

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-400

Pharmacology Review(s)

To: Florence Houn
Director ODE III

From: John Leighton
Associate Director for Pharmacology/Toxicology, ODE III

Subject: NDA 21-400
Levitra (vardenafil)

Date: July 12, 2002

Introduction

Vardenafil is a phosphodiesterase (PDE) 5 inhibitor used to treat male erectile dysfunction. The compound is provided in oral formulations to 20 mg. Specific wording regarding labeling is incorporated into the pharmacology review in the sections for genetic toxicology, reproductive toxicology, and carcinogenicity. A separate labeling review was not provided. Vasculitis and effects on cardiac parameters in animal studies appear related to the pharmacodynamic properties of vardenafil, and these topics are discussed extensively throughout the pharmacology NDA review.

Comments

With few exceptions, the Division review has adequately addressed the nonclinical pharmacology and toxicology of vardenafil. However, several issues identified below appear unresolved.

The Executive CAC recommendations were contingent upon adequacy of a statistical review of the survival and tumor data in mice and submission of historical control data for thymomas in rats. The statistical review was conducted and is provided as Appendix III to the review. It appears that this analysis of survival and tumor data was adequate and supports the conclusion that there is no evidence of carcinogenicity of vardenafil in these studies. Historical control data from the conducting laboratory for thymomas also appears to have been provided by the sponsor. The Division, particularly in light of the recommendations of the Executive CAC, should address whether both these issues have been adequately resolved.

The major metabolite was incorporated into the analysis for the carcinogenicity studies but not the reproductive toxicity studies, as described in the recommended labeling language for the respective topics. The rationale for this difference is not clear. It is recommended that the major metabolite also be incorporated into the exposure analysis for the reproductive toxicity studies.

Vardenafil is present in the milk of lactating rats. This information should be considered for labelling recommendations.

A memorandum of comment or concurrence from the pharmacology team leader was not available at this time.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-400

Review number: 1

Sequence number: 000

Date/type of submission: September 24, 2001/Original

Information to sponsor: Yes () No (x)

Sponsor: Bayer Corporation, 400 Morgan Lane, West Haven, CT 06516

Manufacturer for drug substance: Bayer AG, Business Group Pharma, 51368 Leverkusen, Germany

Reviewer name: Yangmee Shin, Ph.D.

Division name: Div. of Reproductive and Urologic Drug Products, HFD-580

Review completion date: 7/12/02

Drug:

Trade name: Levitra

Generic name: Vardenafil HCl

Code name: BAY 38-9456

Chemical name:

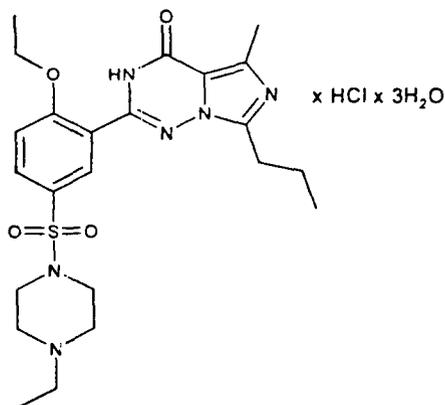
CAS registry number:

Mole file number:

Molecular formula: $C_{23}H_{32}N_6O_4S \cdot HCl \cdot 3H_2O$

Molecular weight: 579.1 g/mole

Structure:



Relevant INDs/NDAs/DMFs: IND

Drug class: Phosphodiesterase (PDE) 5 inhibitor

Indication: Male erectile dysfunction

Clinical formulation: Orange round tablets containing 5, 10 or 20 mg vardenafil HCl, microcrystalline cellulose NF, crospovidone NF, colloidal silicone dioxide NF, and magnesium stearate NF, with hydroxypropyl methylcellulose USP, polyethylene glycol NF, and ferric oxide NF (yellow), ferric oxide NF (red) & titanium dioxide USP as pigments.

Route of administration: Oral

Proposed use: Treatment of erectile dysfunction (ED)

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: BAY 38-9456 is a PDE5 inhibitor for the treatment of ED.

Studies reviewed within this submission:

PHARMACODYNAMICS (Ph-31060, Ph-31083, Ph-30552, R-8008)

SAFETY PHARMACOLOGY (R-8014, R-7995, Ph-30693)

PHARMACOKINETICS

Pharmacokinetics (Ph-28287A, Ph-28636, Ph-28636A, Ph-30012, Ph-30012A)

Absorption (Ph-28563A)

Distribution (Ph-28903A, R-7752, Ph-31210)

Metabolism (Ph-31090, Ph-30797, Ph-30350, Ph-30853, Ph-30868, Ph-31173, Ph-31184)

Excretion (Ph-30981, Ph-30980)

Protein Binding (Ph-28322A, Ph-28322B, Ph-29686, Ph-31046)

Toxicokinetics (Ph-30699, Ph30699A, Ph-30486, Ph-29788, Ph-29788A, Ph-29509A, Ph-29605A, Ph-31176, Ph-31165)

TOXICOLOGY

Acute toxicity study via oral gavage in male & female Wistar rats (Ph-30147/T4069487)

2-Week toxicity study via oral gavage in rats (Ph-27257/T7061884)

Liver enzyme activities of 4-week toxicity study in dogs (Ph-27983/T0062480)

2-Week intravenous toxicity study in beagle dogs (Ph-31239/T0069807)

14-Week toxicity study with 4-week recovery via oral gavage in Wistar rats (Ph-28640A/T5067057)

13-Week toxicity study via drinking water in Wistar rats (Ph-29976/T8062659)

SPECIAL TOXICOLOGY

In vitro interaction study with thyroid peroxidase & iodotyronine deiodinase type I & II-catalyzed reactions (Ph-28209)

4-Week toxicity study via oral gavage in rats for BAY 38-9456 & BAY 41-6484 (Ph-30893)

CARCINOGENICITY

2-Year carcinogenicity study via oral gavage in Wistar rats (Ph-31276)

2-Year carcinogenicity study via drinking water in CD-1 mice (Ph-31279)

REPROTOXICOLOGY

Pre- & postnatal development including maternal function after oral gavage administration in rats (Ph-30851/T5061305)

Studies not reviewed within this submission:

Review #1

PHARMACOLOGY (Ph-31166, Ph-28173, Ph-28174)

SAFETY PHARMACOLOGY (Ph-27670, Ph-27671, Ph-27672, Ph-27193, Ph-27893, Ph-7272, Ph-27168, Ph-27758, Ph-27681, Ph-27757, Ph-27760)

PHARMACOKINETICS

Pharmacokinetics (Ph-28287)

Protein Binding (Ph-28322)

Toxicokinetics (Ph-27992, Ph-27993)

TOXICOLOGY

Acute toxicity in mice & rats after oral & intravenous administration (Ph-28162)

4-Week toxicity via oral gavage in rats (Ph-28163/T5062467)

14-Week toxicity via oral gavage in rats (Ph-28640/T5067057)

13-Week toxicity study in mice via drinking water (Ph-30633/T5067273)

4-Week toxicity via oral gavage in dogs (Ph-28164/T0062480)

GENOTOXICITY

In vitro Ames test (Ph-27811/T2059765)

In vitro chromosome aberration test in Chinese Hamster V79 cells (Ph-28011/T3061475)

In vivo micronucleus test in mice (Ph-27816/T306483)

Review#2

Review & evaluation of rodent carcinogenicity protocols

Review#3

PHARMACOKINETICS

Pharmacokinetics (Ph-28636, Ph-28912, Ph-28510, Ph-29249)

Absorption (Ph-28563)

Protein binding (Ph-29340, Ph-29430)

Distribution (Ph-28710, Ph-29438, Ph-28903)

Metabolism (Ph-28510, Ph-28562)

Toxicokinetics (Ph-28567, Ph-28519, Ph-28960, Ph-28578, Ph-28624, Ph-29509, Ph-20605, Ph-29153)

TOXICOLOGY

14-Week Toxicity via oral gavage in Wistar rats (Ph-28640/T5067057)

13-Week Toxicity via oral gavage in Beagle dogs (Ph-28660)

27-Week Toxicity via oral gavage in Wistar rats (Ph-29532/T9068186)

52-Week Toxicity via oral gavage in Beagle dogs (Ph-29796/T3067488)

GENOTOXICITY

V79-HPRT test for detection of induced forward mutations (Ph-28532/T3059810)

REPROTOXICITY

Fertility & Early embryonic development in rats (Ph-28846/T2060592)

Developmental toxicity in rats (Ph-29093/T2061375)

Developmental toxicity in rabbits (Ph-28625/T4062097)

**APPEARS THIS WAY
ON ORIGINAL**

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: It is recommended that the NDA 21-400 for BAY 38-9456 be approved from a preclinical safety perspective.

B. Recommendation for Nonclinical Studies: Based on the Executive CAC recommendations on carcinogenicity studies, statistical review of the survival and tumor data, and historical control data for thymomas in female rats were provided. The statistical analysis supports the negative carcinogenicity results, and the thymomas were within the historical control range.

C. Recommendations on Labeling: Refer to the labeling comments

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: BAY 38-9456 is a PDE5 inhibitor for the treatment of male erectile dysfunction. Vasodilating cardiovascular activity was a major effect of BAY 38-9456 in rats and dogs. In safety pharmacology studies, dose-dependent decrease in peripheral resistance and systolic blood pressure accompanied by a compensatory increase of heart rate, cardiac contractility and cardiac output was observed in intraduodenal doses of 0.3- to 10 mg/kg in anesthetized dogs. BAY 38-9456 alone (0.1- to 3.0 mg/kg, p.o) or in combination with glyceroltrinitrate (0.5 mg/kg, p.o.) produced more marked reduction of blood pressure/increased heart rate and prolonged duration than sildenafil (0.07- to 2.1 mg/kg, p.o) in conscious dogs. Hematocrit and hemoglobin concentrations were reduced from 1 mg/kg in male rats, possibly as a secondary effect of vasodilation. There was no substantial change in QTc intervals with intraduodenal doses up to 10 mg/kg in anesthetized dogs, but *in vitro* HERG channel activities were inhibited from 1 μ M (at +40 mV). No significant toxicity was observed in the CNS, respiration, GI function, renal system and coagulation at doses studied up to 10 mg/kg. The acute toxic effects observed in single dose studies in rats and mice were most likely due to exaggerated pharmacodynamic response of the drug. Oral LD₅₀ in rats was 250 mg/kg in males and 190 mg/kg in females. In repeated-dose studies, myocardial fibrosis and necrosis resulted in deaths of high-dose rats. In dogs, subepicardial and pericardial edema in the atrium accompanied by an increase in minimal to mild subepicardial inflammatory infiltration was observed in the 1-month study. Mild to moderate periarteritis and arteritis occurred at high dose in the 1-, 3- and 12-month studies possibly secondary to the hypotensive effects of the drug. The periarteritis findings were not progressive in the 1-year chronic study. A NOAEL for any toxicity was considered to be 3 mg/kg in rats and dogs, which produces 2-21 fold of safety margin for the total exposures of the unbound drug (BAY 38-9456+M-1) the human exposure at 20 mg. Adrenal vacuolation and hypertrophy of the pancreas, parotid/submandibular glands and thyroid gland were only observed in rats with a safety margin of 2-12 fold at NOAELs. The acinar hypertrophy of the exocrine glands was considered as an adaptive effect of PDE inhibitors. BAY 38-9456 was negative in *in vitro* and *in vivo* genotoxicity studies. BAY 38-9456 was negative in 2-year carcinogenicity bioassays in rats with exposures up to 180-400 fold and mice up to 21-37 fold higher than in humans. Reproductive studies conducted in rats defined a NOAEL as 100 mg/kg for F0 fertility in Segment I, 18 mg/kg for maternal/developmental toxicity in Segment II, and 8 mg/kg for maternal toxicity, physical F1 development after weaning and F1 fertility and at 1 mg/kg for f1 pre/postnatal development in Segment III. In a rabbit developmental study, a NOAEL was established as 3 mg/kg for maternal toxicity and 18 mg/kg for embryonic development. The retarded fetal skeletal development associated with the reduced placental/fetal weights and severe maternal toxicity was observed at high dose, indicative of developmental toxicity as a consequence of systemic toxicity rather than a teratogenic effect.

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:.....	1
II. SAFETY PHARMACOLOGY:.....	1
III. PHARMACOKINETICS/TOXICOKINETICS:.....	2
IV. GENERAL TOXICOLOGY:.....	5
V. GENETIC TOXICOLOGY:.....	13
VI. CARCINOGENICITY:.....	13
VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:.....	27
VIII. SPECIAL TOXICOLOGY STUDIES:.....	34
IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:.....	37
X. APPENDIX/ATTACHMENTS:.....	44

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY (See also Review #1 in Appendix I)

Primary pharmacodynamics:

Mechanism of action: BAY 38-9456 inhibits cGMP-specific PDE5 by potentiating NO-induced relaxation of corpus cavernosum smooth muscle cells via inhibition of cGMP breakdown.

Drug activity related to proposed indication: Both BAY 38-9456 and M-1 induced erections either after oral (BAY 38-9456; 1-30 mg/kg) or intravenous (BAY 38-9456; 0.3-1 mg/kg, M-1; 1-3 mg/kg) administration in conscious rabbits (PH-31083) with minimal effective oral dose of 1 mg/kg. The main metabolite, M-1 was ~10 fold less potent than the parent compound *in vivo*.

Secondary pharmacodynamics: Vardenafil up to 10 μ M did not inhibit platelet aggregation induced by various agonists. In the presence of a NO donor SNP (0.3 μ M), concentration-dependent enhancement of the antiaggregatory effects of SNP, which was quantitatively similar to the effects of sildenafil, was observed with ≥ 0.1 μ M vardenafil in the presence of various agonist-induced platelet aggregation (R-8008).

Pharmacology summary: BAY 38-9456 is more potent on PDE5 ($IC_{50}=0.7$ nM for human platelets, 0.89 nM for human recombinant PDE5A1) than PDE1 ($IC_{50}=121$ nM) with >100 fold, and than PDE2, 3, 4, 7, 8, 9, 10 and PDE11A (PH-31166A) with a selectivity ratio of >300 fold. BAY 38-9456 is less selective relative to PDE6 ($IC_{50}=11$ nM) with a ratio of 15 fold. The metabolites of BAY 38-9456, BAY 44-5576 (M-1), BAY 44-5578 (M-4) and BAY 44-5577 (M-5) are also potent inhibitors of human recombinant PDE5 with IC_{50} of 3.2, 16 and 18 nM, respectively. The selectivity of M-1 for inhibition of PDE5 over PDE1 and PDE3 was similar to that of BAY 38-9456. The PDE2 and PDE4 were not inhibited by any metabolites up to 10 mM. Sponsor considered the contribution of the metabolites M-1, M-4 and M-5 to the overall efficacy to be 7.2%, 0.06% and 0.6% relative to the parent drug, respectively, based on the PK observed in man (PH-31060). The relatively high doses for the induction of erections in the rabbit (3-30 mg/kg) compared to the effective dose in humans (0.4-1.5 mg/kg) was considered to be the lack of sexual stimulation in the rabbit model.

II. SAFETY PHARMACOLOGY (See Review #1 in Appendix I)

Safety pharmacology summary: As expected from its mechanism of action, BAY 38-9456 exhibited a dose-dependent decrease in peripheral resistance and SBP accompanied by increase of HR, cardiac contractility and cardiac output in anesthetized dogs after single intraduodenal administration at 0.3- to 10 mg/kg. BAY 38-9456 alone (0.1- to 3.0 mg/kg, p.o) or in combination with glyceroltrinitrate (0.5 mg/kg, p.o.) produced more marked reduction of blood pressure/increased heart rate and prolonged duration than sildenafil (0.07- to 2.1 mg/kg, p.o) in conscious dogs (PH-30693). BAY 38-9456 at 3- to 30 mg/kg (p.o.) and sildenafil at 10- to 30 mg/kg (p.o.) equipotently produced a dose-dependent decrease in mean blood pressure and an increase in heart rate in conscious spontaneously hypertensive rats (SHR, PH-30552), suggesting that BAY 38-9456 is 3 fold more potent than sildenafil on blood pressure. QT interval decreased dose-dependently during the tachycardia in conscious and anesthetized dogs. *In vitro* HERG channel activity was blocked by sildenafil or vardenafil similarly to class III antiarrhythmics but at higher concentrations with vardenafil having higher potency than sildenafil (IC_{50} 32 μ M vs. 56 μ M at +40 mV under 10 mM K^+ ; 30 μ M vs. 47 μ M at +20 mV under 4 mM K^+ , R-7995). The threshold concentration (1 μ M at 40 mV) is about 29 fold higher than the peak plasma level in man (34 nM). Sponsor stated that BAY 38-9456 may be a less potent HERG blocker under physiological conditions since the plateau of action potential is depolarized to a lesser degree than +40 mV and for only about 200 ms (as compared to 1000 ms in the 4 mM experiments). Blood cell parameters (erythrocytes, hematocrit, Hb) were decreased dose-dependently (1-10 mg/kg) up to 11% at 10 mg/kg in rats, possibly subsequent to the cardiovascular effects of the drug. BAY 38-9456 showed a dose-dependent inhibition of platelet aggregation in the presence of sodium nitroprusside (SNP) with statistical significance at 1 μ M, which is ~27 fold above the C_{max} of 20 mg human dose. Oral administration of BAY 38-9456 or sildenafil at 3 and 10 mg/kg had no effect on bleeding time in the anesthetized rat. BAY 38-9456 did not enhance the heparin effect on bleeding time, while sildenafil tended to blunt the effect of

heparin without dose-dependency (R-8014). There was a statistically significant increase of travelling distance observed 30 min after 10 mg/kg dosing in rats in the open field test.

