Study title: BSF 208075: A 39-week oral toxicity study in beagle dogs followed by a 20-week recovery period

Key study findings: Compound-related lesions were limited to a non-dose-related increase in bilateral testicular tubular atrophy/aspermatogenesis which showed some recovery during the 20-week recovery period, but persisted in one dog.

Study no.: 2002-6032
Volume #, and page #:
Conducting laboratory and location: [redacted]

Date of study initiation: 4/8/03
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: Lot No. 10011, ≥ [redacted] purity

Methods

Doses: 0, 30, 300 or 600 mg/kg/day
Species/strain: beagle dog
Number/sex/group or time point (main study): 4
Route, formulation, volume, and infusion rate: oral, gavage, 10 mL/kg
Satellite groups used for toxicokinetics or recovery: 2 (recovery)
Age: 7-8 mos.
Weight (nonrodents only): Males: 6.6-8.1 kg 6.9-9.5 kg; Females:

Observation times and results

Mortality: Mortality checks were performed once a day during all phases of the study.

Clinical signs: Clinical signs (ill health, behavioral changes, etc.) were evaluated once daily during all phases of the study.

A detailed clinical examination of each dog was performed one week prior to initiation of treatment, and weekly thereafter during the treatment and recovery periods.

Body weights: Body weights were recorded once prior to randomization, and one week prior to initiation of treatment. During the treatment period, body weights were measured and reported on Day 1, prior to dosing and weekly thereafter during the treatment and recovery periods.

Food consumption: Individual food intake was recorded daily for one week prior to initiation of treatment and daily during the treatment and recovery periods. Average weekly food intake is reported.

Ophthalmoscopy: Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit-lamp) examinations were performed on all animals, following the application of a mydriatic agent, once during the pre-treatment period and again during Week 39 of the treatment period.

Ophthalmic examinations were also performed on all the Recovery animals during Week 20 of the recovery period.

EKG: Electrocardiograms (leads I, II and III, and augmented leads aVR, aVL and aVF) were obtained from all animals once during the pre-treatment period and again during Weeks 26 and 39 of the treatment period. Electrocardiograms were also obtained from the Recovery animals during week 20 of the recovery period.

Electrocardiograms were assessed (qualitatively and quantitatively) for gross changes indicative of cardiac abnormalities (e.g. hypertrophy, ischemia, conduction disturbances, etc.). Heart rate (lead II), sinus rhythm and the atrioventricular rhythms were also assessed. P-R, QRS, QT and QTc intervals were measured. Tabular data of heart rate and ECG intervals were evaluated.

Clinical Pathology

Clinical pathology investigations (hematology, clinical chemistry, coagulation and urinalysis) were performed on all animals once during the pre-treatment period, once during Weeks 13, 26 and 39 of the treatment period, and at the end of the recovery period (Week 20), prior to necropsy.

a. Hematology*

Parameters Examined	
Red blood cell count	Mean Corpuscular Hemoglobin
Hematocrit	Mean Corpuscular Volume
Hemoglobin	Platelet count
White blood cell count	Red Cell Distribution Width
WBC differential (absolute and relative)	Reticulocyte counts
Mean Corpuscular Hemoglobin Concentration	

* EDTA was used as the anticoagulant.

b. Coagulation*

Parameters Examined**
Activated partial thromboplastin time
Prothrombin time

* Citrate was used as the anticoagulant.
** Various additional parameters are automatically recorded by the instrumentation but not reported.

c. Clinical Chemistry (serum)

Parameters Examined	
A/G ratio (calculated)	Creatinine
Alanine aminotransferase	Globulin (calculated)
Albumin	Glucose
Alkaline phosphatase	Phosphorus (inorganic)
Aspartate aminotransferase	Potassium
Bilirubin(total)	Sodium
Calcium	Total protein
Chloride	Triglycerides
Cholesterol (total)	Urea nitrogen

d. Urinalysis

Parameters Examined**	
Bilirubin	pH
Blood	Protein
Color and appearance	Specific gravity
Glucose	Urine sediment
Ketones	Urobilinogen
Leukocytes	Volume
Nitrites	

** Various parameters are automatically recorded by the instrumentation, but not reported.

Gross pathology: The Main animals were sacrificed on the day following their last dose (Day 274), while the Recovery animals were kept for an additional 20 weeks (not dosed), and then euthanized on Day 414. All dogs were sedated with xylazine, and then euthanized by an intravenous administration of an overdose of sodium pentobarbital, followed by the severance of major arteries.

Gross pathology consisted of an external examination, including identification of all clinically recorded lesions as well as a detailed internal examination.

In order to avoid autolytic changes, necropsy was conducted, as soon as each animal died, or euthanized during the study.

Organ weights: The following organs were dissected, trimmed free of fat and weighed:

Adrenals	Kidneys
Ovaries	Testes with epididymides
Brain	Liver
Prostate	Thymus
Heart	Lungs
Spleen	Uterus

Paired organs were weighed together. Relative organ weight (relative to terminal body weight) was calculated and reported.

Histopathology: On completion of the gross pathology examination, the following tissues and organs were retained. Neutral buffered 10% formalin was used for fixation and preservation.

Abnormal Tissues
Adrenal Glands
Animal Identification*
Aorta (Thoracic)
Brain (Cerebral Cortex, Midbrain, Cerebellum and Medulla)
Cecum
Colon
Esophagus
Eyes
Femur & Marrow
Gallbladder
Heart **
Kidneys
Liver (sample of two lobes)
Lung (with bronchi, 2 lobes) @
Lymph Node, Mandibular
Lymph Node, Mesenteric
Mammary Gland (Inguinal)
Nasal Cavities and sinuses (3 levels)##
Optic Nerves
Ovaries
Pancreas
Pituitary
Prostate
Salivary Gland (Mandibular)
Sciatic Nerve
Skeletal Muscle
Skin & Subcutis (Inguinal)
Small Intestine, Duodenum
Small Intestine, Ileum
Small Intestine, Jejunum
Spinal Cord (Cervical)
Spleen
Sternum & Marrow
Stomach
Testes with Epididymides#
Thymus
Thyroid and parathyroids+
Tongue
Trachea
Urinary Bladder
Uterus
Vagina

* Fixation and preservation only.
** Section including ventricles and atria, septum with papillary muscle.
@ Lungs of euthanized animals were infused with formalin.
\+ Parathyroids were examined histologically, only if present in routine sections.
\# Fixed in Davidson's solution. The epididymides were not separated from the testes in order to optimize sectioning and evaluation of the efferent ductules.
Bone was decalcified prior to sectioning.

Toxicokinetics: Blood samples (approximately 2 mL) were collected from all animals on Days 1, 91, 182 and 273 at the following timepoints: prior to dosing, and 30 minutes, 1, 3, 6 and 24 hours after dosing.

Results

Mortality

Two dogs (were sacrificed following clinical signs (rales and shallow respiration) consistent with dosing solution aspiration. No histological evaluations were performed on these animals as both animals were replaced on the study.

One 600 mg/kg dog was found dead on Day 89 and one 300 mg/kg dog was sacrificed on Day 91. Both dogs exhibited rales, inactivity and thin body condition. Due to the dose-dependent nature of these clinical signs as outlined below, an effect of the test agent cannot be ruled out. The mortality could be associated with emesis and aspiratory pneumonia.

Clinical observations

Slight to severe salivation and emesis were noted at all dose levels including controls on multiple occasions, with apparent increase in frequency at 300 and 600 mg/kg/day. In addition, a dose-related increase in the incidence of rales in 1, 8 and 10 animals at 30, 300 and 600 mg/kg/day, respectively that resulted in some animals being observed with coughing, sneezing and/or wheezing.

Other clinical signs observed in the control and treated groups that were not considered dose-related, included (but were not limited to) soft feces, lacrimation and frothing.

Body weights

There were no adverse effects on body weight at any dose level during the treatment or recovery periods in comparison to the controls.

Food consumption

There were no adverse effects on food consumption at any dose level during the treatment or recovery periods.

Hematology

There were no treatment-related effects on hematology parameters at any dose level in comparison to the controls at any of the assessment periods including the recovery period. White Blood Cell count (WBC) was statistically significantly ($p = 0.05$) decreased in males dosed at 30 mg/kg/day in comparison to the controls at Week 13. This decrease was not considered to be treatment-related due to the lack of a dose relationship and the comparable results for this parameter to the pretreatment value for this group. Reticulocyte (percent) was statistically significantly ($p = 0.05$) decreased for males at 300 and 600 mg/kg/day in comparison to the controls at Week 39. The significance of this decrease is not known. During week 13 assessment, WBC (and components of WBC),

and Platelets (PLT) were statistically significantly ($p = 0.05$ or $p = 0.01$) increased in females dosed at 300 mg/kg/day in comparison to the controls. The increase in WBC and PLT were due mainly to the sickness of No. 3503C which was later sacrificed. A statistically significantly ($p = 0.01$) decrease in Red Blood Cell count was observed in females dosed at 300 mg/kg/day in comparison to the controls during Week 13 and was considered to be incidental. A few parameters were observed with incidences of statistically significant increases during the study that were considered to be incidental or not toxicologically important.

Coagulation
There were no treatment-related effects on coagulation parameters at any dose level in comparison to the controls at any of the assessment periods including the recovery period. An observed statistically significant ($p = 0.05$) increase in Activated Partial Thromboplastin time in females dosed at 300 mg/kg/day at week 13 was considered to be incidental.

Clinical Chemistry

There were no treatment-related effects on clinical chemistry parameters at any dose level in comparison to the controls at any of the assessment periods including the recovery period. Statistically significant ($p = 0.05$ or $p = 0.01$) changes were observed in a few parameters during Weeks 26 or 30 in males or females dosed at 30, 300 or 600 mg/kg/day, since these were observed in isolated cases with no dose relationship and the significance of any one parameter was not observed in both sexes, the incidences were considered to be incidental or of no toxicological importance.

Urinalysis

There were no treatment-related effects on the urinalysis parameters at any dose level in comparison to the controls at any of the assessment periods including the recovery period.

Ophthalmoscopy
There were no adverse ocular effects, caused by the administration of BSF 208075 that could be identified during the course of this experiment. Findings observed after 39 weeks of treatment and after the recovery period were observed on the pretreatment ophthalmic examination or are spontaneous and considered normal for the dog population.

Electrocardiography
The morphology and intervals of the P-QRS-T complexes showed no clinically significant differences at weeks 26 or 39 of treatment compared to pre-treatment in all the groups. Only normal variations occurred in relationship to changes in heart rates. Variable increases in mean heart rates were noted in all the groups, including the control. These increases are not consistent with a dose-related effect and persist, although to a lesser degree, through the recovery period. The latter observations suggested that the

increases in heart rates were more related to external cause rather than to an effect of the test article.

Toxicokinetics

There was a statistically significant increase in Cmax between the 30 mg/kg/day dose group and that of the 300 and 600 mg/kg/day dose group throughout the entire 39 week sampling period suggesting BSF 208075 accumulation at doses greater than 30 mg/kg/day. There was no statistically significant difference in Cmax between the 300 mg/kg and 600 mg/kg/day dose groups throughout the entire 39 week sampling period indicating no increased accumulation of BSF 208075 beyond 300 mg/kg/day.

There were no statistically significant differences in BSF 208075 T1/2, between all dose groups, throughout the entire 39 week sampling period suggesting no alteration in T1/2 by increased BSF 208075 dose.

There was a statistically significant increase in $AUC_{0\text{-last}}$ between the 30 mg/kg/day dose group and that of the 300 and 600 mg/kg dose group throughout the entire 39 week sampling period indicating increased BSF 208075 systemic exposure at doses greater than 30 mg/kg/day. There was no statistically significant difference in $AUC_{0\text{-last}}$ between the 300 mg/kg and 600 mg/kg/day dose groups throughout the entire 39 week sampling period indicating no increased systemic BSF 208075 exposure beyond 300 mg/kg/day.

There was no statistically significant alteration in BSF 208075 Mean Residence Time (MRT), between all dose groups, throughout the entire 39 week sampling period suggesting no apparent change in time that a BSF 208075 molecule resides in the body with increasing BSF 208075 dose.

Gross pathology

There were no gross pathological findings related to treatment. The few gross changes observed that included pale and/or dark areas in the lungs, kidneys, small intestines and spleen, were considered incidental in origin as they were either observed in the controls or in isolated cases.

Organ weights

There were no treatment-related effects on the organ weights or organ weights relative to body weights in comparison to the controls.

Histopathology

Focal lesions of testicular atrophy/aspermatogenesis, whether unilaterally or bilaterally distributed, are not uncommon in otherwise normal laboratory Beagles. However, in this study, bilateral lesions occurred only in treated dogs and not in controls and were reversible. Therefore, it must be concluded that a compound-related effect could be

occurring, as is consistent with other compounds of this class. The above testicular histopathology was the only compound-related finding in this study. All other observed microscopic findings had neither the incidence nor severity to suggest they were compound-related.

Dosing formulation

Analysis of BSF 208075 dosing solutions taken over 39-weeks resulted in a recovery range of [redacted] of theory for all dosing solutions. The retention time of the drug material used in the last two dosing time points of the study agreed with the reference standard, thus confirming the identity of the.

CONCLUSION

The oral administration of BSF 208075 to beagle dogs for 39 consecutive weeks at doses of 30, 300 or 600 mg/kg/day, followed by a 20-week recovery period resulted in salivation and rales at 300 and 600 mg/kg/day during the dosing period with complete reversibility during the recovery period. Compound-related lesions were limited to a non-dose-related increase in bilateral testicular tubular atrophy/aspermatogenesis which was reversed during the 20-week recovery period. This testicular histopathology was the only compound-related finding in this study. All other observed microscopic findings had neither the incidence nor severity to suggest they were compound-related. There was a significant increase in Cmax between the 30 mg/kg dose group and that of the 300 and 600 mg/kg/day dose group throughout the entire 39-week sampling period suggesting BSF 208075 accumulation at doses greater than 30 mg/kg/day and there was no significant difference in Cmax between the 300 mg/kg/day and 600 mg/kg dose groups throughout the entire 39 week sampling period indicating no increased accumulation of BSF 208075 beyond 300 mg/kg/day. There were no adverse effects on food consumption, body weights, clinical pathology, ophthalmology and electrocardiograms.

On the basis of the results of this study, the No-Observed-Adverse-Effect-Level (NOAEL) is considered to be < 30 mg/kg/day for males due to the presence of testicular atrophy at the low dose which did not recover after 20 weeks in one animal. The NOAEL for females is considered to be = 30 mg/kg/day due to the presence of rales at the two higher doses, but this respiratory effect was reversible through the recovery period. These NOAEL doses correspond to an AUC of < 3002 and = 2232 ng·h/mL (week 39, free drug) in males and females, respectively.

Study title: BSF 208075: Study for effects on fertility and early embryonic development to implantation after oral administration (gavage) in the Wistar rat

Key study findings:
Treatment of males at 300 mg/kg/day was associated with a reduction of the percentage of mating rats was noted during both pairing phases (with treated as well as with untreated females), this decrease in mating was accompanied in a decrease in fertility indices.

Caesarean section data for treated females showed an increase in pre-implantation loss at 300 mg/kg/day.

Testicular tubular atrophy (focal and diffuse) was observed at all dose levels (NOAEL < 10 mg/kg), although fertility was only affected at 300 mg/kg. These findings did not completely recover after a 13 week recovery period.

Study no.: MDF/DT 0039E [redacted]
Conducting laboratory and location: [redacted]
Date of study initiation: Males: 11/20/00, Females (treated): 1/15/01
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: L0003139, [redacted] purity

Methods

Doses: 0, 10, 30, 100 and 300 mg/kg/day
Species/strain: Rat WIST Hanlbm: WIST (SPF)
Number/sex/group: 22
Route, formulation, volume, and infusion rate: oral, gavage, 10 mL/kg
Study design:

Before treatment initiation the animals (males and females which were treated) and before initiation of the pairing period the females (which remained untreated) were assigned to the different groups randomly. In addition the body weights (recorded on the day of allocation) were taken into consideration in order to ensure similar mean body weights in all groups at initiation of the treatment periods.

Males received the test item for 70 days prior to pairing, during the pairing period and subsequently until they had been dosed for 98 days. Half of the males were then maintained for a further 13 week recovery period without treatment. Males being killed after 98 days treatment were dosed until one day prior to the actual day of necropsy.

The females for the first mating phase received the test item for 14 days prior to pairing, during the pairing period and up to day 6 post-coitum. Females for the second mating phase were not treated.

After the animals had received the test item for 70 days (males) and 14 days (females), the first pairing period was initiated while dosing was continued.

The second pairing period commenced after completion of the pairing period with the treated females. Dosing of the males was continued until all untreated females were mated or after the 14 days pairing period.

During the first and the second pairing period the females were housed with the males (one male: one female) in special automatic mating cages, i.e. with synchronized timing to initiate the nightly mating period, until evidence of copulation was observed. This system reduced the variation in the copulation times of the different females. The females were removed and housed individually if:

a) the daily vaginal smear was sperm-positive, or
b) a copulation plug was observed. This day was designated day 0 post-coitum.

If a treated female was not mated during the first 14-day pairing period, the female was paired with a male of the same group which mated already successfully. If mating was not recorded during this additional pairing period of maximum 14 days the female was sacrificed and the uterus and ovaries preserved in neutral phosphate buffered 4% formaldehyde solution.

If an untreated female was not mated during the first 14-day pairing period, the female was paired with a male of the same group which mated already successfully. If mating was not recorded during this additional pairing period of maximum 14 days the female was sacrificed and the uterus and ovaries preserved in neutral phosphate buffered 4% formaldehyde solution.

Parameters and endpoints evaluated:

Mortality
The animals were checked at least twice daily for any mortalities. Any animal sacrificed or found dead during the study was subjected to macroscopic examination with, for females, emphasis on the uterus and its contents. Specimens of abnormal tissue were fixed in neutral phosphate buffered formaldehyde solution.

Clinical signs:
The animals were observed at least twice daily for signs of reaction to treatment and/or symptoms of ill health.

Body weights:
Body weights of the treated females were recorded daily during the treatment period and also thereafter until day 21 post-coitum. Untreated females were weighed on days 0, 7, 14 and 21 post-coitum.

Body weights of the males were recorded daily during the treatment period and on the day of termination. During the 13 week withdrawal phase males were weighed weekly.

Food consumption:
The food consumption of the males and females was recorded weekly during the pre-pairing and withdrawal periods. No food consumption was recorded during the pairing periods (males and females) or thereafter (males).

Food consumption of all mated females (treated and untreated) was recorded for days 0-7, 7-14 and 14-21 post-coitum.

Termination of the study:
The termination procedures for treated and untreated females was the same.

On day 21 post-coitum, the females were killed by $C0_2$ asphyxiation and the fetuses removed by Caesarean section.

Caesarean section and Post mortem examination, including gross macroscopic post mortem examination of all internal organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed and the data recorded. The uteri (and contents) of all females with live fetuses were weighed at necropsy on day 21 post-coitum to enable the calculation of the corrected body weight gain. The fetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities and allocated to one of the following procedures:

Sectioning/dissection technique:
1) Half of the number of fetuses from each litter were fixed in Bouin's fixative (one fetus per container). Fetuses of groups 1 and 5 (from treated and untreated females) were examined by a combination of serial sections of the head and micro-dissection of the thorax and abdomen. This included detailed examination of the major blood vessels and sectioning of the heart and kidneys. After examination the sections were preserved in a solution of glycerin/ethanol (one fetus per container). Descriptions of any abnormalities and variations were recorded.

Fetuses of groups 2 - 4 (from treated and untreated females) were stored in Bouin's fixative for possible future examination and were only examined in the event of test item-related abnormalities in group 5.

Fetuses with abnormalities were photographed where applicable.

2) The remaining fetuses were placed in a solution of potassium hydroxide for clearing and stained with alizarin red S (modified technique") The skeletons were examined and all abnormal findings and variations were recorded. The specimens were preserved individually in plastic bags.

If no implantation sites were evident, the uterus was placed in an aqueous solution of ammonium sulfide to accentuate possible hemorrhagic areas of implantation sites.

Fetuses with abnormalities were photographed.

Termination of the study - MALES

Necropsy:
Half of the males in each group were killed after 14 weeks of treatment and the remaining males following a withdrawal phase of at least 13 weeks. The termination procedures for all males was the same. At necropsy samples were taken for sperm analysis. Testes were weighed and any macroscopic abnormalities recorded. Organs showing macroscopic changes, testes and epididymides (left and right organs were differentiated) were preserved against the contingency of histological examination. Testes and epididymides were preserved in Bouin's solution. Other tissues were preserved in neutral phosphate buffered 4% formaldehyde solution.

Sperm analyses

Sperm were analyzed for concentration, motility and morphology.

Results

SUMMARY OF PERFORMANCE OF FO ANIMALS

Group Dose (mg/kg)	1 (0)	2 (10)	3 (30)	4 (100)	5 (300)
Number of males					
at initiation	22	22	22	22	22
killed 14 weeks of treatment	11	11	11	11	9 (A)
killed after withdrawal period	11	11	11	11	9 (B)
Number of treated females	22	22	22	22	22
mating	22	21 (C)	21 (C)	22	19 (C)
pregnant	22	21	20 (D)	22	18 (D)
with live fetuses#	22	21	20	21 (E)	18
Number of untreated females	22	22	22	22	22
mating	22	22	22	22	19 (F)
pregnant	22	21 (G)	22	20 (G)	19
with live fetuses#	22	21	21 (H)	20	18 (H)

(A) = Male nos. 96 and 98 were found dead on treatment days 11 and 7, respectively

(B) = Male no. 109 was found dead on treatment day 12, male no. 104 was killed in extremis on treatment day 12

(C) = Female nos. 147*, 165, 204, 210 and 217* did not mate during the two pairing periods.

(D) = Female nos. 168* and 209* were not pregnant

(E) = Female no. 192 had implantation sites only

(F) = Female nos. 314, 319* and 327* did not mate during the two pairing periods

(G) = Female nos. 257*, 289 and 302* were not pregnant

(H) = Female nos. 278* and 315 had implantation sites only

= Females used for the calculation of body weight gain and food consumption during gestation, and for reproduction data, in the following figures and tables.

* = Females paired with males noted with abnormal findings at histopathological examination of testes

Parental data

Mortality and clinical signs

Three males of group 5 were found dead on days 7, 11 and 12 of treatment, respectively and one male was killed in extremis on day 12 of treatment. Two of these animals revealed abscess-like lesions in the abdominal cavity suggesting a traumatic incident. A direct test item-related effect was considered to be unlikely.

There were no more mortalities or clinical findings among the other males in this group. No clinical signs were noted in groups 4 and 2.

The clinical signs observed in males of groups 3 and 1 were considered not to be test item related.

Females There were no mortalities or clinical signs among females.

Food consumption - MALES Pre-pairing treatment period

Food consumption of males in group 5 was significantly reduced during the 30-week pre-pairing period (-13.4% versus vehicle controls) with a very marked decrease during the first recording period (days 1-8 of treatment: 59.0% versus vehicle controls). Food consumption of males in groups 1-4 was similar throughout the pre-pairing period.

Withdrawal period: Food consumption during the withdrawal period was similar in all groups.

- FEMALES

There was no test item-related effect on the food consumption of treated females either during the two week pre-pairing treatment period, or during gestation.

The slight intergroup variation observed in the mean food consumption of untreated females during gestation was considered to be normal background variation.

Body weights – MALES Pre-pairing treatment period

Consistent with the reduced food consumption, there was a test item-related body weight loss during the first recording period for males in group 5 (-9.1% versus +10.2% in vehicle controls).
Body weight development of males group 2-4 gave no indication for a test item-related effect.

Pairing periods:

During both the first pairing period, with treated females, and the second pairing period, with untreated females, mean body weights of males in group 5 remained lower than that of vehicle controls. This difference reflected the divergence from control value observed during the pre-pairing treatment period. However body weight gain was similar to that of vehicle controls during the pairing periods.

As for the pre-pairing period body weight development of males from groups 2-4 was similar to that of vehicle controls.

Withdrawal period

The mean body weights of the retained males (11 per group) showed a similar pattern of differences from the controls at the start of the withdrawal period, as observed during the pairing periods (22 males per group), with lower mean values in group 5 and similar weights in groups 1-4.

Subsequently, during the withdrawal period, males in group 5 showed a higher weight gain than control males (+20.3% versus +13.8% in vehicle controls) resulting in an only

slightly lower body weight for males in group 5 (463g versus 478g in vehicle controls) at the end of the withdrawal period.

Body weights and body weight development of males in groups 1 - 4 was similar throughout the withdrawal period.

Treated FEMALES - Pre-pairing treatment period

Mean body weights of females were closely similar for all groups, throughout the pre-pairing treatment period. Analysis of body weight gain did show some statistically significant intergroup differences, but these were considered to be incidental and not test item related.

Gestation period

There was no test item-related effect on female body weights during gestation up to the cessation of dosing on day 6 post-coitum or afterwards.

Untreated FEMALES

Body weight development af untreated females during gestation was very similar in all groups. There was no indication for any test item-related effects.

Gross pathology - Males

Among males killed at the end of the two pairing periods (after 14 weeks of treatment), there were 6 males in group 5 with macroscopical changes: two had bilateral renal pelvic dilations, one had a unilateral and one a bilateral reduction in testes and epididymal size, with the small testes containing watery fluid in all cases. Two deceased males were noted with advanced autolysis, in one male in conjunction with jejunum and ileum being distended with gas and in one male in conjunction with one bluish discolored testis.

In group 4 three males were noted with unilateral renal pelvic dilation and one male had a unilateral reduction in testis and epididymal size with the affected testis containing watery fluid. In group 3 two males had a unilateral and two males had a bilateral renal pelvic dilation, one male was noted with unilateral reduction in testis size. In group 2 two mates with unilateral respectively bilateral renal pelvic dilation were noted. In vehicle controls there were two males with unilateral renal pelvic dilation and one male with unilateral pelvic dilation in combination with absent left kidney, left testis, left epididymis and seminal vesicle.

At the end of the withdrawal period in group 5, in two males macroscopic changes of the kidneys were noted (unilateral pelvic dilation and bilateral watery cysts, respectively); in one male the stomach mucosa was thickened and red-brownish discolored, additionally the liver was adherent to the stomach. In one male the right testis was noted to be flaccid and in another male of this group the liver was noted to adhere to stomach, spleen,

duodenum and jejunum, additionally both testis were reduced in size and the content of the body cavities were granulated, mucous, fawn and gray white. There were two males with macroscopic findings in group 4: one with a unilateral renal pelvis dilation and one with bilaterally reduced size of testes and epididymides. In both groups 2 and 3 three animals were noted with macroscopic findings each: in each group two males had unilateral renal pelvic dilation and one was noted with bilateral reduction in testes and epididymal size. No macroscopic findings were observed in group 1.

Females There were no macroscopic changes observed at necropsy for treated or untreated females.

Testes weights

There was no effect on mean testes weights, or on testes weights as % of body weight, either for males killed after the pairing phases, or for males killed after the withdrawal phase.

Testis histopathology

The number of altered tubules (more than one vacuole per tubule, with changes ranging from lowering of the germinal epithelium to tubular atrophy and/or tubules with calcified sperm, summarized as tubular atrophy) was determined at each cut level. In the **control** animals, the altered tubule counts were very low. Most of the control testes showed none (30/43), or only 1 (4/43) altered tubule; however 2 to 4 altered tubules were found in 7/43 testes and 5 to 10 altered tubules in 2/43 testes. Although the total tubules were not counted in control testes, the calculated median percentage of affected tubules in relation to total tubules should be about 0 taking into consideration a total number of 2200 to 3000 tubules in this approach.

