Study Title: 13-week repeated dose toxicity (MTD study) after oral administration in the CD-1 mouse. (DRAFT report)

Study no: MPR/PT0115E
Formulation/vehicle: dietary admixture

Methods:

Table 1: Ambrisentan treatment groups for MTD mouse study

Allocation and Target Dose Levels	Group 1* 0 mg/kg/day	Group 2 60 mg/kg/day	Group 3 150 mg/kg/day	Group 4 500 mg/kg/day	Group 5 1250 mg/kg/day
Males	1-18	19-36	37-54	55-72	73-90
Females	91-108	109-126	127-144	145-162	163-180

*Control animals received identical feed without the test item.

Clinical signs and viability/mortality were recorded daily. Food consumption and body weight were recorded weekly during acclimatization and treatment. Fresh batches of feed pellets were prepared weekly, and the test article concentration in feed was adjusted for the individual food consumption and body weight changes to most accurately deliver target doses of drug.

Clinical laboratory measurements
For toxicokinetic determinations. blood samples (approximately 0.6 ml/animal) were collected from animals during week I 1 of treatment under light anesthesia into heparinized tubes at the following time points: ca. 4, 8, 12, 16, 20 and 24 hours. At each time point 3 animals out of 18 animals per dose group and sex were used.

After 13 weeks of dosing was completed, blood samples and urine were collected from all surviving animals for hematology (Table 2), clinical chemistry (Table 3), and urinalysis (Table 4) measurements.

After blood and urine sampling, mice were killed and necropsied then subsequently examined post mortem, and organ weights recorded. Organs and additional tissues were then processed to hematoxylin and eosin stained slides for microscopic histologic evaluation listed in Table 5. The animals were adequately exposed. The systemic exposure to ambrisentan increased in both genders approximately proportional to the dose. At 60, 150 and 1250 mg/kg/day, 2-fold higher systemic exposure was measured in females than in males, whereas at 500 mg/kg/day there was no gender difference.

Table 2: Hematology endpoints for ambrisentan

Erythrocyte count	Reticulocyte count
Hemoglobin	Reticulocyte fluorescence ratios
Hematocrit	Total leukocyte count
Mean corpuscular volume	Differential leukocyte count
Mean corpuscular hemoglobin	
Mean corpuscular hemoglobin concentration	
Platelet count	

Table 3: Clinical chemistry endpoints for ambrisentan

Glucose	Alkaline phosphatase
Urea	Gamma-glutamyl-transferase
Creatinine	Sodium
Bilirubin, total Cholesterol, total	Potassium
Triglycerides	Chloride
Phospholipids	Calcium
Aspartate aminotransferase	Phosphorus
Alanine aminotransferase	Protein, total Albumin
Lactate dehydrogenase	Globulin
Creatine kinase	Albumin/Globulin ratio

Table 4: Urinalysis endpoints for ambrisentan

Volume (18 hours)	Protein
Specific gravity	Glucose
Relat. Density	Ketone
Color	Urobilinogen
Appearance	Bilirubin
pH	Blood
Nitrite	Sediment

Table 5: Organs and tissues analyzed for ambrisentan

Adrenal glands	Ovaries
Aorta	Pancreas
Auricles	Pituitary gland
Brain – including section of medulla/pons, cerebral and	Prostate gland
Cerebellar cortex	Rectum
Cecum	Salivary glands – mandibular and sublingual
Colon	Sciatic nerve
Duodenum	Seminal vesicles
Epididymides (left and right separately)	Skeletal muscle (thigh)
Efferent ducts (left and right separately)	Skin
Esophagus	Spinal cord – cervical, midthoracic, lumbar
Extra-orbital lacrimal gland	Spleen
Eyes with optic nerve	Sternum with bone marrow
Harderian glands	Stomach
Heart	Testes (left and right separately)
Ileum	Thymus
Jejunum	Thyroid gland / parathyroid gland
Kidneys	Tongue
Larynx	Trachea
Liver with gallbladder	Urinary bladder, infused with formalin at necropsy
Lungs, infused with formalin at necropsy	Uterus
Lymph nodes – mandibular and mesenteric	All gross lesions
Mammary gland area	
Nasal cavity	

Results:

Dosing adequacy

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The actual mean dose levels delivered were determined and are presented in Table 6. The daily intake of ambrisentan was acceptably close to the target value for all doses.

Table 6: Target dose versus dose delivered to mice for ambrisentan

Group	Target Dose (mg/kg)	Males		Females	
		Delivered	% of target dose	Delivered	% of target dose
1	0	---	---	---	---
2	60	59.5	99.1	63.9	106.5
3	150	149.0	99.3	160.4	106.9
4	500	486.2	97.2	508.8	101.8
5	1250	1228.3	98.3	1225.6	98.0

Toxicokinetics

AUCs were measured for each dose group receiving test article and are presented in Table 7.

Table 7: Exposure as AUC for mice treated with ambrisentan

Group	Target Dose (mg/kg/day)	AUC (ng•h/ml)	
		Males	Females
2	60	7215	13510
3	150	21897	38582
4	500	75338	82065
5	1250	178089	337498

Viability/Mortality

Nine 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 from 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. At 1250 mg/kg/day blood sampling in animals of poor condition (i.e. 8 females and 1 male) seemed to be the cause of death in these animals. The increased number of deceased animals at the high dose strongly suggested that these rats were more susceptible to blood sampling related mortality suggestive of a test item related effect.

Clinical observations

Overall, female animals were more affected than the males and findings were evident with increasing incidence and severity during the last third of the treatment period. The most prominent findings at 1250 mg/kg/day seen both in males and females were hunched posture, swelling of the abdomen, ruffled fur and poor condition. In addition, single animals showed tremor, ventral recumbency, emaciation and uncoordinated movements. These findings were considered test item-related.

Food consumption

The absolute daily food consumption was slightly to moderately reduced in males and females at 500 and 1250 mg/kg/day, starting in week 8 and 7 in males, respectively, and in week 3 in females. The reduction was dose dependent and more pronounced at the end of the treatment period. This finding was considered test item-related.

Body weight

In males and females at 500 and 1250 mg/kg/day, the body weights were moderately to markedly reduced after the 13-week treatment period (-10.4 and -19.4% in males, respectively, and -13.2 and -26.4% in females, respectively).

The onset of body weight reduction was earlier and more accentuated at 1250 mg/kg/day (week 4 in males and females) than at 500 mg/kg/day (week 9 in males or 8 in females). The overall reduction was more accentuated in females than in males. These findings corresponded with the reduction in the absolute food consumption and were clearly test item-related.

Hematology

Hematological investigations showed differences between control and test item-treated groups as presented in Table 8.

In males at 1250 mg/kg/day, slightly increased red blood cell count, hemoglobin and hematocrit levels were observed. Moreover, slightly increased reticulocyte count and moderately decreased white blood cell count with decreased neutrophils, lymphocytes, eosinophils and monocytes and large unstained cells were noted. In addition, basophils were markedly increased at this dose level. In males and females of this group, slightly decreased platelet counts were recorded.

At 500 mg/kg/day, slightly increased reticulocyte count and slightly decreased white blood cell count with decreased neutrophils, lymphocytes, eosinophils and monocytes were observed in males. In females of this group, slightly decreased platelet counts and slightly increased red blood cell counts were recorded.

At 150 mg/kg/day, slightly decreased white blood cell count with decreased neutrophils, lymphocytes and monocytes were measured in males only.

Some changes in the counts of white blood cells, neutrophils, lymphocytes, eosinophils. monocytes and basophils were only observed as a trend without statistical significance.

These findings were considered test item-related.

Table 8: Difference in percent of the control value (p≤0.05 or p≤0.01)

Dose	Parameter	Males	Females
150 mg/kg/day	WBC	(-42%)	
	Lymphocytes (abs.)	(-43%)	
	Neutrophils (abs.)	-46%	
	Monocytes (abs.)	(-45%)	
500 mg/kg/day	RBC	(+11%)	+15%
	Reticulocytes (abs.)	(+36%)	
	WBC	(-33%)	
	Eosinophils (abs.)	(-30%)	
	Lymphocytes (abs.)	(-28%)	
	Neutrophils (abs.)	(-44%)	
	Monocytes (abs.)	(-58%)	
	Platelets		-20%
1250 mg/kg/day	RBC	+21%	+9%
	Hemoglobin	+21%	(+6%)
	Hematocrit	+17%	
	Reticulocytes (abs.)	+56%	(+16%)
	WBC	-74%	(-26%)
	Basophils (rel.)	+513%	(+160%)
	Large unstained cells	-70%	
	Neutrophils (abs.)	-68%	
	Eosinophils (abs.)	(-54%)	(-43%)
	Lymphocytes (abs.)	-77%	(-38%)
	Monocytes (abs.)	-78%	
	Platelets	-31%	-29%

( ) = statistically not significant

Clinical biochemistry

1250 mg/kg/day

At 1250 mg/kg/day, slightly to moderately increased alanine and aspartate aminotransferase activities, decreased triglycerides, cholesterol and phospholipids,slightly decreased protein in total (with decreased albumin and globulin) levels and increased sodium concentration were noted in males and females (Table 9). In addition, decreased creatinine concentration, increased levels of creatine kinase, lactate dehydrogenase, total bilirubin, phosphate and urea were noted in females at this dose level. These changes did not always reach statistical significance and varied from minimal to moderate severity.

500(mg/kg/day

At 500 mg/kg/day, slightly increased alanine and aspartate anunotransferase activities were evident in females which was also observed as a trend in males at this group. In addition, the females showed slight increases in lactate dehydrogenase (statistical significant) whereas creatine kinase was increased as a trend.

The above findings were considered test item-related.

Table 9: Difference in percent of the control value (p≤0.05 or p≤0.01)

Dose	Parameter	Males	Females
500 mg/kg/day	ASAT	(+21%)	+136%
	ALAT	(+51%)	+161%
	Creatine kinase		(+92%)
	LDH		+129%
1250 mg/kg/day	ASAT	(+73%)	+131%
	ALAT	(+69%)	+268%
	Triglycerides	-58%	-65%
	Cholesterol	-26%	
	Phospholipid	-34%	(-14%)
	Protein (total)	-16%	-21%
	Albumin (total)	-13%	-21%
	Globulin (total)	-20%	-21%
	Sodium	+2%	+2%
	Potassium		+2%
	Phosphate		(+17%)
	Creatinine		-56%
	Creatine kinase		(+138%)
	LDH		(+98%)
	Urea		(+196%)
	Bilirubin (total)		(+105%)

( ) = statistically not significant

Urinalysis

No test item-related changes in urinalysis were evident at all dose levels after the 13-week treatment period.

Organ weights

Due to the different body weight status of individual treatment groups, the absolute organ weights are not comparable with the controls. The organ to body weight ratios reflect a more accurate picture of the significant changes in the organ weights (Table 10).

In males, moderately increased relative adrenal to body weight ratios were evident at 1250 mg/kg/day whereas in females slightly increased liver to body weight ratios were noted. The increased relative testes weights were considered dependent on the body weight loss at 500 and 1250 mg/kg/day. All other changes in organ to body weight ratios after the treatment period were considered incidental changes.

Table 10: Changes in organ to body weight ratios compared to control animals in percent (p≤0.05).

Dose level	Organ	Males	Females
500 mg/kg/day	Testes	+14%	
1250 mg/kg/day	Liver		+19%
	Adrenals	+41%	
	Testes	+17%	

Macroscopic findings

Test item-related findings included primarily signs of the impaired general health condition of deceased or prematurely sacrificed animals with emaciation (at 1250 mg/kg/day), discoloration of the lung (,at 500 and 1250 mg/kg/day), food like contents in the esophagus (at 1250 mg/kg/day), black brown contents and distension by gas in the stomach. duodenum, jejunum (with red discoloration), ileum. cecum and colon (at 500 and 1250 mg/kg/day).

Microscopic findings

A number of microscopic findings were noted at the termination of the 13-week treatment period. From these findings, the following organs were considered to distinguish treated mice from the control mice:
Liver: Minimal to slight diffuse hepatocellular hypertrophy in 5 males and 4 females at 1250 mg/kg/day; minimal centrilobular hepatocellular hypertrophy in 2 males at 150 mg/kg/day, 6 males and I female at 500 mg/kg/day, and 5 males and 5 females at 1250 mg/kg/day; minimal to moderate incidence of single cell necrosis in 4 males at 1250 mg/kg/day; slightly to moderately decreased hematopoiesis in males and females at 1250 mg/kg/day; moderately decreased lymphoid cell infiltration in females at 1250 mg/kg/day; slightly increased diffuse fatty change in males at 1250 mg/kg/day, minimal to slight diffuse fatty change in 1 female at 150 mg/kg/day and 2 females each at 500 and 1250 mg/kg/day: minimal to slight congestion in 1 male each at 150 and 500 mg/kg/day and 4 males at 1250 mg/kg/day, moderately increased congestion in females at 1250 mg/kg/day;
Adrenal cortices: Minimal to slight diffuse hypertrophy of the zona fasciculata in 4 males and 1 female at 500 mg/kg/day, and 11 males and 5 females at 1250 mg/kg/day; slight to moderate decrease in X-zone degeneration in females at 500 and 1250 mg/kg/day; minimal to slight hemangiectasis in 1 female at 500 mg/kg/day and 1 male and 5 females at 1250 mg/kg/day;
Testes: Marked bilateral diffuse tubular atrophy in 1 decedent at 1250 mg/kg/day; slightly increased focal/mufti focal tubular atrophy and vacuolation in males from 60 mg/kg/day; minimal to slight sperm stasis in 2 males at 150 mg/kg/day, 1 male at 500 mg/kg/day, and 2 males at 1250 mg/kg/day;
Epididymides: Aspermia in 1 decedent at 1250 mg/kg/day; oligospertnia in 1 male each at 150 and 1250 mg/kg/day;
Nasal cavity (anterior and intermediate part): Minimally to markedly increased eosinophilic cytoplasmic inclusions with epithelial degeneration in males at 60, 150, 500 and 1250 mg/kg/day and females at 150, 500 and 1250 mg/kg/day; (posterior part): Minimally to markedly increased eosinophilic cytoplasmic inclusions with epithelial degeneration in males at 60, 500 and 1250 mg/kg/day and females at 150, 500 and 1250 mg/kg/day.
Ovaries: Absence of corpora lutea in 3 females at 500 and in 6 females at 1250 mg/kg/day:
Uterus: Minimal to marked atrophy in 10 females at 1250 mg/kg/day;
Heart: Minimal to slight auricular and ventricular dilation in 1 male and 5 female decedents at 1250 mg/kg/day; minimal ventricular dilation in 1 female decedent at 500 mg/kg/day;
Esophagus: Minimal dilation in 1 female at 150, 1 male at 500 and 4 females at 1250 mg/kg/day (decedents only);
Stomach: Moderate glandular stomach ulceration and erosion in 1 male at 1250 mg/kg/day;

Salivary glands (sublingual and/or mandibular glands): Minimal diffuse atrophy in 1 female at 500 mg/kg/day and 1 male and 3 females at 1250 mg/kg/day (decedents only);

Mesenteric lymph node: Minimal to slight lymphoid depletion in 5 males and 1 female at 1250 mg/kg/day; moderately decreased incidence of lymphoid hyperplasia in males treated with 1250 mg/kg/day;

Thymus: Minimally increased incidence of atrophy in males at 500 mg/kg/day; markedly increased incidence of atrophy in males at 1250 mg/kg/day; minimal to moderate atrophy in 3 females at 60 mg/kg/day, 6 females at 500 mg/kg/day and S females treated with 1250 mg/kg/day;

Spleen: Minimal to slight lymphoid depletion in 1 male at 500 mg/kg/day and 2 males and 4 females at 1250 mg/kg/day; minimal atrophy of red pulp in 2 males and 1 female treated with 1250 mg/kg/day; slightly decreased incidence of hematopoiesis in males and females at 1250 mg/kg/day;

All other microscopic findings noted in the organs and tissues were considered to be incidental findings commonly occurring in mice this strain and age.

Conclusions

Oral administration of ambrisentan to [redacted] CD-I(ICR)BR mice at target doses of 60, 150, 500 and 1250 mg/kg/day for 13 weeks resulted in the unscheduled deaths of 9 females and 3 males at1250 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 control females. However, the increased number of deceased animals at the high dose strongly suggested a relation to the effect of the test item. A few deceased animals also revealed an auricular and/ ventricular dilation in the heart. Some of the high-dosed decedents showed minimal to moderate atrophy in different organs such as salivary glands. reproductive organs, spleen, and/or thymus reflecting an impaired general health condition.

The plasma level determination indicated an adequate and dose proportional exposure to the test item. Females showed 2-fold higher systemic exposure at 60, 150 and 1250 mg/kg/day. At 500 mg/kg/day no gender difference was noted.

Test item-related findings were restricted to a slight to moderate reduction of the food consumption and consequent slight to moderate reduction of the body weight at 500 and 1250 mg/kg/day. This was considered a toxic effect and not the consequence of decreased palatability. Clinical signs observed at target doses of 1250 ms/kg/day included mobility disturbances in single animals (hunched posture, tremor, ventral recumbency and uncoordinated movements) and general signs of toxicity (ruffled fur. emaciation, poor condition, swelling of the abdomen). The severity of these clinical findings

was slight to moderate and female animals were more affected than males with increasing incidence and severity during the last third of the treatment period.

Hematological investigations showed test item-related effects on the red and white blood cell parameters, and platelets at 500 and/or 1250 mg/kg/day. Significant changes were noted on red blood cell parameters (higher erythrocyte and hematocrit level, increased hemoglobin levels) as well as an increased absolute reticulocyte count. Decreased white blood cell counts associated with decreased lymphocytes, neutrophils and monocytes were observed from 150 mg/kg/day. Higher basophil and lower platelet counts were noted in animals treated with 500 and 1250 mg/kg/day. No test item-related changes in urinalysis were noted after the treatment period.

Significant test item-related changes in clinical biochemical parameters indicated mainly effects on the liver, kidneys and blood lipids at 500 and 1250 mg/kg/day. Increased aspartate aminotransferase and alanine aminotransferase activities were considered to reflect an effect on the liver correlating histopathologically with minimal to slight diffuse hypertrophy of hepatocytes. Slightly increased urea level and slight effects on the electrolytes (increased phosphate and sodium) suggesting some slight effect on the kidneys, although no correlative pathological changes were noted. Effects on the blood lipids (lower values of phospholipids) correlated with the reduced food consumption. The reduction of triglycerides, bilirubin, cholesterol and protein was seen at 1250 mg/kg/day only. Additional changes were increased activities of lactate dehydrogenase and creature kinase.

Changes in organ/body weight ratios were considered to be more relevant than the absolute values due to the marked decrease in body weight gain in several treatment groups. Test item-related slight to moderate changes in relative organ/body weights were seen at 1250 mg/kg/day (increased weights of the adrenals in males and increased weights of the liver in females). All other changes seen in absolute organ weights and organ brain weight ratios were considered to be due to the reduced body weights.

The gross finding "distended with gas" of "black-brown contents" noted in the gastrointestinal tract of a few animals treated with 500 and 1250 mg/kg/day could not be correlated with findings at the microscopic level.

