physical parameters and reduced body weights. Effect on motor activity in all treated males and in high-dose females with reduced vertical activity during the 1st test and increased vertical activity in F1 males during the 2nd test was seen with reduced body weight. Reduced time course of the behavior with water-M-maze test at high dose during the last passages was considered to be more indicative of decreased motor ability and earlier exhaustion than of a specific effect on learning and re-learning ability. The number of pregnant females that delivered and litter size of f2 pups were reduced in the high-dose F1 animals, possibly due to the systemic effects.

Reproductive and developmental toxicology summary: Reproductive and developmental toxicity studies were conducted for fertility/early embryonic development and pre-/postnatal development including maternal function in rats, and embryo-fetal development in rats and rabbits. In the Segment I, reduced fertility was seen in high-dose males although the values were within the historical range and were not statistically significant. Decreased body weight gain from the mid dose (25 mg/kg) and reduced food consumption at high dose were seen in both sexes during the pre-mating period. In the Segment II, severe maternal toxicity was observed at 100 mg/kg consisting of increased mortality, clinical signs (piloerection, sunken flanks, disturbance of gait and hypoactivity) decreased weight gain, decreased food intake, increased water intake, light/reduced feces, stomach filled with bedding/reddish brown spot in gastric mucosa and myocardial fibrosis/infiltration/edema/necrosis. At the high dose, total resorption occurred in 1 dam, and increased incidence of postimplantation loss and reduced litter size were noted. Placental and fetal weights were reduced in addition to retarded fetal ossification (delayed ossification of phalanges of digits, toes, metacarpals/metatarsals, sternebrae, vertebral arches/bodies, slight enlargement of fontanelles & increased incidence of 4th sternebrae variation) and increased incidence of skeletal variations of asymmetric sternebrae. Fetuses from the high-dose dams (3 fetuses/2 litters) had reduced body weight and malformations of the craniofacial bones (cleft palate/alteration of os palatinum & os exoccipitale or combined with sternal, vertebral & rib findings). The delayed skeletal development seemed to correlate to the reduced fetal and placental weights at high dose, suggesting maternal toxicity/stress rather than a direct drug-induced developmental toxicity. Sponsor considered the malformations to be subsequent to vasodilation by the drug rather than a teratogenic effect since it is known that hemodynamic changes in either dam or fetus could result in an increased incidence of malformation, possibly caused or exaggerated by hypoxia. Fetal exposures of BAY 38-9456 and M-1 reached 4-10% at low dose, 10-20% in mid dose, and were equivalent (96-98%) to maternal plasma concentrations at high dose at 0.5 hr post-dose. In the Segment III study, maternal toxicity (clinical signs, reduced body weights/food intake, myocardial fibrosis) and reduced gestation index were observed at high dose (60 mg/kg), delayed physical development and fertility of F1 was seen from mid dose (8 mg/kg), decreased motor (vertical) activity was noted from low dose, and the number of pregnant females that delivered were reduced in F1 at high dose, possibly due to systemic effects. In a rabbit developmental toxicity study, maternal toxicity was associated with reduced food intake/body weight gain from mid dose (18 mg/kg). There was reduced gestation rate due to 1 total resorption at high dose. Increased postimplantation loss, decreased number of fetuses, and retarded ossification of the 5th medial phalanges of digits/toes and of the 1st cervical vertebral bodies was observed at high dose. A NOAEL for fertility and systemic toxicity was considered to be 100 mg/kg and 6 mg/kg, respectively, in the Segment I study. A NOAEL for both maternal toxicity and embryo/fetal development was determined to be 18 mg/kg in Segment II. A NOAEL was defined as 3 mg/kg for maternal toxicity and 18 mg/kg for developmental toxicity in rabbits. In Segment III, a NOAEL was determined to be 8 mg/kg for maternal effects and physical development after weaning/ fertility of F1, 1 mg/kg for pre/postnatal development of F1, and <1 mg/kg for motor activity of F1.

Reproductive and developmental toxicology conclusions: BAY 38-9456 had no significant effects on fertility or early embryonic development up to 100 mg/kg in Segment I in rats. In Segment II, severe maternal toxicity including mortality and retarded skeletal development parallel to the reduced fetal/placental weights were observed at high dose, indicative of developmental toxicity as a consequence of maternal toxicity rather than a teratogenic effect. In a rabbit developmental toxicity study, maternal toxicity, one total resorption, increased post-implantation loss and an equivocal retardation of ossification were considered to be related to the maternal intoxication. In Segment III, maternal toxicity, reduced gestation index due to prenatal loss, increased stillborn pups/pup mortality (up to Day 4 p.p.), decreased F1 body weight, physical development after weaning and F1 fertility at high dose (60 mg/kg), and pre/postnatal development and motor activity from 8 mg/kg were observed, possibly due to the systemic effects in the dams.

