Nasal cavity	X		X	X
Optic nerves	X	X	X	X
Ovaries	X	X	X	X
Pancreas	X	X	X	X
Parathyroid	X	X	X	X
Peripheral nerve	X	X	X	X
Pharynx	X		X	X
Pituitary	X	X	X	X
Prostate	X	X	X	X
Rectum	X	X	X	X
Salivary gland	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X		X	
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X	X	X	X
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X	X	X	X
Thymus	X	X	X	X
Thyroid	X	X	X	X
Tongue	X	X	X	X
Trachea	X		X	X
Urinary bladder	X	X	X	X
Uterus	X	X	X	X
Vagina	X	X	X	X
Zymbal gland	X		X	X

REPRODUCTIVE TOXICOLOGY:

Fertility and Early Embryonic Development in Rats

Study No: and number: PH-28846, #T2060592
Site and testing facility: Bayer AG, Institute of Toxicology, Wuppertal, Germany
Study initiation date: October 19, 1998
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 503880
Protocol reviewed by Division Yes ( ) No (X):

Methods:

- Species/strain: Wistar rats
- Doses employed: 0, 6, 25, 100 mg/kg/day in 0.5% Tylose (methylhydroxyethylcellulose, 10 mg/kg/bw)
- Route of Administration: Orally by gavage
- Study Design: Male rats were treated for 4 weeks prior to mating and during the mating period. Female rats were treated for 2 weeks prior to mating, during mating and through day 7 of gestation. Cesarean sections were performed on day 14-16 of gestation.
- Number of animals/sex/dosing group: 24/sex/dose
- Parameters and endpoints evaluated: mortality and signs, body weight, food and water consumption, gross pathology of adult animals , reproductive organ weights and histopathology. Female specific data included: vaginal cycles, time to insemination, # of corpora lutea, implantations, live embryos, resorptions.
- Statistical evaluations: ANOVA with Dunnett's posthoc test.

Results:

- Clinical signs: Reddish appearance of ears for 2-4 hours postdose beginning 30 minutes after dosing in all BAY treated groups. Increased salivation in some HD rats (5 males , 1 female). Light colored feces were observed in 16 /24 HD males and 24.24 HD females.
- Mortality: none
- Body weight: Body weight gain in males and females was decreased during the pre-mating period.

Body Weight Gain (g) in BAY 38-9456–treated Rats

Time period	Controls	6 mg/kg/day	25 mg/kg/day	100 mg/kg/day
Males				
Days 1-29	60	53	54	41 (↓ 30%)
Females				
Days 1-8	5.4	6.7	5.3	2.5**
Days 8-15	5.4	5.0	2.8**	4.6
Days 0-7, p.c.[a]	23	20	17.9**	15.5**
Days 7-14, p.c.	27	27	24	25
Total gain	61	58.7	50 (↓ 15%)	47 (↓ 20%)

a. p.c. = postcoitus ** - $P < 0.01$

- Food consumption: Decreased food consumption during first week of study in HD males and females (during pre-mating treatment period).
- Water consumption: Increased in 3/24 MD and 4/24 HD males.
- Toxicokinetics: not performed.
- Fertility in Males
  - In-life observations: Mating index was not affected by treatment. **Fertility index was slightly reduced in high dose males : 88% , 96%, 92%, 75% in C, LD, MD, HD, respectively.**
  -
- Fertility and Early Embryonic Development in Females
- In-life observations: Drug treatment had no effect on estrus cycling or time to insemination.
  - Terminal and Necroscopic evaluations: Drug treatment had no effect on number of corpora lutea, implantation sites, preimplantation loss, or postimplantation loss (see table).

Reproductive Data Obtained at Ceserean Section

Dose, mg/kg/d	0	6	25	100
Number of dams	21	23	22	18[a]
Number of corpora lutea	14.2	13.7	13.4	13.2
Implantations	12.1	12.9	12.5	12.4
Preinplantation loss	2.1	0.9	0.9	0.8
Postimplantation loss	1.0	0.8	0.7	1.4
Viable embryos	11.1	12.0	11.8	11.0

a. only 18 dams due to decreased fertility.

- Terminal and Necroscopic Evaluations:

-Organ weights- there were no drug-related effects on testicular or ovarian weights. 1/24 HD males had decreased prostate and seminal vesicle size upon gross evaluation.

Summary and Evaluation: The only finding in this rat fertility study was decreased fertility in HD rats. The sponsor discounts the finding as being drug-related stating the rate is within the historical control range. However, this explanation is unlikely since testicular degeneration was observed infrequently in rats and dogs treated with BAY 38-9456 in the 3 month oral toxicity studies. In addition, testicular degeneration is a class effect observed with other PDE V inhibitors (IC 351, Viagra). It is recommended that Bayer conduct additional evaluations of spermatogenesis, including evaluation of sperm counts, motility, and morphology in ongoing chronic toxicity studies with BAY 38-9456.

Developmental Toxicity in Rats

Study No: T2061375 (report PH-29093)
Site and testing facility: Institute of Toxicology , Wuppertal, Germany
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Batch number: 503845, 83.7% free base
Protocol reviewed by Division Yes ( ) No (X):
Study initiation: August 4, 1998
Methods:

- Species/strain: Rat, Wistar
- Doses employed: 0, 3, 18, and 100 mg/kg/day in 0.5% Tylose

- Route of Administration: Oral , by gavage, 10 ml/kg
- Study Design: dosing days 6-17 post coitus (pc)
- Number of animals/sex/dosing group: 24/dose main study; 6 /dose for toxicokinetics
- Parameters and endpoints evaluated: clinical signs (daily), body weight (daily), food consumption (3 day), gross and histopathology (pc Day 20 for main and Day 17 for satellite), toxicokinetics via retroorbital venous plexus at 0.5, 2, 7, 24 h after the 11th dose and Day 17 at 0.5 h after the 12th dose, corpora lutea, implantations, uterus and placental weight, early/late resorptions, viable fetuses, sex, fetal weight, external malformations/variations, visceral/skeletal tissue evaluation
- Statistical evaluations: ANOVA followed by Dunnett's post hoc test, Chi Square (correction according to Yates), 2 by N Chi Square follwed by Fisher's exact with Bonferroni correction, Kruskal-
- Wallis test followed by Dunn's, Welch t-test

Results:

- Clinical signs: In addition to the two females with premature deaths (#8402, 8438) another from this group, (#8336) had disturbance of gait and hypoactivity after administration. In addition to the two females with premature deaths, two additional females in this dose group had piloerection for 2-3 days during treatment as well.
- Mortality: One female (#8402) given 100 mg/kg was sacrificed moribund on day 9, another female (#8438) was found dead on day 10. Both females had piloerection, light colored feces, with severely reduced feed intake prior to death together with reduced feces in #8438 with severe body weight loss and staggering gait in #8402. Histopathology findings of the stomach (#8402; bedding in stomach, red-brown foci in gastric mucosa) and heart. Female #8402 had moderate cardiac fibrosis in addition to slight interstitial edema and minimal diffuse inflammatory infiltration. Female #8438 had moderate interstitial edema and slight myocardial necrosis and slight diffuse inflammatory infiltration.

Body Weight (g)	0 mg/kg	3 mg/kg	18 mg/kg	100 mg/kg
Main Group				
Day 20	305	297	295	273**
Gain Day 0-20	92	86	87	61**
Gravid uterus	62	55	57	44**
Carcass (body-uterus weight)	243	242	238	228**
Corrected Wt. change 0-20 days	31	31	30	17**
Satellite Group				
Day 17	261	259	262	238
Gain Day 0-17	49	45	52	30*

- Food consumption: The 100 mg/kg group had a 50% reduction in food intake from Day 6-9 which increased thereafter. Day 18-20 there was a compensatory 50% statistically significant increase. Reduced feces began day 8-9 which correlates with the reduced food intake.

	0 mg/kg	3 mg/kg	18 mg/kg	100 mg/kg
Dilation renal pelvis				3/24 bilateral slight 1/24 unilateral
Point like retractions at caudal kidney				1/24 (#8402)
Myocardial fibrosis (LV or septal)	3/30 minimal		2/30 minimal	8/30 minimal 5/30 mild 3/30 moderate
Myocardial inflamm. infiltration Diffuse				2/30 minimal-mild
Myocardial interstitial edema				2/30 mild-moderate
Myocardial necrosis				1/30 mild

The sponsor considers the kidney findings as unrelated to treatment because they were within historical control range and were not seen in a 4 week study in rats using 6, 25, 100 mg/kg.