Test item-related microscopic findings were noted in the liver, adrenal cortices, testes, epididymdes and nasal cavity. The liver showed diffuse or centrilobular hepatocellular hypertrophy at 150, 500 and 1250 mg/kg/day, in some males the hypertrophy was associated with single cell necrosis. These changes reflect an adaptation process during the 13 weeks of treatment. The increase in diffuse fatty change from 150 mg/kg/day was likely test item-related. The increase in congestion at 1250 mg/kg/day was also attributed to treatment with the test item.

In the adrenal cortices, diffuse hypertrophy of zona fasciculata in mice at 500 and 1250 mg/gk/day and hemangiectasis at 500 (females) and 1250 mg/kg/day was likely test item-related. In the testes, focal tubular atrophies were noted in all groups, but in higher

incidences or severities from 60 mg/kg/day. In most of the cases, tubular atrophy was associated with tubular vacuolation, which was also increased in treated males. Occasionally, sperm stasis was noted in single testicular tubules of males at 150. 500 and 1250 mg/kg/day. The bilateral and diffuse tubular atrophy in one decedent male at 1250 mg/kg/day was associated with an aspermia in the epididymides. All these lesions in the male reproductive system were attributed to the effect of the test item. Increased eosinophilic cytoplasmic inclusions primarily in olfactory epithelium of all parts of the nasal cavity were observed in all test article groups, were increased with dose, and were attributed to the effect of the test item. These inclusions were often associated with epithelial degenerations, which were considered consequent to a marked cellular overload with eosinophilic material.
Additional findings at 500 and/or 1250 mg/kg/day (e.g. in spleen, liver, thymus, mesenteric lymph node, adrenals, uterus. ovaries, salivary glands, esophagus, stomach and heart) were regarded to be associated with the impaired health condition, decreased body weight, premortal or perimortal circulatory disturbances, and/or stress.

> In conclusion, most test item-related findings (clinical signs and findings in clinical pathology and microscopy) were observed from 150 mg/kg/day onwards. In addition, microscopic findings at 60 mg/kg/day in the nasal cavities (eosinophilic inclusions with epithelial degeneration) and testes (focal/multifocal tubular atrophy) were considered to be of minor relevance because of their generally minimal severity.

Based on these data, the No-Observed-Adverse-Effect Level (NOAEL) was considered to be 60 mg/kg/day, corresponding to AUCs of 7215 and 13510 ng x h/ml in male and female mice, respectively.

Study title: BSF 208075 - toxicity study with repeated (6-week treatment) oral administration (feed) to the NMRI mouse (palatab

Key study findings: NOAEL <250 mg/kg/day. The study was performed to establish maximum doses for 13 week studies yet to be conducted. Key target organs were liver, testes and nasal cavity; consistent with previous findings at 4 week gavage dosing.

Study no: DT 2600
Volume #, and page #: v017: p001
Conducting laboratory and location: [redacted]
Date of study initiation: dosing started 9/4/00
GLP compliance: no
QA report: no
Drug, lot #, radiolabel, and % purity: Batch No. L0003139, [redacted] purity
Formulation/vehicle: admixed in dietary pellets

Methods (unique aspects):
Dosing:
Species/strain: mouse, NMRI
#/sex/group or time point (main study): 6/sex/group
Satellite groups used for toxicokinetics or recovery:
Age: 6 weeks
Weight: **M**:22.3-30.6g; **F**: 21.4-29.3g
Doses in administered units: 0, 250, 500, 1000, 2000 mg/kg/day
Route, form, volume, and infusion rate: oral, dietary admixture

Observations and times:
Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: nd
EKG: nd
Hematology: nd
Clinical chemistry: see table for parameters
Urinalysis: nd
Gross pathology: all mice
Organs weighed: brain, heart, liver, kidneys, spleen, thymus, mesenteric lymph node, prostate, testes, ovaries, adrenals
Histopathology: see table for tissues/organs examined
Toxicokinetics: blood from 1 animal/sex/group/timepoint was taken at 4 hr intervals for 24 hr on Days 2-3 and 24-25.
Other:

Results:
Mortality: one female at 500 mg/kg/day
Clinical signs: see table for summary
Body weights: decreased from 500 mg/kg onwards, see table
Food consumption: decreased from 500 mg/kg onwards, see table
Clinical chemistry: see table
Organ weights: dose-dependent increase in heart, adrenals; decrease in thymic and lymph node weight 500 mg/kg and above; summarized in table
Gross pathology: summarized in table
Histopathology: see table for summary; **liver:** centilobular hepatic hypertrophy @ 1000 mg/kg and above; **Adrenals:** diffuse fatty change @ 2000 mg/kg; **nasal cavity:** eosinophilic inclusions in all dose groups, increase severity at 500 mg/kg and above; **testes:** tubular atrophy in controls and all dosed groups, no signs of dose-dependence of effect

Toxicokinetics: see table

Table 7 Main plasma kinetic data of BSF 208075 in male and female mice

Intended daily dose [mg/kg]	250		500		1000		2000	
Sex	M	F	M	F	M	F	M	F
Day 2/3								
Actual dose in Wk 1 [mg/kg/day]	264.9	262.3	509.7	524.8	1008.3	1085.8	2030.5	2057.5
C_{max} [µg/mL]	13.9	6.0	13.3	8.2	26.3	33.6	49.2	69.6
AUC_{24h} [h·µg/mL]	122.4	87.2	187.5	135.6	311.5	394.0	700.7	869.4
AUC/dose [h·µg/mL/(mg/kg)]	0.5	0.3	0.4	0.3	0.3	0.4	0.3	0.4
Day 24/25								
Actual dose in Wk 4 [mg/kg/day]	290.4	304.9	571.0	573.4	983.2	1111.3	2506.5	2326.6
C_{max} [µg/mL]	3.1	5.8	11.9	6.5	13.3	34.5	205.3	54.5
AUC_{24h} [h·µg/mL]	17.1	83.6	139.9	87.8	173.4	335.1	1797.2	521.6
AUC/dose [h·µg/mL/(mg/kg)]	0.1	0.3	0.2	0.2	0.2	0.3	0.7	0.2

Table 4 Blood chemistry parameters

Parameter	Method	Unit
Alanine aminotransferase (ALT)	Optimized kinetic ultraviolet test	U/L
Alkaline phosphatase	Optimized kinetic ultraviolet test	U/L
Total bilirubin	DPD method, color test	µmol/L
Cholesterol	Enzymatic color test, CHOD-PAP method	mmol/L
Triglycerides	Kinetic test, fully enzymatic	mmol/L
Cholinesterase	Kinetic test, acetylthiocholine method	U/L
Glucose	Hexokinase method	mmol/L
Creatinine	Jaffé's test without deproteinization	µmol/L
Urea (BUN)	Kinetic ultraviolet test, urease method	mmol/L
Total protein	Biuret reaction	g/L

Table 5 Organs undergoing histological examination

Organ	Plane, section or organ		Fixative [a]
Brain	Planes		
	I	Lateral olfactorial tract and optic nerve	F
	II	Infundibulum of pituitary	F
	III	Mammillary body	F
	IV	Cerebellum (vermis, paraflocculus)	F
		All sections latero-lateral	F
Pituitary gland			F
Esophagus	Middle part		F
Trachea	Middle part		F
Heart	Left and right ventricles, atria with auricles		F
Lungs	Left and right phrenic lobe, cranial lobe		F
Stomach	Forestomach, fundus, pylorus		F
Small intestine	Duodenum, jejunum, ileum		F
Large intestine	Colon, cecum, rectum		F
Liver	Left and right lateral lobe, caudal lobe		F
Lymph nodes	Mesenteric lymph node		F
Kidneys	Left and right		F
Urinary bladder			F
Seminal vesicle	Left and right seminal vesicle with coagulating gland		F
Prostate	Ventral, dorsal and lateral lobe		F
Testes	Left and right		B
Epididymides	Left and right		B
Efferent ducts	Left and right		B
Ovaries	Left and right		F
Uterus	Left and right horn, body of uterus		F
Vagina			F
Thyroid gland	Left and right lobe		F
Parathyroid glands	Left and right		F
Thymus			F
Adrenal glands	Left and right		F
Spleen			F
Nasal cavity and paranasal sinus	Level III		F
All organs or tissues showing macroscopic alterations			F

[a] 4 % Formaldehyde (F), Bouin's solution (B)

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Table 8 Overall incidence of relevant clinical signs

Clinical sign	Males					Females				
Dose [mg/kg/day]	0	250	500	1000	2000	0	250	500	1000	2000
Respiratory										
- Forced respiration	-	1/6,t	5/6,t	6/6,t	6/6,t	-	-	5/6,t	6/6,t	6/6,t
- Respiratory sounds	-	-	5/6,t	5/6,t	6/6,t	-	-	6/6,t	6/6,t	6/6,t
- Sneezing	-	-	4/6,t	6/6,t	6/6,t	-	2/6,t	5/6,t	6/6,t	6/6,t
Gastrointestinal										
- Pale feces	-	-	-	2/6,t	3/6,t	-	-	-	1/6,t	6/6,t
- Soft feces	-	-	-	1/6,t	1/6,t	-	-	-	-	2/6,t
- Increased defecation	-	-	-	-	6/6,t	-	-	-	-	5/6,t
- Abdominal distension	-	-	-	1/6,t	2/6,t	-	-	-	2/6,t	2/6,t
Skin										
- Rough coat	-	-	1/6,t	1/6,t	4/6,t	-	-	1/6,t	1/6,t	3/6,t
Various										
- Hunch-back gait	-	-	-	-	1/6,t	-	-	-	2/6,t	-
- Eye lesions (external)	3/6,t	2/6,t	1/6,t	4/6,t	3/6,t	-	2/6,t	1/6,t	1/6,p	-

t = transient, p =permanent, - = no finding noted

Table 11 Selected blood chemistry parameters

Dose [mg/kg/day]	0	250	500	1000	2000
MALE	(N=6)	(N=5)	(N=6)	(N=6)	(N=6)
Glucose [mmol/L]	8.54	8.09	9.32	8.43	6.43*
	(100)	*(95)*	*(109)*	*(99)*	*(75)*
Cholinesterase [U/L]	7109	6463	6962	7719	11188*
	(100)	*(91)*	*(98)*	*(109)*	*(157)*
FEMALE	(N=6)	(N=6)	(N=5)	(N=6)	(N=6)
Glucose [mmol/l]	8.69	10.51*	9.96	8.93	7.90
	(100)	*(121)*	*(115)*	*(103)*	*(91)*
Cholinesterase [U/L]	10186	8943	7255	10662	11875
	(100)	*(88)*	*(71)*	*(105)*	*(117)*

Mean values and percent of control (in paranthesis); determined in Week 6
* $p \leq 0.05$ (Dunnett's test)

Table 9 Mean body weights [g]

Dose [mg/kg/day]		Males 0	250	500	1000	2000	Females 0	250	500	1000	2000
Wk 1/2	Mean	34.5	35.8	33.9	32.5	33.1	26.7	26.9	26.9	26.5	27.8
	SD	2.5	1.8	1.9	1.9	2.4	1.6	2.4	2.3	2.7	3.0
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	104	98	94	96	-	101	101	107	104
Wk 2/3	Mean	36.8	38.9	35.3	33.6	31.3*	27.6	27.8	28.9	27.7	26.3
	SD	2.7	2.4	2.5	1.3	4.5	1.5	2.4	1.6	1.4	3.3
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	98	96	90	85	-	100	97	100	95
Wk 3/4	Mean	38.5	37.0	34.5	32.6*	30.2*	28.7	28.4	28.9	28.7	28.7*
	SD	3.2	3.6	3.7	2.2	4.2	1.8	2.5	2.6	2.1	2.5
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	96	90	85	78	-	99	94	90	83
Wk 4/5	Mean	39.1	37.9	34.2	31.2*	27.7*	28.8	27.5	25.7	23.5*	21.9*
	SD	3.2	3.5	4.1	1.9	4.2	2.0	2.9	2.3	1.6	1.2
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	97	87	80	71	-	95	89	82	76
Wk 5/6	Mean	39.6	36.1	33.0*	29.3*	25.7*	28.6	28.2	24.9*	23.8*	22.1*
	SD	3.3	3.3	4.2	2.8	3.9	2.0	2.9	2.3	1.6	1.2
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	91	83	74	67	-	99	87	83	77
Wk 6/7	Mean	41.1	39.4	33.6*	29.4*	27.3*	29.0	29.0	25.5*	22.9*	21.5*
	SD	3.4	4.0	4.2	2.9	3.9	2.0	3.4	2.5	2.2	1.3
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	95	81	71	66	-	97	85	77	72

* p < 0.05 (Dunnett's test)

Table 10 Mean food consumption [g/animal/day]

Dose [mg/kg/day]		Males 0	250	500	1000	2000	Females 0	250	500	1000	2000
Wk 1	Mean	6.5	6.5	6.0	6.0	6.2	5.1	5.1	5.1	5.3	5.3
	SD	0.7	0.6	0.3	0.7	0.4	0.5	0.3	0.4	0.3	0.5
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	100	92	92	95	-	100	100	106	104
Wk 2	Mean	5.6	5.0	5.3	5.0	4.8	4.9	4.5	4.1*	4.6	4.1*
	SD	0.5	0.8	0.5	0.2	0.7	0.6	0.4	0.5	0.2	0.5
	N	6	6	6	6	6	6	6	5	6	6
	% of control	-	89	95	89	86	-	92	84	94	84
Wk 3	Mean	5.9	5.4	5.3	5.0	5.4	5.1	5.1	4.8	4.4	3.9*
	SD	0.2	0.6	0.5	0.7	0.9	0.5	0.5	0.5	0.4	0.5
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	93	91	86	93	-	100	94	86	75
Wk 4	Mean	6.3	5.9	5.3*	4.6*	5.0*	5.7	5.2	4.8*	4.2*	3.0*
	SD	0.4	0.3	0.6	0.4	1.1	0.3	0.6	0.7	0.5	0.6
	N	6	5	6	6	6	6	6	6	6	6
	% of control	-	94	84	73	79	-	91	84	74	68
Wk 5	Mean	6.3	6.2	5.5*	5.3*	5.1*	6.2	5.6	4.8*	5.4*	4.6*
	SD	0.4	0.3	0.4	0.6	0.8	0.8	0.5	0.4	0.5	0.4
	N	6	6	6	6	6	6	5	6	6	5
	% of control	-	98	87	84	81	-	90	77	87	74
Wk 6	Mean	6.1	5.9	5.3*	4.8*	4.9*	6.1	5.4	4.6*	4.6*	4.7*
	SD	0.3	0.6	0.5	0.7	0.4	1.0	0.4	0.4	0.7	0.5
	N	6	6	6	6	6	6	6	6	6	6
	% of control	-	97	87	79	80	-	89	75	74	77
Wk 1 – 6	Mean	6.1	5.8	5.4	5.1	5.2	5.5	5.2	4.7	4.8	4.4
	% of control	-	96	89	84	85	-	95	85	87	80

* p ≤ 0.05 (Dunnett's test)

Table 12 Mean relative organ weights (in relation to body weight)

MALES	Dose [mg/kg/day] Control	250	500	1000	2000
N	6	6	6	6	6
Body weight [g]	41.1	39.7	33.7*	29.8*	27.3*
	(100)	(97)	(82)	(73)	(66)
Brain	1.083	1.128	1.251	1.388*	1.431*
	(100)	(104)	(116)	(128)	(132)
Heart	0.442	0.493	0.551*	0.604*	0.565*
	(100)	(111)	(125)	(136)	(128)
Liver	5.24	6.02*	4.97	5.17	5.56
	(100)	(115)	(95)	(99)	(106)
Kidneys	1.211	1.320	1.335	1.418	1.282
	(100)	(109)	(110)	(116)	(106)
Prostate	0.154	0.177	0.133	0.173	0.125
	(100)	(115)	(87)	(112)	(81)
Testes	0.534	0.574	0.607	0.760*	0.694*
	(100)	(108)	(114)	(142)	(130)
Adrenals	0.009	0.015	0.017	0.028*	0.033*
	(100)	(164)	(191)	(304)	(361)
Thymus	0.111	0.106	0.096	0.072*	0.072*
	(100)	(95)	(86)	(65)	(65)
Spleen	0.327	0.372	0.325	0.337	0.259
	(100)	(114)	(100)	(103)	(82)
Mesenteric lymph node	0.107	0.106	0.099	0.092	0.082
	(100)	(99)	(92)	(86)	(77)
FEMALES	Dose [mg/kg/day] Control	250	500	1000	2000
N	6	6	6	5	6
Body weight [g]	29.8	29.2	25.5*	24.1*	22.3*
	(100)	(98)	(86)	(81)	(75)
Brain	1.525	1.516	1.580	1.770*	1.719
	(100)	(99)	(104)	(116)	(113)
Heart	0.522	0.469	0.543	0.561	0.525
	(100)	(90)	(104)	(108)	(101)
Liver	5.17	4.97	5.13	4.87	5.56
	(100)	(96)	(99)	(94)	(107)
Kidneys	1.164	1.136	1.221	1.290	1.313
	(100)	(98)	(105)	(111)	(113)
Ovaries	0.070	0.088	0.057	0.062	0.109
	(100)	(126)	(82)	(89)	(155)
Adrenals	0.057	0.053	0.051	0.051	0.046
	(100)	(93)	(90)	(90)	(81)
Thymus	0.191	0.256	0.158	0.119*	0.107*
	(100)	(133)	(83)	(62)	(56)
Spleen	0.426	0.475	0.464	0.385	0.356
	(100)	(112)	(109)	(91)	(84)
Mesenteric lymph node	0.145	0.162	0.144	0.100	0.118
	(100)	(111)	(99)	(69)	(81)

() = % of Control
* = p≤ 0.05 (Dunnett's test)

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Table 13 Incidence of macroscopic findings

Organ/finding	Dose (mg/kg/day)				
	Control	250	500	1000	2000
General observation					
-Emaciation	-	-	1M, 1F	2M, 3F	4M, 5F
-Poor general condition	-	-	-	2F	1M, 1F
-All large parenchyma diminished in size	-	-	-	2F	2M, 2F
Stomach					
-Distended by gas	-	-	-	1M, 1F	3M
Small intestine					
-Distended by gas	-	-	2M	3M, 6F	4M, 6F
Large intestine					
-Distended by gas	-	-	-	2M, 4F	2M, 2F

Table 14 Incidence and grading of major histopathological findings in males

Organ/finding		Dose (mg/kg/day)				
		Control	250	500	1000	2000
Liver						
- Centrilobular hepatocellular hypertrophy	Incidence	-	-	-	3	6
	Grading				1.0	1.7
- Hepatocytic glycogen deposits	Incidence	5	6	4	4	2
	Grading	1.6	1.0	1.0	1.0	1.0
Adrenals cortices						
- Diffuse fatty change	Incidence	-	1	2	2	3
	Grading		1.0	1.0	1.0	1.7
Duodenum						
- Dilation	Incidence	-	-	-	1	1
	Grading				1.0	1.0
Jejunum						
- Dilation	Incidence	-	-	-	1	3
	Grading				1.0	1.0
Ileum						
- Dilation	Incidence	-	-	-	2	2
	Grading				1.0	1.5
Cecum						
- Dilation	Incidence	-	-	-	2	1
	Grading				1.5	2.0
Colon						
- Dilation	Incidence	-	-	-	2	-
	Grading				1.5	
Rectum						
- Dilation	Incidence	-	-	-	-	1
	Grading					1.0
Thymus						
- Atrophy	Incidence	-	-	-	1	5
	Grading				1.0	2.0
Testes						
- Focal/multifocal tubular atrophy	Incidence	1	1	2	2	1
	Grading	3.0	3.0	2.0	1.0	2.0
Nasal cavity						
- Cytoplasmic eosinophilic inclusions	Incidence	2	3	5	5	5
	Grading	1.0	1.3	1.4	2.4	2.4