At the end of 14-week treatment period, the number of altered tubules in the treated animals was low in relation to the total number of sectioned tubules. In most testes, the percentage of segmental (focal) tubular atrophy was less than 10 % even in animals with massive (>100 tubules affected) tubular atrophy. The highest percentages above 10 % were noted at the end of the treatment period at the dose level of 300 mg/kg/day (animal No. 92: 15.58 %) and at 30 mg/kg/day (animal No. 50: 14.09 %). Overall, the degree of tubular atrophy displayed a heterogeneous pattern. One animal from group 2 (No. 28), another from group 3 (No. 50) and two animals from group 5 (Nos. 90 and 92) revealed marked (> 50 tubules affected) to massive tubular atrophy, while the majority of the rats treated with BSF 208075 displayed only minimal to slight tubular atrophy with less than 1% of the tubules altered (estimation) assuming about 2200 to 3000 tubules in total. As a rule, the epididymides of these animals revealed no abnormalities.

Several rats of all treatment groups (group 2: No. 37; group 3: No. 58; group 4: Nos. 75 and 82; group 5: Nos. 92 and 99) revealed marked or massive diffuse testicular tubular atrophy (defined as >500 altered tubules per testis) associated with uni- or bilateral aspermia (or marked oligospermia) in the epididymides. Most of these animals were

assigned to the 13-week recovery group (apart from animal No. 76 receiving 100 mg/kg/day and Nos. 92 and 99 receiving 300 mg/kg/day). However, in animal No. 107 (Group 5) with a slight (about 600 altered tubules or 30 % affected tubules) to moderate diffuse tubular atrophy there was no concurrent alteration in the epididymis.

In addition, several testes of terminally sacrificed animals revealed minimal tubular dilation which was also attributed to the administration of the test item BSF 208075.

The total number of altered tubules of all animals was about 40 % lower after the 13-week recovery period compared to the results for the animals at the top dose level (300 mg/kg/day) at the end of the 14-week treatment period. Whereas the number of animals affected was reduced during the drug-free recovery period, the percentages of the affected tubules in relation to total tubules did not reach control values after the 13-week recovery period. The segmental tubular atrophy in the testes was considered to be not completely reversible after 14-week treatment with BSF 208075 followed by a 13-week treatment-free recovery period.

REPRODUCTION DATA

Males and treated females (FIRST PAIRING PERIOD)

There was no test item-related effect on median pre-coital time.

The percentage of rats that mated was slightly reduced in group 5 (86.4% versus 100% in vehicle controls) and consequently the fertility index was also decreased (81.8% versus 100% vehicle controls). These findings were considered to be possibly test item related.

In groups 2 - 4, there was no test item-related effect on the mean reproduction data of males, as assessed by percentage mating or fertility indices. However, in all groups treated with the test item single males were noted with abnormal testes findings at histopathological examination. These abnormal findings were in most cases accompanied by reduced testes weights, abnormal findings during sperm analyses and in some cases also with the inability to cause pregnancy. Since only single males were affected in groups 2 - 5, no statistically significant differences could be determined. But in the absence of any abnormal histopathological findings in testes and epididymides in control males, these findings were considered to be test item related.

Males and untreated females (SECOND PAIRING PERIOD)

There was no test item-related effect on median pre-coital time.

In group 5, as for the first pairing period a reduced percentage of mating rats was noted during the second pairing period (86.4% versus 100% in vehicle controls) with a consequently decreased fertility index (86.4% versus 100% in vehicle controls). These findings were considered to be possible test item related.

There was no test item-related effect on the mean reproduction performance of males in groups 2 - 4, as assessed by percentage mating or fertility indices. But as stated for the first pairing period, the occurrence of single males with a combination of abnormal findings affecting the reproductive data in groups 2 -5 (compared to such findings in the vehicle controls) was considered to be test item related.

Sperm analysis

For males killed after 14 weeks of treatment no treatment-related effect on total sperm count or on sperm motility was noted.

In group 5, the mean percentage of morphologically normal sperm was statistically significantly decreased (91.9% versus 96.1% in vehicle controls) and was below the previous control range (95.0 - 97.7%, 5 studies in Wistar rat, left or right vas deferens). However, this was due to the very low percentage of morphologically normal sperm (75.0%) in a single male (no. 92) of this group (this animal showed a diffuse tubular atrophy in testis and aspermia in the epididymis).

The mean value for isolated normal sperm heads from the right vas deferens for males in group 5 was slightly higher than the controls (3.2% versus 1.6% in controls), with the difference showing statistical significance. A similar finding was observed for the left vas deferens samples, without reaching the level of statistical significance (2.2% versus 1.1% in controls). These differences were considered to be minor in nature but may have been test item related.

For the males killed after the withdrawal period no effect on total sperm count, sperm morphology or on sperm motility was noted that was considered to be test item-related.

In group 3, a decrease in morphologically normal sperm was noted (88.0% versus 96.2% in vehicle controls). This decrease was due to a single male (no. 58 with only 3.0% morphologically normal sperm). Since this male showed a diffuse tubular atrophy in testis and aspermia in the epididymis, this finding was considered to be test item related.

Males treated for 14 weeks

Group (mg/kg)	Male No.	Reproductive performance	Sperm analyses	Testes weights	Histopathology findings
2 (10)	28	mated with female Nos. 138 and 248 (both with 13 live fetuses)	increased percentage of abnormally shaped sperm in left vas deferens		diffuse tubular testes atrophy (bilateral)
3 (30)	50	mated with female Nos. 160 and 270 (15 and 14 live fetuses, resp.)	moderate number of sperm motile in left vas deferens increased percentage of abnormally shaped sperm in left vas deferens	relative weight of left testis reduced	diffuse tubular testis atrophy (unilateral, left)
5 (300)	92	mated with female Nos. 202 and 312 (5 and 12 live fetuses, resp.)	no sperm in left vas deferens, moderate number of sperm motile in right vas deferens increased percentage of abnormally shaped sperm in right vas deferens	relative weight of left testis reduced	diffuse tubular testes atrophy (bilateral)
	99	mated with female No. 209 (not pregnant) and did not mate with female No. 319	no sperm in left and right vas deferens	relative weight of both testes reduced	diffuse tubular testes atrophy (bilateral)

Males treated for 14 weeks (necropsied after 13 weeks of recovery)

Group (mg/kg)	Male No.	Reproductive performance	Sperm analyses	Testes weights	Histopathology findings
2 (10)	37	did not mate with female No. 147, mated with female No. 257 (not pregnant)	no sperm in left and right vas deferens	relative weight of both testes reduced	diffuse tubular testes atrophy (bilateral)
3 (30)	58	mated with female No. 168 (not pregnant) and No. 278 (implantation sites only)	number of sperm markedly reduced (bilaterally) with increased percentage of abnormally shaped sperms (left vas deferens) no motile sperm (bilaterally)	relative weight of both testes reduced	diffuse tubular testes atrophy (bilateral)
4 (100)	82	mated with female No.192 (9 live fetuses) and mated with female No. 302 (not pregnant)	no sperm in left and right vas deferens	relative weight of both testes reduced	diffuse tubular testes atrophy (bilateral)
5 (300)	107	did not mate female Nos. 217 and 327	number of sperm markedly reduced with increased percentage of abnormally shaped sperms (bilaterally) moderate number of sperm motile in left and right vas deferens	relative weight of both testes slightly reduced	diffuse tubular testes atrophy (bilateral)

Caesarean section reproduction data from **treated females**

Among treated females, there was a statistically significant increase in pre-irnplantation loss in group 5 (11.7% of corpora lutea vs. 5.9% in vehicle controls) and a consequent reduction in the number of implantations sites as a percentage of corpora lutea and a slightly reduced number of fetuses per dam (11.3 vs. 12.2 in controls).

Although the mean value of pre-implantation loss in group 5 was well within the historical control range (4.8 - 21.7), it was noted that pre-implantation loss in group 4 was also marginally increased (6.5% of corpora lutea). Therefore, the increase in pre-implantation loss in group 5 was considered to be possibly test item-related.

There was no test item-related effect on corpora lutea number, on post-implantation loss or on the numbers of fetuses.

Caesarean section reproduction data from **untreated females**

For the untreated females there was no test item-related effect on corpora lutea number, pre- implantation loss, the number of implantation sites, post-implantation loss or on the numbers of fetuses.

In the absence of a dose relationship the statistically significantly increased post-implantation loss noted in group 3 (8.1% of implantation sites vs. 4.0% in controls) that was mainly due to increased embryonic resorptions was considered to be incidental.

FETAL DATA

Treated dams

Body weights

No effects on mean fetal weights were noted that were considered to be test item-related. Statistically significantly increased fetal body weights in group 5 (4.9 g vs. 4.7 g in controls) were considered to be a consequence of a slightly lower number of fetuses per dam (11.3 vs. 12.2 in controls). The increased fetal body weight in group 4 (4.9 g) was considered to be incidental.

Sex ratio

No test item-related effects on sex ratios were noted.

External examination

In group 2, there was one fetus with an umbilical hernia. No abnormalities were noted in fetuses of groups 5, 3, 4 and 5.

Visceral examination

Visceral examination was limited to fetuses of groups 1 and 5. During visceral examination of fetuses no abnormal findings that were considered to be test item-related were noted.

Findings were noted in:

66% of examined fetuses (in 22/22 litters) of group 1 and in 53% of examined fetuses (in 18/18 titters) of group 5

The findings noted mainly comprised small additional liver lobe(s) in the median cleft (in 60 and 32% of examined fetuses in groups 1 and 5, respectively) and left-sided urnbiltca1 arteries (12 and 22%).

Skeletal examination of fetuses

During skeletal examination of the fetuses, abnormal findings of the bones were noted in:

7% of examined fetuses (in 6/22 litters) of group 1
4% of examined fetuses (in 5/21 litters) of group 2
2 % of examined fetuses (in 3/20 titters) of group 3
2% of examined fetuses (in 2/21 titters) of group 4
4% of examined fetuses (in 418 litters) of group 5

The findings noted comprised common abnormal findings (abnormally ossified sternebrae; wavy ribs, abnormally ossified or fused vertebral bodies and caudal displacement of pelvic girdle) and showed no indication of an effect of the test item; the overall incidence of fetuses with any skeletal abnormality was slightly lower in groups 2, 3, 4 and 5 than for the controls.

Untreated dams

Body weight

No test item-related effects on mean fetal weights were noted. Statistically significant decreased fetal body weights in group 2 (4.8 g vs. 4.9 g in controls) were considered to be incidental.

Sex ratio

No test item-related effects on sex ratios were noted.

External examination

No fetal abnormalities were noted during external examination.

Visceral examination

Visceral examination was limited to fetuses of groups 1 and 5. During visceral examination of fetuses no abnormal findings that were considered to be test item-related were noted.

Findings were noted in:

53% of examined fetuses (in 21/22 litters) of group 1 and in 49% of examined fetuses (in 18/18 litters) of group 5

The findings noted mainly comprised small additional liver lobe(s) in the median cleft (in 33 and 35% of examined fetuses in groups 1 and 5, respectively) and left-sided umbilical arteries (13 and 15%), consistent with treated females.

Skeletal examination of fetuses

(pp. 231-233) During skeletal examination of the fetuses, abnormal findings of the bones were noted in:

6% of examined fetuses (in 7/22 litters) of group 1
l% of examined fetuses (in 8/21 Jitters) of group 2
6% of examined fetuses (in 6/21 titters) of group 3
3% of examined fetuses (in 3/20 litters) of group 4
5% of examined fetuses (in 4/18 litters) of group 5

The findings noted comprised common abnormal findings (abnormally ossified sternebrae; wavy ribs and abnormally ossified or fused vertebral bodies) and showed no indication of an effect of the test item; the overall incidence of fetuses with any skeletal abnormality was similar in all groups.

Study title: BSF 208075: Study for effects on pre- and postnatal development including maternal function in the Han Wistar rat.

Key study findings:

Clear effects on reproduction data were observed at doses of 45 and 150 mg/kg/day (one total litter loss at 150 mg/kg/day, increase in pup loss from days 0 to 4 post partum, increase in pup mortality between day 5 and day 21 post partum and an increase in mean pup weights after day 14 post partum).

At 45 and 150 mg/kg/day, there was an effect in the F0 generation reproductive phase . (increase in pup mortality), and an impairment on the reproductive capability of the F1 generation (decreased mating and fewer pregnancies, decrease of the fertility index), At necropsy, the males of the F1 generation showed a dose-dependent occurrence of small testes.

Based on this data it can be concluded that the no observed effect level (NOEL) for effects on F0 pregnant females and reproduction data was 15 mg/kg/day. This dose was without adverse effect on the development, behavior, sexual maturation, and reproductive performance of the F1 generation.

Study no.: 38654
Conducting laboratory and location: [redacted]
Date of study initiation: 2/14/06
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: 10011, [redacted] purity

Methods

Doses: 0, 15, 45 and 150 mg/kg/day
Species/strain: Rat Han [redacted]:WIST (SPF)
Number/sex/group: 22 mated females
Route, formulation, volume, and infusion rate: oral, gavage, 10 mL/kg. Dosing was from post coitum Day 15 through weaning of the F1 generation.

Parameters and endpoints evaluated:

Body weight (dams, F0 generation): Daily

Food consumption (F0 generation): Food consumption was recorded for the following intervals: days 0-6, 6-11, 11-15 and 15-21 post coitum, and days 1-4, 4-7 and 7-14 post partum

Body weight (reared F1 generation): Weekly

Body weight and food consumption: On days 0-6, 6-11, and 11-14 of gestation. (dams, F1 generation)

Peri-Postnatal observations: The behavior of dams during parturition was observed, if possible. The day of completed parturition was designated day 0 of lactation (post partum). The duration of gestation was calculated. Nesting and nursing behavior of the dams were observed daily.

The following observations were recorded for F1 offspring at birth and during lactation:

sex of pups and number of newborns with gross abnormalities
number of missing (cannibalized) or dead pups (daily)
number of live and dead pups after parturition and on days 1, 4, 7, 14 and 21 of lactation
abnormal findings in the pups (daily)
individual pup weights and mean body weights by sex on days 1, 4, 7, 14, 21, 28 and 35 post partum

Physical development: The following developmental parameters were monitored and behavioral tests for all F1.pups:

pinna unfolding
incisor eruption
onset of coat development
eye opening
testes descent (males only)
cleavage of balano-praeputial gland (males only)
vaginal opening (females only)

The following behavioral tests were performed:

1) Righting reflex, performed on day 14 post partum (+ 1 day). When held by the tail, the animal would normally lift its head.

An attempt by the animal to achieve this posture constituted a positive reaction.

2) Photophobotaxis, performed on day 21 post partum (+ 1 day). The rat normally prefers a dark environment. To test this behavior, the animals will be kept in the dark for 10 minutes, after which they will be placed in a Y-shaped tube with the choice of dark or tight chambers.

Choice of a dark chamber constituted a positive response.

3) Cliff avoidance, performed on day 21 post partum (+ 1 day). The rat was placed on an elevated platform and its behavior observed.

Remaining on the platform constituted a positive behavior.

4) Palmar grasp ability, performed on day 16 post partum (2 1 day). The rat was tested for its ability to support its own weight by grasping a horizontally-suspended wire.

Supporting its own weight constituted a positive reaction.

5) Negative geotaxis (slope - 45°) performed on day 21 post partum (+ 1 day). The rat was placed on an inclined platform and its behaviouor observed.

Choosing the uphill platform after investigating the downhill platform constituted a positive behavior.

6) Exploratory locomotor pattern in a cylindrical cage (21 cm diameter), performed on day 21 post partum (+ 1 day).

Points were awarded according to the following system: 0 points for no activity, 1 point for exploratory behavior, 2 points for rearing against and climbing the wall of the cage and 3 points for trying to escape.

7) Direct pupillary reflex, performed on day 21 post partum (+ 1 day). Contractibility of the pupils constituted a positive reaction.

8) Hearing ability, performed on day 21 post postpartum (+ 1 day).

Positive Preyer's reflex (pinna reflex) when exposed to a tone of frequency 10 kHz; volume 80 dB, duration 30 msec.

9) Water maze tests (three different tests, a, b and c), performed between days 35 and 43 post partum (± 1 day, respectively) on two male and two female pups per litter (randomly selected).

a) Learning test: The ability to find a staircase in a water labyrinth constituted a positive reaction.

b) Memory test: The ability to remember the position of the staircase after 7 days constituted a positive reaction.

c) Relearning test: The ability to find the staircase after changing the position in the water labyrinth constituted a positive reaction,

F1 Generation

After weaning on day 21 post partum, male and female pups of each litter were separated and the dams killed and autopsied. After all developmental / behavioral tests had been

completed, the F1 generation was selected. 22 male and 22 female pups were randomly selected from each group, from as many different litters as possible.

Reproductive performance F1 animals were paired when they were at least 10 weeks old. Siblings were not paired.

F1 females were sacrificed on day14 post coitum. During necropsy, reproduction data and any macroscopic abnormalities were recorded.

Animals which survived to scheduled necropsy were killed by CO_2 asphyxiation. All animals were necropsied and any macroscopic abnormalities were recorded.

F0 parental females were sacrificed after weaning of the offspring on day 21 post partum.

The uteri of all F0 females were placed in an aqueous solution of ammonium sulphide to accentuate possible hemorrhagic areas of implantation sites.

F1 offspring not selected for reproduction were sacrificed when the developmental parameters and behavioral tests had been completed.

F1 parental females were sacrificed on day 14 post coitum and the reproduction data were recorded. If no implantation sites were evident, the uterus was placed in an aqueous solution of ammonium sulphide, as described above.

F1 males were sacrificed after the F1 females.

Results

F0 in-life:

Group Dose (mg/kg/day)	1 (0)	2 (15)	3 (45)	4 (150)
Female numbers	1 - 22	23 - 44	45 - 65	67 - 88
Number of mated females	22	22	22	22
Non pregnant (A)	1	1	1	2
Total pregnant*	21	21	21	20
Dams giving birth	21	21	21	20
Total litter loss (B)	0	0	0	1
With live pups at day 21 post partum	21	21	21	19

* = Used for calculations of group mean values for food consumption and body weight gain during gestation

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MATERNAL DATA

All females survived until scheduled necropsy. No clinical signs were noted in any dam of the groups 1, 2, 3, and 4 during the gestation and lactation periods.

Food consumption

Mean food consumption in groups 1, 2, 3, and 4 was not affected by the treatment with the test item during the gestation period. In group 4, mean food consumption was statistically significantly decreased from day 1 to day 7 p.p.. In group 3 mean food consumption was statistically significantly decreased from day 1 to day 4 p.p. As these decreases in food consumption were not noted in groups 1 and 2, these findings were considered to be treatment related.

Body weight

During gestation, body weight and body weight gain of groups 1, 2, 3, and 4 were not affected by the treatment with the test item.

During lactation, mean body weight and body weight gain of groups 1, 2, and 3 were not affected by treatment with the test item. Body weight gain of group 4 was statistically significantly decreased between days 4 and 10 p.p. This finding was considered to be correlated to the low food consumption between day 1 and day 7 p.p.

Necropsy findings

No test item related findings were noted at necropsy.

REPRODUCTION DATA

Gestation and parturition

The pregnancy rate was generally high (21, 21, 21, and 20 pregnant females in groups 1, 2, 3, and 4, respectively) and the mean duration of gestation was similar in all groups (21.4, 21.6, 21.6, and 21.7, in groups 1, 2, 3, and 4, respectively).

Post implantation loss

The post-implantation loss was similar in all groups.

The post implantation loss in groups 1, 2, 3, and 4 was high with 11, 14, 17, and 14 litters affected and a total of 32, 29, 44, and 39 lost implantations (numbers in order of ascending dose level respectively). No significance was shown for these differences.

Number of pups at first litter check

In groups 1, 2, 3, and 4, the number of living pups were comparable with 236, 270, 220, and 226 in total and 11.2, 12.9, 10.5, and 11.3 as mean/litter, respectively, and therefore not affected by treatment with the test item.

The number of dead pups per group was comparable (1, 0, 1, and 0 in order of ascending dose level) and not affected by treatment with the test item.

Postnatal loss – Day 0-4 post-partum

The incidence of post-natal loss was increased in group 4 (27.4 % missing or dead compared with 0.8 4k in the vehicle control). This included dam no. 70 with a total litter loss at day 2 p.p. Consequently, the number of living pups was statistically significantly reduced on day 4 p.p. (with 164 in total and 8.2 as mean/litter) when compared with the control group on day 4 p.p. (with 234 in total and 11.1 as mean/litter).

The incidence of post-natal loss was also increased in group 3 (11.8 % compared with 0.8 % in the vehicle control). The number of living pups was not significantly reduced on day 4 p.p. (with 194 in total and 9.2 as mean/litter) when compared with the control group on day 4 p.p. (with 234 in total and 11.1 as mean/litter).

This dose-dependent increase of pup loss was considered to be test item-related.

Groups 1 and 2 showed a post-natal loss of 0.8 and 2.6 %, respectively. These data are within the range of historical control data.

Breeding loss - day 5 - 21 post partum

The pup mortality between days 5 to 21 p.p. was statistically significantly increased in group 4 (26 pups for a toss of 15.9 %) when compared with the control group (1 pup for a loss of 0.4 %). In group 3, six pups were lost during this time period (3.1%). This was also statistically significantly increased compared to the control group, These dose dependent increases were considered to be test item-related.

The number of lost pups in group 2 was similar to that of the control group (each with one lost pup for a loss of 0.4%).

After weaning on day 21 p.p., the total number of living pups/dose group was 233, 262, 188, and 138 in order of ascending dose level, with corresponding mean litter sizes of 11.1, 12.5, 9.0, and -statistically significantly decreased- 7.3 pups/litter.

PUP DATA

External examinations

No meaningful differences were apparent among Groups.

Sex ratio

Sex ratios at first litter check and on day 21 p.p. were unaffected by treatment with the test item. The proportion of males / females at first litter check was 45 / 55, 46 / 54, 50 / 50, and 51 / 49% in order of ascending dose level. On day 21 p.p., the proportion of males to females was 45 / 55, 45 / 55, 45 / 55, and 49 / 51 for groups 1, 2, 3, and 4, respectively.

Body weight

In group 4, statistically significantly increased mean pup weights were noted starting on day 14 p.p, until day 21 p.p. This was due to the statistically significantly increased mean male pup weights that started on day 14 p.p. and lasted until day 35 p.p. The mean female pup weight was not statistically significant but also increased when compared with the control mean female pup weights.

In group 3, statistically significantly increased mean pup weights were observed on days 14 p.p. and 21 p.p. On day 14 p.p., the mean female pup weights were statistically significantly increased while on day 21 p.p. the mean mate pup weights were statistically significantly increased. On days 28 and 35 p.p. the mean pup weights stayed increased when compared with the mean pup weights of the control (statistically not significant).

Mean pup weights in group 2 were similar to those of the vehicle control throughout lactation and during the two-week rearing period after weaning.

Developmental indices

In group 4, the opening of vagina was statistically significantly delayed (33.5 days p.p.) when compared with the control females (32.3 days p.p.)

The majority of developmental indices (pinna unfolding, incisor eruption, onset of coat development, opening of eye, testes descent, and balano-preputial separation) were similar in all groups and gave no indication of a test item-related effect on pup development.

In groups 3 and 4, the opening of eyes were statistically significantly earlier (day 14.1 p.p. and 14.3 p.p., respectively) when compared with the control group (day 14.9 p.p.). These findings lay within the normal range of biological variation.

Behavioral tests

No test item related effects were noted on the different behavioral tests: righting reflex, photo-phobotaxis, cliff avoidance, palmar grasp ability, negative geotaxis, exploratory locomotor activity, papillary reflex, hearing ability, or water maze test.

Clinical signs during lactation and rearing periods (days 0 - 35 post partum)

(pp. 112, 113, 384-422)

No test item related clinical signs in the pups were noted during lactation or during the rearing period after weaning.

Thirty-one pups in group 4 were seen without milk in the stomach between day 2 p.p. to day 6 p.p.. Starting on day 6 p.p. milk was noted in the stomach of eight of these pups. Five-of these survived. Others without milk were missing same days later or were found dead.

F1 GENERATION ANIMALS, REARED FOR REPRODUCTION

All animals selected for reproduction survived until scheduled necropsy. No test item-related clinical signs or signs of discomfort were noted.

Food consumption and body weights, MALES

During the pre-pairing and after pairing periods, food consumption, body weight and body weight gain of groups 2, 3, and 4 were similar to food consumption, body weight and body weight gain of group 1, Thus, no indication of a test item-related effect was noted.

Food consumption and body weights, FEMALES

Pre-pairing period and Gestation period

Mean food consumption of females was similar in all groups and showed no indication of a test item-related effect.

Body weight and body weight gain were similar in all groups and showed no indication of a test item-related effect.

Mating performance:

Group Dose (mg/kg/day) *		1 (0)	2 (15)	3 (45)	4 (150)
Animal per group	males	22	22	22	22
	females	22	22	22	22
Number of mated females (A)		22	22	21	20
Non pregnant (B)		0	0	1	3
Total pregnant**		22	22	20	17
First mating not detected (C)		0	0	1	0
Number of pregnant females (with necropsy on day 14 p.c.)***		22	22	19	17

* = indicates the dosages administered to the females of the F0-generation

** = Used for calculation of group mean values for reproduction data

*** = Used for calculation of group mean values for food consumption, body weight and body weight gain

(A)= Female Nos. 347, 367, and 387 were not mated.

(B) = Female Nos. 366, 373, 376, and 384 were not pregnant.

(C) = Female No. 346 was necropsied later than day 14 p.c. because the first mating was not detected.

REPRODUCTION DATA

Three females, one in group 3 and 2 in group 4 did not mate in the 14-day pairing period. These findings are of uncertain relationship to treatment.

The mean and median pre-coital times were not affected by the test item. Mean pre-coital times were 2.9, 2.9, 2.7, and 3.2 days in order of ascending dose level. Median pre-coital time was 3 days in groups 1, 2, 3, and 4.

The fertility index [(# females achieving a pregnancy / # females paired) x 100] was 100%, 100%, 90.9%, and 77.3% in order of ascending dose level. The decrease in fertility index for groups 3 and 4 was considered test item-related.

The mean corpora lutea counts were 14.3, 13.5, 13.0, and 13.2 in groups 1, 2, 3, and 4, respectively. Pre-implantation loss was 7.0, 6.0, 10.0, and 10.2 %, in order of ascending dose level. Post-implantation loss was 8.8, 6.4, 3.8, and 2.5 % in order of ascending dose level. The mean number of live embryos/group were 12.4, 11.9, 11.3, 11.6 for groups 1, 2, 3 and 4, respectively. These findings showed no indication of a test item-related effect.

Necropsy findings

During necropsy, in group 4, three males were noted with small testes. One male was seen with small testes, small epididymides and small liver and one male had testes with white spots. Five dams in Group 4 (F1) were not pregnant.
All other males and females in group 4 were noted without any abnormal finding.

In group 3, one male was observed with small testes, small epididymides and small liver. Two dams were not pregnant.

All other males and females in group 3 had no abnormal findings. In group 1 and 2, no abnormal findings were noted in any male or any dam.