Labeling recommendations: Vardenafil is not indicated for use in newborns, children or women. There are no adequate and well-controlled trials of vardenafil in pregnant women. Vardenafil was secreted into the milk in lactating rats with an AUC for the milk of more than 10 fold higher than for the plasma. Following a single dose of 3 mg/kg, 3.3% of the administered dose was excreted into the milk within 24 hours. Vardenafil did not impair fertility in male and female rats administered doses up to 100 mg/kg/day for 28 days to males and 14 days to females prior to mating and continuing through day 7 of gestation, a dose producing total AUC values for unbound vardenafil and its major metabolite (in a corresponding subacute toxicity study), of more than 400 times the AUC in humans at the maximum recommended dose of 20 mg. No evidence of specific potential for teratogenicity, embryotoxicity or fetotoxicity was observed in rats and rabbits that received up to 18 mg/kg/day during organogenesis. These doses represent more than 100 times (rat) and 29 times (rabbit) the total AUC values of unbound vardenafil and its major metabolite in humans given the maximum recommended human dose of 20 mg. In the rat pre-and postnatal development trial, no adverse effect was observed at doses up to 8 mg/kg/day given for 36 days. Based on the results of the rat teratogenicity study, an 8 mg/kg/day dose in the pregnant rat is estimated to produce total AUC values for unbound vardenafil and its major metabolite of more than 40 times the human AUC at the MRHD of 20 mg.

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: *In vitro* Interaction with Thyroid peroxidase and Iodotyrosine Deiodinase Type I- and II-catalyzed Reactions

Key study findings: Thyroidal hormone synthesis and the regulation were not a mechanism of BAY 38-9456-induced changes in thyroid- and TSH hormone levels.

Methods: Using solubilized hog thyroid microsomes, interaction with thyroid peroxidase was studied spectrophotometrically. Enzyme release of ^{125}I from the phenolic ring of radiolabeled iodothyronine was used to study interaction with iodothyronine deiodinases using 10,000 g supernatant from male rat liver homogenates for type I deiodinase activity and rat brain microsomes for type II deiodinase.

Results: BAY 38-9456 and sildenafil citrate only marginally inhibited (~10%) thyroid peroxidase (TPO)-catalyzed oxidation of the model substrate guaiacol, which is responsible for organification of iodide and the synthesis of both T_3 and T_4, and type II iodothyronine deiodinase (~20%) catalyzing the local formation of T_3 from T_4 in hypothalamus/pituitary gland for negative feedback, at maximally employable concentration of 300 μM. Both drugs did not affect TPO-catalyzed iodine formation and type I iodothyronine deiodinase (deiodinates T4 to T3 in the periphery like thyroid, liver & kidney) at 300 μM (PH-28209).

Summary/conclusions: BAY 39-9456 neither affected TPO-catalyzed guaiacol oxidation and iodine formation nor inhibited iodothyronine deiodinase I- and II at maximally employable concentration, suggesting that the compound did not interfere with key enzymes of thyroidal hormone synthesis and regulation.

Study title: 4-Week Oral Gavage Toxicity Studies of BAY 38-9456 and BAY 41-6484 in Rats.

Key study findings: Toxicological profiles of both BAY 38-9456 and BAY 41-6484 were similar except for myocardial and kidney findings only seen with BAY 38-9456.

Study no: PH-30893 (T7068463) **Date of study initiation:** May 31, 1999
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, 503891 (84.1% free base)/BAY 41-6484, 504611 (98.2%)
Formulation/vehicle: 0.5% Tylose mucilage
Dosing:
Species/strain: SPF-bred Wistar rats [HsdCpb: WU] **#/sex/group:** 10/sex/group

Satellite groups used for toxicokinetics or recovery: 3/sex/group
Weight: 112-135 g for males, 99-128 g for females **Age:** 5 to 6 weeks
Doses in administered units: 0, 40, 100 mg/kg BAY 38-9456 & 200 mg/kg BAY 41-6484
Route, form, volume, and infusion rate: Oral gavage 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/ Organ weights/Necropsy	Week 4
Urinalysis	Days 3/4 & 23/24 at 16 h
Toxicokinetics	Days 1 & 28