- = No finding noted; Grading = Average grading; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Marked

Table 15 Incidence and grading of major histopathological findings in females

Organ/finding		Dose (mg/kg/day)				
		Control	250	500	1000	2000
Liver						
- Centrilobular hepatocellular hypertrophy	Incidence	-	-	-	2	6
	Grading				1.0	1.8
- Hepatocytic glycogen deposits	Incidence	5	5	4	3	2
	Grading	1.2	1.6	1.3	1.0	1.0
Adrenals cortices						
- Fatty X-zone depletion	Incidence	6	6	5	3	1
	Grading	1.7	2.0	1.2	1.3	1.0
- Diffuse fatty change	Incidence	5	6	6	5	6
	Grading	1.0	1.0	1.0	1.4	1.0
Duodenum						
- Dilation	Incidence	-	-	-	4	4
	Grading				2.0	1.0
Jejunum						
- Dilation	Incidence	-	-	-	2	5
	Grading				2.0	1.2
Ileum						
- Dilation	Incidence	-	-	-	3	4
	Grading				1.3	1.0
Cecum						
- Dilation	Incidence	-	-	-	3	2
	Grading				2.0	2.0
Colon						
- Dilation	Incidence	-	-	-	4	1
	Grading				1.0	2.0
Thymus						
- Atrophy	Incidence	-	-	-	1	-
	Grading				3.0	
Nasal cavity						
- Cytoplasmic eosinophilic inclusions	Incidence	4	5	6	5	5
	Grading	1.0	1.8	3.0	1.8	2.3

- = No finding noted; Grading = Average grading; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Marked

Study Title: LU 208075: Effect on the cardiovascular and respiratory systems (i.v.) in the dog

Key study findings:

Study no: MPF-FG-9910E ([redacted] Study No. 709211
Volume #, and page #: eCTD
Conducting laboratory and location: [redacted]
Date of study initiation: 12/2/98
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. L0002155 [redacted] purity
Formulation/vehicle: in normal saline

Methods:

Doses: 0 or 100 mg/kg
Species/strain: beagle dog
Number/sex/group or time point (main study): 2
Route, formulation, volume, and infusion rate: i.v., 1 mL/kg
Age: 16-19 months
Weight (nonrodents only): Males: 8.7 – 10.4 kg Females: 8.1 – 10.0 kg

Parameters evaluated:

Cardiovascular parameters (systolic and diastolic blood pressure k heart rate):

Recorded continuously throughout the experiment starting at least 10 minutes before dosing and for 4 hours (240 minutes) after dosing. Values obtained at 10- minute intervals as well as a "maximum effect" value (maximal deviation from the before dosing values within the first three minutes after dosing) are reported.

Respiratory parameters (respiratory rate, tidal volume & minute volume):

Recorded continuously throughout the experiment starting at least 10 minutes before dosing and for 4 hours (240 minutes) after dosing. Values obtained at 10- minute intervals as well as a "maximum effect" value (maximal deviation from the before dosing values within the first three minutes after dosing) are reported.

Electrocardiogram:

Monitored continuously (Visual Display Unit) throughout the experiment. Traces were recorded prior to and at 20- minute intervals after the administration of test or control article, P-wave duration and amplitude, P-Q, QRS and Q-T intervals were measured manually from the traces (of lead II) and reported.

Blood velocity & cardiac output:

Once prior to dosing and every 30 minutes after dosing

Blood gas analysis:

Once prior to dosing and every 30 minutes after dosing

Surgical preparation and recording:

Animals were fasted for about 18 hours prior to surgery with access to water *ad libitum*. Animals were weighed and anesthetized. Upon the induction of anesthesia the trachea was intubated, and connected to a pneumotachograph to measure respiratory parameters (respiratory rate, tidal volume and minute volume). A cannula (containing 50 IU/ml heparin) was inserted into the left axillary artery and connected to a pressure transducer to measure cardiovascular parameters (systolic and diastolic blood pressure and heart rate). Both the pneumotachograph and the pressure transducer were connected to a physiological recorder and monitoring system. Blood gas analysis was also performed using an [redacted] blood gas analyzer, on samples obtained from the axillary artery.

Blood flow through the femoral artery was measured using a 7.5 MHz APAT sound probe. The color doppler flow probe was placed at an angle at the artery site. The region with the maximal flow was then identified and marked on the ultrasound screen. A doppler signal was then sent through the vessel along the marked region on the screen and received via the sound probe. The peak height of the blood velocity recorded as a function of time corresponds to the maximal blood velocity. Cardiac output was determined by a thermodilution technique.

Electrocardiograms were obtained using Einthoven (I, II and III) and Goldberger (aVR, aVL and aVF) leads. The P-wave duration and amplitude, P-Q interval, QRS complex and Q-T interval were measured using a representative section of electrocardiogram from lead II, The ECG recordings were taken using a paper speed of 50 mm/s and a 10 mm/mV amplitude. This allowed the traces of lead II to be measured with a resolution of 0.05 mV for the P-wave amplitude and 10 ms for the duration of P-wave as well as the P-Q, QRS and Q-T intervals.

Results:

Following intravenous administration of LU 208075 (100 mg/kg) a statistically significant fall in systolic, diastolic and mean blood pressure was seen within the first 3 minutes after dosing in both male and female animals. The reduction in mean blood pressure was of the magnitude of 25.7 mmHg in males and, at 16.8 mmHg, slightly less in females. At 10 minutes after dosing the effect was diminished in both sexes and had completely disappeared by 20 minutes after administration. No further pharmacologically relevant effects on systolic, diastolic and mean blood pressures were measured in male and female animals for up to 4 hours after dosing. Some statistically significant

differences in systolic blood pressure between LU 208075-treated and vehicle- treated control male animals were seen at isolated time points and in diastolic and mean blood pressures in females., These differences are thought to be spurious as, in absolute terms, the differences were minimal and, when data are presented as delta change (change relative to before dosing values), statistical significance was no longer attained.

In absolute terms, no differences in heart rate were apparent between LU 208075 and vehicle-treated animals. However, over time, a marginal decrease in heart rate was observed in vehicle-treated control male and female animals while in LU 208075-treated animals heart rate tended to remain at pre- dosing values (males) or increase with time (females). The gradual increase in heart rate in female dogs resulted in statistically significant differences from control animals starting at 160 minutes after dosing and continuing to the end of the recording period.

The gradual increase in heart rate towards the end of the experiment in female dogs was accompanied by a concurrent decrease in Q-T interval (mean of between 7.5 and 17.5 ms) which, and since no such changes were evident in control animals, reached statistical significance at 180 minutes after dosing ($p<0.05$). With the exception of this small change, electrocardiograms were generally unaffected following treatment with LU 208075 and no Q-T interval prolongation was observed. The P-wave amplitude in male animals was statistically significantly higher than in control animals throughout the experiment, including prior to dosing.

In male and female dogs treated with LU 208075, blood velocity remained relatively constant throughout the 4 hour post-dosing period whilst in vehicle-treated control animals a gradual fall in blood velocity was measured starting between 120 and 15O minutes after dosing. In males, this resulted in statistically significant differences occurring both in mean absolute values as well as delta change values. In females, differences from control animals failed to reach statistical significance.

Cardiac output also gradually declined with time m the control animals. In males treated with LU 208075, an initial, transient but significant increase in cardiac output was seen shortly after the fall in blood pressure. This was followed by a gradual decline over time, similar to, but to a lesser extent than that seen in the control animals. In females, cardiac output did not increase after treatment with LU 208075, but remained stable for about 90 minutes after dosing. Thereafter a slow decline was observed. No statistically significant differences compared with controls were attained.

Statistically significant differences (maximum change from baseline 0.l pH unit) in blood pH between control and LU 208075-treated animals were evident at several time points in males (60 and 90 minutes, and from 150 through 240 minutes after dosing) and females (60 and 90 minutes after dosing). There were no apparent treatment-induced changes in either pCO_2 or pO_2.

Effects on respiratory parameters were limited to a marginal increase in tidal volume at 30 minutes after dosing in males and a slight increase in respiratory rate at 40, 70 and 80

minutes and in minute volume at 60, 70 and 80 minutes after dosing in females following a small decrease in minute volume shortly after administration ('maximal effect'). These marginal changes in respiration are not considered to be of pharmacological relevance.

Conclusion

A single intravenous dose of LU 208075 at 100 mg/kg b.w. had no marked effects on either the electrocardiogaphic parameters or on respiration in anesthetised dogs.

Transient reductions in blood pressure were measured in male and female animals during the first three minutes following administration of LU 208075 at a dosage of 100 mg/kg b.w.. At between 10 and 20 minutes after dosing these effects were no longer evident. A slight increase in heart rate, particularly in female animals, was observed between 2 and 4 hours after dosing. Marginal reductions in blood pH were measured after treatment. Blood velocity and cardiac output were slightly increased in male animals, starting at 150 minutes after treatment with LU 208075 at 100 mg/kg b.w..

All of the above effects are small in magnitude and either spurious, or adaptive responses to the blood pressure-lowering effects of the compound. In this reviewer's opinion, the findings should not be viewed as adverse effects.

Study title: BSF 208075: 104-Week Oncogenicity (Feeding) Study in the Mouse

Key study findings: Significantly increased mortality in males and females at the high dose leaves this group unsuitable for evaluation. The only tumor finding suggestive of borderline significance is histiocytic sarcoma in female mice. The incidence shows apparent dose-dependence in the low and mid dose females. This tumor was found to be not significant following in-house statistical analysis. All other findings are of similar or lower incidence relative to controls. Analysis of combined similar neoplasms, inclusion or exclusion of toxicokinetic animals and/or the high dose group does not change this conclusion.

Study no.: 851466
Volume #, and page #: eCTD
Conducting laboratory and location:

Date of study initiation: 3/31/04
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: Myogen Lot. No. 10011, [redacted] purity
CAC concurrence: yes

Methods

Doses: 0, 50, 100 or 250 mg/kg/day (see chart below)
Basis of dose selection: MTD
Species/strain: CD-1 mice [redacted] CD-1(ICR)SR), outbred, SPF quality
Number/sex/group (main study): 60
Route, formulation, volume: food admixture
Frequency of dosing: daily
Satellite groups used for toxicokinetics or special groups: 18
Age: 5 weeks, start of dosing
Animal housing: individual caging
Restriction paradigm for dietary restriction studies: none
Dual controls employed: yes
Interim sacrifices: none
Deviations from original study protocol: dosing reduction in Group 5 as detailed below.

Allocation and Target Dose Levels**	Group 1 0 mg/kg/day	Group 2 0 mg/kg/day	Group 3 50 mg/kg/day	Group 4 100 mg/kg/day	Group 5 250/150*/0** mg/kg/day
Males A	1-60	79-138	139-198	217-276	295-354
Males B	61-78		199-216	277-294	355-372
Females A	373-432	451-510	511-570	589-648	667-726
Females B	433-450		571-588	649-666	727-744

A – Animals: Oncogenicity Animals
B - Animals: Plasma Level Animals
* reduced from 250 mg/kg/day to 150 mg/kg/day from week 39 onwards
** In group 5 dosing was stopped on 25 January 2006 for males and on 07 September 2005 for females.
*** the test item was applied as supplied by the sponsor.

Observation times
The following observations were recorded:

Viability / mortality: Twice daily,

Clinical signs: At least once daily incl. a weekly palpation for tissue masses.

Food consumption: Weekly up to week 14, afterwards 2 weeks later, and then every 4 weeks.

Body weights: Weekly up to week 14, afterwards 2 weeks later, and then every 4 weeks.

Ophthalmoscopy: In 15 animals/group and sex (starting with the lowest animal number) during acclimatization. In 15 animals/sex in groups 1 and 5 (starting with the lowest animal no.) in weeks 51/104. At 104 week due to test item- related findings in group 5, 15 animals/sex (starting with the lowest animal no.) of the interim dose groups were also examined.

Clinical lab investigations: Blood samples were collected from all allocation A animals under light anesthesia. The animals were fasted for 18 hrs before blood sampling. 20 ml/kg water was given by gavage before the fasting period. Blood samples were collected from the retro-orbital plexus in the morning hours to reduce biological variation caused by circadian rhythms

Blood samples for blood smears were also taken from all allocation A animals, if feasible.
Blood and urine sampling at week 52: 23 March 2005; at week 105: 29 March 2006

Hematology parameters:

Erythrocyte count
Hemoglobin
Hematocrit
Mean corpuscular volume
Red cell volume distribution width
Hemoglobin concentration distribution width
Platelet (thrombocyte) count
Reticulocyte count
Reticulocyte maturity index
Total leukocyte count

Clinical chemistry parameters:

Glucose	Sodium
Urea	Potassium
Creatinine	Chloride
Bilirubin, total	Calcium
Cholesterol, total	Phosphorus inorganic
Triglycerides	Protein, total
Aspartate aminotransferase	Albumin
Alanine aminotransferase	Globulin
Alkaline phosphatase	Albumin/Globulin ratio
Gamma-glutamyl-transferase	

Urinalysis parameters:

Glucose	Sodium
Urea	Potassium
Creatinine	Chloride
Bilirubin, total	Calcium
Cholesterol, total	Phosphorus inorganic
Triglycerides	Protein, total
Aspartate aminotransferase	Albumin
Alanine aminotransferase	Globulin
Alkaline phosphatase	Albumin/Globulin ratio
Gamma-glutamyl-transferase	

Toxicokinetics:

Blood samples were collected from allocation B animals for the deterrnination of plasma drug concentrations during weeks 2, 52 and 104. Blood sampling at week 2: 07/08 April 2004, at week 52: 24/25 March 2005 and at week 104: 23 March 2006.

Due to the high mortality in group 6, the kinetics in week 104 were not performed in this group. All surviving satellite animals of group 5 were examined histopathologically together with the Allocation A animals.

Pathology

Those animals that died or were sacrificed in a moribund status during the course of the study underwent necropsy as soon as possible.

In week 43 it was decided that all decedent and surviving animals of allocation B be necropsied and histologically examined.

Necropsy at week 84: 08 November 2005 for females of group 5, and hence are treated in this report as interim sacrifice animals (K1), whereas all other animals were scheduled for terminal sacrifice (K0).

Necropsy after 104 weeks: from 29 March 2006 until 07 April 2006

All animals (oncogenicity and toxicokinetic) were necropsied and descriptions of all rnacroscopic abnorrnalities were recorded. All animals surviving to the end of the observation period and all moribund animals have been anaesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination,

Blood was sampled from all moribund animals and at scheduled necropsy via cardiac puncture and CO_2, H CO_2, O_2 and pH has been measured.

Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution, unless otherwise detailed.

Adrenal glands
Aorta
Auricles
Bone (sternum and femur including joint)
Bone marrow (femur)
Brain (medulla/pons, cerebral and cerebellar cortex)
Cecum
Colon
Duodenum
Epididymides (fixed in Bouin's solution)
Esophagus
Eyes w/optic nerve, fixed in Davidson's solution
Gall bladder
Harderian gland, fixed in Davidson's solution
Head, remaining
Heart
Ileum
Jejunum
Kidneys
Larynx
Lacrimal gland, exorbital
Liver
Lungs, filled w/formalin at necropsy
Lymph nodes - mesenteric, mandibular
Mammary gland area
Nasal cavity
Ovaries
Pancreas
Pharynx
Pituitary gland
Prostate gland
Rectum
Salivary glands - mandibular, sublingual
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord - cervical, midthoracic, lumbar
Spleen
Stomach
Testes (fixed in Bouin's solution)
Thymus
Thyroid incl. parathyroid gland, if possible
Tongue
Trachea
Urinary bladder, filled w/formalin at necropsy
Uterus with cervix
Vagina
Zymbal's gland
Gross lesions and tissue masses

Histopathology

Slides of all tissues and organs listed under necropsy from all animals of the controls and high-dose groups as well as from all animals which died spontaneously or were terminated in extremis, and all gross lesions from all animals were examined by the study pathologist.

Test item-related morphological changes in the organs of any high-dose animal did require histologic evaluation of the same organs in the low- and mid-dose groups. All macroscopic findings in the low and mid dose groups did require the histopathologic examination of these tissues for all groups. An increase in the incidence of tumors would

have required to look at the next lower dose group for that tissue as well as for related tissues. In the presence of an excessive decrease on survival in the examined dose group, the corresponding lower dose group had also to be examined.

Results

Mortality:

The viability/mortality of male and female CD-1 mice was markedly affected in high dose males and in females of groups 4 and 5 as summarized below.

MALES GROUP	DOSE	INITIAL NUMBER		FOUND DEAD	MORIBUND SACRIFICE	24-MONTH SACRIFICE	PERCENT SURVIVING
1	0 mg/kg	60	a)	27	5	27	45.76
2	0 mg/kg	60		27	8	25	41.67
3	50 mg/kg	60		24	8	28	46.67
4	100 mg/kg	60		21	12	27	45.00
5	250/150/0 mg/kg	60		30	19	11	18.33**

a) 1 lost for other reason

FEMALES GROUP	DOSE	INITIAL NUMBER	FOUND DEAD	MORIBUND SACRIFICE	24-MONTH SACRIFICE	PERCENT SURVIVING
1	0 mg/kg	60	24	9	27	45.00
2	0 mg/kg	60	30	6	24	40.00
3	50 mg/kg	60	33	9	18	30.00
4	100 mg/kg	60	37	9	14	23.33
5	250/150/0 mg/kg	60	33	18	9#	15.00**

84-week sacrifice

Clinical signs:

There was an increased incidence of hunched posture, poor condition, emaciation, respiratory findings (rales), and ruffled fur in males of group 4 and in both sexes of group 5, and superficial skin lesions and eye findings in male groups 3 and 5. Clinical signs are summarized below.

Detailed weekly observations revealed the following incidences (number of affected animals):

Group / Clinical sign	1 M	2 M	3 M	4 M	5 M	1 F	2 F	3 F	4 F	5 F
No. of animals	78	60	78	78	78	78	60	78	78	78
Recumbency	2	1	3			1	2	1	2	2
Posture, hunched	18	11	15	23	46	13	16	15	24	49
Poor condition	16	16	19	24	47	18	17	21	27	45
Emaciation	14	8	19	20	35	8	11	10	13	40
Respiration, labored / rales	10	8	14	8	40	5	10	8	14	44
Movement, uncoord. Behaviour abnormal	3	6	6	5	2	7	6	4	8	6
Paresis		1	1	1		2		1		
Swellings	6	7	15	7	7	15	10	27	23	9
Ruffled fur / rough coat	23	23	30	32	54	21	21	29	37	56
Hair loss, localized	6	7	7	8	6	14	10	17	19	10
Superficial skin lesion[1)]	13	16	19	32	37	12	13	23	18	12
Mass, crusted / open		2	1	1	1	2	1		2	
Lacrimation[2)]	3	5	2	5	4		1	1		1
Nasal secretion										
Diarrhea			1	1		1		1	1	
Eye lesion, injured, shrunk, missing	7	5	8	8	7	9	5	6	8	3
Eye white, opaque	8	5	21	19	12	12	9	22	15	6
Tail (apex), kinked missing, discolored	1		2	1		1			1	
Head, tilt		1	4	2		2		2	4	1
Vaginal prolapse						2		1	1	1
Spasms, tremor			3							

1) Incl.: crusts, scabbed wounds, scars, bleedings, sores, hemorrh. spots, erythema, discoloration, abscess, watery discharge
2) Incl.: chromodacryorrhea

Body weights:

Significantly lower mean body weights were recorded from week 25 onwards for high dose males and from week 65 onwards for group 4 males. However, no significant differences to the male control group were recorded at treatment end.