BREEDING DATA PER GROUP
F0 GENERATION - ALL DAMS GIVING BIRTH

	GROUP 1 0 MG/KG	GROUP 2 15 MG/KG	GROUP 3 45 MG/KG	GROUP 4 150 MG/KG
LITTERS				
TOTAL	21	21	21	20
DURATION OF GESTATION				
MEAN (+)	21.4	21.5	21.6	21.7
ST.DEV	0.59	0.51	0.50	0.49
N	21	21	21	20
IMPLANTATIONS				
TOTAL	268	299	264	265
MEAN (+)	12.8	14.2	12.6	13.3
ST.DEV	2.91	1.48	2.42	3.09
N	21	21	21	20
POST IMPLANTATION LOSS				
% OF IMPLANTATIONS	11.9	9.7	16.7	14.7
LITTERS AFFECTED (#)	11	14	17	14
TOTAL (#)	32	29	44	39
MEAN (+)	1.5	1.4	2.1	2.0
ST.DEV	2.27	1.53	2.72	1.93
N	21	21	21	20
DEAD PUPS AT FIRST LITTER CHECK				
LITTERS AFFECTED (#)	1	0	1	0
TOTAL	1	0	1	0
MEAN (+)	0.0	0.0	0.0	0.0
ST.DEV	0.22	0.00	0.22	0.00
N	21	21	21	20
LIVING PUPS AT FIRST LITTER CHECK				
% OF MALES / FEMALES (#)	45 / 55	46 / 54	50 / 50	50 / 50
TOTAL	236	270	220	226
MEAN (+)	11.2	12.9	10.5	11.3
ST.DEV	3.77	1.62	3.20	2.77
N	21	21	21	20
POSTNATAL LOSS DAYS 0 - 4 P.P.				
% OF LIVING PUPS	0.8	2.6	11.8	27.4
LITTERS AFFECTED (#)	2	5	8 #	14 ##
TOTAL (#)	2	7	26 ##	62 ##
MEAN (+)	0.1	0.3	1.2	3.1 +
ST.DEV	0.30	0.73	2.36	3.34
N	21	21	21	20
LIVING PUPS DAY 4 P.P.				
TOTAL	234	263	194	164
MEAN (+)	11.1	12.5	9.2	8.2 +
ST.DEV	3.85	1.69	3.30	3.46
N	21	21	21	20
BREEDING LOSS DAYS 5 - 21 P.P.				
% OF LIVING PUPS AT DAY 4 P.P.	0.4	0.4	3.1	15.9
LITTERS AFFECTED (#)	1	1	3	15 ##
TOTAL (#)	1	1	6 #	26 ##
MEAN (+)	0.0	0.0	0.3	1.3 +
ST.DEV	0.22	0.22	0.72	1.03
N	21	21	21	20
LIVING PUPS DAY 21 P.P.				
% OF MALES / FEMALES (#)	45 / 55	45 / 55	45 / 55	49 / 51
TOTAL	233	262	188	138
MEAN (+)	11.1	12.5	9.0	6.9 +
ST.DEV	3.86	1.69	3.46	3.04
N	21	21	21	20

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FERTILITY
F1 GENERATION

FEMALES SCHEDULED FOR CAESAREAN SECTION

	GROUP 1 0 MG/KG	GROUP 2 15 MG/KG	GROUP 3 45 MG/KG	GROUP 4 150 MG/KG
Percentage mating	100.0	100.0	95.5	90.9
Fertility index (%)	100.0	100.0	90.9	77.3 #
Conception rate (%)	100.0	100.0	95.2	85.0
Gestation index (%) (Caesarean section)	100.0	100.0	100.0	100.0

REPRODUCTION DATA SUMMARY
F1 GENERATION

	GROUP 1 0 MG/KG	GROUP 2 15 MG/KG	GROUP 3 45 MG/KG	GROUP 4 150 MG/KG
NUMBER OF DAMS	22	22	20	17
CORPORA LUTEA	314	298	260	225
MEAN (+)	14.3	13.5	13.0 +	13.2
ST.DEV.	1.5	1.9	1.1	1.8
PRE-IMPLANTATION LOSS	22	18	26	23
% OF CORP. LUTEA (#)	7.0	6.0	10.0	10.2
MEAN (+)	1.0	0.8	1.3	1.4
ST.DEV.	1.3	1.0	1.3	1.0
NUMBER OF DAMS AFFECTED	12	11	15	13
IMPLANTATION SITES	292	280	234	202
% OF CORP. LUTEA (#)	93.0	94.0	90.0	89.8
MEAN (+)	13.3	12.7	11.7	11.9
ST.DEV.	2.1	2.2	1.6	2.0
POST-IMPLANTATION LOSS	20	18	9	5
% OF IMPL. SITES (#)	6.8	6.4	3.8	2.5 #
MEAN (+)	0.9	0.8	0.5	0.3
ST.DEV.	0.9	1.2	0.8	0.6
NUMBER OF DAMS AFFECTED	13	11	6	4
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC RESORPTIONS	20	18	9	5
EMBRYOS				
TOTAL EMBRYOS	272	262	225	197
% OF IMPL. SITES (#)	93.2	93.6	96.2	97.5 #
MEAN (+)	12.4	11.9	11.3	11.6
ST.DEV.	2.1	2.8	1.7	2.0
LIVE EMBRYOS	272	262	225	197
DEAD EMBRYOS	0	0	0	0
ABNORMAL EMBRYOS	0	0	0	0

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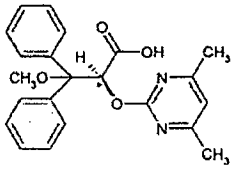
PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: **64,915**
Review number: 2
Sequence number/date/type of submission: **010**, 12/10/02, 26 week rat and dog tox studies (**draft reports**)
Information to sponsor:
Sponsor and/or agent: Myogen, Inc., Westminster, Colorado
Manufacturer for drug substance :

[redacted] have entered into a license agreement whereas [redacted] is responsible for Phase II trials and [redacted] is responsible to supply drug substance and bulk finished product. [redacted] discovered BSF 208075 (also known as LU 208075) and has developed the processes and controls for active pharmaceutical ingredient (API) and the finished dosage forms (film coated tablets). [redacted] is the site where tablets have been manufactured. At the time of the agreement between [redacted] was a division of [redacted] contracted [redacted] to package, test, release and conduct stability trials on the packaged finished product used in the clinical trials.

Reviewer name: William T. Link, Ph.D.
Division name: Cardio-Renal Drug Products DCRDP
HFD #: 110
Review completion date:

Drug:
Trade name: to be determined
Generic name (list alphabetically): to be determined
Code name: **BSF 208075** or **LU 208075**
Chemical name: (S)-2-(4,6-Dimethyl-pyrimidine-2-yloxy)-3-methoxy-3, 3-diphenyl-propionic acid
CAS registry number:
Mole file number:
Molecular formula/molecular weight: 378.43 g/Mol
Structure:



Relevant INDs/NDAs/DMFs: [redacted]

Drug class: endothelin receptor antagonist (ET_A selective)

Indication: pulmonary arterial hypertension

Clinical formulation: [redacted] 5 and 10 mg tablets

Route of administration: oral

Preclinical studies:

This amendment contained **draft** reports of 26 week toxicology studies in rat and dog, reviewed below.

Summary to date:

LU 208075 was evaluated with chronic dosing in rats and dogs for durations up to 13 weeks in GLP toxicology assays. The target organs in both species consist mainly of the liver, kidneys, testes (rat), GI tract and nasal ethmoturbinates (rat). Findings in the liver were confined to hepatocellular hypertrophy and moderate induction of hepatic enzymes. The NOAEL for liver toxicity findings was 40 mg/kg in rats, the more sensitive species. Kidney and GI tract findings were mainly gross observations of dilatation, swelling and reddening, indicative of mild inflammatory processes. The findings in nasal tissue are of uncertain origin, but are dose-dependent and found in the rat only. The dosing was well tolerated in the dog and the NOAEL for toxic signs was much higher than in the rat.

Testicular atrophy was found in mice and rats at moderate incidence, but not in dogs. There is a suggestion of dose-dependence and the findings persist after 4 weeks recovery. The NOAEL for this finding is within the range of expected human exposure in the planned clinical trials. The possibility of effects on male fertility in the affected species has not been evaluated.

Safety Pharmacology

Neurological effects: Both oral and intravenous dosing with BSF 208075 were evaluated for neurological effects. No effects on motor coordination or locomotion were detected in standardized tests. Behavioral effects were limited to small increases in alertness and exploratory activity and not all animals were affected. There were no stimulatory effects as measured by time-to-sleep or sleep duration following hexobarbitone administration.

Changes in EEG indicate disruption of waking, classical sleep and paradoxical sleep patterns. A marked decrease in paradoxical sleep duration was observed in female mice. The relevance to humans is unclear.

There were no discernible effects on neuromuscular transmission.

Cardiovascular effects: A prolonged decrease in blood pressure, consistent with the known pharmacology of this class of compounds was observed in rats and dogs following

oral or iv dosing. An increase in heart rate was variably observed. ECG changes were not observed in treated dogs.

Pulmonary effects: There were no studies evaluating pulmonary performance except respiratory rate monitored in the cardiovascular study. Studies on pulmonary compliance and airflow need to be conducted.

Renal effects: Decreases in sodium, chloride, and calcium to lesser extent, excretion were observed. These probably reflect the decreased renal perfusion pressure associated with the fall in blood pressure.

Gastrointestinal effects: There were no effects on GI motility or secretion following dosing. A dose-dependent increase in bile flow was apparent following intraduodenal and iv dosing.

Genetic toxicology:

LU 208075 was evaluated in the reverse mutation, human lymphocyte, micronucleus, chromosomal aberration and unscheduled DNA synthesis assays. The compound was clearly clastogenic *in vitro* (human lymphocytes), producing both included and excluded gaps at non-cytotoxic levels in a concentration-dependent fashion. No numerical aberrations were observed. All other genetic toxicology assays were adequately performed and were negative.

Reproductive toxicology:

Consistent with the findings in all other ET receptor antagonists, LU 208075 produced craniofacial abnormalities in dose-dependent fashion in both rats and rabbits. NOAELs were not established in two assays in each species and are <15 mg/kg and <7 mg/kg in rats and rabbits respectively. Additionally, rabbits showed increased post-implantation losses with dosing possibly associated with maternal toxicity. NOAELs for maternal toxicity were 150 and 21 mg/kg/day in rats and rabbits, respectively. Effects on fertility have not been evaluated.

Study Title: BSF 208075: 26-Week Repeated Dose Oral Toxicity (Feeding) Study in the Wistar Rat

Key study findings: Morbidity/mortality was observed at 500 mg/kg, a dose which exhibited little toxicity in 13 week studies, indicating duration-dependent toxicity which is manifest between 16 and 20 weeks. Results indicate the toxicity involves cardiovascular failure, probably associated with hypoxia and acidosis resulting from airway obstruction due to nasal turbinate hyperplasia. Clinical signs of labored breathing are consistent with this conclusion.

Study no: [redacted]Study No. 800313, [redacted] MPR/PT 0106 E
Volume #, and page #: *v4.3:p001*
Conducting laboratory and location: [redacted]

Date of study initiation: 7/4/2001
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. Lot No. 10020
Formulation/vehicle: food admixture

Methods:

Species/strain; sex: rat, HanBrl:Wistar (SPF), males and females
Doses: 0 (control), 5, 100 and 500 mg/kg/day
Duration: 26 weeks
Main study No. /group: 12/sex/group
Additional groups: 6/sex/group for 20-week recovery, 9/sex/group for toxicokinetics

Allocation and Target Dose Levels	Group 1* 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day
Males A	1-12	28-39	55-66	82-93
Males B	13-18	40-45	67-72	94-99
Males C1	19-21	46-48	73-75	100-102
Males C2	22-24	49-51	76-78	103-105
Males C3	25-27	52-54	79-81	106-108
Females A	109-120	136-147	163-174	190-201
Females B	121-126	148-153	175-180	202-207
Females C1	127-129	154-156	181-183	208-210
Females C2	130-132	157-159	184-186	211-213
Females C3	133-135	160-162	187-189	214-216

A - Main Study (termination after 26 weeks of treatment)
B – Recovery (termination after 26 weeks of treatment and 20 weeks of recovery)
*Control animals received identical feed without the test item.
C1 to C3 – Toxicokinetics

Parameters examined:

Mortality: twice daily
Clinical signs: daily
Food consumption: weekly
Body weights: weekly

Ophthalmoscopy: pretest, 26 weeks and following recovery (week 46)
Hematology: 4, 13 and 26 weeks, recovery (week 46); the following parameters were measured:

Hematology Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	1
Hemoglobin	HB	mmol/L	1
Hematocrit	HCT	L/L	1
Mean corpuscular volume	MCV	fL	1
Mean corpuscular hemoglobin	MCH	fmol	1
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	1
Platelet count	PLATELETS	G/L	1
Reticulocyte count	RETIC.	%(rel.) T/L(abs.)	2
Reticulocyte fluorescence ratios	HFR = high, MFR = middle, LFR = low	%	2
Nucleated erythrocytes (normoblasts)	NEN	NEN/100WBC	3

Hematology Parameter	Abbreviation	Unit	Instrumentation
Total leukocyte count	WBC	G/L	1
Differential leukocyte count	Diff. WBC Count	%(rel.) G/L(abs.)	3
Red blood cell morphology	TYPE CELL	normal/abnormal	3
Thromboplastin time (=prothrombin time)	PT	sec	5
Activated partial thromboplastin time	APTT	sec	5

Key:
1 [redacted] Multi-Parameter Automated Hematology Analyzer
2 [redacted] Automated Reticulocyte Analyzer
3 [redacted] light microscope and [redacted]
Computer Printer Blood Cell Calculator
4 Cell Classification: BAND. = Band Neutrophil; SEG. = Segmented Neutrophil; EO. = Eosinophil; BASO. = Basophil; LYMPH = Lymphocyte; MONO. = Monocyte; PLAS. = Plasma Cell; OTHER = Blast Cell (undifferentiated)
5 [redacted] Coagulation System

Clinical chemistry: 4, 13 and 26 weeks, recovery (week 46). The following parameters were measured:

Clinical Biochemistry Parameter	Abbreviation	Unit	Instrumentation
Glucose		mmol/L	1
Urea		mmol/L	1
Creatinine		µmol/L	1
Bilirubin, total	BILI. T	µmol/L	1
Lipids total*	LIPIDS T.	g/L	1
Cholesterol, total	CHOLEST. T	mmol/L	1
Triglycerides	TRIGL.	mmol/L	1
Phospholipids	PHOS.LIPID	mmol/L	1
Aspartate aminotransferase	ASAT/GOT	µkat/L (37°C)	1
Alanine aminotransferase	ALAT/GPT	µkat/L (37°C)	1
Lactate dehydrogenase	LDH	µkat/L (37°C)	1
Creatine kinase	CK	µkat/L (37°C)	1
Alkaline phosphatase	ALP	µkat/L (37°C)	1
Gamma-glutamyl transferase	G-GT	nkat/L (37°C)	1
Calcium		mmol/L	1
Phosphorus		mmol/L	1
Sodium		mmol/L	1
Potassium		mmol/L	1
Chloride		mmol/L	1
Protein, total	PROTEIN T.	g/L	1
Protein, electrophoresis		g/L	2
Albumin	ALBUMIN		
Alpha 1-globulin	A1-GLOB.		
Alpha 2-globulin	A2-GLOB.		
Beta globulin	B-GLOB.		
Gamma globulin	G-GLOB.		
Globulin		g/L	2
Albumin/Globulin ratio	A/G RATIO	---	2

Key:
* total lipids were determined on weeks 4 and 13 only
1 [redacted] Discrete Random-Access Analyzer
2 [redacted] Automated Multiparameter Agarose Gel Electrophoresis Processing System and Multitask Scanning Densitometer System

Urinalysis: 4, 13 and 26 weeks, recovery (week 46); the following parameters were measured:

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Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Volume (18-hour)		ml	1
Specific gravity	SPEC. GRAV.	1	2
Osmolality		mmol/kg	3
Color		-	4
Appearance		-	4
pH		-	5
Protein		0 = negative 1 = 0.25 g/L 2 = 0.75 g/L 2 = 1.50 g/L 3 = ≥5.00 g/L	5
Glucose		0 = normal 1 = 3 mmol/L 1 = 6 mmol/L 2 = 17 mmol/L 3 = ≥56 mmol/L	5
Ketone		0 = negative 0 = 0.5 mmol/L 1 = 1.5 mmol/L 2 = 5.0 mmol/L 3 = ≥15 mmol/L	5
Bilirubin		0 = negative 1 = 17 µmol/L 2 = 50 µmol/L 3 = ≥100 µmol/L	5

Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Blood		0 = negative 1 = 15 RBCs/µL 1 = 25 RBCs/µL 2 = 80 RBCs/µL 2 = 150 RBCs/µL 3 = ≥250 RBCs/µL	5
Nitrite		negative / positive	5
Urobilinogen	UROBILI.	0 = normal 1 = 17 µmol/L 2 = 68 µmol/L 3 = 135 µmol/L 3 = ≥ 203 µmol/L	5
Urine sediment	SED. MICRO.	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Red blood cells	RBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
White blood cells	WBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Crystals (Triple phosphate)	TRIP. PHOS	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6

Key:
1 Metabolism cage
2 Refractometer
3 [redacted] Multi-Sample Osmometer
4 Visual observation
5 Reagent-Test-Strip [redacted] used with the [redacted] Semi-Automated Urine Chemistry Analyzer
6 [redacted] light microscope

Histopathology: The following Table lists all tissues collected at necropsy. Those in boldface were examined in control and high dose rats only. Those underlined were examined in all groups.

Adrenal glands
Aorta
Auricles
Bone with bone marrow (femur including articular surface, sternum)
Brain - including section of medulla/pons, cerebral and cerebellar cortex
Cecum
Colon
Duodenum
Epididymides
Ductus deferens
Esophagus
Eyes with optic nerve
Harderian glands
Heart
Ileum
Jejunum
Kidneys
Lacrimal glands
Liver
Lungs, infused with formalin at necropsy
Lymph nodes – mandibular, bronchial, mesenteric
Mammary gland area
Nasal turbinates
Ovaries
Pancreas
Pituitary gland
Prostate gland
Rectum
Salivary glands - mandibular, sublingual, parotid
Sciatic nerve
Seminal vesicles
Skeletal muscle (thigh)
Skin
Spinal cord - cervical, midthoracic, lumbar
Spleen
Stomach
Testes
Thymus
Thyroid gland / parathyroid gland
Tongue
Trachea
Urinary bladder, infused with formalin at necropsy
Uterus/cervix
Vagina
All gross lesions

The following organ weights were recorded on the scheduled dates of necropsy:

Adrenals	Liver	Prostate gland
Brain	Mesenteric lymph nodes	Spleen
Heart	Ovaries	Testes
Kidneys	Pituitary gland	Thymus

The organ to terminal body weight ratios as well as organ to brain weight ratios were determined.

The determination of the terminal body weight was performed immediately prior to necropsy.

Toxicokinetics: Blood for plasma levels of BSF 208075 was collected on Day 2, and Week 13 and 26 of dosing. The times for collection were 0800, 1200, 2000, 2400, 0400 and 0800 hrs.

Results:

Test item intake: was within acceptable limits as follows:

Group	Target Dose	Males		Females	
	(mg/kg)	Value	% of target dose	Value	% of target dose
1	0	—	—	—	—
2	5	4.94	98.8	5.03	100.6
3	100	98.90	98.9	102.08	102.1
4	500	485.55	97.1	507.46	101.5

Mortality: Mortality findings are summarized below. All deaths are considered treatment-related. Deaths in "recovery" groups occurred during the treatment period and not during recovery.

	Group 1 (control) 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day
Main study - **Male**	0/12	0/12	1/12	7/12
Recovery - **Male**	0/6	0/6	1/6	4/6
TK - **Male**	0/9	0/9	0/9	5/9
Main study - **Female**	0/12	0/12	2/12	10/12
Recovery - **Female**	0/6	0/6	1/6	5/6
TK - **Female**	0/9	0/9	1/9	7/9

Clinical signs:

In animals treated with 500 mg/kg/day, moderate clinical signs were noted starting in weeks 10 (females) and 17 (males) of the treatment period and ending at week 6 (rales only) of the recovery period. They were restricted to: hunched posture, uncoordinated movements, breathing disturbances (labored respiration and rales), emaciation, swelling of the abdomen, poor condition, blue skin and ruffled fur. At 100 mg/kg/day, the clinical symptoms were quite similar, but with lower incidence. Starting in weeks 21/19 (males/females) of the treatment period, breathing disturbances (tachypnea, labored respiration and rales), ruffled fur, hunched posture, and emaciation were noted. At both dose levels, all clinical signs were no longer apparent after week 6 of the recovery period.

Food consumption

The absolute daily food consumption was reduced in males and females at 100 and 500 mg/kg/day, starting in week 4 in males (-8.1 and -8.5% at 100 and 500 mg/kg/day, respectively) and in week 7 in females (-7.2 and -10.8% at 100 and 500 mg/kg/day, respectively). The reduction was dose dependent and more pronounced at the end of the treatment period (-15.4 and -30.8% in males at 100 and 500 mg/kg/day, respectively, and -16.7 and -28% in females at 100 and 500 mg/kg/day, respectively).

Several changes in food consumption were evident at 5 mg/kg/day males during the treatment period, but they were considered to be incidental as there were some higher or lower levels when compared with the controls.

During the recovery period, in males at 100 and 500 mg/kg/day, the mean daily food consumption was compensatory increased starting with +25.4% at 100 mg/kg/day and +27.8% at 500 mg/kg/day. This finding persisted at 100 mg/kg/day until week 17 of the recovery period (+21.5%) and at 500 mg/kg/day until the scheduled necropsy (+29.8%) in week 46.

In addition, increased mean daily food consumption was noted in males at 5 mg/kg/day during several weeks of the recovery period. Starting with +11 .8% increased food consumption in week 1 of the recovery period and ending with +19.0% before necropsy.

Body weight

In males and females at 100 and 500 mg/kg/day, the body weights were markedly reduced after the 26 week treatment period (-14.2 and -34.7% in males, respectively, and -14.3 and -22.6% in females, respectively).

The onset of body weight reduction was earlier and more accentuated at 500 mg/kg/day (week 6 in males or 9 in females) than at 100 mg/kg/day (week 14 in males or 18 in females). The overall reduction was more accentuated in males than in females. These findings corresponded with the reduction in the absolute food consumption and were clearly test item-related.

The compensatory higher food consumption in the affected groups during the recovery period led to an increase in body weight gain compared to the controls resulting in

comparable overall body weights after only a few weeks of recovery. In males treated with 5 mg/kg/day, increased body weight (21 - 22%) was measured, which corresponded to the increased food consumption in this period.

Hematology

The hematological investigations showed the following differences between control and test item-treated groups:

500 mg/kg/day:

Red blood cell counts, hemoglobin and hematocrit levels were slightly decreased in males after 4 weeks; slightly increased in both sexes after 13 weeks; markedly increased in males and females after 26 weeks of treatment. Decreased platelet counts were noted in males and females after 13 weeks, and 26 weeks. Slightly decreased mean corpuscular hemoglobin concentration was noted in males after 13 weeks and in females after 26 weeks of treatment. Increased reticulocyte counts were noted in females after 13 and 26 weeks of treatment and in males after 26 weeks of treatment weeks of treatment. A shift towards lower flourescent reticulocytes was noted in females and males after 13 weeks of treatment. White blood cell counts in association with absolute lymphocytes were slightly decreased in males after 13 and 26 weeks.

100 mg/kg/day:

Moderate increased reticulocyte counts with a left shift towards lower fluorescent reticulocytes were noted in females after 13 weeks of treatment, and increased reticulocyte counts were noted in both sexes after 26 weeks of treatment. Red blood cell count, hemoglobin and hematocrit levels were markedly increased in males and females after 26 weeks of treatment. Decreased platelet count and slightly decreased mean corpuscular hemoglobin concentration and were measured in females at the end of the 26 week treatment period.

Hematology changes are summarized below.

The table below shows the test item related differences from the controls in percent.

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)									
Dose	Parameter	Males				Females			
		Week 4	Week 13	Week 26	Week 46	Week 4	Week 13	Week 26	Week 46
100 mg/kg/day	RBC			+14%			+7%	+22%	
	Hemoglobin			+13%				+20%	
	Hematocrit			+15%				+23%	
	MCHC					-2%		-2%	
	HFR						-46%		
	MFR			-25%			-12%	-16%	
	LFR			+11%			+12%		
	Platelets							-23%	
	Retic. (rel.)						+20%	+27%	
	Retic. (abs.)			+32%			+30%	+59%	
500 mg/kg/day	RBC	-4%	+14%	+31%			+14%	+35%	
	Hemoglobin	-3%	+10%	+30%			+12%	+33%	
	Hematocrit	-4%	+11%	+38%			+15%	+40%	
	MCV		-3%	+6%		+4%			
	MCHC			-5%		-3%	-2%	-6%	
	HFR			+89%				+183%	
	MFR		-23%	-19%			-17%		
	LFR		+12%				+10%		
	Platelets		-16%	-50%			-25%	-44%	
	Retic. (rel.)			+67%			+28%	+99%	
	Retic. (abs.)			+110%			+45%	+157%	
	WBC		-17%	-22%					
	Lymphocytes (abs.)		-23%	-28%					

Serum biochemistry

Changes are summarized as follows:

The table below shows the test item related differences from the controls in percent.

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)									
Dose	Parameter	Males				Females			
		Week 4	Week 13	Week 26	Week 46	Week 4	Week 13	Week 26	Week 46
5 mg/kg/day	Bilirubin, total		-27%			-21%			
	Triglycerides	-45%	-28%			-15%		-20%	
	Protein (total)							-5%	
	Chloride		-2%						
100 mg/kg/day	Bilirubin, total	-24%	-42%			-22%	-27%		
	ALP							+88%	
	γ-GT							+196%	
	Triglycerides	-33%							
	Phospholipid			-17%				-31%	
	CK							+42%	
	Protein (total)						-6%	-12%	
	Sodium			+1%				+2%	
	Chloride	-1%	-4%	-5%		-2%	-4%	-5%	
500 mg/kg/day	Bilirubin, total	-29%				-26%			
	ASAT		+31%	+127%			+47%		
	ALAT		+39%	+108%					
	ALP	-17%	+26%	+62%			+69%	+154%	
	γ-GT		+29%	(+22%)			(+49%)		
	Triglycerides	-29%	-25%			-19%			
	Phospholipid		-13%	-19%		-16%	-27%	-37%	
	Cholesterol		-13%				-21%		
	Glucose	-18%	-33%	(-17%)			-16%	-39%	
	LDH			+169%			+66%	(+29%)	
	CK			+123%			+31%	(+44%)	
	Protein (total)	-2%	-4%	-22%			-10%	-15%	
	Urea			+107%					
	Phosphorus	-7%	+9%	+43%			+31%	+95%	
	Sodium	+1%	+1%	+2%			+2%	+4%	
	Chloride	-1%	-6%	-8%		-3%	-6%	-7%	

( ) = statistically not significant

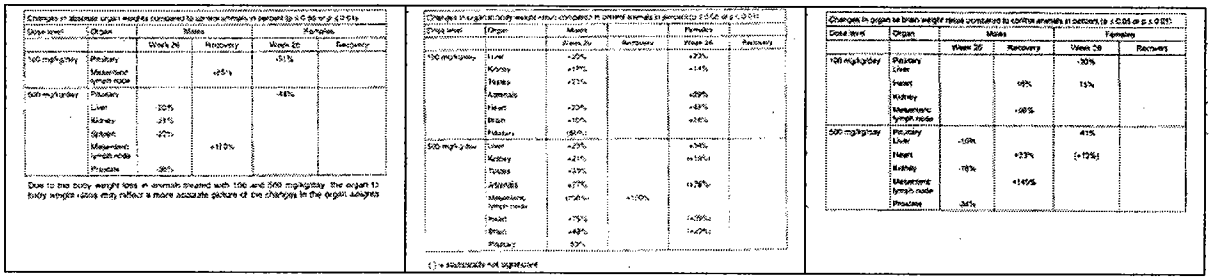
Urinalysis

After 4 weeks of treatment slightly lower urine volume, correlating with higher specific

gravity and higher osmolality, as well as increased ketone levels were noted in males treated with 500 mg/kg/day. After 13 weeks slightly higher specific gravity and higher osmolality was measured in males at 500 mg/kg/day when compared with the controls.

