regulation (U-K) and quality assurance unit was included.

Animals: Beagle dogs (6 months old: males = 8.85-9.0 kg and females = 7.85-9.95 kg).

Drug Batch No.: C6832.7-10

Methods: To determine the maximum tolerated dose of BSZ, dogs (1/sex) were given oral (gavage) dose of 500 mg/kg/day from days 1-3, 1000 mg/kg/day from days 4-6, 2000 mg/kg/day from days 7-9 and 3000 mg/kg/day on day 10 of the study. The volume of administration was fixed at 5 ml/kg. Sponsor indicated that the top dose i.e. 3000 mg/kg/day could not be administered properly due to thick consistency of the dosing solution (600 mg/ml). During the course of study period no toxicity was evident and 2000 mg/kg/day was considered to be maximum "practicable dose". In the 14-day toxicity study dogs (1/sex) was given 2000 mg/kg/day of BSZ orally (gavage). No control group was included in this study. All dogs were observed daily for clinical signs, body weights were recorded weekly and food intakes daily. Urine samples were collected at 0-6 hr. and 6-24 hr. after drug administration on days 1 and 14 of the study to determine BSZ and its metabolites (proof of absorption: results will be reported separately in HE report # 1067/1-1010). At the end of study period dogs were sacrificed and subjected to complete necropsy. Only liver and kidney were weighed. No histopathological examinations were performed.

Results: Only yellow/orange discoloration of the feces was seen in dogs. This is due to the coloration of the test article. In this study there were no control animals, therefore meaningful comparison can not be made. According to sponsor no toxic effects were seen. All dogs (n=2) survive the treatment. A dose level of 2000 mg/kg can be consider as "practical dose" of BSZ to dogs.

Addendum: The toxicokinetic data were not previously submitted. Plasma levels of the drug and its metabolites were not determined. The urinary excretion of BSZ was very low (0.11-0.55%) as compared to total 5-ASA ranging from ~4.21-7.21% of the dose. The urinary excretion of 4-ABA was ~1.92-3.37% of the dose.

## 28-Day Oral (gavage) Toxicity Study in Dogs
## (Report # 7477-1067/2)

Testing Laboratories: _____

Study Started: August 25, 1992

Study Completed: September 29, 1993

GLP Requirements: A Statement of Compliance with the GLP regulations (U.K.) quality assurance unit was included.

Animals: Beagle dogs (6-7 months old: males = 7.0-11.95 kg and females = 6.95-9.35 kg).

Drug Batch No.: C6832.7-10

Methods: Groups of dogs (3/sex/group) were given 0 (1% methylcellulose), 120, 600 and 2000 mg/kg/day of BSZ orally via gavage for 28 days. The volume of administration was 5 ml/kg. All dogs were observed daily for clinical signs and mortality. Body weights were recorded weekly and food and water consumptions were recorded daily. ECG tracing was obtained at pre-test and during week 4 of the study period. Blood samples were collected from jugular vein at pre-test and during week 4 of the study for hematological and serum chemistry tests. Twenty-four hr. urine samples were also collected during the above mentioned time period for urinalysis, during which time animals were fasted. Part of the blood and urine samples were saved for measuring drug and its metabolites levels (results will be reported separately in HE report # 1067/2-1010). At the end of treatment period all animals were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: Yellow/orange feces were seen in treated dogs. This is due to the coloration of the test article.

2. Mortality: One dog from low dose group died on day 1 of the study due to dosing error. This dog was replaced with another naive dog and the treatment was started from day 2 onwards.

3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were seen.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry and Urinalysis: No treatment related effects were seen in serum chemistry tests. Urinary potassium levels were decreased by 30% in males and 63% in females of high dose treated group when compared to control values. Additionally, urinary chloride levels were decreased by 56% in high dose treated females, compared to control values.

6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.

7. Organ Weights: One male dog (#3251) of high dose group had very enlarged prostate (17.1 g vs mean control value of 3.78 +/- 2.8 g). Sponsor considered this not to be treatment related.