A temporarily depressed body weight was recorded for high dose females between weeks 29 and 77.

Food consumption:

Despite a number of statistically significant deviations from control means in all male and female dose groups, there were no clear treatment- and dose-dependent effects. The overall mean food intakes (weeks 1 to 104) were 5.3, 5.4, 5.3, 5.1, and 4.8 (-10% from the mean of the 2 control values) g/animal/day in males and 5.0, 5.3, 4.9, 5.1, and 4.7 (-6%) g/animal/day in female groups i, 2, 3, 4, and 5, respectively. The differences to the control means did not attain a level of statistical significance.

Hematology

Investigations performed at weeks 52 and 104 revealed no relevant changes to hematology parameters.

The following transient changes to hematology parameters (significantly different from control means) were considered incidental, without dose-relationship and/or within the historical control range and, therefore, without toxicological relevance:

lower value for MCHC in group 3 females (week 52)
higher WBC count in group 3 females (week 52)
higher value for Neut (abs.) in group 3 females (week 52)
higher value for Mono (abs.) in group 3 females (week 52)

Clinical chemistry

The following changes (% deviation from control means) were considered related to the treatment with the test item:

		MALES				FEMALES			
Parameter	week	2	3	4	5	2	3	4	5
Creat	52				-48%**				
	105				-14%#				
Na+	52								
	105			+2%*	+3%*				
K+	52				+26%**				+19%#
	105								
Cl-	52				-3%*				
	105				+4%*				
ASAT	52				+54%*				+36%*
	105							+146%##	

Statistical key: */**: p< 0.05 / 0.01 # not significant at 5% level

due to one animal (no. 631) with an extremely high value

Urinalysis

There were no large or relevant changes in urinalysis parameters.

Toxicokinetics:

A summary of the mean toxicokinetic parameters of BSF 208075 is given in the tables below:

Group 3 50 mg/kg/day		Week 2		Week 52		Week 104	
		males	females	males	females	males	females
$C_{average}$	[ng/ml]	221	547	228	549	(918)	(1150)
AUC_{0-24h}	[ng·h/ml]	5334	12556	5047	13166	n.c.	n.c.

Group 4 100 mg/kg/day		Week 2		Week 52		Week 104	
		males	females	males	females	males	females
$C_{average}$	[ng/ml]	626	1032	508	773	(1502)	(2040)
AUC_{0-24h}	[ng·h/ml]	14822	23276	11824	19176	n.c.	n.c.

Group 5 250/150 mg/kg/day		Week 2 [a]		Week 52 [b]		Week 104	
		males	females	males	females	males	females
$C_{average}$	[ng/ml]	2233	3255	898	1479	---	---
AUC_{0-24h}	[ng·h/ml]	51316	79253	19544	36153	---	---

( ): value not reliable, since derived from two time points only
n.c.: not calculated due to insufficient number of data points (two time points only)
---: no plasma data available (no blood sampling was performed)
a: dose in week 2 was 250 mg/kg/day
b: dose in week 52 was 150 mg/kg/day

Gross pathology:

A number of lesions was recorded at statistically significantly decreased incidences (Fisher's Exact Test) in group 5 animals related to early and high death rate including:

focus/foci in the lungs and Harderian glands, discoloration of the Harderian glands and seminal vesicles, - nodules in the lungs, liver and uterus, thickened seminal vesicles, hemorrhagic and watery cysts of the ovaries, cystic wall of the uterus, nodular thickened thymus, thickened mesenteric lymph nodes.

The following gross lesions recorded at statistically increased values in group 4 animals (Fisher s Exact Test) were considered to be treatment-related: - discoloration in lungs red discoloration of complete animal (groups 4 and 5)

Histopathology:

Non-neoplastic:

Autolysis in tissues from mice, which died during the course of the study, was most often well within acceptable limits and thus was not a hindrance to the histological evaluation. Most of the lesions recorded were age-associated and degenerative, inflammatory or proliferative in nature. Although the incidences of some non-neoplastic findings showed statistically significant differences, there was no biological significant finding that could be attributed with the test item in the findings except the organs described below.

Nasal Cavities

Several changes were recorded in the nasal cavity lining respiratory and olfactory epithelium at alt levels consisting of increased incidences and severity of hyaline inclusions (groups 3- 5), increased secretion (groups 3-5) and inflammatory secretion (group 5), respiratory epithelial hyperplasia and metaplasia (groups 3-5) and epithelial degeneration (mainly dorsal meatus) (groups 3-5) or submucosal inflammation (groups 4 and 5). In addition, increased congestion along with increased vascular dilation was recorded in groups 4 and 5. Finding are summarized in the following tables.

Table: Incidences (%) and Mean Severity of Selected Lesions: All Animals incl. Deaths

Group	1		2		3		4		5	
Sex	M	F	M	F	M	F	M	F	M	F
Level 1: Hyaline inclus. (Incidence [%]) M:+<0.0005,F:+<0.0005*	3/1.0	21/1.3	5/1.0	22/1.7	31/1.7 <0.0001 **	43/1.6 0.0012**	61/1.6 <0.0001 **	56/1.6 <0.0001 **	29/1.2 <0.0001 **	66/1.7
Level 1: Inflamm.secret. (Incidence [%]) M:+0.0162, F:+<0.0158*		1/3.0	2/2.0				1/1.0	3/2.0	4/1.7	5/2.0
Level 1: Secretion (Incidence [%]) M:+<0.0005,F:+<0.0005*	6/1.0	6/1.0	12/1.4	5/1.0	35/1.5 <0.0001 **	23/1.5 0.0003**	51/1.7 <0.0001 **	43/1.8 <0.0001 **	59/1.8 <0.0001 **	46/1.4
Level 1: Resp.hyperpl. (Incidence [%]) M:+0.0113,F:+0.0026*	14/1.1	13/1.4	15/1.3	13/1.1	26/1.3 0.0345**	28/1.4 0.0071**	35/1.3 0.0009**	57/1.4 <0.0001 **	29/1.3 0.0135**	29/1.4
Level 1: Epithel.degen. (Incidence [%]) M:+0.0013*					16/1.2 <0.0001 **	7/1.0 0.0059**	28/1.2 <0.0001 **	4/1.0 0.0411**	14/1.1 <0.0001 **	3/1.5
Level 1: Submuc.inflam. (Incidence [%]) M:+0.0043*						1/1.0	9/1.6 0.0007**	3/1.5	6/1.0 0.0136**	2/1.0

* Results of Armitage Trend Test
** Results of Fisher's Exact Test

Group	1		2		3		4		5	
Sex	M	F	M	F	M	F	M	F	M	F
Level 2: Hyaline inclus. (Incidence [%]) M:+<0.0005,F:+<0.0005*	1/1.0	18/1.6	5/1.3	17/1.6	29/1.8 <0.0001 **	49/1.8 <0.0001 **	69/1.8 <0.0001 **	69/2.1 <0.0001 **	63/1.5 <0.0001 **	91/2.3
Level 2: Inflamm.secret. (Incidence [%]) M:+0.0084 F:+<0.0307*		1/3.0	3/2.0			1/2.0	4/1.7	3/1.0	7/1.4 0.0483**	5/1.3
Level 2: Secretion (Incidence [%]) M:<+0.0005,F:+<0.0005*	14/1.5	6/1.3	13/1.4	12/1.4	29/1.5 0.0080**	28/1.6 0.0003**	48/1.8 <0.0001 **	43/1.7 <0.0001 **	59/1.8 <0.0001 **	43/1.5
Level 2: Resp.hyperpl. (Incidence [%]) M:+0.0107,F:+0.0089*	19/1.2	8/1.5	25/1.3	13/1.1	38/1.4 0.0104**	43/1.5 <0.0001 **	43/1.6 0.0013**	54/1.6 <0.0001 **	38/1.4 0.0104**	28/1.4
Level 2: Epithel.degen. (Incidence [%]) M:+<0.0005*		1/1.0			13/1.0 <0.0001 **	5/1.0	19/1.4 <0.0001 **	1/1.0	16/1.2 <0.0001 **	2/1.0
Level 2: Submuc.inflam. (Incidence [%]) M:+0.0485*	3/1.0	3/1.5	5/1.0	3/1.0	3/1.5	7/1.0	9/1.7	21/1.1 <0.0001 **	9/1.5	3/1.0

* Results of Armitage Trend Test
** Results of Fisher's Exact Test

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Group	1		2		3		4		5	
Sex	M	F	M	F	M	F	M	F	M	F
Level 3: Hyaline inclus. (Incidence [%]) M:+<0.0005,F:+<0.0005*	1/1.0	28/1.5	2/1.0	22/1.4	21/1.5 <0.0001 **	38/1.8 0.0414**	51/1.8 <0.0001 **	78/2.1 <0.0001 **	70/1.4 <0.0001 **	88/2.1
Level 3: Resp.hyperpl. (Incidence [%]) F:+<0.0005*	16/1.2	15/1.2	17/1.4	25/1.3	40/1.6 0.0003**	31/1.4	35/1.5 0.0023**	64/2. <0.0001 **	26/1.7	53/1.2
Level 3: Epithel.degen. (Incidence [%]) M:+0.0352,F :+0.0051*	1/1.0			3/1.0	1/1.0	4/1.3	1/1.0	3/2.0	4/1.7	9/1.5
Level 3: Submuc.inflam. (Incidence [%]) F:+0.0031*	1/2.0	3/1.0	2/1.0		6/1.0	5/1.0	11/1.6 0.0055**	9/1.2 0.0212**	3/2.0	11/1.7

* Results of Armitage Trend Test
** Results of Fisher's Exact Test

Testes

Tubular degeneration increased in incidence and severity in all test item-treated groups. In addition, multinuclear giant cells increased in incidence and severity in groups 4 and 5. These findings are consistent with those of other endothelin receptor antagonists and are considered a class effect.

Neoplastic:

Summary findings:

Number of Animals with Neoplasms:

Dose Group	1		2		3		4		4	
Sex	M	F	M	F	M	F	M	F	M	F
Number affected	73	72	60	60	68	75	75	70	70	67
%	53.4	63.9	61.7	66.7	61.8	60.0	46.7	61.4	25.7	25.4

Number of Animals with Benign and Malignant Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Benign	19	26	26	15	26	16	19	21	8	10	98	88
Malignant	28	34	25	34	23	37	20	33	11	7	107	145

Number of Primary Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Primary Tumors	59	80	64	57	56	61	47	69	24	18	250	285

Number of Benign and Malignant Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Benign	27	39	37	16	31	17	22	30	11	11	128	113
Malignant	32	41	27	41	25	44	25	39	13	7	122	172

Survival curves, body weight and body weight gain curves, and complete listing of all neoplastic findings are attached.

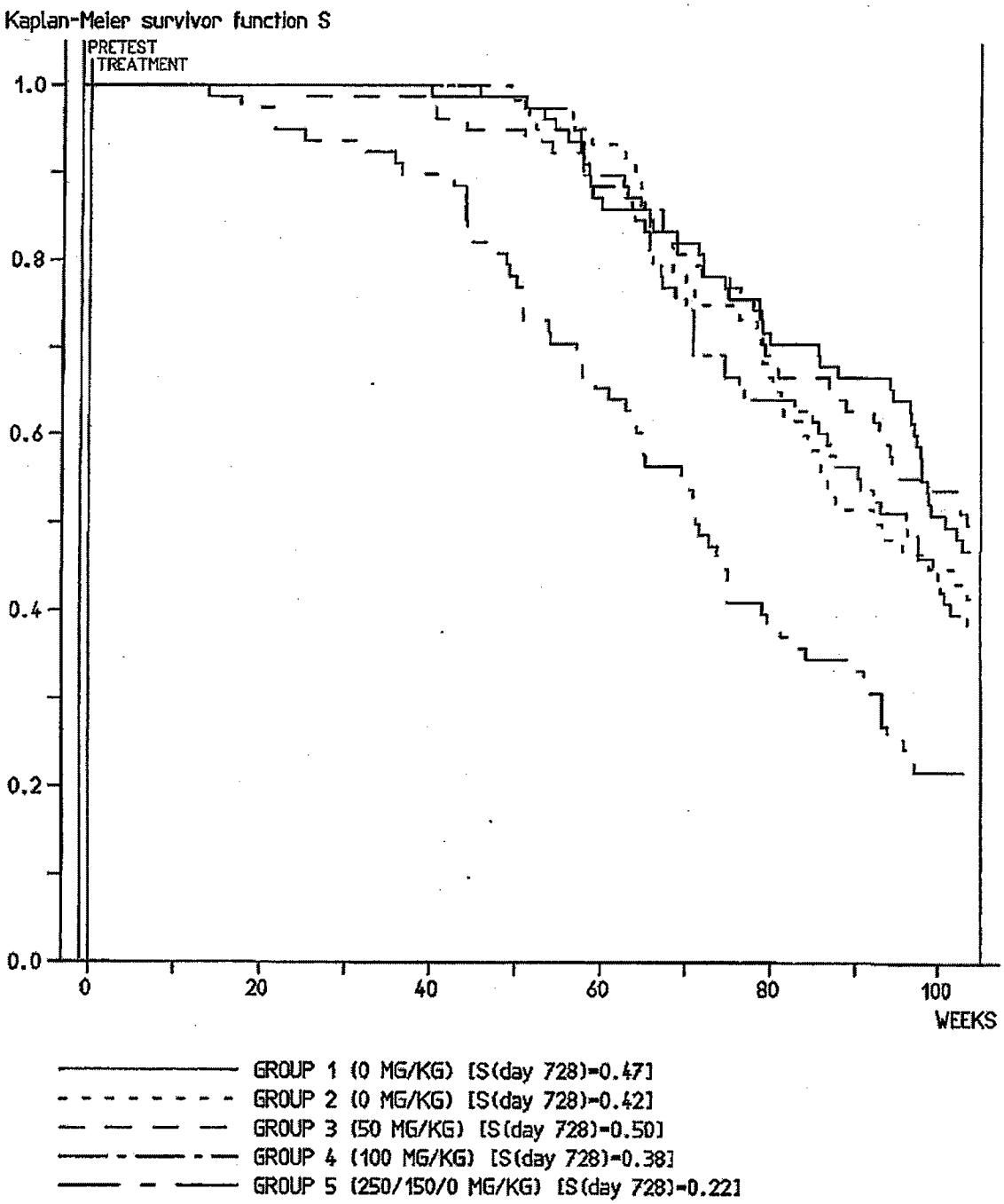
The high dose group was excluded from analysis due to being clearly above a Maximal Tolerated Dose based on the mortality effect in both sexes. Independent, in-house statistical evaluation was conducted following recommendations for combination of similar tumor types within organs, and all permutations of inclusion or exclusion of both the TK animals and the mid dose group were examined. Although the incidence of histiocytic sarcoma of the skin in female mice appeared to be the only finding of borderline significance, upon visual inspection of the data, none of the analysis permutations revealed a significant increase in incidence.

Conclusions

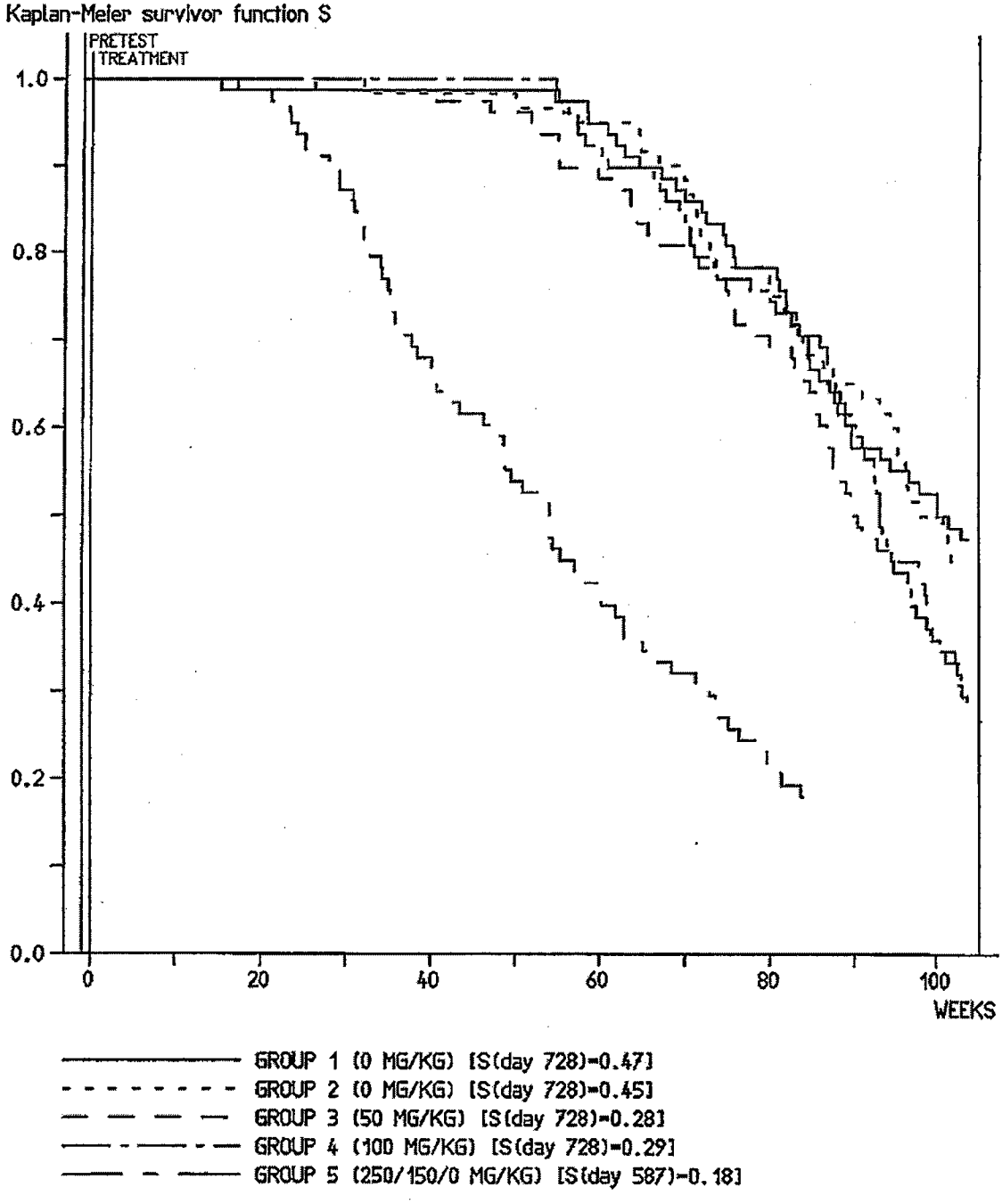
A 2-year, dietary administration study was conducted in CD-1 mice at ExecCAC-recommended dose levels of 50, 100 and 250 mg/kg/day. There were 60 animals/sex/group including each of two control groups. An additional 12 animals/sex/group used for toxicokinetics were included in the analysis for tumorigenic effects.