Organ weights

In males and females, increased liver, heart and kidney/body weight ratios were seen in males and females at 100 and 500 mg/kg/day. In addition, the adrenal gland/body weight ratio was increased in males at 500 mg/kg/day and in females at 100 and 500 mg/kg/day. In males, an increased pituitary gland/body ratio was measured at 500 mg/kg/day. In males, an increase in the testis/body weight ration was observed after 100 and 500 mg/kg/day. Absolute, and relative changes are summarized as follows:



Macroscopic findings:

- General observations: Dark red, reddish or bluish discoloration in 2 males and 6 females at
100 mg/kg/day, and 8 males and 8 females at 500 mg/kg/day; emaciation in 2 males and 3 females at 100 mg/kg/day, and 9 males and 3 females at 500 mg/kg/day.

- Lung: Reddish or dark red discoloration in 1 female at 5 mg/kg/day, and 2 males and 5 females at 500 mg/kg/day.

- Jejunum: Distended with gas in 1 male and 3 females at 100 mg/kg/day and 6 males and 2 females at 500 mg/kg/day.

- Ileum: Distended with gas in 1 male and 3 females at 100 mg/kg/day and 7 males and 8 females at 500 mg/kg/day.

- Cecum: Distended with gas in 1 male and 2 females at 100 mg/kg/day, and 6 males and 8 females at 500 mg/kg/day.

- Colon: Distended with gas in 1 male and 2 females at 100 mg/kg/day and 5 males and 3 females at 500 mg/kg/day.

- Testes: Reduced in size in 1 male at 5 mg/kg/day, 2 males at 100 mg/kg/day, and 6 males at 500 mg/kg/day; flaccid in 1 male at 5 mg/kg/day, 3 males at 100 mg/kg/day, and 4 males at 500 mg/kg/day.

- Epididymides: Reduced in size in I male at 5 mg/kg/day, 2 males at 100 mg/kg/day, and 4
males at 500 mg/kg/day.

- Prostate: Reduced in size in 4 males at 100 mg/kg/day and 7 males at 500 mg/kg/day.

- Seminal vesicles: Reduced in size in 2 males at 100 mg/kg/day and 11 males at 500 mg/kg/day.

- Ovaries: Dark red discoloration in 3 females at 100 mg/kg/day and 8 females at 500 mg/kg/day.

- Uterus: Dark red discoloration in 4 females at 500 mg/kg/day.

- Thymus: Dark red discoloration in 1 control female, 1 male at 5 mg/kg/day, 1 male and 1
female at 100 mg/kg/day, and 2 males and 11 females at 500 mg/kg/day; reduced in size in 1
female at 100 mg/kg/day and 3 males and 2 females at 500 mg/kg/day.

- Mandibular lymph node: Dark red discoloration in 1 control male, 1 male and 1 female at 100 mg/kg/day, and 2 males and 5 females at 500 mg/kg/day.

Microscopic findings:

A number of microscopic findings were noted at the termination of the 26 week treatment period and in decedents (of treatment and recovery groups). From these findings, the following ones were considered to distinguish treated from the control rats:

- Testes: Moderate to massive bilateral diffuse tubular atrophy was seen in 1 male at 5 mg/kg/day, 3 rats at 100 mg/kg/day and in 10 rats at 500 mg/kg/day. This finding was associated with mainly unilateral minimal to moderate tubular mineralization (1 rat each at 5 and 100 mg/kg/day, 4 rats at 500 mg/kg/day), unilateral slight spermatic granuloma (1 rat each at 100 and 500 mg/kg/day), unilateral or bilateral minimal to marked tubular fibrosis (1 rat at 100 and 2 rats at 500 mg/kg/day), or unilateral or bilateral moderate tubular luminal occlusion (1 rat at 100 and 2 rats at 500 mg/kg/day). Minimal focal unilateral tubular atrophy was seen in 1 control rat, minimal to massive focal/multifocal unilateral or bilateral tubular atrophy in 6 rats at 100 mg/kg/day and 5 rats at 500 mg/kg/day.

- Epididymides (mainly decedents): Minimal to moderate atrophy in 1 rat at 5 mg/kg/day, 1 rat at 100 mg/kg/day and in 9 rats at 500 mg/kg/day; aspermia in 3 rats at 100 mg/kg/day and 2 rats at 500 mg/kg/day, in 1 rat at 500 mg/kg/day associated with minimal bilateral luminal mineralization and marked unilateral hemorrhage; oligospermia was observed in 1 rat at 5 mg/kg/day and 8 rats at 500 mg/kg/day; moderate sperm granuloma in 1 rat at 5 mg/kg/day.

- Efferent ducts: Minimal to moderate interductular fibrosis was seen in 1 rat at 5

mg/kg/day, 2 rats at 100 mg/kg/day and in 3 rats at 500 mg/kg/day; minimal to slight luminal mineralization was observed in 1 rat at 5 mg/kg/day and 2 rats each at 100 and 500 mg/kg/day; moderate luminal occlusion was noted in 1 male at 5 mg/kg/day; minimal to slight inflammatory cell infiltration and granuloma were observed in 2 rats at 100 mg/kg/day.

- Seminal vesicles (mainly in decedents): Minimal to moderate atrophy was seen in 2 rats at 100 mg/kg/day, and in 14 rats at 500 mg/kg/day.

- -Small intestine (mainly decedents):
Duodenum: Minimal to slight dilation was observed in 1 female at 100 mg/kg/day and in 2 males and 1 female at 500 mg/kg/day;

Jejunum: minimal to slight dilation was noted in 4 females at 100 mg/kg/day, 5 males and 5 females at 500 mg/kg/day;

Ileum: minimal to slight dilation was noted in 4 females at 100 mg/kg/day, 5 males and 6 females at 500 mg/kg/day.

- Large intestine (mainly in decedents):
Cecum: Minimal dilation was noted in 1 female at 100 mg/kg/day and 5 males and 5 females at 500 mg/kg/day;

Colon: minimal to slight dilation was noted in 1 female at 100 mg/kg/day, 5 males at 500 mg/kg/day.

- Pancreas: Minimal diffuse acinar cell atrophy was seen in 2 females at 100 mg/kg/day and
4 males and 1 female at 500 mg/kg/day.

- Thymus (mainly decedents): Minimal to marked atrophy was observed in 2 males and 5 females at 100 mg/kg/day and in 11 males and 12 females at 500 mg/kg/day.

- Salivary glands: (mainly decedents):

Mandibular salivary gland: minimal to moderate atrophy was noted in 2 males and 4 at 100 mg/kg/day, and in 12 males and 9 females at 500 mg/kg/day;

Sublingual salivary gland: minimal to moderate atrophy was seen in 1 male and 3 females at
100 mg/kg/day and in 6 males and 5 females at 500 mg/kg/day;

Parotid salivary gland: minimal to slight atrophy was noted in 2 males and 3 females at 100
mg/kg/day, and in 8 males and 4 females at 500 mg/kg/day.

- Uterus (mainly decedents): Minimal to moderate atrophy was observed in 4 rats at 100

mg/kg/day and in 12 rats at 500 mg/kg/day.

- Skin/subcutis (mainly decedents): Minimal to moderate hair follicle atrophy was noted in 2 males and 4 females at 100 mg/kg/day and in 10 males and 13 females at 500 mg/kg/day.

- Mammary gland (mainly decedents): Minimal to moderate atrophy was noted in 3 females
at 100 mg/kg/day and in 6 males and 9 females at 600 mg/kg/day.

- Skeletal muscle (mainly decedents): Minimal to slight atrophy was seen in 2 females at 100
mg/kg/day and in 7 males and 4 females at 500 mg/kg/day; minimal to slight myodegeneration in 1 male and female each at 100 and 500 mg/kg/day.

- Prostate gland (mainly decedents): Minimal to moderate atrophy was noted in 3 rats at 100 mg/kg/day and in 13 rats at 500 mg/kg/day.

- Nasal cavity:
Anterior part: Minimal to moderate osseous hyperplasia was noted in 9 males and 10 females at 100 mg/kg/day, and in 16 males and 17 females at 500 mg/kg/day; in 8 males and 6 females at 100 mg/kg/day, and in 10 males and females at 500 mg/kg/day osseous hyperplasia was associated with minimal to slight inflammation.

Intermediate part: minimal to marked osseous hyperplasia was noted in 12 males and 13 females at 100 mg/kg/day, and in 16 males and 17 females at 500 mg/kg/day; in 7 males and 1 female at 100 mg/kg/day, and in 4 males and 3 females at 500 mg/kg/day osseous hyperplasia was associated with minimal inflammation; minimal to slight eosinophilic inclusions in 3 females at 500 mg/kg/day.

Posterior part: minimal to moderate osseous hyperplasia was noted in 6 males and 6 females at 100 mg/kg/day, and 16 males and 17 females at 500 mg/kg/day; in 2 males at 100 mg/kg/day and 1 male at 500 mg/kg/day osseous hyperplasia was associated with minimal inflammation; minimal eosinophilic inclusions in 1 female each at 100 and 500 mg/kg/day.

- Adrenal cortices: Minimal to moderate diffuse hypertrophy of zona glomerulosa was seen in 4 females at 100 mg/kg/day and in 5 males and 14 females at 500 mg/kg/day; moderate increase in hemangiectasis was observed in females at 100 mg/kg/day and males and females at 500 mg/kg/day.

- Ovaries: Minimal to slight stromal cell hyperplasia was noted in 4 rats at 100 mg/kg/day and in 10 rats at 500 mg/kg/day.

- Mesenteric lymph node: Slight to moderate increase of histiocytosis was observed in males and females at 500 mg/kg/day and in females at 100 mg/kg/day.

- Spleen: Slight increase in hematopoiesis was noted in males at 500 mg/kg/day, minimal

to marked hematopoiesis in 3 females at 100 mg/kg/day and 8 females at 500 mg/kg/day; slight to moderate lymphoid depletion in 1 male and 2 females at 100 mg/kg/day, 4 males and 4 females at 500 mg/kg/day.

- Bone marrow: Sternum/Femur: minimal erythroid hyperplasia was seen in 3 females at 100 mg/kg/day and 4 males and 4 females at 500 mg/kg/day.

- Liver: Minimal to slight diffuse hepatocellular hypertrophy was noted in 1 female decedent at 100 mg/kg/day and 4 female decedents at 500 mg/kg/day; moderately decreased incidence of lymphoid cell infiltration in males and females at 100 and 500 mg/kg/day.

- Heart (only decedents): Minimal to slight auricular dilation was noted in 1 male and 1 female at 100 mg/kg/day and 2 males and 4 females at 500 mg/kg/day; in one female at 100
mg/kg/day the auricular dilation was associated with minimal ventricular dilation.

- Lung: Marked multiple foreign body granuloma associated with moderate aspiration pneumonia was observed in 1 male decedent at 500 mg/kg/day. Slight alveolitis was seen in 1 male and 2 females at 500 mg/kg/day. Slight pleural fibrosis was noted in 1 female at 50 mg/kg/day.

- General observations (only decedents): Generalized congestion in 2 males and 3 females at 100 mg/kg/day and in 7 males and 3 females at 500 mg/kg/day.

The generalized congestion, noted under general observations in some decedents, correlated with the macroscopic finding of dark red discoloration of the whole body. In addition, the microscopically noted congestion was also recorded under the various organs and tissues affected.

A number of microscopic findings were noted after an additional 20 week recovery period in surviving recovery animals. From these findings, the following ones were considered to distinguish treated rats from the control rats:

- Testes: Minimal focal unilateral or bilateral tubular atrophy in 1/6 rats of the control group and 2/6 rats at 5 mg/kg/day, minimal to slight focal unilateral tubular atrophy in 2/5 rats at 100
mg/kg/day, and minimal focal bilateral tubular atrophy in 1/2 rats at 500 mg/kg/day.

- Nasal cavity: Anterior part: Minimal osseous hyperplasia in 1/2 male at 500 mg/kg/day; intermediate part: moderate osseous hyperplasia in 1/2 male at 500 mg/kg/day.

Toxicokinetics: Parameters are summarized below:

Main plasma kinetic parameters of BSF 208075 in rats receiving doses of 5, 100, and 500 mg/kg/day

Dose (mg/kg/day)	Parameter	Unit	MALES Days 2/3	MALES Days 85/86	MALES Days 175/176	FEMALES Days 2/3	FEMALES Days 85/86	FEMALES Days 175/176
5	AUC	h*ng/mL	84042.4	9331.6	9607.2	51489.8	5791.0	8025.6
	AUC/dose	(h*ng/mL)/(mg/kg)	21063.3	1948.1	2001.5	11544.8	1204.0	1601.9
	C_{max}	ng/mL	4495.5	498.8	536.7	3420.8	420.4	412.6
	C_{max}/dose	(ng/mL)/(mg/kg)	1126.7	104.1	111.8	767.0	87.4	82.4
	T_{max}	day/h	2/20	85/8	175/20	3/4	85/8	176/4
100	AUC	h*ng/mL	190593.6	138532.2	188408.2	222915.0	209411.6	228234.4
	AUC/dose	(h*ng/mL)/(mg/kg)	1592.9	1483.1	1910.1	1693.5	2232.8	2228.9
	C_{max}	ng/mL	10640.9	9605.3	9699.3	11834.5	17353.5	12868.5
	C_{max}/dose	(ng/mL)/(mg/kg)	88.9	102.8	98.3	89.9	185.0	125.7
	T_{max}	day/h	3/4	85/8	175/12	3/4	85/8	175/20
500	AUC	h*ng/mL	800604.2	781266.4	676742.0	692128.0	1002502.4	932869.0
	AUC/dose	(h*ng/mL)/ (mg/kg)	2026.7	1713.7	1499.2	1549.8	2164.0	1925.0
	C_{max}	ng/mL	41467.9	42168.5	33597.1	47396.2	54460.4	47700.4
	C_{max}/dose	(ng/mL)/ (mg/kg)	105.0	92.5	74.4	106.1	117.6	98.4
	T_{max}	day/h	3/4	86/4	175/8	3/4	86/4	175/20

DISCUSSION

Study Title: BSF 208075: 26-Week Oral (Gavage) Toxicity Study in the Beagle Dog (Draft)

Key study findings:
Study no: — Study No. 756977, [redacted] MPR/PT 0111 E
Volume #, and page #: *v4.1:p001*
Conducting laboratory and location: [redacted]

Date of study initiation: 6/20/2001
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. 10020
Formulation/vehicle: solution in deionized water, pH 7.5

Methods:
Species/strain; sex: Beagle dog, purebred, males and females
Doses: 0 (control), 100, 300 and 900 mg/kg/day
Duration: 26 weeks
Main study No. /group: 4/sex/group
Additional groups: 2/sex/group for 15-week recovery

Parameters examined:
Mortality: twice daily
Clinical signs: twice daily
Food consumption: measured daily, reported weekly
Body weights: weekly
Ophthalmoscopy: pretest, weeks 13 and 25, and following recovery
Electrocardiography: pretest, weeks 4, 13 and 25, and following recovery
Hematology: pretest, weeks 4, 13 and 25, recovery Week 15; the following parameters were measured:

Hematology Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	1
Hemoglobin	HB	mmol/L	1
Hematocrit	HCT	L/L	1
Mean corpuscular volume	MCV	fL	1
Mean corpuscular hemoglobin	MCH	fmol	1
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	1
Platelet count	PLATELETS	G/L	1
Total leukocyte count	WBC	G/L	1
Reticulocyte count	RETIC.	%(rel.); T/L(abs.)	2
Reticulocyte fluorescence ratios	HFR = high, MFR = middle, LFR = low	%	2
Nucleated erythrocytes (normoblasts)	NEN	NEN/100WBC	3
Differential leukocyte count	Diff. WBC Count[4]	1(rel.); G/L(abs.)	3
Red blood cell morphology		normal/abnormal (score)	3

Key:
1 [redacted] Multi-Parameter Automated Hematology Analyzer
2 [redacted] Automated Reticulocyte Analyzer
3 [redacted] light microscope, [redacted] Computer Printer Blood Cell Calculator
4 Cell Classification: BAND = Band Neutrophil; SEG. = Segmented Neutrophil; EO. = Eosinophil; BASO = Basophil; LY&PH. = Lymphocyte; MONO. = Monocyte; PLAS. = Plasma Cell; OTHER = Apoptotic Cell (lymphocytic lysis)

Coagulation Parameter	Abbreviation	Unit	Instrumentation
Thromboplastin time (prothrombin time)	PT	sec	5
Activated partial thromboplastin time	APTT	sec	5
Fibrinogen	FIB.	mg/dL	5

Key:
5 [redacted] Coagulation System

Clinical chemistry: : pretest, weeks 4, 13 and 25, recovery Week 15; the following parameters were measured:

Clinical Biochemistry Parameter	Abbreviation	Unit	Instrumentation
Glucose		mmol/L	1
Urea		mmol/L	1
Creatinine		µmol/L	1
Bilirubin, total	BILI T.	µmol/L	1
Lipids, total*	LIPIDS T.	g/L	1
Cholesterol, total	CHOLEST. T.	mmol/L	1
Triglycerides	TRIGL.	mmol/L	1
Phospholipids	PHOS. LIPID	mmol/L	1
Aspartate aminotransferase	ASAT/GOT	µkat/L (37°C)	1
Alanine aminotransferase	ALAT/GPT	µkat/L (37°C)	1
Lactate dehydrogenase	LDH	µkat/L (37°C)	1
Glutamate dehydrogenase	GLDH	nkat/L (37°C)	1
Creatine kinase	CK	µkat/L (37°C)	1
Alkaline phosphatase	ALP	µkat/L (37°C)	1
Gamma-glutamyl transferase	G-GT	nkat/L (37°C)	1
Iron		µmol/L	1
Calcium		mmol/L	1
Phosphorus		mmol/L	1
Magnesium		mmol/L	1
Sodium		mmol/L	1
Potassium		mmol/L	1
Chloride		mmol/L	1
Protein, total	PROTEIN T.	g/L	1
Protein electrophoresis	PROT. ELECTROPH.[3]	1 (rel.) g/L (abs.)	2

* Measurement of total lipids was not performed during Week 13 of treatment and Week 8 of recovery as this was considered obsolete and the reagents could no longer be obtained.

Key:
1 [redacted] Discrete Random-Access Analyzer
2 [redacted] Automated Multiparameter Agarose Gel Electrophoresis Processing System, [redacted] Multitask Scanning Densitometer System
3 Electrophoretic fractions:
ALBUMIN = Albumin; A1-GLOB. = Alpha 1-globulin; A2-GLOB. = Alpha 2-globulin; B1-GLOB = Beta 1-globulin; B2-GLOB. = Beta 2-globulin; G-GLOB. = Gamma globulin; A/G RATIO = Albumin to Globulin ratio

Urinalysis: : pretest, weeks 4, 13 and 25, recovery Week 15; the following parameters were measured:

Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Specific gravity	SPEC. GRAV.	-	1
Osmolality		mmol/kg	2
Color		-	3
Appearance		-	3
pH		-	4
Protein		0 = negative 1 = 0.25 g/L 2 = 0.75 g/L 2 = 1.50 g/L 3 = ≥5.00 g/L	4
Glucose		0 = normal 1 = 3 mmol/L 1 = 8 mmol/L 2 = 17 mmol/L 3 = ≥56 mmol/L	4
Ketone		0 = negative 0 = 0.5 mmol/L 1 = 1.5 mmol/L 2 = 5.0 mmol/L 3 = ≥15 mmol/L	4
Bilirubin		0 = negative 1 = 17 µmol/L 2 = 50 µmol/L 3 = ≥100 µmol/L	4
Blood		0 = negative 1 = 10 RBCs/µL 1 = 25 RBCs/µL 2 = 50 RBCs/µL 2 = 150 RBCs/µL 3 = ≥250 RBCs/µL	4
Nitrite		negative/positive	4
Urobilinogen	UROBILI.	0 = normal 1 = 17 µmol/L 2 = 68 µmol/L 3 = 135 µmol/L 3 = ≥ 203 µmol/L	4
Urine sediment [6]	SED. MICRO.	normal = 0 abnormal amount 1 (small), 2 (moderate), 3 (large)	5

Key:

1 [redacted] Refractometer
2 [redacted] Multi-Sample Osmometer
3 Visual observation
4 [redacted] Semi-Automated Urine Chemistry Analyzer used with [redacted] Reagent-Test-Strip
5 [redacted] light microscope

Histopathology: The following Table lists all tissues collected at necropsy. Those in boldface were examined in control and high dose rats only. Those underlined were examined in all groups.

Adrenal glands
Aorta
Bone – femur including articular surface
Bone marrow - sternum
Brain – including sections of medulla/pons, cerebral and cerebellar cortex
Ductuli efferentes
Epididymides (fixed in Bouin's solution)
Esophagus
Eyes with optic nerve (fixed in Heidenhain's Susa solution)
Gallbladder
Heart
Kidneys
Large intestine - cecum, colon, rectum
Liver
Lungs, infused with formalin
Lymph nodes – retropharyngeal, mesenteric
Mammary gland area
Nasal cavity (Level III)
Ovaries
Oviducts
Pancreas
Parathyroid gland, if technically possible
Pituitary gland
Prostate gland
Salivary glands – mandibular, parotid, (sublingual)
Sciatic nerve
Skeletal muscle
Skin
Small intestine – duodenum, jejunum, ileum
Spinal cord – cervical, midthoracic and lumbar segments
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid gland
Tongue
Trachea
Urinary bladder
Uterus (with vagina)
All gross lesions

The following organs were weighed before fixation. Paired organs were weighed separately. Relative organ weights were calculated based on the body weight and brain weight. In the organ weight tables, testis/epididymis is abbreviated to testis and thyroid/parathyroid is abbreviated to thyroid due to space limitation.

Adrenal glands (l, r)
Brain (including brainstem)
Heart
Kidneys (l, r)
Liver
Ovaries (l, r)
Pituitary gland
Prostate gland
Spleen
Testes with epididymides (l, r)
Thyroid gland with parathyroid (l, r)

Toxicokinetics: Blood for plasma levels of BSF 208075 was collected on Days 1, 90 and 174 of dosing. The collection times were 0.5, 1, 3, 6 and 24 hrs post dose.

Results:

Toxicokinetics:

Dose mg/kg/day	Parameter	Unit	Day 1	Day 90	Day 177	Day 1	Day 90	Day 177
			MALES			FEMALES		
100	AUC	h*ng/mL	286314	189758	189959	343666	233073	243463
	AUC/dose	(h*ng/mL)/(mg/kg)	2863	1898	1900	3437	2331	2435
	Cmax	ng/mL	161062	115522	112808	176018	148615	145086
	Cmax/dose	(ng/mL)/(mg/kg)	1610.6	1155.2	1128.1	1760.2	1486.1	1450.9
	Tmax	h	0.7	0.7	0.6	0.5	0.5	0.6
300	AUC	h*ng/mL	546621	473599	335554	692251	564981	534397
	AUC/dose	(h*ng/mL)/(mg/kg)	1822	1579	1119	2308	1883	1781
	Cmax	ng/mL	220958	202928	164649	215203	318868	216801
	Cmax/dose	(ng/mL)/(mg/kg)	736.5	676.4	548.8	717.3	1062.9	722.7
	Tmax	h	1.1	0.6	0.6	1.2	0.8	0.8
900	AUC	h*ng/mL	504435	911727	892254	561743	816346	1013824
	AUC/dose	(h*ng/mL)/(mg/kg)	560	1013	991	624	907	1126
	Cmax	ng/mL	206593	325566	323066	231952	338837	331765
	Cmax/dose	(ng/mL)/(mg/kg)	229.5	361.7	359.0	257.7	376.5	368.6
	Tmax	h	0.7	0.8	0.8	0.5	0.8	0.8

The peak plasma concentrations were obtained within one hour after the administration of the test item by gavage. The body exposure to the test item as represented by the AUC and C_{max} values appeared to be independent of sex and treatment duration. The AUC increased with dose except at 900 mg/kg/day on Day 1 but appeared under-proportional in both sexes.

Several plasma samples from Control dogs had quantifiable concentrations of BSF 208275, especially at the sampling on Day 90. No explanation of this contamination was found after extensive research. It was decided that the following sampling period would be divided so that plasma was collected from Control and treated animals on separated days (Days 174 and 177, respectively). There was a clear reduction in the incidence of quantifiable concentrations of BSF 208275 in Controls at this sampling occasion. Overall, the presence of these plasma concentrations, particularly on Day 90, were considered not to be relevant as the levels (including C_{max}) were significantly lower than the those seen in the low dose group.

Two male animals receiving 100 mg/kg/day were not dosed on single occasions during the treatment period. Male No. 10 was not dosed on one day during Week 15 (Day 103) due to labored respiration observed following dosing on the previous day. Dosing recommenced the following day. Male No. 8 was not dosed on seven consecutive days during Week 19/20 (Days 133-139) also due to labored respiration.

Mortality: All animals survived the scheduled treatment or recovery periods.

Clinical signs:
Salivation was observed during the first 6 weeks of the study in animals administered 100 mg/kg/day, frequently throughout the study in animals administered 300 mg/kg/day and generally daily throughout the study in animals administered 900 mg/kg/day.

Occasional incidences of tremor were noted in the majority of animals administered 300 or 900 mg/kg/day. Recumbency and decreased activity were also noted at these dose levels, although with a higher incidence at 900 mg/kg/day. Tremor, recumbency and decreased activity were noted on one occasion during Week 6 and/or 19 in one male and one female receiving 100 mg/kg/day. Decreased activity was also seen on occasional days for two further males receiving 100 mg/kg/day during Week or 19/20.

Increased incidences of vomiting mucus, food and/or clear fluid and watery feces were noted in animals receiving 300 or 900 mg/kg/day in comparison with the Controls. Mucus in the feces was also recorded at a higher incidence in test item treated animals

than in the Controls. These increases in incidence were generally dosage related.

Isolated incidences of breathing noises were noted in animals administered 300 or 900 mg/kg/day.

During the recovery period, occasions of loose or watery feces were observed in animals of all groups. The incidence was generally lower than during treatment but remained higher than the Controls for animals that had been administered 900 mg/kg/day.

The onset of the behavioral changes generally occurred approximately one hour after dosing and persisted for up to approximately four hours. Salivation was also observed before, during and immediately after dosing.

Occasional incidences of red and/or dark feces or feces containing white particles were noted on occasion during the study period in animals across all groups, including the Controls

Food consumption:
Food consumption was unaffected by treatment with the test item. The values were generally comparable with pretest levels throughout the treatment and recovery periods for animals in all groups.

Body weights:
Body weight gain was moderately to markedly reduced, in comparison with the Controls, in males administered 300 mg/kg/day and animals of both sexes administered 900 mg/kg/day and the differences were dosage related. This reduction in body weight gain resulted in lower bodyweights at termination for animals in these groups when compared with the Controls.

During the recovery period, the animals affected by the test item as indicated above, gained a similar or greater amount of weight whereas the weight gain in the remaining groups was lower than during treatment. This resulted in comparable body weights across all dose groups at the end of the recovery period.

The following table compares the percentage increases (calculated from group mean weights) in group mean body weights at the end of the dosing and recovery periods.

Group/ Treatment (mg/kg/day)	% Increase Between Day 1 and Week 27		% Increase Between Day 1 of and the end of the Recovery Period	
	males	females	males	females
1 (0)	33%	24%	-3%	4%
2 (100)	25%	21%	1%	1%
3 (300)	17%	24%	9%	2%
4 (900)	13%	12%	11%	19%

Ophthalmoscopy:

A slightly higher incidence of reddening of sclera and watery and/or mucous occular discharge was noted in animals receiving the test item in comparison with the Controls. This was no longer apparent at the end of the recovery period.

There were no other ophthalmic changes that were considered to be related to treatment with the test item.