Safety pharmacology conclusions: BAY 38-9456 had vasodilating effects in SHR from 3 mg/kg and anesthetized dogs from 0.3 mg/kg, a dose which gives a C_{max} slightly higher than that seen in man following the 20 mg, but had no significant effects on CNS, respiratory, GI and renal systems tested up to 10 mg/kg (p.o.).

III. PHARMACOKINETICS/TOXICOKINETICS (See also Reviews #1/3 in Appendix I)

PK parameters: In men, Bay 38-9456 is rapidly absorbed with T_{max} between 0.5- and 2 hrs and $T_{1/2}$ of 4- to 5 hrs after oral dosing. AUC and C_{max} increase slightly more than dose-proportionality at ≥ 10 mg. There was no accumulation on repeated dosing with AUC_{0-24h} of 76 $\mu g \cdot hr/L$ for BAY 38-9456 & 41.1 $\mu g \cdot hr/L$ for M-1 at steady state (#100196). The mean steady-state volume of distribution was 208 L, indicating extensive tissue distribution. The plasma concentration of the major circulating metabolite, M-1 was approximately 26% that of the parent compound. Major excretion was via the feces (~91 to 95%).

Single Dose PK after [^{14}C]-BAY 38-9456 in Plasma to Male Wistar Rats (PH-28563A)

Route	i.v.	p.o.
Dose, mg/kg	1	1
AUC_{0-24hr} , $\mu g \cdot hr/mL$	2210	222
C_{max} , $\mu g/mL$	10600	99.9
T_{max} , hr	-	0.290
$t_{1/2}$ (apparent), hr	6.62	5.45
$t_{1/2}$, hr	11.0	7.47
Absorption rate, %	-	9.98
AUC_{0-8hr} , $\mu g \cdot hr/mL$	2.13	0.173
$t_{1/2}$ (apparent), hr	1.83	4.46

Geometric means

-, not available

Single Dose PK in Plasma after BAY 38-9456 or [^{14}C]-BAY 38-9456 (1 mg/kg) to Female Beagle Dogs (PH-28636)

Route	i.v.			p.o.			
	0.3	1		0.3	1	3	
Parameters	Unchanged		Radioactive	Unchanged		Radioactive	
AUC_{0-24hr} , $\mu g \cdot hr/L$	137	390	921	31.2	102	615	369
C_{max} , $\mu g/L$	97.3	325	422	9.21	31.7	100	85.7
T_{max} , hr	-	-	-	1.44	1.14	1.14	1.50
$t_{1/2}$, hr	1.53	1.75	49.4	2.11	1.69	39.6	2.90
Bioavailability [#] , %	-	-	-	27.3	26.8	-	32.5
Total Clearance, L/hr/kg	2.19	2.62	-	-	-	-	-
Absorption rate [§] , %	-	-	-	-	-	75.9	-
AUC_{0-8hr} , $\mu g \cdot hr/mL$	133	376	716	28.8	96.7	398	318
$t_{1/2}$ (apparent), hr	1.53	1.75	2.91	2.11	1.69	3.2	1.93

Geometric means

[#]calculated from AUC_{norm} of unchanged BAY 38-9456 after oral to after i.v. (1 mg/kg) of BAY 38-9456

[§]calculated from AUC_{norm} of radioactive BAY 38-9456 after oral to after i.v. (1 mg/kg) of [^{14}C]-BAY 38-9456

-, not available

Single Dose (1 mg/kg) PK of BAY 44-5576 (M-1) in Wistar Rats (PH-30012A)

Drug	BAY 38-9456		BAY 44-5576	
	M	F	M	F
Route	p.o.		i.v.	
AUC_{0-24hr} ($\mu g \cdot hr/L$)	18.9	103	166	-
C_{max} ($\mu g/L$)	10.8	55.7	-	-

T _{max} (hr)	0.333	0.167	-	-
t _{1/2} (hr)	1.37	1.38	1.57	-
Total Clearance (L/hr/kg)	-	-	6.04	-
Bioavailability* (%)	12.1	-	-	-

Geometric means

*calculated by comparison of AUC_{norm} of M-1 after oral administration of BAY 38-9456 and after i.v. of M-1

-: not available

Absorption: After i.d. administration (3 mg/kg) in male bile-duct cannulated rats and i.v. (1 mg/kg) administration of [¹⁴C]-BAY 38-9456 in intact rats, 75.4% and 82.6% of the dose, respectively, were found in bile and urine within 24 hrs. [¹⁴C]-BAY 38-9456 was almost completely absorbed from the intestinal tract up to 24 hrs after i.d. administration with an absorption extent of 91%. A theoretical value for the absorption after i.v. and oral administration of 1 mg/kg was only 9.9%, indicating that a large portion of the amount absorbed from the GI tract was already eliminated pre-systemically (PH-28563A). The bioavailability of M-1 after oral administration of 1 mg/kg BAY 38-9456 to male rats was 12.1%.

Distribution: Oral administration of [¹⁴C]-BAY 38-9456 at 1 mg/kg to male rats exhibited the highest concentrations in the liver>adrenal, kidneys>lungs>testes>skin>plasma, blood>heart>brain with the longest terminal t_{1/2} of 71 hrs for kidneys. Male pigmented rats exhibited the highest concentrations detected in the eyes, liver and pigmented/non-pigmented skin at 24-hr post-dose, in the eyes, liver, kidneys and pigmented skin at 96-hr post-dose, and in the eye and pigmented skin with t_{1/2} of 14 days and 5 days, respectively, indicating affinity to melanin. The results suggest that an oral dose of 1 MBq in male volunteers would result in a radiation exposure of 0.5 mSv for Calculation of Committed Effective Dose Equivalent (CEDE) by the National Radiological Protection Board (R-7752). [¹⁴C]-BAY 38-9456 at 3 mg/kg to pregnant Wistar rats on gestation day 19 was rapidly and thoroughly distributed to maternal organs and tissues (except to brain and cartilage) with the maximum highest radioactivity concentrations 2 hrs post-orally in the maternal liver, spleen and adrenal cortex. Maximum fetal concentrations were found in GI tract, liver, lungs, adrenal cortex, eye and skin. Based on AUCs, most organs and tissues were highly exposed than maternal blood except fetal organs, amniotic fluid, maternal brain and cartilage. The highest exposure was in amnion, maternal liver, spleen and thyroid and mammary glands. The latter indicates secretion of radioactivity with milk. The AUC ratios of fetal blood/maternal blood (0.18) and fetal tissues/maternal blood (0.26) were low, indicating low placental transfer. The AUC in fetal brain was 2.2 fold higher than that in the maternal brain. The elimination was rapid with T_{1/2} of 3- to 10 hrs, but 25 hrs for the amnion. After 24 hr, highest residual radioactivity was found in the maternal thyroid, amnion and mammary glands, and low residues in the fetal adrenals and liver (PH-31210) with no detectable radioactivity in the fetuses after 48 hrs. Within 24 hrs at 3 mg/kg, 3.3% of the radioactivity was excreted into milk in lactating rats, which the AUC was more than 10 fold higher than for the plasma (PH-30980).

Metabolism: Metabolites from oral [¹⁴C]-BAY 38-9456 in plasma were M-1 (BAY 44-5576) formed by N-deethylation as a main metabolite, N-desethyl/des-ethylene derivative M-5 (BAY 44-5577) and M-4 (BAY 44-5578) by degradation of the piperazine ring in all species studied. Formation of the sulfonamide M7 (BAY 50-6599) increased time-dependently in rat and mouse plasma, but was of less importance in non-rodents and human (PH-30797). Unstable glucuronide of M-1 was also identified in human plasma after thermal treatment (PH-31090). The main metabolites from human liver microsomes were M-1, M-4 and M-5 (PH-31184). In human liver microsomes, CYP3A5 (11%), CYP2C8 (6%), 2C9 (6%) and 2C19 (2%) play a considerable role as high-affinity components in the N-deethylation, whereas CYP3A4 predominates (80%) as low-affinity component at higher concentrations. Autoinhibition of CYP3A4-catalyzed biotransformation was observed (PH-31173).

Biotransformation of Oral [¹⁴C]-BAY 38-9456 in Plasma of Mice, Rats, Dogs or Humans

Study #	Species	Dose, mg/kg	Parent	Metabolites	
				Major	Minor
PH-30797	Mouse (M)	3	47→43% (10 min→60 min)	M-7 (11→17%)	M-1 (5-8%), M-5 (5-8%), M-4 (2-3%)
PH-30350	Rat (M)	1	47→6% (20 min→4 hr)	M-1 (22→26%)	M-7 (5-19%), M-4 (1-3%), M-5 (1-5%)

PH-30853	Dog (F)	1	40→34% (2 hr→4 hr)	M-1 (25→27%)	M-4 (6%), M-6 (5-6%), M-5 (2%)
PH-30868	Human (M)	40	36→21% (45 min→4 hr)	M-1 (32→25%)	M-4 (5%), M-5 (1-2%), M-13 (1-2%)

Excretion: After i.v. or oral administration of [¹⁴C]-BAY 38-9456 to male rats or female dogs at 1 mg/kg, the radioactivity was 94-99% via bile/feces within 168 hrs. The radioactivity in the rat was mainly located in the liver and skin by 168 hrs (PH-28563A). After i.v. administration of [¹⁴C]-BAY 38-9456 to male bile-duct cannulated mice (3 mg/kg), rats (1 mg/kg) or dogs (2 mg/kg), the administered dose was excreted in mice with 57% via bile and 6.4% via urine within 7 hrs (PH-30981), in rats with 78% via bile, 4.3% via urine and 6.9% via feces within 24 hrs (PH-28563A), or in dogs with 78% via bile, 13.5% via feces and 6.22% via urine within 168 hrs (PH-28636). After i.d. administration of [¹⁴C]-BAY 38-9456 to male bile-duct cannulated rats at 3 mg/kg, 70% was excreted via bile, 5.3% via urine, 19% via feces and 0.73% in the body excluding GI tract up to 24 hrs. There was a moderate extrabiliary excretion (3.5-13.7%) of the systemically available radioactivity in rats and dogs after i.v. administration.

Plasma protein binding: The unbound fraction of [¹⁴C]-BAY 38-9456 (1 mg/kg) to dog plasma proteins *ex vivo* at 2 hrs was between 14 and 19% similar to those obtained *in vitro*, and was declined to 3- (i.v.) to 5% (p.o.) at 24 hrs, indicating the altered binding characteristics of metabolites found by the time (PH-28636A). Major binding protein fraction in human was albumin, and to a lower extent of α -globulins, β -globulins and LDL (PH-31046). [¹⁴C]-BAY 38-9456 distributed to a moderate degree in to erythrocytes in rats, dogs and men. Influence of highly bound drugs on the free fraction at 1 μ g/mL was minor on altered pH, higher drug concentrations, other protein bound drugs, and non-esterified fatty acid (PH-31046).

***In Vitro* Plasma Protein Binding of [¹⁴C]-BAY 38-9456 & Metabolites at 1 mg/L (PH-29686)**

Species (Strain)	[¹⁴ C]-BAY 38-9456		[¹⁴ C]-BAY 44-5576 (M-1)		[¹⁴ C]-BAY 44-5578 (M-4)	
	Bound, %	Free, %	Bound, %	Free, %	Bound, %	Free, %
Mouse (CD1)	94	6	92	8	94	6
Rat (Wistar, M)	95	5	92	8	73	27
Dog (Beagle, F)	87	13	85 (M)	15 (M)	76	24
Rabbit (NZW, F)	97	3	90	10	77	23
Human (M)	94	6	94	6	82	18

PK/TK summary: Absorption of BAY 38-9456 was rapid and almost completely from the GI tract in rats and dogs. Oral bioavailability was moderate from 7.4- to 28.6% in the rat, from 27- to 33% in the dog at 0.3 to 3 mg/kg, and 14.5% in man at a single 10 mg dose. PK in male rats (1 mg/kg) was influenced by the feeding with longer elimination half-life of 1.6 hrs compared to 0.5 hrs in fasted rats, indicating a prolonged absorption. Volume of distribution at steady state was moderate ranged from 2.0 L/kg in the rat, 2.5 L/kg in man, 5.2 L/kg in the dog, reflecting the species difference in protein binding (94-95% in man/rat, 87% in dog). Oral administration of [¹⁴C]-BAY 38-9456 to male rats at 1 mg/kg was distributed rapidly from blood into organs and tissues with maximum radioactivity at 20 min post-dosing. The highest radioactivity was observed in the liver, adrenals and kidneys. The terminal half-life for the body excluding GI tract was 37 hr, with the longest 71 hr for kidneys. BAY 38-9456 penetrated the blood/brain barrier to a moderate extent and the placental barrier to a low extent in rats. Retention of radioactivity occurred in the pigmented structures (eye-wall, meninges), indicating affinity to melanin. Sponsor stated that melanin binding to the pigmented eye is a common characteristic of compounds in this class, and the affinity has not been associated with ocular toxicity in any of the animal species in long-term toxicity studies. Elimination of the drug occurred exclusively by biotransformation being excreted metabolites. In liver microsomes, unchanged drug and M-1 formed by N-deethylation were the major metabolites in all species tested. CYP3A5 was identified to play a considerable role as a high affinity component in metabolism, and CYP3A4 was predominated in the biotransformation at higher concentrations *in vitro*. Excretion was predominantly via bile and feces in mice, rats and dogs.

IV. GENERAL TOXICOLOGY (See also Reviews #1/3 in Appendix I)

Study title: Acute Oral Gavage Toxicity of BAY 38-7268 (PH-30147) or BAY 38-9456 (PH-30606) in Fasted Wistar Rats

Study #	Dose, mg/kg	LD ₅₀ , mg/kg	Results
PH-30147 (T4069487)	2000 (in demineralized water with 2% Cremophor EL)	>2500 [#]	Mortality- 0/3M, 1/3F (Day 2) Clinical signs- ↓ motility/reactivity, narrowed palpebral fissures, labored breathing, piloerection (F) & uncoordinated gait (F), which occurred 40-min postdosing, & lasted up to Day 5, closed eyes & pale discoloration in the kidney in dead female.
PH-30606 (T5070044)	200 (in 0.5% aqueous tylose solution)	>200 [#]	Mortality- 0/3M, 1/3F (Day 2) Clinical signs- constipation, ↓ motility/reactivity, narrowed palpebral fissures, labored breathing, uncoordinated gait, ↓ salivation (F), which occurred 15-min post-dosing and lasted up to Day 5, autolysis in dead female

[#]According to the OECD guideline No. 423

Summary: The median lethal oral dose in the fasted rat was determined at >200 mg/kg for Bay 38-9456 and >2500 mg/kg for free base of BAY 38-9456 (BAY 38-7268).

Study title: 2-Week Oral Gavage Toxicity in Wistar Rats (PH-27257/T7061884)

Key study findings: BAY 38-9456 produced changes in RBC parameters (≥25 mg/kg in females), urine sediment (≥25 mg/kg in females), myocardial fibrosis/lung congestion and induction of liver monooxygenases at 100 mg/kg in rats for 2-week oral dosing. The 200 mg/kg was lethal to females.