Increased incidences of hunched posture, emaciation and rales were observed in high and mid dose males and high dose females. The high dose male and female groups had their dose lowered to 150 mg/kg/day in week 39 and were taken off drug completely in week 96 (males) or week 76 (females). Effects on survival became evident within the first six months in males and females. Only 11 high dose males, compared with at least 25 in each of the other main study male control and treated groups, survived to scheduled sacrifice at 24 months. None of the main study high dose females survived to 24 months as all 9 surviving members of this group were sacrificed at 84 weeks. Only 14 mid dose females and 18 low dose females survived to 24 months compared with at least 24 females in each of the concurrent control groups. Statistical analysis revealed no evidence of drug-related tumorigenesis, whether or not the high dose groups were included.

SURVIVAL RATE
MALES



SURVIVAL RATE
FEMALES



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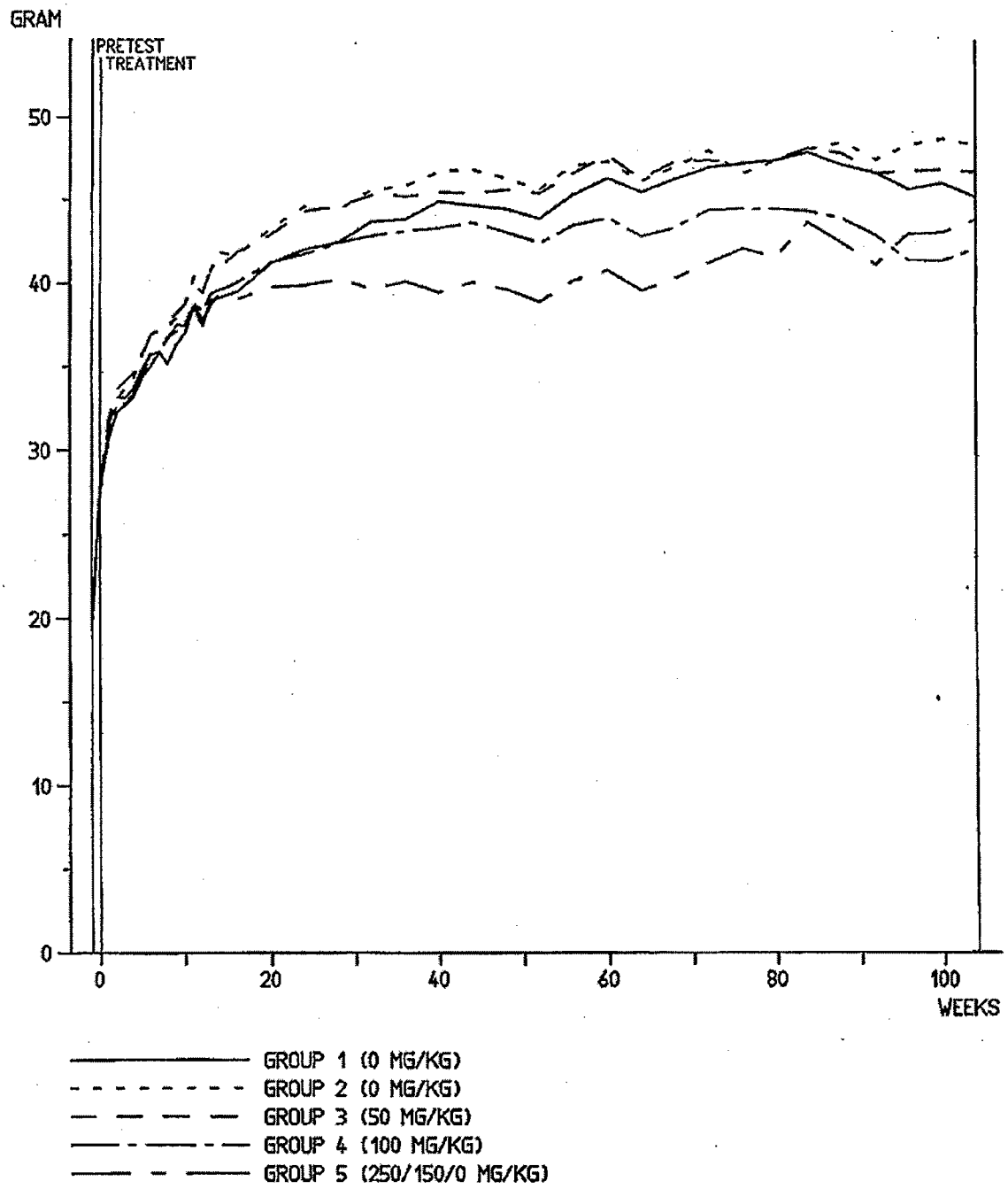
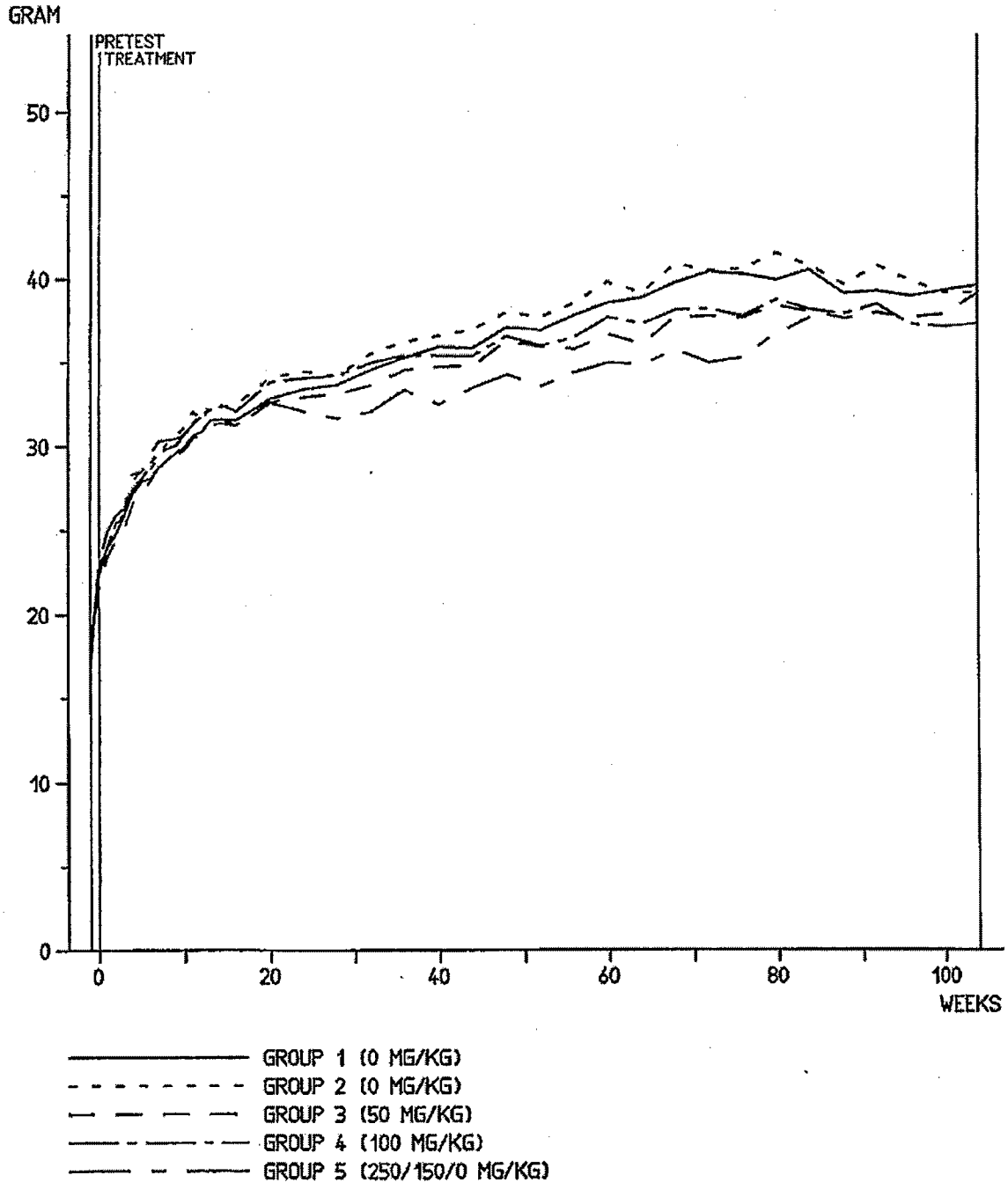
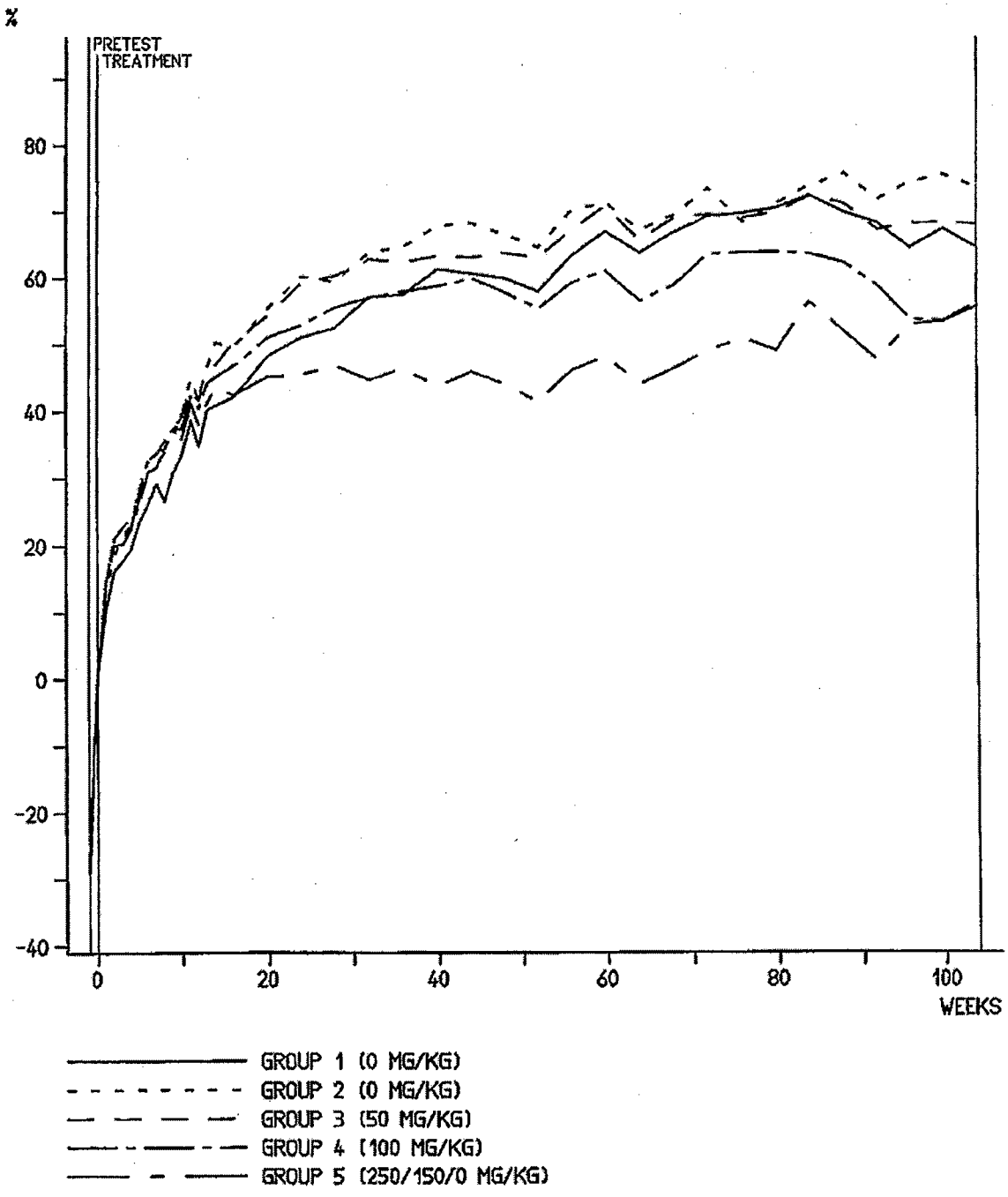
BODY WEIGHTS
MALES



BODY WEIGHTS
FEMALES



BODY WEIGHT GAIN
MALES



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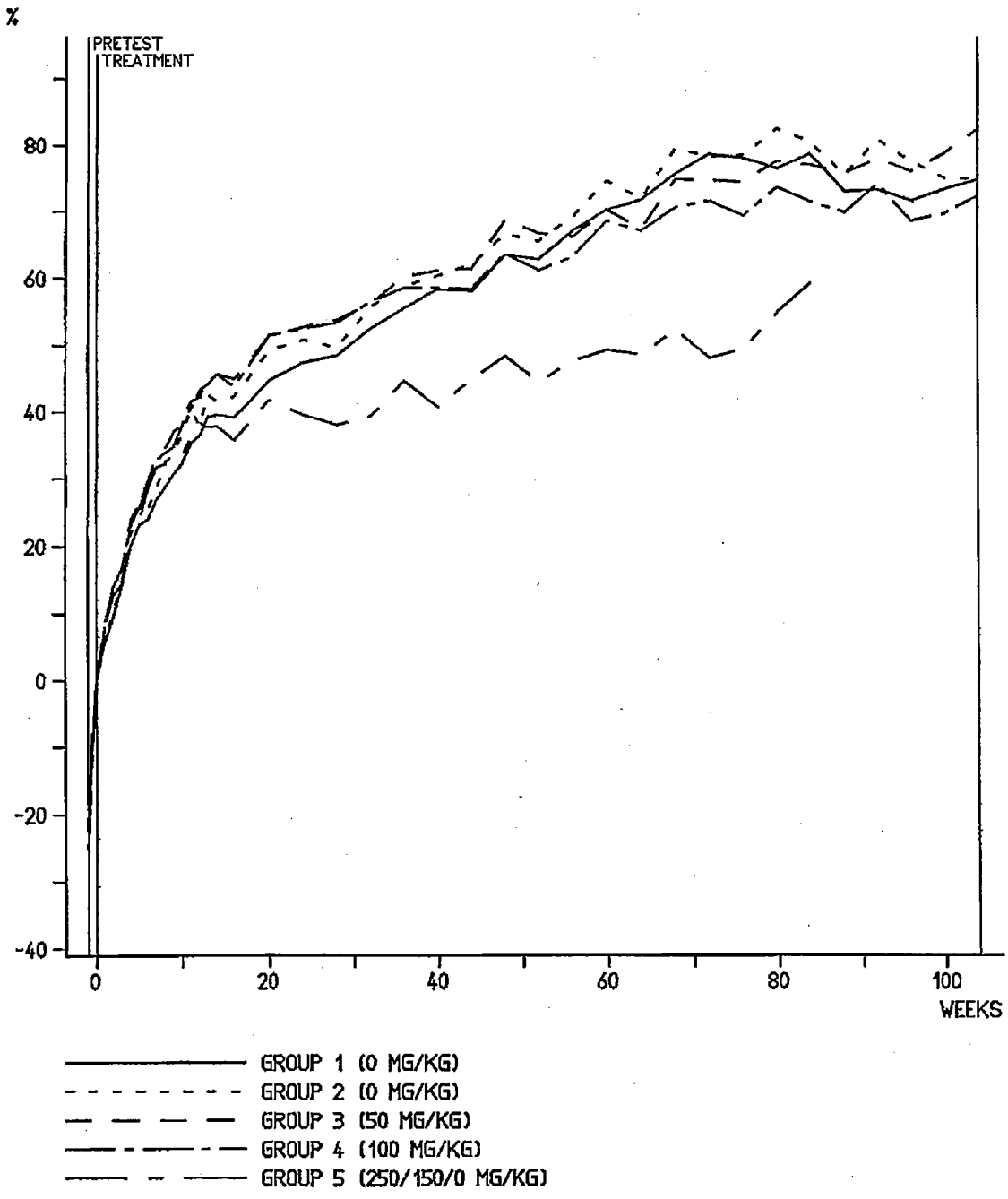
BODY WEIGHT GAIN
FEMALES



PATHOLOGY REPORT PAGE : 69/3692
SUMMARY TABLES [redacted] : 851466

TEST ARTICLE : BSF 208075 *PATHOL. NO.*: 41814 WEK
TEST SYSTEM : MOUSE, 104-Week, Oncogenicity *DATE* : 01-NOV-06
SPONSOR : Myogen, Inc. *PathData@System V6.2b5*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	78	78	60	60	78	78	78	78	78	–
SPINAL CORD, CERVIC. :	68	71	60	60	68	73	75	70	69	–
– Astrocytoma :	–	–	–	–	1	–	–	–	–	–
SPINAL CORD, THORAC. :	68	71	60	60	68	73	75	70	68	–
– Metastatic astrocyt.:	–	–	–	–	1	–	–	–	–	–
HEART :	70	72	60	60	68	75	75	70	69	–
– Metastatic carcinoma:	–	1	–	–	–	–	–	1	–	–
– Metastatic sarcoma :	–	–	–	–	–	2	–	–	–	–
AORTA :	68	72	60	59	68	75	75	70	67	–
– Metastatic carcinoma:	–	1	1	–	–	–	–	1	–	–
LUNG :	69	71	60	60	68	75	75	70	69	–
– Adenoma, alv./bron. :	4	6	13	–	9	1	4	3	2	–
– Carcinoma, alv./bron:	12	6	9	4	9	4	8	5	3	–
– Metastatic carcinoma:	–	–	1	–	–	–	–	–	–	–
– Metastatic sarcoma :	–	–	–	–	–	2	–	1	–	–
STOMACH :	69	71	60	59	68	74	75	69	67	–
– Adenocarcinoma :	–	–	–	–	–	–	–	1	–	–
– Metastatic sarcoma :	1	–	–	–	–	1	–	–	–	–
DUODENUM :	68	70	59	57	68	72	73	66	68	–
– Adenoma :	–	1	–	–	1	–	–	–	–	–
– Adenocarcinoma :	–	–	–	–	1	1	–	–	–	–
– Metastatic sarcoma :	–	–	–	–	–	1	–	–	–	–
ILEUM :	65	69	60	57	65	72	72	66	68	–
– Adenocarcinoma :	–	–	1	–	–	–	–	–	–	–
CECUM :	66	71	60	56	66	73	73	67	68	–
– Adenoma :	–	1	–	–	–	–	–	–	–	–
– Leiomyoma :	–	–	–	–	–	–	1	–	–	–
– Adenocarcinoma :	–	–	–	–	–	–	1	–	–	–
– Metastatic carcinoma:	1	–	–	–	–	–	–	–	–	–

