Results:

1. Achieved Doses: The compound consumption was highly variable (male: low dose = 80.2-152.6 mg/kg/day, mid dose = 333.3-618.3 mg/kg/day and high dose = 684.6-1299.5 mg/kg/day; female: low dose = 81.1-140.0 mg/kg/day, mid dose = 335.1-736.0 mg/kg/day and high dose = 714.5-1098.9 mg/kg/day). During various weeks compound intakes were 60-147% of the intended doses.

Percent Range of Intended Dose

	Low Dose	Mid Dose	High Dose
Males	88-121	89-124	93-130
Females	60-117	68-147	84-111

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

3. Mortality:

Survival Rates (%)

Week	Sex	Control	Low Dose	Mid Dose	High Dose
52	M	93	94	92	96
	F	97	96	96	98
78	M	73	84	76	72
	F	83	80	74	82
83	M	56	62	52	52
	F	66	56	54	66
84	M	14	18	10	10
	F	39	28	24	32

However, in Table 9.1 (page 1677, Vol. 1, of Amendment # 021) sponsor indicated that at the end of study period, the survival rates were 69%, 76%, 68% and 66% in males and 79%, 70%, 68% and 78% in females of control, low dose, mid dose and high dose respectively. Table 3 (pages 1618 and 1622, of Amendment # 021) provides different survival rates than that mentioned above. Hence, there is a lot of discrepancy. Irrespective of which table one uses for analysis, the survival rates were comparable in all groups.

4. Body Weight/Food Consumption/Water Consumption: Body weight gains were not affected by the treatment (body weights of females during week 1-13 were not reported). Throughout the study period food consumptions were comparable in all groups. However, it should be noted that animals were given food approximately 95% of the average daily consumption in the form of "moist ball of the diet" (control diet was moistened with the same amount of water as the treated group). During the first two weeks of treatment both males and females were given food 6 g/mouse/day and then 7.5 g/mouse/day for the remainder of the study period. However, looking at the individual weekly data it is quite evident that male animals consumed more food during weeks 53-82 than that provided to them, which indicated some kind of error in weighing or calculation. Water intakes were not affected by the treatment.

5. Hematology: No treatment related effects were seen.

6. Gross Pathology: No tabulated summary was provided. According to sponsor, no treatment related effects were seen.

7. Histopathology: No treatment related histopathological findings were evident.

This study was conducted by Biorex Pharmaceuticals Ltd. during 1983. Original report was not submitted to us. Ten year later another testing laboratory namely ——— e re-assembled the report for our review. This study was not conducted according to GLP regulations and was not inspected by QUA. Furthermore, ——— does not accept the responsibility for the "recording or integrity" of the raw data. With respect to the report the following deficiencies were noted: (1) test drug was not analyzed for purity during study period, (2) stability of drug was not determined, (3) dose-range study was not conducted to select maximum tolerated dose, (4) sponsor arbitrarily selected 1000 mg/kg/day at the high dose since it represents a multiple of "30 times" the proposed human therapeutic dose. Additionally, sponsor also stated that dose levels were selected "on the basis of the results of a previous study conducted in rats", (5) it is not proper to select dose levels for carcinogenicity study in mouse on the basis of drug toxicity profile in rats and (6) sponsor has not provided proof of absorption of drug &/or its metabolites. In view of the above deficiencies, it is concluded that the study is invalid. Sponsor should be asked to conduct a dose-ranging study in mice to define a MTD and to repeat carcinogenicity study in mouse of same strain.

BX661A: 104 Week Dietary Carcinogenicity Study in Rats
(Report # BIORECON No. 1)

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: March 25, 1985

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 QUA.

Test Species & Strain: Biorex Wistar rats (age not given; males: 172-281 g and females: 168-237 g).

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

Route of Administration: Via diet.

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

Drug Batch No.: 261010

Methods: Sponsor arbitrarily selected 1000 mg/kg/day as the high dose level in carcinogenicity study since it represents a multiple of "30 times" the proposed human therapeutic dose. Groups of rats (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 and serum chemistry tests. Overnight urine samples were also collected at the end of treatment period for urinalysis, during which time animals were fasted. Fecal samples were collected at different time points throughout the study to measure drug and its metabolites (report will be presented later). 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.

Results:

1. Achieved Doses: The compound consumptions were within 10% of the intended doses (male: low dose = 91.7-122.2 mg/kg/day, mid dose = 361.8-509.8 mg/kg/day and high dose = 733.5-1012.9 mg/kg/day; female: low dose = 103.9-124.8 mg/kg/day, mid dose = 426.5-512.6 mg/kg/day and high dose = 855.3-1016.8 mg/kg/day).

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

3. Mortality: The survival rates were comparable in all groups.

Survival Rates (%)					
Week	Sex (M/F)	Control	Low Dose	Mid Dose	High Dose
52	M	90	90	90	82
	F	97	94	96	96
78	M	74	70	62	66
	F	80	80	80	86
104	M	36	34	30	26
	F	43	32	46	48

4. Body Weight/Food Consumption/Water Consumption: Body weight gains were not affected by the treatment. Throughout the study period food consumptions were comparable in all groups. It should be noted here that rats were on restricted feeding regimen. In the form of "moist ball of the diet", male rats were given only 24 g feed with or without drug/rat/day while female received 20 g feed with or without drug/rat/day during the study period. According to sponsor this amount reflects about 95% of the average daily consumption. Compared to control, water consumptions were significantly increased in all treated rats.

5. Hematology: No treatment related effects were seen.

6. Blood Chemistry/Urinalysis: No treatment related effects were seen in serum chemistry tests. Urinary volumes were increased by 59% and 74% in high dose treated males and females respectively, when compared to control values.

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.

This study was conducted by Biorex Pharmaceuticals Ltd. during 1983. Original report was not submitted to us. Ten years later another testing laboratory namely ——— re-assembled the report for our review. This study was not conducted according to GLP regulations and was not inspected by QUA. Furthermore, ——— does not accept the responsibility for the "recording or integrity" of the raw data. With respect to the report following deficiencies were noted: (1) test drug was not analyzed for purity during study period, (2) stability of drug was not determined, (3) 3-month dose-range study was not conducted to select maximum tolerated dose in Biorex Wistar rats (in 14-day palatability oral (diet) study in SD-rats (# 7402-1067/5) sponsor indicated that 2000 mg/kg/day should be the highest dose level for the carcinogenicity study). Furthermore, in 2 week oral toxicity study in Wistar rats, the no effect dose was 1000 mg/kg/day, (4) sponsor arbitrarily selected 1000 mg/kg/day as the high dose level in carcinogenicity study since it represents a multiple of "30 times" the proposed human therapeutic dose and (5) sponsor has not provided proof of absorption of drug &/or its metabolites. In view of the above deficiencies, it is concluded that the study is invalid. Sponsor should be asked to conduct a dose-ranging study in rat to define a MTD and to repeat carcinogenicity study in rat of the same strain.

Addendum: In the method section, the duration of treatment was incorrectly stated as 84 weeks. This study was for 104 weeks.

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT CARCINOGENICITY FACT SHEET

NDA: ——— DRUG CODE #:
CAS #: DATE:
DIVISION(s): HFD-180
DRUG NAME(s): Colazide

SPONSOR: Salix Pharmaceutical, Inc.

TESTING LABORATORY: ———

P/T REVIEWER(s): Tanveer Ahmad, Ph.D.
P/T REVIEW DATE: 6/27/96
CARCINOGENICITY STUDY REPORT DATE: 6/29/95
THERAPEUTIC CATEGORY: Anti-ulcerative Colitis

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION:

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): BSZ was positive in CHL/HGPRT forward gene mutation assay.

RAT CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc):

RAT STUDY DURATION (weeks): 104
STUDY STARTING DATE: 9/16/92
STUDY ENDING DATE: 6/29/95
RAT STRAIN: SD
ROUTE: Via diet
DOSING COMMENTS: Highest tested dose is the maximum tolerated dose (MTD)

No. Rats in Control1 (C1): 60 Control2 (C2): 60
Low Dose (LD): 60 Middle Dose (MD): 60
High Dose (HD): 60 High Dose2 HD2):

RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 120 Rat Middle Dose: 600
Rat High Dose: 2000 Rat High Dose2:
*Dose adjustment during study: yes

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): MTD

RAT CARCINOGENICITY (negative; positive; MF; M; F): Negative

RAT TUMOR FINDINGS: None

RAT STUDY COMMENTS: In 104-week dietary carcinogenicity study in rats, doses of 120, 600 and 2000 mg/kg/day were used. In this study, highest tested dose is the maximum tolerated dose since at this dose level body weight in males and females were 17% and 19% lower than the control body weights respectively. Hence, dose selection was appropriate. Treatment had no significant effect of intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups. Increased incidences of renal pelvic mineralization, tubular dilatation and urothelial hyperplasia were seen in mid and high dose treated males. In high dose treated males there was also an increased incidence of hydronephrosis, urinary bladder distention, cystitis and prostatitis. Significant increase in the incidence of benign pheochromocytoma in adrenal was seen in high dose treated male rats (control 1 = 13.3%, control 2 = 6.7%, low dose = 13.3%, mid dose = 15% and high dose = 30%, $p < 0.001$ [trend test], pairwise: control 1 vs high dose; $p = 0.003$ and control 2 vs high dose; $p = 0.001$). However, rate of incidence of benign pheochromocytoma in adrenal of high dose treated males (30%) was "within laboratory historical control incidence range (10-46%)". No other treatment related neoplastic findings were evident in this study. Hence, BSZ has no carcinogenic potential in SD rats.