Toxicokinetics:	3 mg/kg		18 mg/kg		100 mg/kg	
	Dam (pc Day 16)	Fetus (pc Day 17)	Dam (pc Day 16)	Fetus (pc Day 17)	Dam (pc Day 16)	Fetus (pc Day 17)
AUC_{0-24} (µg h/l) parent	1716	52	9501	456	58713	4281
AUC_{0-24} (µg h/l) M1	313	13	3171	317	14726	14137

Female Fertility/Early Embryonic Development

Terminal and Necroscopic evaluations: The number of females with viable fetuses as a percentage of the number with implantations was reduced in the 18 mg/kg and 100 mg/kg groups, based on one dam with total early resorptions (#8384; 18 mg/kg; one fetus) and female #8325 of the 100 mg/kg group comprising 4 fetuses. The total resorption in the 18 mg/kg group is within historical range whereas treatment relationship cannot be excluded for the 100 mg/kg particular with the associated maternal toxicity and increased postimplantation loss (main, satellite) in this dose group. The postimplantation loss was evident only on a group basis, however the mean vlaue per female was above historical control resulting in a slightly reduced litter size compared to historical data. This in association with the maternal toxicity in this group led the sponsor to conclude that the postimplantation loss was treatment related. The fetal weight reduction in the 100 mg/kg group is above historical control values and is observed in both genders.

Cesarean Section	0 mg/kg	3 mg/kg	18 mg/kg	100 mg/kg
Preimplantation loss/group Loss/F	26 1.2	52** 3.1*	30 1.5	42* 2.3
Implantations/group (F with viable fetuses)	248	183**	218	198*
Implantations %/group %/dam with viable fetuses	92 93	89 88	91 91	78** 76**
Postimplantation loss/group (F with viable fetuses)	19	20	19	44**
Late Resorptions/group (F with viable fetuses)	19	19	19	44*
Placental wt. (g)	0.63	0.7	0.64	0.52**
Fetal Wt. (g)	3.63	3.58	3.69	3.1**

Embryo-fetal Development/Terminal and Necroscopic evaluations: Total fetuses with malformations and affected litters were increased (not significant), above the control and historical range data on a fetal basis (0-4.5%) and on a litter basis (0-40%). In the 100 mg/kg group 3 fetuses out of 2 litters had abnormal shape of the os palatinum and os exoccipitale and one case of cleft palate with related external findings (fetus #101 from female #8332) combined with 2 fetuses with alterations of sternebrae, vertebrae and ribs. The malformations of these underweight fetuses were considered hypoxia related events attributed to the vasodilator activity of this PDE V inhibitor. Retarded skeletal development was observed at the 100 mg/kg level which consisted of statistically significant delayed ossification of phalanges of digits, toes, metacarpals/metatarsals, sternebrae, vertebral arches/bodies, slight enlargement of fontanelles and increased incidence of asymmetric 4th sternebrae variation on a fetal/litter basis. The retarded skeletal development seems to correlate to the reduced fetal body weights and suggests this effect is drug related according to the sponsor.

Summary and Evaluation: Severe maternal toxicity at 100 mg/kg consisting of increased mortality (2/30) decreased weight (and gain), decreased food intake, light and reduced feces, gross pathology of stomach (filled with bedding, reddish brown spot in gastric mucosa) and histopathology of the myocardium. Cardiac histopathology revealed minimal to moderate fibrosis of the myocardium at 100 mg/kg and acute edema, necrosis, diffuse inflammatory infiltration in high dose females (including premature deaths).

One dam in the 100 mg/kg group had a total resorption. This group had an increased incidence of postimplantation loss and reduced litter size. Placental and fetal weights were reduced in the 100 mg/kg group in addition to retarded fetal ossification and increased incidence of skeletal variations (asymmetric sternebrae). Fetuses

from the high dose dams had reduced body weights and 3 fetuses from 2 litters had malformations of the craniofacial bones including cleft palate/alterations of os palatinum and os exoccipitale combined with sternal, vertebral or rib findings together with increased total malformations were considered drug related by the sponsor. Fetal exposures (AUC) were equivalent (96-98%) to maternal at the 100 mg/kg/day group at 0.5 h post dose. The sponsor suggests a NOAEL for maternal and developmental toxicity of 18 mg/kg/day.

Developmental Toxicity Study in Rabbits After Oral Administration

Study No: T4062097 (#025, volumes 9.2 – 9.3)
Site and testing facility: Institute of Toxicology, Wuppertal, Germany
GLP compliance: Yes
QA- Reports Yes (X) No ( ):
Lot and batch numbers: 503845
Protocol reviewed by Division Yes ( ) No (X):
Study initiation date: August 4, 1998

Methods:

- Species/strain: Himalayan female rabbits,
- Doses employed: 0, 3, 18, 90 mg/kg/day BAY 38-9456 in 0.5% Tylose
  Rationale for dose selections not provided, however, a statement is made that 1 F dosed with 100 mg/kg and ½ females dosed with 125 mg/kg had total resorptions in preliminary tolerability studies.
- Route of Administration: orally by gavage
- Study Design: 20 females /dose were administered drug on days 6 to 20 post conception and fetuses were delivered via cesarean section on day 29.
  An additional 3 rabbits/dose were treated for determination of test compound concentrations in dams and fetuses.
- Parameters and endpoints evaluated: signs, body weight, food consumption, # of corpora lutea, implantations,early and late resorptions, uterine and placental weights, live and dead fetuses, fetal sex and weights, external, skeletal, and internal fetal anomolies, maternal toxicokinetics and fetal drug concentrations.
- Statistical evaluations: ANOVA with Dunnett's in cases of significance.

Results:

- Mortality: One control female found dead on day 28. No deaths in BAY treated rabbits.
- Clinical signs: reddened ears and hypoactivity observed in two HD females each lasting 2 hours (2-4 hours postdose) on a single day.
- Body weight- gain modestly decreased (15%) in mid and high dose females
  (Gain day 0-29 = 183, 181, 156, 150 g in C, LD, MD, HD).
- Food consumption: slightly decreased on dosing days 6 – 12 in mid and high dose dams.
- Water consumption – increased in HD females.

- **Fertility and Early Embryonic Development in Females**
  - In-life observations
  - Terminal and Necroscopic evaluations: The number of corpora lutea was significantly reduced in the HD group but was within historical control range.
  - The getation rate was decreased in the HD group due to total (late) resorption in one female. The affected female had decreased food consumption and marked body weight loss from day 5 –22 of gestation (during dosing interval).

Reproductive Endpoints

Dose	Control (0)	3 mg/kg	18 mg/kg	90 mg/kg
Fertility rate	94.4	95.0	100	90
# of corpora lutea	8.6	8.2	8.1	7.2
Preimplantation loss	1.6	0.2	1.0	1.6
Implantations	6.9	8.1	7.1	5.7
Gestation rate	100	100	100	94
Placental weight	4.2	3.9	4.4	4.6
Postimplantation loss (per female)	0.3	0.7	0.8	1.2
Postimplantation Loss/ group	5 (4.2%)	14 (9.2%)	15 (10.6%)	17 (17.3#)**

Reproductive Endpoints Continued

Dose	Control (0)	3 mg/kg	18 mg/kg	90 mg/kg
Number of fetuses	6.6	7.3	6.3	4.8*
% Males	45.8	58.5	50.7	59.2
Fetal wt., g	39.0	35.8	38.5	37.6

* - statistically different from control, P< 0.05 ; ** P< 0.01

a. Gestation rate = % with viable fetuses on day 29.

- Embryo-fetal Development
  - Terminal and Necroscopic evaluations:
- **Dams:** Mononuclear cell infiltration in the myocardium and mural mineralization of the aortic trunk were observed. The incidence and severity did not display a clear dose-related pattern and the sponsor attributes the findings as background lesions.
  Myocardial mononuclear infiltration – 3C, 2 LD, 4 MD, 5 HD
  Aortic mineralization –6 C, 5 LD, 9 MD, 6 HD
  Myocardial fibrosis – 1 HD
- **Offspring:** The incidence of malformations is summarized in the table below. All of the skeletal and internal malformations observed are within the historical control incidence for studies conducted by Bayer in the 1990s. (21 studies).