Electrocardiography:
A slight to marked increase in the amplitude of the P-wave was recorded at two hours after dosing during Weeks 4,13 and 25 in animals receiving 900 mg/kg/day (22 to 72% higher than Control values). A slight increase in the heart rate was also noted after dosing at 900 mg/kg/day in males during Week 4 (36% higher than the Control) and females during Week 13 (24% higher than the Controls). A minimal increase in the Q-T interval was observed during Week 25 for males receiving 300 mg/kg/day (7% higher than Controls) or 900 mg/kg/day (8% higher than Controls). There was no similar difference in the females. These differences were no longer clearly apparent at the end of the recovery period.

Assessment of the traces did not reveal any arrhythmias that were considered to be related to treatment with the test item. The only findings of note comprised occasional incidences of second degree atrioventricular block or ventricular premature complexes. These findings can arise spontaneously and due to their low incidence and the lack of a dosage relationship they were considered not to be related to treatment with the test item.

Other findings which included changes in the S-T junction and segment, prominent Q-waves in Leads I, II and III, changes in polarity of T-wave, variability of the PQ interval and wandering pacemaker are commonly seen at these laboratories and their incidence and severity did not indicate and effect of treatment with the test item.

Hematology:
There were no hematology effects which were considered to be of toxicological significance.

Increases in platelet levels were noted during Week 4 in females and Weeks 13 and 25 in animals of both sexes administered 900 mg/kg/day. Since individual values remained within background ranges and in the absence of other related changes, the observation is considered not to be toxicologically significant.

Serum biochemistry:
Slight reductions in group mean aspartate aminotransferase (61-85% of Control), alanine aminotransferase (59-91% of Control) and creatine kinase (63-87% of Control) activities were recorded in comparison with Control and pretest values from Week 4 onwards in animals receiving 900 mg/kg/day. In addition, total bilirubin (40-66% of Control) levels were slightly low for females receiving this dose level.

Total protein and albumin levels were reduced in animals receiving 900 mg/kg/day from

Week 13 onwards. Associated increases in absolute alpha-2 globulin (males only) and beta-1 globulin (females only) levels and a decrease in beta-2 globulin (females only) were also noted, resulting in reductions in albumin-globulin ratios.

Full or partial recovery was observed in these parameters at the end of the recovery period with the exception of aspartate aminotransferase and creatine kinase activity which remained low for females previously treated at 900 mg/kg/day.

Urinalysis:
There were no effects on urinalysis parameters that were considered to be associated with the test item.

The differences from the Controls in specific gravity and osmolality, which occasionally achieved statistical significance, were inconsistent between the sexes and sampling occasions. In light of the differences that were present at pretest they were considered to reflect normal biological variation.

Organ weights:
The absolute organ weight and/or organ weight relative to body weight for liver, kidney and adrenals were slightly higher for animals which had received 900 mg/kg/day than the Controls following 26 weeks of treatment. The absolute and relative prostate weight was low for males receiving 900 mg/kg/day when compared with the Controls.

The adrenal weights were still higher and the prostate weights were still lower of males previously treated at 900 mg/kg/day in comparison with the Controls at the end of the recovery period. The remaining differences were no longer apparent.

Macroscopic findings:
There were no macroscopic findings that were considered to be related to treatment with the test item. A number of findings were recorded at the end of the treatment or recovery periods but they did not distinguish treated dogs from Controls and were considered to be incidental changes commonly occurring in dogs of this age and strain.

Microscopic findings:
Minimal to slight focal/multifocal tubular basophilia was noted in the kidneys in one male at 300 mg/kg/day and two males and three females at 900 mg/kg/day. The finding was associated with minimal focal tubular cell vacuolation in the male at 300 mg/kg/day. The basophilia was accompanied by minimal to slight simple tubular dilation in both males and one female at 900 mg/kg/day. In addition, minimal lymphoid cell infiltration was observed in one male and all females at 900 mg/kg/day.

Slight to moderate pyelitis was noted in the kidneys of two females at 300 mg/kg/day and three females at 900 mg/kg/day. This was associated with slight to moderate reactive transitional cell hyperplasia in all animals and with minimal corticomedullary mineralization in one of the females.

Slight to moderate inflammation of the urinary bladder occurred in two females at 900 mg/kg/day. This was associated with a minimal to slight reactive transitional cell hyperplasia. Minimal to slight lymphoid cell infiltration was present in one female at 300 mg/kg/day and one female at 900 mg/kg/day.

A minimally increased incidence and/or severity of focal/multifocal tubular atrophy were seen in the testes in one Control dog, three males at 100 mg/kg/day, two males at 300 mg/kg/day, all four males at 900 mg/kg/day. Minimally increased incidence and/or severity of focal/multifocal tubular vacuolation were present in one Control male, four dogs at 100 mg/kg/day two males at 300 mg/kg/day and all four males at 900 mg/kg/day. These findings were bilateral in all animals with the exception of one animal. Minimal to slight bilateral tubular dilation was noted in all males treated with the test item. This was associated with minimal to slight focal/multifocal and unilateral or bilateral presence of intraluminal fluid in three males at 100 mg/kg/day, two males at 300 mg/kg/day and all males at 900 mg/kg/day.

Minimal to slight multifocal atrophy of the fundic glands with less fundic pits and more fibrous tissue was noted in the stomach of one female at 300 mg/kg/day and three females at 900 mg/kg/day.

Minimal to slight purulent inflammation was seen in the nasal cavities of three males at 900 mg/kg/day. This lesion was focal or multifocal and acute in two animals or focal and chronic in the remaining animal.

Minimal atrophy of the thymus was noted in one male at 900 mg/kg/day.

Minimal myeloid hyperplasia was noted in the bone marrow in one female at 300 mg/kg/day and two females at 900 mg/kg/day.

A minimal increase in hematopoiesis was observed in the spleen of one male and one female at 900 mg/kg/day.

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Protocols

Application (IND/NDA) number: IND 64,915 Amendment 034 (SX)
Division: DCRDP
CAS#:
Drug name: BSF 208075 (ambrisentan)
Pharmacological Classification: endothelin receptor antagonist
Sponsor/Applicant: [redacted] US agent for Myogen, Inc.
Sponsor/Applicant contact name: [redacted], Regulatory Manager, [redacted]
Sponsor/Applicant telephone and fax number: Phone: [redacted]
Date submitted (stamp date): 10/14/03
45-day date (from submission stamp date): 11/28/03
P/T Reviewer(s): William T. Link
Date of Exec CAC review: 11/18/03
CAC members: Abby Jacobs, acting chair
Joe Contrera
Lois Freed
Terry Peters
Roswitha Kelly.

A. Summary of Sponsor's Proposal for Review:

Species/strain: rat, Wistar
Number/sex/dose: 50
Route: oral, via food admixture

	male	female
Doses proposed:	10, 20 & 60 mg/kg/d	10, 20 & 60 mg/kg/d
Basis of dose selection:		
MTD	X	X
AUC ratio		
saturation		
MFD		
PD		
other		

Kinetics submitted:	rodent	human
pharmacokinetics	x	x
metabolism		
protein binding		

Notable design features: none

B. Summary of Reviewer's Recommendations to CAC:
The following dose-selection rationale was provided by the sponsor and is presented verbatim:

DOSE SELECTION FOR THE RAT CARCINOGENICITY STUDY FOR AMBRISENTAN COMPARING TO EXPOSURE RESULTS IN HUMANS

Maximum clinical dose

The preliminary results from the dose-ranging Phase 11 clinical trial (AMB-220) of ambrisentan in patients with PAH indicate that the maximal dose expected to be used in future clinical trials will likely be 10 mg/day. The exposures (AUC) at 10 mg from this Phase 11 study subdivided by gender are listed in Table 13.

Table 13: Dose and exposure* of PAH patients administered ambrisentan (10 mg, QD) for 12 weeks

Dose (mg/day)	AUC (ng x h)/mL; Males	AUC (ng x h)/mL; females
10**	15361	10118, 19040 mean = 14579

* = AUC values listed above are calculated from [ambrisentan] in plasma measured as total (bound + free drug)
** = Values in each box are individual patients followed by the mean (females only)

Comparison of rat to human exposures

The AUCs reported in the rat toxicity study, and the AUCs listed in Table 13 for Phase II patients, were generated by a validated bioanalytical assay that measured total (free + protein bound) concentrations of ambrisentan in the appropriate plasma matrix. However, ambrisentan has been demonstrated in vitro to be highly protein bound in the plasma of rats, rabbits, dogs and humans, therefore the active unbound free fraction is the fraction of total drug in plasma considered as available for endothelin receptor binding and biological activity. Protein binding was also found to be concentration independent (0.2 - 2.0 μg per mL of plasma). Accordingly, the respective free fractions of ambrisentan in rats and humans should be used for comparison of exposure between those two species. The in vitro study above determined that the percent of BSF 208075 protein bound for rats and humans was 97.2% and 98.8%, respectively. Percent protein bound for neither rats nor humans was gender specific.

In Table 14, the exposure listings of rat/human AUCs are listed as ratios of free ambrisentan. Rat exposure comparisons to the largest expected human AUC yields the smallest exposure ratios, and the most conservative assessment of risk.

Table 14: Relative exposures using rat/human AUC ratios of free ambrisentan

Species [reference]	Dose	Free [Plasma] AUC (ng x h)/mL; males	Free [Plasma] AUC (ng x h)/mL; females	Ratio to Human AUC; males	Ratio to Human AUC; females
Human [52]	10 mg/day	184	175	1	1
Rats* [51]	5 mg/kg/day	269	224	1.5	1.3
	100 "	5275	6391	28.7	36.5
	500 "	18949	26120	103.0	149.3

* = AUCs above for the mouse 13-week and the rat 26-week toxicity studies were determined at the end of each treatment period, respectively

Proposed doses and rationale for rats

Table 15: Proposed doses, projected exposures and ratios for ambrisentan

Proposed Dose (mg/kg/day)	Projected Rat AUC (ng x h)/mL; males	Projected Rat AUC (ng x h)/mL; females	Projected Ratio rat/human; males	Projected Ratio rat/human; females
10	1132	1053	6.2	6.0
20	1499	1567	8.1	9.0
60	2967	3623	16.1	20.7

The proposed MTD for rats of 60 mg/kg/day (Table 15) is based on the following findings in the 26-week rat toxicity study of ambrisentan. In this investigation, 2 males and 4 females dosed at 100 mg/kg/day died spontaneously or were sacrificed in extremis during the last 5 weeks of the treatment period. Thirty-eight rats in the 500 mg/kg/day high dose groups also died similarly starting at week 14. These rats demonstrated severe adverse clinical signs prior to death or sacrifice that included breathing disturbances, ruffled posture and emaciation. Food consumption was reduced and body weight gain was attenuated at 100 and 500 mg/kg/day compared to control rats. Alkaline phosphatase was significantly increased at doses ≥ 100 mg/kg/day. Primary microscopic histopathological findings with the potential to contribute to morbidity and mortality in rats administered 100 mg/kg/day were observed in the tissues of the nasal cavity. Minimal to marked eosinophilic cytoplasmic inclusions and osseous hyperplasia accompanied by inflammation in the anterior, intermediate and posterior portions of the nasal cavity were found in both genders of rat receiving ≥ 100 mg/kg/day.

Pertinent toxicity information for the rat 26-week toxicity study may appear to recommend a MTD > 100 mg/kg/day for use in the proposed rat carcinogenicity study. However, the progressive nasal effects of ambrisentan in the rat which appears to have contributed to delayed-onset morbidity and mortality in the 26-week study, strongly suggest an MTD for life-time treatment that is below 100 mg/kg/day. The nasal effects of ambrisentan were not associated with morbidity and mortality at doses of 100, 500 and 2000 mg/kg/day in the 13-week study, whereas in the 26-week study, doses of 100 and 500 mg/kg/day exhibited morbidity and mortality that were associated with nasal osseous hyperplasia and signs of systemic hypoxia. Moreover, there is no evidence that the ambrisentan induced nasal osseous hyperplasia and its sequelae had reached a steady state in the 26-week study. Thus the true incidence of delayed-onset mortality could possibly be greater than observed in the 26-week study with the possibility that, with lifetime treatment, doses below 100 mg/kg/day may induce mortality by this mechanism. Accordingly, Myogen is proposing a MTD of 60 mg/kg/day for the high dose in the rat carcinogenicity study. However, if cumulative mortality due to nasal obstructive effects in the 60 mg/kg/day dose group reaches greater than or equal to 25 males and females in combination during the study, this high dose will be reduced to 30 mg/kg/day for the duration of the study. Dose reduction will only occur if the 25 animals have expired between and including week 1 and the 18th month of dosing.

Myogen proposes 20 and 10 mg/kg/day for the middle and low doses of the carcinogenicity study, respectively, for three reasons. First, we expect that these doses

should produce little or no toxicity upon delivery to rats over two years. Secondly, the exposures for these doses are associated with rat/human exposure ratios that represent relevant multiples acceptable for determining risk. Thirdly, these doses are at or above the pharmacodynamically active doses in the rat and in humans. All doses in this study will be administered as a diet admixture because diet was the route of administration in the prior oral dose rat toxicity studies that were used to select the dosing regimens for this proposed study.

	male	female
Doses recommended by reviewer:	10, 20 & 60 mg/kg/d	10, 20 & 60 mg/kg/d

Alternatively, the sponsor may wish to explore the use of other species (hamster?) which may not show the high sensitivity to the nasal effects of ambrisentan. Choice of a different species would necessitate performing a dose-ranging study which should probably for greater than 13 week duration.

C. Basis for Recommendation (experimental details from sponsor's submission):

REVIEW OF NON-CLINICAL PHARMACOLOGY

In vitro preclinical studies with ambrisentan have shown that it is a potent (Ki= 0.63 nmol/L) and selective inhibitor of the endothelin ET_A receptor (77-fold more selective for ET_A over ET_B) with no relevant binding to other receptors. In vitro data using human cardiac ET_A receptors indicated a Ki of 0.81 nmol/L, and a selectivity of 260-fold for the ET_A versus the ET_B receptor in this system.

In a model of acute renal failure in the anesthetized rat, ambrisentan (5 and 10 mg/kg/day, intravenously) enhanced recovery after an ischemic insult as evidenced by the significant increase in creatinine clearance and the decrease in fractional sodium excretion when compared with placebo treated animals. In the anesthetized rat, ambrisentan produced a dose-dependent (1, 3 and 10 mg/kg, orally) and statistically significant ($p<0.05$) inhibition of the increase in blood pressure produced by the intravenous injection of the vasoconstrictor agent big endothelin-1. In conscious, normotensive dogs, ambrisentan (1, 10, and 100 mg/kg, orally) produced a significant decrease in blood pressure at all doses tested. This decrease in blood pressure was still apparent 6 hours postdose in the 10 and 100 mg/kg dose groups. A tendency toward a mild increase in heart rate was seen with administration of 1 and 100 mg/kg doses. However, there was no corresponding increase in heart rate observed with the 10 mg/kg dose. In a pig model of PTCA-induced restenosis, ambrisentan was shown to have a significant antiproliferative effect on the vascular intima when given orally for 4 weeks at 10 and 30 mg/kg.

General / safety pharmacology studies were conducted to evaluate the effects of ambrisentan on in vitro receptor binding, in vitro proliferation of murine spleen cells, and in vivo on the central nervous, cardiovascular and respiratory, gastrointestinal, and genitourinary systems. In studies designed to evaluate the central nervous system activity of ambrisentan, at 100 and 300 mg/kg orally and 10, 30, and 100 mg/kg intravenously, no significant effects were observed. In the

conscious, normotensive rat ambrisentan at an oral dose of 300 mg/kg and at an intravenous dose of 100 mg/kg produced transient increases in blood pressure and heart rate followed by significant and long-lasting decreases in blood pressure with no associated increases in heart rate.

The compound had no effect of the action potential (amplitude, dV/dt or duration) of isolated guinea pig papillary muscles at concentrations up to 10^{-5} mol/L at stimulation frequencies of 0.5 and 1.0 Hz. Ambrisentan had no effect on coagulation parameters nor induced hemolysis in human blood at concentration up to 10^{-5} mol/L. In the isolated rat phrenic nerve-diaphragm preparation, the compound was without effect on neuromuscular transmission at concentrations up to 10^{-5} mol/L. Ambrisentan had little effect on the gastrointestinal system in rats; however, a significant increase in bile flow was observed in the rat at intraduodenal doses of 30 mg/kg and intravenous doses of 10 and 30 mg/kg. A reduction in urine volume was observed in rats at oral doses of 100 mg/kg or intravenous doses of 30 mg/kg. Sodium and chloride excretions were statistically significantly reduced in this study. In a study to evaluate the receptor binding profile, ambrisentan caused no or weak (<50%) inhibition of the specific radioligand binding to over 100 receptors tested at concentrations up to IV mol/L. No relevant antiproliferative or mitogenic effects were observed in murine spleen cells at concentrations of ambrisentan up to 10^{-5} mol/L.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME)

The ADME profile of ambrisentan was assessed in the mouse, rat, rabbit, and dog. Ambrisentan was well absorbed after oral administration in both rats and dogs. The oral bioavailability was 85% and 72% after dosing with 30 mg/kg in rats and dogs, respectively. After oral administration of 30 mg/kg peak concentrations of 32.4 μg/mL were observed at 1 hour postdose in rats and peak concentrations of 43.6 μg/mL were obtained between 0.5 to 2 hours in dogs; mean clearance values were 3.1 and 4.3 mL/min/kg in the rat and dog, respectively. The plasma concentration half-life after oral dosing in rats and dogs was 5.7 hours and 7.9 hours, respectively. No effect on the pharmacokinetic parameters was observed after repeat dosing in the rat, while in the dog the exposure (assessed by AUC) was 1.5-2.4 times higher on day 1 compared with day 28. Tmax was not affected by repeat dosing except when ambrisentan was given twice daily. These data suggest that in the dog, an increase in clearance, a decrease in absorption, or both were occurring with repeat dosing. The results from the four repeat dose studies in dogs suggest higher plasma concentrations were achieved with gelatin capsules than with a solution. Above 500 mg/kg/day, the exposure was generally greater in females than in males.

Ambrisentan, at concentrations up to 20 μg/mL, was highly protein bound ranging from 96.4% to 98.8% (with dog<rabbit<rat<human). At a concentration of 200 μg/mL ambrisentan, the binding decreased to 83.1% to 94.4% in rats, rabbits, and dogs, suggesting saturation of binding sites had occurred. No sex-dependent differences were noted in rabbit, dog, and human plasma, however there was a tendency for higher values in female rats. In human plasma, ambrisentan was

primarily bound to plasma albumin (96.5%). The blood:plasma mean partition coefficient values were between 0.54 and 0.64, corresponding to fractions of ambrisentan in plasma between 90.2% and 95.9%.

The distribution of radiolabeled ambrisentan was assessed in the rat. The tissues with the highest localization of radioactivity apart from the gastrointestinal tract were the liver, plasma, lungs, and kidneys with $t_{1/2}$ values of 17, 11, 14 and 21 hours. In pigmented animals, no higher affinity or prolonged binding to melanin was observed.

Ambrisentan was evaluated for effects on enzyme induction and enzyme inhibition. One induction study suggested that ambrisentan at doses up to 200 mg/kg/day does not possess any relevant induction potential in the liver of rats. In a second study at daily doses of 400 mg/kg/day, a small enhancement of the detoxification capacity of the rat liver was observed. Similarly, there was a low potential for ambrisentan to inhibit the phase I and phase II enzyme activities. In the dog, oral administration of ambrisentan (100, 300, 1000, and 1500 mg/kg/day) for 4 weeks resulted in a dose-dependent increase in pentoxyresorufin-O-dealkylase (PROD, CYP 213) activity of 2.2- to 4.6-fold and benzyloxyresorufin-O-dealkylase (BROD, CYP3A) activity of 1.7- to 5-fold between 100 and 1000 mg/kg/day. Erythromycin-N-demethylase (ERMD) activity was increased 50% to 70% in males and 83% to 143% in females. These effects on liver enzymes were modest with notable changes occurring at doses which were toxic/lethal to the animals. Combined, the data from the rat and dog suggest that ambrisentan administered chronically may have an effect on enzyme induction in the liver, but only at high exposures. The metabolic profile of ambrisentan was evaluated in the mouse, rat, rabbit, dog, and human. In the in vivo studies in mouse, rat, rabbit and dog, unchanged compound predominated in the plasma, urine and feces. Metabolites identified in all species included an ambrisentan glucuronide, O-demethylated derivative, hydroxylated derivative and dihydroxylated derivative, and 4,6-dimethyl-2-hydroxypyridine. From in vitro studies employing isolated liver hepatocytes from dog and human liver samples, the main metabolite identified was an ambrisentan glucuronide.

The excretion profile of ambrisentan was evaluated in the rat and dog. In the rat, the preferred route of excretion was feces (88%) followed by urine (9%). In the dog, the preferred route of excretion was feces (91 %) followed by urine (8%). The majority (~85%) of administered radioactivity was excreted in the first 24 hours via these excretion routes in both species. After intraduodenal administration of 10 mg/kg [^{14}C]-ambrisentan in the dog, approximately 50% of the dose was excreted into the bile over the 8-hour sampling period. The major metabolite identified was the ambrisentan 1-0-acylglucuronide representing approximately 80% of the recovered radioactivity. The stability of the 1-0acylglucuronide toward isomerization was evaluated and it was concluded that the risk of covalent adduct formation during clinical use is minimal at the low systemic exposure of ambrisentan predicted in man.

REVIEW OF NON-CLINICAL TOXICOLOGY *(13 and 26-week rat study reviews attached)*

Ambrisentan was evaluated in single-dose (mice, rats), repeat-dose (mice, rats, dogs), reproduction (rats, rabbits), and genotoxicity (Ames test, chromosomal aberration, unscheduled DNA synthesis, micronucleus) studies.

Single-dose toxicity was evaluated in mice and rats after oral or intravenous dosing. In mice, the oral LD_{50} was 2610-3160 mg/kg and 1000-2150 mg/kg in males and females, respectively, while the intravenous LD_{50} was 750-909 mg/kg for males and females. In the rat, the oral LD_{50} was >4640 mg/kg and 3160-4640 mg/kg in males and females, respectively, while the approximate LD_{50} after intravenous dosing was 464-562 mg/kg in males and females. The clinical signs observed in rats and mice included lassitude, forced respiration, prone position, partial palpebral closure, and convulsions. Necropsy findings revealed congestion in the liver and kidneys.

Repeat-dose toxicity studies were conducted in mice, rats and dogs ranging from 4 to 26 weeks in duration [mouse: 6 weeks (1 study); rat: 4 weeks (4 studies), 13 weeks (1 study); and 26 weeks (1 study); dog: 4 weeks (2 studies), 13 weeks (1 study), and 26 weeks (1 study)]. With the exception of the mouse study and two of the 4-week rat studies, each study contained a treatment-free recovery period of 2-20 weeks. In general, the longest treatment-free recovery periods were associated with the longer treatment period studies (e.g. 20 weeks recovery in the rat and 15 weeks in the dog, respectively, in each of the 26-week studies).

In the mouse, oral administration of ambrisentan for 6 weeks at doses ranging from 250 to 2000 mg/kg/day identified the gastrointestinal tract, liver, adrenal gland and nasal cavity as target organs. Dose-dependent decreases in food consumption at >250 mg/kg/day and body weight at ≥500 mg/kg/day were observed. Overt clinical signs were noted in the respiratory and gastrointestinal tract at ≥500 mg/kg/day. In the anterior and intermediate parts of the nasal cavity, minimally to markedly increased eosinophilic cytoplasmic inclusions with epithelial degeneration were noted at 60, 150, 500 and 1250 mg/kg/day in males and at 150, 500 and 1250 mg/kg/day in females. In the posterior part, minimally to markedly increased eosinophilic cytoplasmic inclusions with epithelial degeneration were noted in males at 60, 500 and 1250 mg/kg/day, and in females at 150, 500 and 1250 mg/kg/day. Centrilobular hepatocellular hypertrophy was present at ≥1000 mg/kg/day, with no associated increases in serum alkaline phosphatase (AP), serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) concentrations. Changes in the adrenal gland at ≥1000 mg/kg/day were judged related to emaciation and poor general health of the animals rather than a direct effect of the treatment. In the testes, a low frequency of focal/multifocal tubular atrophy occurred unilaterally and bilaterally with minimal to moderate severity in both the treated and control groups. Treatment with ambrisentan may slightly increase the incidence of this lesion; however, there is insufficient data to draw a firm conclusion on this point. The NOAEL in mice after oral dosing for 6 weeks was 250 mg/kg/day.

In rats, studies of 4 to 26 weeks duration at oral doses ranging from 1 to 2000 mg/kg/day were conducted. Target organs identified included the gastrointestinal tract, kidney, heart, nasal cavity, testes, adrenal gland and liver. Histopathologic evaluation of the liver

identified dose-related centrilobular hepatocellular hypertrophy at 100 and 500 mg/kg/day in single females in the 26-week study. Hepatic changes were associated with moderate elevations in liver enzymes (AP, AST and ALT). Centrilobular changes were fully reversed by the end of the 20-week treatment-free recovery period in this study.

The FDA has identified evidence that the class of endothelin receptor antagonists has been linked to the development of testicular injury and sterility with chronic use in animals and that where examined, these effects have been irreversible. Ambrisentan administration has been associated with the development of focal/multifocal and diffuse testicular tubular atrophy in the 4-week, 13-week and 26-week rat toxicology studies. In the 26-week study, the testicular lesions (all types of lesions combined) were dose dependent. At the highest doses tested, the testicular lesions occurred in all treated animals. Data from the treatment-free recovery periods indicated a reduced incidence and severity of testicular lesions; however, complete reversal did not occur within the 20-week recovery period. There appeared to be some recovery of the diffuse testicular lesion in these studies. Additional investigation of the mechanism of this toxicity is ongoing.

In the nasal cavity, osseous hyperplasia of the nasal turbinates was observed at doses >100 mg/kg/day in the 26-week rat study, as was delayed-onset (>13 weeks of treatment) mortality. In addition, some animals at these doses showed an incidence of delayed-onset moribund condition that led to the need for early sacrifice. Delayed-onset mortality and the incidence of moribund condition was dose dependent and the incidence of both outcomes was more frequent in females than in males. Animals that died early or that were killed in extremis showed a remarkably consistent constellation of clinical signs comprised of rapid, labored breathing, distended GI tract (gas), hunched posture, rales (defined as audible breathing) and blue-colored extremities. Since osseous hyperplasia, congestion and inflammation of the nasal turbinates was observed in this study and has been noted previously in other toxicity studies with ambrisentan, there was a possibility that the labored respiration observed and rales heard were due to increased upper airway resistance. Examination of the nasal histopathology findings in the 100 and 500 mg/kg/day groups showed that there was clearly a greater incidence and severity of nasal turbinate findings in the animal groups that died spontaneously or were sacrificed moribund compared to animals that survived to terminal sacrifice. Moreover, animals in both dose groups had a profound secondary polycythemia and reticulocytosis that was associated with and perhaps secondary to systemic hypoxemia (blue color of skin and tongue). These data and observations led to the hypothesis that delayed-onset mortality was due to poor pulmonary ventilation caused by a direct effect of the drug on the nasal cavity (increased upper airway resistance) and resultant systemic hypoxemia and that the delay was caused by the time it took for the drug effects in the nasal turbinates to increase to the point where ventilation was severely impacted.

The NOEL for the testicular lesion (most sensitive target organ in the rat) after oral dosing in males for 26 weeks was <5 mg/kg/day (free drug AUC = 269 ng•hr/mL), and the NOEL in females was = 5 mg/kg/day (free drug AUC = 225 ng•hr/mL) due to the nasal osseous hyperplasia. The data from this 26-week study support human safety margins of < 1.5 fold and = 1.3 fold for males and females, respectively, based on a free

drug AUC in human males of 184 ng•hr/mL and an AUC in females of 175 ng•hr/mL after a 10 mg oral dose. These human exposures were determined in PAH patients after 12 weeks of daily dosing with 10 mg of ambrisentan in a Phase II study.