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. Study No.: 1067/6-1050

2. Name of Laboratory: [redacted]

3. Strain: Sprague-Dawley (SD) rats

4. No./sex/group: 60

5. Doses (O, L, M, H): 0, 0, 120, 600 and 2000 mg/kg/day

6. Basis for dose selection stated: Yes

7. Interim sacrifice: No

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
0	48/Malignant Sarcoma (Skin Subcutis)	54/Benign Adenoma (Pituitary)
0	49/Malignant Sarcoma (Skin Subcutis)	39/Malignant Osteosarcoma (Bone)
L	50/Malignant Carcinoma (Prostate)	54/Benign Adenoma (Pituitary)
M	43/Malignant Sarcoma (Skin Subcutis)	42/Malignant Carcinoma (Mammary Gland)
H	54/Benign C-Cell Adenoma (Thyroid)	24/Malignant Nephroblastoma (Kidney)

10. No. alive at termination:

		Male	% Survival	Female	% Survival
(Control 1)	O	25/60	41.7	13/60	21.7
(Control 2)	0	19/60	31.7	17/60	28.3
	L	18/60	30.0	14/60	23.3
	M	13/60	21.7	15/60	25.0
	H	28/60	46.7	27/60	45.0

11. Statistical Methods Used: Two Chi-squared test, pairwise comparisons and "IARC Annex" for the analysis of tumor incidence.

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix 1.

BSZ: 104 Week Dietary Carcinogenicity Study in Rats (Report # 1067/6-1050)

Testing Laboratories: ———

Study Started: September 16, 1992

Study Completed: June 29, 1995

GLP Requirements: A statement of compliance with GLP regulations was included.

Test Species & Strain: Crl:CD(SD)BR rats (42 days old; males: 157-253 g and females: 134-203 g).

No. of Animals: 60/sex/group.

Route of Administration: Via diet.

Dose Levels: 0, 0, 120, 600 and 2000 mg/kg/day.

Drug Batch No.: BN1754H

Methods: In this study dose selection was based on 14-day palatability oral (diet) study (#7402-1067/5) in SD rats in which the highest tested dose (2000 mg/kg/day) was the no effect dose. Sponsor selected 2000 mg/kg/day as the high dose level in carcinogenicity study since it is the "maximum feasible dose which can be given via diet". Groups of rats (60/sex/group) were given BSZ (via diet) at daily doses of 0, 0, 120, 600 and 2000 mg/kg/day for 104 weeks. All animals were observed for clinical signs and mortality daily. Body weights and food consumptions were recorded weekly during the first 16 weeks of treatment and thereafter at 4-week intervals. Just before sacrifice, blood samples were collected from vena cava for hematological tests. Twenty-four hour urine samples were also collected at weeks 2, 23, 50, 75 and 100 of the study measuring BSZ and/or its metabolites (results will be reported by ——— separately under report # 1067/6-1010). All surviving animals were killed at the end of the study period and subjected to complete necropsy. Histopathological examinations were performed on all tissues from control and high dose groups, animals died/killed during study period, all gross lesions, and adrenal and kidneys from all animals. Kaplan-Meier technique and log-rank tests were used to analyze intercurrent mortality and survival rate. Sponsor used two sided chi-squared test, pairwise comparisons and "IARC annex" for the analysis of tumor incidence.

Results:

1. Achieved Doses: The compound consumptions were within 2% of the intended doses (male: low dose = 120.3 +/- 3.6 mg/kg/day, mid dose = 601.6 +/- 18.0 mg/kg/day and high dose = 2001.8 +/- 70.1 mg/kg/day; female: low dose = 120.5 +/- 5.2 mg/kg/day, mid dose = 603.0 +/- 27.7 mg/kg/day and high dose = 2008.4 +/- 84.0 mg/kg/day).

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

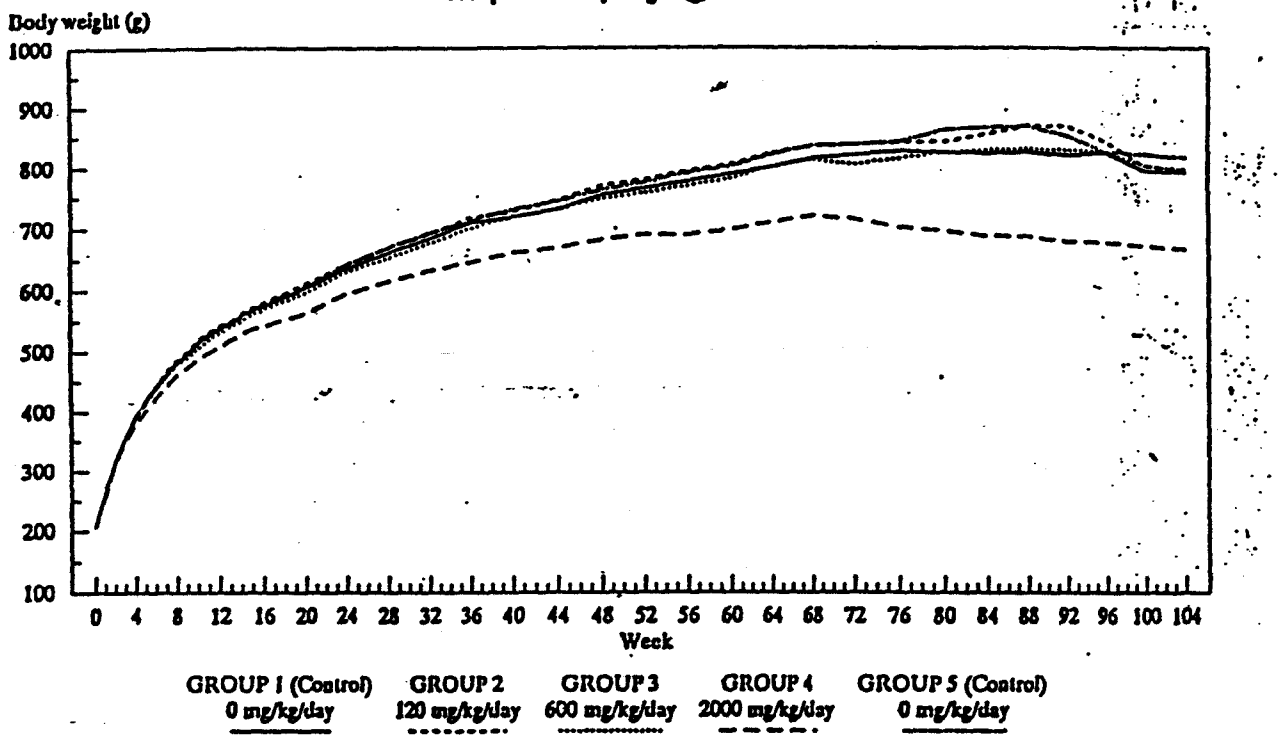
3. Mortality: Treatment had no significant effect on intercurrent mortality rates (see below). At termination survival rates were comparable in all groups (30-47% in males and 22-45% in females).