Methods: SPF-bred Wistar rats [HsdCpb: WU] (126-162 g/4 to 5 weeks for males, 120-149 g/5 to 6 weeks for females) were administered at 0, 3, 25, 200/100, 100 mg/kg (5/sex/group) given in demineralized water for 2 weeks by oral gavage. Mortality/Clinical signs twice daily, Body weights at pre-study phase & once daily post-study phase, food Intake once weekly, clinical chemistry on Days 10/11 (urine, glucose) & Days 14 or 15 (blood, tissue samples), Hematology/Urinalysis/Organ weights/Necropsy on Days 14 or 15, and toxicokinetics on Days 0 & 9 at 0.5, 24 hrs post-dose were observed.

Results: Three females died on Day 2 and one female on Day 3 at 200 mg/kg. The high-dose group was reduced to 100 mg/kg on Day 2, and the dead animals were replaced. Piloerection and reduced motility occurred on the first treatment of 200 mg/kg. Additional signs of accelerated breathing, decreased reactivity, spastic/staggering gait and narrowed eyelids were observed in most of the animals at 200 mg/kg. Significant increase in HB/hematocrit values in females at high dose and in males at all treated groups, and increased hypochromasia in males was found at high dose. All other changes were within historical ranges. Increased hepatic enzyme activities of N-DEM and O-DEM in females correlated with increased liver weights at 100 mg/kg. Decreased urine density combined with increased urine volume/positive blood/erythrocytes/amorphous salts/triplephosphates in urine sediment was noted in the high-dose males or from 25 mg/kg in females. Deceased animals showed autolysis in various tissues, discoloration of the kidneys/spleen/stomach/lungs (with congestion), and mild to moderate interstitial edema of the atrial/ventricular myocardium associated with inflammatory reaction. After 2 weeks of treatment the remaining female from 200 mg/kg and 2/5 females at 100 mg/kg showed mild to moderate interstitial fibrosis of septum wall of the left ventricle without progression of acute myocardial degeneration. Minimal inflammatory infiltration was noted in 4/5 females at high dose. All other findings were considered to be of spontaneous origin. Plasma concentrations increased disproportionately to the drug exposure and declined within 24 hours post-dosing being the elimination faster in males.

Dose, mg/kg	0		3		25		200/100#	
	5M	5F	5M	5F	5M	5F	5M	5F
Mortality								4 [@]
Clinical signs ^a ,								
Piloerection							1	2
Reduced motility							1	2
Accelerating breathing/Spastic gait								2
Body weights	UR	UR	UR	UR	UR	UR	UR	UR
Food consumption, g/day	UR	UR	UR	UR	UR	UR	UR	UR
Clinical chemistry,								
ALP, U/L	645	355	553	324	579	303*	544	312**
Cholesterol, mmol/L	2.49	1.91	2.33	2.05	2.44	1.98	2.21	2.36*
TG, mmol/L	1.07	1.16	1.20	0.82	1.16	0.92	1.07	0.60*
Hematology,								
HB, g/L	138	140	145	147	146*	144	144	150*
HCT, l/L	0.435	0.428	0.458*	0.447	0.456	0.433	0.460*	0.461*
Hypochromasia	1	0	0	0	0	0	2	0
Neutrophils, 10E9/L	0.57	0.42	0.44	0.31	0.41	0.37	0.38	0.49
Liver Biochemistry ^b , Days 14 (m)/15 (f)								
N-DEM, mU/g	148.7	71.3	167.8	79.0	158.3	78.9	129.7	111.6**
O-DEM, mU/g	11.2	8.3	10.9	8.7	10.8	9.6	10.5	12.2**
Urinalysis, Days 10 (m)/11 (f)								
Volume, mL	8.5	5.0	8.4	5.7	8.3	6.5	8.8	9.5
Density, g/L	1018	1020	1017	1016	1017	1012	1015	1010*
Blood	0	0	0	0	0	1	2	1
Erythrocytes	0	0	0	0	0	1	1	1
Amorphous salts	2	1	2	2	2	2	3	2
Triplephosphate	2	1	2	1	2	1	1	2
Organ weights, absolute (mg)								
Heart	828	645	806	646	842	700	827	762
Liver	10648	7422	10726	7703	10876	7976	10840	8746
Spleen	548	369	584	396	577	408	523	426
Thymus	522	442	603	433	569	403	557	391
Epididymides	813	-	812	-	781	-	710	-
Ovaries	-	100	-	101	-	109	-	87
Gross pathology (200 mg/kg),	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=6
Kidney, pale discoloration								(4)
Lung, dark-red discoloration								(4)
Spleen, pale discoloration				4		1		5(1)
Stomach, dark-red								(4)
Histopathology (200 mg/kg),	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=6
Adrenal gland, mononuclear cell infiltration								(1)
Epididymides, mononuclear cell infiltration		-	-	-	-	-	1	-
Esophagus, fibrosis			-	-	-	-		(1)
Heart, infiltration/inflammatory								3(4)
fibrosis/musculature								2(1)
fibrosis/valvular								(1)
leukocytostasis								(2)
edema								(3)
Kidney, tubular necrosis								(1)
Liver, fibrosis							1	
congestion								(3)
glycogen, reduced								(1)
Liver/oro., fatty change/s. cel.		1			1	1	1	
fatty change/centri.						1		2(2)
Lung, collapsed/artificial					1		1	
septal thickening					1		1	1
congestion								3

hemorrhage	1			2
edema				1
intraalveolar macrophages				1
mesoth. hypertrophy				
septal thickening				(1)
Spleen, increased blood content		5	2	1(1)
Stomach, dilated gland/s.	-	-	-	(2)
Thymus, lymphoid cell necrosis	-	-	-	1(1)
Toxicokinetics	no data	no data	no data	no data

*The high dose group was reduced from 200 mg/kg to 100 mg/kg on Day 2 due to the increased mortality, and the dead animals were replaced. The female group included 1 from 200 mg/kg on Day 2 & 5 from 100 mg/kg.

@Three females died on Day 2 at 200 mg/kg, and one female died on Day 3 after 200 mg/kg for 2 days and once with 100 mg/kg.

*Clinical signs occurred from Day 0 at 200 mg/kg until the end of the study except for the spastic gait/accelerated breathing in 1 survived female rat for 2-3 days.

*N-DEM=aminopyrine-N-Demethylase; O-DEM=p-Nitroanisole-O-Demethylase

Statistically significant from control group at p=0.05* & p=0.01**

-: not available

UR: unremarkable

Summary: Administration of BAY 38-9456 to rats for 2 weeks caused elevated RBC parameters at high dose, blood/erythrocytes in urine sediment in the high-dose males or from mid-dose females, myocardial changes and induction of hepatic monooxygenases at 100 mg/kg in females. The myocardial degeneration regressed to myocardial fibrosis after 2-week treatment in females at 100 mg/kg, which predominantly affected septum and left ventricle wall, suggesting left heart failure as cause of death in the deceased rats at 200 mg/kg. Congestion in liver and lungs was considered to be secondary to cardiovascular failure.

Conclusion: A NOAEL was identified as 100 mg/kg in males and 25 mg/kg in females.

Study title: 2-Week Intravenous Toxicity of BAY 38-7268 in Beagle Dogs

Key study findings: Dogs tolerated i.v. dosing up to 0.5 mg/kg with a NOAEL of 0.15 mg/kg.

Study no: PH-31239/T0069807

Date of study initiation: July 31, 2000

Conducting laboratory and location: Institute for Toxicology, BAYER AG, Wuppertal, Germany

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BAY 38-9456, 000510-002

Formulation/vehicle: 0.01% in 50 mL ampoules

Dosing:

Species/strain: Beagle dogs [HsdBor:BEAG]

#/sex/group: 3/sex/group

Weight: 11.9 to 15.3 kg

Age: 51 to 58 weeks

Doses in administered units: 0, 0.05, 0.15 & 0.5 mg/kg

Route, form, volume, and infusion rate: i.v. in a volume of 0.5 to 5.0 mL/kg

Observations and times:

Observations	Times
Mortality/Clinical signs	Once daily
Body weights	Once weekly
Food Intake	Once daily
Ophthalmoscopy/Clinical chemistry/Hematology/Urinalysis	Weeks -2 & 2
ECG	Weeks -2, 1 & 2 (pre-dose & 1h-post-dose)
Organ weights/ Necropsy	Days 14 or 15
Toxicokinetics	Days 1 & 14 at 0.08, 1, 2, 7, & 24 hrs post-dose

Results: Slight decrease of GLUT/ALD/EH and increase of GST were observed at high dose. Hepatic multifocal Kupffer cell foci within the liver parenchyma were observed in all groups with increased severity (slight to moderate) at high dose. Higher prostate weight at high dose was due to 1 male. Posttraumatic changes such as perivascular infiltrates and macrophage accumulation were seen at the application sites in all groups. Sponsor stated that all other findings were within the historical control data in this age group of Beagle dogs.

Dose, mg/kg	0		0.05		0.15		0.50	
	3M	3F	3M	3F	3M	3F	3M	3F
Mortality	0	0	0	0	0	0	0	0
Clinical signs	UR	UR						
Body weights	UR	UR						
Food consumption	UR	UR						
Clinical chemistry, week 2								
ALT, U/L	17.9	18.6	20.6	15.6	24.7	17.8	17.9	32.7
LDH, U/L	43	38	48	40	55	40	44	55
Hematology, week 2								
Eosinophils, 10E9/L	0.26	0.36	0.35	0.46	0.41	0.24	0.44	0.57
Liver Biochemistry#, nmol/g•min								
GST	0.096	0.088	0.102	0.088	0.102	0.112	0.116	0.108
GLUT	2343	560	1913	546	2521	702	1959	500
ALD	10.5	7.2	10.2	6.1	9.3	8.7	8.2	4.9
EH	2774	1414	2406	1365	2724	1668	2372	1226
Urinalysis, week 2								
Volume, mL	344	194	281	299	273	332	355	339
Organ weights, absolute (g)								
Prostate	8.20	-	7.23	-	6.87	-	18.55@	-
Ovary	-	0.940	-	2.583	-	2.240	-	1.863
Uterus	-	5.3	-	27.3	-	17.3	-	16.0
ECG	UR	UR						
Gross pathology,								
Fat		2		1	1			3
Jejunum, area/s.							1	
Femur, area/s.							1	1
Joints, deformation							1	1
Histopathology,								
Kidney, glomerular lipidosis					1			1
Liver, hyperemia		1			1	1	1	
Lung, focal alveolar metaplasia				1	1	1	1	1
focal granulocyte infiltration						1	1	
Veins, front right, focal degeneration							1	
hind right, mononuclear cell infiltration						1		1

#ALD=aldrin epoxidase; EH=epoxide hydrolase; GST=glutathione-S-transferase; GLUT=UDP-glucuronyltransferase

@One male had a significant high value.

-: not available

UR: unremarkable

Toxicokinetics (#T0069807): There were no gender differences in exposure. Plasma concentrations of BAY 38-9456 increased dose-proportionally to low- and mid doses, and slightly over-proportionally to high dose with T_{max} of 5 min. M-1 caused dose-linearity on C_{max} but over-proportionality on AUC with T_{max} within 2 hrs. There was no accumulation or induction. Metabolic ratios for M-1 to BAY 38-9456 were 13-16% for AUC and 4-7% for C_{max} or less than 1% for M-4 to BAY 38-9456.

Dose, mg/kg		0.05	0.15	0.50
BAY 38-9456				
AUC _{0-7h} (µg•hr/L)	Day 1	23.6#	73.8#	315
	Day 10	20.1	65.9	300
C _{max} (µg/L)	Day 1	18.7	56.9	195
	Day 10	14.5	46.9	184
T _{max} (hr)	Day 1	0.0833	0.0833	0.0833
	Day 10	0.0833	0.0833	0.0833
BAY 44-5576 (M-1)				
AUC _{0-7h} (µg•hr/L)	Day 1	-@	9.28	41.1
	Day 10	-@	10.2	43.9

C _{max} ^s (µg/L)	Day 1	0.773	2.16	8.92
	Day 10	0.996	2.49	9.99
T _{max} (hr)	Day 1	1.50	1.83	1.67
	Day 10	1.00	1.67	1.67
BAY 44-5578 (M-4)		-	-	-

3/sex/timepoint as arithmetic means

*4 of 6 values were extrapolated from 2 to 7 hrs

The administered dose refers to the free base.

-@: AUC was not calculated due to sparse data points.

*C_{max} was derived from the concentrations at timepoint 1 & 2 hr post-dose.

-: Plasma concentrations of M-4 were below the LOQ (— µg/L) in most cases except in the 0.5 mg/kg group with — µg/L at 2hr post-dose on Day 1 or — µg/L at 1 & 2 hr post-dose on Day 10, respectively.

Summary and conclusion: There were no treatment-related findings in the intravenous dosing up to 0.5 mg/kg over 2 weeks. A NOAEL was defined as 0.15 mg/kg due to increased severity of multifocal Kupffer cell foci in the liver at high dose.

Study title: 3-Month Toxicity in the Drinking Water of Wistar Rats

Key study findings: A NOAEL of 8 mg/kg was selected due to changes in RBC parameters in all treated groups.

Study no: PH-29976/T8062659

Date of study initiation: May 29, 1998

Conducting laboratory and location: Institute for Toxicology, BAYER AG, Wuppertal, Germany

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BAY 38-9456, 503856/503881

Formulation/vehicle: Demineralized water

Dosing:

Species/strain: SPF-bred Wistar rats [HsdCpb: WU]

#/sex/group: 10/sex/group

Satellite groups used for toxicokinetics or recovery: 3/sex/group for TK

Weight: 150-180 g for males, 108-139 g for females

Age: 5 to 6 weeks (m), 5 to 6 weeks (f)

Doses in administered units: 0, 8, 40 & 200 mg/kg

Route, form, volume, and infusion rate: Via drinking water in a volume of 10 mL/kg

Observations and times:

Observations	Times
Mortality/Clinical signs	Twice daily
Body weights/Food Intake	Weekly
Clinical chemistry/Hematology/Urinalysis	Week 12
Organ weights/Necropsy	Week 14
Toxicokinetics	Days 5, 6, 22, 23, 89 & 90

Results: No treatment-related mortality occurred. Body weight reduction was statistically significant at 200 mg/kg in males from Week 4 with 18%, and in females at 40 mg/kg from Weeks 7 to 11 with 8% or at 200 mg/kg from Week 0 with 12%. Dose-dependent reduction in water intake was noted in all treated groups, and was pronounced in high-dose males with 26% and in females with 31%, possibly due to palatability. Dose-dependent decrease (erythrocytes, HB, HCT) and increase (MCV, MCH) in RBC parameters were observed. Higher urine volume with lower pH was shown in the high-dose males. All other changes were within historical ranges. Relative liver weights were increased at high dose, and TG was decreased from mid dose. Significant increase in N-DEM, O-DEM and CYP450 was observed in the high-dose males and from mid-dose females. Dose-dependent increase in EROD and decrease in ALD activities were noted in males. Marked enzyme induction was found for the EH and GST. Dose-dependent increase in kidney weight and urea paralleled with basophilic tubules with increased severity in males or incidence in females from mid dose, indicating regeneration following initial tubular degeneration. Myocardial fibrosis and higher frequency of mononuclear cell infiltration associated with increased relative heart weight at high dose possibly as a result of the pharmacological effect of the drug. Follicular cell hypertrophy/colloidal vacuolation in the thyroid glands in high-dose females with increased incidence and/or severity correlated with increased T3/T4 levels and TSH. Acinar hypertrophy in the parotid gland/submandibular gland with increased incidence and

severity was characterized by an increase in cell size and enhanced cytoplasmic vesicular structure and basophilia, and considered as an adaptive effect of PDE inhibitors. Plasma concentrations of BAY 38-9456 or M-1 generally increased over-proportionally to dose levels. Exposure was significantly higher in females with slight accumulation of the parent drug on repeated dosing. Ratio of M-1 to BAY 38-9456 was 55-190% in males and 30-53% in females.