Malformation	Controls	3 mg/kg	18 mg/kg	90 mg/kg
Number of fetuses/ group	N = 113	N= 139	N = 127	N = 81
Malposition of forelimbs		4 (4)	2 (2)	1
Cardiac septal defect			1	
Malformation of heart/major vessel		1		
Fusion/bifurcation of ribs	3 (3)		2 (2)	4 (4)
Missing lumbar vertebra	4 (1)		2 (1)	

( ) – number of litters affected

There were no significant differences in the number of fetuses with malformations , % of malformed fetuses /group, number of litters with malformations, or % of malformed litters /group.

Toxicokinetics (Study PH-29153): Data is presented as geometric mean±SD, Gestation Day 20; Note: µg/l = ng/ml

Dose	Maternal Plasma (µg/l)		Fetal Tissue (µg/kg)	
Mg/kg	BAY 38-9456	BAY 44-5576	BAY 38-9456	BAY 44-5576
3	14±1.9	39±1.2	3.7±2	<2
18	378±2.3	803±1.9	93±2	7.8±1.4
90	5446±1.1	11486±1.2	2237±1.1	138±1.3

Summary and Evaluation: The sponsor suggests that maternal toxicity could not be exluded at the 18 mg/kg (one female) and was evident at 90 mg/kg. One total resorption was most likely due to distincet systemic toxicity in the affected female at the 90 mg/kg level. A marginally increased postimplantation loss and an equivocal retradation of ossification was evident at the 90 mg/kg level. Therefore the sponsor suggests a maternal NOAEL =3 mg/kg/day with a developmental NOAEL=18 mg/kg/day.

GENETIC TOXICOLOGY:

V79-HPRT Test for Detection of Induced Forward Mutations

Study No: T 3059810

Study Type: Invitro assay to detect point mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 Chinese hamster lung cells.

Amendment # 018, Volume # 7.5 and Page # 1:

Conducting Laboratory: Bayer AG, PH-PD Toxicology, Carcinogenicity and Genotoxicity

Date of Study Initiation/completion: Trial 1- 10/9/98; Trial 2 – 10/30/98

GLP Compliance: Yes

QA- Reports Yes (X) No ( ):

Drug Lot Number: 503836

Study Endpoint: V79 cells are unable to use 6-thioguanine(6-TG) for DNA synthesis due to a mutation at the HPRT locus. Mutagens or promutagens which induce forward mutations at the HPRT locus enable V79 cells to utilize 6-TG. Therefore, the study endpoint is an increase in colony formation in the presence of 6-TG.

Methodology:

- Strains/Species/Cell line: V79 Chinese hamster lung cells (doubling time of 10-14 hours)
- Dose Selection Criteria:
  - Basis of dose selection: Concentrations up to 320 ug/ml were tested both with and without S9 activation. No cytotoxicity was observed but significant precipitation of BAY 38-9456 was observed at concentrations ≥ 250 ug/ml.
  - Range finding studies:
- Test Agent Stability:
- Metabolic Activation System: S9 fraction from Aroclor 1254-induced male SD rats purchased from [redacted] lot # 85681.
- Controls:
  - Vehicle: BAY-38-9456 was dissolved in DMSO. Concentrations up to 40 ug/ml did not change pH or osmolality of the medium.
  - Negative Controls: 1% DMSO
- Positive Controls: Ethyl methanesulfonate (EMS) 900 ug/ml was used in nonactivated samples. Dimethylbenzanthracene (DMBA) in DMSO, 20 ug/ml was used in samples with metabolic activation.
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: V79 cells (4×10^6) exposed to BAY 38-9456 for 5 hours, then washed with PBS. 1.5×10^6 cells were cultured in a flask and cells were plated to 8 petri dishes (200 cells /plate for cloning efficiency and 3×10^5 cells for mutant selections). Cells were grown for 7 days and colonies fixed, stained with Geimsa and counted for 6-TG resistant colonies.
  - Doses used in definitive study: 10, 20, 40, 80, 160, 320 ug/ml

- Analysis:
  - No. plates analyzed: 2 flask of cells were treated per dose. Treated cells from each flask were then transferred to 1 flask and 8 plates. Two trials were performed.
  - Counting method: Automatic colony counting (instrument not specified)
  - Cytotoxic endpoints: cloning efficiency, cell growth and survival
  - Genetic toxicity endpoints/results: Mutant frequency.
- Statistical methods: Weighted ANOVA followed by pairwise comparisons To vehicle control for significantly different groups using Dunnett's test.

Criteria for Valid Assay:

1. cloning efficiency > 50%,
2. Spontaneous mutant frequency of vehicle controls $< 25 \times 10^{-6}$
3. Positive control induces an average mutant frequency > 3 times vehicle controls
4. High dose should produce low relative survival (0-30%) or should result in precipitation in the medim.

Criteria for Positive Assay: Concentration-related increase in mutant frequency is observed which is reproducible in duplicate assays.

Results:

- Study Validity: All criteria were met and therefore, assay is valid.
- Study Outcome: Cytotoxicity was not observed as evidenced by the absence of decreases in relative survival or growth. No increases in frequency of forward mutations was observed in V79 cells treated with BAY 38-9456. The positive controls , EMS and DMBA, induced significant increases in the number of HPRT revertant colonies.
- Data from the two separate trials are presented below.

BAY 38-9456 without Metabolic Activation

Treatment without S9	Relative Growth, (% Vehicle Control)		Mutant Frequency x 10^6	
	Trial 1	Trial 2	Trial 1	Trial 2
Negative Control (water)	153, 115	105, 89	1.5, 2.5	0.8, 0.6
Vehicle Control (1% DMSO)	100, 100	100, 100	1.2, 0.6	1.1, 0.7
Positive Control , EMS 900ug	35, 85	54, 51	**624, 354**	**147, 199**
BAY 38-9456, ug/ml				
10	64, 153	72, 56	0.6, 0.6	0.5, 0.9
20	70, 196	93, 63	0.6, 1.3	2.9, 1.1
40	57, 137	80, 48	0.5, 1.2	0.5, 2.2
80	67, 126	88, 66	1.5, 0.6	4.8, 1.1
160	78, 119	90, 100	0.6, 1.5	1.3, 4.3
320	26, 126	76, 37	2.3, 1.2	0.7, 0.4

BAY 38-9456 with Metabolic Activation

Treatment with S9	Relative Growth, (% Vehicle Control)		Mutant Frequency x 10^6	
	Trial 1	Trial 2	Trial 1	Trial 2
Negative Control (water)	146, 120	138, 140	0.7, 1.3	0.5, 0.6
Vehicle Control (1% DMSO)	100, 100	100, 100	0.5, 0.7	0.8, 0.9
Positive Control DMBA, 20ug	66, 122	70, 120	**60, 76**	**83, 48**
BAY 38-9456, ug/ml				
10	81, 141	66, 114	0.4, 3.7	0.9, 0.7
20	90, 156	66, 164	0.5, 0.5	0.7, 0.6
40	101, 76	71, 188	2.1, 0.5	0.3, 1.1
80	106, 89	72, 143	1.2, 0.6	0.4, 0.4
160	45, 107	60, 108	4.5, 0.8	0.4, 0.8
320	88, 83	61, 55	2.5, 0.9	0.4, 1.2

Summary: BAY 38-9456 was concluded to be non-mutagenic in incubations with and without metabolic activation.

OVERALL SUMMARY AND EVALUATION: BAY 38-9456 is a cyclic GMP-phosphodiesterase type V inhibitor similar to sildenafil citrate (Viagra), being developed for treatment of erectile dysfunction.

BAY 38-9456 is rapidly metabolized to the active metabolite BAY 44-5576 (M1) in all species. The half life of the metabolite is significantly longer than the parent and therefore contributes to the biological activity. BAY 38-9456 is extensively bound to plasma proteins 93-95% in mice, rats, humans and 87-88% in dogs and 96-97% in rabbits.