PATHOLOGY REPORT PAGE : 70/3692
SUMMARY TABLES : 851466

TEST ARTICLE : BSF 208075 *PATHOL. NO.*: 41814 WEK
TEST SYSTEM : MOUSE, 104-Week, Oncogenicity *DATE* : 01-NOV-06
SPONSOR : Myogen, Inc. *PathData@System V6.2b5*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	78	78	60	60	78	78	78	78	78	–
COLON :	66	71	60	57	67	73	74	67	68	–
– Adenocarcinoma :	1	–	–	–	–	–	–	–	–	–
RECTUM :	68	71	60	58	68	74	74	68	68	–
– Leiomyosarcoma :	–	–	–	–	–	–	1	–	–	–
LIVER :	69	72	60	60	67	74	74	67	68	–
– Adenoma hepatocell. :	9	–	10	–	10	–	6	–	–	–
– Carcinoma hepatocel.:	4	1	4	–	3	–	4	–	2	–
– Hemangioma :	2	1	2	–	2	–	–	–	1	–
– Cholangioma :	1	–	–	–	–	–	–	–	–	–
– Hemangiosarcoma :	–	1	1	–	1	1	1	–	1	–
– Metastatic sarcoma :	1	–	–	–	–	–	–	–	1	–
– Metastatic carcinoma:	–	–	–	1	–	1	–	–	–	–
GALLBLADDER :	68	71	59	58	66	71	71	69	67	–
– Papilloma :	–	–	1	–	–	–	–	–	–	–
PANCREAS :	67	69	57	55	67	71	73	66	63	–
– Islets cell adenoma :	–	–	–	–	–	–	–	1	–	–
– Hemangiosarcoma :	1	–	–	–	–	–	–	–	–	–
– Metastatic sarcoma :	–	–	–	–	–	1	–	–	–	–
KIDNEYS :	69	70	60	60	68	75	75	70	68	–
– Hemangioma :	–	–	–	–	–	–	–	–	1	–
– Tubular adenoma :	1	–	–	–	–	–	–	1	–	–
– Tubular carcinoma :	–	–	–	–	–	–	–	–	1	–
– Metastatic carcinoma:	–	–	–	–	–	–	–	1	–	–
TESTES :	69	–	60	–	68	–	75	–	68	–
– Leydig cell tumor :	1	–	–	–	–	–	2	–	–	–
– Hemangioma :	–	–	–	–	1	–	1	–	–	–
– Rete carcinoma :	–	–	1	–	–	–	–	–	–	–
EPIDIDYMIDES :	69	–	60	–	68	–	75	–	68	–
– Malignant schwannoma:	–	–	–	–	–	–	1	–	–	–
– Metastatic sarcoma :	1	–	–	–	–	–	–	–	–	–

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TEST SYSTEM : MOUSE, 104-Week, Oncogenicity *DATE* : 01-NOV-06
SPONSOR : Myogen, Inc. *PathData@System V6.2b5*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	78	78	60	60	78	78	78	78	78	-
PROSTATE GLAND :	68	-	59	-	68	-	75	-	67	-
- Adenoma :	-	-	1	-	1	-	-	-	-	-
- Adenocarcinoma :	-	-	1	-	-	-	-	-	-	-
- Metastatic sarcoma :	1	-	-	-	-	-	-	-	-	-
SEMINAL VESICLES :	68	-	60	-	68	-	75	-	68	-
- Granular cell tumor :	-	-	-	-	-	-	1	-	-	-
- Metastatic sarcoma :	1	-	-	-	-	-	-	-	-	-
BULBOURETHRAL GLANDS :	1	-	-	-	1	-	-	-	1	-
- Hemangioma :	-	-	-	-	1	-	-	-	-	-
OVARIES :	-	71	-	59	-	74	-	69	-	-
- Hemangioma :	-	1	-	-	-	-	-	-	-	-
- Luteoma :	-	-	-	1	-	-	-	-	-	-
- Cystadenoma :	-	3	-	2	-	1	-	5	-	-
- Cystadenocarcinoma :	-	-	-	-	-	1	-	-	-	-
- Metastatic sarcoma :	-	-	-	-	-	1	-	-	-	-
UTERUS :	-	71	-	60	-	75	-	69	-	-
- Endometrial polyp :	-	1	-	-	-	-	-	-	-	-
- Stromal polyp :	-	5	-	3	-	6	-	3	-	-
- Adenoma :	-	2	-	-	-	1	-	1	-	-
- Hemangioma :	-	1	-	1	-	4	-	4	-	-
- Leiomyoma :	-	2	-	-	-	-	-	2	-	-
- Leimyosarcoma :	-	1	-	2	-	2	-	3	-	-
- Adenocarcinoma :	-	-	-	1	-	-	-	1	-	-
- Hemangiosarcoma :	-	-	-	-	-	-	-	1	-	-
- Stromal sarcoma :	-	2	-	1	-	5	-	2	-	-
- Metastatic carcinoma:	-	-	-	-	-	1	-	-	-	-
CERVIX :	-	71	-	60	-	74	-	68	-	-
- Stromal polyp :	-	-	-	1	-	-	-	1	-	-
- Leiomyoma :	-	3	-	1	-	-	-	2	-	-
- Metastatic sarcoma :	-	-	-	-	-	-	-	1	-	-

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NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	78	78	60	60	78	78	78	78	78	–
PITUITARY GLAND :	68	69	60	60	64	73	71	69	68	–
– Adenoma: p.anterior :	–	3	–	1	–	–	–	2	–	–
THYROID GLAND :	68	71	57	60	68	74	74	69	66	–
– Follicular adenoma :	–	–	1	–	–	–	–	–	–	–
PARATHYROID GLANDS :	42	49	40	35	40	45	43	44	35	–
– Carcinoma :	–	–	1	–	–	–	–	–	–	–
ADRENAL CORTICES :	69	71	60	59	67	74	74	69	69	–
– A-cell adenoma :	–	–	–	–	–	2	–	–	–	–
– B-cell adenoma :	2	–	2	–	1	–	1	–	–	–
– Adenoma :	1	1	3	1	–	–	2	–	1	–
– Carcinoma :	–	–	–	1	–	1	–	–	–	–
– Metastatic sarcoma :	1	–	–	–	–	1	–	–	–	–
ADRENAL MEDULLAS :	61	70	58	56	65	73	74	69	66	–
– Pheochromocytoma b. :	–	–	–	2	–	1	–	–	–	–
– Pheochromocytoma m. :	–	–	–	1	–	1	–	–	–	–
HEMOLYMPHORET. SYS. :	70	71	60	60	67	74	75	70	68	–
– Plasmacytoma :	–	1	–	–	–	–	1	–	–	–
– Histiocytic sarcoma :	4	2	1	4	1	6	–	9	1	–
– Malignant lymphoma :	9	20	8	23	8	15	5	12	3	–
– Granulocyt.leucemia :	–	–	–	–	–	1	–	–	–	–
SPLEEN :	68	71	58	58	67	71	74	68	65	–
– Hemangioma :	–	–	–	–	1	–	1	–	–	–
– Hemangiosarcoma :	–	1	–	–	–	–	1	–	1	–
– Metastatic sarcoma :	–	–	–	–	–	1	–	–	–	–
BONE MARROW-FEMORAL :	69	69	60	60	67	72	74	67	68	–
– Hemangioma :	–	–	1	–	–	–	–	–	–	–
THYMUS :	59	64	50	52	51	65	66	61	55	–
– Thymoma, lymph.,ben.:	1	2	–	1	–	–	–	–	1	–
– Metastatic carcinoma:	–	1	–	–	–	–	–	1	–	–

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NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	78	78	60	60	78	78	78	78	78	–
MESENT. LYMPH NODE :	65	67	53	52	58	58	65	58	52	–
– Hemangioma :	–	–	1	–	–	–	–	–	–	–
– Hemangiosarcoma :	–	–	–	1	–	–	–	–	–	–
– Metastatic sarcoma :	1	–	–	–	–	–	–	–	–	–
HARDERIAN GLANDS :	69	71	60	60	68	74	75	70	68	–
– Adenoma :	5	5	2	2	4	1	2	3	4	–
– Carcinoma :	–	–	–	–	–	1	–	1	–	–
MAMMARY GLAND :	68	70	59	58	67	71	70	67	66	–
– Adenocarcinoma :	–	4	–	1	–	1	–	2	–	–
– Adenoacanthoma :	–	–	–	1	–	–	–	–	–	–
SKIN/SUBCUTIS :	67	70	59	59	68	73	74	69	67	–
– Keratoacanthoma :	–	1	–	–	–	–	1	–	–	–
– Squamous carcinoma :	–	2	–	–	–	–	–	–	–	–
– Basal cell carcinoma:	–	–	–	–	1	–	1	–	–	–
– Fibrosarcoma :	–	–	–	1	–	1	–	–	–	–
– Malignant schwannoma:	–	–	–	–	–	–	1	–	1	–
– Osteosarcoma :	–	–	–	–	–	–	–	2	–	–
– Metastatic sarcoma :	1	–	–	–	–	–	–	–	–	–
– Histio.sarcoma:skin :	–	–	–	–	–	1	–	–	–	–
BONE :	1	–	–	–	1	1	–	1	–	–
– Osteoma :	–	–	–	–	–	–	–	1	–	–
– Osteosarcoma :	–	–	–	–	–	1	–	–	–	–
AUDITORY MEATUSES :	–	1	1	–	–	–	3	1	1	–
– Squamous papilloma :	–	–	–	–	–	–	–	–	1	–
BODY CAVITIES :	6	8	2	11	5	12	5	10	3	–
– Sarcoma, NOS :	1	–	–	–	–	1	–	–	–	–
– Metastatic sarcoma :	–	–	–	–	–	1	–	–	–	–
– Metastatic carcinoma:	1	–	–	–	–	–	–	–	–	–

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TEST SYSTEM : MOUSE, 104-Week, Oncogenicity *DATE* : 01-NOV-06
SPONSOR : Myogen, Inc. *PathData@System V6.2b5*

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K1, INCL. DEATHS

DOSE GROUP:	01		02		03		04		05	
SEX :	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	—	—	—	—	—	—	—	—	—	78
LUNG :	—	—	—	—	—	—	—	—	—	66
— Adenoma, alv./bron. :	—	—	—	—	—	—	—	—	—	3
— Carcinoma, alv./bron:	—	—	—	—	—	—	—	—	—	2
— Carcinoma; broncho. :	—	—	—	—	—	—	—	—	—	1
— Metastatic sarcoma :	—	—	—	—	—	—	—	—	—	1
UTERUS :	—	—	—	—	—	—	—	—	—	64
— Stromal polyp :	—	—	—	—	—	—	—	—	—	1
— Hemangioma :	—	—	—	—	—	—	—	—	—	1
— Leiomyoma :	—	—	—	—	—	—	—	—	—	2
HEMOLYMPHORET. SYS. :	—	—	—	—	—	—	—	—	—	62
— Malignant lymphoma :	—	—	—	—	—	—	—	—	—	3
THYMUS :	—	—	—	—	—	—	—	—	—	52
— Thymoma, lymph.,ben.:	—	—	—	—	—	—	—	—	—	3
HARDERIAN GLANDS :	—	—	—	—	—	—	—	—	—	63
— Adenoma :	—	—	—	—	—	—	—	—	—	1

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Study title: 104-week oncogenicity (feeding) study in the rat.

Key study findings:

Body weight gain and food consumption were dose-dependently reduced for both sexes at the mid and high dose levels ($p<0.01$), and hunched posture, labored respiration, rales and emaciation were evident in these groups before the end of the first year of dosing. The high and mid-dose male and female groups had their doses lowered to 40 and 20 mg/kg/day, respectively, in week 51. The high dose males and females were taken off drug completely in weeks 69 and 93, respectively. Effects on survival of these groups became evident within the first 6 months. Only 13 (of the 50 main study) high dose males and 12 high dose females and 17 mid-dose males and 21 mid-dose females survived to scheduled termination (remaining animals of these groups died or were sacrificed in extremis) compared with at least 35/sex in each of the other (control and low dose) groups.

The only evidence of ambrisentan-related carcinogenicity was a positive trend ($p<0.025$) for the combined incidence of benign basal cell tumor and basal cell carcinoma of skin/subcutis in male rats when the high dose group was eliminated from the analysis (1 animal with each tumor at the mid-dose, none in any other group, $p<0.025$) and the occurrence of mammary fibroadenomas in male rats of that same high dose group (4 animals with the tumor in that group, none in any other male group, $p<0.05$, pairwise comparison with controls).

Study no.: 851465
Volume #, and page #: eCTD
Conducting laboratory and location:

Date of study initiation: 1/5/04
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: Lot. No. 10011, [redacted] purity
CAC concurrence: yes

Methods

Doses: 0, 10, 30 or 60 mg/kg/day (see chart below)
Basis of dose selection: MTD
Species/strain: rat, Wistar
Number/sex/group (main study): 50
Route, formulation, volume: food admixture
Frequency of dosing: daily
Satellite groups used for toxicokinetics or special groups: 12
Age: 5 weeks
Animal housing: individual cages
Drug stability/homogeneity: evaluated

Dual controls employed: yes

Deviations from original study protocol: dosing reduction in Groups 4 and 5 as below:

Allocation And Target Dose Levels	Group 1 0 mg/kg/day	Group 2 0 mg/kg/day	Group 3 10 mg/kg/day	Group 4 30/20* mg/kg/day	Group 5 60/40*/0** mg/kg/day
Males A	1-50	63-112	113-162	175-224	237-286
Males B	51-62		163-174	225-236	287-298
Females A	299-348	361-410	411-460	473-522	535-584
Females B	349-360		461-472	523-534	585-596

A – Animals: Oncogenicity Animals
B – Animals : Plasma Level Animals

*Due to the high mortality the dose levels were reduced to 20 (Group 4) and 40 (Group 5) mg/kg/day starting on December 23, 2004. ** Discontinuation of treatment in high dose males at week 69 and in females at week 93.

Observation times

The following observations were recorded:

Viability / mortality: Twice daily.

Clinical signs: At least once daily including a weekly palpation for tissue masses.

Food consumption: Weekly up to week 15, except for weeks 16 and 17, every 4 weeks thereafter.

Body weights: Weekly up to week 15, in week 17, and every 4 weeks thereafter.

Ophthalmoscopic examinations: Ophthalmoscopic examinations were performed in allocation A animals, in 15 animals/group/ sex (starting with the lowest animal number) during acclimatization. In 15 animals/sex in groups 1 and 5 (starting with the lowest animal no.) in weeks 52/104, at 104 week due to test item-related findings in group 5, 15 animals/sex (starting with the lowest animal no.) of the interim dose groups were also examined.

Hematology parameters:

Erythrocyte count RBC	Mean corpuscular hemoglobin concentration MCHC
Hemoglobin HB	Hemoglobin concentration distribution width KDW
Hematocrit HCT	Reticulocyte maturity index (low, medium, high fluorescence) L RETI, M RETI, H RETI
Mean corpuscular volume MCV	Leukocyte count, total WBC
Red cell volume distribution width	Differential leukocyte count

RDW	
Mean corpuscular hemoglobin MCH	Neutrophils NEUT
Reticulocyte count RETI	Lymphocytes LYMPH
Eosinophils EOS	Monocytes MONO
Basophils BASO	Large unstained cells LUC
Platelet count PLATELET	

Clinical chemistry

Glucose	Aspartate aminotransferase EC 2.6.1.1'	Potassium
Urea	Alanine aminotransferase EC 2.6.1.2	Chloride
Creatinine	Lactate dehydrogenase EC 1.1.1.27	Calcium
Bilirubin, total	Glutamate dehydrogenase EC 1.4.1.3	Protein, total
Cholesterol, total	Alkaline phosphatase EC 3.1.3.1	Albumin
Triglycerides	Gamma-glutamyl transferase EC 2.3.2.2	Globulin
Phospholipids	Creatine kinase EC 2.7.3.2	A1burnin/Globulin Ratio
Phosphorus	Sodium	

Urinalysis

Urine volume (18-hour)	Glucose
Specific gravity	Ketones
Color	Urobilinogen
Appearance	Bilirubin
pH	Erythrocytes
Nitrite	Leukocytes
Protein	

Toxicokinetics

Blood samples from allocation B animals for the determination of plasma drug concentrations were collected during weeks 2, 52, and 104. Due to high mortality in group 5, additional samples were collected in week 65 in all groups (including surviving 1-2 group 5 animals per sex and dose and timepoint at week 65); treatment of group 5 animals was terminated prematurely, so toxicokinetic samples were collected in week 104 only in groups 1, 3 and 4.

Pathology

All animals (oncogenicity and kinetic) were necropsied and descriptions of all macroscopic abnormalities recorded. The following organs/tissues were examined:

Adrenal glands	Ovaries
Aorta	Pancreas
Auricles	Pituitary gland
Bone (sternum and femur including joint)	Prostate gland
Bone marrow (femur)	Rectum
Brain (medulla/pons, cerebral and cerebellar cortex)	Salivary glands - mandibular, sublingual
Cecum	Sciatic nerve
Colon	Seminal vesicles
Duodenum	Skeletal muscle
Epididymis	Skin
Esophagus	Spinal cord - cervical, midthoracic, lumbar
Exorbital lachrymal gland	Spleen
Eyes with optic nerve	Stomach
Harderian gland	Testes
Heart	Thymus
Ileum	Thyroid including parathyroid gland if possible
Jejunum	Tongue
Kidneys	Trachea
Larynx	Urinary bladder
Liver	Uterus with cervix
Lungs	Vagina
Lymph nodes – mesenteric, mandibular	Zymbal's gland
Mammary gland area	Gross lesions and tissue masses
Nasopharyngeal tissue	

Results

Mortality:

A total of 208 (110 males and 98 females) of the 500 allocation A animals either died spontaneously or were sacrificed in extremis prior to scheduled necropsy after 104 weeks. Increased mortality was evident in both sexes of Groups 4 & 5, as follows:

Group (mg/kg/day)	1 (0)		2 (0)		3 (10)		4 (40/20)		5 (60/40)	
Sex	M	F	M	F	M	F	M	F	M	F
Number at start	50	50	50	50	50	50	50	50	50	50
Spontaneous death	8	7	8	8	4	3	17	19	23	31
Killed in extremis	3	4	7	3	10	6	16	10	14	7
Total premature	11	11	15	11	14	9	33	29	37	38
Scheduled necropsy	39	39	35	39	36	41	17	21	13	12
Survival rate (%)	78%	78%	70%	78%	72%	82%	34%**	42%**	26%**	24%**

** $p \leq 0.01$ (Cox regression model test)
Lit. Cox D.R.: Regression models and life tables. J Roy Stat Soc B 1972; 34:187–220

Clinical signs:

The incidence of hunched posture, labored respiration, rales, and emaciation was increased by treatment in groups 4 and 5 (both sexes), starting in the second half of the first year of treatment. Rales were displayed by most animals in group 4, and all animals in group 5 (both sexes); the other clinical signs affected a minority of animals in each group.

Body weights:

Body weight and body weight gain were dose-dependently reduced in groups 4 and 5 (both sexes) throughout treatment. In group 5, body weight and body weight gain recovered to control levels on cessation of treatment (males in week 69, females in week 93).

At study end (week 104), mean body weights were significantly ($p<0.01$) lower than controls (group 1) in groups 4 and 5 (-31% and -11%, respectively, in males; -19% and -18% in females),

Food consumption:

Food consumption was dose-dependently reduced in group 4 and 5 (both sexes) throughout treatment. In group 5, the food consumption recovered to control levels immediately on cessation of treatment (males in week 69, females in week 93).