Dose, mg/kg	0		8		40		200	
	10M	10F	10M	10F	10M	10F	10M	10F
Mortality	0	1*	0	1*	0	1*	1*	0
Clinical signs	UR	UR	UR	UR	UR	UR	UR	UR
Body weights, week 13 (g)	386	222	383	221	388	211	318**	195**
Food consumption	UR	UR	UR	UR	UR	UR	UR	UR
Water intake, g/day/animal	26.2	20.1	25.9	19.2	24.5	17.1	19.4	13.8
Clinical chemistry, week 12								
TG, mmol/L	1.72	1.69	1.98	1.60	1.56	1.07**	0.81*	0.69**
Urea, mmol/L	7.38	7.22	7.60	7.73	8.29	7.79	9.16**	11.12**
Total bilirubin, μ mol/L	1.4	1.4	1.5	1.3	1.6	1.3	1.8**	1.4
T3, nmol/L	1.75	1.74	1.94*	1.83	2.06**	1.85	2.07**	1.86
T4, nmol/L	48	45	50	50	59**	50	63**	52
TSH, μ g/L	7.68	2.03	6.00	2.37	8.14	1.91	9.62	3.80*
Hematology, week 12								
Erythrocytes, $10E12/L$	8.98	8.63	8.97	8.15**	8.47*	7.95**	8.04**	7.47**
HB, g/L	153	150	150	141**	146	143*	143**	137**
HCT, l/L	0.476	0.459	0.468	0.432**	0.451*	0.433**	0.442**	0.418**
MCV, fl	53.1	53.2	52.3	53.0	53.2	54.5	54.9	56.0*
MCH, pg	17.1	17.4	16.8	17.4	17.2	18.0	17.7	18.4*
Liver biochemistry ^e , week 14								
N-DEM, mU/g	147.1	69.9	128.9	66.5	127.0	79.7	166.9*	121.2**
O-DEM, mU/g	12.7	9.6	13.0	10.2	12.9	12.9	18.6*	18.3**
CYP450, nmol/g \cdot min	44.8	38.0	42.4	37.4	45.3	40.4	54.0*	41.1**
EROD, nmol/g \cdot min	0.30	0.42	0.42	0.17	0.56*	0.22	0.61	0.61
ALD, nmol/g \cdot min	129.7	22.5	102.0	12.8**	85.2*	14.1*	44.7**	27.3
EH, nmol/g \cdot min	350	201	330	114*	425	145	526	387**
GST, μ mol/g \cdot min	143	122	152	122	163	128	165**	141*
GLUT, nmol/g \cdot min	641	486	572	271	629	288	716	549
Urinalysis, week 12								
Volume, mL	12.8	10.2	16.3	13.3	14.2	7.2	17.2	9.9
pH	7.7	7.2	7.6	7.3	7.4	7.3	6.8*	7.5
Organ weights, absolute (g)								
Brain	2073	1872	1997	1901	2011	1767	1936*	1768
Liver	13894	7935	13617	8513	13983	8298	12979	9319**
Kidney	2208	1387	2334	1432	2474*	1512	2547**	1941**
Spleen	707	480	728	458	686	499	595*	461
Thymus	375	286	369	332	346	329	295*	265
Gross pathology	UR	UR	UR	UR	UR	UR	UR	UR
Histopathology,								
Heart, myocardial fibrosis							1	1
mononuclear cell infiltration	1		1		1		4	2
Kidney, basophilic tubules	8	1	7	1	10	3	9	7
Thyroid gland, follicular cell hypertrophy	7	2	9	1	5	3	7	8
colloidal vacuolation	3		9	1	5	2	4	4
Parotid gland, acinar hypertrophy	1				1	1	8	8
Submandibular glands, acinar hypertrophy							2	
Toxicokinetics, Day 22/23, 3/sex/group								
AUC _{0-21h} (μ g \cdot hr/L)			-	935	816	5463	11830	61743
C _{max} (ng/mL)			29.8	98.4	88	636	1591	6614
T _{max} (hrs)			12	21	21	15	-	15

#Deaths were causally related to blood collection.

@ECOD=7-ethoxycoumarin deethylase; EROD=7-ethoxyresorufin deethylase; ALD=aldrin epoxidase; EH=epoxide hydrolase;

GST=glutathione-S-transferase; GLUT=UDP-glucuronyltransferase; N-DEM=aminopyrine-N-demethylase; O-DEM=*p*-nitroanisole-O-demethylase

Statistically significant from control group at $p=0.05^*$ & $p=0.01^{**}$

Summary: A NOAEL was determined as 8 mg/kg due to dose-dependent changes of RBC parameters in all treated groups.

Toxicology summary: Oral LD₅₀ of BAY 38-9456 was 1000 mg/kg in mice, and 190-250 mg/kg in rats. The acute toxic effects observed in rats and mice are most likely due to an exaggerated pharmacodynamic response, which results in low blood pressure and in death. Repeated dose toxicity studies were conducted in rats, mice and dogs. Major findings were the cardiovascular effects and testicular degeneration in both rats and dogs, vasculitis in dogs, vacuolation of the adrenal cortex and acinar hypertrophy in pancreas/thyroid/submandibular/parotid glands in rats. In rats, deaths occurred at high doses secondary to myocardial damage. Vasodilating properties of BAY 38-9456 led to myocardial fibrosis/necrosis and subepicardial changes in the atrium consisting of subepicardial/pericardial edema, partly accompanied by an increase in minimal to mild subepicardial inflammatory infiltration in dogs. In the 1-month study, the subepicardial edema occurred from mid dose (10 mg/kg) in males and low-dose females with increased incidence and severity. Minimal to mild edema of the adventitial layer of the subepicardial arteries from mid dose and combined with subepicardial inflammatory cell infiltration were found in high-dose males. Slight arteritis/periarteritis in the right ventricle was noted in 1 of each male and female at high dose. In the 3-month study, minimal to moderate periarteritis/arteritis were observed in multiple cardiac lesions at high dose (3/6M, 1/6F). Minimal to slight periarterial edema was found in 1 of each male and female at high dose in the 1-year study, indicating no aggravation over time. Exposures at NOAELs for the arteritis findings corresponded to about 11 fold in the 1- and 3-month studies, and 150 fold in the 1-year study the human exposure at the maximum recommended dose of 20 mg. These effects associated with hypotension/tachycardia, and were considered secondary to the hemodynamic effects of the drug. Testicular degeneration/atrophy was observed in the 3-month studies in rats and dogs, but did not progress in the chronic studies with no/low incidence. Follicular cell hypertrophy in the thyroid was observed in the 1- and 3-month rat studies at high doses more pronounced in females corresponding to higher exposures. The hypertrophy was noted only in one high-dose female in the 6-month study, and did not markedly progress in the 2-year carcinogenicity study in females. The incidence, however, was statistically significant in the high-dose males in the 2-year study. The findings were often associated with altered T3/T4 or TSH levels, but were reversible during a 4-week recovery period in the 3-month study. An *in vitro* examination showed no indication of the interference with the thyroid enzymes, suggesting that the thyroidal changes be neither a direct effect on the thyroid nor the pituitary, but be an adaptive response secondary to increased hepatic thyroxin elimination. Sponsor considered the findings were not toxicologically relevant since (1) the effects were comparable to sildenafil; (2) did not reflect a direct effect at the thyroid gland level; (3) occurred only at high doses in the rat, which is known to be more sensitive than other species; (4) did not show a progression with prolongation of treatment; and (5) did not induce malignant tumors in a 2-year carcinogenicity study. Diffuse acinar hypertrophy in the pancreas was seen in the 1-, 3-, 6-month and 2-year studies at high doses. Male rats exhibited a focal acinar atrophy associated with interstitial fibrosis, a macrophageal pigment deposition and/or a mononuclear cell infiltration with increased incidence in the 3-month study at high dose and in the 6-month study from mid dose (15 mg/kg). Diffuse acinar hypertrophy in the salivary gland/parotid gland were found in the 1-month study at ≥ 25 mg/kg (PH-28163A), in the 3-month study at ≥ 25 mg/kg, in the 6-month at 75 mg/kg (F), and in the 2-year study at 15 (M)/25 (F) mg/kg. Acinar hypertrophy in the exocrine glands was considered to be an adaptive effect of PDE5 inhibitors. Glomerulosa vacuolation in the adrenal glands (large/small vesicles) was noted in the 1-month study at ≥ 40 mg/kg (PH-30893), in the 6-month study at ≥ 3 mg/kg (small vesicles) and in the 2-year study at 75 (M)/25 (F) mg/kg. All these findings occurred at >10-fold exposure of the human therapeutic exposures.

Toxicology conclusions: A NOAEL for any toxicity was considered to be 3 mg/kg in rats and dogs, which produces exposure of the unbound drug of 2-21 fold greater than the human exposure at 20 mg.

Histopathology Inventory for NDA #21-400

Species	2.wk Rat	2.wk Dog	1.mo Rat	1.mo Dog	3.mo Mouse	3.mo Rat	3.mo Dog	6.mo Rat	1.yr dog	2.yr Mouse	2.yr Rat
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X	X	X	X	X	X	X
Bone Marrow smear											
Bone (femur)	X	X	X	X	X	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X	X	X	X	X	X	X
Cervix											
Colon	X	X	X	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X	X	X	X
Epididymis	X*	X*	X*	X*	X	X	X	X	X*	X	X
Esophagus	X	X	X	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X	X	X	X
Fallopian tube											
Gall bladder		X		X			X		X	X	
Gross lesions	X	X	X	X	X	X	X	X	X*	X	X
Harderian gland	X		X		X	X		X		X	X
Heart	X*	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Ileum	X	X	X	X	X	X	X	X	X	X	X
Injection site											
Ileum	X	X	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland	X		X		X	X		X		X	X
Larynx	X	X	X	X	X	X	X	X	X	X	X
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X*	X	X*	X	X	X*	X	X*	X	X
Lymph nodes, cervical											
Lymph nodes, mandibular	X	X	X	X	X	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X	X	X	X
Mammary Gland	X		X		X	X	X	X	X	X	X
Nasal cavity		X		X					X		
Optic nerves	X	X	X	X	X	X	X	X	X	X	X
Ovaries	X*	X*	X	X*	X*	X*	X*	X*	X*	X*	X*
Pancreas	X	X*	X	X*	X	X	X*	X	X*	X	X
Parathyroid	X	X	X	X	X	X	X	X	X	X	X
Peripheral nerve											
Pharynx		X		X					X		
Pituitary	X	X	X	X	X	X	X*	X	X*	X	X
Prostate	X	X*	X	X*	X	X	X*	X	X*	X	X
Rectum	X	X	X	X	X	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X	X	X	X	X	X
Seminal vesicles	X		X		X	X		X		X	X
Skeletal muscle	X	X	X	X	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X	X	X	X	X	X	X
Stomach	X	X	X	X	X	X	X	X	X	X	X
Testes	X*	X*	X*	X	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X	X*	X*	X	X*	X	X
Thyroid	X	X*	X	X*	X	X	X*	X	X*	X	X
Tongue	X	X	X	X	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X	X	X	X
Uterus	X	X*	X	X*	X	X	X*	X	X*	X	X
Vagina	X	X	X	X	X	X	X	X	X	X	X
Zymbal gland	X		X		X	X		X		X	X
Ureter/Urethra			X		X	X		X		X	X

X, histopathology performed
 *, organ weight obtained

V. GENETIC TOXICOLOGY (See also Review #1/3 in Appendix I)

Genetic toxicology summary and conclusions: BAY 38-9456 was negative in the *in vitro* Ames test, chromosome aberration assay and induced forward mutation, and the *in vivo* mouse micronucleus assay.

Labeling recommendations: Vardenafil was negative in *in vitro* bacterial and Chinese hamster V79 cell assays to detect mutagenicity, and in an *in vitro* assay (Chinese hamster V79 cells) and in an *in vivo* mouse micronucleus test to detect clastogenicity.

VI. CARCINOGENICITY:

Study title: 2-Year Carcinogenicity Study via Drinking Water in CD-1 Mice

Key study findings: BAY 38-9456 was negative in 2-year mouse carcinogenicity study up to 1000 ppm.

Study number: PH-31279 (T7068012) Date of study initiation: 3/3/99
 Conducting laboratory and location: Institute of Toxicology, BAYER AG, Wuppertal, Germany QA report: yes (x) no ()
 GLP compliance: yes
 Drug, lot #, and % purity: BAY 38-9456, #503986, 84% as free base
 CAC concurrence: Study protocol and dose selection were approved by the Executive CAC on 2/9/99.

Study Type: 2-year rodent bioassay Number/sex/group: 50/sex/group
 Species/strain: SPF-bred Cd1 mice/Crl:CD1(ICR)BR Formulation/vehicle: Drinking water
 Age at start of study: 7-8 weeks; 28-35 g (M), 22-28 g (F)
 Animal housing: Individually
 Drug stability/homogeneity: Assessed

Methods:
 Doses: 0, 40, 200 & 1000 ppm; 0, 7.0, 31.9 & 150.5 mg/kg (M); 0, 8.5, 42.1 & 193.4 mg/kg (F)
 Basis of dose selection: PK endpoint for 14-week dose range-finding study (PH-30633)
 Restriction paradigm for dietary restriction studies: N/A
 Route of administration: Orally via drinking water Frequency of drug administration: Weekly
 Dual controls employed: No Interim sacrifices: M; 28-18-27-21, F; 33-32-33-29
 Satellite PK or special study group(s): 5/sex/timepoint for PK Deviations from original study protocol: N/A
 Statistical methods: Exact Fisher test and Peto's trend test for pathology

Observations and times:

Observations	Times
Clinical Signs	Twice daily (detailed weekly)
Body Weights	Weekly
Food Consumption	Every 4 weeks
Water Intake	Weekly up to 13 week/Every 4 weeks thereafter
Hematology	Weeks 52/53, 78/79 & 104
Organ weights/Pathology	Week 104
Toxicokinetics	Days 5, 364 & 735 at 14 or 15 hr/Days 112/113 at 0, 3, 6, 9, 12, 15, 18, 21 & 24 hr

Results: No treatment-related mortality/body weight/food consumption were indicated at the end of the study. Intercurrent deaths were equally distributed throughout the dose groups. There exhibited significantly lower body weights in the high dose males at some time points, which are of no account (see Appendix III for graphical presentation). These animals mostly died of local or systemic tumor growth, inflammatory changes or amyloidosis. Water intake was markedly reduced at 1000 ppm with a maximum of 24% in Week 76 in males or 22% in Week 85 in females. Cumulative and mean daily water intake was about 10% less in the high dose group than controls. Higher incidence of piloerection, girth and accelerated breathing were observed from 200 ppm females.

Dose, ppm	0		40		200		1000	
	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	28	33	18	32	27	32	21	29
Clinical signs,								

Piloerection	14	16	11	11	14	19	12	21
Increased girth	10	18	8	19	14	21	5	28
Accelerated breathing	3	6	1	9	5	9	4	12
Eye, reduced size	1		3		1	2	4	1
opacity	2		5	1	6	3	4	3
redness of eyelids	1		2	1	2	1	3	1
Body weights	UR							
Food consumption	UR							
Water intake, g/animal/day, week 105	6.58	7.08	5.83	7.21	6.28	6.34	5.32	6.17
Hematology, Week 104								
Band neutrophils, %	1.1	0.9	0.8	0.8	0.5	1.5	0.5	1.1
Atypical lymphocytes, %	2.0	1.8	1.5	1.5	2.0	1.9	1.0*	1.6
Organ weights, mg								
Adrenals	10	11	6	11	7	11	6	10
Liver	2560	2043	2336	2304	2380	2020	2289	2249
Spleen	129	238	146	277	123	228	141	350
Kidneys	837	514	847	560	831	547	978	582

Statistically significant from control group at p=0.05*

UR- unremarkable

Toxicokinetics: Plasma concentrations were measured in the present study at single points on Days 5, 365 and 735 (PH-31176) and in a separate study at AUC_{0-24h} on Days 5 and 112/113 (PH-29788). Exposures of BAY 38-9456 and M-1 were higher in females than in males at high dose on Days 364 and 835. M-4 had 3-fold difference on Day 735. M-1 and M-4 reached up to 24% and 8% of the plasma concentrations of the parent drug on Day 112/113, respectively. BAY 38-9456; AUC increased proportionally and C_{max} increased over-proportionally at high dose. Decrease in the plasma concentrations of the high dose group on Day 364 in males (36%) and from the mid dose group on Day 735 for both sexes (34-20 % in males, 43-74% in females) was considered due to variable individual data. Lower exposure was observed in the high dose males on Day 735 (174 µg/L) than on Day 112/113 (333 µg/L). Sponsor explained the difference due to drinking behavior and short half-life of the drug. Slight accumulation occurred on repeated dosing. M-1 & M-4; AUC and C_{max} increased slightly over-proportionally at higher doses on Day 112/113. No accumulation occurred on repeated dosing.