Phosphodiesterase V inhibitors are associated with disseminated arteritis and testicular atrophy. Arteritis has been demonstrated in multiple species including rat, dog and monkey. Testicular atrophy has been shown in dogs. The 14-week rat study shows drug related deaths in 4/10 rats given 125 mg/kg/day secondary to myocardial lesions. Non-reversible myocardial fibrosis was observed in 2/10 M, 9/10 F from the main study and 2/10 M and 8/10 F from the 4 week recovery group. Myocardial lesions were not observed at ≤25 mg/kg/day in males and ≤5 mg/kg/day in females (Cmax <1000 ng/ml; 16-40X human Cmax based on a therapeutic dose of 20 mg). Hematologic changes (RBC,WBC) and increased adrenal, kidney, liver and testes weights were observed in rats given 125 mg/kg/day. The hematologic changes were reversible after a 4 week recovery period. Female rats were more severely effected than males since exposures were higher than males with equivalent dosing. This is attributable to the significant sexual dimorphism in the metabolism of BAY 38-9456 in rats due to sex differences in CYP 3A4, the major enzyme involved in metabolism of BAY 38-9456. Sex differences in kinetics were not observed in mice, dogs and are not expected in humans. Seminiferous tubule degeneration/atrophy was observed in 1/10 M at 25 mg/kg/day and 1/10 M at 125 mg/kg/day. Acinar atrophy of the pancreas, parotid and submandibular glands were observed with increased incidence at doses ≥25 mg/kg/day. Retinal atrophy and/or degeneration of the optic nerve were observed in 2/10 M and 1/10 F.

In the 27 week rat study 1/20 females given 75 mg/kg/day died on Day 5 with evidence of myocardial damage. This represents an AUC exposure of >180X human. High dose females (75 mg/kg/day) had increased myocardial fibrosis (5/20). Minimal increases in hemoglobin and mean corpuscular hemoglobin concentrations with mild increases in leukocyte and lymphocyte counts were observed in rats of both genders given 75 mg/kg/day. Dose related increases in heart, kidney, and liver weights were observed at 15 mg/kg/day, (10%) and 75 mg/kg/day (15-30%). High dose rats of both genders had histopathology including vacuolization of the adrenal zona glomerulosa, acinar hypertrophy of the pancreas and salivary glands. The mid dose of 15 mg/kg/day (>7X human exposure) was considered the NOAEL for the 6 month study.

In the 13 week dog toxicity study, ¼ male dogs receiving ≥3 mg/kg/day and ¼ given 30 mg/kg/day had corneal opacity and discoloration. Urinalysis revealed an increased frequency of significant blood in the urine of treated dogs. Heart and liver weights were mildly increased in males given ≥10 mg/kg/day and females given 30 mg/kg/day. Thymic weights were decreased in males given 30 mg/kg/day. Histopathologic findings were observed with increased frequency in treated dogs as follows:

- **Heart:** minimal to mild periarteritis /arteritis and intramural edema were observed at multiple locations in the hearts of mid and high dose dogs (right and left atria and ventricles, papillary muscle). May be secondary to hemodynamic changes since it was observed in dogs dosed with 10 and 30 mg/kg/day, doses associated with significant hemodynamic changes, but not in dogs dosed with 1 or 3 mg/kg/day in which hemodynamic effects were absent. Decreased total peripheral resistance (30-50%), hypotension and reflex tachycardia were observed in dogs dosed single or multiple s of BAY 38-9456 ≥3 mg/kg (>6 X human therapeutic exposures with 40 mg). The hypotensive effect was more severe with first dose in some dogs.
- **Kidney:** karyomegaly was observed in the renal proximal tubules of treated dogs. Bayer states it is a familial finding in their colony, but historical rates were not provided and the finding was only observed in treated dogs.
- **Liver:** a dose-related increase in the incidence but not the severity (minimal to mild) of cytoplamic inclusions was observed.
- **Eyes:** increased incidence of vacuolization of the lens in treated dogs :33%, 75%, 63%, 87%, and 55 % at 0, 1, 3, 10, 30 mg/kg/day, respectively . Severity was minimal at lower doses and mild in HD dogs.
- **Focal hemorrhages:** were observed more frequently in the lungs, mesenteric lymph nodes, tonsils, spleen, and brain stem of treated dogs, even at the two lowest dose levels which did not produce significant hemodynamic changes.
- **Aorta** – intramural fiber disorganization observed at all doses.
- **Pancreas**- increased apoptosis and atrophy at all doses.
- Testes- focal degeneration of seminiferous tubule germinal epithelium.

The pathologic findings are of concern since they were observed even at the two lowest dose levels (1, 3 mg/kg/day) that produce drug exposures equal to or less than therapeutic. However, the toxicities were observed with daily dosing while clinical use is "as needed" and protein binding was less extensive in dogs than other species, therefore, free drug concentrations are twice as high in dogs as in other species.

Fewer dogs are effected in the 52 week study with testicular atrophy than in the 3 month study suggesting that the testicular toxicity is not progressive. Those affected (¼ HD, MD) occurred at doses ≥10 mg/kg/day (≥10 X human exposures). The testicular toxicity observed in dogs is less severe than for other PDE V inhibitors since it occurred less frequently and only at significant multiples (19X) of the human therapeutic exposures. Sperm analysis was not warranted in the clinical trial. Histopathological evidence of arteritis was not observed, although one male and female given 30 mg/kg/day had evidence of cardiac periarterial edema. Significant dose related decreases (>20 mm Hg) in systolic and diastolic blood pressure were observed 2h post dose in dogs of both sexes given ≥10 mg/kg/day. However significant, dose related increases in heart rate occurred at 10 mg/kg/day (35-40%) and 30 mg/kg/day (50-80%). The low dose (3 mg/kg/day) had no effect on BP or HR.

Decreased fertility in rats was observed at doses ≥ 100 mg/kg/day. The sponsor discounts this finding as being within historical control range. However testicular atrophy with oligo-/aspermia has been associated with this drug class and has been demonstrated at this dose level in rat toxicity studies (14 & 27 week). Maternal toxicity at 100 mg/kg consisting of increased mortality (2/30) decreased weight (and gain), decreased food intake, gross pathology of stomach (filled with bedding, reddish brown spot in gastric mucosa) and histopathology of the myocardium consisting of minimal to moderate fibrosis at 100 mg/kg with acute edema, necrosis and diffuse inflammatory infiltration which included premature deaths. One dam in the 100 mg/kg group had a total resorption. This group had an increased incidence of postimplantation loss and reduced litter size. Fetuses from the 100 mg/kg/day dams had reduced body weights and placental weights in addition to retarded fetal ossification and increased incidence of skeletal variations (asymmetric sternebrae). Fetuses (n=3) from 2 litters had malformations of the craniofacial bones including cleft palate/alterations of os palatinum and os exoccipitale combined with sternal, vertebral or rib findings. PK studies in pregnant rats suggests that the fetal tissue is exposed to 96-98% of maternal

dose at the 100 mg/kg/day level. The sponsor suggests a NOAEL for rat maternal and developmental toxicity of 18 mg/kg/day.

Maternal toxicity in rabbits at the 18 mg/kg dose was evident with the death of 1/20 dams. One total resorption was most likely due to systemic toxicity in the affected female at the 90 mg/kg level. A marginally increased postimplantation loss and an equivocal retardation of ossification was evident at the 90 mg/kg level. Therefore the sponsor suggests a maternal NOAEL =3 mg/kg/day with a developmental NOAEL=18 mg/kg/day in rabbit.

An in vitro assay to detect point mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 Chinese hamster lung cells demonstrates no proclivity of BAY 38-9456 to induce point mutations either with/without metabolic activation.

Clinical Relevance of Safety Issues: The testicular atrophy and disseminated arteritis are the toxicities of concern for phosphodiesterase V inhibitors. The testicular atrophy is found in dogs at exposures ≥10X achieved with a 20 mg human dose following daily dosing for one year. Overall the incidence is lower in the chronic dog study than in the 3 month duration study, suggesting that the testicular atrophy is not progressive with BAY38-945. Seminiferous tubule degeneration/atrophy was observed in 1/10 male rats given 25 mg/kg/day and 1/10 males given 125 mg/kg/day in the 14 week study (not dose dependent) and without incidence in the 27 week rat toxicity study. Therefore clinical sperm assessment is not warranted at this time. Myocardial lesions in rat, indicative of possible arteritis (no hemodynamics provided) are seen at 16-40X human Cmax values based on a 20 mg therapeutic dose. The chronic (1 yr.) dog study does not show histopathology indicative of arteritis although significant hypotension and tachycardia are observed with doses ≥3 mg/kg/day. The 13 week dog study shows evidence of arteritis with hemodynamic changes at dose ≥3 mg/kg./day (>12X human exposure with a 20 mg therapeutic dose).

