2.6.4.10 Tables and figures to include comparative TK summary

The following studies are reviewed in the Toxicology section (Section 2.6.6) including repeat-dose toxicity (2.6.6.3) and reproductive and developmental toxicology (Section 2.6.6.6) studies. These studies are the following: 6-month repeat dose in rats (Study No. DS03260/930011282), intravenous study of fertility and early embryonic development in rats (Study No. DN0128/930000931), intravenous study of embryo-fetal development in rats (DN01009/930000517), intravenous study in rabbits (ND01014/930000804), and 9-month repeat dose study in dogs (DS03196/930012300).

Species (Duration)	Dose (mg/kg)	Sex	Cmax (ng/mL) range		AUC_{0-t} (ng h/mL) range		Ratio of animal to human exposure
			Day 1	End of study	Day 1	End of study	AUC
Rat Single dose	10	M	6422	NA	3864	NA	1.6
		F	8384	NA	8156	NA	3.4
	25	M	19066	NA	11980	NA	5.0
		F	20524	NA	28476	NA	12.0
	30	M	24414	NA	19269	NA	8.0
		F	25054	NA	34563	NA	14.3
Rat 6-month	0.7	M	105	338	351	400	0.2
		F	118	473	400	455	0.2
	3.0	M	1801	2215	1165	1701	0.7
		F	1788	1748	1906	1490	0.6
	6.7	M	5532	6299	4055	5051	2.1
		F	1680	2209	2910	3303	1.4
Rat Non-pregnant females (GD 6-15)	0.02	M	8.2	2.5	NC	NC	NC
		F	NA	3.8	NA	NA	NA
	0.06	M	12	8.7	14.0	29.0	0
		F	13.8	8.6	15.6	34.4	0
	0.2	M	102	157	136	181	0.07
		F	106	70	170	153	0.06
Rat Pregnant females (GD 7-19)	0.02	F	3.4	5.1	NC	NC	NC
	0.08	F	19	13.6	NA	41.7	0.02
	0.3	F	134	209	192	267	0.11
Rabbit Pregnant females (GD 7-19)	0.01	F	NC	2.2	NC	NC	NC
	0.03	F	3.2	6.4	NC	10.6	0
	0.11	F	6.2	13.8	NC	78.2	0.03
	0.3	F	25.5	NA	21.8	NA	NA
Dog Single dose	0.5	M	218	NA	258	NA	0.10
		F	-	NA	-	NA	-
	5	M	5118	NA	6925	NA	2.9
		F	-	NA	-	NA	-
Dog 9-month	0.1	M	26a	104	26	62a	0.02
		F	55	171	NC	119b	0.05
	0.5	M	366	399	351	389	0.2
		F	453	337	338	309	0.1
	0.75	M	NA *	1394	NA	1075	0.4
		F	NA	1085	NA	816	0.3
	0.9	M	1582	NA	1175	NA	NA
		F	891	NA	780	NA	NA

- Human exposure AUC value for Q21 days IV is 2406 ng hr/mL.
- For all studies, ratio of animal to human exposure was calculated from end of study AUC values except single dose studies which were taken from Day 1 AUC values.
- NC = not calculated due to plasma concentrations being < LLOQ.(<2.0 ng/mL)

- NA = not available
- [a] n = 2 animals
- [b] n = 1 animal

Single dose dog study:

- Mean toxicokinetic parameters are combined across gender (n=4) in dog study.

6-month rat study:

- Systemic exposure after intermittent (q3w) dosing was approximately dose proportional.
- Cmax values from males at the HD (6.7 mg/kg) were 3-fold higher compared to females on Days 1 and 190.
- Mean $T_{1/2}$ were not provided by the Sponsor.

9-month dog study:

- Due to excessive toxicity at the 0.9 mg/kg dose, the high-dose was lowered to 0.75 mg/kg. Day 1 reflects the 0.9 mg/kg dose.
- Mean $T_{1/2}$ were not provided by the Sponsor.
- Systemic exposure was more than dose proportional.
- No accumulation of exposure was observed after the intermittent dosing schedule.
- There were no apparent gender-related differences.

Rabbit study:

- Cmax and AUC values taken during GD7 and GD19.
- Animals at 0.3 mg/kg/day were only dosed on GD7.
- AUC was not calculated since either none or only one of two quantifiable data point(s) were observed (all remaining time points were <LLOQ).

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See section 2.6.4.10

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The general toxicology of ixabepilone has been examined in rats and dogs using the IV and oral routes of administration. Single dose studies were conducted in the rat and dog using the IV and PO routes. Primary toxicities were identified in these studies and toxic doses of ixabepilone were reached. Findings included effects on GI, hematopoietic and male reproductive. Repeat-dose toxicity was examined in the rat and dog using the IV route.

In the pivotal six month rat study (20 rats/sex/group for the control and HD and 10 rats/sex/group for the low and mid-dose), ixabepilone was administered once-every 21 days for 6 months at doses of 0, 0.7, 3, and 6.7 mg/kg/day. Three (3) satellite groups consisting of 9 rats/sex/group were utilized for toxicokinetic (TK) evaluations on Days 1, 106, and 190. Rats were dosed at the same volume and dosing regimen as the main study treatment groups. All rats (with the exception of HD) received a total of 10 doses (Q21xD10 on Days 1, 22, 43, 64, 85, 106, 127, 148, 169, and 190). Terminal and recovery necropsies were conducted on Days 196 and 225, respectively.

Due to severe limb dysfunction and poor physical condition at the HD (6.7 mg/kg), these animals did not receive the scheduled dose on Day 85 to allow additional time for recovery from toxicity. Therefore, animals in this dose group received a total of only 9 doses throughout the study. Drug related mortalities occurred in 1 M at the at the MD (Day 113) and 10 males and 13 females at the HD (Days 75 to 195 for males and

Days 7 to 196 for females). During the 4-week recovery period, mortality occurred in 4 F at the HD.

Drug related clinical findings observed for animals that died prior to study termination and/or euthanized *in extremis* included thin appearance, decreased activity, hunched posture, audible, and difficult breathing, black material around the eyes and mouth, moribundity, skin cold to touch, impaired limb function, and/or loss of righting reflex. The cause of death in the one male at the MD and 4 males and 2 females at HD was inflammation/septicemia that was secondary to drug-related immunosuppression (bone-marrow hypocellularity) and/or GI toxicity. According to the Sponsor, the cause of death of the remaining animals could not be definitively determined. However, macroscopic and microscopic evaluation showed these animals had toxicity compatible with immunosuppression (thymic and bone-marrow atrophy) and/or compromise of the GI mucosal barrier. For all remaining rats at the MD and HD, clinical signs included impaired righting reflex and limb function, lacrimation, thin appearance, hunched posture, red or black material around the eyes and/or the nose, cold to touch, tail swelling/discoloration, sparse or absent hair, brown and/or yellow hair discoloration (in females). Additional clinical signs at the HD included splayed limbs, loss of righting reflex, swelling of the face and/or limbs, tail rigid and portions missing, decreased activity, unkempt appearance, and slow breathing. At the HD, splayed limbs were first noted around Week 5 and progressed to impairment of limb function and righting reflex. Most clinical findings at the MD and HD were not apparent by the end of the recovery period with the exception of scabs and skin redness on the tail, sparse hair coat and black material around the eyes.

Reversible decreases in body weight were observed in MD males (92% of control at Week 26) and HD males and females (68-76% of control at Week 26). Partially reversible decreases in food consumption were seen in HD males and females (68-86% of control at Week 25).

Hematology and clinical chemistry findings were seen mostly at the MD and HD groups. These included a reversible decreases in LEU (mainly LYMPH and NEUT), EOS, MONO, and RETIC at 5 days post-dose. There was reversible decreases in TP, TRIGLY, and CHOL and reversible to partially reversible decreases in CREA and ALB. Partially reversible increases in AST and ALT were also observed at Day 111 and termination. No drug-related effects on coagulation or urinalysis were observed at any dose levels.

As with clinical pathology findings, macroscopic pathology findings were limited mostly to MD and HD rats. Drug-related findings were dose related in incidence and severity and included atrophy of testes and thymus and ulceration/scabs of the injection sites. At the HD group there was an enlargement of spleen and mandibular lymph nodes, atrophy of epididymis and seminal vesicles, and erosions of the glandular stomach. Findings at the injection site, glandular stomach and thymus in HD animals were less severe at the end of the recovery period.

Due to mortality in HD rats during the treatment phase (no organ weights were available for HD rats at the terminal necropsy), organ weight changes were limited to LD and MD animals only. Organ weight changes at the MD included a decrease in absolute and relative weights of the thymus (34-36%), testes (60%), and uterus (42%). These

findings were also present during the recovery phase with the addition of a decrease in prostate gland weight in HD recovery animals (62%).

Histopathology findings in HD animals that died or were euthanized *in extremis* during included decreased ossification of the femoral growth plate; axonal/myelin degeneration in cervical, thoracic, and lumbar spinal cord; atrophy and myofiber degeneration/necrosis in skeletal muscle (which was considered to be secondary to severe limb dysfunction); acinar atrophy of the salivary glands; bilateral atrophy of seminal vesicles; and acute to chronic active inflammation of injection sites.

Drug-related histopathology findings at the end of treatment at the MD and HD animals were dose related in incidence and severity and included cellular depletion and regenerative hyperplasia in bone marrow; thymic atrophy; lymphoid depletion and necrosis of lymphoid tissues; increases in extramedullary hematopoiesis in the spleen; axonal/myelin degeneration in sciatic nerve; single-cell necrosis and reactive hyperplasia of the glandular mucosa of the gastrointestinal tract; atrophy of testes (severe with degeneration), epididymis, prostate gland (with inflammation), uterus and vaginal epithelium; and single-cell necrosis in female mammary glands.

During the recovery phase, drug-related changes at the MD were still present in the same organs at terminal necropsy, however, there was a decreased incidence and severity indicating a partial recovery with the exception of testis and peripheral nerves. In HD animals, however, the incidence and severity of toxicity of some organs were still present by the end of the recovery period. Affected organs included bone and bone marrow, lymphatic organs, sciatic nerve, spinal cord, cervical and lumbar dorsal root fibers, skeletal muscle, glandular and nonglandular stomach, glandular mucosa of the gastrointestinal tract, mandibular and parotid salivary glands, testes (also at MD), epididymis (also at MD), seminal vesicles, prostate gland, uterus, vagina, and injection site.

In the pivotal nine month dog study (6 dogs/sex/group for the Control and HD and 4 dogs/sex/group for the MD and LD), ixabepilone was administered once-every 21 days for 6 months at doses of 0, 0.1, 0.5, and 0.9 mg/kg/day. Dogs received a total of 14 doses (Q21xD14 on Days 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, 232, 253, and 27). Terminal and recovery necropsies were conducted on Days 281 and 302, respectively. Due to severe toxicity that occurred after the first dose which resulted in the deaths of 3 HD dogs (1 M on Day 6, 1 M on Day 7 and 1 F on Day 4), these animals were replaced and dosed at a reduced dose of 0.75 mg/kg throughout the study starting on Day 22. An additional death occurred in one male dog (Day 7) at the reduced high dose of 0.75 mg/kg. This animal was not replaced.

As stated, mortality occurred in 2 M and 1 F at the 0.9 mg/kg dose and 1M at the 0.75 mg/kg dose. Cause of death for the 1 M at the 0.9 mg/kg was related to aspiration pneumona characterized by necrosis, hemorrhage, inflammation, and bacterial colonization in the lungs, tonsillar necrosis, and associated dysphagia. In the other male dogs, the cause of death was associated with minimal single-cell necrosis of the tubular epithelium of kidneys and lesions in the GI. The cause of death in the female dog was related to severe thymic atrophy and lesions in the GI. And finally, the cause of death for the one male dog at the revised dose of 0.75 mg/kg dose was related to necrotizing pneumonia, secondary to bone-marrow hypocellularity and severe tonsillar necrosis and dysphagia.

For all other animals, transient clinical signs occurred at all dose levels (including Control animals) during or shortly after dosing. They included the following: increased and decreased activity; tremors; tonic convulsions (1 F only); salivation; vocalization; emesis; soft and/or mucoid feces; swelling of the feet and/or limbs; red discoloration of the gums; skin warm to touch; and red discoloration of the skin (erythema). The Sponsor concluded that these findings were attributed to the Cremophor vehicle since findings occurred during or shortly after dosing and have been previously been reported in other studies in which Cremophor was part of the vehicle. Most of the clinical findings, however, were reversible during the recovery period. Other clinical signs that were not vehicle related at the 0.5 and 0.75 dose groups included sparse hair and umkempt appearance in females, red or yellow feces, few/absent feces, and yellow material in the pan in both M and F. These findings were also reversible.

A transient decrease in body weights in all groups following dose administration was also observed. A dose related, reversible decrease in food consumption was observed in males at both the mid and high dose. Food consumption values decreased in HD females only.

Hematology and clinical chemistry findings were seen mostly at the mid and revised high-dose groups (0.5 and 0.75 mg/kg, respectively). These included a reversible decrease in LEU counts (primarily NEUT, MONO, and EOS) at 5-days after dosing. A reversible decrease in ERYTHRO, HGB, BASO (M only), and RETIC, were also seen in HD M and F. A non-significant increase in fibrinogen was also seen in both M and F 5-days post-dose. In addition, reversible increases in ALT and decreases in TP and K were observed in HD M and F.

Organ weights changes were limited to HD male animals. They included decreases in absolute and relative weights of testes and epididymis. These changes were still present at the end of the recovery phase. Other changes included a reversible decrease in absolute and relative thymus weights in both HD males and females.

Histopathology findings in both M and F at the MD and HD included: minimal reactive hyperplasia in the gallbladder epithelium; minimal to mild increases in extramedullary hematopoiesis in spleen; and slight Kupffer cell pigmentation in the liver. All these findings were reversible. Other findings included a partially reversible, dose-related (incidence and/or severity) minimal to severe cell depletion and granulocytic to mixed hyperplasia of bone marrow. Minimal to moderate degeneration/atrophy of the seminiferous tubules of the testes; minimal to severe, dose-related oligospermia/germ cell debris with single-cell necrosis and reactive hyperplasia of the ductal epithelium in the epididymis.

Histopathology findings specifically at the HD include the following: increased severity of thymic atrophy with lymphoid depletion in spleen, mandibular, mesenteric and tracheobronchial lymph nodes, gut-associated lymphoid tissue (GALT), and nictitans glands; minimal increases in extramedullary hematopoiesis in adrenal glands; enteropathy (including single-cell necrosis and reactive hyperplasia) of the intestinal tract and minimal to mild axonal/myelin degeneration in nerves at injection sites. All findings with the exception of axonal/myelin degeneration in nerves at the injection site and changes in the epididymis were reversible and/or partially reversible at the end of the recovery period.

Genetic toxicology:

Ixabepilone was tested for mutagenicity and clastogenicity in the *in vitro* Ames test and cytogenetics study in primary human lymphocytes and in the *in vivo* rat bone marrow micronucleus assay. Ixabepilone was not mutagenic in an Ames reverse bacterial mutagenicity assay using *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537 and *Escherichia coli* strain WP2uvrA at concentrations up to 5000 µg/plate. Ixabepilone, in the absence or presence of S-9 metabolic activation, was not clastogenic to dividing human lymphocytes at concentrations ranging from 250 to 2000 µg/mL. However, ixabepilone did induce an increase incidence of polyploid lymphocytes at concentrations of 1000 and 2000 µg/mL.

In the *in vivo* rat micronucleus assay, 3 daily IV doses of ixabepilone induced micronuclei at doses of ≥0.625 mg/kg/day suggesting an effect on the mitotic spindle, consistent with the *in vitro* findings in human lymphocytes and its mechanism of action.

Carcinogenicity:

Two-year carcinogenicity studies with ixabepilone were not conducted.

Reproductive toxicology:

Fertility studies in the rat were conducted with ixabepilone treated at 0.02, 0.06, and 0.2 mg/kg/day. Treated males were mated with treated females. Females were dosed for 2 weeks prior to mating through GD 7, and were Cesarean-sectioned and evaluated for pregnancy on GD 16. Males were dosed for 2 weeks prior to mating until the scheduled necropsy (45 daily doses). No effects were seen on mating and fertility when rats were treated prior to and throughout breeding. Mating and fertility indexes were comparable to controls. No overt paternal toxicity occurred at LD and MD, but paternal toxicity (decreased body-weight and food consumption) was observed at the HD of 0.2 mg/kg.

When the female rats were dosed with ixabepilone (0.02, 0.06, and 0.2 mg/kg) during breeding and through the first seven days of gestation, maternal toxicity (decreased body-weight, body weight gain, and food consumption) was observed at the HD of 0.2 mg/kg. No changes in mating or fertility indexes were observed. However, ixabepilone did have an effect on fertility and early embryo development. At the HD, there were significant changes on corpora lutea (decrease from 16.8 to 13.0), number of implantations (decrease from 15.1 to 6.6), pre and post implantation loss (increase from 9.4 to 47.6 and 5.2 to 80.3, respectively), and number of viable embryos (decrease from 14.3 to 0.6). In addition, there was a dose-related increased incidence of embryo lethality, as evidenced by an increase in resorptions, at the MD and HD.

The pivotal rat embryo-fetal development study (0.02, 0.08, and 0.3 mg/kg) did not show any teratogenic effects of ixabepilone. Maternal (body weight loss and decreased food consumption) and embryo-fetal (resorptions, decreased fetal bodyweights, and reduced ossification of caudal vertebrae, sternebrae, and metacarpals) toxicity occurred at the high-dose of 0.3 mg/kg dose.

In the pivotal embryo-fetal development rabbit study (0.1, 0.03, and 0.11 mg/kg), there was a lack of drug-related toxicity at the high dose of 0.11 mg/kg. Therefore, 2 additional groups of pregnant rabbits were evaluated at doses of 0 and 0.3 mg/kg. Maternal (deaths) and embryo-fetal (resorptions) toxicity occurred at 0.3 mg/kg dose.

This dose also caused a marked increase in resorptions in litters including 10 litters consisting of resorbed conceptuses. Since all does died at the 0.3 mg/kg dose, no information was available for the C-section data, litter observations, and offspring data. As to maternal toxicity, it was evident in the significantly decreased body weight loss and food consumption seen at the 0.3 mg/kg dose group through the dosing period. No drug-related findings of malformations were seen in the offspring at doses of 0.1, 0.3 and 0.11 mg/kg.

Special toxicology: No studies reviewed

2.6.6.2 Single-dose toxicity:

Main findings in single dose rat studies:

- Oral study at doses of 0, 20, 45, 75, and 100 mg/kg resulted in dose-dependent mortality at ≥20 mg/kg.
- IV study at doses of 0, 10, 25, and 30 mg/kg resulted in dose-dependent mortality at ≥ 25 mg/kg.
- In both studies, drug related toxicities first appeared 2 to 3 days following dose administration.
- In both studies, target organs of toxicity included: GI, hematopoietic (bone marrow), lymphoid, and peripheral-nervous and male reproductive system.
- In IV study, mortality occurred on Days 1 and 5-9 at MD (25 mg/kg) and Days 5-13 at HD (30 mg/kg).
- In IV study, morbundity and death were attributed to drug-related depletion of the bone marrow and lymphoid organs, and toxic enteropathy.
- In oral study, GI and lymphoid organ toxicity was related to mortality.

Main findings in single dose dog studies:

- Single oral dose at 0, 0.5, and 2.5 mg/kg. Dose of 2.5 mg/kg was toxic with severe GI toxicity and death.
- Single IV dose at 0, 0.5, and 5 mg/kg. Dose of 5 mg/kg was toxic with severe GI toxicity and all dogs were sacrificed moribund on Day 3.
- In both studies, target organs of toxicity included: GI and hematopoietic.
- GI was considered the major cause of moribundity.

2.6.6.3 Repeat-dose toxicity

Main findings in 5-day IV rat study:

- Rats received daily doses of BMS-247550 at 0, 0.25, 0.5, 1, 2, and 4 mg/kg.
- Mortality at doses ≥ 1 mg/kg.
- The 0.5 mg/kg dose showed reversible changes in body weight, food consumption, clinical chemistry parameters, and organ weights.
- Clinical signs appeared 2-3 days after dosing and females were more affected than males.
- Target organs of toxicity included GI, hematopoietic/lymphoid tissues and male reproductive system.

Main findings in 2-week IV rat study:

- Rats received 14 daily doses of BMS-247550 at 0, 0.5, 0.12, and 0.3 mg/kg/day.
- No mortality
- Clinical findings at the high dose appeared during week 2 and included decreases in body weight, food consumption and changes in hematology clinical chemistry parameters.
- Mild clinical toxicities were observed at all other dose levels and female rats were more severely affected compared to male rats.
- Target organs of toxicity were similar to other studies and included the hematologic, lymphoid, GI, hepatic, and male reproductive organs.
- Additional finding of growth plate lesions characterized by loss of bony trabeculae on the diaphyseal surface of the femoral growth plate was observed in HD females.

Main findings in 1-month intermittent-dose (QWx5) IV rat study:

- Rats received BMS-247550 doses of 0, 0.1, 1 and 2 mg/kg.
- No mortality.
- Most treatment-related changes occurred in after 1-week of treatment and were limited to MD and HD groups.
- Clinical signs included partially reversible decreases in body weights, body-weight gains, and food consumption, and, in females, increased food consumption during the recovery period.
- Hematopoietic effects included reversible dose-related decreases in RCM, HEM, LYMPH, RETIC, and bone marrow and lymphoid depletion.
- Target organ toxicity included reversible hematopoietic/lymphoid, GI, hepatic, peripheral neuropathy, and delayed testicular toxicity.

Main findings in 5-day IV dog study:

- Dogs received daily doses of BMS-247550 at 0, 0.015, and 0.15 mg/kg
- Clinical findings at the HD included alopecia and single-cell necrosis of the gall bladder epithelium.

Main findings in 2-week IV dog study:

- Dogs received daily doses of BMS-247550 at 0.05, 0.09, or 0.15 mg/kg.
- No mortality.
- Clinical toxicity occurred at HD during Week 2 and included body-weight loss and decreased food consumption.
- Drug-related microscopic changes were dose related in incidence and severity, and included single-cell necrosis of the epithelium of the gallbladder, renal tubules, prostatic urethra and epididymis, and degeneration of the seminiferous tubules of the testes.

Main findings in 1-month intermittent-dose (QWx5) IV dog study:

- Dogs received BMS-247550 doses of 0, 0.1, 0.4, or 0.75 mg/kg.
- No mortality.
- Clinical findings included inappetence, prostration, thin appearance, few/absent or black feces, hunched posture, and partially reversible decreases in body weights and food consumption.
- Target organs of toxicity included hematopoietic/lymphoid, gastrointestinal, and liver.