TK Parameters of BAY 38-9456, M-1 & M-4 via Drinking Water in Mice on Day 112/113 (PH-29788)

Dose, ppm	40		200		1000	
	M	F	M	F	M	F
BAY 38-9456, mg/kg	6	8	30	37	136	169
AUC _{0-24h} (µg•hr/L)	65.6	148	365	808	2118	3541
C _{max} (µg/L)	6.80	18.7	51.3	130	334	626
T _{max} (hr)	24 [#]	15	15	12	15	15
BAY 44-5576 (M-1), mg/kg	5.66	7.54	28.3	34.9	128	159
AUC _{0-24h} (µg•hr/L)	-	-	-	106	408	824
C _{max} (µg/L)	1.36	2.74	5.68	10.9	49.2	118
T _{max} (hr)	24 [#]	15	15	12	9	15
BAY 44-5578 (M-4), mg/kg	5.68	7.58	28.4	35.0	129	160
AUC _{0-24h} (µg•hr/L)	-	-	17.4	21.5	163	135
C _{max} (µg/L)	1.21	0.75	1.37	2.08	28.3	16.1
T _{max} (hr)	18	12	15	12	9	15

*Day 113

The administered doses refer to the free base BAY 38-7268.

n=5/sex/timepoint as geometric means

Plasma Concentrations of BAY 38-9456, M-1 & M-4 via Drinking Water in Mice (PH-31176)

Dose, ppm	40		200		1000	
	M	F	M	F	M	F
BAY 38-9456						
C (µg/L) Day 5, 15 hr	1.86	2.95	136	75.3	1379	1482
Day 364, 14 hr	19.1	31.4	109	190	380	1752
Day 735, 14 hr	4.03	4.22	31.6	21.9	174	847
BAY 44-5576 (M-1)						
C (µg/L) Day 5, 15 hr	-	1.11	12.6	14.5	172	198
Day 364, 14 hr	1.13	2.48	8.34	29.9	47.2	391
Day 735, 14 hr	-	1.20	3.82	4.08	31.2	175
BAY 44-5578 (M-4)						
C (µg/L) Day 5, 15 hr	<0.5	<0.5	2.51	2.35	35.2	36.1
Day 364, 14 hr	<0.5	<0.5	2.00	3.26	12.2	29.5
Day 735, 14 hr	<1.0	-	0.959	1.35	8.66	28.5

The administered doses refer to the free base BAY 38-7268.

n=5/sex/timepoint as geometric means

-, not available

Gross pathology: The incidence of uterine dilation, consistency-changes and nodules increased markedly at high dose. Other findings were sporadically distributed and were considered to be incidental. Table below summarizes the macroscopic findings with increased incidence.

Dose, ppm	0		40		200		1000	
	50M	50F	50M	50F	50M	50F	50M	50F
Heart, enlarged					1		2	1
Lung, collapsed, slight area	1 3	4	2 2	4	5 3	1	3 2	2 4
Liver, area enlarged	1	2 6	4 1	5 5	1 3	3	3 3	1 3
Stomach, thickened area	1 2	2	2 1		2		4 1	2 3
Spleen, small size						2	3	
Eyes, turbidity size, small	1 1		3 1	2	2 1	3 1	3	3 2
Duodenum, change-in-contents						1		2
Uterus, consistency-change	-	5	-	11	-	15	-	18
dilation	-	1	-	6	-	5	-	10
nodule	-	9	-	8	-	9	-	16

Non-neoplastic findings: Historical control data from the conducting laboratory were not provided. Most of the findings were associated with the age of the animals, which were degenerative, inflammatory or proliferative in nature. Some of the findings were statistically reduced or increased. Reduced incidence of the atrophies in the testes and inflammation/aspermia/epithelial vacuolation in the epididymides was observed at high dose. In the kidneys, the incidence of basophilic cortical tubules in females and focal fibrosis in males was significantly increased at high dose with concomitant increase of kidney weight. The increase in basophilic tubules was regarded as incidental due to the equally distributed grades of the lesion without clear dose correlation. Focal fibrosis was minimal to slight and occurred mostly unilaterally. Hematopoiesis in the liver was significantly increased in the high dose females without apparent increase in the other organs where hematopoiesis was recorded (mandibular/mesenteric lymph nodes, spleen, bone marrow). Dose-dependent increase of focal medullary hyperplasia in the adrenal glands was observed in females (0-2-3-5), and was statistically significant at high dose. The incidence was generally minimal to slight, unilateral, and close to the historical range from the Registry of Industrial Toxicology Animal-Data (RITA) according to the sponsor. However, the neoplasm of the adrenal medulla, benign medullary tumor was found sporadically in females (0-0-1-0). Stomach cyst, lung congestion and fatty degeneration/subcapsular cell hyperplasia in the adrenal

gland cortex were statistically significantly decreased in females. In males, diffuse atrophy/Leydig cell atrophy in the testes, aspermia/epithelial vacuolation/inflammation in the epididymides, subcapsular cell hyperplasia in the adrenal gland cortex, lacrimal gland atrophy, and eye inflammation were statistically significantly reduced at high dose. Frequencies of amyloidosis and inflammation in the various organs were significantly reduced in the high dose group. Sponsor stated that all other findings were incidental and occurred with the range of historical data of the strains. Table below summarizes the microscopic findings with increased incidence.

Dose, ppm	0		40		200		1000	
	49M	49F	50M	50F	49M	48F	49M	50F
Heart, arteritis/periarteritis	1	4	3	2	4	1	4	1
myocardial degeneration					1		2	
myocardial fibrosis	4	1	8	4	7	2	7	3
Lung, alveolar macrophages	11	8	7	10	13	14	15	14
cystic glands	1		3	1	5	1	1	2
congestion	7	7	3	2	3	3	6	1 ^a
Liver, congestion				1	1	1	2	2
focal necrosis/degeneration	7	4	4	5	2	5	2	8
hematopoiesis		2	2	5		4	1	10 ^b
single cell necrosis/degeneration	1	1	3	1	2	3	3	2
Nose, atrophy			2		4		1	2
granulocytosis			1				2	
hyaline inclusions	3	3	6	3	4	2	3	7
Trachea, mononuclear infiltration	3		7	2	6	2		2
Tongue, edema					1			2
Stomach, cyst	1	10	1	4	5	8	3	2 ^c
gld. stomach erosion	2	2	2	2	3	1	1	6
Gall bladder, mononuclear infiltration		7	2	1	1	1	3	3
Kidneys, basophilic tubules	32	21	32	27	32	19	35	31 ^d
focal fibrosis	3		7		4	1	10 ^c	
papillary mineralization	6	1	2	2	4	3	4	5
Epididymides, aspermia	8	-	8	-	10	-	1 ^f	-
epithelial vacuolation	5	-	6	-	1	-	0 ^g	-
inflammation	5	-	0 ^e	-	2	-	0 ^h	-
Testes, tubular atrophy, diffuse	14	-	13	-	13	-	6 ⁱ	-
Leydig cell atrophy, differentiated	4	-	1	-	1	-	0 ^j	-
tubular mineralization	9	-	6	-	13	-	3	-
Vagina, prolapsed uterus	-		-	1	-		-	2
Thyroid gland, follicular ectasis	14	9	13	15	16	10	18	15
Spleen, lymphocytolysis	1	1	2		2	1	1	3
lymphoid hyperplasia	4	2	5	3	4	6	3	5
Thymus, angiectasis		1		1		1		3
lymphoid hyperplasia	1	17	3	10	2	8	4	10
Mesent. lymph node, hematopoiesis		1	1	2	1	4	1	2
histiocytosis		5	4	5	3	2	3	4
lymphoid hyperplasia	10	4	8	7	4	9	5	9
plasmacytosis		2	4		2	1	2	1
Mand. lymph nodes, hematopoiesis	1			3	1	2		3
histiocytosis	1	2				1	4	3
lymphoid hyperplasia	3	2					3	5
Salivary glands, basophilic hypertrophy, foc.	1		2	4	2		3	1
hypertrophy, parotid gland							2	
Harderian glands, glandular ectasis	1	4	2	5	2	1	3	1
Lacrimal glands, Harderian gland alteration	2		2	2		5		6
atrophy	8	6	10	4	4	5	2 ^k	6

Adrenal glands, cortical atrophy	11	2	21	5	16	5	17	4
hematopoiesis		2		4		3	2	1
cortical hypertrophy	3	2	9	1	5		8	2
fatty degeneration	13	28	23*	23	18	23	14	18 ^l
focal medullary hyperplasia	1		3	2		3	1	5 ^m
subcapsular cell hyperplasia A	22	47	17	45	16	44	13*	40 ⁿ
Zymbal glands, atrophy		1	1	1	1		3	
inflammation	1	2	6	1	2	1	4	
Femur, cystic structure			1		2		3	1
increased hematopoiesis	4	4	5	4	6	6	3	7
Sternum, increased hematopoiesis	4	3	5	4	6	6	2	7
Eyes, retinal atrophy	1	7	4	4		4	3	7
inflammation	6		1	1	2	1	0*	

Statistically significant from control group at p=0.05* (Exact Fisher test)
 p=0.0359^a, 0.0062^b, 0.0239^c, p=0.0336^d, p=0.0244^e, p=0.0052^f, p=0.0082^g, p=0.0475^h, p=0.0174ⁱ, p=0.0495^j, p=0.0107^k, p=0.0336^l,
 p=0.0212^m or p=0.0062ⁿ (Non-survival adjusted trend test)

Neoplastic findings: Historical control data from the conducting laboratory were not provided. All tumor incidences were evenly distributed without dose-correlation and within/ close to the historical control data. Incidences of hemangiomas and hemangiosarcomas were frequently encountered in various locations (mostly in liver and uterus) with slightly increased total incidence in the high dose females.

Incidence of Hemangiomas & Hemangiosarcomas

Dose (ppm)	0		40		200		1000	
	9M	9F	50M	50F	9M	8F	9M	50F
Hemangiomas, liver	1	3	4	1	2	2	1	
spinal cord					1			
spleen					1			
uterus/cervix		1		3		1		2
mesent. lymph node		1				1		
femur								1
skin		1						
All sites for hemangiomas	1	6	4	4	4	4	1	3
Hemangiosarcomas, Liver	2	1	1			1		
Spinal cord		1						
Uterus/Cervix	-		-	1	-	1	-	2
Spleen								1
Skeletal muscle								1
All sites for hemangiosarcomas	2	1	1	1	0	2	0	4

Bronchiolo-alveolar adenomas and carcinomas were one of the most frequent tumors in the present studies. The incidence of adenomas and carcinomas was increased at all doses without dose-relatedness in the distribution and in the preceding stage of hyperplasia.

Neoplastic Findings in the Lung

Dose (ppm)	0		40		200		1000	
	9M	9F	50M	50F	9M	8F	9M	50F
Adenoma, bronchiolo-alveolar	6	2	12	3	11	6	12	4
Carcinoma, bronchiolo-alveolar	4	3	3	4	6	3	5	3
Carcinoma, metastasis	1	1	1	4	3	1	1	2
Adenomas+Carcinomas	10	5	15	7	17	9	17	7

Incidence of hepatocellular adenomas was slightly increased from mid-dose males and was observed in the high dose females without statistical significance.

Neoplastic Findings in the Liver

Dose (ppm)	0		40		200		1000	
	9M	9F	50M	50F	49M	48F	49M	50F
Carcinoma, hepatocellular	7		3		6		5	
Adenoma, hepatocellular	4		5		7		7	1
Adenomas+Carcinomas	11		8		13		12	1

Diffuse tubular and Leydig cell atrophies were significantly reduced at high dose, whereas Leydig cell hyperplasia and Leydig cell tumor were observed with increased incidence at high dose.

Neoplastic/Non-neoplastic Findings in the Testes

Dose (ppm)	0		40		200		1000	
	9M	9F	50M	50F	49M	48F	49M	50F
Leydig cell hyperplasia, differentiated		16		20		16		22
Leydig cell tumor (B)		1		0		3		3

In the pituitary, adenomas of the pars distalis were more common in females than in males at all treated groups.

Neoplastic/Non-neoplastic Findings in the Pituitary Gland

Dose (ppm)	0		40		200		1000	
	49M	49F	47M	50F	49M	48F	49M	50F
Cyst	5		1	1		2	3	3
Single cell necrosis								1
Adenoma, pars distalis	2		1	4		2		3
Carcinoma, pars distalis		1						
Adenomas+Carcinomas	2	1	1	4	0	2	0	3

Adrenal cortex adenomas were encountered mostly in males without dose-correlation.

Neoplastic/Non-neoplastic Findings in the Adrenal Gland

Dose (ppm)	0		40		200		1000	
	9M	9F	50M	50F	49M	48F	49M	50F
Subcapsular cell hyperplasia A	22	47	17	45	16	44	13*	40*
Adenoma, subcapsular cell		1	2		2		2	
Adenoma, cortical			2		1		3	

Statistically significant from control group at $p=0.05^*$ (Exact Fisher test)
 $p=0.0052^*$ (Exact Peto's trend test)

In the uterus, dilation, consistency-changes and nodules were increased at the high dose level. Various histopathological lesions were related to the gross findings. The most frequent finding of cystic endometrial hyperplasia in the present study can lead to cystic uteri and increased uterine size. Sponsor considered the increased incidence of gross findings as incidental since the historical data for cystic uteri were in the same range as consistency-changes in the present study. Various tumor types in the uterus occurred sporadically without dose correlation.

Neoplastic/Non-neoplastic Findings in the Uterus/Uterine Cervix

Dose (ppm)	0		40		200		1000	
	9F	9F	50F	50F	48F	48F	50F	50F
Consistency-change	5		11		15		18	
Dilation	1		6		5		10	
Nodule	9		8		9		16	
Cystic endometrial hyperplasia	34		41		39		41	
Angiomatous hyperplasia					2		1	
Adenocarcinoma			1				1	

Hemangioma	1	3	1	2
Hemangiosarcoma		1	1	2
Leiomyoma	4	3	6	5
Tumor NOS				1

Other rare tumors were found in the stomach (1/49, F), cecum (1/49, M), skin (1/49, F) and skeletal muscle (1/49, M). The incidence of histiocytic sarcoma was increased in the high-dose females, but was close to historical control data from the RITA database (4-12%) according to the sponsor. The incidence in males displayed a negative trend. In the ovaries, various tumor types occurred sporadically without dose correlation.

Other Neoplastic Findings

Dose, ppm	0		10		200		1000	
	9M	9F	50M	50F	9M	9F	9M	9F
Heart, metastatic sarcoma	1							1
Stomach, adenoma			1					1
Cecum, adenoma							1	
Hemolymphoreticular system, histiocytic sarcoma	2	4	2	4	0	3	0	7
malignant lymphoma	4	7	3	10	6	11	5	12
Harderian glands, adenoma	5	3	9	4	7	4	7	1
Mandibular lymph node, metastatic carcinoma								1
Skin, fibrous histiocytoma	1							1
Vagina, sarcoma, metastasis	-		-		-		-	1
Skeletal muscle, rhabdomyosarcoma							1	
Ovary, cystadenoma (B)	-		-	3	-	2	-	1
granulosa cell tumor (B)	-		-	1	-	2	-	1

*p=0.0360 (Exact Peto's trend test)

OVERALL INTERPRETATION AND EVALUATION

Adequacy of the carcinogenicity study and appropriateness of the test model: Mortality rate (36 to 66%) was similar among treatment groups at the end of the study. The total number of animals with tumors, benign/malignant tumors or metastases was not significantly affected with doses in scheduled or unscheduled deaths for both sexes. The number of mice with more than one primary neoplasm was increased in females with 18.4% (vehicle), 34.0% (LD), 31.3% (MD) and 30.0% (HD) without dose-relatedness, but not in males. Changes of body weight and food intake were not affected in both males and females except intermittent lower body weights in the high dose males than controls. Cumulative and mean daily water consumption was significantly reduced at high dose (~10%) compared to controls. Sponsor measured the AUC in a separate study on Day 112/113, and the plasma concentrations at a single timepoint were measured in the present study. Decrease in the plasma concentrations of the high dose group on Day 364 in males (36%) and from the mid dose group on Day 735 for both sexes (34-20% in males, 43-74% in females) was considered due to variable individual data rather than enzyme induction. Also, lower exposure was observed in the high-dose males on Day 735 (174 µg/L) than on Day 112/113 (333 µg/L). Sponsor explained the difference due to drinking behavior. The total AUCs of the unbound drug (BAY 38-9456+M-1) produced approximately 21 fold in males and 37 fold in females the human therapeutic exposure at 20 mg, which gives similar multiples based on mg/m² at corresponding doses of 123 and 160 mg/kg in Week 105.