INTERCURRENT MORTALITY RATES

Male Rats										
Weeks	Control 1	%	Control 2	%	Low Dose	%	Mid Dose	%	High Dose	%
0 - 58	1/60	1.7	7/60	11.7	5/60	8.3	8/60	13.3	4/60	6.7
59 - 78	13/59	22.0	8/53	15.1	11/55	20.0	12/52	23.1	7/56	12.5
79 - 104	21/46	45.6	26/45	57.8	26/44	59.1	27/40	67.5	21/49	42.8
Terminal kill	25	----	19	----	18	----	13	----	28	----
Survival Rate	---	41.7	---	31.7	---	30.0	---	21.7	---	46.7
Female Rats										
0 - 58	1/60	1.7	3/60	1.1	2/60	3.3	2/60	3.3	3/60	5.0
59 - 78	12/59	20.3	2/57	3.5	12/58	20.7	8/58	13.8	10/57	17.5
79 - 104	34/47	72.3	28/45	17.8	32/46	69.6	35/50	70.0	20/47	42.5
Terminal kill	13	----	17	----	14	----	15	----	27	----
Survival Rate	---	21.7	---	28.3	---	23.3	---	25.0	---	45.0

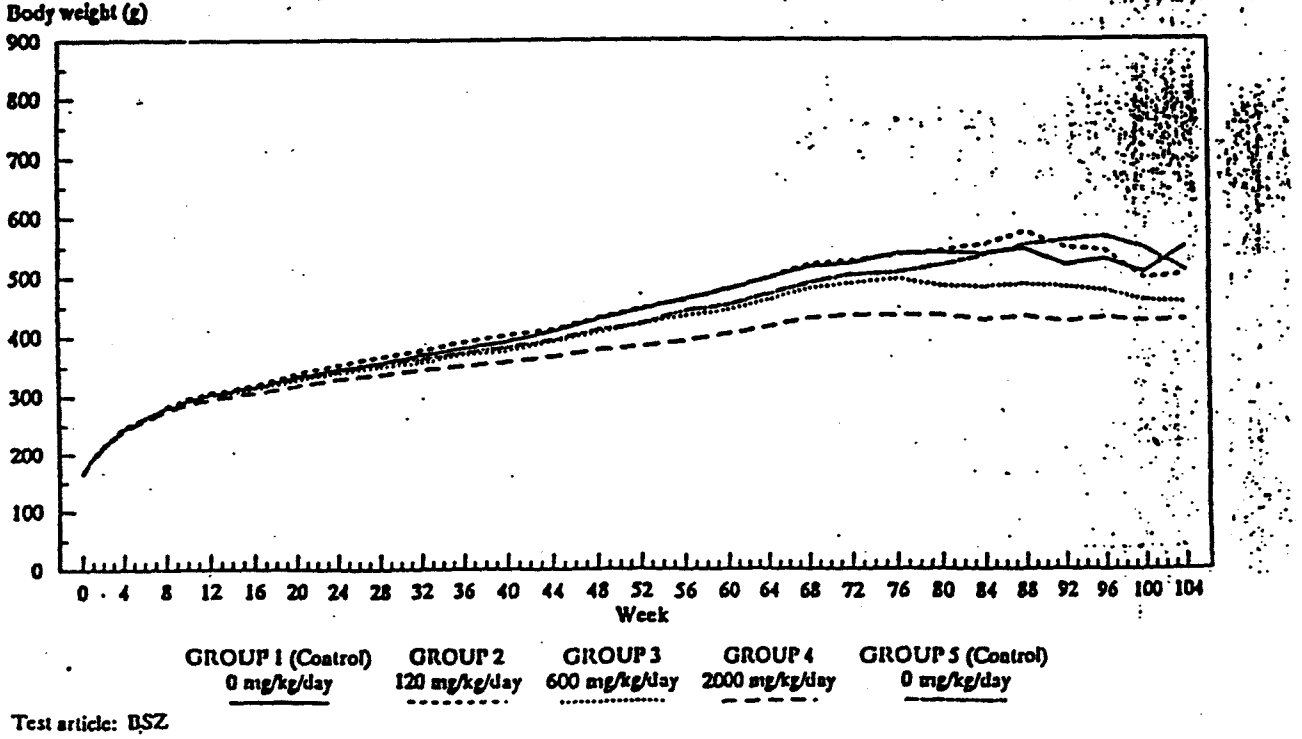
4. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, absolute body weights in low, mid and high dose treated females were about 5%, 14% and 19% lower than the combined control body weights. At the end of treatment period, the absolute body weights in high dose treated males were about 17% lower than the combined control values. Treatment had no significant effect on food consumptions.

FIGURE 3
Group mean body weight (g) - males



Test article: BSZ

FIGURE 4
Group mean body weight (g) - females



Test article: BSZ

MEAN BODY WEIGHT (g) OF MALE RATS					
Weeks	Control 1	Control 2	Low Dose	Mid Dose	High Dose
1 (start)	207.4	209.4	207.3	206.4	209.7
13	546.1	543.9	549.2	539.5	518.0
52	765.2	773.9	779.0	757.4	689.9
104	789.6	814.3	794.8	794.6	665.3
MEAN BODY WEIGHT (g) OF FEMALE RATS					
1 (start)	168.8	165.7	165.3	167.1	168.5
13	305.5	304.8	309.9	303.1	297.9
52	445.8	421.5	447.8	420.7	384.8
104	549.1	509.9	502.7	456.6	427.3

FOOD CONSUMPTION (g/animal/week) IN MALE RATS					
WEEKS	Control 1	Control 2	Low Dose	Mid Dose	High Dose
1	192	190	182	193	185
13	187	183	189	189	189
52	181	184	179	184	184
104	196	197	195	208	193
FOOD CONSUMPTION (g/kg/day) IN FEMALE RATS					
1	131	130	134	137	131
13	131	131	134	130	126
52	144	136	142	140	134
104	194	145	158	145	158

5. Hematology: No treatment related effects were seen.

6. Gross Pathology: Distention of the cecum in high dose treated males (control 1 = 2/60, control 2 = 1/60, low dose = 1/60, mid dose = 3/60 and high dose = 38/60) and females (control 1 = 1/60, control 2 = 1/60, low dose = 0/60, mid dose = 1/60 and high dose = 24/60) were seen. Additionally, in high dose treated males, significant increase in renal pelvic dilatation (control 1 = 4/60, control 2 = 2/60, low dose = 3/60, mid dose = 2/60 and

high dose = 11/60) and distention of urinary bladder (control 1 = 5/60, control 2 = 5/60, low dose = 2/60, mid dose = 4/60 and high dose = 13/60) were also seen.

9. Histopathology:

Non-neoplastic Findings: Increased incidences of renal pelvic mineralization, tubular dilatation and urothelial hyperplasia were seen in mid and high dose treated males. In high dose treated males there was also an increased incidence of hydronephrosis, urinary bladder distention, cystitis and prostatitis.

Non-Neoplastic Findings						
Site/Type	Sex (M/F)	Control 1	Control 2	Low Dose	Mid Dose	High Dose
Kidney:						
Pelvic Mineralization	M	7/60	7/60	10/60	22/60	46/60
	F	46/60	45/60	54/60	57/60	51/60
Tubular Dilatation	M	2/60	3/60	2/60	9/60	11/60
	F	0/60	1/60	2/60	6/60	6/60
Hydronephrosis	M	5/60	2/60	4/60	5/60	9/60
	F	0/60	0/60	0/60	1/60	2/60
Urothelial Hyperplasia	M	9/60	7/60	11/60	22/60	47/60
	F	25/60	25/60	30/60	42/60	50/60
Bladder:						
Distention	M	6/59	5/59	2/42	5/47	12/60
	F	3/59	0/60	1/47	2/45	1/60
Cystitis	M	2/59	1/59	3/42	1/47	7/60
	F	0/59	0/60	0/47	1/45	0/60
Prostate:						
Prostatitis	M	26/60	26/59	19/42	23/49	42/60
	F	---	---	---	---	---

Neoplastic Findings: Significant increase in the incidence of benign pheochromocytoma in adrenal was seen in high dose treated male rats (control 1 = 13.3%, control 2 = 6.7%, low dose = 13.3%, mid dose = 15% and high dose = 30%, $p < 0.001$ [trend test], pairwise: control 1 vs high dose; $p = 0.003$ and control 2 vs high dose; $p = 0.001$). However, rate of incidence of benign pheochromocytoma in adrenal of high dose treated males (30%) was "within laboratory historical control incidence range (10-46%)". Hence, BSZ has no carcinogenic potential in SD rats.

In this study, highest tested dose is the maximum tolerated dose since at this dose level body weight in males and females were 17% and 19% lower than the control body weights respectively. Hence, dose selection was appropriate. Treatment had no significant effect of intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups. Increased incidences of renal pelvic mineralization, tubular dilatation and urothelial hyperplasia were seen in mid and high dose treated males. In high dose treated males there was also an increased incidence of hydronephrosis, urinary bladder distention, cystitis and prostatitis. Significant increase in the incidence of benign pheochromocytoma in adrenal was seen in high dose treated male rats (control 1 = 13.3%, control 2 = 6.7%, low dose = 13.3%, mid dose = 15% and high dose = 30%, $p < 0.001$ [trend test], pairwise: control 1 vs high dose; $p = 0.003$ and control 2 vs high dose; $p = 0.001$). However, rate of incidence of benign pheochromocytoma in adrenal of high dose treated males (30%) was "within laboratory historical control incidence range (10-46%)". No other treatment related neoplastic findings were evident in this study. Hence, BSZ has no carcinogenic potential in SD rats.

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 (0.063-1.15% of the dose) as compared to total 5-ASA (~0.96% to 30.1%) and NASA (4.6-14.14%). The urinary excretion of 4-ABA and NABA was ~0.314-7.66% and 0.58-4.09% of the dose, respectively.