The effects of ambrisentan were evaluated in studies of 4-week and 13-week duration in the dog with doses ranging from 80 to 1500 mg/kg/day. Deaths occurred at 1500 mg/kg/day. However, dogs were also administered ambrisentan at 100, 300, and 900 mg/kg/day for 26 weeks with no mortality. In the two shorter studies, histopathologic evaluation identified the gastrointestinal tract, kidneys, and heart as target organs primarily at doses >1000 mg/kg/day. Compared to the previous dog toxicity studies of shorter duration described above, dogs treated for 26 weeks demonstrated a focal/multifocal testicular tubular atrophy that was observed in all treatment groups. These testicular lesions were observed in one of 2 male dogs in the recovery group dosed at 900 mg/kg/day, but not in any recovering dogs from the other treatment groups. Slight fundic region atrophy was observed in the stomach of 3 females dosed at 900 mg/kg/day and 1 female at 100 mg/kg/day. Kidney effects were similar to those seen in the studies of shorter duration, but there were no cardiac findings after 26 weeks of dosing. Minimal to slight inflammation was observed in the nasal cavities of 3 high dose males. With the exception of focal/multifocal testicular atrophy, no microscopic findings distinguished treated dogs from control dogs at the end of the 15-week recovery period. In the 13 and 26-week studies there were no unusual urinalysis, hematology or clinical chemistry effects of ambrisentan except for decreases in AST and ALT associated with a mild increase in liver weight.

From the 26-week study, the NOAEL for males was < 100 mg/kg/day based on the testicular lesions. This dose has a corresponding free drug AUC = 6840 ng•hr/mL. The NOAEL for females was = 100 mg/kg/day as a result of the kidney and stomach lesions present at doses ≥ 300 mg/kg/day; free drug AUC = 8766 ng•hr/mL. The dose of 100 mg/kg/day and accompanying AUCs associated with minimal and reversible canine toxicity in male and female dogs can be used to calculate human safety margins of < 37 fold and = 50 fold for males and females, respectively, based on a free drug AUC in human males of 184 ng•hr/mL and an AUC in females of 175 ng•hr/mL after a 10 mg oral dose. These human exposures were determined in PAH patients after 12 weeks of daily dosing with 10 mg of ambrisentan in the Phase 11 study.

The effect of ambrisentan on fertility and embryo-fetal development was assessed in rats, and in rats and rabbits, respectively, after oral dose administration. In the rat and rabbit embryo-fetal studies, abnormalities of the lower jaw, tongue, and/or palate were consistently observed in the fetus at all doses. The NOAELs for fetal toxicity in rats and rabbits were <15 mg/kg/day and <7 mg/kg/day, respectively (lowest doses tested). The NOAELs in these studies for maternal toxicity were 150 mg/kg/day and 21 mg/kg/day, in rats and rabbits, respectively. The FDA has indicated that teratogenicity is a class effect of endothelin receptor antagonists.

In a 14-week rat fertility study, a low incidence (1/11 animals) of male infertility was observed at the highest dose tested (300 mg/kg/day). Sperm abnormalities were observed at this and lower doses. Infertility was associated with bilateral diffuse testicular atrophy. A low incidence of unilateral diffuse testicular tubular atrophy and

epididymal aspermia was also observed at most but not all doses. The focal/multifocal tubular lesion was also observed in this study at most doses, but the incidence was very low (1/11 animals/group). Following a 13-week treatment-free recovery period in this study, the incidence of the focal/multifocal testicular tubular lesion declined to zero, suggesting that this lesion is reversible. However, the incidence of the diffuse lesion following the recovery period was somewhat increased compared to that observed at the end of the treatment phase (1/11 animals in each treatment dose group). In addition, the treated animals showing the diffuse lesion bilaterally after the recovery period were infertile. Thus there is no evidence that the diffuse lesion and its associated infertility were reversible over the 13-week treatment-free recovery period.

The genotoxicity of ambrisentan was assessed in the Ames test, *in vitro* chromosome aberration assay, rat unscheduled DNA synthesis and rat micronucleus assay. Mutagenicity was observed only in the chromosome aberration test, in which concentrations > 525 ug/mL produced only structural chromosome aberrations. There were no mutagenic effects observed with ambrisentan in either of the *in vivo* genotoxicity studies.

There are currently three toxicology studies underway with ambrisentan: 1) a 9-month dog chronic toxicity study, 2) a rat 15-week study with a 20-week drug-free recovery period to assess the reversibility of male fertility impairment, and 3) a small rat exploratory study to assess the effect of ambrisentan on serum bile salt levels.

APPENDIX

Study Title: BSF 208075: 13-Week Repeated Dose Toxicity (Feeding) Study in the Wistar Rat

Key study findings: NOAEL was not established due to findings of testicular atrophy at 10 mg/kg and above. Hepatocellular hypertrophy with mild elevation of AST, ALT and GGT at doses of 100 mg/kg and above. Increases in kidney, heart and liver weights at 100 mg/kg and above.

Study no: [redacted] Study No. 793528, [redacted] MPF/DT 0032 E
Volume #, and page #: *v3.2:p001*
Conducting laboratory and location: [redacted]
Date of study initiation: 12/20/2000
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. Lot No. L0004663
Formulation/vehicle: food admixture

Methods:

Species/strain; sex: rat, HanBrl:Wistar (SPF), males and females
Doses: 0 (control), 10, 100, 500 and 2000 mg/kg/day
Duration: 13 weeks
Main study No. /group: 12/sex/group
Additional groups: 6/sex/group for 8-week recovery, 9/sex/group for toxicokinetics

Allocation and Target Dose Levels	Group 1* 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day	Group 5 2000 mg/kg/day
Males A	1-12	28-39	55-66	82-93	109-120
Males B	13-18	40-45	67-72	94-99	121-126
Males C1	19-21	46-48	73-75	100-102	127-129
Males C2	22-24	49-51	76-78	103-105	130-132
Males C3	25-27	52-54	79-81	106-108	133-135
Females A	136-147	163-174	190-201	217-228	244-255
Females B	148-153	175-180	202-207	229-234	256-261
Females C1	154-156	181-183	208-210	235-237	262-264
Females C2	157-159	184-186	211-213	238-240	265-267
Females C3	160-162	187-189	214-216	241-243	268-270

A Main Study (termination after 13 weeks of treatment)
B Recovery (termination after 13 weeks of treatment and 8 weeks of recovery)
*Control animals received identical feed without the test item.
C1 to C3 – Satellite animals for toxicokinetical evaluations

Parameters examined:

Mortality: twice daily
Clinical signs: daily
Food consumption: weekly
Body weights: weekly
Ophthalmoscopy: pretest, 13 weeks and following recovery
Hematology: 4 and 13 weeks, recovery (21 weeks); the following parameters were measured:

Hematology Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	1
Hemoglobin	HB	mmol/L	1
Hematocrit	HCT	L/L	1
Mean corpuscular volume	MCV	fL	1
Mean corpuscular hemoglobin	MCH	fmol	1
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	1
Platelet count	PLATELETS	G/L	1
Reticulocyte count	RETIC.	%(rel.) T/L(abs.)	2
Reticulocyte fluorescence ratios	HFR = high, MFR = middle, LFR = low	%	2
Nucleated erythrocytes (normoblasts)	NEN	NEN/100WBC	3

Hematology Parameter	Abbreviation	Unit	Instrumentation
Total leukocyte count	WBC	G/L	1
Differential leukocyte count	Diff. WBC Count[4]	1(rel.) G/L(abs.)	3
Red blood cell morphology	TYPE CELL	normal/abnormal	3
Thromboplastin time (=prothrombin time)	PT	sec	5
Activated partial thromboplastin time	APTT	sec	5

Key:
1 [redacted] Multi-Parameter Automated Hematology Analyzer
2 [redacted] Automated Reticulocyte Analyzer
3 [redacted] light microscope and Computer Printer Blood Cell Calculator
4 Cell Classification: BAND. = Band Neutrophil; SEG. = Segmented Neutrophil; EO. = Eosinophil; BASO. = Basophil; LYMPH = Lymphocyte; MONO. = Monocyte; PLAS. = Plasma Cell; OTHER = Blast Cell (undifferentiated)
5 Instrumentation Laboratory (IL) ACL 300 Coagulation System

Clinical chemistry: 4 and 13 weeks, recovery (21 weeks). the following parameters were measured:

Clinical Biochemistry Parameter	Abbreviation	Unit	Instrumentation
Glucose		mmol/L	1
Urea		mmol/L	1
Creatinine		µmol/L	1
Bilirubin, total	BILI. T	µmol/L	1
Lipids total*	LIPIDS T.	g/L	1
Cholesterol, total	CHOLEST. T	mmol/L	1
Triglycerides	TRIGL.	mmol/L	1
Phospholipids	PHOS.LIPID	mmol/L	1
Aspartate aminotransferase	ASAT/GOT	µkat/L (37°C)	1
Alanine aminotransferase	ALAT/GPT	µkat/L (37°C)	1
Lactate dehydrogenase	LDH	µkat/L (37°C)	1
Creatine kinase	CK	µkat/L (37°C)	1
Alkaline phosphatase	ALP	µkat/L (37°C)	1
Gamma-glutamyl transferase	G-GT	nkat/L (37°C)	1
Calcium		mmol/L	1
Phosphorus		mmol/L	1
Sodium		mmol/L	1
Potassium		mmol/L	1
Chloride		mmol/L	1
Protein, total	PROTEIN T.	g/L	1
Protein, electrophoresis		g/L	2
Albumin	ALBUMIN		
Alpha 1-globulin	A1-GLOB.		
Alpha 2-globulin	A2-GLOB.		
Beta globulin	B-GLOB.		
Gamma globulin	G-GLOB.		
Globulin		g/L	2
Albumin/Globulin ratio	A/G RATIO	---	2

Key:
* total lipids were determined on weeks 4 and 13 only
1 [redacted] Discrete Random-Access Analyzer
2 [redacted] Automated Multiparameter Agarose Gel Electrophoresis Processing System and Multitask Scanning Densitometer System

Urinalysis: 4 and 13 weeks, recovery (21 weeks); the following parameters were measured:

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On Original

Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Volume (16-hour)		ml	1
Specific gravity	SPEC. GRAV.	1	2
Osmolality		mmol/kg	3
Color		-	4
Appearance		-	4
pH		-	5
Protein		0 = negative 1 = 0.25 g/L 2 = 0.75 g/L 2 = 1.50 g/L 3 = ≥5.00 g/L	5
Glucose		0 = normal 1 = 3 mmol/L 1 = 8 mmol/L 2 = 17 mmol/L 3 = ≥56 mmol/L	5
Ketone		0 = negative 0 = 0.5 mmol/L 1 = 1.5 mmol/L 2 = 5.0 mmol/L 3 = ≥15 mmol/L	5
Bilirubin		0 = negative 1 = 17 µmol/L 2 = 50 µmol/L 3 = ≥100 µmol/L	5

Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Blood		0 = negative 1 = 15 RBCs/µL 1 = 25 RBCs/µL 2 = 80 RBCs/µL 2 = 150 RBCs/µL 3 = ≥250 RBCs/µL	5
Nitrite		negative / positive	5
Urobilinogen	UROBILI.	0 = normal 1 = 17 µmol/L 2 = 68 µmol/L 3 = 135 µmol/L 3 = ≥ 203 µmol/L	5
Urine sediment	SED. MICRO.	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Red blood cells	RBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
White blood cells	WBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Crystals (Triple phosphate)	TRIP. PHOS	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6

Key:
1 Metabolism cage
2 [redacted] Refractometer
3 [redacted] Multi-Sample Osmometer
4 Visual observation
5 Reagent-Test-Strip / [redacted] used with the [redacted] Semi-Automated Urine Chemistry Analyzer
6 [redacted] light microscope

Histopathology: The following Table lists all tissues collected at necropsy. Those in boldface were examined in control and high dose rats only. Those underlined were examined in all groups.

Adrenal glands
Aorta
Auricles
Bone with bone marrow (femur including articular surface, sternum)
Brain - including section of medulla/pons, cerebral and cerebellar cortex
Cecum
Colon
Duodenum
Epididymides
Ductus deferens
Esophagus
Eyes with optic nerve
Harderian glands
Heart
Ileum
Jejunum
Kidneys
Lacrimal glands
Liver
Lungs, infused with formalin at necropsy
Lymph nodes – mandibular, bronchial, mesenteric
Mammary gland area
Nasal turbinates
Ovaries
Pancreas
Pituitary gland
Prostate gland
Rectum
Salivary glands - mandibular, sublingual, parotid
Sciatic nerve
Seminal vesicles
Skeletal muscle (thigh)
Skin
Spinal cord - cervical, midthoracic, lumbar
Spleen
Stomach
Testes
Thymus
Thyroid gland / parathyroid gland
Tongue
Trachea
Urinary bladder, infused with formalin at necropsy
Uterus/cervix
Vagina
All gross lesions

The following organ weights were recorded on the scheduled dates of necropsy:

Adrenals	Liver	Prostate gland
Brain	Mesenteric lymph nodes	Spleen
Heart	Ovaries	Testes
Kidneys	Pituitary gland	Thymus

The organ to terminal body weight ratios as well as organ to brain weight ratios were determined.

The determination of the terminal body weight was performed immediately prior to necropsy.

Toxicokinetics: Blood for plasma levels of BSF 208075 was collected on Days 2, 29 and 91 of dosing. The times for collection were 0800, 1200, 2000, 2400, 0400 and 0800 hrs.

Results:

Test item intake: was within acceptable limits as follows:

Group	Target Dose	Males		Females	
	(mg/kg/day)	Nominal Value	% of target dose	Nominal Value	% of target dose
1	0	---	---	---	---
2	10	9.53	95.3	9.75	97.5
3	100	95.2	95.2	95.9	95.9
4	500	471	94.2	483	96.6
5	2000	1926	96.3	1966	98.3

Mortality: Mortality findings are summarized below. All deaths are considered treatment-related.

	Group 1 (control) 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day	Group 5 2000 mg/kg/day
Main study - Male	0/12	0/12	0/12	1/12	0/12
Recovery - Male	0/6	0/6	0/6	0/6	0/6
TK - Male	0/9	0/9	0/9	0/9	0/9
Main study - Female	0/12	0/12	0/12	0/12	2/12
Recovery - Female	0/6	0/6	0/6	0/6	0/6
TK - Female	0/9	0/9	0/9	2/9	2/9

Clinical signs: Minor clinical signs of ruffled fur, soft feces and emaciation were seen in Group 3 and above, mainly towards the end of the treatment period. In addition, single animals exhibited exophthalmos, breathing or motility (abnormal gait or posture) disturbances. Females of Group 5 (decedents) showed additional signs of lateral recumbency, head tilt, skin cyanosis, sedation and tremor. The incidence and severity of clinical signs declined or disappeared during the recovery period.

Toxicokinetics: Parameters are summarized below:

Dose (mg/kg/day)	Parameter	Unit	Days 2/3	Days 29/30	Days 91/92
MALES					
10	AUC	h x ng/ml	13598	17886	17507
	AUC/dose	h x ng/ml/(mg/kg)	1865.3	1783.3	1693.1
	C_{max}	ng/ml	741.7	1155	934.6
	T_{max}	day/h	3/8	29/8	91/8
100	AUC	h x ng/ml	132301	152067	130831
	AUC/dose	h x ng/ml/(mg/kg)	1782.3	1550.4	1313.6
	C_{max}	ng/ml	9989	8032	9792
	T_{max}	day/h	3/8	30/4	92/8
500	AUC	h x ng/ml	607039	632274	657360
	AUC/dose	h x ng/ml/(mg/kg)	1614.9	1309.2	1379.8
	C_{max}	ng/ml	35196	38485	32857
	T_{max}	day/h	2/8	29/24	91/24
2000	AUC	h x ng/ml	1798878	1718847	2660246
	AUC/dose	h x ng/ml/(mg/kg)	1196.2	908.4	1332.7
	C_{max}	ng/ml	150498	102307	133205
	T_{max}	day/h	2/8	30/4	91/12
FEMALES					
10	AUC	h x ng/ml	7630	8327	10412
	AUC/dose	h x ng/ml/(mg/kg)	931.6	842.8	989.7
	C_{max}	ng/ml	487.3	685.0	647.9
	T_{max}	day/h	2/24	29/8	92/4
100	AUC	h x ng/ml	110817	116287	138825
	AUC/dose	h x ng/ml/(mg/kg)	1353.2	1125.2	1335.8
	C_{max}	ng/ml	5860	6038	8336
	T_{max}	day/h	2/20	30/8	91/8
500	AUC	h x ng/ml	604933	619008	988273
	AUC/dose	h x ng/ml/(mg/kg)	1470.8	1188.4	1972.2
	C_{max}	ng/ml	34970	32568	58107
	T_{max}	day/h	¾	30/4	91/24
2000	AUC	h x ng/ml	1815255	1942645	3170881
	AUC/dose	h x ng/ml/(mg/kg)	1098.8	948.0	1555.4
	C_{max}	ng/ml	98467	112166	154216
	T_{max}	day/h	2/20	29/8	92/4

Food consumption:

The absolute food consumption was reduced in males of groups 3 (-l%), 4 (-6%) and 5 (-10%) beginning from around week 6 of treatment onwards until the end of the treatment period. This reduction was dose dependent and was more pronounced at week 13 (-8% in group 3, -22% in group 4 and -24% in group 5). The females of groups 3 (up to +9%), 4 (up +9%) and 5 (up to +12%) showed a tendency to higher absolute food consumption in the beginning of the treatment period (week 3-6). From week 9, a reduction of the absolute food consumption was evident in groups 4 and 5 (-20% in group 4 and -18% in group 5 at week 13). The relative food consumption was generally slightly higher in group 4 (+2% in males, +1% in females) and 5 (+l% in males, +2% in females) for males and females. This corresponded mainly with the reduction in body weights and body weight gain of these groups.

During the recovery period there was a general tendency towards compensatory higher food consumption in males and females of groups 3 (+ 9% in males, +7% in females), 4 (+2% in males, +1 % in females) and 5 (+1 % in males, +2% in females).

Body weights:

Body weights (BW) and body weight gain (BWG) of groups 4 and 5 were markedly reduced in males (-20% BW, -27% BWG in group 4 and -33% BW, -50% BWG in group 5) and females (-10% BW, -21% BWG in group 4 and -18% BW, -42% BWG in group 5) after 13 weeks of treatment. The onset of this finding was earlier in males than in females. Also the overall reduction was more accentuated in males than in females. A tendency towards lower body weights was also measurable in group 3 males (-5%) at the

end of the treatment period. These findings corresponded with the reduction in the absolute food consumption and were clearly test item-related.

The compensatory higher food consumption in the affected groups during the recovery period led to increasing body weight gains during this period. The body weights of the females of group 3 reached those of the control group after 3 weeks of recovery, whereas males in groups 4 (-9%) and 5 (-12%) still showed significantly lower weights at the end of the recovery period.

Ophthalmoscopy:

No test item-related effects were evident during the ophthalmoscopic examinations after the treatment and recovery periods. All findings which were recorded in the high dose animals (corneal opacity, persistent hyaloid vessel in vitreous body and/or persistent pupillary membrane) were considered to be incidental as they were also recorded in controls and during the pretest.

Hematology:

The hematological investigations showed the following differences between control and test item treated groups:

Males of groups 4 and 5 showed higher erythrocyte count and hematocrit as well as lower mean corpuscular hemoglobin concentration and platelet counts at the end of the treatment period. The reticulocyte flourescence ratios were right shifted towards higher middle and low flourescence ratio in males of groups 3, 4 and 5 at the end of the treatment period. In addition, the activated partial thromboplastin time was prolonged in males of groups 3, 4 and 5 and the thromboplastin time in males of groups 4 and 5.

Females of groups 4 and 5 showed higher erythrocyte count, hemoglobin and hematocrit as well as lower mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelets at the end of the treatment period. The relative and absolute reticulocyte counts were higher in group 5 females and the reticulocyte flourescence ratios were right shifted towards higher middle and low flourescence ratios in females of groups 3, 4 and 5 at the end of the treatment period.

None of these changes were seen at the end of the recovery period, confirming the reversibility of these finding after an 8 week treatment-free recovery. Findings are summarized in the following Table:

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)					
Group Dose	Parameter	Males		Females	
		Week 4	Week 13	Week 4	Week 13
Group 3 100 mg/kg/day	APTT	+ 9%	+7%	-	-
Group 4 500 mg/kg/day	Erythrocyte count	-	+18%	-	+19%
	Hemoglobin	-	-	-	+14%
	Hematocrit	-	+18%	-	+15%
	MCH	-	-	-	-4%
	MCHC	-	-3%	-	-1%
	Platelet counts	-	-19%	-	-23%
	APTT	+9%	+11%	-	-
	Thromboplastin time	+5%	+8%	-	-
Group 5 2000 mg/kg/day	Erythrocyte count	-	+10%	-	+17%
	Hemoglobin	-	-	-	+11%
	Hematocrit	-	+11%	-	+15%
	MCH	-	-	-	-6%
	MCHC	-	-4%	-	-4%
	platelet counts		-22%	-	-25%
	APTT	+14%	+9%	-	-6%
	Thromboplastin time	+6%	+9%	-	-6%
	Reticulocyte counts	-	-	-	+67%

Clinical chemistry:

Males of group 5 had lower glucose values after 4 and 13 weeks of treatment. Urea was increased in group 5 males after 13 weeks of dosing. The blood lipids (total lipids, cholesterol total, triclycerides, phospholipids) showed a tendency to lower values in males of groups 4 and 5.

Further changes included increased aspartate aminotransferase and alanine aminotransferase in group 5 males after 4 and 13 weeks and increased lactate dehydrogenase (LDH) in group 4 and 5 males after 4 weeks of treatment. Creatine kinase (after 4 weeks) and alkaline phosphatase levels (after 13 weeks) were increased in males of group 5. Phosphorus and sodium were higher in group 3, 4 and 5 males after 4 and 13 weeks. Chloride was lower in group 4 after 4 weeks and in groups 4 and 5 after 13 weeks. Total protein was lower in group 4 after 4 weeks and in group 4 and 5 after 13 weeks. Differences in the protein electrophoresis in groups 3, 4 or 5 males were mainly restricted to $\alpha 1$ globulin reduction and/or β globulin increase. The minor changes in the protein electrophoresis were generally neither time nor dose related, within the range of the historical data and therefore considered to be without toxicological relevance.

Females of group 5 had lower glucose and higher urea values after 13 weeks of treatment. Further changes included increased aspartate aminotransferase and alanine aminotransferase activities in group 5 females after 13 weeks. Lactate dehydrogenase was increased in group 4 and 5 females, whereas creatine kinase and alkaline phosphatase as well as gamma-glutamyl-transferase were increased in group 5 females after 13 weeks of treatment. Sodium was higher in females of groups 3, 4 and 5 after 4 weeks and in group 4 and 5 after 13 weeks. Phosphorus levels were increased in females of groups 3 and 4 after 4 weeks and in group 3, 4 and 5 after 13 weeks. Potassium was lower in group 3, 4 and 5 females after 4 weeks and higher after 13 weeks. Chloride was higher in group 3 females after 4 and lower after 13 weeks, lower in group 4 and 5 females after 4 and 13 weeks. Total protein was also lower in group 3, 4 and 5 females. Differences in the protein electrophoresis in all females were mainly restricted to $\alpha 1$ globulin reduction and/or β globulin increase. The minor changes in

the protein electrophoresis were generally neither time nor dose related, within the range of the historical data and therefore considered to be without toxicological relevance.

After the recovery period, males of groups 4 and 5 showed moderately lower total triglycerides, slightly higher phosphorus levels, slightly lower albumin levels moderate to marked increased lactate dehydrogenase activity. The latter finding was also noted in females of group 5. These findings were considered to be persistent test item-related effects and all other differences noted were considered to be incidental. Findings are summarized as follows:

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)

Group Dose	Parameter	Males		Females	
		Week 4	Week 13	Week 4	Week 13
Group 3 100 mg/kg/day	Phosphorus	+8%	-	+9%	+20%
	Sodium	+1%	-	+1%	(+1%)
	Potassium	-	-	-	(+6%)
	Chloride	-	-3%	+1%	-2%
	Total protein	-4%	-4%	-	(-3%)
Group 4 500 mg/kg/day	LDH	(+33%)	(+5%)	-	+57%
	Total lipids	-13%	-14%	-	-
	Cholesterol, total	-17%	-19%	-	-
	Triglycerides	-12%	-18%	-	-
	Phospholipids	-13%	-10%	-	-
	Phosphorus	+16%	+26%	+9%	+35%
	Sodium	+1%	+2%	+1%	+2%
	Potassium	-	-	-	(+6%)
	Chloride	-1%	-4%	-1%	-5%
	Total protein	-4%	-6%	-	-6%
	Albumin (rel.)	-	+3%	-	-3%
Group 5 2000 mg/kg/day	Glucose	-10%	-35%	-	-19%
	Urea	-	+31%	-	+29%
	LDH	+87%	(+15%)	-	+114%
	Creatine kinase	+35%	-	-	+51%
	Alkaline phosphatase	-	+25%	-	+101%
	Gamma-GT	-	-	-	+65%
	Total lipids	(-7%)	-12%	-	-
	Cholesterol, total	(-7%)	(-14%)	-	-
	Triglycerides	(-23%)	-30%	-	+48%
	Phospholipids	(-5%)	(-9%)	-	-
	ASAT	+12%	+39%	-	+34%
	ALAT	+30%	+59%	+11%	(+31%)
	Phosphorus	+12%	+20%	(+6%)	+33%
	Sodium	+2%	+3%	+2%	+4%
	Potassium	-	-	-	+18%
	Chloride	-	-4%	(-1%)	-5%
	Total protein	-	-6%	-	-8%
	Albumin (rel.)	-	+3%	-3%	-9%

() increase or decrease not statistically significant

Urinalysis:

After 4 weeks of treatment, males of group 5 showed a lower urine volume and corresponding higher specific gravity and osmolality. After 13 weeks lower urine volumes were seen in groups 3, 4 and 5 but without correlating changes in specific gravity and osmolality.

Histopathology findings:

Organ weights:

There were several differences in absolute and relative (to body or brain weight) organ weights between test item treated animals of groups 4 and 5 and control animals. Absolute pituitary, liver, kidney and spleen weights of group 4 and 5 males were lower than in the corresponding control males. Additionally the absolute brain and thymus weights of group 5 males were reduced. Absolute pituitary, liver, kidney and ovary weights of group 5 females were lower than in the corresponding control females whereas only the absolute pituitary weights of group 4 females were reduced. Differences from the controls which were noted in individual organ weights of the other

dose groups were without a dose relationship and were therefore considered to be incidental.

Due to the marked difference in the body weights between control and test item treated animals, the relative organ weight ratios reflected a more realistic picture of the test item-related changes:

The organ to body weight ratios of group 4 and 5 males showed increased brain, heart, liver, kidney, adrenal, testes and prostate values. Group 3 males showed increased heart, liver, kidney and prostate values. Group 4 and 5 females showed increased brain and heart values relative to the body weights.

The organ to brain weight ratios showed lower pituitary and liver values in group 4 and 5 males and a lower spleen ratio in group 5 males. In females, lower pituitary ratios occurred in group 4 and 5 females. Differences from the controls which were noted in individual organ weights of the other dose groups were without a dose relationship and were therefore considered to be incidental.