Results: One female from main study died on Day 10 caused by a malapplication of the drug. Clinical signs of discolored feces from Day 11 at ≥40 mg/kg and reddish skin on Days 3 to 5 at 100 mg/kg were observed. Slight decrease in body weights was noted in high-dose males treated with BAY 38-9456. Hematology and clinical chemistry parameters except for increased creatinine for BAY 41-6484 were within the historical controls. Differences found in urinalysis were regarded as chance since they were not observed in a previous study. Increased liver weights were observed in females. Hepatic enzyme induction of EH, GST, EROD, ECOD and ALD (female only) was dose-dependent and significant at high dose treated with BAY 38-9456. GLUT in females were significantly increased at high dose. BAY 41-6484 increased ECOD, ALD, and all phase II enzymes (EH, GST, GLUT) significantly. Other CYP450 subtypes of 16β-OHT, 2β-OHT parallel to 6β-OHT (CYP3A) were elevated dose-dependently treated with BAY 38-9456 and sildenafil. Myocardial fibrosis, reduced centriacinar glycogen content in the liver and increased renal basophilic tubules (mostly unilateral) were observed in the high-dose females treated with BAY 38-9456. Diffuse acinar hypertrophy was observed in the pancreas at 100 mg/kg and in the parotid glands/submandibular glands from 40 mg/kg. The hypertrophy was characterized by an increase in cell size and in basophilia of the cytoplasm with increased incidence and severity. Increased vacuolation of zona fasiculata/glomerulosa in the adrenal glands was seen at high dose. In BAY 41-6484-treated rats, reduced hepatic glycogen content, follicular cell hypertrophy in the thyroid gland and adrenal gland vacuolation (small vesicles) were also noted.

Dose, mg/kg	**Control**		**BAY 38-9456**				**BAY 41-6484**	
	0		40		100		200	
	10M	10F	10M	10F	10M	10F	10M	10F
Mortality								1*
Clinical signs,								
Discolored feces				10	10	10		
Reddish skin					10	10		
Body weights, Day 30/31 (g)	286	178	275	173	258	175	275	174
Food consumption, Week 4 (g)	21.0	15.7	19.9	13.2	19.4	13.8	20.6	13.0
Water intake, Week 4 (g)	26.1	21.8	27.8	21.3	27.1	27.4	29.9	19.7
Clinical chemistry, Week 4								
ALP, U/L	544	307	543	279	**437****	**274**	505	278
GLDH, U/L	4.2	0.2	**1.7**	0.0	**1.5**	0.3	**0.6****	0.0
LDH, U/L	104	66	63	55	71	53	**58**	**40****
CK, U/L	113	115	**97**	102	77	107	**68**	**65***
Cholesterol, mmol/L	1.80	1.60	1.97	1.77	1.94	1.63	2.13*	2.01**
Creatinine, μmol/L	47	42	50	41	46	42	**56**	**59****
Urea, mmol/L	7.66	7.47	7.01	7.81	8.09	**9.00****	7.22	7.58
Protein, g/L	61.3	62.5	62.9	61.0	58.6	**58.4****	61.2	62.1
K^+, mmol/L	5.1	4.3	5.2	4.3	5.0	5.0**	5.1	4.6*
T3, mmol/L	2.01	1.99	2.15	2.25**	2.12	2.25**	2.21	2.05
T4, mmol/L	58	47	56	59	64	**64****	63	54
Hematology	UR	UR	UR	UR	UR	UR	UR	UR
Liver biochemistry$^{@}$, Week 4								
CYP450, nmol/g	42.4	41.0	**44.7**	42.3	**45.7***	39.4	**50.9****	**47.5****
N-DEM, mU/g	152.4	80.9	144.0	81.0	125.5*	88.2	156.6	**111.5****