RECOMMENDATIONS:

External Recommendations (to sponsor):

1. Pharmacokinetics parameters should be provided as arithmetic mean, not geometric means for all studies
2. Evaluation of spermatogenesis, including sperm counts, motility and morphology is recommended in any ongoing non-rodent chronic toxicity studies with BAY 38-9456

Reviewer signature/team leader signature of Concurrence: /S/ 8/16/00

/S/ 8/21

cc: HFD580/Davis-Bruno/Jordan/Colangelo

Appendix II

Executive CAC
February 9, 1999

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD- 900, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Alternate Member
Jeri El-Hage, Ph.D., HFD-580, Reviewer
Author of Draft: Jeri El-Hage

The following reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review (#2).

IND # : [redacted]
Drug: BAY 38-9456
Sponsor: Bayer Pharmaceuticals Division, West Haven, CT

Mouse Carcinogenicity Protocol and Dose Selections

CD-1 mice will be administered 0, 40, 200 and 1000 ppm BAY-38-9456 orally via the drinking water for 2 years. A 14-week dose-finding study conducted in CD-1 mice utilizing the same doses and route of administration revealed no toxicity that might impact on 2-year survival. The sponsor selected the high dose for use in the mouse carcinogenicity study based upon an AUC exposure multiple for parent drug in mice which is greater than 25-30 times the AUC exposure to BAY 38-9456 in men receiving the highest proposed therapeutic dose of 40 mg. The doses to be used in the mouse carcinogenicity study are expected to result in parent drug AUC exposures 1, 4-5 and > 60 times the human AUC with the 40 mg dose. The excess exposure at the highest dose of 1000 ppm should allow for any differences in the pharmacokinetics of BAY-38-9456 observed in women, the elderly, or with multiple dosing.

The use of AUC ratios for dose selection is considered generally acceptable for this study since: 1) BAY 38-9456 tested negative in a standard genotoxicity battery, 2) the in vitro metabolite profile is qualitatively similar in mice and humans, and 3) mice , rats, and humans display comparable drug protein binding (93-95%). However, AUC data for mice was calculated for day 22-23 only. Plasma concentration data collected on days 5 and 89 of the 14-week study demonstrated that concentrations of BAY 38-9456 in mice treated with the highest dose of 1000 ppm were 4 to 5-fold lower on day 89 than on day 5. There is some concern that the AUC exposures calculated from day 22 may over estimate the AUC exposures in mice by several fold during sustained dosing. Thus, the exposure margin may not exceed 25-fold.

Rat Carcinogenicity Protocol and Dose Selections

Bayer proposed dose levels of 0, 3, 15 and 75 mg/kg/day BAY 38-9456 be administered to Wistar rats (n = 50/sex/dose) orally by gavage for two years. The 13-week dose-finding study examined the effects of 1, 5, 25 and 125 mg/kg/day. Deaths were observed in 2/10 males and 3/10 females receiving 125 mg/kg/day. The deaths in the female rats were described as occurring secondary to moderate myocardial fibrosis. Histopathologic examinations suggest the deaths in the males were caused by gavage errors. The sponsor lowered the high dose to 75 mg/kg/day in both sexes based on the observed deaths in the dose-finding study. The reduction in the high dose is acceptable since the parent drug AUC exposure with 75 mg/kg/day should still exceed 25 times the human therapeutic exposure. In males these doses are expected to produce multiples ½, 5 and > 30 times human exposures to parent drug. Due to a sexual dimorphism in drug metabolism, parent drug exposures in females rats are higher than in males receiving comparable doses. Female rats treated with 25 and 125 mg/kg/day had increased heart weights and myocardial fibrosis which were dose-related in incidence and severity. The increased incidence and severity of myocardial

findings and drug-related deaths in female rats can be explained by the higher drug exposures in females. The drug-related myocardial lesions in females were observed with lower doses when administered for a longer duration (100 mg/kg/day for 1 month vs 25 mg/kg/day for 3 months). The 75 mg /kg/day dose in female rats would be anticipated to produce myocardial lesions which would impact on survival in a two year study. Thus, it was concluded by the committee that the proposed doses for females exceeded the MTD.

The protocol design of both carcinogenicity studies appears adequate with the exception that the sponsor does not have toxicokinetics data for the dose levels to be used in the rat study. The sponsor plans to conduct histopathologic evaluation of tissues from all treatment groups for both carcinogenicity studies.

Executive CAC Recommendations and Conclusions

Regarding the two-year mouse carcinogenicity study:

1. The Committee concurred with the dose selections of 40, 200 and 1000 ppm BAY 38-9456 in drinking water based upon achieving AUC multiples with the highest dose > 25 times the anticipated human AUC exposure. The concurrence is contingent upon demonstration that the exposure (AUC) to parent and metabolites remains at least 25-fold the human exposure at the therapeutic dose following prolonged treatment.
2. Kinetic data (AUC) for the parent drug and the major active metabolite (M1) at a time point after at least 3 months of dosing will be essential to confirm that total AUC exposures are greater than 25 times human exposure with the highest therapeutic dose. This could be done as part of the carcinogenicity study or as a separate study employing the doses used in the carcinogenicity study. Unless the AUC data is collected at study termination if the carcinogenicity study animals are used , we recommend the toxicokinetics samples be collected from satellite groups or a separate study to avoid mortality associated with blood sampling in the main study groups.

Regarding the two-year rat carcinogenicity study:

1. The Committee concurred with the dose selections of 3, 15 and 75 mg/kg/day BAY 38-9456 orally by gavage in male rats based upon achieving AUC multiples with the highest dose > 25 times the anticipated human AUC exposure.
2. Due to the sexual dimorphism in drug metabolism in rats with consequent higher drug exposures and greater myocardial toxicity in females, the Committee could not concur with the proposed doses for females. The committee could concur if doses of 3, 10 and 25 mg/kg/day BAY 38-9456 were used to treat female rats in the 2-year carcinogenicity study.
3. Since the kinetics are not linear and AUC has not been determined for the dose levels to be used, it is also advised that toxicokinetics (AUC) data for parent drug and the M1 metabolite be collected for the dose levels used in the rat carcinogenicity study at study termination, in satellite groups, or as a separate study.

/S/

Joseph DeGeorge, Ph.D., Chair
Cc: IND [redacted] HFD-580 IND
HFD-580/ Colangelo/ElHage/Jordan
HFD-024/ Seifried

Executive CAC
4/16/02
Mouse/Rat Carcinogenicity Study

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abigail Jacobs, Ph.D., HFD-540, alternate Member
Robin Huff, Ph.D., Alternate Member
Alex Jordan, Ph.D., Team Leader
Yangmee Shin, Ph.D., Presenting Reviewer

Author of Draft: Yangmee Shin

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: 21-400
Drug Name: BAY 38-9456
Sponsor: Bayer Corporation, West Haven, CT

Background: BAY 38-9456 is a PDE5 inhibitor for the treatment of erectile dysfunction. The major metabolite M-1 displays similar potency as the parent drug. The sponsor conducted 2-year carcinogenicity bioassays in rats by oral gavage and in mice by drinking water.

Mouse Carcinogenicity Study: The results were negative, pending statistical survival and tumor data analysis. The AUC's of the high dose of 1000 ppm for the unbound total drug (MAY 38-9456+M-1) produced approximately 21 fold in males and 37-fold in females the human exposure at the maximum clinical dose of 20 mg.

Rat Carcinogenicity Study: Benign thymomas in female rats were statistically significant by a trend test at p=0.025 but were not statistically significant by a pairwise test at mid- and high dose with p=0.2475 and p=0.1175, respectively. The sponsor stated that the tumors were not biologically significant due to lack of pre-neoplastic hyperplasias, non-malignancy, occurrence in only one sex and were within the range of historical controls (10%). The committee noted that the historical control data were not submitted and requested that these be submitted. The AUCs of the high dose of 75 mg/kg in males and 25 mg/kg in females for the unbound total drug (MAY 38-9456+M-1) produced >180 fold the human exposure at the maximum clinical dose of 20 mg. Statistical analysis of survival and tumor data is ongoing.