Evaluation of Tumor findings: Hemangiomas and hemangiosarcomas were frequently encountered in various locations (mostly in liver and uterus) with slightly increased total incidence in the high-dose females. Histiocytic sarcomas were slightly increased in the high-dose females (4-4-3-7) but were close to historical control range from the RITA database (4-12%) according to the sponsor. Other rare tumors were found in the stomach (1/49, F), cecum (1/49, M), skin (1/49, F) and skeletal muscle (1/49, M) at high dose.

Study title: 2-Year Oral Gavage Carcinogenicity Study in Wistar Rats

Key study findings: BAY 38-9456 was negative in rat carcinogenicity study up to 75 mg/kg in males and 25 mg/kg in females.

Study number: PH-31276 (T5067624)

Date of study initiation: 2/22/99

Conducting laboratory and location: Institute of Toxicology, BAYER AG, Wuppertal, Germany

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: BAY 38-9456, #503986, 84% as free base

CAC concurrence: Study protocol and dose selection were approved by the Executive CAC on 2/9/99.

Study Type: 2-year rodent bioassay

Species/strain: SPF-bred Wistar rats/Cpb:WU

Age at start of study: 6-7 weeks; 158-198 g (M), 123-160 g (F)

Number/sex/group: 50/sex/group

Animal housing: Individually

Formulation/vehicle: Suspension in 0.5% Tylose

Drug stability/homogeneity: Assessed

Methods:

Doses: 0, 3, 15 & 75 mg/kg (M); 0, 3, 10 & 25 mg/kg (F)

Basis of dose selection: PK endpoint for 13-week dose range-finding study (PH-28640)

Restriction paradigm for dietary restriction studies: N/A

Route of administration: Oral gavage

Frequency of drug administration: Daily

Dual controls employed: No

Interim sacrifices: M; 13-18-16-14, F; 20-26-19-14

Satellite PK or special study group(s): 3/sex/timepoint for PK

Deviations from original study protocol: N/A

Statistical methods: Exact Fisher test and Peto's trend test for pathology

Observations and times:

Observations	Times
Clinical Signs	Twice daily (detailed weekly)
Body Weights	Weekly
Food Consumption	Weekly up to 13 week, thereafter every 4 weeks
Water Intake	Every 4 weeks
Clinical Chemistry	Weeks 27, 53, 79 & 104
Hematology	Weeks 27, 53, 59/60, 79 & 104
Urinalysis	Weeks 27, 53/54, 79 & 104
Organ weights/Ophthalmology/Pathology	Week 104
Toxicokinetics	0.5 & 24 hr at Week 0/0.5 & 1 hr at Week 5/0.5 (M), 1 (F) & 24 hr at Week 50/ 0.5, 1, 2, 4, 7 & 24 hr at Week 102

Results: Dose-mortality trend for female rats was statistically significant due to higher mortality in control females. Mean body weights were significantly decreased in the high dose males (~10%) and females (~7%) than in controls at termination. Water intake was increased in the high dose males (~18%) resulting in elevated urine volume/pH and corresponding decrease in urine density/protein. There was a decrease in age-related lens alterations such as water clefts/opacities in the posterior lens capsular and the sum of lens opacities in the high dose males and in all treated females. Transient changes of RBC parameters (MCV, MCH, HCT, HB, thrombocytes) in Week 27 were interpreted as adaptive. WBC parameters of leukocytes, lymphocytes and monocytes were increased dose-dependently being marked in females. Neutrophils were increased in the high dose females. Markedly elevated urea/inorganic phosphate and reduced T3 levels were observed at high dose. The elevated inorganic phosphate at high dose was considered as a result of an elevated renal reabsorption of phosphate secondary to the hemodynamic effects of the drug. Significantly increased weights of liver/adrenals at high dose and reduced spleen weights in the high dose males were noted.

Dose, mg/kg	0		3		15		75		25	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	13	20	18	26	16	19	14	14 [#]		
Clinical signs, week 104										
Bloody muzzle		2	5	1	3		4	1		
Inflammation			3	5	5	4	4	1		
Body weights, week 103 (g)	505	317	504	309	502	311	456 ^{**}	295 [*]		
Food consumption, week 101	UR	UR								

Water intake, g/animal/day, week 101	22.3	21.6	23.7	22.0	23.6	22.0	26.2	22.2
Ophthalmology, %								
Water clefts	22.5	9.5	16.2	4.2	30.6	1.6	10.7	2.6
Lens opacity, capsular posterior, diffuse	50.0	30.2	41.2	8.3	52.8	3.2	28.0	3.9
Sum lens opacity	67.5	42.9	48.5	22.9	65.3	8.1	30.7	13.2
Hematology, week 104 (10E9/L)								
Leukocytes	8.35	5.64	8.61	6.08	9.48	6.75**	9.38	11.25**
Neutrophils	1.64	1.64	2.09	1.62	1.97	1.55	1.58	5.07
Lymphocytes	5.94	3.52	5.67	3.96	6.63	4.65**	6.95*	5.58**
Monocytes	0.47	0.30	0.54	0.32	0.54	0.35	0.55	0.38
Clinical chemistry, week 104								
Urea, mmol/L	8.36	5.36	9.60	6.0	7.20	5.64	10.65	6.67**
Total bilirubin, μ mol/L	2.0	1.6	2.1	1.4	1.7	1.2	1.6	1.1
T3, nmol/L	1.70	1.49	1.56	1.30	1.50	1.29	1.26**	1.22
T4, nmol/L	44	45	41	42	44	43	50	37
TSH, μ g/L	3.52	2.16	3.39	2.36	3.33	2.50	3.91	2.78
Inorganic phosphate, mmol/L	1.48	1.28	1.48	1.37	1.45	1.30	1.69	1.44
Urinalysis, week 104								
Volume, mL	7.1	4.4	6.3	5.8	5.5	7.4*	21.9**	7.2*
Density, g/L	1034	1027	1040	1025	1045	1027	1017**	1021
pH	7.0	6.8	6.8	6.5	7.1	6.7	8.0**	6.9
Protein, g/L	9.31	2.37	10.88	2.12	8.40	1.97	1.53**	1.36
Protein volume, mg	71.8	9.3	70.3	8.6	44.4	12.4	33.1	9.3
Blood, #	0	0	0	2	1	1	1	1
Organ weights, mg								
Liver	17555	10372	17297	10696	17368	11113	19404*	11558*
Spleen	1167	777	1049	729	1068	699	970*	808
Adrenals	67	74	67	81	93	77	88	83

Statistically significant from control group at $p=0.05^*$ or $p=0.01^{**}$ (Exact Fisher Test)

* $p=0.0487$ by Cox test and $p=0.0382$ by Kruskal-wallis test

Toxicokinetics: Exposure of the parent drug was generally lower in males than in females based on the dose-normalized parameters. The AUC and C_{max} were over-proportionally to low/medium doses and were proportionally to high dose. M-1 reached the same extent as the parent drug in males and 10- to 30% in females. M-4 was 1- to 3%. Accumulation of the parent and M-1 upon repeated administration of vardenafil was explained as a result of reduced clearance and hepatic first pass in the aging rats.

TK Parameters of BAY 38-9456, M-1 & M-4 via Oral Gavage to Rats on Week 102 (PH-31165)

Dose, mg/kg	3		15		75		25	
	M	F	M	F	M	F	M	F
BAY 38-9456								
AUC _{0-24h} (μ g \cdot hr/L)	689#	1117	5364#	6657	27426#	17324		
C_{max} (μ g/L)	377	327	5274	2739	6475	3964		
T_{max} (hr)	1.0	0.5	0.5	0.5	0.5	0.5		
BAY 44-5576 (M-1)								
AUC _{0-24h} (μ g \cdot hr/L)	288#	169#	2219	1480	18948	4917		
C_{max} (μ g/L)	163	41.4	525	342	2408	612		
T_{max} (hr)	0.5	0.5	1.0	0.5	1.0	4.0		
BAY 44-5578 (M-4)								
AUC _{0-7h} (μ g \cdot hr/L)	7.58 ^s	9.84 [@]	35.8	45.2	405	104		
C_{max} (μ g/L)	4.82	2.85	15.1	16.9	121	22.4		
T_{max} (hr)	0.5	0.5	1.0	0.5	1.0	0.5		

The administered doses refer to the free base BAY 38-7268.

n=3/sex/timepoint as geometric means

#The AUC_(0-24h) was extrapolated from 7 to 24 hr.

^sThe AUC_(0-7h) was extrapolated from 2 to 7 hr.

[@]The AUC_(0-7h) was extrapolated from 4 to 7 hr.

Gross pathology findings: Increased incidence of discoloration of the lungs was observed in all treated males. This finding corresponded histologically to the foreign body granulomas, which were considered as a secondary effect due to an altered composition of the saliva following the distinct hypertrophy of the salivary/parotid glands, after which the process of deglutition was disturbed. Marked reduction of surface-change, discoloration and cysts were associated with decreased severity of chronic progressive nephropathy in the high dose males of the kidneys. All other necropsy findings were distributed sporadically among the groups. Table below summarizes the macroscopic findings with increased incidence.

Dose, mg/kg	0		3		15		10		75		25	
	50M	50F										
General, skinny	2		3	1	1							2
Lungs, discoloration	2	4	4	3	4	2	9	3				
nodule	1			1			2	4				
slightly collapsed		1			1		3					
Stomach, area	2	1	1		2	1						3
Liver, distinct lobulation enlarged	3	5	2	3	5	1	7	1				
1					1		2					
Pancreas, consistency-change	1			1		2						2
Kidneys, discoloration	12	5	8	2	6	1	2	2				
surface-change	17	2	17	2	12		3	1				
cyst	6	1	7		6	1	3					
Urinary bladder, dilation			3		1	1	1	1				
Adrenal gland, area		5		3		1	1	1	1			1
discoloration				1	1	1	2					
Spleen, swollen				3	1	1	1	3				
Thymus, nodule		1			2	2	2	2				
Body cavities, fluid	1		1	3	1	2	4	1				

Non-neoplastic findings: Treatment-related findings of hypertrophies of the mucous submandibular salivary gland and acinar parotid gland were statistically significant in the mid- and high-dose males or in the high dose females without increase in proliferative lesion. Concomitantly, basophilic hypertrophic focus and intermingled fat cells in the parotid glands were markedly decreased in high dose males. Mononuclear cell infiltration in the lacrimal glands was also reduced in the high dose group. Increased incidence of the foreign body granulomas in the lungs corresponded to discoloration in the gross findings in the high-dose males, and was considered as a secondary effect due to an altered composition of the saliva following the hypertrophy of the salivary and parotid glands. The increase in submandibular hypertrophy of the salivary gland and the acinar hypertrophy of the parotid gland was observed from the mid dose. Acinar hypertrophy of the salivary glands was considered to be an adaptive effect of PDE inhibitors, and there was no increase in any proliferative lesion over 2-year period. Basophilic hypertrophic foci and intermingled fat cells in the parotid gland were significantly decreased in the high-dose males. In adrenal glands, a shift from normally observed focal hypertrophy of zona glomerulosa cells to an increased occurrence of diffuse hypertrophy and/or thickening of the zona glomerulosa was observed at high dose. This finding was frequently accompanied by an increased vacuolation of the zona glomerulosa cells. Decreased incidences of some age-related findings were found. Concomitant decrease in proliferative (focal hyperplasia) or degenerative (focal fatty change, zona fasciculata vacuolation, cortical degeneration) lesions as common in aged rats was seen at high dose. In the thyroid glands, a significant positive trend was observed for the hypertrophy of the follicular cell epithelium, whereas hyperplasia of the diffuse C-cell and follicular cell was markedly decreased in high-dose males. Increased incidence and severity of colloidal alteration (clumping) were observed from the mid dose group with a positive trend. Some of the age-related findings were decreased. Marked reduction of retinal atrophy was noted in all treated groups more obvious in females. Decreased incidence of water clefs and lens opacity in the eye was noted from low-dose females and in high-dose males. Bile duct hyperplasia and hepatocellular vacuolation were markedly decreased at high dose, whereas hepatocytic hypertrophy was increased in the high dose males. Decreased incidence and severity of progressive cardiomyopathy occurred at high dose. There was a marked reduction of surface-change, discoloration and cysts associated with a decreased severity of chronic progressive nephropathy in the high-dose males. Arteritis/periarteritis was less

frequent in the tongue, pancreas and most obvious in the testes in the high dose males. Incidence of hepatocytic hypertrophy and peri-insular hypertrophy in the pancreas had a positive trend in the high-dose males. Focal tubulostromal hyperplasia in the ovaries, which markedly increased at high dose. Sponsor considered the tubulostromal hyperplasia mainly located at the ovarian hilus (where the common old age type and the papillary type are located) to be an incidental event since no increased incidence of tubulostromal tumors were seen in the treatment groups with a late onset although the counts exceeded historical range. Table below summarizes the microscopic findings with increased incidence.

Dose, mg/kg	0		3		15		10		75		25	
	49M	50F	50M	50F	50M	50F	50M	50F	50M	49F	50M	49F
Spinal cord, vacuolation, lumbar hypercellularity/BM	21	19	19	15	26	16	25	30	1	5	8	6
Heart, myocarditis	1	1	3		1		4	2	40	35	37	26
progressive myocardopathy					43	29	33 ^a	31		1		
vascular mineralization			3		1		1					
Nasal cavity, inflammation	3			1	11	2	7	3	4	1	3	
glandular degeneration, ser. hyperplasia, nasolacr.	4	1	3	2	6		10	1	2			
Lungs, bronchiolar hyperplasia	1	2	4		2	1	3					
alveolar edema			3	1	1		6	1				
foreign body granuloma	2	2	4	2	5	7	12 ^{**b}	10 ^{*c}				
hyperemia/congestion	4	4	5	5	5	1	7	1				
Tongue, arteritis/periarteritis	5	3	6	1	1	1	0 ^d					
Stomach, glandular cyst	1	3	4	2	1	1	1	6				
Rectum, luminal dilation	2	2	1	1		5	2	6				
Liver, regenerative hyperplasia	1				2	1		1				
basal focus/NOS	1		1			1	1	2				
hepatocytic hypertrophy	1						5 ^e	1				
bile duct hyperplasia	38	26	35	22	37	21	25 ^{**f}	15 ^{*g}				
hepatocellular vacuolation	6	5	9	7	4	2	0 ^h	2				
Pancreas, hyperplasia, acinar							2	1				
peri-insular hypertrophy	4	2	1	1	2		9 ⁱ	1				
arteritis/periarteritis	2	1	4		1							
Kidneys, transit. hyperplasia	16	7	20	9	19	9	22	11				
chronic nephropathy	45	31	44	24	46	23	42	27				
Ureters, dilation			1			1		2				
Ovaries, tubulostromal hyperplasia	-	2	-	3	-	3	-	8 ^j				
Cervix, stromal hyperplasia	-		-	1	-	2	-	3				
focal hyperkeratosis	-		-	1	-	2	-	2				
Testes, Leydig cell hyperplasia	4	-	6	-	8	-	10	-				
arteritis/periarteritis	17	-	14	-	14	-	1 ^{**#}	-				
Epididymides, granuloma inflammation		-		-		-	4	-				
mononuclear infiltration	3	-	11	-	7	-	9	-				
Prostate, focal inflammation	22	-	23	-	31	-	31	-				
Uterus, thrombosis	-		-	2	-		-	2				
Parathyroid glands, focal hyperplasia					2		2					
Adrenal gland, cortical degeneration	7	37	3	36	3	39	2	23 ^k				
glomerulosa hypertrophy, focal	28	35	32	31	25	29	0 ^{**#}	13 ^{**#}				
glomer. hypertrophy/thickening, diffuse	1				4	3	31 ^{**#}	18 ^{**#}				
glomerulosa vacuolation	1						37 ^{**#}	8 ^{**#}				
focal fatty change, fasciculata	26	5	22	4	19	2	2 ^{**#}	2				
increased vacuolation, fasciculata	11		11		8	2	3 ^l	4				
mononuclear infiltration	3	2	2	1	1	3	3	4				
cortical hyperplasia	6	7	8	2	7	2	1	0 ^{**m}				
Mesent. lymph nodes, sinus histiocytosis	5	14	9	20	11	14	8	17				
blood resorption	4	1	7		4	1	6	4				
focal fibrosis							4	1				

pigment deposition	2	2	1	1	4	3		
Mandib. lymph nodes, plasmocytosis	18	8	17	7	15	17	24	13
Salivary gland, submandibular acinar hypertrophy		7	2	7	15**	8	28***#	15*n
Parotid glands, acinar hypertrophy	1	2	1		24**	4	48***#	32***#
basophilic hyperplasia, focus	14	23	13	28	15	24	5**o	29
intermingled fat cell	32		30		25	2	9***#	
interstitial edema	1	3	2	2	1	7	5	2
Harderian glands, chronic inflammation	13	9	15	10	8	15	10	19
Lacrimal glands, mononuclear infiltration	41	22	37	15	36	17	30**p	10* ^q
Pituitary gland, cyst, pars intermed.	1	1	1		3	2	3	5
Thyroid gland, diffuse C-cell hyperplasia	35	38	33	38	36	43	21***#	38
follicular epith. hypertrophy	12	4	14	4	11	3	34***#	10*
colloidal alteration	30	7	35	5	32	13	38	14*
follicular cell hyperplasia	4		3	3	1	1	2	3
Mammary gland, galactocele		3		3		2		6
diffuse hyperplasia		15	1	17		19	1	18
Femur, hypercellularity, BM	2	9	2	10	3	8	2	15
myelofibrosis, BM				1	1	1	1	2
Sternum, hypercellularity, BM	2	7	1	10	1	4	1	14
Thymus, vascular hypertrophy			1		4		4	
Eyes, retinal atrophy	24	24	14*	6**	14*	3**	3***#	4***#
Body cavities, peritonitis				3				2