O-DEM, mU/g	11.2	9.6	12.8	10.2	11.5	12.7**	14.4**	14.0**
ECOD, nmol/g•min	7.3	4.8	**7.6**	5.9	**9.3***	**5.9**	**12.1****	**6.3***
EROD (CYP1A1), nmol/g•min	0.46	0.44	0.43	**0.48**	**0.71***	**0.74***	0.65	0.53
ALD, nmol/g•min	166.0	22.3	**139.3**	**27.5**	129.2	**30.8***	**130.9***	**36.3****
EH, nmol/g•min	366	288	**391**	**469****	**555****	**561****	**801****	**1094****
GST, µmol/g•min	71	65	**78****	**78****	**91****	**85****	**109****	**131****
GLUT, nmol/g•min	683	420	555*	**606****	653	**561***	**927****	**1128****
7α-OHT, nmol/g•min	4.4	7.3	4.5	10.3	5.2	7.5	**6.4**	7.7
6β-OHT (CYP3A), nmol/g•min	32.7	2.0	**41.0**	**5.8**	**50.8**	**9.3**	**93.1**	**18.5**
16α-OHT, nmol/g•min	49.3	2.3	51.2	3.9	43.6	3.8	45.3	**4.5**
16β-OHT, nmol/g•min	0.8	0.9	1.1	1.4	**1.8**	**1.7**	**2.0**	**2.1**
2α-OHT, nmol/g•min	28.7	-	**27.7**	-	**22.2**	-	**21.3**	-
2β-OHT, nmol/g•min	4.0	0.6	**5.1**	**2.2**	**6.8**	**4.3**	**13.3**	**10.4**
Urinalysis, Day 23/24								
Volume, mL	14.7	13.4	**13.6**	9.6	**11.0**	**21.3***	11.9	10.1
Protein, g/L	0.77	0.18	**0.67**	0.19	**0.59**	**0.34***	0.74	0.18
Creatinine, mmol/L	4.38	2.87	**4.18**	3.65	**3.91**	**1.56***	4.18	3.38
N-Acetyl-β-Glucosaminidase (NAG)	11.04	11.93	11.77	12.50	13.51	**6.43***	12.31	11.77
Protein volume, mg	9.7	2.3	**8.2**	1.7	**6.2****	4.9	**7.8**	**1.6**
Creatinine volume	54	33	**51**	**30**	**40****	**29**	**43***	**29**
NAG volume	142	138	145	103**	137	124	134	**102****
γGT volume, U	8.45	1.65	8.46	1.17	6.65	1.69	**6.23**	**1.04***
Alanine aminopeptidase	630	92	**597**	70	**479***	103	**442***	**53****
NAG/Creatinine, U/mmol	2.60	4.17	**2.85**	3.41**	**3.40****	4.44	**3.08**	**3.53***
AAP/Creatinine, U/mmol	11.58	2.78	11.84	2.35	12.00	3.67	10.26	**1.85****
LDH/Creatinine, U/mmol	2.21	2.26	2.20	2.29	**3.22****	**15.71**	**3.25****	2.62
Organ weights, absolute (mg)								
Liver	12969	7668	12729	**7963**	12463	**9369****	13978	**9664****
Kidney	1925	1201	1890	1215	1814	**1336***	1965	1299
Thymus	648	379	**595**	392	**523***	368	630	374
Gross pathology	UR	UR	UR	UR	UR	UR	UR	UR
Histopathology,								
Lungs, congestion (marked)								1
vascular mineralization (minimal)								1
Heart, myocardial fibrosis (minimal/marked)					1	8		
mononuclear cell infiltration (minimal)							1	1
Liver, mononuclear cell infiltration (minimal)	1			1	1	2	1	
reduced glycogen/centriacinar (minimal)						2		6
centrilobular hepatocellular hypertrophy								1
congestion (slight)								1
Kidney, basophilic cortical tubules (minimal)	4	1	1	1	2	8	3	
mononuclear cell infiltration (minimal)	1	1		1	2	3	1	2
pelvic dilation (slight)						1		
congestion (moderate)								1
incipient autolysis (slight)								1
Stomach, erosions (minimal)							1	
Pancreas, acinar hypertrophy (slight)					2	1		
Thymus, hemorrhage (slight)							1	
lymphocytolysis (slight)								1
Spleen, lymphoid depletion (slight)								1
Parotid gland, acinar hypertrophy (slight/moderate)			3	10	10	10		1
acinar vacuolation (minimal)		2	3		1			
Submandibular glands, acinar hypertrophy (slight)				7	1	9		
Lacrimal glands, Harderian metaplasia (slight)					2			
inflammation (posttraumatic)						1		
Skeletal muscle, regeneration (minimal)								1
Tongue, inflammation/periglandular (slight)						1		