Hematology:

The following changes to hematology parameters recorded at the 52- and 105-week assessments were considered related to the treatment with the test item:

		MALES				FEMALES			
Parameter	week	2	3	4	5	2	3	4	5
RBC	52			+7%#	+11%#			+10%*	+17%**
	105			+20%**				+11%*	+7%#
Hb	52			+8%#	+14%#			+9%#	+19%**
	105			+21%**				+8%#	
Hct	52				+20%**			+10%#	+19%**
	105			+25%**				+9%*	
MCV	52				+9%*				
	105								-5%**
RDW	52								+18%#
	105				-7%#				
MCH	52								
	105								-6%**
MCHC	52				-6%**				
	105								
Reti abs.	52				+185%*				
	105			+40%#					
L-Reti	52				-11%#				
	105								+13%#
M-Reti	52				-12%+				-15%#
	105								-13%+
H-Reti	52				+141%#				
	105			+44%#	+44%#				
WBC	52				-14%#				
	105			-47%**	-25%#				
Neut abs.	52								
	105			-49%#					
Eos abs.	52			-37%*	-54%**				
	105			-63%**	-20%#				

		MALES				FEMALES			
Parameter	week	2	3	4	5	2	3	4	5
Baso abs.	52				+2-fold				
	105								
Lympho abs.	52			-11%#	-19%*				
	105			-46%**	-14%#				
Mono abs.	52								
	105			-32%#	-20%#				
Plt.	52			-22%**	-32%**				-10%#
	105			-36%**	-18%#			-12%#	

Statistical key: */**: p< 0.05 / 0.01 (Dunnett) # not significant at 5% level + p< 0.05 (Steel Test)

Clinical chemistry:

The following changes to clinical biochemistry parameters recorded at the 52- and 104-week assessments were considered related to the treatment with the test item:

		MALES				FEMALES			
Parameter	week	2	3	4	5	2	3	4	5
Gluc	49								
	52								-11%#
	105			-20%**	-15%*			-17%**	
Urea	49								
	52				+24%#				
	105			+56%**	+15%#		+26%*	+31%**	+31%**
Creat	49								
	52				-9%#				
	105			-4%#					
Prot	49				-7%*				
	52				-7%**			-7%**	-8%**
	105								
Alb	49								
	52							-8%**	-15%**
	105								
Glob	49				-14%*				
	52				-15%**				
	105				-9%#				
A/G	49								
	52				+18%*				-20%*
	105								

		MALES				FEMALES			
Parameter	week	2	3	4	5	2	3	4	5
Chol	49							-33%*	-22%#
	52						-26%*	-35%**	-26%*
	105				-17%#		-31%**	-27%*	-18%#
Trigly	49		-36%*	-62%**	-63%**			-35%#	-51%*
	52		-26%#	-46%*	-27%#		-24%**	-23%**	-36%**
	105						-59%*	-67%**	-63%*
Phos-Lip	49			-21%#	-33%**				
	52				+16%#		-23%**	-34%**	-30%**
	105				-15%#		-29%**	-31%**	
Na+	49				+2.0%				
	52			+2.0%**	+3.0%**	+1.0%*			+2.0%**
	105								
K+	49				+11%#				
	52			+11%**	+12%**			+7%#	+11%**
	105			+26%**					
Cl-	49				-1.0%#				
	52			-3.0%**	-4.0%**			-2.0%*	-4.0%**
	105			-6.0%**				-3.0%**	
PO4-In	49								
	52			+12%*	+20%**				+13.0%*
	105			+22%**			+19.0%**	+17.0%*	+15.0%*
ALP	49								
	52				+33%**				+53%**
	105								
CK	49								
	52				-21%#				
	105								

Statistical key: */**: p< 0.05 / 0.01 (Dunnett) # not significant at 5% level + p< 0.05 (Steel Test)

Urinalysis:

Urinalysis data indicated no remarkable findings at week 52 and after 104 weeks of treatment for the animals of the treated groups.

Toxicokinetics:

A summary of toxicokinetic parameters (means) of BSF 208075 is given in the tables below:

Group 3 10 mg/kg/day	Week 2		Week 52		Week 65		Week 104	
	males	females	males	females	males	females	males	females
$C_{average}$ [ng/ml]	626	369	698	1327	739	663	1527	1104
AUC_{0-24h} [ng·h/ml]	14672	8629	16497	28921	16962	14544	34823	24621

Group 4 30/20 mg/kg/day	Week 2		Week 52		Week 65		Week 104	
	males	females	males	females	males	females	males	females
$C_{average}$ [ng/ml]	2080	1451	1648	1917	1508	2004	2406	2872
AUC_{0-24h} [ng·h/ml]	48108	33117	39418	47292	35335	47512	55639	68672

Group 5 60/40 mg/kg/day	Week 2		Week 52		Week 65		Week 104	
	males	females	males	females	males	females	males	females
$C_{average}$ [ng/ml]	3899	2631	2937	3651	3702	2611	n.c.	n.c.
AUC_{0-24h} [ng·h/ml]	93970	61228	65270	87658	84453	64973	n.c.	n.c.

n.c.: not calculated (due to high mortality, no samples were available)

Gross pathology:

At necropsy, the following findings, considered to distinguish treated rats form controls, were recorded:
Heart: round heart/distended auricles in 1 female of group 1, in 3 males and 2 females in group 4, and 2 males and 1 female in group 5.
Lungs: gray-white foci 3 males and 4 females in group 1, 7 males and 2 females of group 2, 7 males and 4 females of group 3, 13 males and 12 females of group 4, and 10 males and 23 females of group 5.
Testes: flaccid in 4 rats of group 1, 3 rats of group 3, 23 rats of group 4, and 19 rats of group 5.

Histopathology:

Non-neoplastic findings:

Nasal Cavities: There was an increased incidence of a cystic/hemorrhagtc change (cysts were recorded within the mucosa) in males and females of groups 3-5. In addition, there was an increased incidence of Steno's gland degeneration in both sexes of groups 3 to 5.

Lung: An increased in alveolar histiocytosis in rnales of groups 4 and 5 and fernales of group 5 was noted. Oral Cavity: There was a high occurrence of dental dysplasia in the incisors of males and females of groups 3-5.
Testes and Epididymides: Testicular tubular degeneration increased in incidence and severity in all test item-treated groups. This was associated with an increased incidence of aspermia/oligospermia in Groups 4 to 5 rat epididymides.
Spleen: Increased hematopoiesis was diagnosed in males of groups 4 and 5.

Neoplastic findings: Summary of tumor findings

Number of Animals with Neoplasms:

Dose Group	1		2		3		4		5	
Sex	M	F	M	F	M	F	M	F	M	F
Number affected	42	50	39	42	50	51	29	40	17	24
%	67.7	80.7	78.0	84.0	80.7	82.3	46.8	64.5	25.8	38.7

Number of Animals with Benign and Malignant Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Benign	34	48	38	41	43	49	25	36	13	20	153	194
Malignant	13	7	5	7	12	11	7	7	4	5	41	37

Number of Primary Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Primary Tumors	60	95	53	87	76	103	42	62	19	36	250	383

Number of Benign and Malignant Neoplasms:

Dose Group	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Benign	44	87	48	79	62	91	34	55	14	31	202	343
Malignant	16	8	5	8	14	12	8	7	5	5	48	40

Full listing of all neoplastic findings (organ/group/sex) are attached.

Conclusions

A 2-year, dietary administration study was conducted in Wistar rats at ExecCAC-recommended dose levels of 10, 30 and 60 mg/kg/day. There were 50 animals/sex/group including each of two control groups. An additional 12 rats/sex/group used for toxicokinetics were included in the analysis for tumorigenic effects.

Body weight gain and food consumption were dose-dependently reduced for both sexes at the mid and high dose levels ($p<0.01$), and hunched posture, labored respiration, rales and emaciation were evident in these groups before the end of the first year of dosing. The high and mid-dose male and female groups had their doses lowered to 40 and 20 mg/kg/day, respectively, in week 51. The high dose males and females were taken off drug completely in weeks 69 and 93, respectively. Effects on survival of these groups became evident within the first 6 months. Only 13 (of the 50 main study) high dose

males and 12 high dose females and 17 mid-dose males and 21 mid-dose females survived to scheduled termination (remaining animals of these groups died or were sacrificed in extremis) compared with at least 35/sex in each of the other (control and low dose) groups.

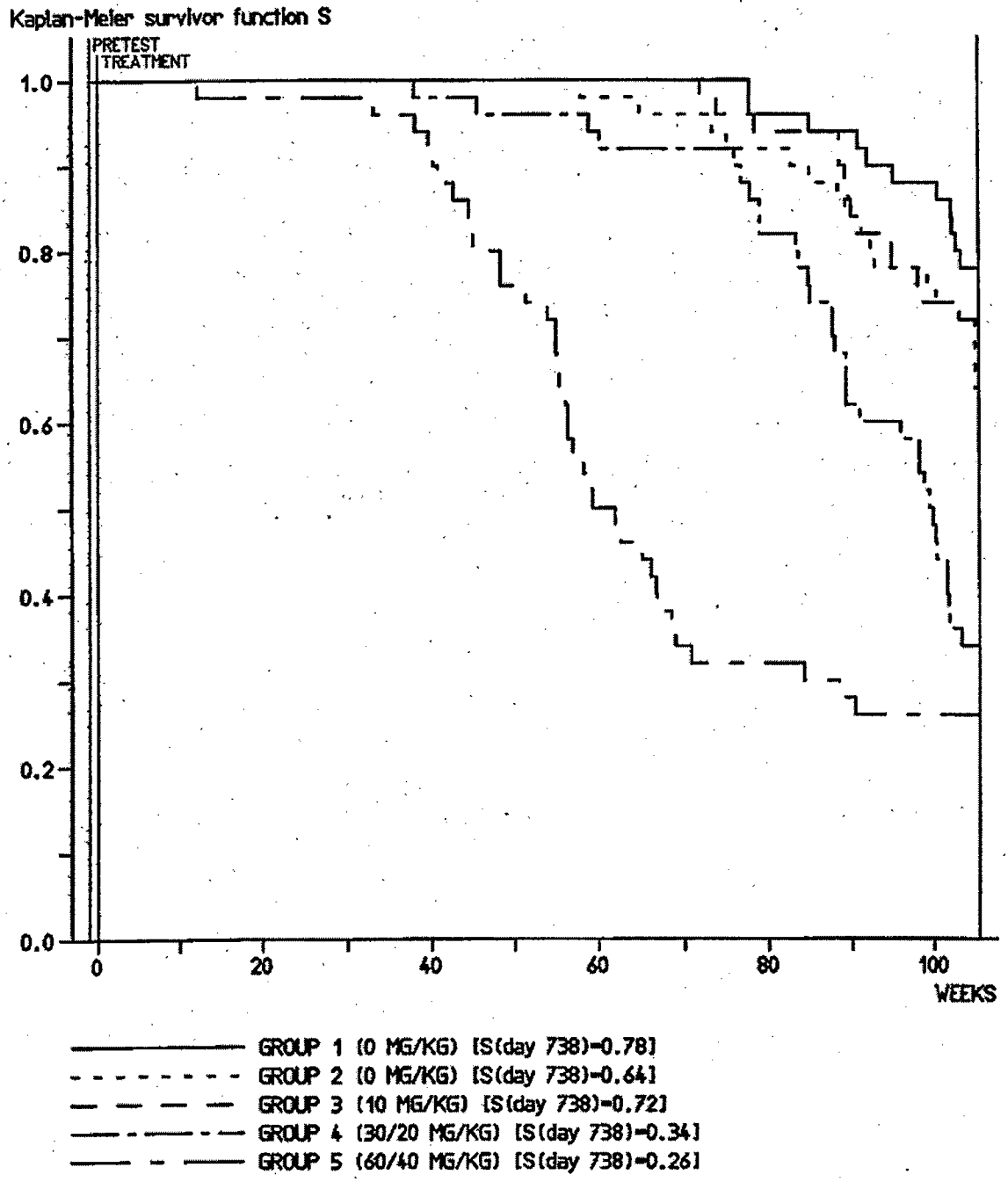
Dosing in the high and, possibly, mid dose groups was considered above the MTD for both sexes based on survival and body weight decrement. Clinical signs, hematology and histopathology findings are all consistent with suffocation in these affected rats. Statistical analysis was therefore conducted with exclusion of the high dose, and combination of like tumor types within organs as recommended in FDA guidances.

The only evidence of ambrisentan-related carcinogenicity was a positive trend ($p<0.025$) for the combined incidence of benign basal cell tumor and basal cell carcinoma of skin/subcutis in male rats when the high dose group was eliminated from the analysis (1 animal with each tumor at the mid-dose, none in any other group, $p<0.025$).

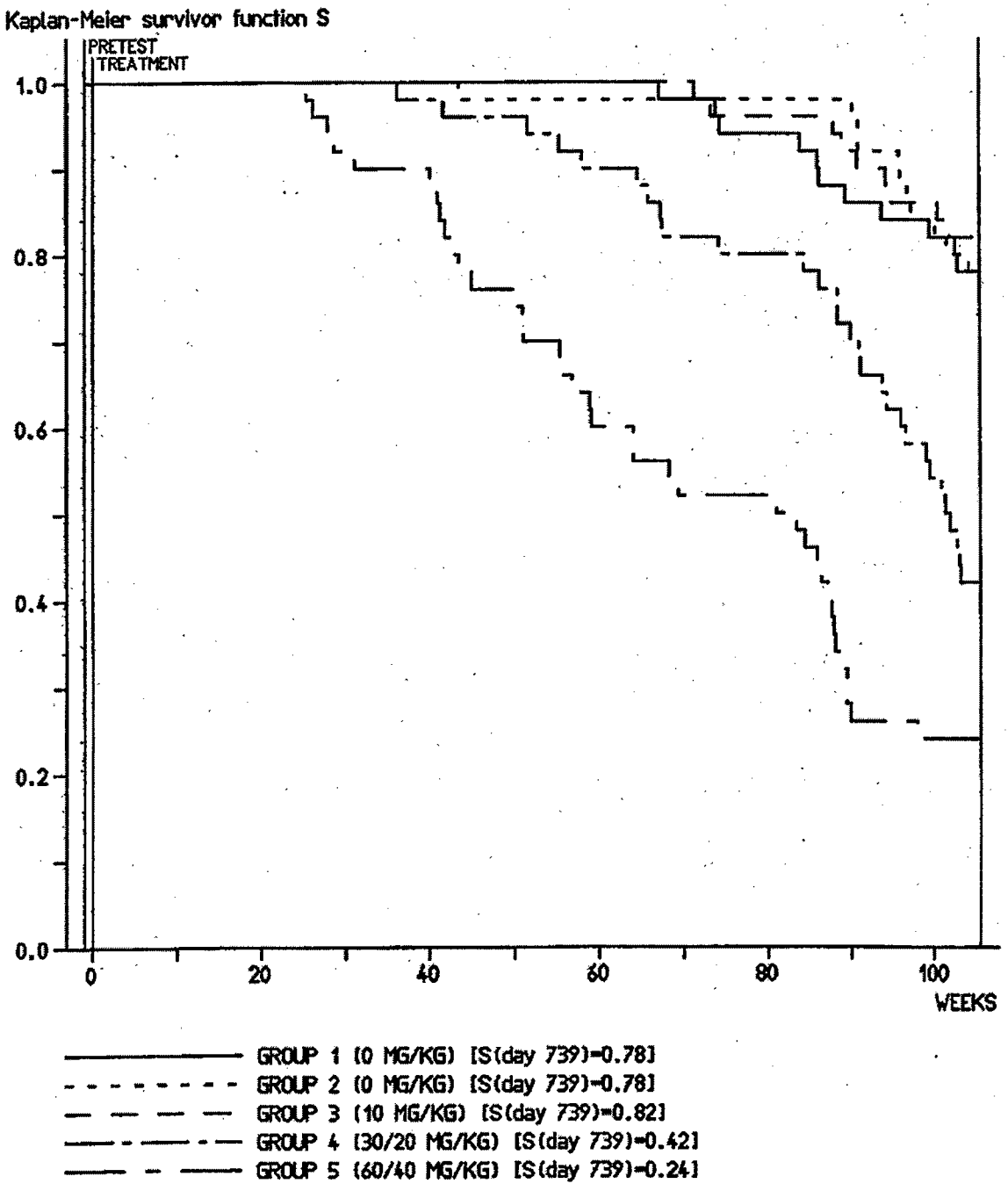
When the high dose group was included in the analysis, the incidence of mammary fibroadenomas in male rats of that same high dose group (4 animals with the tumor in that group, none in any other male group, $p<0.05$, pairwise comparison with controls) was considered significant.

The relevance of these findings is not certain to this reviewer. Both findings occurred only in the context of a seemingly unrelated toxicity and lethality which may be rodent-specific as they are obligate nose-breathers. Conversely, the findings could represent the threshold of a neoplastic response in skin which could occur in the absence of toxic signs in non-rodent species. Both these findings should be included in labeling and presented in the context this toxicity.