Study title: Six-month Intermittent-dose (Q21Dx10) intravenous toxicity study in rats

Key study findings:

- The high-dose of 6.7 mg/kg caused severe toxicity and mortality throughout the study and recovery period.
- Drug related mortalities occurred in 1 M at MD (3 mg/kg) and 14 M and 13 F at HD (6.7 mg/kg).
- Major target organ toxicities included bone marrow, lymphoid tissue, gastrointestinal tract, peripheral nerves, and male and female reproductive organs.
- Most organ findings were partially or completely reversible with the exception of testis and peripheral nerves at the mid-dose while most changes were not recoverable at the high-dose of 6.7 mg/kg.

Study no.:	DS03260
Volume #, and page #:	Module 4.2.3.2
Conducting laboratory and location:	
Date of study initiation:	September 8, 2006
GLP compliance:	yes
QA report:	yes (X) no ()
Drug, lot #, and % purity:	BMS-247550 Lot No.: BMS-247550 Purity:
Formulation/vehicle:	40% polyethylene glycol 300 5% BMS-purified (cleaned) Cremphor®EL 5% ethanol 50% (v/v) 50 mM phosphate buffer

Dose justification: See single and repeat dose studies

Methods (Unique study design or methodology):

- Terminal and recovery necropsies were conducted on Days 196 and 225, respectively.
- Due to severe toxicity at the HD, animals in this group did not receive the drug dose on Day 85 to allow additional time for recovery of toxicity. Therefore, these animals received a total of 9 instead of 10 doses

Dosing:

Species/strain:	HSD: Sprague Dawley (SD) rat
#/sex/group or time point (main study):	10/sex/dose (10 sex/dose for recovery in Cont. and HD)
Satellite groups used for toxicokinetics:	9/sex/dose
Age:	8 weeks
Weight:	M: 175-350 g/F: 150-175 g
Route, formulation, volume, and infusion rate:	IV bolus (tail vein) Dose volume of 5 mL/kg Infusion rate of 2 mL/min
Doses in administered units:	0, 0.7, 3, 6.7 mg/kg/day
Schedule:	q21d x 10 on D 1, 22, 43, 64, 85, 106, 127, 148, 169, and 190

Observations and times:

Mortality:	Twice daily during pretest, treatment, and recovery
Clinical signs:	Once/week during the study
Body weights:	At least once pretest; once prior to dosing, and weekly during treatment and recovery
Food consumption:	Weekly during treatment and recovery
EKG	Not conducted
Ophthalmoscopy:	Once pretest, during Week 13, and prior to terminal (Day 195) and recovery (Day 224) necropsies.
Hematology:	Days 48, 63, 111, 126, 153, 168, 195 (terminal) and 224 (recovery)
Clinical chemistry:	Days 111, 195 (terminal) and 224 (recovery)
Coagulation	Prior to terminal (Day 195) and recovery (Day 224) necropsies
Urinalysis:	Days 111, 195 (terminal) and 224 (recovery)
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Days 195(terminal) and 224 (recovery).
Organ weights:	Days 195 (terminal) and 224 (recovery)
Histopathology:	All dose groups (terminal and recovery) Adequate Battery: yes (X), no ( )—explain Peer review: yes (X), no ( ) See Histopath Table, tissues examined in the control and high dose. Tissues in low and mid dose were evaluated to determine the no effect level.
Toxicokinetics:	1 min, 1, 3, 6, 9, 12, and 24 hours post-dose on Day 1, 106, and 190.

Results

Mortality:

Gender	Male		Female
Dose (mg/kg/day)	3	6.7	6.7
Treatment			
No. of animals	10	20	20
No. of deaths	1	14[a]	13
Day of study	113	75-195	7-196
Recovery			
No. of animals	9	6	7
No. of deaths	-	1[a]	4
Day of study	-	224	197-201, 213

[a]= Four males (drug treatment) and 1 M (recovery) died during blood collection.

Clinical signs – **Main Study**:

Index	No. of animals affected							
Gender	**Males**				**Females**			
Dose (mg/kg/day)	0	0.7	3	6.7	0	0.7	3	6.7
No. of animals	20	10	10	20	20	10	10	20
Weeks 1-28								
Animal husbandry								
– Teeth broken			3	5				
Behavior activity								
– Activity decreased	1		1	3				2
– Salivation	1			1			1	1
– Righting reflex impaired			1	2				3
– Righting reflex lost	1			3				1
Excretion								
– Feces soft	1	1		3				
– Material in pan/bedding, red				3			1	

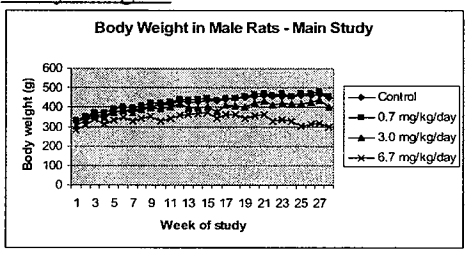
External appearance								
– material around eyes, black	2		6	3			8	3
– material around nose, black				11			5	15
– material around nose, red						1	3	4
– posture hunched			1	6				4
– swelling		1	1	8				4
– tail missing – portion				8			1	4
– thin	1		1	2			1	10
– lacrimation					1		3	
– limb function impaired				18				16
– limbs splayed								13
Pelage/skin				4				
– hair sparse			1	18			10	12
– hair discolored, yellow							6	12
– scabbed area	1	2	3	13			2	14
– skin cold to touch			1	3				4
– skin discolored, red		1	3	11				9
– unkempt appearance				3			2	2
Respiration								
– breathing, slow				2				2

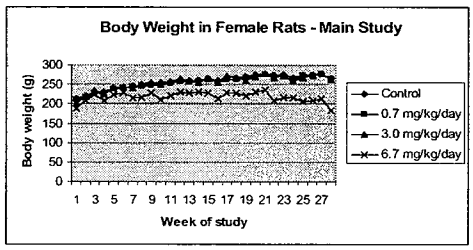
Clinical signs – **Recovery**:

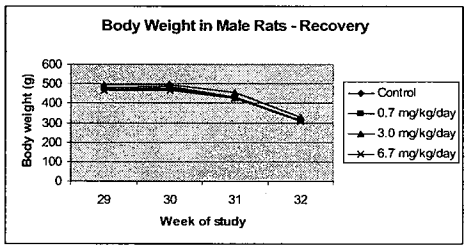
Index	No. of animals affected				
Gender	**Males**			**Females**	
Dose (mg/kg/day)	0.7	3.0	6.7	3.0	6.7
No. of animals	5	5	6	5	7
Weeks 29-32					
Animal husbandry					
– teeth broken			2		
Behavior activity					
– salivation			1		
– activity deceased					1
– righting reflex right					1
Excretion					
– material in pan/bedding, red			1		
– urine discolored, red			1		

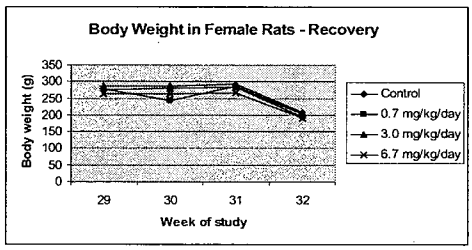
External appearance					
– limb function impaired			5		6
– limbs splayed			1		2
– material around nose, black					1
– posture hunched			1		1
– swelling			1		1
– tail missing – portion			3		2
– thin					3
Pelage/skin					
– hair discolored, brown			1		1
– hair discolored, yellow			1		2
– hair sparse		1	5		3
– scabbed area	1	2	3		4
– skin discolored, red	1	1	1		1
– unkempt appearance			1		1
Eye/ocular					
– eye discolored, cloudy				1	1
– eye swollen				1	1

Body weights:



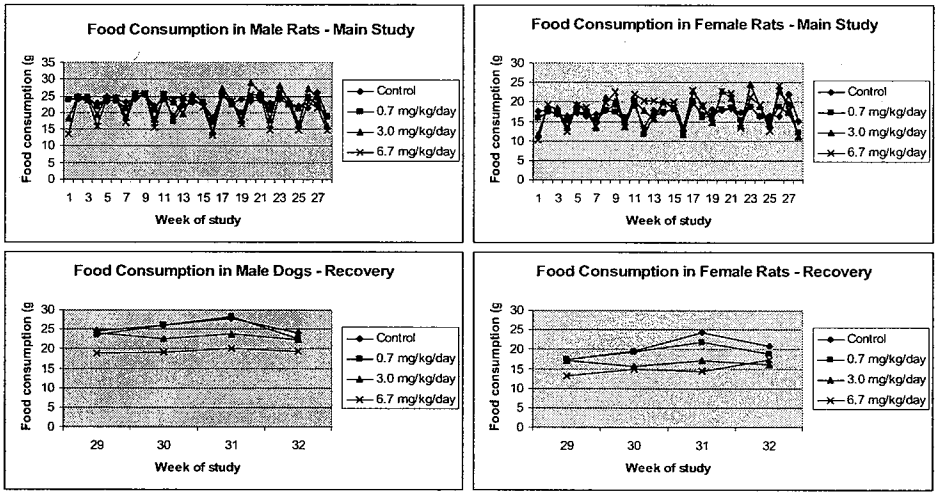






Appears This Way
On Original

Food consumption:



Ophthalmoscopy: unremarkable

EKG: unremarkable

Hematology:

Index	% Control					
Gender	**Males**			**Females**		
Dose (mg/kg/day)	0.7	3	6.7	0.7	3	6.7
Day 48 (5 D after 3rd injection)						
No. of animals	5	5	5	5	5	5
LEU						-53**
RETIC		-47*	-64**	+44**	-83**	-89**
NEUT		-78**	-95**		-91**	-94**
LYMPH			-61**			-48**
MONO		-66*	-61*			
EOS		-66**	-88**			
BASO			-60*			
Day 63 (1 D before 4th injection)						
No. of animals	5	5	5	5	5	5
LEU		-24*	-28*			+73**
LYMPH			-41**			
NEUT					+73*	+484**
MONO						+242**
EOS						+312**

Day 111 (5 D after 6th injection)						
No. of animals	10	9	19	10	9	12
LEU		-56**	-61**		-34**	-68**
RETIC		-40*	-62**		-65**	-76**
NEUT		-90**	-93**		-92**	-91**
LYMPH		-48**	-55**			-65**
MONO		-85**	-81**		-78**	-86**
EOS			-85**			
Day 126 (1 D before 7th injection)						
No. of animals5	5	5	5	5	5	5
RETIC		+45*	+46*			
NEUT			+528*			
LYMPH			-35**			
EOS		+110*				
Day 153 (5 D after 8th injection)						
No. of animals	5	5	5	5	5	5
LEU			-62**		-43*	-64**
RETIC		-72**	-65*		-86**	-80**
NEUT		-94**	-86**		-88**	
LYMPH			-58**			-58**
MONO		-85**			-79*	
EOS		-66**			-80*	
Day 168 (1 D before 9th injection)						
No. of animals	5	5	5	5	5	5
RETIC		+74*	+87*			
NEUT			+117*			+174*
Day 195 (5 D after 10th injection)						
No. of animals	10	9	5	10	9	8
LEU	-20**	-49**	-73**		-44**	-76**
RETIC	+22**	-75**	-89**		-74**	-90**
NEUT		-91**	-90**		-92**	-89**
LYMPH	-17*	-39**	-70**		-33*	-74**
MONO	-40**	-86**	-89**		-83**	-91**
EOS		-74**		-36*		
Day 225 (End of recovery period)						
No. of animals	5	5	5	5	5	3
NEUT			+267**			
LYMPH			-38**			

– ** = $p \leq 0.01$; * = $p \leq 0.05$

Clinical chemistry:

Index	% Control			
Gender	**Males**		**Females**	
Dose (mg/kg/day)	3	6.7	3	6.7
Day 111 (5 D after 6th injection)				
No. of animals	9	19	10	15
ALP		-23**		-26**
CREA	-30**	-28**	-12**	-28**
TP	-6*	-6**	-6**	-10**
TRIGLY		-39*	+26**	-17*
Day 195 (5 D after 10th injection)				
No. of animals	9	7	10	9
PHOS				+25**
AST				+160**
ALT		+105**	+114**	+101**
CREA	-22**	-41**		-46**
TP	-7**	-16**		-18**
ALB		-17**		
TRIGLY		-74**		-40*
CHOL			-29**	-39**
Day 225 (End of recovery period)				
No. of animals	5	5	5	3
AST		+34**		+78**
ALT				+32**
CREA		-44**		-37**
PHOS				+33**
ALB				-23**

– ** = $p \leq 0.01$; * = $p \leq 0.05$

Coagulation: unremarkable
Urinalysis: unremarkable

Gross pathology – **Early deaths**:

Index	**No. of animals affected**	
Gender	Males	Females
Dose (mg/kg/day)	6.7	6.7
No. of animals	10	13
Epididymides		
– small	2	
Heart		
– focus/foci, tan	1	
– thickened (mild/severe)	2	

Injection site, tail		
– abrasion	1	1
– absent, portion	1	
– dermatitis, chronic	5	1
– scab	1	3
– ulcer		1
Joint		
– abscess	1	
Kidneys		
– focus/foci, tan	2	
Large intestine, cecum		
– distended with gas		1
Lymph node, mandibular		
– discoloration, red	1	
– enlarged	1	
Seminal vesicles		
– small	1	
Skin, subcutis		
– edema	1	3
Spleen		
– enlarged		1
Small intestine, jejunum		
– discoloration, black	1	
Stomach, glandular		
– discoloration, black	3	1
– discoloration, red	2	1
– focus/foci, red	1	4
Tail		
– dermatitis, chronic	1	
Testes		
– small (mild)	8	
– soft (mild/moderate)	5	
Tooth/teeth		
– absent/broken/malocclusion	1	1
Thymus gland		
– small		2
Uterus with cervix		
– cyst		1

Gross pathology – **Terminal Sacrifice**:

Index	No. of animals affected					
Gender	**Males**			**Females**		
Dose (mg/kg/day)	0	0.7	3	0	0.7	3
No. of animals	8	5	4	10	5	5
Testes						
– small			1			
– soft			1			
Thymus gland						
– small						1

– Due to mortality at the 6.7 mg/kg dose in both M and F, no animals were available for Terminal gross pathology. See "Early deaths" gross pathology.

Gross pathology – **Recovery**:

Index	No. of animals affected					
Gender	**Males**			**Females**		
Dose (mg/kg/day)	0.7	3	6.7	0	3	6.7
No. of animals	5	5	5	10	5	3
Injection site, tail						
– abrasion	1		1			3
– abrasion, portion			1			
Lymph node, popliteal						
– enlarged			1			
Skeletal muscle, biceps femoris						
– abscess			1			
Stomach, glandular						
– discolored						1
– foci			1			1
Testes						
– soft		5	4			
– small			1			
Thymus						
– small						1
Uterus with cervix						
– enlarged				2	1	

Organ weights – **Terminal Sacrifice:**

Index	% Control	
Gender	Males	Females
Dose (mg/kg/day)	3	3
No. of animals	4	5
Liver		
– body weight		+30**
– brain weight		+18**
Pituitary		
– body weight	+21*	-17**
– brain weight		-24**
Testes		
– body weight	-57**	
– brain weight	-60**	
Thymus		
– brain weight	-34*	-38*
Uterus w/cervix		
– body weight		-39**
– brain weight		-44**

- ** = $p \leq 0.01$; * = $p \leq 0.05$
- Due to mortality at the 6.7 mg/kg dose in both M and F, no animals were available for organ weights determination for the terminal sacrifice.

Organ weights – **Recovery:**

Index	% Control	
Gender	Males	Females
Dose (mg/kg/day)	6.7	6.7
No. of animals	5	3
Adrenal		
– body weight	+64*	+32*
Brain		
– body weight		+30**
– brain weight	+56**	
Heart		
– body weight	+23**	+36**
– brain weight	-20**	
Kidneys		
– body weight	+25**	+29**
– brain weight	-20**	
Liver		
– brain weight	-34**	

Pituitary		
– body weight	+38**	-24*
– brain weight		-41**
Prostate w/seminal vessels		
– brain weight	-62**	
Spleen		
– body weight	+26**	
Testes		
– body weight	-51**	
– brain weight	-68**	
Thymus		
– brain weight	-67**	
Thyroid/parathyroid		
– body weight	+42**	
Uterus w/cervix		
– body weight		-46*
– brain weight		-58**

– ** = $p \leq 0.01$; * = $p \leq 0.05$

Histopathology – **Early deaths:**

Index	No. of animals affected			
Gender	Males			Females
Dose (mg/kg/day)	0	3	6.7	6.7
No. of animals	2	1	10	13
Adrenal glands				
Bacterial colonies				
– *minimal*		1	1	
Hypertrophy, focal cortical				
– *minimal*	1			
Fatty change, focal cortical				
– *minimal*				1
Hematopoiesis, Extramedullary				
– *minimal*				1
Hyperplasia, diffuse				
– *mild*				1
Bone marrow, femur				
Atrophy				
– *minimal*				2
– *mild*			2	
– *moderate*	1	1	1	2
– *severe*			6	6
Hyperplasia, granulocytic				
– *minimal*				1
– *mild*			1	1
– *moderate*				1
Hyperplasia, mixed				
– *mild*			3	

Bone marrow, sternum				
Atrophy				
– *minimal*				2
– *mild*			2	2
– *moderate*	1	1	2	
– *severe*			4	6
Hyperplasia, granuloctyic				
– *minimal*			1	
– *mild*			1	2
– *moderate*				1
Hyperplasia, mixed				
– *minimal*			1	
– *mild*			2	
Bone, femur				
Decreased ossification				
– *minimal*			4	2
– *mild*			4	1
Bone, sternum				
Adhesion/inflammation/fibrosis/ pleural				
– *mild*			1	
Fibrosis				
– *mild*				1
Cervical dorsal root fibers				
Degeneration, axonal/myelin				
– *minimal*			1	1
Coagulating glands				
Inflammation, acute				
– *minimal*			1	
Epididymides				
Granuloma, spermatic				
– *minimal*			1	
– *mild*		1	1	
– *moderate*			1	
Hyperplasia, reactive				
– *minimal*			5	
Mineralization				
– *minimal*			1	
Necrosis, single cell				
– *minimal*			4	
Oligospermia/germ cell debris, bilateral				
– *mild*			1	
– *moderate*			3	
– *severe*	1		10	
Polyarteritis				
– *minimal*			3	
Eyes, optic nerve				
Degeneration, anonal/myelin				
– *minimal*			1	
– *mild*	1			

Heart				
Adhesion				
– *mild*			1	
Bacterial colonies				
– *minimal*				1
– *mild*		1	2	
– *moderate*			2	
Cardiomyopathy				
– *minimal*			1	
– *mild*	1			
Hemorrhage				
– *mild*			1	
Inflammation, acute				
– *mild*			2	
– *severe*			1	
Inflammation, subacute				
– *mild*				1
– *moderate*				1
Mineralization, myofiber				
– *minimal*		1		
– *mild*	1			
Mineralization, vascular				
– *mild*	1			
Necrosis				
– *minimal*		1		
– *mild*			3	
Thrombus				
– *mild*			1	1
– *moderate*			2	
Injection site, tail				
Bacterial colonies				
– *minimal*			3	3
– *mild*			5	2
Edema				
– *minimal*				1
Exudate, epidermal surface				
– *minimal*			4	1
– *mild*			4	1
– *moderate*			1	2
Fibrosis				
– *minimal*			1	1
– *mild*			1	
Hemorrhage				
– *minimal*			1	1
– *mild*				2
Hyperplasia, epidermal				
– *minimal*			6	3
– *mild*				1
Inflammation, subacute				
– *minimal*			2	
– *mild*			3	

Injection site, tail (con't)				
Inflammation, chronic				
– *minimal*			3	1
– *mild*				1
Inflammation, chronic-active				
– *minimal*			2	1
– *mild*				1
– *severe*			1	
Proliferation, fibro-osseous				
– *mild*				1
Thrombus				
– *minimal*			1	2
Ulcer				
– *minimal*			3	
– *mild*			3	2
– *severe*			1	2
Kidneys				
Bacterial colonies				
– *minimal*		1	2	1
Mineralization				
– *mild*	1			
Mineralization, vascular				
– *minimal*	1			
Infiltration, lymphocytic				
– *minimal*				1
Mineralization, pelvic				
– *mild*				1
Mineralization, tubular				
– *minimal*				3
Nephropathy, chronic progressive				
– *minimal*		1	9	6
– *mild*	1		4	
– *moderate*			1	
– *severe*	1			
Pyelonephritis, bilateral				
– *mild*			1	
Pyelonephritis, unilateral				
– *mild*			1	
Lacrimal glands, exorbital				
Bacterial colonies				
– *minimal*		1		
Inflammation, chronic				
– *minimal*	1			
Metaplasia, Harderian				
– *minimal*	1		10	5
– *mild*	1		4	

Large intestine, cecum				
Erosion/ulcer				
– *moderate*			1	
Congestion				
– *minimal*				1
– *mild*			1	1
Hyperplasia, reactive				
– *minimal*			1	
– *mild*			2	
Dilatation, glandular				
– *minimal*			2	1
Edema				
– *mild*			1	1
– *moderate*			1	
Inflammation, acute				
– *minimal*			1	
Inflammation, subacute				
– *minimal*				1
– *mild*			1	
Necrosis, single cell				
– *minimal*			4	7
Large intestine, colon				
Dilation, glandular				
– *minimal*			1	
– *mild*			2	1
Hyperplasia, reactive				
– *minimal*			1	1
– *mild*			1	
Mineralization, vascular				
– *minimal*	1			
Necrosis, single cell				
– *minimal*			4	9
Large intestine, rectum				
Dilation, glandular				
– *minimal*				1
Hyperplasia, reactive				
– *minimal*			2	
Necrosis, single cell				
– *minimal*			7	9
Liver				
Bacterial colonies				
– *minimal*		1		
Hematopoiesis, extramedullary				
– *minimal*				1
Hypertrophy, kupffer cell				
– *minimal*			1	1
Inflammation, chronic				
– *minimal*	1	1	11	9
Mineralization, vascular				
– *minimal*	1			
Necrosis, focal				
– *minimal*			1	