REPRODUCTIVE TOXICITY:

The reproductive toxicity studies were previously submitted to ——— and reviewed on June 10, 1994. The review is reproduced below.

REPRODUCTIVE TOXICITY:

Oral Segment I. Fertility and General Reproductive Performance Study in Rats (Report # 93/GY007/0862)

Testing Laboratories: ———

Study Started: October 5, 1992

Study Completed: December 20, 1993

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Animals: Male (6-7 weeks old, 182-225 g) and female (10-11 weeks old, 203-248 g) CD Sprague Dawley rats.

Drug Batch No.: 261R29

Methods: In this study, the dose selection was based on preliminary oral Segment I and III study in rats (study # 92/GYS003/1081) in which doses of 500, 1000 and 2000 mg/kg/day were used. The male rats were treated from 15 days prior to mating and throughout the mating, gestation, lactation until they were sacrificed (approximately 4 days after postpartum). Increased incidence of salivation was seen in mid and high dose treated rats. At the highest tested dose, no effects were seen on fertility and general reproductive performance of rat. Sponsor selected 2000 mg/kg/day as the highest dose for the main study. In the main study, groups of 36 male and 36 female rats were given orally (gavage) 0 (water), 120, 600 and 2000 mg/kg/day of BSZ. The volume of administration was fixed at 12 ml/kg. The male rats were treated from 71 days prior to mating and throughout the mating phase and until they were sacrificed. Females were treated for 15 days prior to mating and throughout mating, gestation, lactation until they were sacrificed (approximately 24 days after postpartum). Parents were observed daily for mortality and toxic signs. Body weights and food/water consumptions were recorded weekly. Additicnally, dams were weighed on days 0, 3, 6, 10, 13, 17 and 20 of gestation. The mating performance and fertility of both sexes were evaluated. About 23 pregnant rats were sacrificed on day 20 of gestation, and was examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. Fetuses

were eviscerated and alternate fetuses from each litter were examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining dams (13/group) were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. The offspring were reared by the dams until weaning. Following delivery, the dams were checked daily for clinical signs, body weight (days 1, 4, 7, 11, 14, 18, 21 and 25 of lactation), food and water consumptions (weekly) were recorded. On day 25 of post-partum all dams were sacrificed and necropsied, and examined as mentioned above. Postnatal body weight changes of the pups were recorded until the age of 25 days. On lactation day 4, litters with more than 8 pups were culled to 4 males and 4 females. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pinna detachment, tooth-eruption, eyelid separation, visual and auditory function tests, testes descent, vaginal opening, learning ability test and open field test). At day 35 of post partum, 26 pairs of the animals per group were selected for F1/F2 generation study. At 10 weeks of age they were continuously mated and study was repeated as mentioned above except animals were not treated. F_2 generation were examined for abnormalities and then killed on day 25 of post partum.

Results: Increased incidence of salivation was seen in high dose treated rats. One male from control group was found dead after vehicle administration during study period. By the end of treatment period, the body weight gains were reduced by 4-9% in treated males when compared to the control values while in females body weight gains were decreased by 8% in high dose treated females during gestation period. No treatment related effects no food consumption was seen. Throughout the study period significant increases in water intakes were seen in high dose treated rats. The estrous cycle of the female rats revealed no differences between the control and treated groups and 92-97% rats mated within the first four days of pairing. Mating performance (100%), conception rate (94-100%) and fertility index (94-100%) were comparable in all groups.

Dams Sacrificed at Day 20:

No treatment related gross lesions were seen in female rats of F_0 generation. There were no significant changes in pregnancy parameters (# of corpora lutea, # of implants, pre- and post implantation loss, litter size, sex ratios, and mean fetal weights). No treatment related major malformation was seen in fetuses except increased incidences of bilateral hydronephrosis were seen in treated groups (control = 0.0%, low dose = 0.5%, mid dose = 1.2% and high dose = 2.2% [in 3 litters], historical control: mean = 0.3% and range = 0-1.7%). Other anomalies found in the study considered not to be treatment related because they were not dose related, incidences were very low and most of them falls within the historical range.

Dams allowed to deliver: No significant differences in the gestation period between the groups were noted. The implantation sites and litter sizes were lower in the high dose treated groups compared to the controls but it was not statistically significant. From day 1 onwards there were no significant effects on viability indices, postnatal development and differentiation were comparable in all groups. There was no significant effect on fertility test and mating performance test of F_1-generation rats. No treatment related gross lesions were seen in rats of F_1 generation. Physical development were comparable in all groups, and no drug related gross lesion were seen in the F_2 pups at necropsy.

Segment I Fertility and General Reproductive Performance Study in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# Mated	36	36	36	36
% Pregnant	100	97	94	100
Dams Sacrificed at Day 20				
# of Dams Examined	23	22	21	23
# of Corpora Lutea/dam	17.5 ± 2.2	18.5 ± 3.0	17.9 ± 1.5	17.3 ± 1.7
# of Implants/dam	16.7 ± 1.7	17.0 ± 3.2	16.8 ± 3.2	16.9 ± 2.6
Pre-implantation Loss/dam (%)	5.2	8.6	6.6	3.5
Post-implantation Loss/dam (%)	6.8	3.8	6.2	6.9
# of Early Resorptions	1.09 ± 1.04	0.64 ± 0.80	1.05 ± 1.02	1.17 ± 1.08
# of Late Resorptions	0.04 ± 0.21	0.0	0.0	0.0
Placental Wt. (g)	0.52 ± 0.02	0.51 ± 0.01	0.51 ± 0.04	0.49 ± 0.01
# of Fetuses/dam	15.5 ± 2.0	16.3 ± 3.0	15.8 ± 3.5	15.7 ± 2.9
Fetal Wt. (3)				
Male	3.81 ± 0.08	3.89 ± 0.09	3.79 ± 0.07	3.85 ± 0.07
Female	3.62 ± 0.08	3.67 ± 0.13	3.57 ± 0.08	3.63 ± 0.09
Sex Ratio (M/F)	0.87	1.01	1.08	0.98
Dams Allowed to Deliver				
# of Dam Examined	13	13	13	13
Gestation Index (%)	100	100	100	100
Viability Index (day 4) (%)	98	97	99	98
Lactation Index (day 25) (%)	99	100	100	99

Gestation Index = No. of live litters born X 100/no. of pregnant.
Viability Index (day 4) = No. of live pups on day 4 X 100/no. of live pups on day 1.
Lactation Index (day 25) = No. of live pups on day 25 X 100/no. of live pups on day 4 post culling.

In conclusion, there were no abnormal effects on the fertility and mating performance of the treated male and female rats at doses up to and including 2000 mg/kg/day of BSZ.

Oral Segment II. Teratology Study in Rats
(Report # 93/GYS006/054)

Testing Laboratories: [redacted]

Study Started: September 8, 1992

Study Completed: September 28, 1993

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: CD Sprague-Dawley pregnant rats

No. of Animals: 32 pregnant rats/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 120, 600 and 2000 mg/kg/day

Drug Batch No.: 261R29

Methods: The selection of the doses were based on the preliminary Segment II teratology study (92/GYS004/1079) in rats in which oral doses of 500, 1000 and 2000 mg/kg/day were used. Pregnant rats were treated from day 6-15 of gestation. Increased incidence of salivation was seen in high dose treated rats. During gestation period body weights were decreased by 12-16% in treated rats. No treatment related effects were seen at necropsy (day 20 of gestation). Based on these results sponsor selected 2000 mg/kg/day as the highest dose for the main study. In the main study, pregnant rats were given oral doses of 120, 600 and 2000 mg/kg from day 6 to 17 day of gestation. Control animals received the vehicle (water) throughout the same period. The volume of administration was fixed at 12 ml/kg. Pregnant dams were observed daily for mortality and clinical signs. Body weight (days 0, 3, 6, 18 and 20 of gestation and 1, 4, 7, 11, 14, 18, 21 and 25 of lactation) and food consumption (0-2, 3-5, 6-8, 9-11, 12-15, 16-17 and 18-19 of gestation) were recorded. Two/third pregnant rats were sacrificed on day 20 of gestation, and were examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. Approximately one-half of the fetuses eviscerated and examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining 1/3 of the dams were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. On lactation day 4, litters with more

than 8 pups were culled to 4 males and 4 females. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pinna detachment, tooth-eruption, eyelid separation, visual and auditory function tests, testes descent, vaginal opening, learning ability test, neuromuscular function test and open field test). On day 25 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. At day 35 of post partum, 20 pairs of the animals per group were selected for F1/F2 generation study. At 10 weeks of age they were continuously mated. Cesarean section was performed on the F_1 dams on day 20 of gestation, uterine contents and fetuses were examined and preserved in acetone and Bouin's fluid.