Executive CAC Recommendations and Conclusions:

Rat:
The Committee noted that the study was adequate and had received prior CAC concurrence.

The incidence of thymomas was positive by the trend test, but was not significantly different from controls by pairwise comparison. The Committee concluded that the study will be considered negative if the sponsor submits information on the historical control range for thymomas in female rats from the testing laboratory and the incidence of thymomas is within the historical range.

Mouse:
The Committee noted that the study was adequate and had received prior CAC concurrence.

The Committee felt that the study was negative.

/S/

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-580
/Alex Jordan, HFD-580
/Yangmee Shin, HFD-580
/Eufrecina De-Guia, HFD-580
/Adele Seifried, HFD-024

Appendix III

Statistical Review and Evaluation
Carcinogenicity

NDA No:	21-400
Applicant:	Bayer
Trade Name:	Nuviva
Pharmacologist:	Yangmee Shin , Ph.D. (HFD-580)
Statistical Reviewer:	Moh-Jee Ng (HFD-715)

Summary

- In the 2-year mouse and rat studies, there was no statistically significant positive trend in survival and no statistically significant difference in survival distributions among treatment groups for both males and females. There was no statistically significant positive linear trend in tumor incidence rates detected for both males and females.

1. Introduction

This reviewer evaluated the oncogenic potential of Bay 38-9456 that was administered orally to the test animals via drinking water for over 105 weeks. This report includes the results of the analyses of the survival and tumor data.

2. Studies Designs

The study designs of mice and rats are summarized in the following Table.

Table 1
Summary of Study Designs

Species	Mice	Rat
Study Number	PH-31279 (T7068012)	PH-31276 (T5067624)
Strain	Crl: CD-1(CR) BR	SPF-bred Wister Rats
Route of Administration	Oral	Oral
Frequency of Drug Administration	Daily	Daily
Dose Unit	mg/kg/day	mg/kg/day
Dose Level (Control, Low, Medium, High)	0, 7.0, 31.9. 150.5 for male 0, 8.5, 42.1, 193.4 for female	0, 3, 15, 75 for male 0, 3, 10, 25 for female
Number of Animals/sex/per treatment group	50 males/group 50 females/group	50 males/group 50 females/group
Length of Study	24 months	24 months

In each of these experiments there were one control group and three treated groups known as low, medium, and high. The dose levels for the treatment groups were 0, 40, 200 and 1000 ppm for mice, in an average drug intake of 7, 31.9 and 150.5 mg/kg in males and 8.5, 42.1 and 193.4 mg/kg in females over 105 weeks. Male rats received the nominal dose levels 0, 3, 15 and 75 mg/kg/day whereas female rats received 0, 3, 10, and 25 mg/kg/day. There were 50 animals of each sex in each treatment group. All surviving males and females were necropsied following a minimum of 104 weeks of dosing. The

terminal sacrifice started at and after weeks 105.

3. Sponsor's Tumor Analyses and Findings

The sponsor performed a statistical evaluation of all neoplastic findings using an exact trend test of Mehtal et al (1984, 1992). The actual doses were used as weight. Fixed time intervals at weeks 0-52, 53-78, 79-91, 92-104, and terminal sacrifice were used. The sponsor used the PathData-System and combined with the statistical program to perform all calculations. One-tailed p-values of $p < 0.05$ are considered as statistically significant and $p < 0.01$ as highly significant. The sponsor also performed a one-tailed Fisher's exact test (pairwise comparisons of control groups vs. dose groups).

The sponsor listed the following findings in its reports.

In survival analysis:

- No significant differences in the mortality among the treated groups were detected when compared to the control for both mice and rats.
- For mice, 94/200 males and 127/200 females; for rats, a total of 61/200 males and 79/200 females died or were killed in extremis before termination of the study.

In tumor analysis:

- No significant positive linear trends in incidence rate in tumor data were detected for both mice and rats.
- There were increases in incidence of hemangiomas, hemangiosarcomas and histiocytic sarcomas in female mice at high dose, none of these were statistically significant.
- There was an increase in incidence of benign thymoma (4, 4, 3, 7 p=0.025) in female rats but a reduction in males (2, 2, 0, 0). The sponsor indicated that the tumors were incidental due to the late onset of the tumor.

The sponsor concluded that there was no carcinogenic potential treated with BAY 38-9456 for mice and rats in both males and females over 105 weeks.

4. Reviewer's Evaluation

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor, using the programs written by Dr. Ted Guo of Division of Biostatistics II. The primary statistical methods used were described by Peto *et al.* (1980), and Lin and Ali (1994). These methods adjust differences in animal mortality and take the fatal or prevalence context of observation of the tumor into consideration. The intervals used for the adjustment of mortality were 0-52, 53-78, 79-91 and 92-103 weeks and terminal sacrifice for animals. The actual doses were used as weights in the analyses.

The statistical analyses of carcinogenicity study data consisted of two parts, namely, the survival data analysis and the tumor data analysis. The survival data analysis was: 1) to

examine the differences in survival distributions among the treatment groups (homogeneity test); and 2) to determine if there is a positive trend in the proportion of deaths with respect to the dose levels (Trend test). Two statistical tests were used in the survival data analysis: the Cox test and the generalized Kruskal-Wallis test. The theoretical background of these tests was described by Lin and Ali (1994) and Thomas *et al* (1977).

The tumor data analysis was to determine if there is a positive trend in the proportions of a selected tumor type in a selected organ/tissue with respect to the dose levels. The tumors were classified as either fatal (lethal) or non-fatal (non-lethal), according to Peto *et al* (1980). The reviewer applied the death-rate method to fatal tumors and the prevalence method to non-fatal tumors. For tumors that caused death for some, but not for all, animals, a combined test was performed.

A rule for adjusting the effect of multiple testings proposed by Haseman (1983) can be used to adjust for the effect of multiple testings in pairwise comparisons. Haseman's rule says that rare tumors should be tested at 0.05 level of significance and common tumors should be tested at 0.01 level of significance. A similar rule proposed by the Office of Biostatistics, CDER/FDA for trend tests was used in this review for tests for positive trend. The rule states that in order to keep the overall false-positive rate at the nominal level of approximately 0.1, tumor types with spontaneous tumor rates of 1% or less (rare tumors) should be tested at 0.025 significance level, otherwise (common tumors) at 0.005 significance level (Lin and Rahman, 1998).

4.1 Evaluation of Carcinogenicity Study on Mice

This reviewer's evaluation comprises the following components:

- Survival data analysis
- Tumor data analysis

4.1.1 Survival Data Analysis of Mice

The survival data analysis determines whether the dose-mortality trend in mortality is statistically significant. A positive result indicates that mortality increases as the dose level increases.

- Tables 2 and 3 present the cumulate percentages of death by dose group for female and male, respectively. The time interval "Final Kill 104-105" presents the terminal-sacrifice interval.
- Figures 1 and 2 present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female and male, respectively.
- Tables 4 and 5 present results of test for dose-mortality trend for female and male using the methods described in the paper "Trend and Homogeneity Analysis of Proportions and Life Table Data" version 2.1, by Donald G. Thomas, National Cancer Institute.

Table 2
Cumulative Percentages of Death in Female Mice

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR	0-52	50	4	46	92	8
	53-78	46	9	37	74	26
	79-91	37	10	27	54	46
	92-103	27	10	17	34	66
	FINALKILL104-106	17	17	0		
LOW	0-52	50	3	47	94	6
	53-78	47	9	38	76	24
	79-91	38	8	30	60	40
	92-103	30	12	18	36	64
	FINALKILL104-106	18	18	0		
MED	0-52	50	3	47	94	6
	53-78	47	6	41	82	18
	79-91	41	11	30	60	40
	92-103	30	12	18	36	64
	FINALKILL104-106	18	18	0		
HIGH	0-52	50	1	49	98	2
	53-78	49	8	41	82	18
	79-91	41	8	33	66	34
	92-103	33	12	21	42	58
	FINALKILL104-106	21	21	0		