Lumber dorsal root fibers				
Degeneration, axonal/myelin				
– *minimal*			2	1
– *mild*			3	4
– *moderate*				2
Lung				
Adhesion/inflammation/fibrosis/ pleural				
– *minimal*			2	
Bacterial colonies				
– *minimal*			1	1
– *mild*			1	
Foreign material				
– *minimal*			2	
Inflammation, acute				
– *minimal*				
– *mild*			2	
Inflammation, subacute				
– *minimal*			1	1
– *mild*				1
Mineralization, vascular				
– *minimal*	1			
Thrombus				
– *minimal*			1	1
– *mild*			1	1
– *moderate*			1	
Lymph node, mandibular				
Bacterial colonies				
– *minimal*			2	2
– *mild*				1
Depletion, lymphoid				
– *mild*				1
Erythrocytosis/ erythrophagocytosis, sinus				
– *minimal*			1	
Histiocytosis, sinus				
– *minimal*			7	4
Hyperplasia, lymphocyte/plasmacyte				
– *minimal*			3	2
– *mild*			6	
Inflammation, acute				
– *minimal*			2	1
Necrosis, lymphoid				
– *minimal*			4	7
– *mild*				2

Lymph node, mesenteric				
Depletion, lymphoid				
– *minimal*				2
– *mild*				7
Erythrocytosis/ erythrophagocytosis, sinus				
– *minimal*	1		3	4
– *moderate*		1		
Histiocytosis, sinus				
– *minimal*		1	4	8
– *mild*			6	3
Inflammation, acute				
– *mild*	1			
Necrosis, lymphoid				
– *minimal*			8	10
– *mild*				1
Mammary gland				
Necrosis, single cell				
– *minimal*				1
Nerve, sciatic				
Degeneration, axonal/myelin				
– *minimal*	2	1		2
– *mild*			14	8
– *moderate*				3
Ovaries				
Mineralization				
– *minimal*				1
Peyer's Patch				
Depletion, lymphoid				
– *minimal*			2	7
– *mild*			1	
Necrosis, lymphoid				
– *minimal*			2	4
– *mild*				1
Prostate gland				
Atrophy				
– *minimal*			1	
– *mild*			4	
Bacterial colonies				
– *minimal*		1		
Inflammation, acute				
– *mild*			1	
Inflammation, chronic				
– *minimal*			1	
– *mild*			2	
– *moderate*			2	
Mineralization				
– *minimal*	1	1	11	

Salivary gland, mandibular				
Atrophy, acinar				
– *minimal*			2	1
– *mild*			3	5
– *moderate*				3
Bacterial colonies				
– *mild*				1
Mineralization, vascular				
– *minimal*	1			
Necrosis, single cell				
– *minimal*			2	
– *mild*			1	
Salivary gland, parotid				
Atrophy, acinar				
– *minimal*			3	1
– *mild*				3
– *moderate*			7	4
– *severe*				4
Hypertrophy				
– *minimal*				
– *mild*				2
– *moderate*			2	
Mineralization, vascular				
– *minimal*	1			
Seminal vesicles				
Atrophy				
– *minimal*			1	
– *mild*			2	
– *moderate*			5	
– *severe*			1	
Skeletal muscle, biceps femoris				
Atrophy				
– *minimal*			2	1
– *mild*			1	1
– *moderate*			1	
Degeneration/necrosis, myofiber				
– *minimal*			1	3
– *mild*			2	1
Skin				
Edema				2
– *mild*			1	
Necrosis, single cell				
– *mild*				1
Mineralization, vascular				
– *minimal*	1			
Small intestine, duodenum				
Hyperplasia, reactive				
– *minimal*			1	4
– *mild*			2	
Necrosis, single cell				
– *minimal*			5	9

Small intestine, ileum				
Hyperplasia, reactive				
– *minimal*			2	1
– *mild*			2	
Mineralization, vascular				
– *minimal*	1			
Necrosis, single cell				
– *minimal*			4	9
Small intestine, jejunum				
Hyperplasia, reactive				
– *minimal*			1	1
– *mild*			2	
Necrosis, single cell			5	
– *minimal*				4
Spinal cord, cervical				
Bacterial colonies				
– *minimal*			1	
Degeneration, axonal/myelin				
– *minimal*			5	5
Inflammation, meningeal				
– *minimal*			1	
Spinal cord, lumbar				
Bacterial colonies				
– *minimal*			1	
Degeneration, axonal/myelin				
– *minimal*			5	1
Inflammation, meningeal				
– *minimal*			1	
Spinal cord, thoracic				
Degeneration, axonal/myelin				
– *minimal*			4	4
Spleen				
Bacterial colonies				
– *minimal*	1		1	
Depletion, lymphoid				
– *minimal*		1	4	1
– *mild*			6	5
– *moderate*	1		1	6
Hematopoiesis, extramedullary, increased				
– *minimal*			1	
– *mild*			2	1
– *moderate*		1	2	
– *severe*			1	
Hyperplasia, monocyte/macrophage				
– *minimal*			2	3
– *mild*	2		2	4
Inflammation, acute				
– *minimal*			1	
Necrosis, lymphoid				
– *minimal*			3	8

Stomach, glandular				
Erosion/ulcer				
– *minimal*			4	7
– *mild*			2	
Gastropathy, uremic				
– *mild*	1			
Hyperplasia, reactive				
– *minimal*			1	
Necrosis, single cell				
– *minimal*			3	3
Testes				
Degeneration/atrophy, seminiferous tubules				
– *minimal*		1		
– *moderate*	1			
– *severe*			14	
Mineralization				
– *minimal*			8	
– *mild*			4	
Mineralization, vascular				
– *minimal*	1			
Thymus gland				
Atrophy				
– *minimal*		1	1	
– *mild*		4	1	
– *moderate*	1		1	1
– *severe*			11	11
Hyperplasia, lymphoid				
– *minimal*			1	
Uterus with cervix				
Atrophy				
– *minimal*				3
– *mild*				9
Dilation, gland/lumen				
– *mild*				1
Vagina				
Atrophy				
– *minimal*				4
– *mild*				7

Histopathology – **Terminal Sacrifice:**

Index	No. of animals affected					
Gender	Males			Females		
Dose (mg/kg/day)	0	0.7	3	0	0.7	3
No. of animals	8	5	4	10	5	5
Bone marrow, femur						
Hyperplasia, granulocytic						
– *minimal*			4			3
– *mild*						2
Atrophy			1			
– *mild*			1			
Bone marrow, sternum						
Hyperplasia, granulocytic						
– *minimal*			2			3
– *mild*			2			2
Atrophy			1			
– *mild*			1			
Epididymides						
Hyperplasia, reactive						
– *minimal*		1	4			
Necrosis, single cell		2	4			
– *minimal*		2	4			
Oligospermia/germ cell debris, bilateral						
– *minimal*	1					
– *severe*	1		4			
Polyarteritis						
– *mild*			1			
Eyes, optic nerve						
Hemorrhage						
– *minimal*				1		
Degeneration, anonal/myelin	3			1		
– *minimal*	2			1		
– *mild*	1					
Injection site, tail						
Inflammation, acute						
– *minimal*	1					
Inflammation, chronic						
– *minimal*		2		4	1	1
Thrombus						
– *minimal*			2			
Kidneys						
Nephropathy, chronic progressive						
– *minimal*	4			8		
– *mild*	3			1		
– *moderate*	1					
Mineralization,tubular						
– *minimal*				1		

Lacrimal glands, exorbital						
Granuloma						
– *minimal*		1				
Inflammation, chronic						
– *minimal*	4	5	1	3		
Metaplasia, Harderian						
– *minimal*	4	1	3	8		3
– *mild*	4	3	1			
Large intestine, colon						
Dilatation, glandular						
– *minimal*			1			
Necrosis, single cell						
– *minimal*						1
Larynx						
erosion/ulcer						
– *minimal*			1			
Exudate, luminal						
– *minimal*	1					
Inflammation, acute						
– *minimal*			1			
Foreign material						
– *mild*			1			
Liver						
Inflammation, chronic						
– *minimal*	8		4	10		5
Lumber dorsal root fibers						
Degeneration, axonal/myelin						
– *minimal*					1	1
Lymph node, mandibular						
Eerythrocytosis/ erythrophagocytosis, sinus						
– *minimal*	1		3	2		1
Hyperplasia, lymphocyte/plasmacyte						
– *minimal*					1	1
– *mild*	1					3
Lymph node, mesenteric						
Erythrocytosis/ erythrophagocytosis, sinus						
– *minimal*	1	1	1			2
Histiocytosis, sinus						
– *minimal*			2			5
Polyarteritis						
– *minimal*			1			
Nerve, sciatic						
Degeneration, axonal/myelin						
– *minimal*	6	4	1	7	3	2
– *mild*			3			3
Ovaries						
Cyst					1	
– *minimal*					1	

Prostate gland						
Atrophy						
– *minimal*		1				
– *mild*			1			
Mineralization						
– *minimal*	7	5	4			
Skin						
Necrosis, single cell						
– *minimal*					1	1
Salivary gland, parotid						
Inflammation, chronic						
– *minimal*	2			3		
Hypertrophy						
– *minimal*				3		
Seminal vesicles						
Inflammation, subacute						
– *minimal*		1				
Small intestine, duodenum						
Necrosis, single cell						
– *minimal*			3			4
Small intestine, ileum						
Necrosis, single cell						1
– *minimal*			1			1
Small intestine, jejunum						
Necrosis, single cell						
– *minimal*			1			2
Spleen						
Hyperplasia, monocyte/macrophage						
– *minimal*						2
Testes						
Degeneration/atrophy, seminiferous tubules, bilateral						
– *minimal*	1					
– *mild*			1			
– *moderate*	1					
– *severe*			3			
Mineralization						
– *minimal*	1	1	3			
Thymus gland						
Atrophy						
– *minimal*	8	1		7	3	
– *mild*		4		3	2	
– *moderate*			1			
– *severe*			2			
Uterus w/ cervix						
Atrophy						
– *minimal*						3
Vagina						
Atrophy						
– *minimal*						2
– *mild*						1

Histopathology – **Recovery:**

Index	No. of animals affected							
Gender	**Males**				**Females**			
Dose (mg/kg/day)	0	0.7	3	6.7	0	0.7	3	6.7
No. of animals	10	5	5	5	10	5	5	3
Adrenal glands								
Hypertrophy, focal cortical								
– *minimal*	1			1	1			
Bone marrow, femur								
Hyperplasia, mixed								
– *minimal*				2				
– *mild*				2				
Atrophy								
– *mild*								1
Bone marrow, sternum								
Hyperplasia, mixed								
– *minimal*				2				
– *mild*				2				
Atrophy								
– *mild*								1
Bone, femur								
Decreased, ossification								
– *minimal*				1				1
– *mild*				1				1
Epididymides								
Granuloma, spermatic								
– *minimal*				1				
– *mild*				2				
Hyperplasia, reactive								
– *minimal*			5	4				
Necrosis, single cell								
– *minimal*			4	4				
Oligospermia/germ cell debris, bilateral								
– *severe*			5	5				
Polyarteritis								
– *minimal*				1				
Eyes, optic nerve								
Hemorrhage								
– *minimal*	1							
Degeneration, anonal/myelin	4			1				
– *minimal*	3							
– *mild*	1			1				
Injection site, tail								
Bacterial colonies								
– *mild*		1						3
Exudate, epidermal surface								
– *minimal*								
– *mild*		1						2
– *severe*								1
Hyperplasia, epidermal								
– *minimal*				1				
– *mild*								3

Injection site, tail (con't)								
Inflammation, chronic								
– *minimal*	1	1		1				
– *mild*				1				
Inflammation, chronic-active								
– *minimal*				1				
– *moderate*								2
– *severe*								1
Thrombus								
– *minimal*	1							
Hemorrhage								
– *minimal*								1
Ulcer								
– *mild*								1
– *moderate*								1
– *severe*								1
Kidneys								
Mineralization, pelvic								
– *minimal*	1							
Mineralization, tubular								
– *minimal*	1			1	3			2
Nephropathy, chronic progressive								
– *minimal*	5			5	7			3
– *mild*	4				3			
– *moderate*	1							
Lacrimal glands, exorbital								
Granuloma								
– *minimal*								
Inflammation, chronic								
– *minimal*	6	5	3		4			1
– *mild*	1							
Metaplasia, Harderian								
– *minimal*	8	1	2	5	9	4	3	2
– *mild*	1	2	2					
– *severe*	1	1	1					
Liver								
Inflammation, chronic								
– *minimal*	10		5	5	10		5	3
Lumber dorsal root fibers								
Degeneration, axonal/myelin								
– *minimal*		1					2	
– *mild*								2
– *moderate*								1
Lung								
Histiocytosis, alveolar								
– *minimal*	3			1	4			2
Hemorrhage								
– *minimal*	1				1			
Hyperplasia,bronchiolar-alveolar								
– *minimal*	1							
Inflammation, acute								
– *minimal*	1							
Inflammation, granulomatous								
– *minimal*	1			1				1

Lung (con't)								
Inflammation, subacute								
– *minimal*	3				1			
Lymph node, mandibular								
Erythrocytosis/								
erythrophagocytosis, sinus								
– *minimal*	4		1					
Hyperplasia,								
lymphocyte/plasmacyte								
– *minimal*			1	1		1		
– *mild*	1	1						
Inflammation, acute								
– *minimal*				1				
Lymph node, mesenteric								
Erythrocytosis/								
erythrophagocytosis, sinus								
– *minimal*	1						1	1
Polyarteritis								
– *minimal*			1					
Nerve, sciatic								
Degeneration, axonal/myelin								
– *minimal*	10	5	2		6	4	1	
– *mild*			3	4			4	2
– *severe*				1				1
Prostate gland								
Atrophy								
– *minimal*		1	1					
– *mild*			3	2				
– *moderate*				1				
Inflammation, chronic								
– *mild*				2				
Inflammation, chronic-active								
– *moderate*				1				
Mineralization								
– *minimal*	8	3	4	4				
Salivary gland, mandibular								
Atrophy, acinar								
– *minimal*				1				1
Salivary gland, parotid								
Hypertrophy								
– *minimal*					2		1	
– *mild*				4				3
– *moderate*				1				
Inflammation, chronic								
– *minimal*	1							
Inflammation, subacute								
– *minimal*			1					
– *mild*			1					
Atrophy, acinar								
– *minimal*								1

Seminal vesicles								
Atrophy								
– *minimal*				3				
– *mild*				1				
– *moderate*				1				
Skeletal muscle, biceps femoris	1							
Degeneration/necrosis, myofiber								
– *minimal*	1						2	
Inflammation, chronic-active								
– *severe*				1				
Regeneration								
– *minimal*					1			
Spinal cord, cervical								
Degeneration, axonal/myelin								
– *minimal*				3				2
– *mild*								1
Spinal cord, lumbar								
Degeneration, axonal/myelin								
– *minimal*				3				2
Spinal cord, thoracic								
Degeneration, axonal/myelin								
– *minimal*				3				3
Spleen								
Hyperplasia, monocyte/macrophage								
– *minimal*				1				
– *mild*							1	
Stomach, nonglandular								
Edema								
– *mild*				2				
Erosion/ulcer								
– *minimal*				1				
– *mild*				1				
Inflammation, acute								
– *mild*				2				
Testes								
Degeneration/atrophy, seminiferous tubules, bilateral								
– *minimal*			5	5				
Degeneration/atrophy, seminiferous tubules, bilateral								
– *minimal*	1							
Mineralization								
– *minimal*			3	2				
– *mild*				1				
Thymus gland								
Atrophy								
– *minimal*	2	2				2		
– *mild*	8	3	2		10	3	1	1
– *moderate*			3	3			4	2
– *severe*				2				

Uterus w/ cervix								
Atrophy								
– *minimal*							1	1
– *mild*								2
Dilatation, gland/lumen								
– *minimal*					1			
– *mild*					3		1	1
Vagina								
Atrophy								
– *minimal*								3

Study title: Nine-month Intermittent-dose (Q21Dx14) intravenous toxicity study in dogs

Key study findings:

- The high-dose of 0.9 mg/kg resulted in a high level of mortality. A reduced high-dose of 0.75 mg/kg was associated with severe toxicity throughout the study recovery period.
- Drug related mortalities occurred early in the study and mostly at the 0.9 mg/kg dose. Mortality was seen 2 M and 1 F at the 0.9 mg/kg. In addition, 1 M at the 0.75 mg/kg dose died.
- Major target organ toxicities included peripheral nerves at the injection site, bone marrow, lymphoid tissues, GI tract, and testes.
- Most organ toxicities at the mid-and high-dose were partially or completely reversible with the exception of testes and peripheral nerves (at the injection site).
- Axonal degeneration of nerves at the injection sites was considered to be a localized effect due to the absence of similar lesions in other sections of nerves in treated animals.

Study no.: DS032196
Volume #, and page #: Module 4.2.3.2
Conducting laboratory and location:

Date of study initiation: November 3, 2003
GLP compliance: yes
QA report: yes (X) no ()
Drug, lot #, and % purity: BMS-247550
Lot No.: BMS-247550
Purity:

Formulation/vehicle: 40% polyethylene glycol 300
5% BMS-purified (cleaned) Cremphor®EL
5% ethanol
50% (v/v) 50 mM phosphate buffer

Dose justification: See single and repeat dose studies

Methods (unique study design or methodology)

1. Due to severe toxicity (resulting in deaths) that occurred at the high-dose of 0.9 mg/kg, a reduced dose of 0.75 mg/kg was administered to all remaining high-dose animals starting on Day 22 and throughout the study.
2. Terminal and recovery necropsies were conducted on Days 281 and 302, respectively.

Dosing:

Species/strain:	beagle dog
#/sex/group or time point (main study):	6/sex/dose (Cont. and HD) 4/sex/dose (LD and MD)
Satellite groups used for toxicokinetics:	4-6/sex/dose
Age:	7 months
Weight:	M: 6-10 kg/ F: 5-7 kg
Route, formulation, volume, and infusion rate:	IV (cephalic/saphenous vein) Dose volume of 0.72 mL/kg Infusion rate of 2 mL/min
Doses in administered units:	0, 0.1, 0.5, and 0.9→0.75
Schedule:	q21d x 14 on D 1, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, 232, 253, and 274

Observations and times:

Mortality:	Twice daily during pretest, treatment, and recovery
Clinical signs:	Once/week during the study
Body weights:	At least once pretest; once prior to dosing, and weekly during treatment and recovery
Food consumption:	Weekly during treatment and recovery
EKG	Once retest, Day 1 and during Weeks 39 (terminal) and 43 (recovery)
Ophthalmoscopy:	Once pretest, during Week 13, and prior to terminal (Day 281) and recovery (Day 302) necropsies.
Hematology:	Pretest, Days 48, 63, 132, 147, 216, 231, 281 (terminal) and 302 (recovery)
Clinical chemistry:	Pretest, Days 48, 63, 132, 147, 216, 231, 281 (terminal) and 302 (recovery)
Coagulation	Pretest, Days 48, 63, 132, 147, 216, 231, 281 (terminal) and 302 (recovery)
Urinalysis:	Pretest, during Weeks 13, 26, and prior to terminal (Day 281) and recovery (Day 302) necropsies.
Gross pathology:	Conducted on all animals including those found dead, euthanized in extremis, and those euthanized on Days 281 (terminal) and 302 (recovery).
Organ weights:	Days 281 (terminal) and 302 (recovery)

Histopathology:	All dose groups (terminal) and Cont. and HD (recovery) Adequate Battery: yes (X), no ( )—explain Peer review: yes (X), no ( ) See Histopath Table, tissues examined in the control and high dose. Tissues in low and mid dose were evaluated to determine the no effect level.
Toxicokinetics	Days 1, 3, 6, 9, 12, and 24 hrs from start of infusion on Days 1, 127 and 274

Results

Mortality:

Gender	Male		Female
Dose (mg/kg/day)	0.75	0.9	0.9
No. of animals	6	6	6
No. of deaths	1	2	1
Day of study	7	6 and 7	4

- Animals that were found dead at the 0.9 mg/kg dose were replaced using dogs from the same shipment of animals.
- 1 replacement male dog was found dead on Day 7 of the study. This animal was **not** replaced.