Results:

Dams Sacrificed at Day 20:

No significant effect on body weight or food consumptions were seen in treated rats. The number of corpora lutea, the number of implants, pre and post implantation loss, weights of fetuses and sex ratio did not show any significant difference between the treated groups and the control group. External examinations were normal except 3 fetuses from one litter of high dose group had rounded heads and shortened snout. and no such finding was reported in the historical control data. Visceral examination of F_1 fetuses revealed no treatment related effects except in high dose group one fetus had unilateral slight microphthalmia and another fetus innominate artery very reduced. These findings were not reported in the historical control data. Sponsor considered this finding not to be treatment related because it happened only in one litter. No skeletal abnormalities were observed.

Segment II Teratology Study in Rats				
Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# of Dam Examined	20	21	21	20
# of Corpora Lutea/dam	17.9 ± 2.0	17.8 ± 2.5	17.6 ± 2.1	17.7 ± 1.7
# of Implants/dam	16.5 ± 2.0	16.5 ± 2.9	16.0 ± 2.2	16.6 ± 1.5
# of Pre-implantation Loss (%)	7.8	7.5	9.2	7.8
# of Post-implantation Loss (%)	4.8	5.2	6.9	2.1
# of Early Resorptions	0.80 ± 0.89	0.81 ± 0.90	1.10 ± 1.05	0.35 ± 0.59
# of Late Resorptions	0.0	0.05 ± 0.22	0.0	0.0
# of Live Fetuses/dam	15.7 ± 2.1	15.7 ± 3.0	14.9 ± 2.4	16.3 ± 1.6
Fetal Wt. (g)				
Male	3.74 ± 0.07	3.73 ± 0.10	3.73 ± 0.08	3.77 ± 0.08
Female	3.56 ± 0.07	3.60 ± 0.07	3.54 ± 0.09	3.59 ± 0.07
Sex Ratio (M/F)	1.04	0.95	1.05	0.92
Morphological Findings of Fetuses				
External Examination				
# of Fetuses (litters) Examined	314 (20)	329 (21)	312 (21)	325 (20)
Rounded Head & Shortened Snout	0 (0)	0 (0)	0 (0)	3 (1)
Visceral Anomalies				
# of Fetuses	155 (20)	159 (21)	149 (21)	160 (20)
Unilateral Slight Microphthalmia	0 (0)	0 (0)	0 (0)	1 (1)
Innominate Artery Very Reduced	0 (0)	0 (0)	0 (0)	1 (1)
Skeletal Anomalies				
# of Fetuses (litter) Examined	159 (20)	170 (21)	163 (21)	165 (20)
Major Anomalies	0 (0)	0 (0)	0 (0)	0 (0)
Minor Anomalies	0 (0)	0 (0)	0 (0)	0 (0)

Dams Allowed to Deliver: No significant differences in the gestation period between the groups were noted. The litter size, survival rates were comparable in all groups. Body weight gains of the pups were decreased by 7% in high dose group. There were no significant effects on postnatal development and differentiation. At necropsy of F_1-generation at termination revealed no abnormalities. There was no significant effect on fertility test and mating performance test of F_1-generation rats. External examination of F_2 fetuses revealed no abnormality.

Thus no teratogenic effects at dosage up to 2000 mg/kg/day was observed. The postnatal development and the fertility of the offspring were comparable in all groups.

Oral Segment II. Teratology Study in Rabbits (Report # 93/GYS009/0802)

Testing Laboratories: [redacted]

Study Started: November 17, 1992

Study Completed: December 14, 1993

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: New Zealand White Rabbits (18-26 weeks old, 3.31-5.04 kg)

No. of Animals: 15 pregnant females/group

Route of Administration: Oral (gavage)

Dose Levels: 120, 600, 2000 mg/kg/day (10 ml/kg)

Drug Batch No.: 261R29

Methods: The selection of the doses were based on the preliminary Segment II teratology study (92/GYS002/0010) in rabbits in which oral doses of 500, 1000 and 2000 mg/kg/day were used. Pregnant rabbits were treated from day 6-19 of gestation. Increased incidence of reduced or loose feces were seen in mid and high dose treated rabbits. One high dose treated rabbit was found dead and the cause of death was "most" likely drug related. Additionally, one high dose treated female aborted during the study period. During treatment period body weights were decreased by 82% along with about 42% reduction in food consumptions in high dose treated females. No treatment related effects were seen at necropsy (day 29 of gestation). Based on these results sponsor selected 1200 mg/kg/day as the highest dose for the main study. In the main study, pregnant rabbits were given oral doses of 120, 600 and 1200 mg/kg from day 6 to 19 day of gestation. Control animals received the vehicle (water) throughout the same period. The volume of administration was fixed at 10 ml/kg. Pregnant dams were observed daily for mortality and clinical signs. Pregnant rabbits were observed daily during pregnancy and their weights were recorded daily. Food intake were recorded during days 1-5, 6-12, 13-19, 20-23 and

24-28 of the study. All surviving dams were sacrificed at day 29 of gestation, and were examined for the number of corpora lutea, the number of implants, number of early/late resorptions, number of live/dead fetuses and identification of any malformed fetuses or uterine abnormalities. Live fetuses were weighted, sexed and examined for external abnormalities. All fetuses were eviscerated and two-third fetuses were examined for skeletal malformations and variations, and one-third of the fetuses were examined for visceral abnormalities. Detailed histopathology of the rabbits that died during the study was also performed.

Results: One female from low dose group was killed in extremis and cause of death could not be established with certainty. Three females (1 from control group, 1 from mid dose group and 1 from high dose group) aborted during study period. These abortions were considered not to be treatment related. The overall rate of pregnancies significantly decreased in mid and high dose treated rabbits (control = 100%, low dose = 100%, mid dose = 60% and high dose = 67%). Body weight gain were decreased by 14% and 28% during the gestation period 0-20 at 600 and 1200 mg/kg/day respectively, when compared to the control values. However, during post dosing period the body weight gains were increased in animals treated with mid and high dose levels, such that by termination day 29 their body weights were comparable to that of controls. Food intakes were also decreased by 11% during the dosing period at 1200 mg/kg/day dose levels. In females which survive to term the number of corpora lutea, number of implants, number of live fetuses and rate of dead or resorbed fetuses did not show any significant difference between the treated groups and the control group. Fetal and placental weights showed no effects of the treatment. No treatment related abnormalities were observed on external skeletal and visceral examinations in any group.

Segment II Teratology Study in Rabbits				
Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# Inseminated	15	15	15	15
# of Deaths	0	1	0	1
# Not Pregnant	0	0	6	5
# of Pregnant	15	14	9	10
% Pregnant	100	100	60	67
# of Abortion	1	0	1	1
# of Dam Examined	15	14	9	10
# of Corpora Lutea/dam	9.3 ± 1.7	10.1 ± 2.3	9.4 ± 2.2	10.6 ± 2.2
# of Implants/dam	8.6 ± 2.7	8.2 ± 2.5	8.4 ± 2.4	9.1 ± 1.8
# of Pre-implantation Loss (%)	7.7	19.0	10.7	14.6
# of Post-implantation Loss (%)	6.7	10.4	6.0	13.4
# of Early resorptions	0.3 ± 0.5	0.4 ± 0.6	0.5 ± 0.7	0.8 ± 0.9
# of Late Resorptions	0.3 ± 0.5	0.5 ± 0.7	0.0	0.4 ± 0.7
# of Live Fetuses/dam	8.0 ± 2.6	7.4 ± 2.4	7.9 ± 2.6	7.9 ± 2.3
Fetal Wt. (g)				
Male	40.8 ± 2.6	42.7 ± 2.7	42.9 ± 2.2	41.6 ± 2.2
Female	40.5 ± 2.5	41.2 ± 1.8	42.9 ± 2.0	39.1 ± 3.3
Sex Ratio (M/F)	1.05	0.90	0.79	1.13
Morphological Findings of Fetuses				
External/Visceral Anomalies				
# of Fetuses (litters) Examined	112 (14)	103 (14)	63 (8)	71 (9)
Abnormalities	0 (0)	0 (0)	0 (0)	0 (0)
Skeletal Anomalies				
# of Fetuses (litter) Examined	76 (14)	73 (14)	43 (8)	49 (9)
Major Anomalies	0 (0)	0 (0)	0 (0)	0 (0)
Minor Anomalies	0 (0)	0 (0)	0 (0)	0 (0)

Due to "apparent effect on the maintenance of pregnancy in females receiving 600 mg/kg/day and above", sponsor repeated part of the experiment. In this repeat test three groups were included in which pregnant rabbits were given oral doses of 0 (vehicle), 300 and 600 mg/kg from day 6 to 19 day of gestation.

This experiment had few problems, such as older rabbits were used and were not in an active growth phase (even control rabbits lost their weights during gestation period). Hence no conclusion can be made about this partial repeat study.