Statistically significant from control group at p=0.05*, p=0.01** (Exact Fisher Test) or p<0.0005* (Non-survival adjusted trend test) p=0.0287*, p=0.0005^b, p=0.0011^c, p=0.0080^d, p=0.0007^e, p=0.0010^f, p=0.0183^g, p=0.0024^h, p=0.0023ⁱ, p=0.0084^j, p=0.0007^k, p=0.0070^l, p=0.0107^m, p=0.0082ⁿ, p=0.0055^o, p=0.0080^p, p=0.0150^q, p=0.0122^r or p=0.0091^s (Non-survival adjusted trend test)

Neoplastic findings: Historical data were not provided. There were no remarkable changes in the number of benign/malignant tumors, metastases or treatment-related increase in the rats. In decedents, there was a slight increase in the number of animals with more than one primary neoplasm at all treated groups (M; 8.1/11.1/18.8/28.6%, F; 15.0/42.3/63.2/30.8). All tumors were evenly distributed among the groups in both sexes and did not achieve statistical significance except for benign thymoma in females. Benign thymomas were seen from mid-dose females, achieving statistical significance at p=0.025 with a positive trend test but not with a pair-wise test. Sponsor considered the tumors were incidental due to the late onset of the tumor, the lack of increased incidence of preneoplastic hyperplasia, the lack of malignancy or increased occurrence in the other sex, and the data within the sponsor's historical range of 10%.

Neoplastic/Non-neoplastic Findings in the Thymus

Dose, mg/kg	0		3		15		75		25	
	9M	50F	50M	50F	50M	50F	50M	50F	50M	19F
Nodule		1			2	2	2	2		2
Lymphoid hyperplasia					1	1	2			
Tubular hyperplasia	2	20	3	20	3	16	2	24		
Site present	1	1	1				3	2		
Benign thymoma	1				1	2				3*
Malignant thymoma				1						
Combined thymomas	1			1	1	2				3

*Statistically significant from control group at p=0.0251 (Exact Peto's trend test)

Incidence of pituitary adenomas or carcinomas of the pars distalis was slightly decreased with negative trend in females. The diagnosis was based on invasion either into the brain or the sphenoid bone. Most of the adenocarcinomas and the adenomas led to an unscheduled death.

Neoplastic Findings in the Pituitary Gland

Dose, mg/kg	0		3		15		75		25	
	9M	50F	50M	50F	50M	50F	50M	50F	50M	19F
Adenoma, pars distalis	12	16	10	19	8	21	7	14		

Carcinoma, pars distalis	2	3	1	1	0*			
Adenomas+Carcinomas	12	18	10	22	9	22	7	14

*p=0.0465 (Exact Peto's trend test)

Uterine neoplasms occurred in a high incidence treated with BAY 38-9456. Uterine adenocarcinomas generally showed an aggressive growth pattern invaded or metastasized in to a number of other organs frequently seen in the lungs, while invasion was observed mainly in the abdominal cavity such as pancreas, mesenterium, liver, kidneys, ovaries, etc. Rare tumor of stromal sarcoma was observed infrequently from the mid dose (1/50).

Neoplastic/Non-neoplastic Findings in Uterus

Dose mg/kg	0		3		10		25	
	50F	50M	50F	50M	50F	50M	50F	50M
Endometrial hyperplasia			3		1		1	
Stromal hyperplasia							1	
Endometritis	2		9		2		4	
Mixed Muellierian tumor							1	
Adenoma	1				1		3	
Stromal sarcoma					1		1	
Stromal polyp	7		11		9		9	

Increased incidence of follicular cell adenomas in the thyroid gland occurred in the high dose males without increased incidence of proliferative lesion of the follicular epithelium. Rare tumors of brain oligodendroglioma, tongue squamous carcinoma, uterine cervix granular cell tumor, sebaceous adenoma/lipoma in the skin, and lipoma (abdominal)/schwannoma (thoracic)/odontoma (oral) in the body cavity were noted at high dose sporadically.

Other Neoplastic Findings

Dose mg/kg	0		3		15		10		25	
	9M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Brain, oligodendroglioma									1	
Heart, infiltrated site				1					1	
Tongue, squamous carcinoma									1	
Lungs, metastasis		3		5		5				7
Stomach, invaded sites				2		2				1
Liver, invaded site		1	1	3		4				4
Pancreas, invaded site		1		3		4				3
Ovaries, granular cell tumor	-		-		-		-		-	1
invaded site	-		-	2	-		-		-	1
Uterine cervix, granular cell tumor	-		-	1	-		-		-	1
Mesenteric lymph node, hemangioma	2		2		2		1		1	
Mammary gland, fibroadenoma		5		7		8				7
Thyroid gland, follicular adenoma	1					1		2		
Zymbal glands, keratoacanthoma									1	
Skin, sebaceous adenoma									1	
lipoma									1	
Body cavity, lipoma									1	
schwannoma									2	
odontoma										1

OVERALL INTERPRETATION AND EVALUATION:

Adequacy of the carcinogenicity study and appropriateness of the test model: Mortality rate was reduced with a negative trend in females due to higher mortality in control group. Body weight was markedly suppressed in both sexes (~10%), while food intake was unchanged at all groups, suggesting palatability is

not the reason for the weight loss. The total number of animals with tumors was higher in females (73.6%) than in males (55.4%). The number of decedent rats with more than one primary neoplasm was increased in both males (8.1/11.1/18.8/28.6%) and females (15.0/42.3/63.2/30.8%) without dose-dependency. Rats were exposed to unbound drug (BAY 38-9456+M-1) at high dose with 180-400 fold the human therapeutic exposure at 20 mg.

Evaluation of Tumor findings: Benign thymomas in female rats were statistically significant by trend test at $p=0.025$, but not by pairwise test. Sponsor considered the tumors were incidental due to the late onset seen in animals of the terminal sacrifice, the lack of increased incidence of pre-neoplastic hyperplasias, the lack of malignancy or increased occurrence in the other sex, and the range within historical control from the laboratories (10%).

Adenomas of the pars distalis in the pituitary gland were decreased at high dose, and the incidence of the carcinomas had a negative trend ($p=0.0405$) in females. Uterine neoplasms occurred at higher frequency, adenocarcinomas being the most frequent tumor type followed by stromal polyps without statistical significance. The uterine adenocarcinomas generally invaded or metastasized into other organs, frequently seen in the lungs. Rare tumors of brain oligodendroglioma, tongue squamous carcinoma, cervix granular cell tumor, sebaceous adenoma/lipoma in the skin, and lipoma/schwannoma/odontoma in the body cavity were noted at high dose sporadically.

SUMMARY AND CONCLUSIONS:

Adequacy of the Studies and Appropriateness of the Test Model: There were no significant effects of drug treatment on survival, body weight, food consumption/water intake, hematology, clinical chemistry, or pathology to negatively impact the validity of the studies. High doses in mice produced total plasma exposures of 21 fold in males and 37 fold in females, and in rats 180-400 fold the human exposures at a 20 mg clinical dose.

Major Tumor Findings:

Non-neoplastic findings: In mice, dose-dependent increase of focal medullary hyperplasia of the adrenal cortex was observed in females (0-2-3-5), and the incidence was statistically significant at high dose. Sponsor stated that the findings were rated as minimal to slight (moderate degree in 1 of each at mid- and high doses), found unilaterally, and close to the historical range from the RITA control data (0-8%). Neoplastic findings of the adrenal medulla consisted of sporadic benign medullary tumors in females (0-0-1-0), suggesting that correlation to treatment is unlikely. Other treatment-related non-neoplastic findings were observed in the previous sub-chronic/chronic studies. There were some age-related reduced findings at high dose in both species, which were not considered to be toxicologically relevant, but rather beneficial.

Neoplastic findings: The only statistical significant tumor finding was the benign thymomas in female rats with significantly positive trend at $p=0.025$ but not significant by the pair-wise test. The tumors had late onset only seen in animals of the terminal sacrifice, showed no increased incidence of pre-neoplastic hyperplasias, lacked malignancy or increased occurrence in males, and were within the range (10%) of the historical control from the laboratory. The incidence of histiocytic sarcoma was increased in the high-dose female mice (4-4-3-7), but was close to historical control data from the RITA database (4-12%) according to the sponsor. The incidence in males displayed a negative trend (2-2-0-0). Other rare tumors in mice were found in the stomach (1/49, F), cecum (1/49, M), skin (1/49, F) and skeletal muscle (1/49, M) at high dose. Rare tumors of brain oligodendroglioma (1/50HD, M), tongue squamous carcinoma (1/50HD, M), uterine cervix granular cell tumor (1/50MD, 1/49HD, F), sebaceous adenoma (1/50HD, M)/lipoma (1/50HD, M) in the skin, and lipoma (1/50HD, M)/schwannoma (2/50HD, M)/odontoma (1/49HD, F) in the body cavity were noted sporadically.

Biological significance: Treatment of BAY 38-9456 for 2 years resulted in a statistically significant hypertrophy of the parotid and salivary glands from mid-dose rats. Hypertrophy of the adrenal, thyroid and

pancreas was statistically significant at the high dose in the 2-year study. The findings were characterized as slight in severity, and observed in the previous repeated dose studies. However, no increase in any proliferative lesion was observed in the present study. In the adrenal glands, a shift from normally observed focal hypertrophy of zona glomerulosa cells to an increased occurrence of diffuse hypertrophy and/or thickening of the zona glomerulosa was observed at high dose over the 2-year period. In contrast, proliferative (focal hyperplasia) and degenerative (focal fatty change, zona fasciculata vacuolation, cortical degeneration) lesions of the adrenal cortex, common in aged rats, were observed less frequently at high dose. Thyroidal changes were observed in the 1-, 3- and 6-month and 2-year studies. The findings were reversible during 4-week recovery period for the 3-month study, did not progress with prolongation of treatment in the 6-month study, and did not induce malignant tumors in a 2-year study. An *in vitro* study demonstrated that BAY 38-9456 did not interfere with enzymes of thyroid hormone homeostasis (thyroid peroxidase, type I- and II iodothyronine deiodinases), indicating that a direct effect either on the thyroid or on the pituitary is unlikely. In *in vivo* comparative toxicity study of sildenafil and BAY 38-9456 (PH-30893), toxicological profiles of BAY 38-9456 were similar when taking into account the higher exposure of the animals with BAY 38-9456 in terms of PDE5 inhibitory activity and relative to human exposure (see pp39). Sponsor considered that BAY 38-9456 has a low potential for adverse effects on the thyroid and thyroid hormone metabolism in the rat since the findings are comparable to sildenafil, and occur only at high doses. Thus, the toxicological relevance to humans was regarded as minimal since the findings may reflect an adaptive response, which is most likely rat specific, and occur at exposure levels greater than those in humans.

Carcinogenicity summary: BAY 38-9456 was negative in mouse carcinogenicity study at 40, 200 and 1000 ppm, an averaged 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. Total exposures of the unbound drug (BAY 38-9456+M-1) at a NOAEL of 200 ppm produced 3- to 8-fold the exposure at a maximum clinical dose of 20 mg (76 $\mu\text{g}\cdot\text{hr}/\text{L}$ for BAY 38-9456 & 41.1 $\mu\text{g}\cdot\text{hr}/\text{L}$ for M-1 at steady state $\text{AUC}_{0-24\text{h}}$ from #100196).

BAY 38-9456 was negative in rat carcinogenicity study treated with up to 75mg/kg in males and 25 mg/kg in females. Total $\text{AUC}_{0-24\text{h}}$ of unbound drug (BAY 38-9456+M-1) at a NOAEL of 3 mg/kg produced 8- to 10-fold the human exposure at a maximum clinical dose of 20 mg (76 $\mu\text{g}\cdot\text{hr}/\text{L}$ for BAY 38-9456 & 41.1 $\mu\text{g}\cdot\text{hr}/\text{L}$ for M-1 at steady state $\text{AUC}_{0-24\text{h}}$ from #100196).

Carcinogenicity conclusions: BAY 38-9456 was negative in the rat carcinogenicity study by oral gavage at doses, which gave up to 180-400 fold higher exposure in rats than in men taking the maximum clinical dose. BAY 38-9456 was negative in the mouse carcinogenicity study where the animals were dosed via drinking water at doses which gave up to 21-37 fold higher exposure of the unbound drug (BAY 38-9456+M-1) in mice than in humans taking a 20 mg dose.

Recommendations for further analysis: None

Labeling Recommendations:

[

DRAFT

]

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY (See also Review #3 in Appendix I)

Study title: Pre- and Postnatal Development Including Maternal Function in Rats

Key study findings: A NOAEL was established for maternal effects as 8 mg/kg/day, for pre-/postnatal development of F1 as 1 mg/kg/day, for physical development of F1 after weaning as 8 mg/kg/day, for motor activity of F1 as <1 mg/kg/day and for fertility of F1 as 8 mg/kg.

Study no.: PH-30851 (T5061305)

Date of study initiation: 12/8/1998

Conducting laboratory and location: Institute of Toxicology, BAYER AG, Wuppertal, Germany

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: BA38-9456, #503845, 84% as free base

Formulation/vehicle: 0.5% methylhydroxyethylcellulose (Tylose MH 300® G4)/demineralized water

Methods:

Species/strain: SPF-bred Wistar rats/Cpb:WU

Number/sex/group: 25/female/group

Doses employed: 0, 1, 8, 60 mg/kg

Route of administration: Oral gavage

Study design: Female rats were treated from Day 6 post coitum (p.c.) to Day 21 post partum (p.p.), & mated 1(M) to 2(F).

Parameters and endpoints evaluated: F0; mortality/clinical signs (twice daily), body weight/water intake (daily), food intake (Days 0-6/6-11/11-16/16-20 p.c., 0-7/7-14 p.p.), reproductive parameters (fertility/gestation/rearing indices), duration of pregnancy, course of birth, lactation behavior, implantation sites, gross pathology & histopathology (heart), f1 pups (Days 0-43); mortality/clinical signs (once daily), body weight/sex ratio (Days 4/7/14/21), food intake (Days 0-6/6-11/11-16/16-20 p.c., 0-7/7-14 p.p.), reproductive parameters (fertility/gestation/rearing indices), external malformations, physical development (pinnae detachment/fur development/eruption of the incisors/eye opened/normal gait/separation of prepuce/opening vagina), reflex & behavior (surface righting/negative geotaxis/pupillary reflex/hearing ability), motor activity (Day 22), learning & memory (Weeks 5-6), & gross pathology. F1 (Weeks 5-14); mortality/clinical signs/water intake (twice daily), body weight (weekly), food intake (weekly during prepartum/gestation 0-7/7-14/14-20 p.c.), motor activity (week 14), reproductive parameters (fertility/gestation/insemination indices), duration of pregnancy, implantation sites, gross pathology & histopathology. f2 pups; mortality, sex distribution, clinical signs, external malformations & body weight.