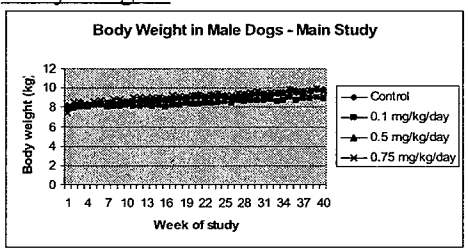
Clinical signs:

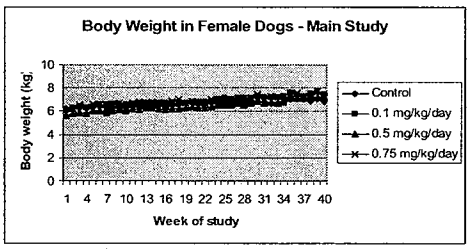
Index	No. of animals affected							
Gender	Males				Females			
Dose (mg/kg/day)	0	0.1	0.5	0.75	0	0.1	0.5	0.75
No. of animals	6	4	4	6	6	4	4	6
Behavior/Activity								
– Activity decreased				2	1		1	4
– Behavior aggressive		1	1			1		
– Salivation	6	3	4	5	6	3	4	6
– Tremors	1	1		1	1			
– Vocalization	4	3	4	3	2	3	1	4
Excretion								
– Emesis	2		1	2		1		2
– Feces discolored, black				1				1
– Feces discolored, red				1				2
– Feces, few/absent				3	1			2
– Feces mucoid	2	2		4			1	2
– Feces soft	6	4	4		6	3	4	6
– Feces watery	1							2
– Material in pan/bedding, red	2		1	4	1			3
– Material in pan/bedding, yellow				1				1

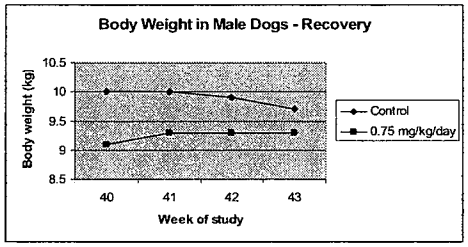
External appearance								
– Discharge, brown						1		1
– Discharge, red			1		6	4	3	6
– Gums discolored, red				1		2		
– Limb function impaired			1	1				3
– Swelling			1	3	1	1		4
– Vulva enlarged						2		3
Pleage/skin								
– Abrasion(s)	1		1	2	1		1	3
– Erytherma			3	4		2		2
– Hair sparse							1	2
– Laceration			1			1		
– Skin discolored, red	5	4	4	5	6	3	4	6
– Skin warm to touch						1	1	2
– Unkempt appearance							1	1

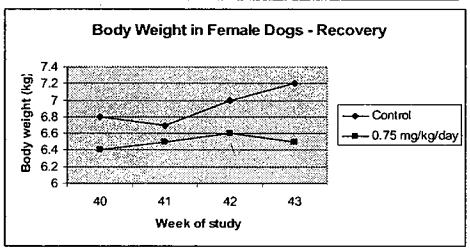
– Note: Recovery animals - unremarkable

Body weights:



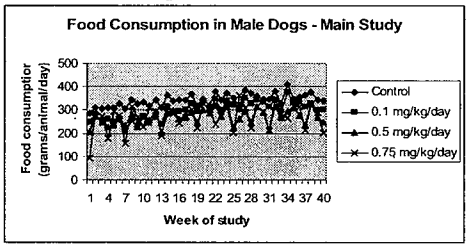


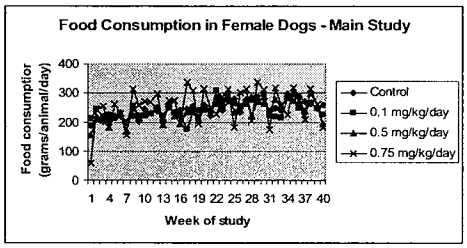


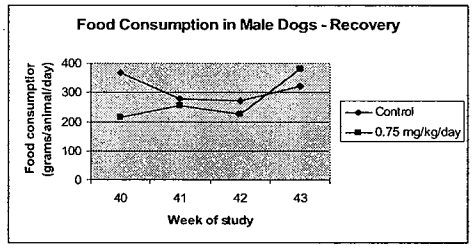


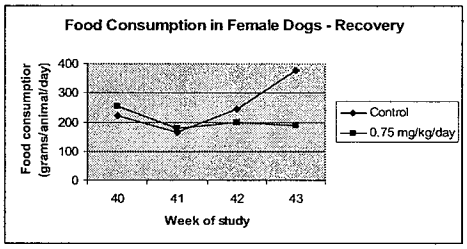
Appears This Way
On Original

Food consumption:









Ophthalmoscopy: unremarkable

EKG: unremarkable

Hematology:

Index	% Control					
Gender	**Males**			**Females**		
Dose (mg/kg/day)	0.1	0.5	0.75	0.1	0.5	0.75
No. of animals	4	4	6	4	4	6
Day 48 (5 D after 3rd injection)						
LEU		-42**	**-72			-56*
ERYTHRO			-12*			-17**
HGB			-10*			-17**
RETIC			-92**			
NEUT		-68**	-91**			-77**
MONO			-85**			
EOS			-76**			
BASO			-62*			
Day 63 (1 D before 4th injection)						
ERYTHRO				+16*		
HGB				+13*		
LYMPH				+29**		
Day 132 (5 D after 7th injection)						
LEU		-53**	-72**			-66**
ERYTHRO			-16**	+19**		-8*
HGB			-14**	+16**		-9*
RETIC			-96*			
NEUT		-76**	-92**			-87**
MONO			-85**			
EOS		-65**	-86**			
BASO			-52			

Day 147 (1 D before 8th injection)						
ERYTHRO				+16**		
HGB				+14**		
LYMPH				+40*		
Day 216 (5 D after 11th injection)						
LEU		-70**	-78**		-67**	-68**
ERYTHRO				+15*		
HGB				+13*		
RETIC		-80**	-91**			
NEUT		-86**	-95**			-89**
MONO		-77**	-91**			
EOS		-77**	-84**			
BASO			-57**			
Day 231 (1 D before 12th injection)						
BASO		+40*				
LYMPH				+48*		
Day 281 (5 D after 14th injection)						
LEU		-55**	-61**		-50**	-50**
ERYTHRO			-13**			-13*
HGB			-12**		-10*	-15**
NEUT		-73**	-89**			-75*

- ** = $p \leq 0.01$; * = $p \leq 0.05$
- Note: Recovery animals - unremarkable

Clinical chemistry:

Index	% Control		
Gender	**Males**		**Females**
Dose (mg/kg/day)	0.5	0.75	0.1
No. of animals	4	4	4
Day 132 (5 D after 7th injection)			
TRIGLY			+52**
K			+14**
Day 147(1 D before 8th injection)			
K			+13**
Day 216 (5 D after 11th injection)			
TRIGLY			+45*
K			+10*
Day 231(1 D before 12thinjection)			
K			+10**
Day 281 (5 D after 14th injection)			
TRIGLY	+46*	+42*	+62*

- ** = $p \leq 0.01$; * = $p \leq 0.05$
- Note: Recovery animals - unremarkable

Coagulation: unremarkable

Urinalysis: unremarkable

Gross pathology – **Early deaths**:

Index	No. of animals affected		
Gender	**Males**		**Females**
Dose (mg/kg/day)	0.75	0.9	0.9
No. of animals	1	2	1
Injection site, vein, left forelimb/arm			
– Discoloration, red, moderate		1	
Lung			
– Discoloration, red, moderate		1	
– Consolidated, moderate	1		
– Consolidated, severe	1		
Thymus gland			
– Discoloration, red, moderate		1	
– Small, moderate		1	
Small intestine, duodenum			
– Discoloration, red, moderate		1	1
Small intestine, jejunum			
– Discoloration, red, moderate		1	1

Gross pathology – **Terminal Sacrifice**:

Index	No. of animals affected			
Gender	**Males**		**Females**	
Dose (mg/kg/day)	0.1	0.5	0	0.5
No. of animals	4	4	4	4
Heart				
– Cyst, mild		1		
Injection site, vein, left forelimb/arm				
– Scab, mild				1
Pituitary gland				
– Cyst, mild	1	1	1	
Small intestine, duodenum				
– Discoloration, red, mild				1

– Note: Recovery animals - unremarkable

Organ weights:

Index	% Control
Gender	**Males**
Dose (mg/kg/day)	0.75
No. of animals	4
Terminal	
Epididymis	
– Brain weight	-19
– Body weight	-12
Testes	
– Brain weight	-31
– Body weight	-27
Thymus	
– Brain weight	-41
– Body weight	-35
Recovery	
Epididymis	
– Brain weight	-21
– Body weight	-22
Testes	
– Brain weight	-40
– Body weight	-38

– No statistical analysis were performed

Histopathology – Early deaths:

Index	No. of animals affected		
Gender	**Males**		**Females**
Dose (mg/kg/day)	0.75	0.9	0.9
No. of animals	1	2	1
Adrenal glands			
Hyperplasia, diffuse			
– *mild*			1
Bone marrow, femur			
Atrophy			
– *minimal*		1	
– *moderate*	1		
– *severe*			1
Hyperplasia, granulocytic			
– *mild*	1		
– *severe*		1	

Bone marrow, rib			
Atrophy			
– *minimal*		1	
– *mild*	1		
– *moderate*		1	
– *severe*			1
Hyperplasia, granulocytic			
– *moderate*	1		
– *severe*		1	
Bone marrow, sternum			
Atrophy			
– *mild*	1		
– *severe*			1
Hyperplasia, granulocytic			
– *severe*	1		
Epididymides			
Hyperplasia, reactive			
– *minimal*	1	2	
Necrosis, single cell			
– *minimal*	1	1	
– *mild*		1	
Gallbladder			
Hyperplasia, reactive			
– *minimal*	1		
Heart			
Hemorrhage			
– *minimal*	1		
Degeneration, myofiber			
– *minimal*			1
Mineralization, myofiber			
– *minimal*			1
Injection site, vein, left forelimb/arm			
Hemorrhage			
– *mild*		1	1
Inflammation, granulomatous			
– *minimal*		1	1
Vasculitis			
– *mild*			1
Injection site, vein, right forelimb/arm			
Hemorrhage			
– *minimal*		1	
Kidneys			
Bacterial colonies			
– *minimal*		1	
Mineralization, tubular			
– *minimal*	1	2	1
Inflammation, granulomatous			
– *minimal*			1
Necrosis, single cell			
– *minimal*		1	

Large intestine, cecum			
Depletion, lymphoid			
– *mild*		1	1
– *moderate*	1		
Dilation, glandular			
– *minimal*		1	1
Hyperplasia, reactive			
– *minimal*			1
Necrosis, single cell			
– *minimal*		1	1
Large intestine, colon			
Dilatation, glandular			
– *minimal*		2	
– *mild*			1
Inflammation, subacute			
– *minimal*		1	
Hyperplasia, reactive			
– *minimal*			1
Necrosis, single cell			
– *minimal*			1
Large intestine, rectum			
Abscess			
– *mild*			1
Depletion, lymphoid			
– *mild*	1	1	
– *moderate*			1
Hemorrhage			
– *mild*		1	
Hyperplasia, reactive			
– *mild*			1
Necrosis, lymphoid			
– *minimal*		1	
Lung			
Bacterial colonies			
– *severe*	1		
Hemorrhage			
– *severe*	1	1	
Inflammation, acute			
– *mild*	1		
Necrosis			
– *severe*	1	1	
Lymph node, mandibular			
Depletion, lymphoid			
– *minimal*			1
Lymph node, mesenteric			
Depletion, lymphoid			
– *mild*		1	
– *moderate*			1
– *severe*	1		
Erythrocytosis/ erythrophagocytosis, sinus			
– *severe*			1

Lymph node, tracheobronchial			
Depletion, lymphoid			
– *minimal*		1	
– *mild*			1
– *moderate*	1		
Erythrocytosis/ erythrophagocytosis, sinus			
– *moderate*		1	
– *severe*	1		
Nictitans glands			
Depletion, lymphoid			
– *minimal*	1		
– *mild*		1	
Palatine tonsil			
Necrosis			
– *mild*		1	
– *severe*		1	
Pancreas			
Depletion, secretory			
– *minimal*	1	1	
– *mild*			1
– *moderate*			
Parathyroid gland			
Cyst			
– *minimal*	1		
– *mild*			1
Peyers patch			
Depletion, lymphoid			
– *mild*		2	1
– *moderate*	1		
Pituitary gland			
Cyst			
– *minimal*		1	1
Salivary gland, sublingual			
Estasia			
– *mild*		1	
Inflammation, acute			
– *minimal*		1	
Mineralization			
– *minimal*	1	1	
Small intestine, duodenum			
Atrophy, mucosal			
– *mild*			1
Dilatation, glandular			
– *minimal*	1	2	
– *moderate*			1
Hyperplasia, reactive			
– *minimal*		1	
– *moderate*			1
Inflammation, subacute			
– *minimal*			1
Necrosis, single cell			
– *minimal*	1		1

Small intestine, ileum			
Depletion, lymphoid			
– *mild*		1	
– *moderate*	1		1
Dilatation, glandular			
– *mild*			1
Hyperplasia, reactive			
– *minimal*		1	
– *mild*			1
Necrosis, single cell			
– *minimal*			1
Small intestine, jejunum			
Atrophy, mucosal			
– *mild*			1
Depletion, lymphoid			
– *mild*			1
Dilatation, glandular			
– *moderate*			1
Hyperplasia, reactive			
– *moderate*			1
Necrosis, single cell			
– *minimal*			1
Spleen			
Depletion, lymphoid			
– *mild*	1		1
Testes			
Degeneration/atrophy, seminiferous tubules, bilateral			
– *minimal*		2	
Thymus gland			
Atrophy			
– *severe*	1	2	1

Histopathology – **Terminal Sacrifice:**

Index	No. of animals affected							
Gender	**Males**				**Females**			
Dose (mg/kg/day)	0	0.1	0.5	0.75	0	0.1	0.5	0.75
No. of animals	4	4	4	3	4	4	4	4
Adrenal glands								
Vacuolar change	1	2		1	1	1	1	
– *minimal*	1	2		1		1	1	
– *mild*					1			
Hematopoiesis, extramedullary								1
– *minimal*								1
Bone marrow, femur								
Hyperplasia, mixed								
– *minimal*			2	2		1	1	3
Bone marrow, rib								
Hyperplasia, mixed								
– *minimal*			3	2				
– *mild*			1	1			1	4

Bone marrow, sternum								
Hyperplasia, mixed			3	3			3	4
– *minimal*			3	1			2	2
– *mild*				1			1	2
– *moderate*				1				
Epididymides								
Necrosis, single cell		1	4	3				
– *minimal*		1	3	1				
– *mild*			1	2				
Oligospermia/germ cell debris, bilateral			1	3				
– *mild*			1					
– *moderate*				2				
– *severe*				1				
Oligospermia/germ cell debris, unilateral			1					
– *minimal*			1					
Gallbladder								
Hyperplasia, reactive			2					
– *mild*			2					
Vacuolation	2	2		1				
– *minimal*	2	2						
– *mild*				1				
Dilatation, glandular						1		1
– *minimal*								1
– *mild*						1		
Infiltration, lymphoctic								1
– *minimal*								1
Heart								
Hematocyst, valvular			1					
– *mild*			1					
Infiltration, mononuclear cell			1					
– *minimal*			1					
Injection site, vein, left forelimb/arm								
Degeneration, axonal/myelin				2		1	1	2
– *minimal*				1		1	1	2
– *mild*				1				
Inflammation, chronic	1		1		1	1	4	1
– *minimal*	1		1		1	1	3	1
– *mild*							1	
Inflammation, granulomatous	1		3		3	2	1	2
– *minimal*	1		3					
Erosin/ulcer							1	
– *mild*							1	
Exudae, epidermal surface							1	
– *mild*							1	
Hemorrhage							1	
– *minimal*							1	

Injection site, vein, right forelimb/arm								
Degeneration, axonal/myelin			1	1				
– *minimal*			1	1				
Exudate, epidermal surface		1		1				
– *minimal*		1		1				
Hyperplasia, epidermal				1				
– *minimal*				1				
Inflammation, granulomatous	1		3		2	1	2	
– *minimal*	1		3		2	1	2	
Inflammation, subacute	1							
– *minimal*	1							
Kidneys								
Dilatation, tubular								1
– *minimal*								1
Lipidosis, glomerular							1	
– *minimal*							1	
Hematopoiesis, extramedullary				1				
– *minimal*				1				
Infiltration, lymphocytic		1			1			
– *minimal*		1			1			
Mineralization, tubular	4	4	4	3	3	4	4	4
– *minimal*	4	4	4	3	3	4	4	4
Inflammation, subacute			1					
– *minimal*			1					
Pigment,tubular	3	3	3	1	1	2	1	3
– *minimal*	3	3	3	1	1	2	1	3
Regeneration, tubular				1				
– *minimal*				1				
Vacuolation, tubular					3	4	4	4
– *minimal*					3	4	4	4
Large intestine, cecum								
Dilatation, glandular							1	1
– *minimal*							1	1
Large intestine, rectum								
Dilatation, glandular	1		1					
– *minimal*	1		1					
Inflammation, granulomatous					2			1
– *minimal*					2			1
Liver								
Hematopoiesis, extramedullary	2		2	3	2	2	3	4
– *minimal*	2		2	1	2	2	3	3
– *mild*				2				1
Inflammation, chronic	4	4	4	3	4	4	3	3
– *minimal*	4	4	4	3	4	4	3	3
Pigment, increased kupffer cell							2	1
– *minimal*							2	1
Lung								
Inflammation, chronic							1	
– *minimal*							1	
Inflammation, subacute						1		
– *minimal*						1		

Lymph node, mandibular								
Hematopoiesis, extramedullary	1							
– *minimal*	1							
Macrophage, pigmented	1	2	2	2				
– *minimal*	1	2	2	2				
Erythrocytosis/								
erythrophagocytosis, sinus					1	1		
– *minimal*					1	1		
Macrophages, pigmented					3	1		2
– *minimal*					3	1		2
Lymph node, mesenteric								
Erythrocytosis/								
erythrophagocytosis, sinus	3	1	3	2	1	1		3
– *minimal*	3	1	3	2	1	1		3
Lymph node, tracheobronchial								
Erythrocytosis/								
erythrophagocytosis, sinus	1	1	1	1	3	2	2	
– *minimal*	1	1	1	1	3		2	
– *mild*						1		
Macrophages, pigmented								1
– *minimal*								1
Nerve, sciatic								
Degeneration, axonal/myelin		1	1	1	1	1	1	
– *minimal*		1	1	1	1	1	1	
Ovaries								
Mineralization							2	1
– *minimal*							2	1
Palatine, tonsil								
Hyperplasia, lymphoid				1				1
– *mild*				1				1
Mineralization		3	2	2			1	
– *minimal*		3	2	2			1	
Parathyroid glands								
Cyst								
– *minimal*	2	2		1	3	1	2	
Pancreas								
Depletion, secretory								
– minimal					1	1		1
Pituitary gland								
Cyst								
– *minimal*	1	2		1			1	
– *mild*	1		1	1	1		2	1
Hyperplasia, focal, pars distalis								
– *minimal*								1
Salivary gland, parotid								
Inflammation, subacute								
– *minimal*	2	1			2		1	

Salivary gland, sublingual								
Inflammation, subacute								
– *minimal*	3	1		1				
Mineralization								
– *minimal*	1	1		2		3	2	3
Atrophy								
– *minimal*						2		
Skin								
Hyperplasia, epidermal								
– *minimal*			1	1				
– *mild*						1		
Inflammation, chronic								
– *minimal*			1			1		
Inflammation, granulomatous								
– *minimal*			1	1		1		
Small intestine, duodenum								
Dilatation, glandular								
– *minimal*	1	2	2	2	1	1	3	4
Small intestine, jejunum								
Dilatation, glandular								
– *minimal*				1				1
Spleen								
Hematopoiesis, Extramedullary, increased								
– *minimal*			3				2	
– *mild*				3				3
Mineralization								
– *minimal*			1					
Testes								
Degeneration/atrophy, seminiferous tubules, bilateral								
– *minimal*			2					
– *mild*			1	1				
– *moderate*			1	2				
Hypoplasia, unilateral								
– *minimal*	2	1						
– *mild*	1	1	1					
Spermatid retention								
– *minimal*			1					
Thymus gland								
Atrophy								
– *minimal*						1		
– *mild*	2	1	3		2		3	2
– *moderate*	2	3	1	1	2	2	1	2
– *severe*				2				
Cyst								
– *minimal*	2		2	2		1		3
Hyperplasia, lymphoid								
– *minimal*								4

Histopathology –**Recovery:**

Index	No. of animals affected			
Gender	**Males**		**Females**	
Dose (mg/kg/day)	0	0.75	0	0.75
No. of animals	2	2	2	2
Adrenal glands				
Vacuolar change	2	2	1	2
– *minimal*	2	2	1	2
Bone, sternum				
Hyperplasia, mixed		1		
– *minimal*		1		
Bone marrow, sternum				
Hyperplasia, mixed				2
– *minimal*				2
Epididymides				
Hyperplasia, reactive		2		
– *minimal*		2		
Necrosis, single cell		2		
– *minimal*		1		
– *mild*		1		
Mineralization		1		
– *minimal*		1		
Injection site, vein, left forelimb/arm				
Inflammation, granulomatous				1
– *minimal*				1
Injection site, vein, right forelimb/arm				
Degeneration, axonal/myelin		1		
– *minimal*		1		
Inflammation, granulomatous		1	2	1
– *minimal*		1	2	1
Kidneys				
Dilatation, tubular		1		
– *minimal*		1		
Lipidosis, glomerular			1	
– *minimal*			1	
Mineralization, tubular	2	2	2	2
– *minimal*	2	2	2	2
Pigment, tubular	2	2		2
– *minimal*	2	2		2
Vacuolation, tubular	1		2	2
– *minimal*	1		2	2

Large intestine, rectum				
Inflammation, subacute	1			
– *minimal*	1			
Abscess				1
– *minimal*				1
Liver				
Hematopoiesis, extramedullary				
– *mild*		2	1	2
Inflammation, chronic	2	2	2	2
– *minimal*	2	2	2	2
Lymph node, mandibular				
Infiltration, neutrophil		1		1
– *minimal*		1		1
Erythrocytosis/ erythrophagocytosis, sinus	1	1		
– *minimal*	1	1		
Macrophages, pigmented		2		2
– *minimal*		2		2
Lymph node, mesenteric				
Erythrocytosis/ erythrophagocytosis, sinus	2	1	1	2
– *minimal*	2	1	1	2
Lymph node, mandibular				
Infiltration, neutrophil				1
– *minimal*				1
Macrophages, pigmented				2
– *minimal*				2
Lymph node, tracheobronchial				
Erythrocytosis/ erythropagocytosis, sinus		1	1	
– *minimal*		1	1	
Infiltration, neutrophil		1		1
– *minimal*		1		1
Hematopoiesis, Extramedullary			1	
– *minimal*			1	
Nerve, sciatic				
Degeneration, axonal/myelin				1
– *minimal*				1
Ovaries				
Mineralization				1
– *minimal*				1

Parathyroid glands				
– Cyst	1	1	1	1
– *minimal*	1	1	1	
– *mild*				1
Pancreas				
Depletion, secretory			1	
– minimal			1	
Palatine tonsil				
Mineralization			1	2
– *minimal*			1	2
Pituitary gland				
Cyst		1		
– *minimal*		1		
Salivary gland, mandibular				
Inflammation, subacute				1
– *minimal*				1
Salivary gland, sublingual				
Mineralization	2		2	2
– *minimal*	2		2	2
Inflammation, subacute				1
– *minimal*				1
Skeletal muscle, biceps feroris				
Inflammation, grandulomatous				1
– *minimal*				1
Skin				
Inflammation, granulomatous			1	
– *minimal*			1	
Small intestine, duodenum				
Dilatation, glandular	1	2	1	1
– *minimal*	1	2	1	
– *mild*				1
Spleen				
Fibrosis	1			
– *minimal*	1			
Hematopoiesis, Extramedullary, increased		2		2
– *minimal*		2		2
Macrophages, pigmented	1			
– *minimal*	1			

Testes				
Degeneration/atrophy,				
seminiferous tubules, bilateral	1	2		
– *mild*	1			
– *moderate*		2		
Hypoplasia, unilateral		1		
– *minimal*		1		
Thymus gland				
Atrophy	2	2	2	2
– *mild*		2	1	1
– *moderate*				1
– *severe*	2		1	
Cyst	1	1	2	2
– *minimal*	1		1	2
– *mild*			1	
Tongue				
Inflammation, subacute		1		
– *minimal*		1		
Urinary bladder				
Mineralization				
– *minimal*				1

Histopathology inventory (optional)

Study	**DS03260**	**DS03196**
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear		X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X*	X*
Colon	X	X
Duodenum	X	X
Epididymis	X	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		X
Gall bladder		X
Gross lesions	X	X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	
Larynx	X	X
Liver	X*	X*
Lungs	X	X*
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		X
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X*
Parathyroid	X	X*
Peripheral nerve		
Pharynx		X
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X*
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X

Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: BMS-247550: Ames Reverse-Mutation Study in *Salmonella* and *E. coli*

Key findings:
- The test article was not mutagenic when evaluated at up to and including the top concentration of 5000 µg/plate.
- However, minimal cytotoxicity was observed only in the full mutation assay in test strain *Salmonella typhimurium* strain TA1537 (in the absence of S-9 activation) at a concentration of 5000 µg/plate.