Figure 1
Kaplan-Meier Survival Functions for Female Mice

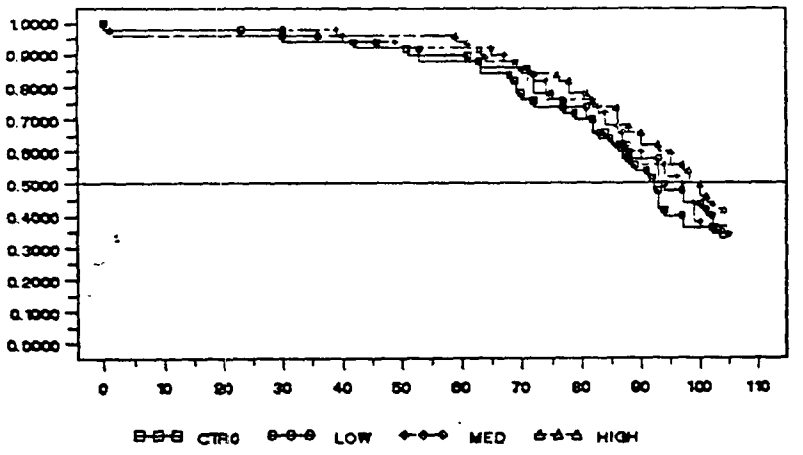
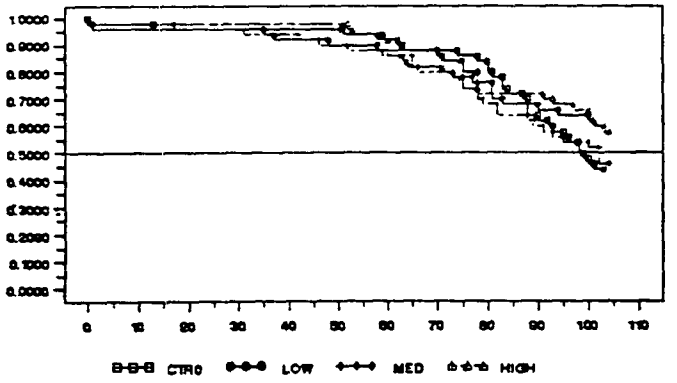


Table 3
Cumulative Percentages of Death in Male Mice

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR	0-52	50	2	48	96	4
	53-78	48	5	43	86	14
	79-91	43	11	32	64	36
	92-103	32	10	22	44	56
	FINALKILL104-106	22	22	0		
LOW	0-52	50	4	46	92	8
	53-78	46	6	40	80	20
	79-91	40	6	34	68	32
	92-103	34	2	32	64	36
	FINALKILL104-106	32	32	0		
MED	0-52	50	5	45	90	10
	53-78	45	7	38	76	24
	79-91	38	8	30	60	40
	92-103	30	7	23	46	54
	FINALKILL104-106	23	23	0		
HIGH	0-52	50	1	49	98	2
	53-78	49	12	37	74	26
	79-91	37	1	36	72	28
	92-103	36	7	29	58	42
	FINALKILL104-106	29	29	0		

Figure 2
Kaplan-Meier Survival Functions for Male Mice



The dose-mortality trend tests for female mice (presented in Table 4) and male mice (presented in Table 5) are not statistically significant using the Cox test and the Kruskal-Wallis test.

Table 4
Results of Tests for Dose-Mortality trend for Female Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Depart from Trend	0.1757	0.9159	0.3435	0.8422
Dose-Mortality Trend	1.1427	0.2851	1.4291	0.2319
Homogeneity	1.3183	0.7248	1.7726	0.6209

Table 5
Results of Tests for Dose-Mortality trend for Male Mice

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Depart from Trend	3.6259	0.1632	2.4402	0.2952
Dose-Mortality Trend	0.4151	0.5194	0.2871	0.5921
Homogeneity	4.0410	0.2571	2.7272	0.4356

4.1.2 Tumor Data Analysis for Mice

The tumor data analysis determines whether the dose-tumor positive linear trend in tumor incidence is statistically significant. This reviewer tested this trend for every organ and tumor combination with the data provided by the sponsor. This reviewer analyzed the dose-tumor trend between the control group and 3 treated groups. The daily doeses 0, 40, 200, and 1000 mg/kg/day were used as weights for those tests. The time intervals used for the adjustment of mortality were 0-52, 53-78. 79-91, 91-104 weeks, and terminal sacrifice. The resulting p-values are compared against the p-value cutoff point set by the FDA procedures.

This reviewer performed an additional statistical analysis combining hemangiomas and combining hemangiosarcomas in all organs. Tables 6 contains incidence rates of the combined tumor types of all the tumor types.

Table 6
Results of Trend Tests of Combined hemangiomas and Hemangiosarcoms in all organ for Male and Female Mice

Organ	Tumor	Tumor-Bearing Animal	P-Value
Female			
Combined hemangiomas (510423)		6, 4, 4, 3	0.8359[1]
Combined hemangiosarcoma (580011)		2, 1, 2, 4	0.1037[1]
Male			
Combined hemangiomas (460022)		1, 4, 4, 1	0.8383[1]

[1]: Using Asymptotic p-value, since the overall tumor type is both fatal and incidental with spontaneous tumor

rates of more than 1%, it should be tested at 0.005 significant level.

The results of tumor analysis are as follows:

- The survival in low dose female mice was lower than those in the other groups.
- No significant positive linear trend in incidence rates in tumor data in both males and females was detected.
- No statistically significant positive trend in tumor incidence in hemangiomas and hemangiosarcomas in all organs combined in both males and females.

4.1.3 Conclusion of Mouse Study

In the 2-year mouse study, there was no significant positive trend in survival and statistically significant difference in survival distributions among differences in survival between treatment groups in both females and males. There was no statistically significant positive linear trend in incidence rates in tumor data in both males and females, no positive trend in tumor incidence in hemangiomas and hemangiosarcomas in all organs combined in both males and females.

4.1.4 Evaluation of Validity of the Study Designs

This reviewer's analysis did not find any tumor type with a significant positive trend in the mouse study. However, before drawing the conclusion that the drug is not carcinogenic in mice, it is important to look into the following two issues as pointed out in the paper by Haseman (1984). The two issues are:

1) Were enough animals exposed to a drug for a sustained amount of time to the risk of late developing tumors?
2) Were dose levels high enough to pose a reasonable tumor challenge to the tested animals?

This is no consensus among experts regarding the number of animals and the length of time at risk, although most carcinogenicity studies are designed to run for two years with fifty animals per treatment group. The following are some rules of thumb regarding these two issues suggested by experts in this field: Haseman (1984) investigated the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3F1 mice conducted at the National Toxicology Program (NTP). It was found that, on an average, approximately 50% of the animals in the high dose group survived the two-year study period. Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals in the high dose group, between weeks 80-90, would be considered as a sufficient number of animals under an adequate exposure. However, the percentage can be lower or higher if the number of animals used in each treatment/sex group is larger or smaller than 50 so that there will be between 20-30 animals still alive during these weeks. In addition, Chu, Cueto, and Ward (1981) suggested that "To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year." It appears, from the above sources, that the proportions of survival at 52 weeks, 80-90 weeks, and two years are of interest in determining the adequacy of exposure and number of animals at risk.

For the adequacy of the chosen dose levels, it is generally accepted that the high dose should be close to the MTD (maximum tolerated dose). Chu, Cueto, and Ward (1981) suggested the following rules on this issue:

I) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dose group relative to the controls." or

II) "The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical." or

III) "In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

Bart, Chu, and Tarone (1979) stated that the mean body weight curves over the entire study period should be taken into consideration with the survival curves, when adequacy of dose levels is to be examined. In particular, "usually, the comparison should be limited to the early weeks of a study when no or little mortality has yet occurred in any of the groups. Here a depression of the mean weight in the treated groups is an indication that the treatment has been tested on levels at or approaching the MTD."

Based on the above suggestions and recommendations, this reviewer examined the validity of the experimental design of the mouse study.

Analysis of Mice Survival and Body Weight Data

The following are the summary survival data of mice for the high dose group at weeks 52, 91, and ends of the studies.

Survival data for High Dose of Mice

Sex	End of 52 Weeks	End of 91 Weeks	End of Study Weeks
Male	98%	72%	58%
Female	98%	66%	42%

The survival rates at week 91 for both male and female mice (72% and 66%) are sufficient to provide adequate exposure.

The following table summarizes the percentages of weight gain as compared to control groups for mice.