Mammary gland, site present	3	4			4	1	4	
Adrenal glands, vacuolation/small/zona fasciculata	1		1		6	1	6	
vacuolation/large/zona glomerulosa				1	3	10		
vacuolation/small/zona glomerulosa			5	10	10	9	10	9
congestion								1
Thyroid gland, follicular cell hypertrophy (minimal)	1				1	2	4	2
thymic ectopia(minimal/moderate)		2	2	2	2	2		1
Epididymides, sperm granuloma (slight)		-		-		-	1	-
Uterus, dilation (slight)	-		-		-	3	-	
Vagina, estrus phase of the estrus cycle	-	3	-		-	5	-	3
proestrus phase of the estrous cycle	-	1	-		-	2	-	2
Spinal cord, degeneration of skeletal muscle fibers								1

*One female died at Day 10 caused by a gavage error.
@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; OHT=hydroxyl testosterone
Statistically significant from control group at p=0.05* or p=0.01**

Reevaluation of the salivary glands (parotid/submandibular/sublingual glands) revealed minimal to moderate acinar hypertrophy of the parotid glands from 25 mg/kg, and minimal to slight acinar hypertrophy of the submandibular glands from 25 mg/kg in females or at 100 mg/kg in males treated with BAY 38-9456 for 4 weeks.

Reevaluation of the Parotid- and Submandibular Glands with BAY 38-9456 (PH-28163A)

Dose, mg/kg	0		6		25		100	
	10M	10F	10M	10F	10M	10F	10M	10F
Parotid glands, acinar hypertrophy					5	9	10	10
Submandibular glands, acinar hypertrophy						9	7	10

Toxicokinetics (PH-30486): Plasma concentrations of BAY 38-9456 and M-1 generally increased dose-proportionally in males or under-proportionally in females being higher exposure. M-4 had a similar dose-dependence on Day 1, but on Day 28 AUC increased over-proportionally. No accumulation was observed on repeated dosing for both BAY 38-9456 and BAY 41-6484. The M-10 metabolite of BAY 41-6484 was about 3 fold higher in males than in females. M-1 reached 60% in males and 20% in females of BAY 38-9456, while M-4 accounted for only 1-2% of the levels of BAY 38-9456. M-10 reached 100% in males and 12% in females of BAY 41-6484 except Day 28 of 536%. Dose-normalized data demonstrate that the sum of exposure (parent & metabolite) was similar for the two drugs (vardenafil 100 mg/kg, sildenafil 142.4 mg/kg).

Dose, mg/kg		40		100		200	
		M	F	M	F	M	F
		BAY 38-9456				**BAY 41-6484**	
AUC_{0-24h} (μg•hr/L)	Day 1	8913	27712	26112	53751	37799	98321
	Day 28	2629	27696	31190	58395	5847	85916
C_{max} (μg/L)	Day 1	2032	4975	5250	8137	3023	15520
	Day 28	1367	6799	4803	11392	845	12145
T_{max} (hr)	Day 1	1.0	1.0	0.5	0.5	2.0	2.0
	Day 28	0.5	0.5	0.5	1.0	0.5	1.0
		BAY 44-5576 (M-1)				**BAY 35-5992 (M-10)**	
AUC_{0-24h} (μg•hr/L)	Day 1	3857	5279	13046	9176	37859	12287
	Day 28	3328	5492	18558	11317	31340	9991
C_{max} (μg/L)	Day 1	675	595	1370	1077	4801	1565
	Day 28	1239	867	3680	1358	3335	1156
T_{max} (hr)	Day 1	1.0	7.0	7.0	7.0	7.0	7.0
	Day 28	0.5	2.0	0.5	4.0	7.0	4.0
		BAY 44-5578 (M-4)					
AUC_{0-7h} (μg•hr/L)	Day 1	190	126	512	171		

	Day 28	46.4	123	198	484	
C_{max} (µg/L)	Day 1	50.6	21.4	116	31.4	
	Day 28	25.5	32.8	62.2	81.6	
T_{max} (hr)	Day 1	1.0	4.0	0.5	7.0	
	Day 28	0.5	2.0	0.5	2.0	

3/sex/timepoint as geometric means
The administered doses refer to the free base for BAY 38-9456 or citrate form for BAY 41-6484.