Results:

F0 maternal generation: Treatment-related maternal effects included piloerection, sunken flanks, high stepping gait around the time of parturition, transiently increased water intake, intermittently light colored feces, reduced food intake from start up to Day 14 p.p., minimal to mild myocardial fibrosis, reduced gestation/rearing indices, lactating behavior, and postnatal pup losses at high dose. One female of the control group was sacrificed moribund at the end of lactation Day 20 with and distinct body weight loss. Reddish discoloration of pinnae appeared ~30 min post-dosing and persisted for 1 to 4 hours in all treated groups during gestation and from mid dose during lactation, which were attributable to the pharmacological activity of vasodilation. One mid-dose female showed sunken flanks and piloerection with signs of severely affected health (somnolence/increased respiratory frequency/pale eyes and at necropsy on Day 1 p.p. with reduced size of kidneys/pale liver) around the time of parturition, which was assumed for a spontaneous gestational toxicosis. Statistically significant mean body weight loss occurred from 1st week during gestation up to Day 14 p.p. of lactation at high dose. During the 2nd and 3rd week of lactation, compensatory increase occurred resulting in a marginally increased overall body weight gain during entire lactation period at high dose. Food consumption was significantly reduced from Day 6 p.c. up to Day 14 p.p. Increased water intake was noted in 2 females at high dose either shortly before or after the day of parturition. Histopathological examination in the hearts showed minimal to mild myocardial fibrosis at high dose (11/25). Fertility index did not differ up to 60 mg/kg, but gestation/rearing indices were decreased at high dose. One high-dose female showed implantation sites at necropsy but either did not deliver (total resorption of 2 implants) or cannibalize the pups. However, increased prenatal loss, reduced live birth index, reduced survival of pups up to Day 4 p.p. (viability index) and maternal toxicity can not be excluded on the reduced gestation index at high dose. A reduced lactation behavior was assumed at high dose due to few pups without milk spots, clinical signs (pale/discolored skin) and reduced viability of f1 pups.

Dose, mg/kg	0 25F	1 25F	8 25F	60 25F
Mortality	1*			
Clinical signs,				
Skin/fur, reddish discolored pinnae, Days 6-24 p.c.		8	25	25
Days 0-21 p.p.			6	21
encrusted wound, Days 6-24 p.c.				1
piloerection, Days 0-21 p.p.			1	2
Light colored feces, Days 6-24 p.c.				21
Days 0-21 p.p.				16
Sunken flanks, Days 0-21 p.p.	1		1	2
Body weight gain (g/day), 6-20 p.c.	93.5	86.7	98.8	75.0**
0-20 p.c.	116.8	109.5	124.0	96.6**

0-7 p.p.	26.2	18.1*	21.6	14.1**
7-14 p.p.	9.5	15.5*	15.0	19.5**
14-21 p.p.	-3.0#	-5.1	-1.2	7.7**
Food consumption (g/day), 6-11 p.c.	20.01	20.05	20.33	16.23**
11-16 p.c.	21.31	20.87	22.25	19.78*
16-20 p.c.	24.08	23.51	24.76	21.86**
0-7 p.p.	40.90	38.10	40.25	35.72**
7-14 p.p.	58.07	56.26	58.68	53.81*
Water consumption, #, increased, Days 6-24				1
Days 0-21				1
decreased, Days 0-21				1
Gross pathology, Day 21 p.p.	UR	UR	UR	UR
Histopathology, Day 45/48				
Heart, fibrosis				11
mononuclear cell infiltration			1	2
Fertility index ¹ , %	88	96	88	92
Gestation index ² , %	100	100	100	95.7
Rearing index ³ , %	100	100	95.7	90.9
Duration of pregnancy, days	22.77	22.38*	22.45	22.23**
Course of birth	UR	UR	UR	UR
Lactation behavior	UR	UR	UR	1
No. of implantation sites	12.14	11.92	12.50	12.55
Prenatal loss/litter	0.45	0.96	0.68	1.64*

Statistically significant from control group at p=0.05* or p=0.01*

#One female was sacrificed moribund at the end of lactation Day 20 with and distinct body weight loss.

¹% of females with implantation sites/inseminated

²% of females with delivering pups/implantation sites

³% of females with reared pups/delivered

f1 pup generation (from birth to Day 43): Treatment-related findings in the f1 pups included clinical signs of pale/discolored and cold skin from mid dose, and at high dose together with lack of milk spot/alterd respiration, delayed pre/postnatal development from mid dose, and reduced live birth/viability/litter size/body weight development at high dose. Two pups of the mid dose and one pup of the high dose were sacrificed in moribund on Day 17 p.p., Week 5 of rearing and Day 17 p.p., respectively. The pup in the high dose group showed clinical signs of deeper breathing, hypomotility, cold skin, altered body position, and at necropsy empty intestines/alterations of the bladder (tightly filled with clear fluid, dark sediment). Sum of missing/died pups was increased at high dose. Increased incidence of pale/discolored and cold skin, and lack of milk was observed from mid-dose group. Sponsor considered the increased incidence of clinical signs at high dose was related to reduced viability of F1 pups since live birth index and survival rate up to Day 4 p.p. were slightly reduced at high dose. Other clinical signs were within historical control data. Reduced litter size at birth, but not on Day 4 p.p. after culling, was observed at 60 mg/kg with increased prenatal loss, but the treatment-related effect on surviving litters was not considered. Live birth and viability indices were reduced below the range of historical control data at high dose, and overall number of stillborn pups and pups lost up to Day 4 p.p. were statistically significantly increased. Mean body weights were statistically significantly decreased from birth to the end of the rearing period in the high dose group with more pronounced effects during the rearing period and in males. Treatment-related retarded physical development was observed for fur and teeth from mid dose, and other signs of pinnae detachment, eye opening, development of normal gait and preputial separation were as well delayed at high dose. All parameters studied except vaginal opening were statistically significantly delayed at high dose consistent with markedly reduced body weights. Delayed preputial separation was regarded as a sign of retarded physical development and not as an effect on sexual maturation, due to the concomitant weight loss of males on the day of preputial separation. Statistically significant findings of motor activity were observed in all treated male groups without dose-dependency (total distance, movement time) or occurrence at a different time pattern within the individual groups (horizontal activity). Sponsor considered the effects were incidental due to the wide range of biological variability in the historical control data. Vertical activity (rearing behavior) was decreased on Day 22 p.p. with most pronounced during the 1st 20 min in the low- and mid-dose groups, and persisted during the overall period at

high dose. A 2nd test in Week 14 (see table on pp 34) showed increased vertical activity with marked weight loss in the high-dose males only. Altered motor activity in all treated groups was considered to be treatment-related from 8 mg/kg due to dose relationship. At high dose the reduced activity paralleled with the body weight loss, dose relationship, duration of the activity and delayed physical development of f1 pups. Reduced learning/relearning behavior after the 6th passage (right step ladder) and the 4th to 6th passage (left step ladder) in the high dose group was lay in the historical range, and considered to be as a result of decreased motor ability/exhaustion. Most necropsy findings were scattered through all groups except that one pup of the high dose had alterations in the bladder and the intestines.

Dose, mg/kg	0		1		8		60	
	M	F	M	F	M	F	M	F
Mortality, pups (litters), After Day 0 p.p., missing	5(4)		1(1)		7(3)		20(9)	
found dead					9(3)		2(2)	
killed <i>in extremis</i>					2(2)		1(1)	
Days 1-4	4		1		15*		20**	
Days 0-4	11		3		19		26**	
Days 0-21	12		3		20		29**	
Postnatal survival (live pups/litter), Day 0	11.64		10.67		11.41		10.32	
Live birth index, %	99.59		97.21		95.80		91.71	
Stillborn, litters	1		7		9		11**	
Viability index ¹ , %	95.82		98.74		89.75		82.67	
Lactation index ² , %	UR		UR		UR		UR	
Clinical signs, pups (litters)								
Head, tilted			4(3)		6(3)		1(1)	
Hypomotility	1(1)						2(2)	
Lying on side							1(1)	
Pale skin	5(4)		1(1)		10(5)		18(11)	
Bluish discolored skin					2(1)		1(1)	
Greyish discolored skin							1(1)	
Milk spot not detectable	2(1)				3(2)		4(4)	
Hematoma at the snout			2(2)		6(6)		1(1)	
on the head					2(2)		1(1)	
at the nose			2(2)		1(1)		1(1)	
on the neck							1(1)	
Cold to touch	5(3)				13(5)		7(7)	
Gasping breathing							1(1)	
Deeper breathing							1(1)	
Labored breathing							1(1)	
Deviation of hind limb							1(1)	
Body weight (g), n=21-24, Day 0 p.p.	6.64	6.31	6.35	6.15	6.49	6.09	5.56**	5.28**
Day 4 preculling	10.60	10.21	10.22	10.07	10.29	9.79	8.72**	8.28**
Day 4, postculling	10.63	10.23	10.24	10.05	10.27	9.73	8.66**	8.30**
Day 21 p.p.	47.06	45.27	45.08	44.34	46.27	45.49	39.78**	39.00**
Physical development, Days after birth								
Pinnae detachment	2.15		2.44*		2.39		2.87**	
Fur development	9.21		9.20		9.58*		10.37**	
Incisor eruption	10.02		10.10		10.64**		11.63**	
Eyes opened	16.33		16.75*		16.46		17.03**	
Normal gait	17.33		17.72*		17.72*		18.22**	
Preputial separation	28.79		29.02		29.14		29.57*	
Body weight at preputial separation (g)	80.78		78.52		79.95		72.54**	
Vaginal opening	UR		UR		UR		UR	
Reflex & behavior ³ , n=24	UR		UR		UR		UR	
Motor activity, 3-4 min, Day 22 p.p.	n=22	n=22	n=23	n=24	n=21	n=21	n=20	n=20
Horizontal activity	462.5	410.6	380.1**	404.8	417.8	432.6	376.2**	460.1
Total distance, cm	179.0	153.2	125.2**	132.5	150.2	139.4	126.9*	165.6

Movement time, sec	17.5	15.6	13.2*	13.3	17.3	16.1	13.1*	18.0
Vertical activity	20.4	17.6	10.9*	19.5	11.2*	23.8	5.7**	12.6
Water-M-maze test, Weeks 5-6, %								
Step ladder, right, 6 th passage	97.7		97.9		100.0		92.5	
left, 4 th passage	81.8		80.9		71.4		72.5	
5 th passage	84.1		85.1		78.6		67.5	
6 th passage	84.1		78.7		85.7		75.0	
Gross pathology, pups (litters), Day 43								
Kidney, dilation of renal pelvis	6(5)		9(6)		9(5)		8(6)	
Bladder, dark sediment/filled with clear fluid							1(1)	
Intestines, empty							1(1)	
Eyes, microphthalmia	1(1)		1(1)		2(2)		2(2)	

Statistically significant from control group at $p=0.05^*$ or $p=0.01^{**}$

One female was excluded due to death on Day 20 p.p.

¹Survival index to Day 4, p.p.

²Survival index from Day 4 p.p. to Day 21 p.p.

³Surface righting, negative geotaxis, hearing test, & pupillary reflex were tested.

F1 generation (from Weeks 5 to 14): Treatment-related findings included reduced food intake/body weight gain/gestation index/fertility at high dose, and motor activity from low dose. Body weights and body weight changes were markedly reduced during the entire rearing, pre-mating and mating period in the high dose-males. The effect was considered to be the consequence of reduced body weight at birth and reduced body weight gain during the pre-weaning period. Reduced body weight gain during gestation in the high dose females was probably related to the reduced litter size. Mean food consumption was reduced statistically significantly during the end of 1st gestation (Days 14-20 p.c.) or during the overall gestation period in the high-dose females, and was considered to be due to the reduced litter size. No gross pathology findings were observed except 2 females each of the mid- and high doses showed uni- and/or bilateral dilation of renal pelvis. Two females in the high dose group delivered no pups but showed implantation sites in the uterus after 2 matings. Due to the low number of pregnant females delivered and reduced litter size at 1st mating at high dose, 2nd mating was performed, and the 2nd mating produced more litters than in the control group, which results in higher fertility rate. Since 2 matings were performed for the control and the high-dose groups, gestation period for resorption of 2 litters or number of implantation sites could not be evaluated. Sponsor considered the treatment relationship is unlikely for the gestation index since the data lay in the historical range, but can not be excluded as a consequence of the systemic effects of treatment on the F1 generation.

Dose, mg/kg	0		1		8		60	
	24M	24F	24M	24F	24M	24F	24M	24F
Clinical signs,								
Head, tilted	1	2	2	2	5	2	5	3
Piloerection						1		1
Body weight (g), Week 5-14, pre-mating	267.8	130.1	285.0	126.6	272.3	131.3	259.5	130.8
Week 14, pre-mating	379.0	227.3	397.3	222.5	380.2	226.5	360.7	221.0
Body weight change (g), Days 0-20 p.c., 1st mating		111.2		105.6		110.4		102.4
Days 0-20 p.c., 2 nd mating		107.4		-		-		97.5*
Food intake (g/day), Week 13, pre-mating	UR		UR		UR		UR	
Days 14-20, 1 st mating		23.58		22.74		23.59		22.22*
Days 14-20, 2 nd mating		24.39		-		-		23.23*
Motor activity⁴, 51-60 min, Week 14, n=24								
Horizontal activity	971.0	-	982.9	-	1081.8	-	1271.6	-
Total distance, cm	353.7	-	364.0	-	344.5	-	484.2	-
Movement time, sec	38.1	-	38.3	-	37.2	-	48.6	-
Vertical activity	198.8	-	191.8	-	205.1	-	276.7*	-
Body weight	381.5		398.8*		380.4		362.8*	
Fertility index¹, %, 1st mating		91.7		95.7		87.0		79.2
2 nd mating		66.7						79.2
Gestation index², %		100.0		100.0		100.0		100.0

Insemination index ³ , %, 1 st mating	100.0	95.8	95.8	100.0
2 nd mating	100.0	-	-	100.0
Implantation sites, /litter, 1 st mating	-	12.45	12.70	-
Gross pathology, Week 32				
Pancreas, yellowish fatty tissue enlargement				1
Kidney, dilation of renal pelvis			2	2
Uterus, 2 implantation sites after 2 matings				1
9 implantation sites after 2 matings				1
Histopathology, Week 15				
Heart, mononuclear cell infiltration	1			1 3

Statistically significant from control group at p=0.05*

One female was excluded due to death on Day 20 p.p.

¹No. of mated females which littered

²No. of females with pups at birth/with implantation sites

³No. of females inseminated/mated

-; not available

f2 pup generation: Litter size was reduced at high dose although the data were within the historical range. Consequence of the systemic effects of treatment on the F1 generation was not completely excluded. Incidence of microphthalmia/anophthalmia marginally increased at high dose, but was regarded as incidental since the findings are the most common spontaneous malformations of the strain, and was comparable to historical data.

Dose, mg/kg	0	1	8	60
Clinical signs, pups (litters)				
1 st mating, milk spot not detectable	1(1)	3(2)	11(3)	3(3)
eye, anophthalmia		1(1)		2(2)
2 nd mating, hematoma		-	-	1(1)
eye, anophthalmia	1(1)	-	-	2(2)
microphthalmia		-	-	1(1)
Body weights	UR	UR	UR	UR
Litter size, 1 st mating, living pups/litter	10.91	10.77	10.85	9.74
pups with malformations	0.23	0.27	0.15	0.33
2 nd mating, living pups/litter	10.88	-	-	9.21*
pups with malformations	0.06	-	-	0.16
Sex ratio	UR	UR	UR	UR

Statistically significant from control group at p=0.05*

-; not available

UR-unremarkable

Summary: Treatment-related maternal effects included piloerection, sunken flanks and high stepping gait around the time of parturition, transiently increased water intake, intermittently light colored feces and reduced food intake from start of treatment up to Day 14 p.p. at high dose. Maternal body weight loss after start of treatment and subsequently reduced body weight gain during gestation was followed by a compensatory increase during lactation. Minimal to mild myocardial fibrosis was observed at high dose. Gestation index, prenatal loss, number of stillborn pups and mortality of f1 pups up to Day 4 were increased at high dose, resulting in decreased live birth index, litter size, and rearing index. Reduced lactation behavior was assumed at high dose due to few pups without milk spots, clinical signs (pale/discolored/cold skin, labored/gasping breathing) and reduced viability of f1 pups. Clinical signs of pale/discolored/cold skin, and lack of milk spot were observed from mid-dose pups with increased incidence. Perinatal mortality was evident at high dose with decreased live birth index and viability index up to Day 4 p.p. Body weights and body weight changes were markedly reduced from birth, during the entire rearing, pre-mating and mating period in the high-dose F1 males. In F1 females, mean body weight loss was evident from birth to the end of the rearing period and during gestation probably related to the reduced litter size. Treatment related effects on delayed development of fur and incisor eruption were observed from mid dose, and all parameters of physical development were more pronounced at high dose except vaginal opening. Delayed preputial separation of F1 males at high dose was regarded as a sign of retarded physical development due to other