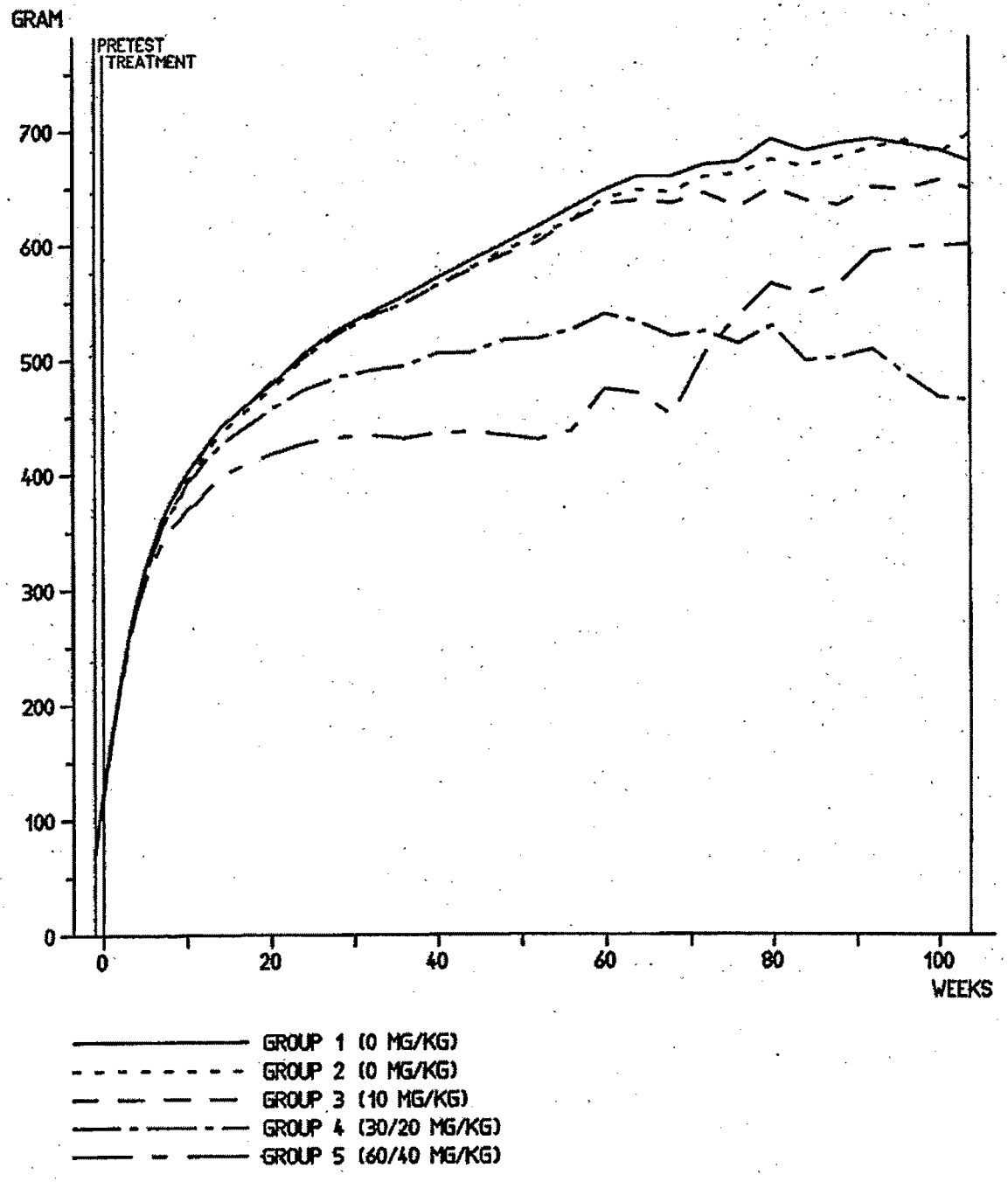
SURVIVAL RATE
AFTER 104 WEEKS / MALES



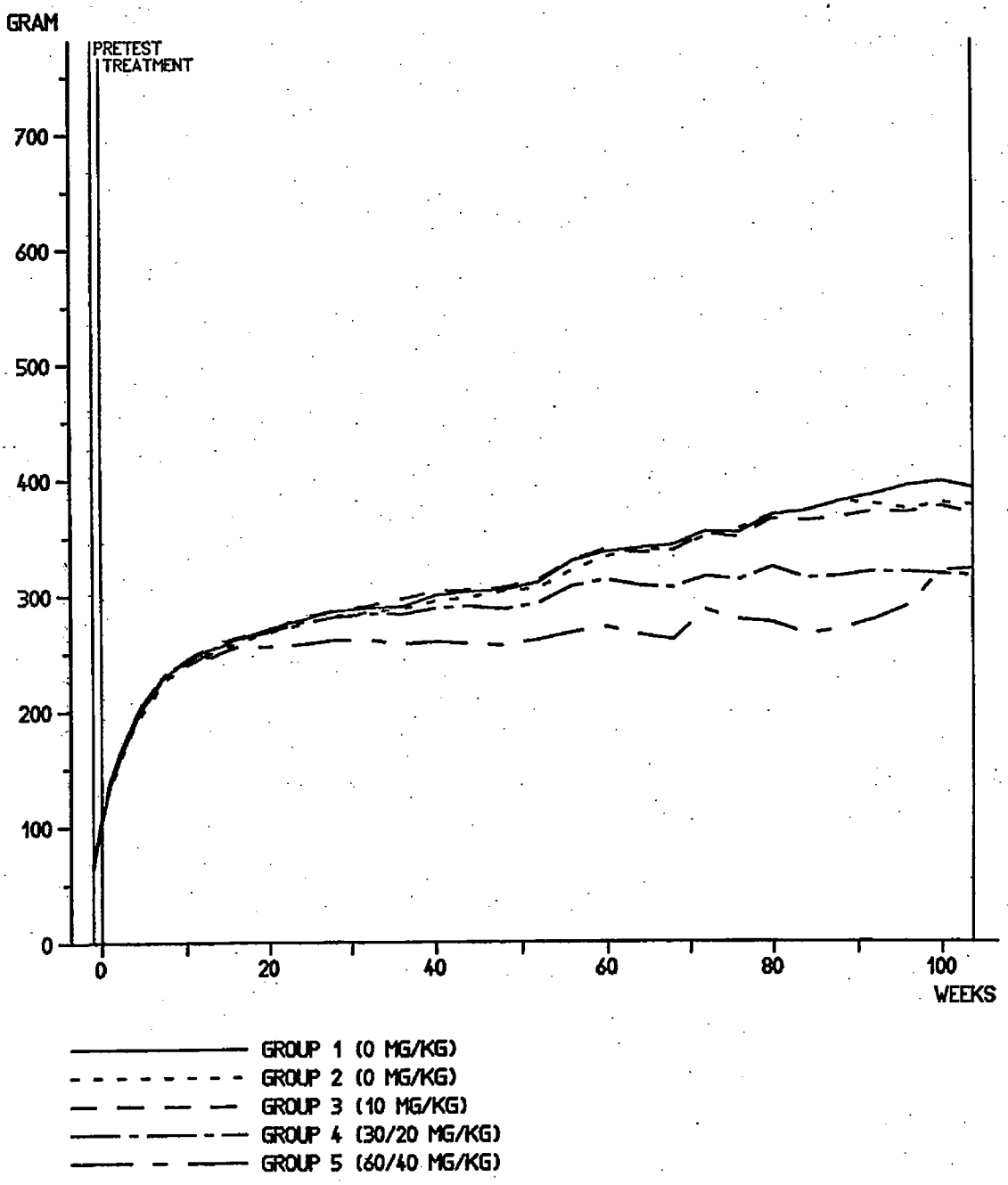
SURVIVAL RATE
AFTER 104 WEEKS / FEMALES



BODY WEIGHTS
AFTER 104 WEEKS MALES



BODY WEIGHTS
AFTER 104 WEEKS FEMALES



PATHOLOGY REPORT
SUMMARY TABLES

PAGE [redacted] PAT: 41/1732
: 851465

TEST ARTICLE : BSF 208075
TEST SYSTEM : RAT, 104 WEEKS, ORAL
SPONSOR : MYOGEN

PATHOL. NO.: 10027 HJC
DATE : 02-NOV-06
PathData®System V6.2b5

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0), Incl. Deaths

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	62	50	62	62	62
HEART No. Examined	62	50	53	53	56
- Benign endocardial schwannoma	-	-	1	1	-
LIVER No. Examined	62	50	56	55	53
- Adenoma: hepatocellular	-	2	1	1	-
- Hepatocellular carcinoma	-	-	1	-	-
- Cholangiocellular carcinoma	-	-	1	-	-
MESENT. LYMPH NODE No. Examined	62	50	54	52	50
- Hemangioma	-	1	3	2	-
- Hemangiosarcoma	1	1	-	-	1
HEMOLYMPHORET. SYS. No. Examined	62	50	53	53	56
- Malignant lymphoma (not otherwise specified)	1	-	-	-	2
- Malignant fibrous histiocytoma	1	-	-	-	-
- Histiocytic sarcoma	-	-	1	-	-
JEJUNUM No. Examined	62	50	53	53	54
- Adenoma	1	-	-	-	-
LUNG No. Examined	62	49	54	55	56
- Alveolar/bronchiolar adenoma	1	1	-	-	-
- Metastasis of carcinoma	1	-	-	-	-
- Metastasis of sarcoma	1	-	-	-	-
THYMUS No. Examined	60	50	53	53	52
- Benign thymoma	1	2	3	-	-
- Malignant thymoma	1	-	-	-	-
- Papilloma in ductal remnant	-	-	-	1	-
TESTES No. Examined	62	50	53	56	52
- Benign Leydig cell tumor	1	3	-	-	-
- Metastasis of sarcoma	-	-	-	1	-
EPIDIDYMIDES No. Examined	62	50	53	55	52
- Malignant mesothelioma	-	-	1	-	-
PAROTID GLANDS No. Examined	-	-	1	-	-
- Adenocarcinoma	-	-	1	-	-
SUBLINGUAL GLANDS No. Examined	61	49	52	48	56
- Carcinoma; anaplastic	1	-	-	-	-
MANDIBULAR GLANDS No. Examined	61	49	52	49	56
- Malignant Schwannoma	-	-	-	1	-
PANCREAS No. Examined	61	50	53	53	55
- Islet cell adenoma	2	2	2	-	1
- Islet cell carcinoma	2	-	-	1	-
- Acinar cell adenocarcinoma	-	-	-	-	1

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control=01,02,
Group 01, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 02, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 03, 10 MG/KG, males: BSF 208075 (10 mg/kg)
Group 04, 30/20 MG/KG, males: BSF 208075 (30 mg/kg)
Group 05, 60/40/0 MG/KG, males: BSF 208075 (60 mg/kg)

PATHOLOGY REPORT PAGE PAT: 42/1732
SUMMARY TABLES : 851465

TEST ARTICLE : BSF 208075 PATHOL. NO.: 10027 HJC
TEST SYSTEM : RAT, 104 WEEKS, ORAL DATE : 02-NOV-06
SPONSOR : MYOGEN PathData@System V6.2b5

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0), Incl. Deaths

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	62	50	62	62	62
MANDIB.LYMPH NODES No. Examined	58	48	53	51	54
- Fibroma	1	-	-	-	-
- Metastasis of carcinoma	-	-	1	-	-
THYROID GLAND No. Examined	62	50	52	53	51
- C-cell adenoma	6	5	5	2	1
- C-cell carcinoma	-	-	1	-	-
- Follicular cell adenoma	-	6	5	-	-
- Follicular cell carcinoma	-	1	-	-	-
PARATHYROID GLANDS No. Examined	57	50	47	44	38
- Adenoma	2	-	-	1	-
ADRENAL CORTICES No. Examined	62	50	53	55	57
- Adenoma	-	2	-	-	-
ADRENAL MEDULLAS No. Examined	62	50	53	55	57
- Benign pheochromocytoma	2	-	2	1	2
- Malignant pheochromocytoma	1	1	-	1	-
- Ganglioneuroma	1	-	-	-	-
SKIN/SUBCUTIS No. Examined	62	50	58	57	55
- Squamous cell carcinoma	1	-	2	-	-
- Keratoacanthoma	2	2	7 *	5	-
- Lipoma	2	-	-	-	-
- Fibroma	-	1	5 *	2	-
- Benign basal cell tumor	-	-	-	1	-
- Carcinoma: basal cell	-	-	-	1	-
- Sebaceous squamous cell carcinoma	1	1	-	-	-
- Carcinoma: sebaceous cell (Zymbal's gland)	-	-	-	-	1
- Benign Schwannoma	-	-	-	1	-
- Malignant Schwannoma	-	-	1	1	-
MAMMARY GLAND No. Examined	62	50	53	54	55
- Fibroadenoma	-	-	-	-	4 *
CEREBRUM No. Examined	62	50	54	54	57
- Granular cell tumor	2	-	-	-	-
- Astrocytoma	1	-	-	-	-
PROSTATE GLAND No. Examined	62	50	55	54	55
- Adenoma	-	-	-	1	-
- Adenocarcinoma	1	-	2	-	-
PITUITARY GLAND No. Examined	62	50	55	55	54
- Adenoma of pars distalis	20	19	23	12	6 **
NASAL CAVITY IV No. Examined	62	50	61	62	56
- Adenoma	-	-	1	-	-

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control=01,02,
Group 01, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 02, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 03, 10 MG/KG, males: BSF 208075 (10 mg/kg)
Group 04, 30/20 MG/KG, males: BSF 208075 (30 mg/kg)
Group 05, 60/40/0 MG/KG, males: BSF 208075 (60 mg/kg)

PATHOLOGY REPORT PAGE PAT: 43/1732
SUMMARY TABLES : 851465

TEST ARTICLE : BSF 208075 PATHOL. NO.: 10027 HJC
TEST SYSTEM : RAT, 104 WEEKS, ORAL DATE : 02-NOV-06
SPONSOR : MYOGEN PathData@System V6.2b5

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0), Incl. Deaths

Sex	Males				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	62	50	62	62	62
ORAL CAVITY No. Examined	62	50	62	62	61
- Squamous cell papilloma	-	-	1	-	-
ZYMBAL'S GLANDS No. Examined	48	41	54	49	41
- Zymbal's gland adenoma	-	-	-	1	-
- Zymbal's gland carcinoma	1	-	1	-	-
BODY CAVITIES No. Examined	4	4	7	7	1
- Hemangioma	-	-	-	1	-
- Hemangiosarcoma	-	-	-	1	-
- Lipoma	-	-	-	1	-
- Malignant Schwannoma	-	1	-	-	-
- Osteosarcoma	-	-	-	1	-
- Myxosarcoma	-	-	1	-	-
- Malignant mesothelioma	-	-	-	1	-
LYMPH NODES No. Examined	4	1	5	1	3
- Hemangioma	-	-	2	-	-
- Metastasis of carcinoma	-	-	2	-	-
PREPUTIAL GLANDS No. Examined	-	1	-	-	-
- Sebaceous adenoma	-	1	-	-	-
PARANASAL SINUSES No. Examined	62	50	60	62	57
- Malignant neurinoma	2	-	-	-	-
- Squamous cell carcinoma	-	-	1	-	-

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control=01,02,
Group 01, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 02, 0 MG/KG, males: BSF 208075 (0 mg/kg)
Group 03, 10 MG/KG, males: BSF 208075 (10 mg/kg)
Group 04, 30/20 MG/KG, males: BSF 208075 (30 mg/kg)
Group 05, 60/40/0 MG/KG, males: BSF 208075 (60 mg/kg)

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PATHOLOGY REPORT PAGE PAT: 44/1732
SUMMARY TABLES : 851465

TEST ARTICLE : BSF 208075 PATHOL. NO.: 10027 HJC
TEST SYSTEM : RAT, 104 WEEKS, ORAL DATE : 02-NOV-06
SPONSOR : MYOGEN PathData®System V6.2b5

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (K0), Incl. Deaths

Sex	Females				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	62	50	62	62	62
LIVER No. Examined	61	50	56	57	53
- Contact metastasis	-	-	-	1	-
- Adenoma: hepatocellular	-	1	-	1	-
- Cholangioma	-	1	-	-	-
MESENT. LYMPH NODE No. Examined	61	49	56	53	39
- Hemangioma	2	-	-	-	-
- Hemangiosarcoma	-	1	-	-	-
- Plasmocytoma	-	-	-	-	1
HEMOLYMPHORET. SYS. No. Examined	62	50	55	56	55
- Malignant lymphoma (not otherwise specified)	1	-	-	-	1
- Histiocytic sarcoma	1	-	-	-	-
STOMACH No. Examined	61	49	55	54	46
- Contact metastasis	-	-	-	-	1
LUNG No. Examined	62	50	54	57	54
- Metastasis of carcinoma	-	-	-	1	1
- Metastasis of sarcoma	-	-	-	-	1
THYMUS No. Examined	62	50	55	52	51
- Benign thymoma	8	3	9	2	3
- Malignant thymoma	2	-	-	1	2
OVARIES No. Examined	61	49	58	54	44
- Benign granulosa cell tumor	6	4	6	3	4
- Benign thecoma	-	1	1	-	-
PAROTID GLANDS No. Examined	-	1	-	-	-
- Adenocarcinoma	-	1	-	-	-
PANCREAS No. Examined	61	49	55	54	42
- Contact metastasis	-	-	-	1	1
- Islet cell adenoma	3	4	3	-	-
- Islet cell carcinoma	-	-	1	-	-
MANDIB.LYMPH NODES No. Examined	60	48	56	56	49
- Metastasis of carcinoma	-	1	-	-	-
THYROID GLAND No. Examined	60	50	54	56	51
- C-cell adenoma	5	5	3	1	- *
- Follicular cell adenoma	1	3	5	-	-
ADRENAL MEDULLAS No. Examined	62	49	57	57	54
- Benign pheochromocytoma	1	-	2	-	1
- Malignant pheochromocytoma	1	1	-	-	-

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control=01,02,
Group 01, 0 MG/KG, females: BSF 208075 (0 mg/kg)
Group 02, 0 MG/KG, females: BSF 208075 (0 mg/kg)
Group 03, 10 MG/KG, females: BSF 208075 (10 mg/kg)
Group 04, 30/20 MG/KG, females: BSF 208075 (30 mg/kg)
Group 05, 60/40/0 MG/KG, females: BSF 208075 (60 mg/kg)

PATHOLOGY REPORT
SUMMARY TABLES

PAGE PAT: 45/1732
: 851465

TEST ARTICLE : BSF 208075
TEST SYSTEM : RAT, 104 WEEKS, ORAL
SPONSOR : MYOGEN

PATHOL. NO.: 10027 HJC
DATE : 02-NOV-06
PathData@System V6.2b5

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL SACRIFICE GROUP (KO), Incl. Deaths

Sex	Females				
Dose Group	01	02	03	04	05
No. Animals per Dose Group	62	50	62	62	62
SKIN/SUBCUTIS No. Examined	60	50	58	56	52
- Squamous cell papilloma	-	1	-	-	-
- Squamous cell carcinoma	-	-	-	1	-
- Keratoacanthoma	-	-	1	1	-
- Basal cell carcinoma	-	-	-	1	-
- Sebaceous cell adenoma	-	-	1	1	-
- Malignant Schwannoma	-	-	-	1	-
MAMMARY GLAND No. Examined	61	49	57	57	48
- Fibroadenoma	18	18	11 *	11 *	4 **
- Adenoma	2	1	-	-	-
- Adenocarcinoma	1	2	7 *	1	1
CEREBRUM No. Examined	62	50	55	56	56
- Oligodendroglioma	1	-	-	-	-
UTERUS No. Examined	61	49	59	58	45
- Stromal polyp	5	7	11	7	4
- Adenoma	-	-	2	1	1
- Adenocarcinoma	1	1	2	1	-
- Hemangioma	-	-	1	-	-
- Squamous cell carcinoma	-	1	-	-	-
- Stromal cell sarcoma	-	-	1	-	-
CERVIX No. Examined	61	49	56	56	45
- Fibroma	-	-	1	-	-
- Stromal polyp	2	-	-	-	-
- Stromal cell sarcoma	-	1	-	-	-
- Malignant Schwannoma	-	-	-	-	1
- Squamous cell carcinoma	-	-	1	-	-
EXORBITAL LACR.GLDS.No. Examined	61	49	54	56	49
- Hemangioma	-	-	-	-	1
PITUITARY GLAND No. Examined	61	50	57	59	54
- Adenoma of pars distalis	31	30	34	27	12 **
ZYMBAL'S GLANDS No. Examined	36	25	51	49	34
- Contact metastasis	-	-	-	1	-
BODY CAVITIES No. Examined	5	2	3	3	2
- Neurofibroma	-	-	-	1	-
- Contact metastasis	-	-	-	1	1
LYMPH NODES No. Examined	1	1	2	3	1
- Metastasis of sarcoma	-	-	-	-	1
- Metastasis of carcinoma	-	-	1	1	-

One-Sided Exact Fisher Test: *) p<=0.05; **) p<=0.01; Control=01,02,
Group 01, 0 MG/KG, females: BSF 208075 (0 mg/kg)
Group 02, 0 MG/KG, females: BSF 208075 (0 mg/kg)
Group 03, 10 MG/KG, females: BSF 208075 (10 mg/kg)
Group 04, 30/20 MG/KG, females: BSF 208075 (30 mg/kg)
Group 05, 60/40/0 MG/KG, females: BSF 208075 (60 mg/kg)

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/s/

William Link
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PHARMACOLOGIST

Albert Defelice
5/1/2007 03:30:10 PM
PHARMACOLOGIST