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: No treatment related effects were seen.

In this study, the highest tested dose (2000 mg/kg/day) was the no effect dose and no target organ of toxicity was identified.

Addendum: The toxicokinetic data were not previously submitted. The results indicated that maximum plasma levels of BSZ was reached within 2 hours. The median $C_{max}$ on Day 1 was 0.595, 1.55 and 3.65 nmol/ml, respectively, in the low, mid and high dose groups. The plasma levels declined progressively with time and still measurable at 8 hours (low and mid doses) and 24 hours (high dose). There was no apparent accumulation of BSZ over time. The urinary recovery of BSZ was <1% of the dose.

The median AUC of SBZ, 5-ASA and 4-ABA was summarized in the following table.

Median AUC of SBZ, 5-ASA and 4-ABA (nmol•h/ml)

| Dose (mg/kg/day) | SBZ | | 5-ASA | | 4-ABA | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 27 | Day 1 | Day 27 | Day 1 | Day 27 |
| 120 | 2.4 | 3.1 | 179 | 158 | 22.1 | 19.1 |
| 600 | 8.6 | 9 | 468 | 1124 | 66.9 | 166 |
| 2000 | 14 | 29 | 1113 | 1135 | 102 | 111 |

The urinary recovery for 4-ABA was 0.5-5.2% of the dose. The urinary recovery for 4-ASBA was 1.3-10.5%.

The median plasma AUC of NASA was 7.30, 26.6 and 28.6 nmol•h/ml on day 27 and urinary recovery was 0.054-0.57% of the dose. Plasma and urinary levels of NABA were below the limit of detection.

## 52-Week Oral (gavage) Toxicity Study in Dogs (Report # 1067/3-1050)

Testing Laboratories: [redacted]

Study Started: December 8, 1992

Study Completed: June 13, 1994

GLP Requirements: A Statement of Compliance with the GLP regulations (U.K.) quality assurance unit was included.

Animals: Beagle dogs (5-7 months old: males = 6.40-8.95 kg and females = 5.15-7.35 kg).

Drug Batch No.: C6632.7-13, BN 1754H, BN E6632.7D-03/01.

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Methods: Groups of dogs (6/sex/group) were given 0 (1% methylcellulose), 120, 600 and 2000 mg/kg/day of BSZ orally via gavage for 52 weeks. The volume of administration was 5 ml/kg. Two dogs/sex/group were used for 26-week interim sacrifice. All dogs were observed daily for clinical signs and mortality. Body weights were recorded weekly and food and water consumptions were recorded daily. ECG tracing was obtained at pre-test and during weeks 26 and 52 of the study period. Blood samples were collected from jugular vein at pre-test and during weeks 1, 6, 13, 26, 38 and 51 of the study for hematological and serum chemistry tests. Twenty-four hr. urine samples were also collected at pre-test and during weeks 1, 6, 13, 24, 38 and 50 for urinalysis, during which time animals were fasted. Part of the blood and urine samples were saved for measuring drug and its metabolites levels (results will be reported separately in HE report # 1067/2-1010). At the end of treatment period all animals were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: Yellow/orange vomit were seen in treated dogs.

2. Mortality: None.

3. Body Weight/Food Consumption/Water Consumption: The initial and final body weights of control males were 7.36 ± 0.75 kg and 10.76 ± 0.79 kg and the corresponding weights of control females were 6.18 ± 0.60 kg and 9.05 ± 0.47 kg respectively. Food consumptions in control males and females were 2549 ± 118 g/animal/week and 2203 ± 332 g/animal/week respectively. No treatment related effects were seen.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry and Urinalysis: No treatment related effects were seen in serum chemistry tests. Urinary sodium levels were increased by 43%, 109% and 326% in low, mid and high dose treated males and by 70% and 85% in mid and high dose treated females when compared to control values. Sponsor attributed this to the high sodium content of the drug.