Summary: Treatment of rats with BAY 38-9456 (40- & 100 mg/kg free base) or BAY 41-6484 (142.4 mg/kg free base) produced an increase in hepatic enzyme induction. The activities were more pronounced in females with parallel increase in liver weight. In BAY 38-9456-treated rats, myocardial fibrosis predominantly occurred in the high-dose females possibly due to the vasodilatory properties of the drug. Renal basophilic tubules in the high-dose females associated with increased organ weight and urea level, were seen unilaterally, indicating the equivocal toxicological relevance. Minimally reduced centriacinar hepatocellular glycogen content was observed in females treated with either BAY 38-9456 or BAY 41-6484. Vacuolation of adrenal glands of BAY 38-9456 or BAY 41-6484 was considered to result from increased synthesis of mineralo-corticoids to compensate for vasodilatation and reduced blood pressure caused by the compound. Microscopic findings of the salivary glands revealed minimal to moderate acinar hypertrophy of parotid glands and submandibular glands at 25- and 100 mg/kg. Dose-normalized sum of exposure (parent+M-1) in 100 mg/kg vardenafil and 142.4 mg/kg sildenafil was similar. Table below is taken directly from the sponsor's submission.

PK of BAY 38-9456 and BAY 41-6484 at steady state: Comparison of Exposure in Rats and Human

Parameters at steady state	Male rat Vardenafil 40 mg/kg	Male rat Vardenafil 100 mg/kg	Male rat Sildenafil 142 mg/kg	Human Vardenafil 20 mg	Human Sildenafil# 100 mg
Parent Compound+					
$AUC_{0\text{-}24h}$ [µg*h/l]	2629	31190	5847	75.7	1686
multiple of human exposure	**35**	**412**	**3**	(1)	(1)
Major Metabolite++	M-1	M-1	M-10	M-1	M-10
$AUC_{0\text{-}24h}$ [µg*h/l]	3328	18558	31340	41.1	801
AUC adjusted +++	924	5155	31340	11.4	801
Sum of parent and major metabolite (adjusted)	3553	36345	37187	87.1	2487
Multiple of human exposure (adjusted for PDE5 inhibitory potency)	**81**	**417**	**15**	(1)	(1)

\# Sildenafil data are taken from FDA (3/27/1998): Pharmacological Reviews of Application 020895(sildenafil), NDA# 20-895
\+ Free base of vardenafil and sildenafil, respectively
++ M-1 (BAY 44-5575) of vardenafil and M-10 (BAY 35-5992/UK103,320) of sildenafil respectively
+++ Adjusted for pharmacological activity (PDE5 inhibition) relative to parent compound:
M-1 (BAY 44-5575) is 3.6 times less potent *in vitro* than vardenafil
M-10 (UK103,320) is equipotent to sildenafil (FDA (3/27/1998): Pharmacological Reviews of Application 020895 (sildenafil), NDA# 20-895; p.42)

Conclusion: Toxicological profile of BAY 38-9456 or BAY 41-6484 was considered to be 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).

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

BAY 38-9456 is a PDE5 inhibitor for the treatment of erectile dysfunction. PDE5 inhibition by vardenafil leads to increased cGMP in the corpus cavernosum, allowing for smooth muscle relaxation and inflow of blood into the penis resulting in erection. BAY 38-9456 inhibited PDE5 isolated from human platelets and human recombinant PDE5A1 at IC_{50} of 0.7 nM (sildenafil 6.6 nM) and 0.89 nM (sildenafil 8.5 nM), respectively. The inhibitory effect on PDE5 is >100 fold greater than on other PDEs except for PDE6 which was a ratio of 15. In an *in vivo* rabbit model, the effective minimal oral dose for an erection was 1 mg/kg.

Safety pharmacology studies showed dose-dependent decrease in peripheral resistance with increase in heart rate, cardiac contractility and cardiac output at 0.3 to 10 mg/kg in anesthetized dogs possibly secondary to vasodilation. QT intervals were decreased dose-dependently during the tachycardia. Similar hypotensive effects occurred at 3 to 30 mg/kg in female SHR. *In vitro* HERG channel activity and platelet aggregation were blocked from 1 μM. Number of erythrocytes, hematocrit and hemoglobin concentration were dose-dependently reduced from 1 to 10 mg/kg in male rats, possibly as a secondary effect of vasodilation. Psychomotor activity was statistically increased at 10 mg/kg in rats. All other studies on CNS, respiration, GI tract, renal function or coagulation up to 10 mg/kg, did not produce any relevant findings.