Thus maternal toxicity was seen at 600 and 1200 mg/kg/day dose level. Additionally, mid and high dose levels adversely affected the pregnancy rates. Sponsor should be asked to conduct additional experiments to find the mechanism responsible for decrease in pregnancy rate. However, there was no evidence of a teratogenic potential.

Oral Segment III. Perinatal and Postnatal Study in Rats (Report # 93/GYS008/0618)

Testing Laboratories: _____

Study Started: September 29, 1992

Study Completed: September 28, 1993

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: CD Sprague-Dawley pregnant rats

No. of Animals: 22 pregnant rats/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 120, 600 and 2000 mg/kg/day

Drug Batch No.: 261R29

Methods: In this study, the dose selection was based on preliminary oral Segment I and III study in rats (study # 92/GYS003/1081) in which the highest tested dose (2000 mg/kg/day) had no effect on fertility and general reproductive performance of rat. Sponsor selected 2000 mg/kg/day as the highest dose for the main study. In the main study, Pregnant rats were given oral (gavage) doses of 0 (vehicle: water; 12 ml/kg), 120, 600 and 2000 mg/kg/day of BSZ from day 15 of gestation to day 25 after parturition. All dams were observed for clinical signs daily, body weights were recorded on days 0, 6, 12 and 15 of post coitum and daily thereafter till the termination of the study. Food consumptions were recorded between days 0-2, 3-5, 6-8, 9-11, 12-14, 15-17 and 18-19 of gestation and 1-3, 4-6, 7-10, 11-13, 14-17, 18-20, and 21-24 of lactation. All dams were allowed to

litter normally and raise their pups to weaning. The number of live/dead pups were recorded, and the live pups were weighed and sexed. On day 25 of post partum all dams were sacrificed and necropsied, and examined externally and internally for abnormalities. Postnatal body weight changes of the pups were recorded until the age of 25 days. On lactation day 4, litters with more than 8 pups were culled to 4 males and 4 females. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (righting reflex, pinna detachment, tooth-eruption, eyelid separation, visual and auditory function tests, testes descent, vaginal opening, learning ability test and open field test). At day 35 of post partum, 20 pairs of the animals per group were selected for F1/F2 generation study. At 10 weeks of age they were continuously mated and cesarean section was performed on the F_1 dams on day 20 of gestation, uterine contents and fetuses were examined and preserved in acetone and Bouin's fluid.

Results: Throughout gestation and lactation period, no abnormalities were seen in clinical signs, body weight gains, food and water consumptions of F_0 dams. Length of gestation was comparable in all groups. No abnormalities were observed at autopsy of F_0 dams which would be attributed to treatment. No drug related effects were seen in the F_1 pups during postnatal period, development and reproductive performance, and no external abnormalities were observed in the F_2 fetuses. Thus no adverse effect were seen in rats following oral dose of up to 2000 mg/kg/day of BSZ during perinatal and postnatal period.

MUTAGENICITY:

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

GENETIC TOXICOLOGY:

Ames Test

Testing Laboratories: ———

———

Dates Studies Started and Completed: Not given

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

Concentration Employed: BX661A: 1- 1000 mcg/plate; and 4-Aminobenzoylalanine and 5-Aminosalicylic acid (1:1): 1-1000 mcg/plate.

Solvent Control: Water or DMSO

Positive Control: 2-anthramine (10 mcg/plate), 9-aminoacridine (40 mcg/plate), 2-nitrofluorene (2 mcg/plate), 4-nitroquinoline-n-oxide (0.5 mcg/ ml), and N-Methyl-N-nitro-N-nitrosoguanidine (2 mcg/plate).

Source of Metabolic Activation: S-9 mix

Drug Batch No.: Not given.

Criteria of Positivity: Criteria of positivity was not mentioned.

Results: Experimental detail was not given. The summary table indicated that the drug (BX661A) was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). However, when 1:1 mixture of 4-Aminobenzoylalanine and 5-Aminosalicylic acid (the two moiety of the parent drug) were used as the test substance, then 2-3 fold increase in the number of revertant colonies above the solvent control were seen in strain TA 1538 in the presence of S-9 mix. This indicates that the mixture of two moieties of the parent drug is mutagenic in this test. Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 mix). The findings in this test should not be considered final since many experimental details are lacking.

Number of Revertant Colonies in Strain TA 1538

Treatment	-S9-Mix	+S9-Mix
Solvent	8	7
1:1 mixture of 4-Aminobenzoylalanine and 5-Aminosalicylic acid		
1 mcg/plate	23	10
10 mcg/plate	11	21
100 mcg/plate	9	14
1000 mcg/plate	6	8
Positive Control	100	1470

Ames Test
(Report # 1067/40)

Testing Laboratories: ———

Dates Studies Started and Completed: April 14, 1993 and June 1, 1993.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537; and E. coli strains WP2 pKM101 and WP2 UVrA pKM101.

Concentration Employed: Experiment 1 (plate incorporation method): 8 - 5000 mcg/plate; Experiment 2 (preincubation method, 1 hr 37C): 1000-5000 mcg/plate

Solvent Control: Water and dimethyl sulfoxide (DMSO)

Positive Control: 2-nitrofluorene (50 mcg/plate), NaN_3 (2 mcg/plate), 9-aminoacridine (50 mcg/plate), 4-nitroquinoline-n-oxide (2, 4 mcg/ ml), and 2-aminoanthracene (5 mcg/plate).

Source of Metabolic Activation: ——— induced rate liver microsomal enzymes (S-9 mix).

Drug Batch No.: BN 1754H

Criteria of Positivity: When Dunnett's test gave a significant response ($p < \text{or} = 0.01$) and the data set showed a significant correlation then the test substance is considered positive provided results were reproducible.

Results: In all of the tester strain, the increase in the number of revertant colonies was less than 2 fold above the solvent control value and Dunnett's test was not significant. Hence drug was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

In Vitro Human Lymphocyte Chromosome Aberration Test (Report # 89/BEB001/0468)

Testing Laboratories: ———

Dates Study Started and Completed: May 2, 1989 and November 13, 1989.

Cells Employed: Cultured human lymphocytes cells.

Concentration Employed: 200-5000 mcg/ml

Solvent Control: Water, ethanol

Positive Control: Cyclophosphamide (6 mcg/ml) and chlorambucil (2 mcg/ml).

Source of Metabolic Activation: Rate liver microsomal enzymes

Drug Batch No.: 261015

Methods: Human lymphocytes cultured cells were treated with BSZ in the presence and absence of metabolic activator (S9 Mix). Cells were harvested at 24 hours after the start of treatment (cells in the presence of S9-Mix were treated only for 3 hours then washed and incubated for an additional 21 hours). The chromosomal aberrations were analyzed in cell sampled at 24 hr at three dose levels (200, 1000 and 5000 mcg/ml) and 100 metaphases were examined per treatment group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations compared to control then the compound is genotoxic.

Results: A concentration of 1000 and 5000 mcg/ml of BSZ in the absence of S-9 mix was cytotoxic in this test (mitotic inhibition = 57-59%). In the presence of S-9 mix no significant inhibition of mitotic activity was seen at the tested concentrations. Irrespective of the presence or absence of metabolic activation, treatment with up to 5000 mcg/ml of BSZ did not produce any significant increase in chromosomal aberration over the value obtained for the control group. The positive controls chlorambucil and cyclophosphamide produced about 11-15% and 24-55% chromosomal aberration respectively in this test. Thus BSZ had no clastogenic activity in this in vitro cytogenetic test.

In Vitro Human Lymphocyte Chromosome Aberration Test (Report # 1076/44)

Testing Laboratories: _____

Dates Studies Started and Completed: April 26, 1993 and July 16, 1993.

Cells Employed: Cultured human lymphocytes cells.

Concentration Employed: 43.6-5000 mcg/ml

Solvent Control: Water, Dimethyl sulfoxide (DMSO)

Positive Control: 4-Nitroquinoline-1-oxide (1.25-5.0) and cyclophosphamide (12.5-25 mcg/ml).

Source of Metabolic Activation: Rate liver microsomal enzymes (S-9 mix).

Drug Batch No.: BN1754H

Methods: Human lymphocytes cultured cells were treated with BSZ in the presence and absence of metabolic activator (S9 mix). Cells were harvested at 20 or 44 hours after the start of treatment (cells in the presence of S9-mix were treated only for 3 hours then washed and incubated for additional 17 or 41 hours). Additionally 3 hr pulse treatment followed by a 17 hr recovery period in the absence of S-9 mix was also performed. The chromosomal aberrations were analyzed in cell sampled at 20 hr at three dose levels (2113, 3250 and 5000 mcg/ml). The highest concentration selected for analysis at this time produced about 38% and 29% mitotic inhibition in the absence and presence of S-9 mix respectively. At 40 hr, the effect of single concentration (5000 mcg/ml) was assessed and at this time drug produced about 30% and 29% mitotic inhibition in the absence and presence of S-9 mix respectively. At the end of experiment 100 metaphases were examined per treatment group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations compared to control and proportion of aberrant cells at such data points exceed the normal range then the compound is genotoxic.