6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment related effects were seen.

7. Organ Weights: No treatment related effects were seen.

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: No treatment related effects were seen.

In this study, the highest tested dose (2000 mg/kg/day) was the no effect dose and no target organ of toxicity was identified.

Addendum: The toxicokinetic data were not previously submitted. Plasma levels of the drug and its metabolites were not determined. The urinary excretion of BSZ was very low (0.062-0.152%) as compared to total 5-ASA ranging from ~4.18% to 11.97% of the dose. The urinary excretion of 4-ABA was ~1.6-6.7% of the dose.

**SPECIAL TOXICITY:**

A special toxicity study was previously submitted to ____ and reviewed on July 12, 1996. The reviews are reproduced below.

**Chemoprevention of Intestinal Tumor Formation in B6-Min/+ Mice by Balsalazide**

Methods: B6-Min/+ mouse is heterozygous for APC (Min), a mutant allele of the mouse homologue of the human APC gene. According to sponsor Min/+ mouse is genetically analogous to human syndrome Familial Adenomatous polyposis (FAP) which "expresses a similar defect in the APC gene". This mouse is used to test the efficacy of BSZ as a chemopreventive agent against the development of intestinal tumor formation. Groups of female B6-Min/+ mice (55 days old, n=4/group) were given 62.5, 125 and 250 mg/kg/day of BSZ via drinking water for 90 days. At the end of treatment period mice were killed, entire small intestine from pyloric sphincter to cecum were isolated and tumors were counted under dissecting microscope.

Results: BSZ inhibited formation of genetically predisposed intestinal tumors in dose dependent manner.

Table I.
Effect of Balsalazide on Mean Total Intestinal Tumor Number in the Min Mouse

| | Control | 62mg/kg | 125mg/kg | 250mg/kg |
|---|---|---|---|---|
| Mean Tumors | 22.44 | 12.56 | 9.33 | 6.33 |
| Stdev | 8.82 | 6.97 | 4.69 | 2.45 |
| 95% CI | 16.6-28.2 | 8.0-17.11 | 6.3-12.4 | 4.7-7.9 |
| Wilcoxon Rank Sum | | p=0.041 | p=0.0046 | p=0.0018 |

CARCINOGENICITY:

The carcinogenicity studies were previously submitted to ——— and reviewed on June 10, 1994 and July 12, 1996. The reviews are reproduced below.

**BX661A: 18-Month Dietary Carcinogenicity Study in Mice**
**(Report # ———)**

**This study was conducted by Biorex Pharmaceuticals Ltd. and the original report was not submitted to us. ——— re-assembled the report and submitted it for our review. Furthermore, ——— does not accept the responsibility for the "recording or integrity" of the raw data.**

Testing Laboratories: Biorex Pharmaceuticals Ltd.

Study Started: May 9, 1983

Study Completed: Not given.

Re-Assembled Report Date: November 5, 1993

GLP Requirements: No Statement of Compliance with GLP regulations was included. Study was not inspected by QAU.

Test Species & Strain: Biorex Albino mice (age not given; males: 24-33 g and females: 20-27 g).

No. of Animals: 50/sex/group (control group had 70 mice of each sex).

Route of Administration: Via diet.

Dose Levels: 0, 120, 500 and 1000 mg/kg/day.

Drug Batch No.: 26/0/0

Methods: Groups of mice (50/sex/treatment group and 70/sex in control group) were given BSZ (via diet) at daily doses of 120, 500 and 1000 mg/kg/day for 84 weeks. All animals were observed for clinical signs and mortality daily. Body weights, food consumptions and water intakes were recorded weekly. Just before sacrifice, blood samples were collected from abdominal aorta for hematological examinations (differential leucocyte count). All surviving animals were killed at the end of the study period and subjected to complete necropsy and histopathological examinations. Cochran-Armitage and Fisher-Irwin test were used to analyze intercurrent mortality and survival rate. No statistical analysis was done for tumor data.