Study no.: ——— '930000015

Volume #, and page #:

Conducting laboratory and location:

Date of study initiation: February 13, 2001
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: BMS-247550-01
Lot #: N031C-247550-01
Purity: ———

Methods

Strains/species/cell line:
Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537
E. coli (tryptophan) strain WP2urA

Concentrations used in definitive study:
50, 160, 500, 1600 and 5000 µg/plate

Basis of concentration selection:
Dose selection was based on preliminary range-finding study (non GLP) with TA98, TA100, TA1535, TA1537, and WP2 uvrA strains in both the presence and absence of S-9 metabolic activation. BMS-247550 was evaluated at six nominal concentrations ranging from 16-5000 µg/plate. No cytotoxicity was observed in any of the tester strains up to and including 5000 µg/plate. Therefore, this dose was selected at the high concentration for this study.

Negative controls:
DMSO

Positive controls:

Strain	Without S9	With S9
TA98	2-nitrofluorene	2-aminoanthrace
TA100	Sodium azide	2- aminoanthrace
TA1535	Sodium azide	2- aminoanthrace
TA1537	9-aminoacridine	2- aminoanthrace
WP2 vurA	MMS	2-aminoanthrace

Note: The Sponsor characterized the S-9 with benzo(a)pyrene and 2-aminoanthracene.

Incubation and sampling times:
Incubated for 48 hours

Results

Study validity:
- Three replicate plates used in the confirmatory study
- Revertant colonies were counted using a MiniCount™ Automated Colony Counter and examined for effects on the growth of the bacterial background lawn.
- Criterion for a positive result are defined as follows:
    1. A two-fold increase in the mean number of revertants per plate above the negative control in strains TA98, TA100, and WP2uvrA.
    2. A three-fold increase in the mean number of revertants per plate above the negative control in strains TA1535 and TA1537.
    3. Increases in revertant counts for all strains must be related to increases in test-article concentration in order to warrant the designation of positive.
    4. A positive response in one tester strain either with or without exogenous metabolic activation is sufficient to designate the test article as a bacterial mutagen.
- The negative and positive control values were within the historical control data ranges
- Study design is valid.

Study outcome:

- A concentration related reproducible statistically significant increase in revertant colony frequency was recorded in the TA98, TA100, TA1535, TA1537, and WP2uvrA strains with metabolic activation at concentrations of 5000 µg/plate and higher (Table 1).
- Test material caused a visible reduction in background bacteria growth in all strains with and without S9 activation at concentrations of 5000 µg/plate and above (Table 2).

Table 1

BMS-247550: Summary

Mean Histidine$^+$ and Tryptophan$^+$ Revertant Counts from the Full Assay

In the Presence of S-9 Metabolic Activation

Mean ± Standard Deviation[1]

Test Article	Concentration (µg/plate)	TA98 Mean ± SD[1]	TA100 Mean ± SD[1]	TA1535 Mean ± SD[1]	TA1537 Mean ± SD[1]	WP2 uvrA Mean ± SD[1]
DMSO	100 µl	21 ± 4	117 ± 11	9 ± 2	9 ± 5	32 ± 7
BMS-247550	50	19 ± 7	123 ± 4	9 ± 4	6 ± 4	34 ± 15
	160	19 ± 4	127 ± 10	6 ± 4	4 ± 1	35 ± 5
	500	20 ± 3	109 ± 18	11 ± 4	9 ± 0	33 ± 2
	1600	19 ± 3	121 ± 5	6 ± 3	7 ± 1	32 ± 6
	5000	19 ± 6	110 ± 5	7 ± 1	8 ± 5	31 ± 7
2-Aminoanthracene	2.5	1809	2363	367	216	
2-Aminoanthracene	10					658

In the Absence of S-9 Metabolic Activation

Mean ± Standard Deviation[1]

Test Article	Concentration (µg/plate)	TA98 Mean ± SD[1]	TA100 Mean ± SD[1]	TA1535 Mean ± SD[1]	TA1537 Mean ± SD[1]	WP2 uvrA Mean ± SD[1]
DMSO	100 µl	18 ± 4	94 ± 7	7 ± 3	4 ± 2	32 ± 7
BMS-247550	50	17 ± 3	105 ± 11	8 ± 3	5 ± 3	33 ± 9
	160	15 ± 4	111 ± 28	5 ± 2	4 ± 0	28 ± 5
	500	17 ± 2	108 ± 3	6 ± 3	7 ± 2	29 ± 4
	1600	15 ± 2	95 ± 8	6 ± 3	7 ± 4	37 ± 6
	5000	21 ± 8	100 ± 13	5 ± 2	7 ± 3	32 ± 1
2-Nitrofluorene	2	805				
Sodium azide	2		912	769		
9-Aminoacridine	100				688	
Methyl methane-sulfonate	2.5 µl/plate					778

Appears This Way
On Original

Table 2
Bacterial Background Lawn Evaluation from
the Full Assay with BMS-247550

In the Presence of S-9 Metabolic Activation[a]

BMS-247550	TA98	TA100	TA1535	TA1537	WP2 uvrA
50	0[b]	0	0	0	0
160	0	0	0	0	0
500	0	0	0	0	0
1600	0	0	0	0	0
5000[c]	0	0	0	0	0

In the Absence of S-9 Metabolic Activation[a]

BMS-247550	TA98	TA100	TA1535	TA1537	WP2 uvrA
50	0[b]	0	0	0	0
160	0	0	0	0	0
500	0	0	0	0	0
1600	0	0	0	0	0
5000[c]	0	0	0	1	0

[a]Concentrations in terms of µg of test article/0.1ml/plate

[b]Treated plates were compared to the negative controls and graded on a scale of 0-4

[c]Visible test-article precipitate present on culture plates

Grading system:
0 = No reduction of the bacterial background lawn
1 = Minimal reduction of the bacterial background lawn
2 = Moderate reduction of the bacterial background lawn
3 = Marked reduction of the bacterial background lawn
4 = Complete annihilation of the bacterial background lawn

Appears This Way
On Original

Study title: BMS-247550: Cytogenetics study in primary human lymphocytes.

Key findings:
- The test article was not clastogenic when evaluated at concentrations ranging from 62.5 to 2000 µg/ml.
- Minimal increase in the incidence of polyploidy cells in cultures at concentrations of 1000 and 2000 µg/ml.

Study no.: [redacted] 930000096

Volume #, and page #:

Conducting laboratory and location: [redacted]

Date of study initiation: October 13, 2003
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: BMS-247550-01
Lot #: N031C
Purity: [redacted]

Methods:

Strains/species/cell line:
Human peripheral blood was obtained on February 12, 2001 via veinpuncture from two healthy male donors at the Health Services Facility of Bristol Myers Squibb, Syracuse, New York.

Concentrations used in definitive study:

24-Hr. Exposure Without S9	62.5, 125, 250 and 500 µg/ml
5-Hr. Exposure With S9	250, 500, 1000, and 2000 µg/ml

Basis of concentration selection:

Dose selection was based on preliminary range-finding study with the test article both in the presence and absence of rat-liver (S-9) enzymes. BMS-247550 was evaluated at ten concentrations ranging from 0.7 to 4410 µg/ml. In the absence of S-9, cytotoxicity was observed at concentrations of 440, 880, and 4410 µg/ml with reductions of 42, 87, and 70% in the mitotic indices, respectively, when compared to control. No cytotoxicity was observed at the 55, 110, and 220 µg/ml concentrations. At the 0.7 to 28 µg/ml concentration, an increase in the mitotic index of 60% (at the 0.7 µg/ml dose) to 13% (at the 28 µg/ml) was observed. An increase in polyploidy was also seen at doses where there was no cytotoxicity (Table 1).

In the presence of S-9, no cytotoxicity was observed at all concentrations levels and the mitotic index was comparable to controls. However, there was a minimal increase in the incidence of polyploid cells, which was not concentration dependent. To further define the solubility of BMS-247550, an additional solubility assay was conducted using similar concentrations. A slight precipitate formed at 1100 µg/ml

concentration while a moderate precipitate formed at 2200 μg/ml level. Therefore, the high concentration for the present study was set to be 500 μg/ml in the absence of S-9 while the highest concentration in the presence of S-9 enzymes was 2000 μg/ml.

Negative controls:
DMSO

Positive controls:

24-Hr. Exposure Without S9	mitomycin C
5-Hr. Exposure With S9	cyclophosphamide

Incubation and sampling times:
Incubated for 72 hours

Results

Study validity:

- Three replicate plates used in the confirmatory study
- Revertant colonies were counted using a MiniCount™ Automated Colony Counter and examined for effects on the growth of the bacterial background lawn
- Criterion for a positive result are defined as follows:
    1. The mitotic index for the negative-control must exceed 2%.
    2. The positive control cultures must exhibit an increase in chromosome-aberration frequency that is statistically significant at the 5% level.
    3. The percentage of damaged metaphases in the negative-control cultures must not exceed 6% (as an average).
    4. The test article, at least at the highest dose, should exhibit some cytotoxicity (i.e., reduced mitotic index). If no cytotoxicity is observed at the highest dose, but the test article is either at its limit of solubility, or its dosing concentration limit (i.e., 10 mM or 5000 μg/ml), or its limit or volume (20%), the assay will be considered acceptable.
- The negative and positive control values were within the historical control data ranges
- Study design is valid.

Study outcome:

24-hr exposure without S-9 metabolic activation:

- No increases in the frequencies of metaphase cells bearing chromosome aberrations were seen at any concentration.
- At the highest concentration (500 μg/ml), a 45% reduction in mitotic index occurred.
- An increase in polyploidy in cultures treated with BMS-247550 at concentrations $\leq$250 μg/ml. However, this increase is typically seen in microtublin stabilizing agents.

5-hr exposure with S-9 metabolic activation:

- There were no increases in the frequencies of metaphase cells bearing chromosome aberrations at any concentration.
- At the highest concentration (2000 μg/ml), an 18% reduction in mitotic index was observed.

- A precipitate was observed in the culture medium at ≥1000 µg/ml.
- A minimal increase in the incidence of polyploidy cells in cultures treated with the test-article at concentrations ≥1000 µg/ml.

TABLE I

Range-Finding Results with BMS-247550

NOMINAL CONCENTRATION	BMS-247550 -S-9 24 hr			BMS-247550 +S-9 5 hr		
	Mitotic Index (%)[1]	%PP[2]	%ER[3]	Mitotic Index (%)[1]	%PP[2]	%ER[3]
Untreated	10.7	0.0	0.0	13.3	0.3	0.3
DMSO	9.1	0.4	0.0	13.4	1.1	0.0
0.7 µg/ml	14.5	0.6	0.0	12.3	1.6	0.0
7 µg/ml	13.3	1.1	0.0	11.2	2.0	0.0
14 µg/ml	11.1	2.1	0.0	11.7	1.3	0.0
28 µg/ml	10.3	5.1	0.0	11.9	0.3	0.0
55 µg/ml	9.2	5.8	0.0	13.2	0.0	0.0
110 µg/ml	9.6	5.5	0.0	14.4	0.0	0.3
220 µg/ml	7.8	8.5	0.0	14.4	0.0	0.4
440 µg/ml	5.3	2.4	0.0	14.6	0.8	0.0
880 µg/ml (P_1)	1.2	0.0	0.0	12.8	1.5	0.0
4410 µg/ml (P_2)	2.7	0.0	0.0	12.9	3.4	0.0

The mitotic index, polyploidy, and endoreduplication values are mean percentages based on results from two separate culture flasks treated with the test article as indicated. Bristol-Myers Squibb personnel evaluated the mitotic indices and the incidence of polyploidy or endoreduplication.

[1] The mitotic index is the average frequency of mitotic cells among blast transformed lymphocytes. The values are calculated from evaluating 1000 mononuclear blast transformed lymphocytes from each culture flask.

[2] %PP = Polyploidy is the average frequency of polyploid cells calculated from the evaluation of the number of mitotic cells per 1000 cells from each culture flask.

[3] %ER = Endoreduplication is the average frequency of cells calculated from the evaluation of the number of mitotic cells per 1000 cells from each culture flask.

P_1 = Precipitate noted in culture medium when dosed then appeared to go into solution.

P_2 = Precipitate noted in culture medium throughout the exposure period.

TABLE 2

BMS-247550
Cytogenetic Data Summarized by Concentration

TREATMENT				ABERRATIONS							
	MITOTIC INDEX MEAN ± SEM	%PP[1]	CELLS SCORED	CELLS WITH ABERRATIONS MEAN (%) ± SEM	ABS/CELL[2] ± SEM	CHROMATID BKS[3]	CHROMATID EXC[4]	CHROMOSOMAL BKS[3]	CHROMOSOMAL EXC[4]	CHROMOSOMAL >10[5]	TOTAL ABS[6]
DMSO	10.6% ± 1.0%	0.0	200	0.0 ± 0.0	0.00 ± 0.00	0	0	0	0	0	0
BMS-247550 (µg/ml) 24-HOUR EXPOSURE WITHOUT S-9											
62.5	10.4% ± 1.4%	8.3	200	0.5 ± 0.5	0.01 ± 0.01	1	0	0	0	0	1
125	9.8% ± 0.8%	7.3	200	0.5 ± 0.5	0.01 ± 0.01	0	1	0	0	0	1
250	9.3% ± 1.3%	9.4	200	0.0 ± 0.0	0.00 ± 0.00	0	0	0	0	0	0
500	5.8% ± 1.6%*	0.8	200	1.0 ± 1.0	0.01 ± 0.01	2	0	0	0	0	2
MITOMYCIN C (µg/ml)											
0.1	7.0% ± 0.4%**	0.0	200	29.0 ± 3.4**	0.37 ± 0.03	39	33	2	0	0	74
DMSO +S-9	14.3% ± 0.9%	1.1	200	0.5 ± 0.5	0.01 ± 0.01	0	1	0	0	0	1
BMS-247550 (µg/ml) 5-HOUR EXPOSURE WITH S-9											
250	16.0% ± 0.3%	0.8	200	2.0 ± 1.4	0.03 ± 0.02	2	3	0	0	0	5
500	15.9% ± 1.3%	1.0	200	0.5 ± 0.5	0.01 ± 0.01	1	0	0	0	0	1
1000 (P_1)	15.9% ± 0.7%	2.0	200	0.5 ± 0.5	0.01 ± 0.01	0	1	0	0	0	1
2000 (P_2)	11.7% ± 0.9%*	1.7	200	0.0 ± 0.0	0.00 ± 0.00	0	0	0	0	0	0
CYCLOPHOSPHAMIDE (µg/ml) +S-9											
4	13.3% ± 1.1%	0.3	200	27.5 ± 5.1**	0.46 ± 0.07	48	36	7	1	0	92

* Denotes significantly different from appropriate control at $P < 0.05$ by student's "t" test.
** Denotes significantly different from appropriate control at $P < 0.01$ by student's "t" test.

1. Average frequency of polyploid cells (total number of polyploid cells divided by the number of mitotic cells).
2. Total aberrations divided by the total number of metaphases evaluated.
3. Total of all breaks including chromatid and isochromatid type.
4. Total of all exchanges including interchanges and intrachanges.
5. Total cells observed with more than 10 separate aberrations.
6. Total aberrations observed for the treatment (sum of the individual totals + 10X the >10 frequency).

P_1 = Precipitate noted in culture medium when dosed then appeared to go into solution.
P_2 = Precipitate noted in culture medium.

Study title: BMS-247550: Intravenous micronucleus study in rats

Key findings:
- The test article was genotoxic at doses of ≤ 0.625 mg/kg/day.

Study no.: ——— /930006844

Volume #, and page #:

Conducting laboratory and location: ———

Date of study initiation: October 13, 2003
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: BMS-247550-01
Lot #: 44794-097-22
Purity: ———

Methods:

Strains/species/cell line:
Hds-Sprague Dawley®SD®(Br)

Doses used in definitive study:
0.3125, 0.625, and 1.25 mg/kg/day dosed on Days 1-3

Basis of dose selection:
Dose selection was based on intravenous neuropathy and dose range-finding study in rats. In the neuropathy study, female rats were administered 0.42, 0.8, and 1.25 of BMS-247550 for three consecutive days. At the HD, all rats died or were sacrificed moribund 2-5 days after receiving the last treatment. No mortality occurred at the LD and MD. In the dose-range finding study, rats (2/sex/dose) were dosed 0, 0.25, 0.5, 0.75, 1, 1.25, and 1.5 mg/kg/day of BMS-247550 for three consecutive days at approximately 24-hour intervals by intravenous injection. Rats were observed immediately predose, 1 hour after each dose, and daily for toxic signs and mortality throughout the study. In addition, rats were observed 24-hours after the last drug administration. Clinical signs included red staining at the injection site after the first dose in at all groups except the 0.75 and 1.5 mg/kg/day. At the HD, hyperactivity was observed immediately after the first dose in both males and females and immediately after the second dose in female only. At the 0.75 mg/kg dose, one male rat was also hyperactive after the third dose. And finally, one male rat had red nasal discharge 1 hour after the second dose at the 0.5 mg/kg group (Table 1 and 3). Based upon the results from the above studies, the Sponsor selected doses for the micronucleus assay to be 0.3125 0.625, and 1.25 mg/kg/day in both male and female rats. In addition, the assay included a second high-dose group of 3/rats/sex/dose for mortalities that may occur throughout the study.

Negative controls:
20% PEG 300; 10% Cremophor EL™, 10% ethanol and 60% phosphate buffer

Positive controls:
Cyclophosphamide (CP)

Incubation and sampling times:
Bone marrow aspirated from rats after euthanized on Day 4, 24-hrs after last drug administration

Results:

Study validity:
- Slides were scored for micronuclei by eye.
- Criterion for a positive result was the detection of a statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response.
- The positive control value induced statistically significant increases in micronucleated PCEs compared to controls (mean 0.98 vs 0.27).
- The negative control value was within the historical control data ranges.
- Study design and findings are valid.

Study outcome:
No mortality occurred in the study. Drug related clinical signs were observed in HD animals and included slight hyperactivity and liquid feces. Clinical observations are presented in Table 2. Marked bone-marrow toxicity occurred in the males and females at the 0.625 and 1.25 mg/kg/day dose with statistically significant decreases in the PCE: NCE ratios that ranged from 42 to 92%. In both dose groups, the PCE: NCE ratio for the males was 0.57 and 0.09, respectively, vs. 1.11 in the vehicle control. The frequencies of MN-PCE in the bone marrow of rats were statistically significantly increased when compared to the vehicle control group. In both dose groups, the mean frequencies of MN-PCE in the bone marrow were 0.44 and 0.82%, respectively, in the male rats (vs. 0.06% in the respective vehicle control group), and 0.45 and 0.55%, respectively, in the female rats (vs. 0.05% in the respective vehicle control group). Results are presented in Table 6.

Table 1: Clinical Observations for the Dose Range-finding Study with BMS-247550

Target Dose Level (mg/kg/day)	Sex	Animal ID	Time After Dosing: Day 1/Dose 1 IPD	Day 1/Dose 1 1 hour PD	Day 2 a.m.	Day 2/Dose 2 IPD	Day 2/Dose 2 1 hour PD	Day 3 a.m.	Day 3/Dose 3 IPD	Day 3/Dose 3 1 hour PD	Day 4 a.m.
Vehicle Control	M	3464	0	2	0	0	0	0	0	0	0
		3469	0	0	0	0	0	0	0	0	0
	F	3477	0	0	0	0	0	0	0	0	0
		3478	0	0	0	0	0	0	0	0	0
0.25	M	3466	0	0	0	0	0	0	0	0	0
		3472	0	0	0	0	0	0	0	0	0
	F	3474	0	0	0	0	0	0	0	0	0
		3476	0	2	0	0	0	0	0	0	0
0.5	M	3465	0	2	0	0	0	0	0	0	0
		3468	0	2	0	0	3	0	0	0	0
	F	3480	0	2	0	0	0	0	0	0	0
		3481	0	2	0	0	0	0	0	0	0
0.75	M	3467	0	0	0	0	0	0	1	0	0
		3470	0	0	0	0	0	0	0	0	0
	F	3475	0	0	0	0	0	0	0	0	0
		3482	0	0	0	0	0	0	0	0	0
1.0	M	3471	0	2	0	0	0	0	0	0	0
		3473	0	0	0	0	0	0	0	0	0
	F	3479	0	0	0	0	0	0	0	0	0
		3483	0	0	0	0	0	0	0	0	0
1.25	M	3484	0	0	0	0	0	0	0	0	0
		3485	0	2	0	0	0	0	0	0	0
	F	3489	0	2	0	0	0	0	0	0	0
		3490	0	0	0	0	0	0	0	0	0
1.5	M	3486	1	0	0	0	0	0	0	0	0
		3487	1	0	0	0	0	0	0	0	0
	F	3488	0	0	0	0	0	0	0	0	0
		3491	1	0	0	1	0	0	0	0	0

Key: 0: normal, 1: hyperactive, 2: red stain at the injection site, 3: red nasal discharge
IPD: immediately postdose, PD = postdose.
Vehicle Control: 20% PEG 300, 10% Cremophor EL™, 10% ethanol and 60% phosphate-buffer (50 mM, pH 7.4)

Table 2: Clinical Observations for the Micronucleus Study with BMS-247550

Target Dose Level (mg/kg/day)	Animal ID	Day 1 Dose 1 IPD	Day 1 Dose 1 1 Hr PD	Day 2 am Obs.	Day 2 Dose 2 IPD	Day 2 Dose 2 1 Hr PD	Day 3 am Obs.	Day 3 Dose 3 IPD	Day 3 Dose 3 1 Hr PD	Day 4 am Obs.
1.25 Males	3666	1	0	0	0	0	0	0	0	0
	3667	1	0	0	0	0	0	0	0	0
	3668	0	0	0	0	0	0	0	0	0
	3669	0	0	0	0	0	0	0	0	0
	3670	0	0	0	0	0	0	0	0	0
	3686	0	0	0	0	0	0	0	0	0
Secondary Males	3723	0	0	0	0	0	0	0	0	2
	2724	0	0	0	0	0	0	0	0	0
	3725	0	0	0	0	0	0	0	0	0
1.25 Females	3702	0	0	0	0	0	0	0	0	0
	3730	0	0	0	0	0	0	0	0	0
	3731	0	0	0	0	0	0	0	0	2
	3732	0	0	0	1	0	0	0	0	0
	3718	0	0	0	0	0	0	0	0	2
	3721	0	0	0	0	0	0	0	0	0
Secondary Females	3733	0	0	0	0	0	0	0	0	0
	3727	0	0	0	0	0	0	0	0	0
	3728	0	0	0	0	0	0	0	0	0

Key: 0: Normal, 1: slightly hyperactive; 2: liquid feces was observed at the time of harvest
IPD: immediately post dosing last animal, PD: post dosing last animal, Obs.: Observations.