Results: Irrespective of the presence or absence of metabolic activation, treatment with up to 5000 mcg/ml of BSZ did not produce any significant increase in chromosomal aberration over the value obtained for the control group. The positive controls gave expected results. Thus BSZ had no clastogenic activity in this in vitro cytogenetic test.

Main mutation assays - treatment means in the absence of S-9 mix

Treatment (ug/ml)	First mutation assay		Second mutation assay	
	Plating efficiency	Mutation frequency[a]	Plating efficiency	Mutation frequency[a]
Distilled water (0)	57.1	4.0	86.4	8.3
Balsalazide Sodium (8)	63.2	3.5	107.1	5.5
Balsalazide Sodium (40)	60.7	0.3	75.5	28.3
Balsalazide Sodium (200)	39.2	1.0	82.1	6.5
Balsalazide Sodium (1000)	61.1	9.2	61.6	14.1
Balsalazide Sodium (5000)	64.0	2.4	84.4	11.6
EMS (1000)	59.3	160.0	91.4	89.9
DMBA (10)	47.7	9.5	90.1	20.6

a - per 10^5 survivors

Main mutation assays - treatment means in the presence of S-9 mix

Treatment (ug/ml)	First mutation assay		Second mutation assay	
	Plating efficiency	Mutation frequency[a]	Plating efficiency	Mutation frequency[a]
Distilled water (0)	87.0	5.4	113.2	7.9
Balsalazide Sodium (8)	57.8	3.1	113.7	13.4
Balsalazide Sodium (40)	80.7	2.3	80.6	17.1
Balsalazide Sodium (200)	74.2	10.9	89.7	18.1
Balsalazide Sodium (1000)	96.9	10.7	101.0	6.6
Balsalazide Sodium (5000)	68.6	3.1	108.9	17.0
DMBA (10)	110.6	84.9	85.8	69.1

a - per 10^5 survivors

In Vivo Genotoxicity Study of BSZ by the Oral Route in the Mouse Micronucleus Test (Report # 89/BEB003/0434)

Testing Laboratories: [redacted]

Dates Study Started and Completed: April 26, 1989 and July 10, 1989.

Test Species: CD-1 Mice.

No. of Animals: 5 animals/sex/group.

Route of Administration: Oral

Dose Levels: 200, 1000 and 5000 mg/kg (10 ml/kg).

Drug Batch No.: 261015

Basis of Dose Selection: Dose levels are based on the preliminary toxicity study.

Negative Control: Water (10 ml/kg)

Positive Control: Chlorambucil (30 mg/10 ml/kg)

Methods: Animals were given a single dose at 24, 48 (control and high dose) or 72 (control and high dose) hours prior to sacrifice and preparation of the bone marrow (positive control animals were sacrificed at 24 hr after chlorambucil administration). On the Giemsa-stained slides, 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei.

Results: BSZ did not induce an increase of micronucleated polychromatic and normochromatic erythrocytes in mice bone marrow. In contrast, the % of micronucleated polychromatic erythrocytes in chlorambucil treated group was markedly higher than the negative control. These findings suggest that BSZ is not mutagenic in this test system.

Thymidine Kinase Mutation Test in Mouse Lymphoma L5178Y Cells (Fluctuation Assay) (Report # 1067/59-1052)

Testing Laboratories: [redacted]

Dates Study Started and Completed: March 6, 1995 and August 2, 1995.

Strain Employed: L5178Y $TK^{+/-}$ mouse lymphoma cells.

Concentration Employed: 250-4373.2 mcg/ml.

Solvent Control: Tissue culture medium (RPMI 1640).

Positive Control: 4-Nitroquinoline-1-oxide (NQO: 0.05 and 0.1 mcg/ml) and benzo(a)pyrene (BP: 2 and 3 mcg/ml).

Source of Metabolic Activation: [redacted] induced Sprague Dawley rat liver microsomal enzymes.

Drug Batch No.: 3G2901

Methods: Two experiments were conducted. In experiment 1, concentrations of 500, 1000, 2000 and 4373.2 mcg/ml were used and in experiment 2, concentrations of 1500, 2500, 3500 and 4373.2 mcg/ml were used. At the top dose, the relative survival rates in experiment 1 were 114.0% and 98.6% in the absence and presence of S-9 mix respectively. The relative survival rates in experiment 2 at the top dose were 95.1% and 109.9% in the absence and presence of S-9 mix respectively. Cells were incubated for 3 hr, then cells were washed, resuspended in media and viability rates were determined on day 8 of the study. Cultures selected for mutation assay (i.e. TFT resistance assessment) were allowed to express for 2 days and then scored 12 days later. The test substance was considered to be mutagenic if the mutation frequency at 1 or more dose was significantly greater than that of the negative control and it was dose related and reproducible.

Results: Irrespective of the treatment with metabolic activation system (S-9 Mix), no increase in mutant colonies was seen in this study. Negative and positive controls gave the expected results. Thus, the drug is not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

Genotoxicity Studies of 4-ABA ("carrier molecule"):

Thymidine Kinase Mutation Test in Mouse Lymphoma L5178Y Cells (Fluctuation Assay)
(Report # 1067/54-1052)

Testing Laboratories: ———

Dates Study Started and Completed: March 2, 1995 and June 29, 1995.

Strain Employed: L5178Y $TK^{+/-}$ mouse lymphoma cells.

Concentration Employed: 260 - 2080 mcg/ml.

Solvent Control: Tissue culture medium (RPMI 1640).

Positive Control: 4-Nitroquinoline-1-oxide (NQO: 0.05 and 0.1 mcg/ml) and benzo(a)pyrene (BP: 2 and 3 mcg/ml).

Source of Metabolic Activation: ——— induced Sprague Dawley rate liver microsomal enzymes.

Drug Batch No.: SCS 939

Methods: Two experiments were conducted. In experiment 1, concentrations of 260, 520, 1040 and 2080 mcg/ml were used and in experiment 2, concentrations of 500, 1000, 1500 and 2080 mcg/ml were used. At the top dose, the relative survival rates in experiment 1 were 75.3% and 98.6% in the absence and presence of S-9 mix respectively. The relative survival rates in experiment 2 at the top dose were 95.8% and 100% in the absence and presence of S-9 mix respectively. Cells were incubated for 3 hr, then cells were washed, resuspended in media and viability rates were determined on day 8 of the study. Cultures selected for mutation assay (i.e. TFT resistance assessment) were allowed to express for 2 days and then scored 12 days later. The test substance was considered to be mutagenic if the mutation frequency at 1 or more dose was significantly greater than that of the negative control and it was dose related and reproducible.

Results: Irrespective of the treatment with metabolic activation system (S-9 Mix), no increase in mutant colonies was seen in this study. Negative and positive controls gave the expected results. Thus, the 4-ABA is not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

In Vitro Human Lymphocyte Chromosome Aberration Test (Report # 1067/57)

Testing Laboratories: ———

Dates Studies Started and Completed: March 3, 1995 and June 14, 1995.

Cells Employed: Cultured human lymphocytes cells.

Concentration Employed: 41.3 - 2080 mcg/ml.

Positive Control: 4-Nitroquinoline-1-oxide (1.25-5.0) and cyclophosphamide (12.5-25 mcg/ml).

Solvent Control: Tissue culture medium (RPMI 1640), Dimethyl sulfoxide (DMSO).

Source of Metabolic Activation: ——— 4 induced Sprague Dawley rate liver microsomal enzymes.

Drug Batch No.: SCS 939

Methods: Human lymphocytes cultured cells were treated with 4-ABA in the presence and absence of metabolic activator (S-9 mix). Cells were harvested at 20 or 44 hours after the start of treatment (cells in the presence of S-9 mix were treated only for 3 hours then washed and incubated for additional 17 or 41 hours). Additionally 3 hr pulse treatment followed by a 17 hr recovery period in the absence of S-9 mix was also performed. The chromosomal aberrations were analyzed in cell sampled at 20 hr at three dose levels (1170, 1560 and 2080 mcg/ml). The highest concentration selected for analysis at this time produced about 61% and 14% mitotic inhibition in the absence and presence of S-9 mix respectively. At 44 hr, the effect of single concentration (2080 mcg/ml) was assessed and at this time drug produced about 52% and 18% mitotic inhibition in the absence and presence of S-9 mix respectively. At 3+17 hr (in the absence of S-9 mix), the highest tested concentration produced 42% mitotic inhibition. At the end of experiment 200 metaphases were examined per treatment group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations compared to control and proportion of aberrant cells at such data points exceed the normal range and if reproducible then the compound is genotoxic.