After the recovery period, males of group 5 had still higher heart (+21 %) and kidney (+16%) values. Relative organ weights showing changes from control are summarized below:

Changes in organ to body weight ratios in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)

Group Dose	Parameter	Males		Females	
		Week 13	Week 21	Week 13	Week 21
Group 3 100 mg/kg/day	Heart	+18%	-	(+11%)	-
	Liver	+10%	-	-	-
	Kidney	+10%	-	-	-
	Prostate	+20%	-	-	-
	Adrenal	(+14%)	-	-	-
	Testes	(+10%)	-	-	-
Group 4 500 mg/kg/day	Brain	+29%	-	+8%	-
	Heart	+29%	-	+21%	-
	Liver	(+4%)	-	-	-
	Kidney	+16%	-	-	-
	Prostate	+25%	-	-	-
	Adrenal	+21%	-	-	-
	Testes	+34%	-	-	-
Group 5 2000 mg/kg/day	Brain	+39%	-	+18%	-
	Heart	+29%	+21%	+32%	-
	Liver	+10%	-	-	-
	Kidney	+29%	+16%	-	-
	Prostate	+20%	-	-	-
	Adrenal	+36%	-	-	-
	Testes	+49%	-	-	-

() increase or decrease not statistically significant

Gross findings:

Test item-related changes included distended cecum (containing digested food) in group 4 and 5 animals, dilation of the duodenum and colon in group 5, red discoloration of the mesenteric lymph node in groups 4 and 5, size reduction of the uterus in females of group 4 and 5, flaccid testes in each one male of group 3 and 5 and dark red discoloration of the ovaries in females in groups 4 and 5. All other organs and animals were without test item-related macroscopic findings.

Microscopic findings:

The following findings recorded at the end of the treatment period were considered to distinguish treated rats from the control rats:

Liver

A minimal to slight diffuse hepatocellular hypertrophy was present in 2/12 males and 3/12 females of group 4, and all 12 males and 10/12 females of group 5. A moderately decreased incidence of hepatocytic glycogen deposits was noted in females of group 4 and animals of group 5.

Spleen

A minimally increased severity of hemopoiesis was seen in males of both groups 4 and 5, and a minimally to slightly increased incidence and/or severity of hemopoiesis were diagnosed in females of both groups 4 and 5.

Mesenteric lymph node

A minimal to moderate congestion was noted in each 1/12 males and females of group 4, and 5/12 males and 8/10 females of group 5. A minimal to slight histiocytosis was seen in 2/12 males and 3/10 females of group 5.

Duodenum

A minimal dilation was diagnosed in 9/12 males and 4/12 females of group 4, and 8/12 males and 9/12 females of group 5. A minimal villous hypertrophy was noted in 2/12 males and 4/12 females of group 4, and 7/12 males and 8/12 females of group 5.

Jejunum

A minimal to slight dilation was seen in 5/12 males and 2/12 females of group 5.

Ileum

A minimal to slight dilation was seen in 3/12 males and 2/12 females of group 5.

Cecum

A minimal dilation was noted in 8/12 males and 3/12 females of group 4, and 10/12 males and 7/12 females of group 5.

Colon

A minimal dilation was diagnosed in 5/12 males and 4/12 females of group 5.

Thymus

A minimal to marked atrophy was found in each 3/12 males and females of both groups 4 and 5.

Testes

A marked bilateral diffuse tubular atrophy was noted in 1/12 males (no.114) of group 5. This atrophy was associated with a slight unilateral spermatic granuloma. Focal/multifocal tubular atrophy was present in each 3/12 males of groups 2 and 3, 6/12 males of group 4, and 3/12 males of group 5. The severity of this finding was minimal in group 2 and minimal to moderate in groups 3, 4 and 5. A tubular dilation was seen in 2/12 males of group 4 and 7/12 males of group 5. The severity of this finding was minimal to slight.

Epididymides

An aspermia was found in each 1/12 males of groups 3 (no.59) and group 5 (no. 114). An unilateral sperm granuloma was noted in 1/12 males (no.115) of group 5. The severity of this finding was moderate.

Ovaries

A minimal to slight stromal cell hyperplasia was seen in 5/12 females of group 4 and 10/12 females of group 5. A minimal to slight congestion was noted in 5/12 females of group 4 and 2/12 females of group 5.

Adrenal cortex

A minimal to slight diffuse hypertrophy of the zona glomerulosa was noted in 9/12 males and 5/12 females of group 4, and 9/12 males and all females of group 5. A slightly increased incidence of diffuse fatty change was diagnosed in males of groups 4 and 5.

Uterus/cervix

A minimal myometrial atrophy was present in 3/12 females of group 4, and 4/12 females of group 5.

Bronchial lymph node

A minimal to slight congestion was noted in 1/12 males and 2/12 females of group 5.

Nasal cavity

Anterior part: A minimal to moderate osseous hyperplasia was seen in 3/12 males and 4/12 females of group 3, 6/12 males and 8/12 females of group 4, and 11/12 males and 8/12 females of group 5. The osseous hyperplasia was associated with a minimal to slight focal to multifocal chronic inflammation in all these animals.

Intermediate part: A minimal to moderate osseous hyperplasia was noted in 2/12 males and 4/12 females of group 3, 5/12 males and 9/12 females of group 4, and each 9/12 males and females of group 5. The osseous hyperplasia was associated with a minimal focal to multifocal chronic inflammation in 2/12 males and 3/12 females of group 3, 4/12 males and 3/12 females of group 4, and 5/12 males and 2/12 females of group 5.

Posterior part: A minimal to moderate osseous hyperplasia was found in 2/12 males and 3/12 females of group 3, 11/12 males and all 12 females of group 4, and all 12 males and females of group 5.

Recovery group findings

A number of findings were noted after an additional 8-week recovery period of eight weeks. From these findings, the following ones distinguished treated rats from the control rats:

Testes

A marked bilateral diffuse tubular atrophy was noted in 1/6 males of group 03. This atrophy was associated with moderate bilateral spermatic granulomas. An unilateral marked diffuse tubular atrophy was noted in 1/6 males of group 3 and 1/6 males of group 4. Minimal focal / multifocal tubular atrophy was seen in 2/6 males in group 1, 1/6 males in group 2, 3/6 males in group 3, 2/6 males in group 4 and 4/6 males in group 5.

Epididymides

An aspermia was found in 2/6 males of group 3 and 1/6 males of group 4.

Nasal cavity

Anterior part: A minimal osseous hyperplasia was seen in 1/6 males of group 5.

Intermediate part: A minimal osseous hyperplasia was noted in each 2/6 males and females of group 5.

Posterior part: A minimal osseous hyperplasia was found in each 2/6 males and females of group 4, and 2/6 males and 1/6 females of group 5.

Other organs/tissues

A number of findings were diagnosed in the other organs and tissues examined after termination of the 13-week treatment period or an additional recovery period. Their incidence, severity, and morphologic appearance did not distinguish treated rats from the control rats.

Conclusions:

Oral administration of BSF 208075 to Wistar rats at target doses of 10, 100, 500 and 2000 mg/kg/day for 92-94 days resulted in the unscheduled deaths of one male and two females of group 4 and of four group 5 females, which were found dead or killed in extremis during the second part of the treatment or during the first days of the recovery period. In one group 4 male the moderate diffuse hemorrhage in the lung was likely the cause of death. In two females of group 5 the thymus was markedly atrophic without any hints of the cause of death. Although the cause of death was not evident in most of these animals or could not be assessed morphologically (4/7 animals were TK satellite animals), the death or moribundity of all of them was considered to be test-item-related.

The plasma level determination indicated an adequate and dose proportional exposure to the test item. No gender-specific changes and no accumulation were evident.

No test item-related changes in ophthalmoscopy and in urinalysis were noted after the treatment and recovery periods.

Test item-related findings were restricted to a slight to moderate reduction of the food consumption and consequent slight to marked reduction of the body weight of groups 3, 4 and 5. This was considered a toxic effect and not the consequence of decreased palatability. Clinical signs observed at target doses of 100 mg/kg/day or more included respiratory (dyspnea) and gastrointestinal findings (soft feces), eye lesions (exophthalmus), motility disturbances (abnormal gait or posture) and general signs of toxicity (ruffled fur and emaciation). The severity of these clinical findings was slight to moderate.

Hematological investigations showed test item related effects on the red blood cell parameters, platelets and coagulation time in males and/or females of groups 3 and/or 4 and 5. Significant changes were noted on red blood cell parameters with consequent influence on reticulocytes (higher erythrocyte and hematocrit count, lower mean corpuscular hemoglobin concentration or mean corpuscular hemoglobin, increased

hemoglobin levels, right-shifted low fluorescence reticulocytes, increased absolute reticulocyte count). In addition, lower platelet counts and prolonged coagulation times were noted from group 3 onwards.

Significant test item-related changes in clinical biochemical parameters indicated mainly effects on the liver, kidneys and blood lipids in groups 3 and/or groups 4 and 5. Lower glucose levels, increased aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl-transferase are considered to reflect an effect on the liver. Increased urea level and effects on the electrolytes (increased phosphorus, potassium and sodium, lower chloride) suggesting some toxic effect on the kidneys although - apart from kidney weight increases - no further pathological changes were noted. Effects on the blood lipids (lower values of total cholesterol, triglycerides and phospholipids) can only be partially correlated with the reduced food consumption. Additional changes were increased activities of alkaline phosphatase, lactate dehydrogenase, creatine kinase and lower total protein.

Changes in relative organ/body weights were considered to be the most relevant ones due to the marked decrease in body weight gain in several treatment groups. Test-item-related changes in relative organ/body weights were seen in group 3 and/or 4 and 5 males (increased weights of the heart, liver, kidneys, adrenals and prostate). These changes were minimal in the liver (males only), and slight to moderate in the heart, kidneys, adrenals and prostate. After the recovery period males of group 5 had still slightly higher heart and kidney values.

In the group 5 males showing gross findings of dilation of duodenum, cecum and colon, the changes correlated microscopically with a dilation of the respective organs and were considered test item-related. The macroscopic finding of reddish discoloration of the mesenteric lymph node in animals at 500 and/or 2000 mg/kg correlated with congestion and/or histiocytosis and was likely test item-related. The flaccid testes in one group 3 and 5 rat each correlated with moderate focal or marked diffuse tubular atrophy. The dark red discoloration in the ovaries of some group 4 and 5 rats correlated microscopically with congestion and the reduction of uterine size with a myometrial atrophy.

Microscopic findings in terminally sacrificed animals were noted in the liver, spleen, duodenum, testes, epididymides, ovaries, adrenal cortices, and nasal cavity. In the liver, a diffuse hepatocellular hypertrophy was present in rats of groups 4 and 5. The spleen revealed an increased hemopoiesis in group 4 and 5 females. In the duodenum, a villous hypertrophy was seen in group 4 and 5 animals.

The testes showed focal tubular atrophies in all treatment groups, but without a dose-relation. Nevertheless, this type of atrophy was considered to be test-item-related. The focal/multifocal tubular atrophy in 3 group 2 animals was only of minimal severity and not associated with epididymidal changes. The bilateral and diffuse tubular atrophy in one group 5 mate, associated with a testicular spermatic granuloma and an aspermia in the epididymides, was attributed to the effect of the test item. In addition, the testes

revealed a tubular dilation in group 4 and 5 males, which was also considered test-item-related as well as the sperm granuloma in the epididymides of another group 5 male.

Stromal cell hyperplasia in the ovaries was observed in one group 3 and several group 4 and 5 females; the single incidence in group 3 was regarded incidental. In most of these females congestion was also present. In the adrenal cortices, an increase in diffuse fatty change was present in group 4 and 5 males. Furthermore, a diffuse hypertrophy of zona glomerulosa was noted in animals of groups 4 and 5 and was considered test item-related.

Osseous hyperplasia was present in all parts of the nasal cavity in groups 3-5. In the anterior part, the osseous hyperplasia was in all animals associated with focal/multifocal chronic inflammation, whereas in the intermediate part, only about half of the animals revealed also an inflammation, and in the posterior part, no inflammation was seen.

After 8 weeks of recovery, the findings in the liver, spleen, duodenum, ovaries, and adrenal cortices were fully reversed, whereas findings in the testes/epididymides and nasal cavity were still present but less prominent. Two males of group 3 and one male of group 4 revealed a unilateral or bilateral diffuse tubular atrophy of testes, associated with an aspermia in the epididymides. In the nasal cavity, a minimal osseous hyperplasia was noted in a few animals of groups 4 and/or 5.

A number of degenerative, inflammatory, reactive, and/or neoplastic lesions were noted after treatment or the recovery period such as progressive myocardiopathy, tubular basophilia in kidneys, lymphoid cell infiltration in various organs, alveolar histiocytosis in the lung, retinal atrophy, and tubular cell adenoma or tubular cell carcinoma or nephroblastoma in the kidneys. These were isolated incidences with no evidence of dose-relatedness.

Based on the results of this study, the No-Observed-Adverse-Effect-Level (NOAEL) was less than 10 mg/kg /day based on testicular findings at 10 mg/kg (AUC 17.5 and 10 µghr/mL for M and F, respectively). Testicular findings were not present at this dose level in recovery animals. The findings described above are consistent with those of previously reviewed 4-week studies, where liver hypertrophy, increased kidney and heart weights, testicular atrophy and osseous hyperplasia of the nasal cavity were reported.

Study Title: BSF 208075: 26-Week Repeated Dose Oral Toxicity (Feeding) Study in the Wistar Rat

Key study findings: Morbidity/mortality was observed at 500 mg/kg, a dose which exhibited little toxicity in 13 week studies, indicating duration-dependent toxicity which is manifest between 16 and 20 weeks. Results indicate the toxicity involves cardiovascular failure, probably associated with hypoxia and acidosis resulting from airway obstruction due to nasal turbinate hyperplasia. Clinical signs of labored breathing are consistent with this conclusion.

Study no: [redacted] Study No. 800313, [redacted] MPR/PT 0106 E
Volume #, and page #: *v4.3:p001*
Conducting laboratory and location: [redacted]
Date of study initiation: 7/4/2001
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. Lot No. 10020
Formulation/vehicle: food admixture

Methods:

Species/strain; sex: rat, HanBrl:Wistar (SPF), males and females
Doses: 0 (control), 5, 100 and 500 mg/kg/day
Duration: 26 weeks
Main study No. /group: 12/sex/group
Additional groups: 6/sex/group for 20-week recovery, 9/sex/group for toxicokinetics

Allocation and Target Dose Levels	Group 1* 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day
Males A	1-12	28-39	55-66	82-93
Males B	13-18	40-45	67-72	94-99
Males C1	19-21	46-48	73-75	100-102
Males C2	22-24	49-51	76-78	103-105
Males C3	25-27	52-54	79-81	106-108
Females A	109-120	136-147	163-174	190-201
Females B	121-126	148-153	175-180	202-207
Females C1	127-129	154-156	181-183	208-210
Females C2	130-132	157-159	184-186	211-213
Females C3	133-135	160-162	187-189	214-216

A - Main Study (termination after 26 weeks of treatment)
B – Recovery (termination after 26 weeks of treatment and 20 weeks of recovery)
*Control animals received identical feed without the test item.
C1 to C3 – Toxicokinetics

Parameters examined:

Mortality: twice daily
Clinical signs: daily
Food consumption: weekly
Body weights: weekly
Ophthalmoscopy: pretest, 26 weeks and following recovery (week 46)
Hematology: 4, 13 and 26 weeks, recovery (week 46); the following parameters were measured:

Hematology Parameter	Abbreviation	Unit	Instrumentation
Erythrocyte count	RBC	T/L	1
Hemoglobin	HB	mmol/L	1
Hematocrit	HCT	L/L	1
Mean corpuscular volume	MCV	fL	1
Mean corpuscular hemoglobin	MCH	fmol	1
Mean corpuscular hemoglobin concentration	MCHC	mmol/L	1
Platelet count	PLATELETS	G/L	1
Reticulocyte count	RETIC.	%(rel.) T/L(abs.)	2
Reticulocyte fluorescence ratios	HFR = high, MFR = middle, LFR = low	%	2
Nucleated erythrocytes (normoblasts)	NEN	NEN/100WBC	3

Hemstology Parameter	Abbreviation	Unit	Instrumentation
Total leukocyte count	WBC	G/L	1
Differential leukocyte count	Diff. WBC Count[4]	%(rel.) G/L(abs.)	3
Red blood cell morphology	TYPE CELL	normal/abnormal	3
Thromboplastin time (=prothrombin time)	PT	sec	5
Activated partial thromboplastin time	APTT	sec	5

Key:
1 Multi-Parameter Automated Hematology Analyzer
2 [redacted] Automated Reticulocyte Analyzer
3 [redacted] light microscope and Computer Printer Blood Cell Calculator
4 Cell Classification: BAND. = Band Neutrophil, SEG. = Segmented Neutrophil, EO. = Eosinophil, BASO. = Basophil, LYMPH = Lymphocyte, MONO. = Monocyte, PLAS. = Plasma Cell, OTHER = Blast Cell (undifferentiated)
5 [redacted] Coagulation System

Clinical chemistry: 4, 13 and 26 weeks, recovery (week 46). The following parameters were measured:

Clinical Biochemistry Parameter	Abbreviation	Unit	Instrumentation
Glucose		mmol/L	1
Urea		mmol/L	1
Creatinine		µmol/L	1
Bilirubin, total	BILI. T	µmol/L	1
Lipids total*	LIPIDS T.	g/L	1
Cholesterol, total	CHOLEST. T	mmol/L	1
Triglycerides	TRIGL.	mmol/L	1
Phospholipids	PHOS.LIPID	mmol/L	1
Aspartate aminotransferase	ASAT/GOT	µkat/L (37°C)	1
Alanine aminotransferase	ALAT/GPT	µkat/L (37°C)	1
Lactate dehydrogenase	LDH	µkat/L (37°C)	1
Creatine kinase	CK	µkat/L (37°C)	1
Alkaline phosphatase	ALP	µkat/L (37°C)	1
Gamma-glutamyl transferase	G-GT	nkat/L (37°C)	1
Calcium		mmol/L	1
Phosphorus		mmol/L	1
Sodium		mmol/L	1
Potassium		mmol/L	1
Chloride		mmol/L	1
Protein, total	PROTEIN T.	g/L	1
Protein, electrophoresis		g/L	2
Albumin	ALBUMIN		
Alpha 1-globulin	A1-GLOB.		
Alpha 2-globulin	A2-GLOB.		
Beta globulin	B-GLOB.		
Gamma globulin	G-GLOB.		
Globulin		g/L	2
Albumin/Globulin ratio	A/G RATIO	---	2

Key:
* total lipids were determined on weeks 4 and 13 only
1 Discrete Random-Access Analyzer
2 [redacted] ; Automated Multiparameter Agarose Gel Electrophoresis Processing System and Multitask Scanning Densitometer System

Urinalysis: 4, 13 and 26 weeks, recovery (week 46); the following parameters were measured:

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Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Volume (16-hour)		ml	1
Specific gravity	SPEC. GRAV.	1	2
Osmolality		mmol/kg	3
Color		-	4
Appearance		-	4
pH		-	5
Protein		0 = negative	5
		1 = 0.25 g/L	
		2 = 0.75 g/L	
		2 = 1.50 g/L	
		3 = ≥5.00 g/L	
Glucose		0 = normal	5
		1 = 3 mmol/L	
		1 = 6 mmol/L	
		2 = 17 mmol/L	
		3 = ≥56 mmol/L	
Ketone		0 = negative	5
		0 = 0.5 mmol/L	
		1 = 1.5 mmol/L	
		2 = 5.0 mmol/L	
		3 = ≥15 mmol/L	
Bilirubin		0 = negative	5
		1 = 17 µmol/L	
		2 = 50 µmol/L	
		3 = ≥100 µmol/L	

Urinalysis Parameter	Abbreviation	Unit/Score	Instrumentation
Blood		0 = negative	5
		1 = 15 RBCs/µL	
		1 = 25 RBCs/µL	
		2 = 80 RBCs/µL	
		2 = 150 RBCs/µL	
		3 = ≥250 RBCs/µL	
Nitrite		negative / positive	5
Urobilinogen	UROBIL.	0 = normal	5
		1 = 17 µmol/L	
		2 = 68 µmol/L	
		3 = 135 µmol/L	
		3 = ≥ 203 µmol/L	
Urine sediment	SED. MICRO.	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Red blood cells	RBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
White blood cells	WBC	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6
Crystals (Triple phosphate)	TRIP. PHOS	normal = 0 abnormal amount = 1 (small), 2 (moderate), 3 (large)	6

Key:
1 Metabolism cage
2 [redacted] Refractometer
3 Multi-Sample Osmometer
4 Visual observation
5 Reagent-Test-Strip [redacted] used with the [redacted] Semi-Automated Urine Chemistry Analyzer
6 [redacted] light microscope

Histopathology: The following Table lists all tissues collected at necropsy. Those in boldface were examined in control and high dose rats only. Those underlined were examined in all groups.

Adrenal glands
Aorta
Auricles
Bone with bone marrow (femur including articular surface, sternum)
Brain - including section of medulla/pons, cerebral and cerebellar cortex
Cecum
Colon
Duodenum
Epididymides
Ductus deferens
Esophagus
Eyes with optic nerve
Harderian glands
Heart
Ileum
Jejunum
Kidneys
Lacrimal glands
Liver
Lungs, infused with formalin at necropsy
Lymph nodes – mandibular, bronchial, mesenteric
Mammary gland area
Nasal turbinates
Ovaries
Pancreas
Pituitary gland
Prostate gland
Rectum
Salivary glands - mandibular, sublingual, parotid
Sciatic nerve
Seminal vesicles
Skeletal muscle (thigh)
Skin
Spinal cord - cervical, midthoracic, lumbar
Spleen
Stomach
Testes
Thymus
Thyroid gland / parathyroid gland
Tongue
Trachea
Urinary bladder, infused with formalin at necropsy
Uterus/cervix
Vagina
All gross lesions

The following organ weights were recorded on the scheduled dates of necropsy:

Adrenals	Liver	Prostate gland
Brain	Mesenteric lymph nodes	Spleen
Heart	Ovaries	Testes
Kidneys	Pituitary gland	Thymus

The organ to terminal body weight ratios as well as organ to brain weight ratios were determined.

The determination of the terminal body weight was performed immediately prior to necropsy.

Toxicokinetics: Blood for plasma levels of BSF 208075 was collected on Day 2, and Week 13 and 26 of dosing. The times for collection were 0800, 1200, 2000, 2400, 0400 and 0800 hrs.

Results:

Test item intake: was within acceptable limits as follows:

Group	Target Dose	Males		Females	
	(mg/kg)	Value	% of target dose	Value	% of target dose
1	0	—	—	—	—
2	5	4.94	98.8	5.03	100.6
3	100	98.90	98.9	102.08	102.1
4	500	485.55	97.1	507.46	101.5

Mortality: Mortality findings are summarized below. All deaths are considered treatment-related. Deaths in "recovery" groups occurred during the treatment period and not during recovery.

	Group 1 (control) 0 mg/kg/day	Group 2 5 mg/kg/day	Group 3 100 mg/kg/day	Group 4 500 mg/kg/day
Main study - **Male**	0/12	0/12	1/12	7/12
Recovery - **Male**	0/6	0/6	1/6	4/6
TK - **Male**	0/9	0/9	0/9	5/9
Main study - **Female**	0/12	0/12	2/12	10/12
Recovery - **Female**	0/6	0/6	1/6	5/6
TK - **Female**	0/9	0/9	1/9	7/9

Clinical signs:

In animals treated with 500 mg/kg/day, moderate clinical signs were noted starting in weeks 10 (females) and 17 (males) of the treatment period and ending at week 6 (rales only) of the recovery period. They were restricted to: hunched posture, uncoordinated movements, breathing disturbances (labored respiration and rales), emaciation, swelling of the abdomen, poor condition, blue skin and ruffled fur. At 100 mg/kg/day, the clinical symptoms were quite similar, but with lower incidence. Starting in weeks 21/19 (males/females) of the treatment period, breathing disturbances (tachypnea, labored respiration and rales), ruffled fur, hunched posture, and emaciation were noted. At both

dose levels, all clinical signs were no longer apparent after week 6 of the recovery period.

Food consumption

The absolute daily food consumption was reduced in males and females at 100 and 500 mg/kg/day, starting in week 4 in males (-8.1 and -8.5% at 100 and 500 mg/kg/day, respectively) and in week 7 in females (-7.2 and -10.8% at 100 and 500 mg/kg/day, respectively). The reduction was dose dependent and more pronounced at the end of the treatment period (-15.4 and -30.8% in males at 100 and 500 mg/kg/day, respectively, and -16.7 and -28% in females at 100 and 500 mg/kg/day, respectively).

Several changes in food consumption were evident at 5 mg/kg/day males during the treatment period, but they were considered to be incidental as there were some higher or lower levels when compared with the controls.

During the recovery period, in males at 100 and 500 mg/kg/day, the mean daily food consumption was compensatory increased starting with +25.4% at 100 mg/kg/day and +27.8% at 500 mg/kg/day. This finding persisted at 100 mg/kg/day until week 17 of the recovery period (+21.5%) and at 500 mg/kg/day until the scheduled necropsy (+29.8%) in week 46.

In addition, increased mean daily food consumption was noted in males at 5 mg/kg/day during several weeks of the recovery period. Starting with +11 .8% increased food consumption in week 1 of the recovery period and ending with +19.0% before necropsy.

Body weight

In males and females at 100 and 500 mg/kg/day, the body weights were markedly reduced after the 26 week treatment period (-14.2 and -34.7% in males, respectively, and -14.3 and -22.6% in females, respectively).

The onset of body weight reduction was earlier and more accentuated at 500 mg/kg/day (week 6 in males or 9 in females) than at 100 mg/kg/day (week 14 in males or 18 in females). The overall reduction was more accentuated in males than in females. These findings corresponded with the reduction in the absolute food consumption and were clearly test item-related.

The compensatory higher food consumption in the affected groups during the recovery period led to an increase in body weight gain compared to the controls resulting in comparable overall body weights after only a few weeks of recovery. In males treated with 5 mg/kg/day, increased body weight (21 - 22%) was measured, which corresponded to the increased food consumption in this period.

Hematology

The hematological investigations showed the following differences between control and test item-treated groups:

500 mg/kg/day:

Red blood cell counts, hemoglobin and hematocrit levels were slightly decreased in males after 4 weeks; slightly increased in both sexes after 13 weeks; markedly increased in males and females after 26 weeks of treatment. Decreased platelet counts were noted in males and females after 13 weeks, and 26 weeks. Slightly decreased mean corpuscular hemoglobin concentration was noted in males after 13 weeks and in females after 26 weeks of treatment. Increased reticulocyte counts were noted in females after 13 and 26 weeks of treatment and in males after 26 weeks of treatment weeks of treatment. A shift towards lower flourescent reticulocytes was noted in females and males after 13 weeks of treatment. White blood cell counts in association with absolute lymphocytes were slightly decreased in males after 13 and 26 weeks.

100 mg/kg/day:

Moderate increased reticulocyte counts with a left shift towards lower fluorescent reticulocytes were noted in females after 13 weeks of treatment, and increased reticulocyte counts were noted in both sexes after 26 weeks of treatment. Red blood cell count, hemoglobin and hematocrit levels were markedly increased in males and females after 26 weeks of treatment. Decreased platelet count and slightly decreased mean corpuscular hemoglobin concentration and were measured in females at the end of the 26 week treatment period.

Hematology changes are summarized below.

The table below shows the test item related differences from the controls in percent.