Table 3: Summary Results of the Dose Rangefinding Assay

Assay No.: 25596-0-454OECD
Test Article: HMS-247550
Initiation of Dosing: 20 October 2003

Treatment	Dose (mg/kg/day)	Harvest Time (hour)	Ratio PCE:NCE Mean ± S.E. Males	Ratio PCE:NCE Mean ± S.E Females
Vehicle Control	(2 mL/kg)	24	0.94 ± 0.06	0.97 ± 0.01
Test Article	0.25	24	0.89 ± 0.13	0.98 ± 0.00
	0.50	24	0.85 ± 0.04	0.99 ± 0.02
	0.75	24	0.62 ± 0.05	0.76 ± 0.07*
	1.00	24	0.40 ± 0.01*	0.35 ± 0.07*
	1.25	24	0.43 ± 0.15*	0.45 ± 0.05*
	1.50	24	0.27 ± 0.05*	0.22 ± 0.03*

* Significantly less than the corresponding vehicle control, p≤0.05.
Vehicle Control: 20% PEG 300, 10% Cremophor EL™, 10% ethanol, and 60% phosphate buffer (50 mM, pH 7.4)
PCE: Polychromatic erythrocyte

Table 6: Micronucleus Data Summary Table

Best Possible Copy

Assay No.: 25596
Test Article: HMS-247550-247560
Initiation of Dosing: 03 Nov 2003

TREATMENT	DOSE	HARVEST TIME (Hour)	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. MALES	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. FEMALES	RATIO PCE:NCE MEAN ± S.E. MALES	RATIO PCE:NCE MEAN ± S.E. FEMALES
Controls						
Vehicle	Vehicle	24	0.06 ± 0.02	0.05 ± 0.02	0.98 ± 0.04	1.11 ± 0.15
Positive	CP 60 mg/kg	24	0.98 ± 0.23*	0.27 ± 0.05**	0.84 ± 0.07	0.72 ± 0.07***
Test Article	0.3125 mg/kg/day	24	0.10 ± 0.03	0.09 ± 0.03	1.08 ± 0.06	1.02 ± 0.16
	0.625 mg/kg/day	24	0.44 ± 0.10*	0.45 ± 0.15**	0.57 ± 0.03***	0.31 ± 0.04***
	1.25 mg/kg/day	24	0.82 ± 0.11*	0.55 ± 0.11*	0.09 ± 0.02***	0.09 ± 0.02***

* Significantly greater than the corresponding vehicle control, p≤0.01.
** Significantly greater than the corresponding vehicle control, p≤0.05.
*** Significantly less than the corresponding vehicle control, p≤0.05.

Vehicle Control: 20% PEG 300, 10% Cremophor EL™, 10% ethanol, and 60% phosphate buffer (50 mM, pH 7.4)
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

2.6.6.5 Carcinogenicity – No studies conducted

2.6.6.6 Reproductive and developmental toxicology

Appears This Way On Original

Fertility and early embryonic development

Study title: BMS-247550: Intravenous study of fertility and early embryonic development in rats

Key study findings:

- No adverse effect on mating or fertility.
- ↓ BW, BW gain (females only), and FC were observed in HD M and F.
- Drug related effects on offspring (↑ resorptions) were observed in HD F.
- ↓ in corpora lutea and ↑ in pre- and post-implantation loss was observed in HD F.

Study no.:	[redacted] 930000931
Volume #, and page #:	
Conducting laboratory and location:	[redacted]
Date of study initiation:	17 August 2001
GLP compliance:	Letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	BMS-247550, Lot# NO36C-24755001, [redacted]

Methods

Doses:	0.02, 0.06, and 0.2 mg/kg/day
Species/strain:	[redacted] CD®(SD)IGS BR VAF/Plus
Number/sex/group:	25/sex/dose
Route, formulation, volume, infusion rate:	Slow bolus intravenous injection in 10% USP ethanol, 10% Cremphor EL, 20% polyethylene glycol 300 in 50 mM 60% phosphate buffer (pH 7.4) at a dose volume of 1 mL/kg.
Satellite groups used for toxicokinetics:	10/sex/dose on D 1 and D 10 at 5 minutes, 1, 3, 6, 12, and 24 hours after drug administration.
Study design:	Males and females dosed 1x/daily beginning 15 days before the cohabitation period. Doses for female rats continued until gestation day GD 7. Females euthanized on GD 16. Males were dosed until study termination (Day 46).
Parameters and endpoints evaluated:	Males: in-life observations, body weight, food consumption, mating, fertility, necropsy, organ weights and toxicokinetics. Females: in-life observations, body weight, gross examination of uterus and cervix, uterus weight, corpora lutea, implantations, resorptions, placental morphology, live and dead fetuses and toxicokinetics.

Dose justification

2-week intravenous study in rats [redacted]

- BMS-247550 given once daily at 0.05, 0.12, and 0.3 mg/kg/day.
- Drug-related toxicity at HD included decreased body weight gain (higher in F) and food consumption.
- Histological findings included single cell necrosis of seminal vesicle epithelium at ≥0.05 mg/kg, single cell necrosis of epididymal ductular epithelium and decreased seminal vesicle/prostate weights at the HD.

10-day intravenous range-finding study in pregnant rats [redacted]

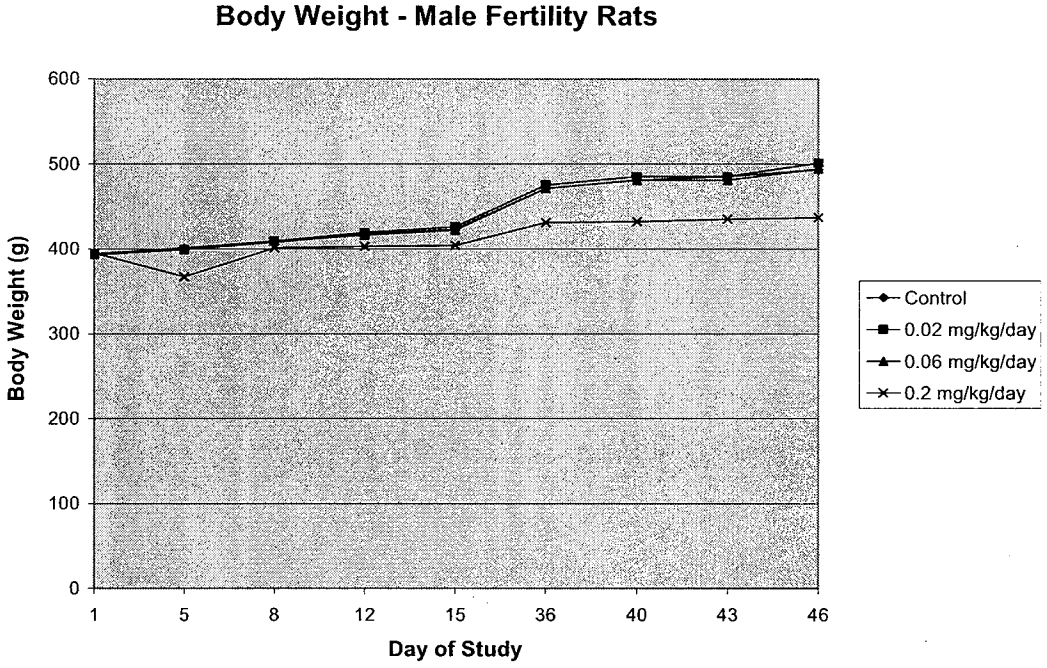
- BMS-247550 given once daily on GD6 through GD15 at 0, 0.05 (LD), 0.15 (MD1), 0.3 (MD2), and 0.5 mg/kg/day (HD).
- No mortality.
- Clinical sign was emaciation at the HD.
- Mild to marked decrease in maternal body weight gain (including body weight losses at ≥0.3 mg/kg) and food consumption was observed at ≥0.15 mg/kg.
- Embryo-fetal death (resorptions) with associated decreases in litter size at ≥0.3 mg/kg.
- Drug-related decrease in fetal body weights at ≥0.3 mg/kg.

Results

Mortality: No drug-related mortality.

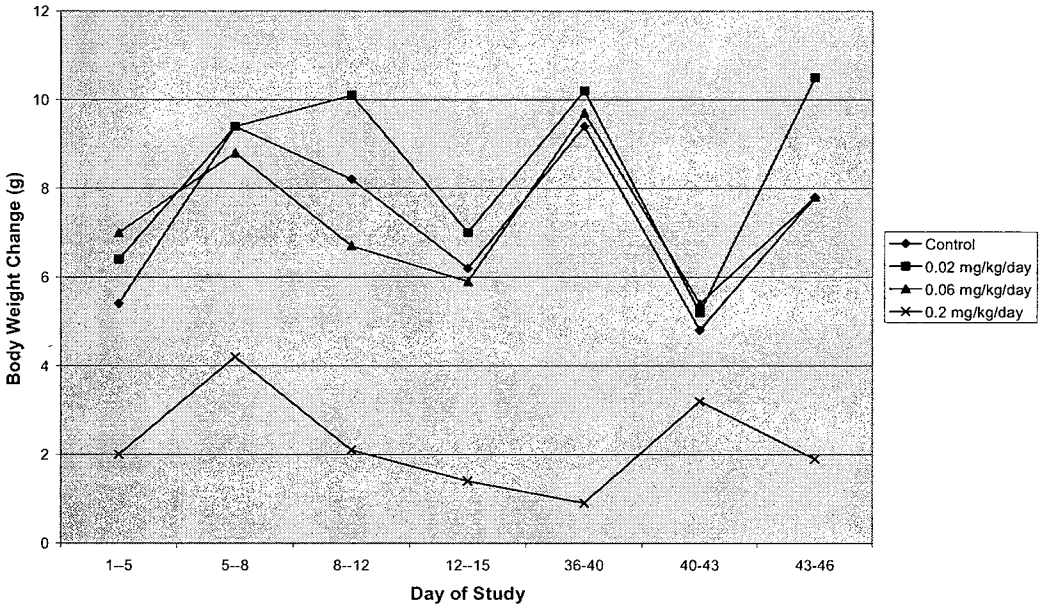
Clinical signs: unremarkable

Body weight:
There were no drug-treated changes in body weight and body weight gain at the LD and MD groups (0.02 and 0.06 mg/kg). All HD male rats showed a statistically significant reduction in body weight (starting on Day 12) and even a greater change in body weight gain starting on Day 5 and throughout the study. For female rats, body weights were taken during precohabitation and then during gestation periods. On the last day of precohabitation, Day 15, body weights of the HD females were significantly lower than the control rats. During gestation period, body weights of the HD females were significantly lower than the control rats throughout the study. During both periods, body weight gains (including body weight losses during the first two weeks of dosing) were decreased compared to control rats in all HD rats.

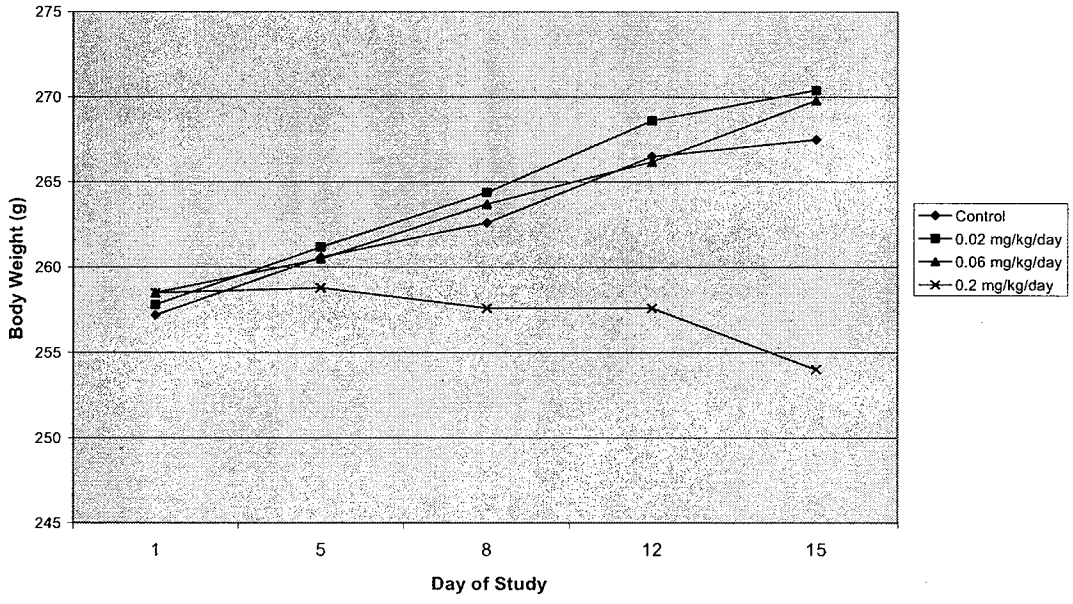


Appears This Way
On Original

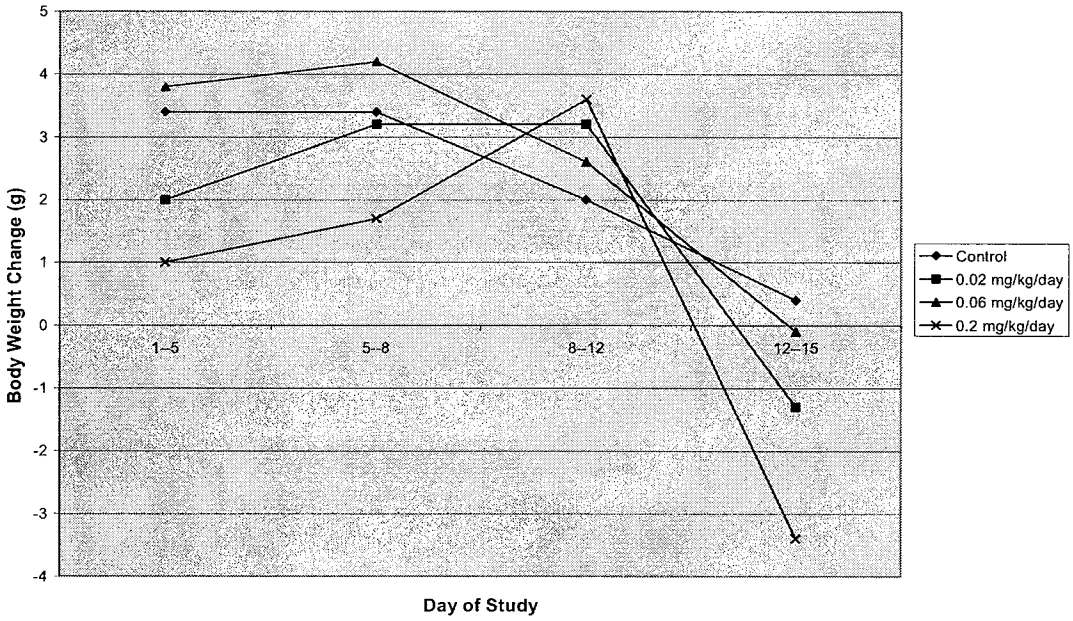
Body Weight Change - Male Fertility Rats



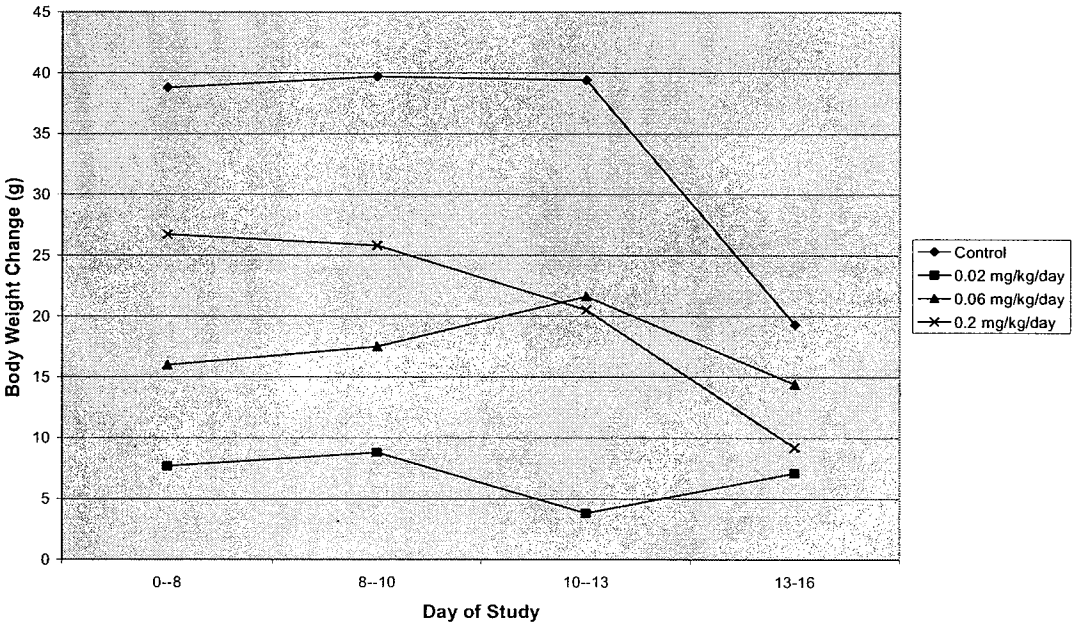
Body Weights in Female Rats - Precohaitation



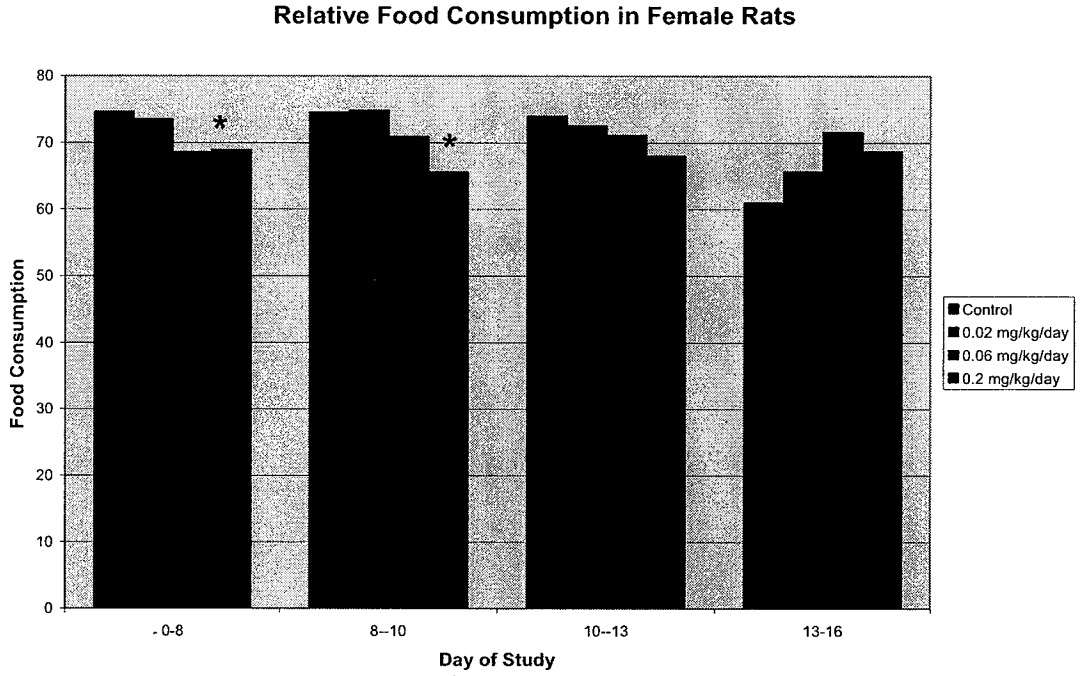
Body Weight Changes in Female Rats - Precohabitation



Body Weight Change in Female Rats - Gestation



Food consumption:
There were no drug-treated changes in food consumption at the LD and MD groups (0.02 and 0.06 mg/kg). All the HD male rats showed significant reductions in food consumption throughout the study, with the most significant difference occurring on the last day of precohabitation, Day 15. Significantly lower food consumption was seen in HD females during the Days 0-8 (the first week of gestation) and then during Days 8-10. Significant data points are shown with an asterisk.



Toxicokinetics:
The Sponsor's table below shows the pharmacokinetics of BMS-247550 in the male and non-pregnant female rats. From the table below, minimal exposures of BMS-247550 were present on Day 1 with exposures falling below the limit of detection of the assay (<0.2 ng/mL) for AUC values. For those values that were detected, the data show that the AUC and Cmax values increased with increasing dose of BMS-247550 of the first and tenth day of dosing. No gender related differences were apparent in male (previous review) and non-pregnant females. $T_{1/2}$ values were not provided by the Sponsor.

Male Rats				
Dose (mg/kg/day)	Cmax (ng/mL)		AUC_{0-t}[a] (ng-h/mL)	
	DS 1	DS 10	DS 1	DS 10
0.02	8.23	2.51	NC-LLOQ[b]	NC-LLOQ[b]
0.06	12.0	8.67	14.0	28.9
0.2	102	157	136	181

NonPregnant Female Rats				
Dose (mg/kg/day)	Cmax (ng/mL)		AUC_{0-t}[a] (ng·h/mL)	
	DS 1	DS 10	DS 1	DS 10
0.02	NA[c]	3.81	NA[c]	NA[c]
0.06	13.8	8.56	15.6	34.4
0.2	106	69.6	170	153

DS = Day(s) of study DG = Day(s) of gestation

a. AUC calculated from time zero to the last quantifiable timepoint, ranging from 3 to 24 hr.

b. NC-LLOQ = Not calculated - Lower limit of quantification. AUC was not calculated from the single quantifiable data point at 5 minutes postdose (all remaining timepoints <LLOQ).

c. NA = No data available due to failed analytical runs.

Necropsy: Unremarkable

Fertility parameters:

Mating parameters, presented in the table below, show that drug exposure did not significantly impact the ability of male and female rats to breed, to impregnate the untreated females, or the amount of time it took until mating.

Dose (mg/kg/day)	Control	Low Dose 0.02 mg/kg	Mid Dose 0.06 mg/kg	High Dose 0.2 mg/kg
No. of animals	25	25	25	25
Males				
Mating Index (%)	100	100	92	100
Fertility Index (%)	92	100	100	100
Days to Mating	3.0	3.0	3.8	3.2
Females				
Mating Index (%)	100	100	100	100
Fertility Index (%)	92	100	100	100
Days to Mating	3.0	3.0	3.4	3.2

The pregnancy parameters for female rats are presented in the table below. As the data show, treatment of female rats with HD BMS-247550 during breeding and through the first 7 days of gestation led to significant effects, primarily in the HD group, on the number of corpora lutea, implantations, resorptions, viable embryos and percentage of pre-implantation and post-implantation loss.