Mean Body Weight Gain for Mice

Sex	Groups	Mean Body Weight (grams)		Mean Body Weight Gain	% Differences in MBWG
		Beginning Study	End of Study		
Male	Control	32	43	37.5	
	Low	31	42	36.5	-3
	Medium	31	42	36.5	-3
	High	32	42	37.0	-1
Female	Control	25	37	31.0	
	Low	25	38	31.5	2
	Medium	25	37	31.0	0
	High	25	38	31.5	2

The body weight gain data presented in the above table suggested that the high dose used for female mice weight might be under MTD according to the criterion proposed by Chu, Cueto, and Ward (1981). The above evaluation of validity of the study designs was based on the information contained in the data of body weight gain and mortality of the mouse study. The information about clinical signs and histopathologic effects attributed to the drug should also be included in the final evaluation.

4.2 Evaluation of Carcinogenicity Study on Rats

This reviewer's evaluation comprises the following components:

- Survival data analysis
- Tumor data analysis

4.2.1 Survival Data Analysis of Rats

The survival data analysis determines whether the dose-mortality trend in mortality is statistically significant. A positive result indicates that mortality increases as the dose level increases.

- Tables 7 and 8 present the cumulate percentages of death by dose group for female and male, respectively. The time interval "Final Kill 104-106" presents the terminal-sacrifice interval.
- Figures 3 and 4 present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female and male, respectively.
- Tables 9 and 10 present results of test for dose-mortality trend for female and male using the methods described in the paper "Trend and Homogeneity Analysis of Proportions and Life Table Data" version 2.1, by Donald G. Thomas, National Cancer Institute.

Table 7

Cumulative Percentages of Death in Female Rats

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR L	0-52	50	4	46	92	8
	53-78	46	4	42	84	16
	79-91	42	4	38	76	24
	92-103	38	8	30	60	40
	FINALKILL104-106	30	30	0		
LOW	0-52	50	4	46	92	8
	53-78	46	8	38	76	24
	79-91	38	5	33	66	34
	92-103	33	8	25	50	50
	FINALKILL104-106	25	25	0		
MED	53-78	50	5	45	90	10
	79-91	45	7	38	76	24
	92-103	38	7	31	62	38
	FINALKILL104-106	31	31	0		
HIGH	53-78	50	5	45	90	10
	79-91	45	3	42	84	16
	92-103	42	6	36	72	28
	FINALKILL104-106	36	36	0		

Figure 3
Kaplan-Meier Survival Functions for Female Rats

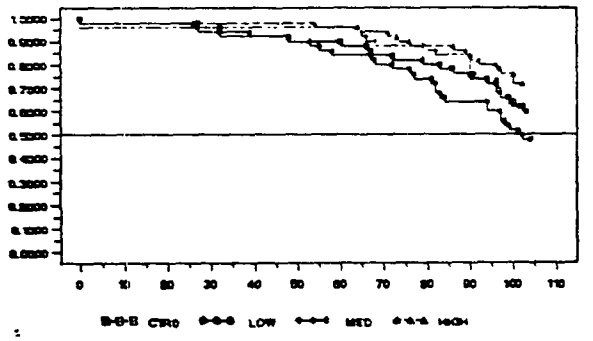
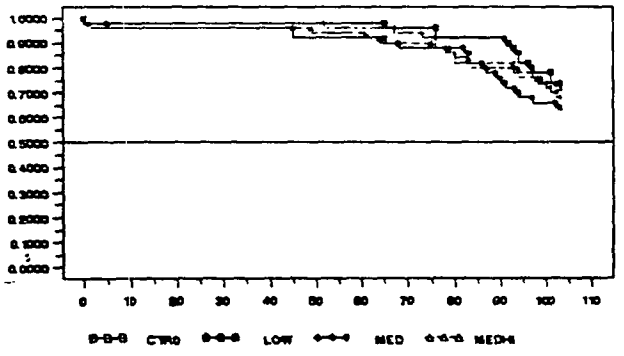


Table 8
Cumulative Percentages of Death in Male Rats

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR0	53-78	50	2	48	96	4
	79-91	48	2	46	92	8
	92-103	46	9	37	74	26
	FINALKILL104-106	37	37	0		
LOW	0-52	50	2	48	98	4
	53-78	48	3	45	90	10
	79-91	45	8	37	74	26
	92-103	37	5	32	64	36
	FINALKILL104-106	32	32	0		
MED	0-52	50	1	49	98	2
	53-78	49	5	44	88	12
	79-91	44	3	41	82	18
	92-103	41	7	34	68	32
	FINALKILL104-106	34	34	0		
HIGH	0-52	50	2	48	98	4
	53-78	48	3	45	90	10
	79-91	45	3	42	84	16
	92-103	42	6	36	72	28
	FINALKILL104-106	36	30	0		

Figure 4
Kaplan-Meier Survival Functions for Male Rats



The dose-mortality trend for female rats (presented in Table 9) is statistically significant using the Cox test and the Kruskal-Wallis test and the dose mortality trend for male rats (presented in Table 10) is not statistically significant using the Cox test and the Kruskal-Wallis test .

Table 9
Results of Tests for Dose-Mortality trend for Female rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Depart from Trend	2.1714	0.3377	2.4405	0.2952
Dose-Mortality Trend	3.8849	0.0487	4.2940	0.0382
Homogeneity	6.0563	0.1089	6.7345	0.0809

Table 10
Results of Tests for Dose-Mortality trend for Male rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Depart from Trend	1.6647	0.4350	2.0447	0.3598
Dose-Mortality Trend	0.0789	0.7788	0.0327	0.8564
Homogeneity	1.7436	0.6273	2.0774	0.5565

4.2.2 Tumor Data Analysis for Rats

The tumor data analysis determines whether the dose-tumor positive linear trend in tumor incidence is statistically significant. This reviewer tested this trend for every organ and tumor combination with the data provided by the sponsor. This reviewer analyzed the dose-tumor trend betweem the control group and 3 treated groups. The daily doeses 0, 3, 10, and 25 mg/kg/day for females and 0, 3, 15, and 75 mg/kg/day for males were used as weights for those tests. The time intervals used for the adjustment of mortality were 0-52, 53-78. 79-91, 91-103 weeks, and terminal sacrifice. The resulting p-values are compared against the p-value cutoff point set by the FDA procedures.

The results of tumor analysis are as follows:

- The sponsor claimed that there was a statistically significant positive dose-response relationship (p=0.025) in incidence rate of benign thymoma in thymus in female rats. However, since the tumor type has a spontaneous tumor rate of 1% or less, the positive dose-response relationship with an asymptotic p-value of 0.0261 is considered as not statistically significant by the Office of Biostatistics decision rule

4.2.3 Conclusion of Rat Study

In the 2-year rat study, there was no significant positive trend in survival and no statistically

significant difference in survival distributions among treatment groups in males. However, The dose-mortality trend for famale rats was statistically significant using the Cox test and the Kruskal-Wallis test. There was no statistically significant positive linear trend in incidence rates in tumor data in both males and females. Since this reviewer's analysis did not detect any tumor type with a significant positive trend in the rat study, it is necessary to evaluate the validity of the study design using the criteria described in section 4.1.4 in the following analysis of survival and body weight data.

Analysis of Rat Survival and Body Weight Data

The following are the summary survival data of rats for the high dose group at weeks 52, 91, and ends of the studies.

Survival data for High Dose of Mice

Sex	End of 52 Weeks	End of 91 Weeks	End of Study Weeks
Male	100%	84%	72%
Female	98%	84%	72%

The survival rates at week 91 for both male and female mice (94% and 84%) are sufficient to provide adequate exposure.

The following table summarizes the percentages of weight gain as compared to control groups for rats.

Mean Body Weight Gain for Mice

Sex	Groups	Mean Body Weight (grams)		Mean Body Weight Gain	% Differences in MBWG
		Beginning Study	End of Study		
Male	Control	182	505	343.5	
	Low	182	504	343.0	-0.2
	Medium	180	502	341.0	-0.7
	High	180	456	318.0	-7
Female	Control	140	317	228.5	
	Low	139	309	224.0	-2
	Medium	138	311	224.5	-2
	High	139	295	217.0	-5

The high dose for male and female rats had 7% and 5% weight gain decrement. The body weight gain data suggested for high doses used for male and female rats were close to MTD according to the criterion proposed by Chu, Cueto, and Ward (1981). The above evaluation of validity of the study designs was based on the information contained in the data of body weight gain and mortality of the mouse study. The information about clinical signs and histopathologic effects attributed to the drug should also be included in the final evaluation.

5. References

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