BAY 38-9456 was almost completely and rapidly absorbed from the GI tract in rats and dogs. Absorption in man was rapid after oral administration with T_{max} of 0.5-2 hrs and mean elimination half-life of 4-5 hrs. Due to a considerable first-pass effect, mean absolute oral bioavailability in man is about 15%. The oral bioavailability in rats and dogs was 7.4-28.6% and 27-33%, respectively. The volume of distribution at steady state (V_{ss}) was moderate, ranging at 2.0 L/kg in rats, 2.5 L/kg in man and 5.2 L/kg in dogs, reflecting the species differences in protein binding. Gender-dependent PK was not seen in dogs and mice, but rats had 8-13 fold higher exposure and longer elimination half-life in females than in males after a single dose (1 mg/kg).

After subchronic and chronic oral administration of BAY 38-9456, exposure of parent drug was higher in female rats than in males due to sexual dimorphism of CYP3A4. The higher exposure in females may account for the more pronounced effects observed in the toxicity studies. AUC increased over-proportionally with dose, and a tendency to accumulate was noted. In pregnant rats, plasma concentrations were in the same range as in the non-pregnant rats. Fetal tissue concentrations amounted to 10-98% of the corresponding maternal concentrations in rats and to 25-41% in rabbits. There was no significant indication of accumulation on repeated dosing in dogs. In mice, exposure was decreased on repeated dosing, but was not considered to be an enzyme induction. The exposure of M-1 and M-4 increased over-proportionally to dose in rats and dogs without accumulation on repeated dosing. Metabolic ratios (M-1 to parent) of AUC were 41-108% in male rats, 14-28% in female rats, 46-86% in dogs and 6-22% in mice. In the rat embryotoxicity study, fetal concentrations of M-1 was dose-dependent ranging from 4-96% of the corresponding maternal plasma concentration. Based on the PK observed in man, overall efficacy of metabolites is predicted to be small with 7.2% (M-1), 0.06% (M-4) and 0.6% (M-5) relative to BAY 38-9456.

[^{14}C]-BAY 38-9456 was distributed rapidly after oral administration to rats, and eliminated within 24 hrs in all tissues except liver and kidneys which had residual radioactivity after 168 hrs. The highest radioactivity was measured in the liver, adrenal glands and kidneys in rats with the longest terminal half-life of 71 hrs for kidneys. BAY 38-9456 distributed to a moderate degree into erythrocytes. Total radioactivity penetrated the blood/brain barrier to a moderate extent and the placental barrier of rats to a low extent. Higher radioactivity in mammary glands indicate that the drug and/or metabolites may be secreted into milk. Higher exposure in the fetal brain was observed than in maternal brain. The half-life of elimination from the eye in pigmented rats was around 14 days.

Metabolism is the major clearance mechanism for BAY 38-9456. Biotransformation in all species involves either N-deethylation (M-1), oxidation (M-13), opening (M-15) or further degradation (M-4, M-5, M-6, M-7 and M-16) of the piperazine ring. The key enzymes for the metabolism are CYP3A4 and CYP3A5. Metabolite M-1 was found in considerable amounts (22-32% of radioactivity) in plasma of all species investigated including human. N-glucuronide conjugate of M-1 was also found in human plasma after thermal treatment.

BAY 38-9456 and M-1 were highly bound to plasma proteins and independent on concentration and gender. The unbound fraction was 4.7-6.7% in man, 12.2-12.8% in dogs, 4.5-6.0% in rats, 2.9-4.2% in rabbits and 5.7-7.2% in mice.

Excretion occurred rapidly mainly via the biliary/fecal route and to a lesser extent via the kidney in mice, rats, dogs and men. Sponsor indicated that 57.8%, 39.6%, 77.2% and 78.1% of the dose could be attributed to known structures in rat, mouse, dog and human excreta, respectively for analyzing urine and bile or feces. BAY 38-9456 penetrated the placental barrier to a low extent in rats and marginally (3.3%) excreted into milk of lactating rats.