Dose (mg/kg/day)	Control	Low Dose 0.02 mg/kg	Mid Dose 0.06 mg/kg	High Dose 0.2 mg/kg
Number corpora lutea	16.8	17.9	16.5	13.0*
Number implantations	15.1	15.6	14.2	6.6*
Pre-implantation loss (%)	9.4	12.0	13.1	47.6*
Number of live conceptuses	14.3	15.0	12.3	0.6*
Number of resorptions	0.8	0.6	1.9	5.9*
Post-implantation loss (%)	5.2	3.6	13.7	80.3*

*Statistically significant compared to control ($p \leq 0.01$)

Embryo-fetal development

Study title: BMS-247550: Intravenous study of embryo-fetal development in rats.

Key study findings:

- Decrease in maternal BW (including BW loss) and FC at the HD.
- Embryo-fetal death with decreases in litter size, fetal BW and ossification was observed at the HD.

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

Date of study initiation:	January 23, 2001
GLP compliance:	Letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	BMS-247550, Lot# C024A-24755001, [redacted]
Methods	
Doses:	0, 0.02, 0.08, and 0.3 mg/kg/day
Species/strain:	[redacted]CD®(SD)IGS BR VAF/Plus
Number/sex/group:	25/female/dose
Route, formulation, volume, infusion rate:	Slow bolus intravenous injection in 10% USP ethanol, 10% Cremphor EL, 20% polyethylene glycol 300 in 50 mM 60% phosphate buffer (pH 7.4) at a dose volume of 1 mL/kg.
Satellite groups used for toxicokinetics:	10/female/dose on GD 6 and GD 15 at 5 minutes, 1, 3, 6, 12, and 24 hours after drug administration.
Study design:	F0 females dosed from GD6-15, with the day of mating noted as GD1. Females then euthanized on GD20.
Parameters and endpoints evaluated:	Females: in-life observations, body weight, food consumption, gross necropsy, uterus weight, corpora lutea, implantations, resorptions, gross placental morphology, live and dead fetuses, fetal weights, and toxicokinetics. F1 rats: fetal examinations (malformations and alterations).

Dose justification

10-day intravenous range-finding study in pregnant rats [redacted]

- BMS-247550 given once daily on GD6 through GD15 at 0, 0.05, 0.15 (MD1), 0.3 (MD2), and 0.5 mg/kg/day.
- No mortality.
- Clinical sign was emaciation at the HD.
- Mild to marked decrease in maternal body weight gain (including body weight losses at ≥0.3 mg/kg) and food consumption was observed at ≥0.15 mg/kg.
- Embryo-fetal death (resorptions) with associated decreases in litter size at ≥0.3 mg/kg.
- Drug-related decrease in fetal body weights at ≥0.3 mg/kg.

Results

Mortality (dams):

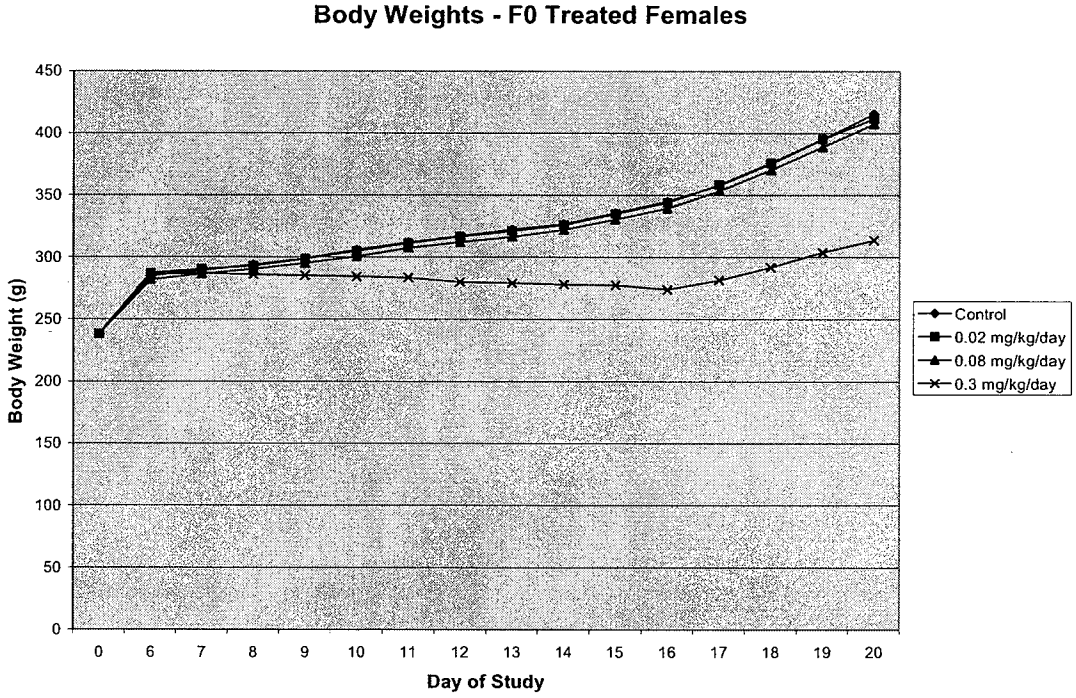
No maternal deaths during the study.

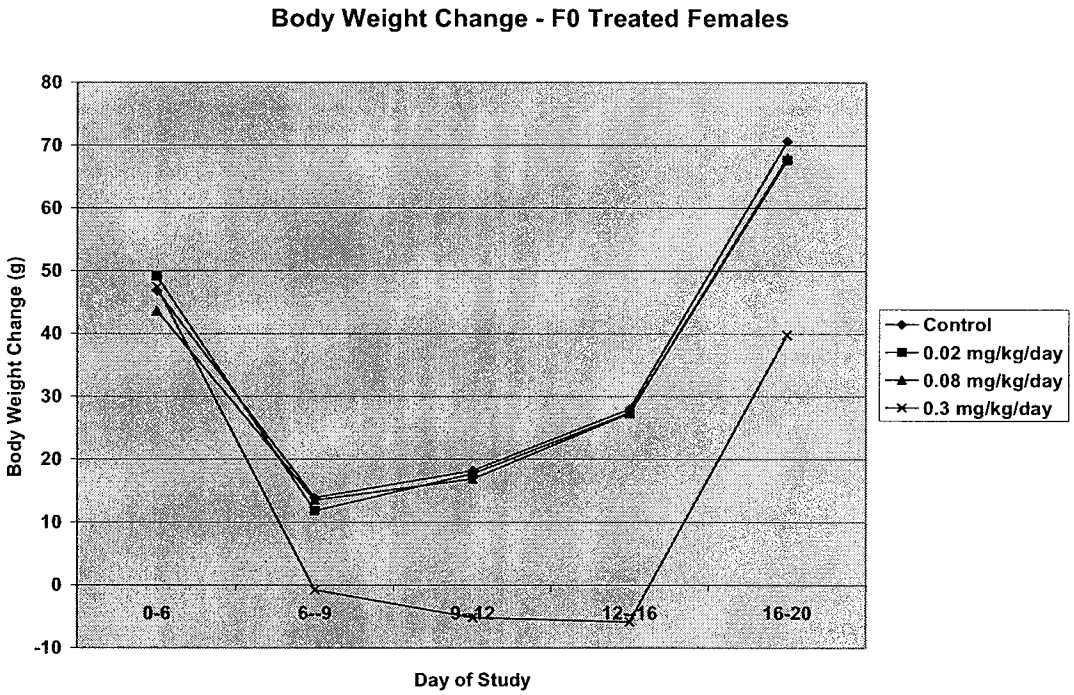
Clinical signs (dams):

Red perivaginal substance (associated with resorbed litters) and scant feces at HD.

Body weight (dams):

Body weights of the dams were adversely affected by BMS-247550 treatment, as is depicted in the graph below. At the HD, a decrease in maternal body weight gain (including body weight losses) was observed during the dose and postdose periods.

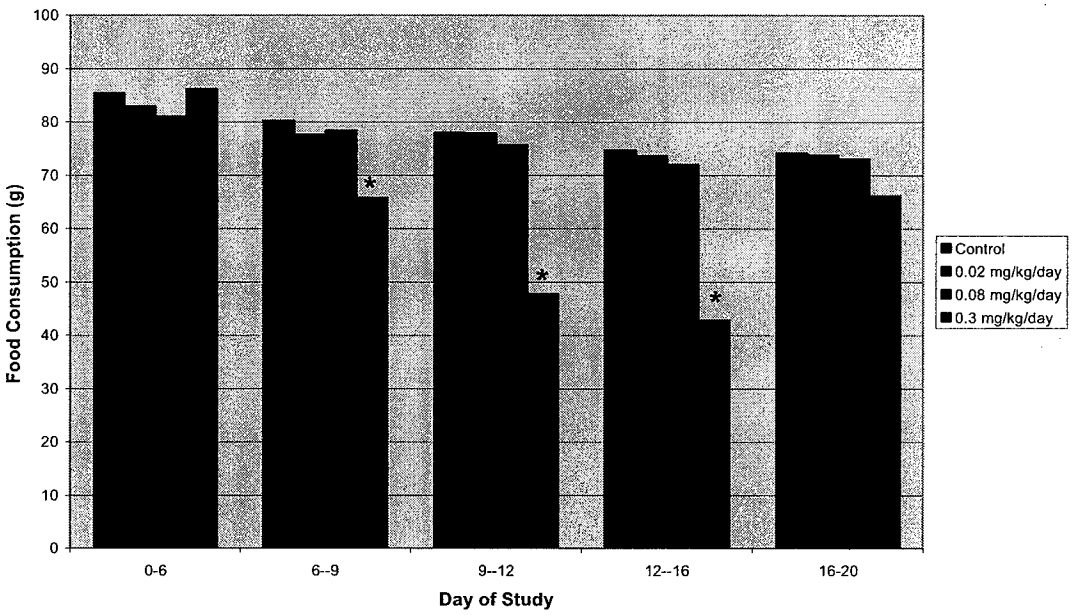




Appears This Way
On Original

Food consumption (dams):
The food consumption data, like the body weights, show that the HD rats were adversely affected by BMS-247550 treatment with a decrease in food consumption compared to all other dose groups. This is seen in the graph, presented below. Significant data points are shown with an asterisk.

Relative Food Consumption - F0 Treated Females



Toxicokinetics:
The Sponsor's table below shows the pharmacokinetics of BMS-247550 in the pregnant rats. From the table below, minimal exposures of BMS-247550 were present on Day 1 with exposures falling below the limit of detection of the assay (<0.2 ng/mL) AUC values. For those values that were detected, the data show that the AUC and Cmax values increased in a greater than dose proportional manner.

Pregnant Female Rats				
Dose (mg/kg/day)	Cmax (ng/mL)		AUC_{0-t}[a] (ng·h/mL)	
	DG 6	DG 15	DG 6	DG 15
0.02	3.41	5.10	NC-LLOQ[b]	NC-LLOQ[b]
0.08	19.0	13.6	NA[c]	41.7
0.3	134	209	192	267

DS = Day(s) of study DG = Day(s) of gestation

a. AUC calculated from time zero to the last quantifiable timepoint, ranging from 3 to 24 hr.
b. NC-LLOQ = Not calculated - Lower limit of quantification. AUC was not calculated from the single quantifiable data point at 5 minutes postdose (all remaining timepoints <LLOQ).
c. NA = No data available due to failed analytical runs.

Terminal and necroscopic evaluations: C-section data:
Results of the uterine examinations are presented in the table below. HD dams had a statistically significant increase in resorptions (both early and late), post implantation loss, and number of live fetuses. Although there was an increase in these parameters, there were no dead fetuses reported.

Dose (mg/kg/day)	Control	Low Dose 0.02	Mid Dose 0.08	High Dose 0.3
Number corpora lutea	17.8	17.1	17.4	18.4
Number implantations	16.0	14.8	14.8	15.1
Pre-implantation loss	10.0	13.4	13.6	16.6
Resorptions	0.5	0.2	0.4	8.8*
– Early	0.5	0.2	0.4	8.2*
– Late	0	0	0	0.6*
Post-implantation loss	3.2	1.5	2.6	58.1*
Litter sizes Number of live fetuses	15.4	14.5	14.4	6.3*

* - Statistically significant compared to control ($p \leq 0.01$)

Litter observations (Caesarean-delivered fetuses):
The table below presents the uterine parameters for the control and BMS-247550 dose groups. A statistically significant decrease in live fetuses and fetal body weights was observed at the HD.

Dose (mg/kg/day)	Control	Low Dose 0.02	Mid Dose 0.08	High Dose 0.3
Litters with one or more live fetus	25	24	24	18
Number of implantations	16	14.8	14.8	15.0
Live fetuses	15.4	14.5	14.4	8.8*
Percent live male fetuses/litter	51.5	44.6	47.5	56.2
Live fetal body weight (g)/litter				
– Mean pup weight	3.70	3.59	3.74	2.91*
– Mean male weight	3.78	3.66	3.82	3.03*
– Mean female weight	3.61	3.53	3.68	2.85*
Percent dead or resorbed conceptuses/litter	3.2	1.5	2.6	41.8*

*Statistically significant compared to control ($p \leq 0.01$)

Offspring

Litters exposed *in utero* to BMS-247550 were compared to litters exposed to the vehicle. Incidences of malformations (irreversible changes not common in this strain of rats) and variations (common findings in this strain and reversible delays or accelerations in development) were recorded for gross, soft tissue and skeletal alterations.

Fetal gross alterations:

- No significant findings

Fetal soft tissue alterations:

- No significant findings
-

Fetal skeletal alterations:

- Statistically significant drug effect was observed in the sternal centra as litter and fetal incidence increased in the HD group when compared to control.

Dose (mg/kg/day)	Control	Low Dose 0.02	Mid Dose 0.08	High Dose 0.3
Sternal centra: Incompletely ossified				
– Litter incidence (%)	4.0	8.3	25.0	33.9*
– Fetal incidence (%)	1.0	1.6	3.9	7.1*

*Statistically significant compared to control ($p \leq 0.05$)

Fetal ossification sites (C-delivered live fetuses GD 20):

Precocious ossification is also seen in this study, evident by decreases in incidences of ossification sites per fetus per litter. The table below shows statistically significant reductions in ossification of the caudal vertebrate, sternebrae, and metacarpals at the HD.

Dose (mg/kg/day)	Control	Low Dose 0.02	Mid Dose 0.08	High Dose 0.3
Vertebrae				
– Caudal	4.8	4.5	4.9	4.3*
Sternum				
– Sternal centers	3.8	3.6	3.8	3.4*
Forelimb b				
– Metacarpals	3.7	3.6	3.7	3.3*

*Statistically significant compared to control ($p \leq 0.05$)

Study title: BMS-247550: Intravenous study of embryo-fetal development in rabbits

Key study findings:

- Due to a lack of toxicity at HD, the study was extended to include two additional groups of presumed pregnant rabbits at doses of 0 and 0.3 mg/kg. The 0.3 mg/kg dose was expected to produce maternal toxicity.
- HD (0.3 mg/kg) led to severe toxicity resulting in mortality of all does.
- HD caused maternal toxicity as evidenced by a significant decrease in body weights (including body weight loss) and food consumption.
- HD caused marked increase in resorptions in litters including 10 litters consisting of resorbed conceptuses.

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

Date of study initiation (initial and extended study):	Part 1: January 19, 2001 Part 2: April 20, 2001
GLP compliance:	Letter included and signed
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	BMS-247550, Lot# C024A-24755001, [redacted]
Methods	
Doses (initial and extended study):	Initial: 0, 0.1, 0.03, and 0.11 mg/kg/day Extended: 0 and 0.3 mg/kg/day
Species/strain:	Rabbit/Hra:NZW SPF
Number/sex/group:	20 females/dose
Route, formulation, volume, infusion rate:	Slow bolus intravenous injection in 10% USP ethanol, 10% Cremphor EL, 20% polyethylene glycol 300 in 50 mM 60% phosphate buffer (pH 7.4) at a dose volume of 1 mL/kg and injection rate of 0.5 mL/min.
Satellite groups used for toxicokinetics:	5 females/dose on GD 7 and GD 19 at 5 minutes, 1, 3, 6, 12, and 24 hours after drug administration.
Study design:	Pregnant rabbits were dosed on GD 7 – 19 inclusive with the day of mating identified as GD0. Rabbits were euthanized and C-sectioned on GD 29.

Parameters and endpoints evaluated: In-life observations, body weight, food consumption, gross necropsy, uterine examination (corpora lutea, implantations, resorptions, live fetuses, post-implantation loss), fetal examinations (external, visceral, skeletal) and toxicokinetics.

Dose justification

13-day intravenous range-finding study in pregnant rabbits [redacted]

- BMS-247550 was administered once daily via intravenous injection on GD7 through GD19 to pregnant rabbits at 0, 0.04, 0.08 (MD1), 0.15 (MD2), and 0.3 mg/kg/day.
- Mortality (9 does) occurred at doses 0.15 mg/kg and higher during GD12 - GD16.
- Two drug-related abortions occurred during the study.
- Drug-related decrease in maternal body weight gain (including body weight losses) at ≥0.15 mg/kg and food consumption at ≥0.08 mg/kg.

Results

Mortality:

No mortality was observed in initial study. In the extended study, the following mortalities were seen at the HD of 0.3 mg/kg:

- 18 does were found dead
- 1 doe was euthanized in moribund condition
- 1 doe was terminated following abortion

Clinical signs:

No drug related clinical signs were observed in initial study. Clinical signs in extended study that were associated with BMS-247550 administration, at the HD of 0.3 mg/kg, in animals that were found dead, euthanized or terminated include the following:

- Perinasal substance
- Emaciation
- Decreased activity
- Dyspnea
- Scant feces
- Soft or liquid feces

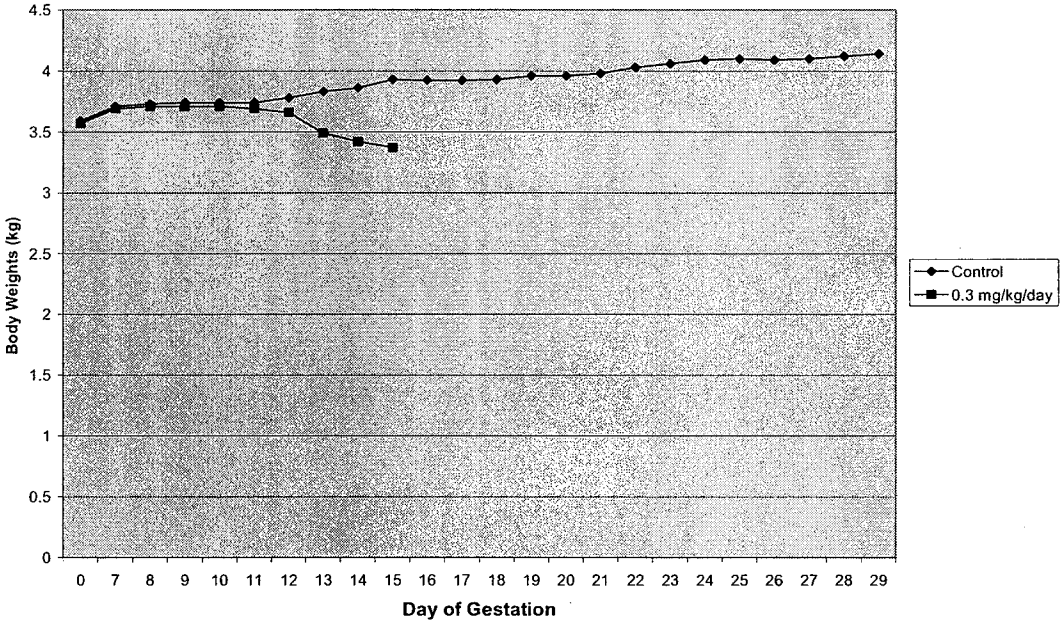
Abortions:

One HD rabbit was terminated following abortion. Details are shown in table below:

Rabbit #	Day aborted		Clinical and gross observations
Rabbit 5939	GD 16	2 right late resorptions 6 left late resorptions 9 total resorptions	Scant feces, ↓ body weight and food consumption, large spleen

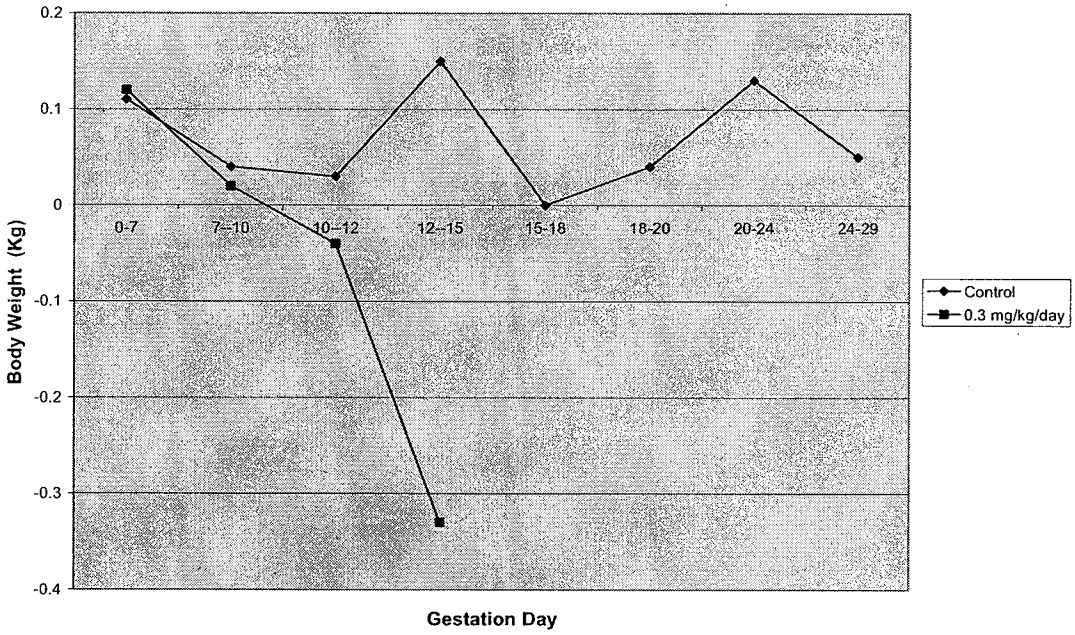
Body weight:
No drug related changes in maternal body weights were observed in initial study. Maternal body weight changes occurred in extended study at the HD of 0.3 mg/kg. The graph below shows the body weights (including body weight gains) of the pregnant rabbits throughout gestation. From GD 13 until GD 15, the body weights of HD rabbits were significantly lower than weights of the control rabbits. In addition, statistically significant body weight gains (including body weight losses) were observed in HD rabbits compared to control. As shown in the graph, information (both body weight and body weight gain) is not available for pregnant rabbits from GD 16 to GD 29 since these rabbits were found dead, euthanized in moribund condition, and/or terminated following abortion.