Results: In the absence of metabolic activation, 4-ABA produced significant increase in chromosomal aberration (chromatid deletions, chromatid exchanges and chromosome deletions) over the value obtained for the control group in 20 hr sampling time point in high dose group (both experiment). In high dose group, multiple aberrations were seen in some high dose treated cells. Furthermore, frequencies of aberrant cells (without gap) at high dose in experiment 2 was higher than that seen in historical range (sponsor's laboratory 0-3% [n=43]). Additionally, numerical aberrations (such as polyploidy) also exceeded the historical negative control range (sponsor's laboratory 0-3% [n=43]). Cultures treated in the presence of S-9 mix had normal frequencies of aberrant cells at both sampling times. Negative and positive controls gave the expected results:

20 hour Treatment of Human Lymphocytes in the Absence of S-9 Mix

Treatment (mcg/ml)	Experiment 1 Cells Aberr. (-gap)	Experiment 2 Cells Aberr. (-gap)
Control	1/200	1/200
1019	1/200	-----
1170	-----	2/200
1456	2/200	-----
1560	-----	4/200
2080	6/200*	8/200*

* = $p<0.05$

Thus, 4-ABA had clastogenic activity in this in vitro cytogenetic test.

Addendum: It is noted that 4-ABA significantly increased the chromosomal aberration as compared to the control at high concentration (2080 µg/ml) in both experiments (Experiments 1 and 2) when sampled at 20 hours in the absence of S-9. Although it was not statistically significant, the increase in the chromosomal aberration was also seen at lower concentrations and this was dose dependent. The mitotic index was ~24% and 61% in Experiments 1 and 2, respectively.

4-ABA: Bacterial Reverse Mutation Test
(4L198)

Testing Laboratory: ———

Dates Started and Completed: June 30, 1994 and September 30, 1994.

Compliance with GLP and QAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential mutagenic effects of 4-ABA, the reverse mutation assay (Ames test) was conducted using the preincubation method in four strains salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and one strain of E. Coli (WP2uvrA) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of 4-ABA were tested: 313, 625, 1250, 2500 and 5000 μg/plate with and without S-9. Positive controls (N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, sodium azide, 2-anthramine and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide) were also tested. The result was considered positive if the test substance induced at least two-fold increases in revertant colonies compared to the control in a concentration dependent manner.

Results: The results indicated that 4-ABA did not significantly increase the colonies. The positive controls, however, significantly induced increase in the colonies compared to the solvent controls.

In conclusion, the results suggest that 4-ABA was not mutagenic in this test system.

The following genetic toxicity studies were previously submitted to ——— and reviewed on July 11, 1996. The review is reproduced below.

Genotoxicity Studies of N-acetyl-4-ABA:

Ames Test
(Report # 1067/056-1052)

Testing Laboratories: ———

Dates Studies Started and Completed: March 2, 1995 and June 29, 1995.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537; and E. coli strain WP2 UVrA.

Concentration Employed: Experiment 1 (plate incorporation method): 8 - 5000 mcg/plate; Experiment 2 (treatment in the presence of S-9 included a preincubation step [1 hr 37C]): 1000-5000 mcg/plate.

Solvent Control: Water and dimethyl sulfoxide (DMSO).

Positive Control: 2-nitrofluorene (50 mcg/plate), NaN_3 (2 mcg/plate), 9-aminoacridine (50 mcg/plate), 4-nitroquinoline-n-oxide (2 mcg/ ml), and 2-aminoanthracene (5 mcg/plate).

Source of Metabolic Activation: ——— 4 induced rate liver microsomal enzymes (S-9 mix).

Drug Batch No.: SCS 936

Criteria of Positivity: When Dunnett's test gave a significant response ($p < \text{or} = 0.01$) and the data set showed a significant correlation then the test substance is considered positive provided results were reproducible.

Results: In all of the tester strain, the increase in the number of revertant colonies was less than 2 fold above the solvent control value and Dunnett's test was not significant. Hence N-acetyl-4-ABA was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

Thymidine Kinase Mutation Test in Mouse Lymphoma L5178Y Cells (Fluctuation Assay) (Report # 1067/55-1052)

Testing Laboratories: ———

Dates Study Started and Completed: March 2, 1995 and June 29, 1995.

Strain Employed: L5178Y $TK^{+/-}$ mouse lymphoma cells.

Concentration Employed: 312.5 - 2500 mcg/ml.

Solvent Control: Tissue culture medium (RPMI 1640).

Positive Control: 4-Nitroquinoline-1-oxide (NQO: 0.05 and 0.1 mcg/ml) and benzo(a)pyrene (BP: 2 and 3 mcg/ml).

Source of Metabolic Activation: —— induced Sprague Dawley rate liver microsomal enzymes.

Drug Batch No.: SCS 936

Methods: Two experiments were conducted. In experiment 1, concentrations of 312.5, 625, 1250 and 2500 mcg/ml were used and in experiment 2, concentrations of 1000, 1500, 2000 and 2500 mcg/ml were used. At the top dose, the relative survival rates in experiment 1 were 91.8% and 108.1% in the absence and presence of S-9 mix respectively. The relative survival rates in experiment 2 at the top dose were 92.4% and 109.8% in the absence and presence of S-9 mix respectively. Cells were incubated for 3 hr, then cells were washed, resuspended in media and viability rates were determined on day 8 of the study. Cultures selected for mutation assay (i.e. TFT resistance assessment) were allowed to express for 2 days and then scored 12 days later. The test substance was considered to be mutagenic if the mutation frequency at 1 or more dose was significantly greater than that of the negative control and it was dose related and reproducible.

Results: Irrespective of the treatment with metabolic activation system (S-9 Mix), no increase in mutant colonies was seen in this study. Negative and positive controls gave the expected results. Thus, N-acetyl-4-ABA is not mutagenic at the tk locus of L5178Y mouse lymphoma cells.

In Vitro Human Lymphocyte Chromosome Aberration Test
(Report # 1067/058-1052)

Testing Laboratories: ——

Dates Studies Started and Completed: March 3, 1995 and June 29, 1995.

Cells Employed: Cultured human lymphocytes cells.

Concentration Employed: 49.43-2500 mcg/ml

Positive Control: 4-Nitroquinoline-1-oxide (1.25-5.0) and cyclophosphamide (12.5-25 mcg/ml).

Solvent Control: Tissue culture medium (RPMI 1640), Dimethyl sulfoxide (DMSO).

Source of Metabolic Activation: —— induced Sprague Dawley rate liver microsomal enzymes.

Drug Batch No.: SCS 936

Methods: Human lymphocytes cultured cells were treated with N-acetyl-4-ABA in the presence and absence of metabolic activator (S-9 mix). Cells were harvested at 20 or 44 hours after the start of treatment (cells in the presence of S-9 mix were treated only for 3 hours then washed and incubated for additional 17 or 41 hours). Additionally, 3 hr pulse treatment followed by a 17 hr recovery period in the absence of S-9 mix was also performed. The chromosomal aberrations were analyzed in cell sampled at 20 hr at three dose levels (1225, 1750 and 2500 mcg/ml). The highest concentration selected for analysis at this time produced about 43% (in repeat experiment: 18%) and 17% (in repeat experiment: 25%) mitotic inhibition in the absence and presence of S-9 mix respectively. At 44 hr, the effect of single concentration (2500 mcg/ml) was assessed and at this time drug produced about 0% and 17% mitotic inhibition in the absence and presence of S-9 mix respectively. At 3+17 hr (in the absence of S-9 mix), the highest tested concentration produced 22% mitotic inhibition. At the end of experiment, 200 metaphases were examined per treatment group. If a statistically significant increases in proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations compared to control and proportion of aberrant cells at such data points exceed the normal range and if reproducible then the compound is genotoxic.

Results: In one out of 2 experiments, in the absence of metabolic activation, N-acetyl-4-ABA produced significant increase in chromosomal aberration over the value obtained for the control group in 20 hr sampling time point. However, frequencies of aberrant cells (without gap) were within the historical range (sponsor's laboratory 0-3% [n=43]). Negative and positive controls gave the expected results.

20 hour Treatment of Human Lymphocytes in the Absence of S-9 Mix

Treatment (mcg/ml)	Experiment 1. Cells Aberr. (-gap)	Experiment 2 Cells Aberr. (-gap)
Control	3/200	0/200
1225	0/200	-----
1406	-----	4/200*
1750	2/200	-----
1875	-----	3/200
2500	1/200	6/200**

* = $p<0.05$, ** = $p<0.01$

Thus N-acetyl-4-ABA had no clastogenic activity in this in vitro cytogenetic test.

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Draft Labeling
(not releasable