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)									
Dose	Parameter	Males				Females			
		Week 4	Week 13	Week 26	Week 46	Week 4	Week 13	Week 26	Week 46
100 mg/kg/day	RBC			+14%			+7%	+22%	
	Hemoglobin			+13%				+20%	
	Hematocrit			+15%				+23%	
	MCHC					-2%		-2%	
	HFR						-46%		
	MFR			-25%			-12%	-16%	
	LFR			+11%			+12%		
	Platelets							-23%	
	Retic (rel.)						+20%	+27%	
	Retic (abs.)			+32%			+30%	+59%	
500 mg/kg/day	RBC	-4%	+14%	+31%			+14%	+35%	
	Hemoglobin	-3%	+10%	+30%			+12%	+33%	
	Hematocrit	-4%	+11%	+38%			+15%	+40%	
	MCV		-3%	+8%		+4%			
	MCHC			-5%		-3%	-2%	-6%	
	HFR			+89%				+183%	
	MFR		-23%	-19%			-17%		
	LFR		+12%				+10%		
	Platelets		-16%	-50%			-25%	-46%	
	Retic. (rel.)			+67%			+28%	+99%	
	Retic. (abs.)			+110%			+45%	+157%	
	WBC		-17%	-22%					
	Lymphocytes (abs.)		-23%	-28%					

Serum biochemistry

Changes are summarized as follows:

The table below shows the test item related differences from the controls in percent.

Difference in Percent of the Control Value ($p \leq 0.05$ or $p \leq 0.01$)									
Dose	Parameter	Males				Females			
		Week 4	Week 13	Week 26	Week 46	Week 4	Week 13	Week 26	Week 46
5 mg/kg/day	Bilirubin, total		-27%			-21%			
	Triglycerides	-45%	-28%			-15%		-20%	
	Protein (total)							-5%	
	Chloride		-2%						
100 mg/kg/day	Bilirubin, total	-24%	-42%			-22%	-27%		
	ALP							+88%	
	γ-GT							+196%	
	Triglycerides	-33%							
	Phospholipid			-17%				-31%	
	CK							+42%	
	Protein (total)						-6%	-12%	
	Sodium			+1%				+2%	
	Chloride	-1%	-4%	-5%		-2%	-4%	-5%	
500 mg/kg/day	Bilirubin, total	-29%				-26%			
	ASAT		+31%	+127%			+47%		
	ALAT		+39%	+108%					
	ALP	-17%	+26%	+62%			+69%	+154%	
	γ-GT		+29%	(+22%)			(+49%)		
	Triglycerides	-29%	-25%			-19%			
	Phospholipid		-13%	-19%		-16%	-27%	-37%	
	Cholesterol		-13%				-21%		
	Glucose	-18%	-33%	(-17%)			-16%	-39%	
	LDH			+169%			+66%	(+29%)	
	CK			+123%			+31%	(+44%)	
	Protein (total)	-2%	-4%	-22%			-10%	-15%	
	Urea			+107%					
	Phosphorus	-7%	+9%	+43%			+31%	+95%	
	Sodium	+1%	+1%	+2%			+2%	+4%	
	Chloride	-1%	-6%	-8%		-3%	-6%	-7%	

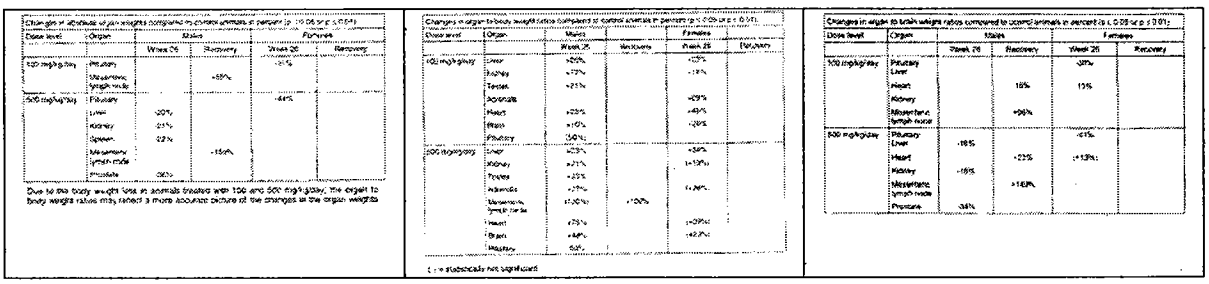
( ) = statistically not significant

Urinalysis

After 4 weeks of treatment slightly lower urine volume, correlating with higher specific gravity and higher osmolality, as well as increased ketone levels were noted in males treated with 500 mg/kg/day. After 13 weeks slightly higher specific gravity and higher osmolality was measured in males at 500 mg/kg/day when compared with the controls.

Organ weights

In males and females, increased liver, heart and kidney/body weight ratios were seen in males and females at 100 and 500 mg/kg/day. In addition, the adrenal gland/body weight ratio was increased in males at 500 mg/kg/day and in females at 100 and 500 mg/kg/day. In males, an increased pituitary gland/body ratio was measured at 500 mg/kg/day. In males, an increase in the testis/body weight ration was observed after 100 and 500 mg/kg/day. Absolute, and relative changes are summarized as follows:



Macroscopic findings:

- General observations: Dark red, reddish or bluish discoloration in 2 males and 6 females at
100 mg/kg/day, and 8 males and 8 females at 500 mg/kg/day; emaciation in 2 males and 3 females at 100 mg/kg/day, and 9 males and 3 females at 500 mg/kg/day.

- Lung: Reddish or dark red discoloration in 1 female at 5 mg/kg/day, and 2 males and 5 females at 500 mg/kg/day.

- Jejunum: Distended with gas in 1 male and 3 females at 100 mg/kg/day and 6 males and 2 females at 500 mg/kg/day.

- Ileum: Distended with gas in 1 male and 3 females at 100 mg/kg/day and 7 males and 8 females at 500 mg/kg/day.

- Cecum: Distended with gas in 1 male and 2 females at 100 mg/kg/day, and 6 males and 8 females at 500 mg/kg/day.

- Colon: Distended with gas in 1 male and 2 females at 100 mg/kg/day and 5 males and 3 females at 500 mg/kg/day.

- Testes: Reduced in size in 1 male at 5 mg/kg/day, 2 males at 100 mg/kg/day, and 6 males at 500 mg/kg/day; flaccid in 1 male at 5 mg/kg/day, 3 males at 100 mg/kg/day, and 4 males at 500 mg/kg/day.

- Epididymides: Reduced in size in I male at 5 mg/kg/day, 2 males at 100 mg/kg/day, and 4
males at 500 mg/kg/day.

- Prostate: Reduced in size in 4 males at 100 mg/kg/day and 7 males at 500 mg/kg/day.

- Seminal vesicles: Reduced in size in 2 males at 100 mg/kg/day and 11 males at 500 mg/kg/day.

- Ovaries: Dark red discoloration in 3 females at 100 mg/kg/day and 8 females at 500 mg/kg/day.

- Uterus: Dark red discoloration in 4 females at 500 mg/kg/day.

- Thymus: Dark red discoloration in 1 control female, 1 male at 5 mg/kg/day, 1 male and 1
female at 100 mg/kg/day, and 2 males and 11 females at 500 mg/kg/day; reduced in size in 1
female at 100 mg/kg/day and 3 males and 2 females at 500 mg/kg/day.

- Mandibular lymph node: Dark red discoloration in 1 control male, 1 male and 1 female at 100 mg/kg/day, and 2 males and 5 females at 500 mg/kg/day.

Microscopic findings:

A number of microscopic findings were noted at the termination of the 26 week treatment period and in decedents (of treatment and recovery groups). From these findings, the following ones were considered to distinguish treated from the control rats:

- Testes: Moderate to massive bilateral diffuse tubular atrophy was seen in 1 male at 5 mg/kg/day, 3 rats at 100 mg/kg/day and in 10 rats at 500 mg/kg/day. This finding was associated with mainly unilateral minimal to moderate tubular mineralization (1 rat each at 5 and 100 mg/kg/day, 4 rats at 500 mg/kg/day), unilateral slight spermatic granuloma (1 rat each at 100 and 500 mg/kg/day), unilateral or bilateral minimal to marked tubular fibrosis (1 rat at 100 and 2 rats at 500 mg/kg/day), or unilateral or bilateral moderate tubular luminal occlusion (1 rat at 100 and 2 rats at 500 mg/kg/day). Minimal focal unilateral tubular atrophy was seen in 1 control rat, minimal to massive focal/multifocal unilateral or bilateral tubular atrophy in 6 rats at 100 mg/kg/day and 5 rats at 500 mg/kg/day.

- Epididymides (mainly decedents): Minimal to moderate atrophy in 1 rat at 5 mg/kg/day, 1 rat at 100 mg/kg/day and in 9 rats at 500 mg/kg/day; aspermia in 3 rats at 100 mg/kg/day and 2 rats at 500 mg/kg/day, in 1 rat at 500 mg/kg/day associated with minimal bilateral luminal mineralization and marked unilateral hemorrhage; oligospermia was observed in 1 rat at 5 mg/kg/day and 8 rats at 500 mg/kg/day; moderate sperm granuloma in 1 rat at 5 mg/kg/day.

- Efferent ducts: Minimal to moderate interductular fibrosis was seen in 1 rat at 5 mg/kg/day, 2 rats at 100 mg/kg/day and in 3 rats at 500 mg/kg/day; minimal to slight luminal mineralization was observed in 1 rat at 5 mg/kg/day and 2 rats each at 100 and 500 mg/kg/day; moderate luminal occlusion was noted in 1 male at 5 mg/kg/day; minimal to slight inflammatory cell infiltration and granuloma were observed in 2 rats at 100 mg/kg/day.

- Seminal vesicles (mainly in decedents): Minimal to moderate atrophy was seen in 2 rats at 100 mg/kg/day, and in 14 rats at 500 mg/kg/day.

- -Small intestine (mainly decedents):
Duodenum: Minimal to slight dilation was observed in 1 female at 100 mg/kg/day and in 2 males and 1 female at 500 mg/kg/day;

Jejunum: minimal to slight dilation was noted in 4 females at 100 mg/kg/day, 5 males and 5 females at 500 mg/kg/day;

Ileum: minimal to slight dilation was noted in 4 females at 100 mg/kg/day, 5 males and 6 females at 500 mg/kg/day.

- Large intestine (mainly in decedents):
Cecum: Minimal dilation was noted in 1 female at 100 mg/kg/day and 5 males and 5 females at 500 mg/kg/day;

Colon: minimal to slight dilation was noted in 1 female at 100 mg/kg/day, 5 males at 500 mg/kg/day.

- Pancreas: Minimal diffuse acinar cell atrophy was seen in 2 females at 100 mg/kg/day and
4 males and 1 female at 500 mg/kg/day.

- Thymus (mainly decedents): Minimal to marked atrophy was observed in 2 males and 5 females at 100 mg/kg/day and in 11 males and 12 females at 500 mg/kg/day.

- Salivary glands: (mainly decedents):

Mandibular salivary gland: minimal to moderate atrophy was noted in 2 males and 4 at 100 mg/kg/day, and in 12 males and 9 females at 500 mg/kg/day;

Sublingual salivary gland: minimal to moderate atrophy was seen in 1 male and 3 females at
100 mg/kg/day and in 6 males and 5 females at 500 mg/kg/day;

Parotid salivary gland: minimal to slight atrophy was noted in 2 males and 3 females at 100
mg/kg/day, and in 8 males and 4 females at 500 mg/kg/day.

- Uterus (mainly decedents): Minimal to moderate atrophy was observed in 4 rats at 100 mg/kg/day and in 12 rats at 500 mg/kg/day.

- Skin/subcutis (mainly decedents): Minimal to moderate hair follicle atrophy was noted in 2 males and 4 females at 100 mg/kg/day and in 10 males and 13 females at 500 mg/kg/day.

- Mammary gland (mainly decedents): Minimal to moderate atrophy was noted in 3 females
at 100 mg/kg/day and in 6 males and 9 females at 600 mg/kg/day.

- Skeletal muscle (mainly decedents): Minimal to slight atrophy was seen in 2 females at 100
mg/kg/day and in 7 males and 4 females at 500 mg/kg/day; minimal to slight myodegeneration in 1 male and female each at 100 and 500 mg/kg/day.

- Prostate gland (mainly decedents): Minimal to moderate atrophy was noted in 3 rats at 100 mg/kg/day and in 13 rats at 500 mg/kg/day.

- Nasal cavity:
Anterior part: Minimal to moderate osseous hyperplasia was noted in 9 males and 10 females at 100 mg/kg/day, and in 16 males and 17 females at 500 mg/kg/day; in 8 males and 6 females at 100 mg/kg/day, and in 10 males and females at 500 mg/kg/day osseous

hyperplasia was associated with minimal to slight inflammation.

Intermediate part: minimal to marked osseous hyperplasia was noted in 12 males and 13 females at 100 mg/kg/day, and in 16 males and 17 females at 500 mg/kg/day; in 7 males and 1 female at 100 mg/kg/day, and in 4 males and 3 females at 500 mg/kg/day osseous hyperplasia was associated with minimal inflammation; minimal to slight eosinophilic inclusions in 3 females at 500 mg/kg/day.

Posterior part: minimal to moderate osseous hyperplasia was noted in 6 males and 6 females at 100 mg/kg/day, and 16 males and 17 females at 500 mg/kg/day; in 2 males at 100 mg/kg/day and 1 male at 500 mg/kg/day osseous hyperplasia was associated with minimal inflammation; minimal eosinophilic inclusions in 1 female each at 100 and 500 mg/kg/day.

- Adrenal cortices: Minimal to moderate diffuse hypertrophy of zona glomerulosa was seen in 4 females at 100 mg/kg/day and in 5 males and 14 females at 500 mg/kg/day; moderate increase in hemangiectasis was observed in females at 100 mg/kg/day and males and females at 500 mg/kg/day.

- Ovaries: Minimal to slight stromal cell hyperplasia was noted in 4 rats at 100 mg/kg/day and in 10 rats at 500 mg/kg/day.

- Mesenteric lymph node: Slight to moderate increase of histiocytosis was observed in males and females at 500 mg/kg/day and in females at 100 mg/kg/day.

- Spleen: Slight increase in hematopoiesis was noted in males at 500 mg/kg/day, minimal to marked hematopoiesis in 3 females at 100 mg/kg/day and 8 females at 500 mg/kg/day; slight to moderate lymphoid depletion in 1 male and 2 females at 100 mg/kg/day, 4 males and 4 females at 500 mg/kg/day.

- Bone marrow: Sternum/Femur: minimal erythroid hyperplasia was seen in 3 females at 100 mg/kg/day and 4 males and 4 females at 500 mg/kg/day.

- Liver: Minimal to slight diffuse hepatocellular hypertrophy was noted in 1 female decedent at 100 mg/kg/day and 4 female decedents at 500 mg/kg/day; moderately decreased incidence of lymphoid cell infiltration in males and females at 100 and 500 mg/kg/day.

- Heart (only decedents): Minimal to slight auricular dilation was noted in 1 male and 1 female at 100 mg/kg/day and 2 males and 4 females at 500 mg/kg/day; in one female at 100
mg/kg/day the auricular dilation was associated with minimal ventricular dilation.

- Lung: Marked multiple foreign body granuloma associated with moderate aspiration pneumonia was observed in 1 male decedent at 500 mg/kg/day. Slight alveolitis was seen in 1 male and 2 females at 500 mg/kg/day. Slight pleural fibrosis was noted in 1 female at 50 mg/kg/day.

- General observations (only decedents): Generalized congestion in 2 males and 3 females at 100 mg/kg/day and in 7 males and 3 females at 500 mg/kg/day.

The generalized congestion, noted under general observations in some decedents, correlated with the macroscopic finding of dark red discoloration of the whole body. In addition, the microscopically noted congestion was also recorded under the various organs and tissues affected.

A number of microscopic findings were noted after an additional 20 week recovery period in surviving recovery animals. From these findings, the following ones were considered to distinguish treated rats from the control rats:

- Testes: Minimal focal unilateral or bilateral tubular atrophy in 1/6 rats of the control group and 2/6 rats at 5 mg/kg/day, minimal to slight focal unilateral tubular atrophy in 2/5 rats at 100
mg/kg/day, and minimal focal bilateral tubular atrophy in 1/2 rats at 500 mg/kg/day.

- Nasal cavity: Anterior part: Minimal osseous hyperplasia in 1/2 male at 500 mg/kg/day; intermediate part: moderate osseous hyperplasia in 1/2 male at 500 mg/kg/day.

Toxicokinetics: Parameters are summarized below:

Main plasma kinetic parameters of BSF 208075 in rats receiving doses of 5, 100, and 500 mg/kg/day

Dose			MALES			FEMALES		
(mg/kg/day)	Parameter	Unit	Days 2/3	Days 85/86	Days 175/176	Days 2/3	Days 85/86	Days 175/176
5	AUC	h*ng/mL	84042.4	9331.6	9607.2	51489.8	5791.0	8025.6
	AUC/dose	(h*ng/mL)/(mg/kg)	21063.3	1948.1	2001.5	11544.8	1204.0	1601.9
	C_{max}	ng/mL	4495.5	498.8	536.7	3420.8	420.4	412.6
	C_{max}/dose	(ng/mL)/(mg/kg)	1126.7	104.1	111.8	767.0	87.4	82.4
	T_{max}	day/h	2/20	85/8	175/20	3/4	85/8	176/4
100	AUC	h*ng/mL	190593.6	138532.2	188408.2	222915.0	209411.6	228234.4
	AUC/dose	(h*ng/mL)/(mg/kg)	1592.9	1483.1	1910.1	1693.5	2232.8	2228.9
	C_{max}	ng/mL	10640.9	9605.3	9699.3	11834.5	17353.5	12868.5
	C_{max}/dose	(ng/mL)/(mg/kg)	88.9	102.8	98.3	89.9	185.0	125.7
	T_{max}	day/h	3/4	85/8	175/12	3/4	85/8	175/20
500	AUC	h*ng/mL	800604.2	781266.4	676742.0	692128.0	1002502.4	932869.0
	AUC/dose	(h*ng/mL)/ (mg/kg)	2026.7	1713.7	1499.2	1549.8	2164.0	1925.0
	C_{max}	ng/mL	41467.9	42168.5	33597.1	47396.2	54460.4	47700.4
	C_{max}/dose	(ng/mL)/ (mg/kg)	105.0	92.5	74.4	106.1	117.6	98.4
	T_{max}	day/h	3/4	86/4	175/8	3/4	86/4	175/20

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Protocols

Application (IND/NDA) number: IND 64,915 Amendment 036 (SX)
Division: DCRDP
CAS#:
Drug name: BSF 208075 (ambrisentan)
Pharmacological Classification: endothelin receptor antagonist
Sponsor/Applicant: [redacted] US agent for Myogen, Inc.
Sponsor/Applicant contact name: [redacted], Regulatory Manager, [redacted]
Sponsor/Applicant telephone and fax number: [redacted]

Date submitted (stamp date): 12/19/03
45-day date (from submission stamp date): 2/2/04
P/T Reviewer(s): William T. Link
Date of Exec CAC review: 1/20/04
CAC members: Abby Jacobs, acting chair
Joe Contrera
Terry Peters
Roswitha Kelly.

A. Summary of Sponsor's Proposal for Review:

Species/strain: mouse, CD-1
Number/sex/dose: 60, additional 18/group for TK analysis
Route: oral, via food admixture

	male	**female**
Doses proposed: mg/kg/d	30, 75 & 180 mg/kg/d	30, 75 & 180
Basis of dose selection:		
MTD	X	
X		
AUC ratio	_____	_____
saturation	_____	_____
MFD	_____	_____
PD	_____	_____
other	_____	_____
Kinetics submitted:	**rodent**	
human		
pharmacokinetics	x	x
metabolism	_____	_____
protein binding	_____	_____

Notable design features: none

B. Summary of Reviewer's Recommendations to CAC:

	male	female
Doses recommended by reviewer: mg/kg/d	30, 75 & 180 mg/kg/d	30, 75 & 180

In this reviewer's opinion, the argument made for an MTD of 180 mg/kg/d is sound. Clearly, 500 mg/kg/d was not tolerated, even at 13 weeks treatment. One could argue that a dose around 300-400 mg/kg might still be used, however, the difference is not great and there were minor signs of toxicity at 150 mg/kg for 13 weeks. It is difficult to predict that life-threatening nasal turbinate hyperplasia may result in mice as it did in rats. As in rats, a 13 week study may not be sufficient to rule out the possibility. There are some indicators that could be predictive, such as the histopathology of the nasal turbinates, but the causal relationship has not been established.

This reviewer is unconvinced by the arguments regarding exposure ratios. It is not clear which comparison (free or total) is more appropriate when the binding and possible displacement by other drugs are not clearly characterized.

C. Basis for Recommendation (experimental details from sponsor's submission):

The proposed MTD for mice of 180 mg/kg/day is based on the 13-week MTD mouse study of ambrisentan. In that investigation. 9 females and 3 males at 1250 mg/kg/day, 1 female and 1 male at 500 mg/kg/day, 1 female and 2 males at 150 mg/kg/day and 2 females at the control group were found dead during the last 4 weeks of the treatment period. Most of these deaths occurred after blood sampling for toxicokinetics or clinical biochemistry. Animals from the control, 150 and 500 mg/kg/day group did not show any adverse signs and died on the day of blood sampling. However, the increased number of deceased animals at the high dose strongly suggested a relation to the effect of the test item. At 1250 mg/kg/day, blood sampling in animals of poor condition (i.e. 8 females and 1 male) might have been the cause of death in these animals.

Food consumption was reduced and body weight gain was attenuated compared to control in mice administered ≥ 500 mg/kg/day, although animals receiving 150 mg/kg/day appeared to be unaffected. Neutrophils, lymphocytes and monocytes were reduced in males administered ≥ 150 mg/kg/day, and these hematological effects were more pronounced in the higher dose groups. Microscopic histopathological findings at ≥ 150 mg/kg/day included hepatic centrilobular hypertrophy and slight hepatic congestion, and minimal to marked eosinophilic cytoplasmic inclusions with epithelial degeneration in the anterior, intermediate and posterior portions of the nasal cavity. The nasal effects were common to both genders and were observed in some mice receiving 60 mg/kg/day.

An additional consideration for MTD close selection in mice is the nasal effect of ambrisentan, which clearly contributed to an unusual morbidity and mortality in the **rat** 26-week study. The mortality effects of ambrisentan were sufficiently delayed in the rat such that a prior 13-week rat toxicity study did not predict the morbidity and mortality occurring in the subsequent 26-week study, which was presumably due to respiratory obstruction. Furthermore, mice receiving 500. 1000, and 2000 mg/kg/day of ambrisentan in a six-week repeated-dose palatability study, demonstrated the same constellation of indicators pointing to an evolving nasal related respiratory inflammation as was reported for the rat (see 6-week mouse palatability study, attached). By the end of 13 weeks of ambrisentan administration, both genders of mice in the MTD study that were administered 150 mg/kg/day did not demonstrate the clinical or clinical pathology signs related to a fulminating respiratory obstruction, but these animals did show eosinophilic cytoplasmic inclusions and epithelial degeneration in all parts of the nasal cavity. These nasal histopathological effects in mice are similar in description to the pre-osseous hyperplasia nasal observations reported in the 26-week rat study. Therefore, it may be reasonable to assume that ambrisentan could eventually elicit nasal obstructive effects in the mouse as severe as those respiratory effects seen in the rat from 15 - 26 weeks, if given daily dosing for a duration of 2 years. Although nasal osseous hyperplasia was not observed in mice treated for 13 weeks, the histopathological changes in the nasal cavity of the mouse reported for that duration of ambrisentan dosing could be viewed as a precursor of impending osseous hyperplasia. Even though osseous hyperplasia was not observed in the mouse, the inflammatory response described above for the tissues of the nasal cavity may have produced a sufficient respiratory obstruction to initialize a hypoxic response, as demonstrated by the 36% and 56% increase in absolute reticulocytes measured in those mice at week 13 receiving 500 and 1250 mg/kg/day, respectively (Table 8, 13-week mouse study, attached).

The mouse and rat results common to ambrisentan leads Myogen to propose a MTD of 180 mg/kg/day for mice due to the developmental potential for the toxicity based endpoint of respiratory obstruction secondary to nasal cavity osseous hyperplasia. This MTD is predicted to produce a minimum toxic effect over the course of two years of dosing in the proposed mouse carcinogenicity study. Doses of > 180 mg/kg/day could generate a cumulative exposure of sufficient magnitude to produce nasal related respiratory obstructive effects leading to premature morbidity and mortality. This proposed MTD dose of 180 mg/kg/day may still produce some degree of mortality due to respiratory obstruction. If cumulative morbidity and mortality in the 180 mg/kg/day dose group begins to approach a significant portion of that group, Myogen plans to reduce the dose to 100 mg/kg/day.

The middle and low doses of 75 and 30 mg/kg/day, respectively, were selected because they should produce little or no toxicity upon delivery to mice over two years. All doses in this study will be administered as a diet admixture because diet

was the route of administration in the prior orally dosed mouse toxicity studies that were used to select the dosing regimens for this proposed study.

Comparison of mouse to human exposures

The AUCs reported in the mouse MTD 13-week toxicity study, and the AUCs listed above for Phase II patients, were generated by a validated bioanalytical assay that measured total (free + protein bound) concentrations of ambrisentan in the appropriate plasma matrix. However, ambrisentan has been demonstrated in vitro to be highly protein bound in the plasma of rats, rabbits, dogs and humans. Therefore, the active unbound free fraction is the fraction of total drug in plasma considered as available for endothelin receptor binding and biological activity. Protein binding was also found to be concentration independent (0.2 - 2.0 μg per ml- of plasma). Accordingly, the respective free fractions of ambrisentan in mice and humans could be used for comparison of exposure between those 2 species (see note below). The protein binding study reported the human plasma binding value for ambrisentan was 98.8%. A more recent *in vitro* study determined that the percent of ambrisentan protein bound by mice is 91.8%.

Preliminary results from the dose-ranging Phase II clinical trial (AMB-220) of ambrisentan in patients with PAH [52] indicate that the planned maximum dose is 10 mg/day. The exposures (AUC) at 10 mg from this Phase II study subdivided by gender are listed in Table 11 below.

Table 11: Dose and exposure* of PAH patients administered ambrisentan (10mg, QD) for 12 weeks

Dose (mg/day)	AUC (ng x h)/mL; Males	AUC** (ng x h)/mL; females
10	15361	10118, 19040 mean = 14579

* = AUC values listed above are calculated from [ambrisentan] in plasma measured as total (bound + free drug)
** = Values in each box are individual patients followed by the mean (females only)

In Table 12, the exposure listings of mouse/human AUCs are listed as ratios of free (calculated) ambrisentan. Mouse exposure comparisons to the largest expected human AUC yields the smallest exposure ratios, and the most conservative assessment of risk.

**Reviewer's note:* It should be recognized that ratio calculation using free ambrisentan, while possibly more relevant than using total, yields a ~10-fold ratio by itself when total plasma ambrisentan concentration is identical in man and mouse. Therefore the ratios presented below may be largely overestimated. Without information on the reversibility of binding and the ease of displacement of bound drug, assumptions regarding the free fraction should be viewed with caution.

Table 12: Relative exposures using mouse/human AUC ratios of free ambrisentan

Species [reference]	Dose	Free [Plasma] AUC (ng x h)/mL; males	Free [Plasma] AUC (ng x h)/mL; females	Ratio to Human AUC; males	Ratio to Human AUC; females
Human [52]	10 mg/day	184	175	1	1
Mice* [63]	60 mg/kg/day	595	1107	3.2	6.3
	150 "	1797	3164	9.8	18.1
	500 "	6177	6731	34	38
	1250 "	14603	27675	79	158

* = AUCs above for the mouse 13-week toxicity study were determined at the end of each treatment period

Proposed doses and exposure in carcinogenicity study: (using **free** ambrisentan)

Table 13: Proposed doses, estimated exposures and exposure ratios for free ambrisentan

Proposed Dose (mg/kg/day)	Estimated Mouse AUC (ng x h)/mL; males	Estimated Mouse AUC (ng x h)/mL; females	Estimated Ratio mouse/human; males	Estimated Ratio mouse/human; females
30	396	1175	2.2	6.7
75	929	1722	5.0	9.8
180	2166	2993	11.8	17.1