Maternal Rabbit Weights During Gestation - Extended Study



Appears This Way
On Original

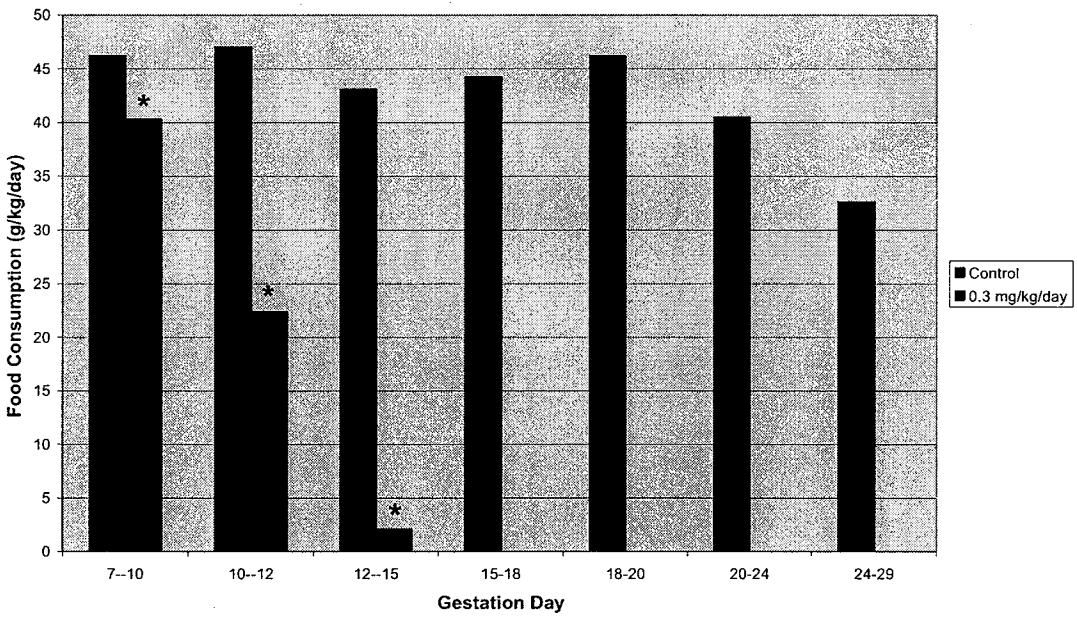
Maternal Body Weight Change During Gestation - Extended Study



Appears This Way
On Original

Food consumption:
No drug related changes in maternal body weights were observed in initial study. Maternal body weight changes occurred in extended study at the HD of 0.3 mg/kg. The graph below shows the food consumption, relative to the rabbits' body weights. Throughout drug administration, the HD rabbits ate significantly less food, both absolute amounts and relative to body weight, than did the control rabbits (significant data points are shown with an asterisk). As shown in the graph, information is not available for pregnant rabbits from GD 16 to GD 29 since these rabbits were found dead, euthanized in moribund condition, and/or terminated following abortion.

Maternal Felative Food Consumption During Gestation - Extended Study



Appears This Way
On Original

Toxicokinetics:

The Sponsor's table below shows the pharmacokinetics of BMS-247550 in the pregnant rabbits. Due to the limited number of detectable time-points collected, AUC values were not calculated for most dose groups. In instances where AUC was reported, the last quantifiable time points ranged from 3 to 24 hours, preventing comparisons of AUC values. Thus, dose proportionality of exposures based on AUC could not be determined. Mean Cmax values were less than dose proportional between 0.01 and 0.11 mg/kg and greater than dose proportional between 0.11 and 0.3 mg/kg. On Day 19, Cmax values increased approximately 2-fold at 0.03 and 0.11 mg/kg.

Dose (mg/kg/day)	Cmax (ng/mL)		AUC_{0-t}[a] (ng-h/mL)	
	DG 7	DG 19	DG 7	DG 19
0.01	LLOQ[b]	2.21	LLOQ[b]	NC-LLOQ[c]
0.03	3.25	6.44	NC-LLOQ[c]	10.6
0.11	6.20	13.8	NC-LLOQ[c]	78.2
0.3	25.5	NA[d]	21.8	NA[d]

DG = Day of gestation

a. AUC(0-t) = area under the plasma concentration vs. time curve from time 0 to the last quantifiable time point. t=3 h for 0.03 mg/kg/day on DG 19, 3 h for 0.3 mg/kg on DG 7, and 24 h for 0.11 mg/kg/day on DG 19.

b. LLOQ = All values below lower limit of quantification (<2.0 ng/mL).

c. NC-LLOQ = Not calculated - Lower limit of quantification. AUC was not calculated from the 1 or 2 quantifiable timepoints (all remaining timepoints <LLOQ).

d. NA = Not applicable. Rabbits given BMS-247550 at 0.3 mg/kg were dosed on DG 7 and euthanatized after the final blood collection on DG 8.

Necropsy findings:

Gross malformations were observed at the HD (0.3 mg/kg) and were limited to the spleen, liver, stomach, GI, and lungs. They include the following observations:

- Large spleen in 3/20 rabbits
- Tan lobes in liver in 1/20 rabbits
- Red/black areas fundic region of stomach in 1/20 rabbits.
- Dark red in cecum in 2/20 rabbits
- Dark red lobes in lungs in 1/20 rabbits

Terminal and necroscopic evaluations – C-section data:
Results from uterine examinations in the initial part of the study are presented in the table below. All doses did not adversely affect the parameters measured.

Dose (mg/kg/day)	Control	Low Dose 0.01	Mid Dose 0.03	High Dose 0.11
Number corpora lutea	10.5	10.2	10.1	10.9
Number implantations	9.5	7.8	8.6	9.3
Pre-implantation loss (%)	9.7	23.7	13.9	14.1
Resorptions	0.7	0.7	0.7	0.7
– Early	0.5	0.6	0.4	0.4
– Late	0.2	0.2	0.4	0.3
Post-implantation loss (%)	7.8	9.1	8.1	8.4
Number of live fetuses	8.8	7.2	7.9	8.6
Number of dead fetuses	0	0	0	0
Litter size				
– Live fetuses	8.8	7.2	7.9	8.6
– Dead fetuses	0	0	0	0

The Sponsor's table below shows uterine contents and litter data for individual rabbits at the HD (0.3 mg/kg) that were euthanized in morbid condition, and/or terminated following abortion. As shown in the table, all does were pregnant with 10 of these litters consisting of entirely (100%) of resorbed conceptuses.

TABLE 3 (PAGE 2): UTERINE CONTENTS AND LITTER DATA FOR INDIVIDUAL RABBITS THAT WERE EUTHANIZED IN MORIBUND CONDITION, DIED, ABORTED OR DELIVERED

GROUP DOSE (MG/KG/DAY) a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	L	T	IMPLANTATIONS R	L	T	RESORP/STUNTED b R	L	A	T	RESORPTIONS c R	L	A	T
V 0 (VEHICLE)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VT 0.3	5921	FOUND DEAD ON DAY 14 OF GESTATION	4	6	10	4	6	10	0	0	0	0	4	6	0	10
	5923	FOUND DEAD ON DAY 13 OF GESTATION	5	6	11	4	2	6	4	3	0	91	0	0	0	0
	5925	EUTHANIZED IN MORIBUND CONDITION ON DAY 15 OF GESTATION	1	5	6	1	3	4	0	0	0	0	1	3	0	4
	5926	FOUND DEAD ON DAY 16 OF GESTATION	5	5	10	5	4	9	0	0	0	0	5	4	0	9
	5928	FOUND DEAD ON DAY 13 OF GESTATION	2	9	11	2	8	10	2	9	0	100	0	0	0	0
	5929	FOUND DEAD ON DAY 18 OF GESTATION	5	4	9	5	4	9	1	1	0	2	4	3	0	7
	5930	FOUND DEAD ON DAY 15 OF GESTATION	5	6	11	5	6	11	2	5	0	7	3	1	0	4
	5931	FOUND DEAD ON DAY 18 OF GESTATION	7	4	11	7	4	11	6	4	0	10	1	0	0	1
	5932	FOUND DEAD ON DAY 16 OF GESTATION	3	6	9	3	6	9	0	0	0	0	3 ELR	6 LLR	0	9
	5936	FOUND DEAD ON DAY 19 OF GESTATION	1	7	8	1	7	8	1	6	0	75	0	1	0	1

R = RIGHT L = LEFT T = TOTAL A = ADJUSTED LR = LATE RESORPTION
a. Days 7 through 19 or 14 of gestation.
b. See the individual fetal alterations table (Appendix 8) for fetuses with gross external and soft tissue alterations.
c. Early resorptions, unless noted otherwise.
d. Early developmental age precluded evaluation.

GROUP DOSE (MG/KG/DAY)a	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	L	T	IMPLANTATIONS R	L	T	RESORP/FETUSES b R	L	A	T	RESORPTIONS c R	L	A	T
VI 0.3 mort.	5931	FOUND DEAD ON DAY 13 OF GESTATION	9	4	11	9	4	7	2	4	0	7	0	0	0	0
	5933	FOUND DEAD ON DAY 23 OF GESTATION	6	3	9	5	3	8	0	0	0	0	5	3	0	8
	5935	FOUND DEAD ON DAY 14 OF GESTATION	9	6	7	3	4	7	0	0	0	0	3	4	0	7
	5934	FOUND DEAD ON DAY 15 OF GESTATION	8	3	8	5	3	8	0	0	0	0	5	3	0	8
	5936	FOUND DEAD ON DAY 13 OF GESTATION	4	5	9	3	5	8	2	5	0	8	0	0	0	0
	5936	FOUND DEAD ON DAY 14 OF GESTATION	8	3	8	5	3	8	0	0	0	0	5	3	0	8
	5937	FOUND DEAD ON DAY 14 OF GESTATION	5	6	11	4	0	4	0	0	0	0	4	0	0	4
	5938	FOUND DEAD ON DAY 15 OF GESTATION	8	3	6	3	3	6	2	0	0	2d	3	3	0	6
	5939	ABORTED ON DAY 15 OF GESTATION	2	7	9	2	7	9	0	0	0	0	ELR	E ALR	1	9
	5940	FOUND DEAD ON DAY 12 OF GESTATION	8	3	11	7	3	10	7	3	0	10d	0	0	0	0

R = RIGHT L = LEFT T = TOTAL A = ABORTED LR = LATE RESORPTION
a. Days 7 through 13 or 14 of gestation.
b. See the individual fetal alterations table (Appendix 4) for fetuses with gross external and soft tissue alterations.
c. Early resorptions, unless noted otherwise.
d. Early developmental age precluded evaluation.

Litter observations (Caesarean-delivered fetuses):

The table below presents uterine parameters for the control and BMS-247550 dose groups in the initial study. No information is available for HD rabbits in the extended study as they were found dead, euthanized in moribund condition, and/or terminated following abortion. The doses in the initial study did not adversely affect the parameters measured.

Dose (mg/kg/day)	Control	Low Dose 0.01	Mid Dose 0.03	High Dose 0.11
Litters with one or more live fetus	17	20	17	18
Number implantations	9.5	7.8	8.6	9.3
Live fetuses	8.8	7.2	7.9	8.6
Percent live male fetuses/litter	50.4	52.9	49.1	46.2
Live fetal body weights/litter				
– Mean pup weights	43.85	46.99	42.41	41.79
– Mean male weight	45.10	46.57	43.56	42.64
– Mean female weights	42.88	46.40	41.70	41.53

Offspring:
The table below shows the incidence of any type of alteration in the initial study. No effect was seen for all dose groups examined. No information is available for HD rabbits in the extended study as they were found dead, euthanized in moribund condition, and/or terminated following abortion.

Dose (mg/kg/day)	Control	Low Dose 0.01	Mid Dose 0.03	High Dose 0.11
Number of pups with any alterations (%)	5 (29.4)	8 (40.0)	7 (41.2)	9 (50.0)
Number of litters with at least one pup w/ any alterations (%)	7 (4.7)	13 (9.1)	12 (8.9)	14 (9.1)
Percent of fetuses with any alterations/litter	5.6	7.7	9.6	8.9

2.6.6.7 Local tolerance No studies reviewed

2.6.6.8 Special toxicology studies No studies reviewed

2.6.6.9 Discussion and Conclusions
The general toxicology program has adequately addressed the safety of ixabepilone with appropriate animal models and dosing ranges and regimens. The primary toxicities of ixabepilone involve tissues having rapid cell division and include the GI, hematopoietic and lymphoid systems, and the male reproductive system. In rats, peripheral neuropathy was also a prominent effect. Although severe axonal/myelin degeneration in sciatic nerve was mainly seen in rats, minimal localized axonal degeneration of nerves at the injection sites was also noted in HD dogs in the 9-month study. Ixabepilone-induced toxicities were generally reversible following a 1-month, post dose recovery period, except for delayed testicular effects in rats and dogs and peripheral neuropathy in rats.

Histopathological changes in the bone marrow of rats and dogs were noted and were dose related in incidence and severity. In rats, severe cellular depletion and regenerative hyperplasia and in dogs minimal to severe cell depletion and granulocytic to mixed hyperplasia was present.

In the battery of genotoxicity studies, ixabepilone was not mutagenic or clastogenic *in vitro*. However, ixabepilone was clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus study.

Ixabepilone did not affect mating or fertility in fertility and early embryonic development studies. However, drug effects were present as evidenced by increased resorptions, reduced number of corpora lutea and increased pre and post-implantation loss. In development studies (Segment II), embryo-fetal toxicity (resorptions, abortions, decreased fetal body weights) in rats and rabbits occurred only at doses that also caused maternal toxicity. Therefore, administration of ixabepilone during pregnancy may pose a risk for fetal toxicity.

2.6.6.10 Tables and Figures – See review of individual studies

2.6.7 TOXICOLOGY TABULATED SUMMARY

Repeat Dose Toxicology Studies				
Species	**Duration/route**	**Doses (mg/kg/d)**	**Doses ($mg/m^2/d$)**	**Significant findings**
Rat	6-month/ intravenous infusion	0.7	4.2	Clinical signs: material around nose, scabbed area, and red discolored skin. Histopathology: minimal to mild atrophy of the prostate and minimal single-cell necrosis and reactive hyperpasia in the epididymis and minimal to mild single-cell necrosis of hair follicles in the skin of females.
		3	18	Mortality: 1 M. Clinical signs: reversible sparse/absent hair, yellow discoloration and unkept appearance (F only). Irreversible impaired limb function and righting reflex, lacrimation, and black/red material around eyes/nose. BW: reversible ↓ (M only). FC: Reversible ↓ (F only). Hematology: reversible ↓ in LEU (NEUT and LYMPH), HGB, RETIC, MONO, and EOS. Clinical chemistry: reversible ↓ CREA, TP, CHOL (F only) and reversible ↑ in ALT (F only). Organ weights: reversible ↑ liver (F only) and pituitary (M only) and ↓ testes, thymus, uterus and pituitary (F only) weights. Pathology: reversible decrease size of thymus (1 F) and testes and irreversible softening of testes. Histopathology: reversible cellular depletion /regenerative hyperplasia in bone marrow; thymic atrophy; lymphoid depletion/necrosis of spleen and lymph nodes; single-cell necrosis and reactive hyperplasia of most glandular mucosal sections of GI; and atrophy of uterus/vaginal epithelium. Irreversible axonal/myelin degeneration in sciatic nerve; atrophy of testes (with degeneration); and epididymis.
		6.7	40.2	Mortality: 10 M and 14 F during treatment period and 4 F during recovery. Clinical signs: thin appearance, hunched posture, audible and difficult breathing, black material around the eyes and mouth, skin cold to touch, skin discoloration on the tail, hind limb dysfunction, activity decreased and/or loss of righting reflex. Unkempt appearance, hair discoloration, splayed limbs/impaired limb function and thin appearance (F only) still present during recovery. BW and FC: Partially reversible ↓. Hematology: partially reversible ↓ in LEU (NEUT and LYMPH). Reversible ↓ HGB, RETIC, MONO, and EOS. Clinical chemistry: reversible ↓ K, ALP, TRIGLY, CREA, TP, CHOL (F only) and partially reversible ↑ ALT and AST. Organ weights: no organ weights were obtained during treatment at HD due to mortality. However recovery animals, ↑ in adrenal, brain, heart, kidneys, pituitary, spleen and thyroid; ↓ liver, prostate gland with seminal vesicle, testes, thymus, and uterus with cervix weights. Pathology: decrease size in thymus (F) testes, epididymides, seminal vesicle, soft testes, black discoloration of glandular stomach, enlarged spleen (1 F) and lymph node (1M), adipose depletion (1 F). Irreversible softening of testes, injection site abrasions, red foci/discoloration in glandular stomach, reduced size of thymus

Repeat Dose Toxicology Studies				
Species	**Duration/route**	**Doses (mg/kg/d)**	**Doses ($mg/m^2/d$)**	**Significant findings**
				(1 F), abscess formation in skeletal muscle. Histopathology: Reversible enlargement of spleen and mandibular lymph nodes, and erosions of glandular stomach; decreased ossification of femoral growth plate; axonal/myelin degeneration in cervical, thoracic and lumbar spinal cord; atrophy and myofiber degeneration/necrosis in skeletal muscle; acinar atrophy of salivary glands; bilateral atrophy of seminal vesicles; and acute to chronic active inflammation at injection sites. Irreversible atrophy of epididymis and seminal vesicles; cellular depletion /regenerative hyperplasia in bone marrow; thymic atrophy; lymphoid depletion/necrosis of spleen and lymph nodes; single-cell necrosis and reactive hyperplasia of most glandular mucosal sections of GI; and atrophy of uterus/vaginal epithelium.
Dog	9-month/ intravenous infusion	0.1	2	No significant findings
		0.5	10	Clinical signs: reversible hair sparse and unkempt appearance (F only). BW and FC: Reversible ↓ BW and FC (M only). Hematology: reversible ↓ LEU (NEUT and MONO), and EOS 5 days post-dose. Organ weights: reversible ↓ absolute/relative testes weights. Histopathology: reversible minimal to moderate degeneration/atrophy of seminiferous tubules of testes; minimal to severe, oligospermia/germ cell debris with single-cell necrosis/reactive hyperplasia of the ductal epithelium in epididymis; and minimal to mild axonal/myelin degeneration in nerves at injection sites.
		0.9→0.75	18→15	Mortality: 2 M and 1 F at 0.9 mg/kg and 1 M at 0.75 mg/kg. Clinical signs: reversible hair sparse and unkempt appearance (F only); feces colored red or yellow or few/absent and yellow material in the pan. BW and FC: reversible ↓. Hematology: reversible ↓ LEU (NEUT and MONO), EOS, BASO, RETIC, HGB, and ERYTHR. Clinical chemistry: reversible ↑ K and TRIGLY. Organ weights: Reversible ↓ absolute/relative thymus (M only) and irreversible ↓ testes and epididymis weights. Histopathology: Reversible minimal to moderate degeneration/atrophy of seminiferous tubules of testes; minimal to severe, oligospermia/germ cell debris with single-cell necrosis/reactive hyperplasia of the ductal epithelium in epididymis. Reversible thymic atrophy with lymphoid depletion in spleen, mandibular, mesenteric and tracheobronchial lymph nodes, gut-associated lymphoid tissue (GALT), and nictitans glands; partially reversible (incidence and/or severity) minimal increases in extramedullary hematopoiesis in adrenal glands; and reversible enteropathy (including single-cell necrosis and reactive hyperplasia) of the intestinal tract. Irreversible minimal to mild axonal/myelin degeneration in nerves at injection sites.

Genetic Toxicology Studies				
Study		**Concentration/ Doses**		**Significant findings**
Bacterial mutagenesis		50-5000 µg/plate		Negative
Human peripheral lymphocytes		62.5-2000 µg/mL		Negative
In vivo rat micronucleus		0.3125-1.25 mg/kg/day		Positive
Reproductive Toxicology Studies				
Species	**Duration/route**	**Doses (mg/kg/d)**	**Doses ($mg/m^2/d$)**	**Significant findings**
Rat	Fertility and early embryo/slow bolus intravenous injection. Males dosed 2 weeks premating to D45. Females dosed 2 weeks premating until GD7.	0.02, 0.06, and 0.2	0.12, 0.36, and 1.2	No adverse effect on mating or fertility at ≥0.02 mg/kg. ↓ BW, BW gain (F only), and FC (M and F) at 0.2 mg/kg. Significant ↑ resorptions, pre-and post-implantation loss and ↓ number of corpora lutea at 0.2 mg/kg.
Rat	Embryo-fetal/slow bolus intravenous injection. Females dosed GD 6-15.	0.02, 0.08, and 0.3	0.12, 0.48, and 1.8	HD (0.3 mg/kg): decrease in maternal BW (including BW loss) and FC; embryo-fetal death with decreases in number of live fetuses and fetal BW; significant ↑ resorptions and post-implantation loss; abnormalities included reduced ossification of caudal vertebrae, sternebrae, and metacarpals.
Rabbit	Embryo-fetal/slow bolus intravenous injection. Females dosed GD 7-19.	0.01, 0.03, 0.11 and 0.3	0.12, 0.36 1.32 and 3.6	Due to a lack of toxicity at 0.11 mg/kg, study was extended to two additional groups of rabbits at 0 and 0.3 mg/kg. HD (0.3 mg/kg) led to severe toxicity resulting in mortality of all does. Maternal toxicity included: significant ↓ in BW (including BW loss) and FC. ↑ resorptions in litters including 10 litters with resorbed conceptuses. No significant findings at 0.01, 0.03, and 0.11 mg/kg dose.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
The non-clinical program of ixabepilone identified the target areas of toxicity to be the, GI, hematopoietic and lymphoid systems, and the male reproductive systems. It was genotoxic *in vivo* but not teratogenic. Though not teratogenic, there is a significant amount of embryo-fetal toxicity in rats and rabbits at doses that also caused maternal toxicity. Therefore, administration of the drug may pose potential risk for fetal toxicity.

Unresolved toxicology issues (if any): None

Recommendations: None

Suggested labeling:
Presented in a separate labeling review

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Robeena Aziz
10/1/2007 05:47:30 PM
PHARMACOLOGIST

John Leighton
10/2/2007 08:32:50 AM
PHARMACOLOGIST