Toxicology studies were conducted for single dose in mice and rats, and for repeated dose in mice, rats and dogs. Oral LD_{50} for vardenafil HCl (BAY 38-9456) was 1000 mg/kg in mice and 190-250 mg/kg in rats, and >2500 mg/kg for vardenafil free base in rats. The free base of BAY 38-9456 appeared to be less toxic to rats by the oral route. Sponsor stated that the differences in the study protocol and/or systemic availability may account for the result. In the repeated studies, dosing was adjusted to give the respective concentration of the free base. The most prominent toxicological findings treated with BAY 38-9456 were the effects on cardiovascular system. QT intervals were dose-dependently decreased up to 14% at high dose, which was associated with increased heart rate in response to the reduced systolic/diastolic blood pressure. Flushing and decreased blood pressure/increased heart rate were observed from 10 mg/kg in the 1-, 3- and 12-month dog studies. However, the hemodynamic effects were observed from 0.3 mg/kg in the single-dose cardiovascular study. Cardiovascular effects were not assessed in rats, but drug-related myocardial fibrosis or necrosis correlated with increased heart weight was observed at high doses in the 2-week, 1-, 3- and 6-month rat studies. The myocardial damage was still present at the end of the recovery period in the 3-month study, and resulted in deaths in high-dose female rats in the 2-week, 3- and 6-month studies. High dose dogs also had focal fibrosis in the left ventricular wall or myocardial necrosis in the 1-month study. Subepicardial and pericardial edema in the atrium occurred from mid dose (10 mg/kg) with increased incidence and severity. Periarteritis/arteritis were observed in multiple cardiac locations at high dose in the 3-month dog study. The periarterial edema was found in 1 of each sex at high dose in the 1-year study, suggesting no aggravation over time. Sponsor considered the myocardial lesions as secondary to the hemodynamic effects of the drug. However, a study that the arteritis was prevented with specific pharmacologic or physiologic antagonists was not conducted to demonstrate the drug-induced arteritis as a result of an exaggerated pharmacologic effect. Total exposures of the unbound drug (parent+M-1) at a NOAEL of 3 mg/kg for the effects gave 2-21 fold the exposure at the maximum recommended human dose (MRHD) of 20 mg.

Testicular atrophy/degeneration is a major concern for PDE5 inhibitors. Oligospermia/debris in the epididymides was observed from mid dose (10 mg/kg) with increased incidence in the 3-month dog study. In the rat, testes degeneration/tubular atrophy was found in one of each at mid- and high doses. The incidence was not seen in the 6-month rat study at high dose and, occurred in one of each at mid- and high doses in the 1-year dog study, suggesting that the testicular atrophy was not progressive over time. The male dog at high dose (30 mg/kg) in the 1-year study showed a moderate bilateral multifocal degeneration of the germinal epithelium in the testes with a subsequent increase in epididymal spermatic debris.

Hypertrophy of the pancreas, submandibular/parotid glands and thyroid were observed only in rats, and considered to be the result of the pharmacology of PDE5 inhibitors. Follicular cell hypertrophy in the thyroid was observed as a sign of thyroid activation. In the 1-month study, increased incidence and severity of colloidal vacuolation in the thyroid was observed at high dose (100 mg/kg), whereas minimal to slight hypertrophy was found from mid-dose females (25 mg/kg). Increased incidence of follicular cell hypertrophy and colloidal vacuolation was observed in the 3-month study at high dose (125 mg/kg) and from 25 mg/kg, respectively. The findings were found in a high-dose female (75 mg/kg) in the 6-month study and in the 2-year study at high dose (75 mg/kg M, 25 mg/kg F). The hypertrophy was reversible during the 4-week recovery period in the 3-month study, and was only seen in 1 female in the 6-month study. Over the 2-year period, there was no malignant tumor induction. Non-reversible and slight increase in T3/T4 in high-dose females and dose-dependent increase in thyroid stimulating hormone (TSH) in all treated male groups were seen in the 3-month study. In the 6-month study, dose-dependently elevated T3/T4 in all treated male groups and slight increase in TSH at high dose were observed. In the 2-year study, T3 levels were dose-dependently decreased with statistical significance in high-dose males, but the changes of T4 and TSH were not remarkable. In comparison with sildenafil in a 4-week study (PH-30893), BAY 38-9456 equipotently elevated T3/T4 and other CYP450-dependent monooxygenase and glutathione-S-transferase (GST) activities. The increase in UDP-glucuronyltransferase (GLUT), 6β-hydroxyl testosterone (6β-OHT for CYP3A) and epoxide hydrolase (EH) activity was more pronounced in sildenafil-treated rats. BAY 38-9456 did not inhibit the key enzymes for thyroidal hormone synthesis and regulation *in vitro* (PH-28209). Sponsor stated that the thyroidal changes were neither a direct effect on the thyroid nor the pituitary, but reflect an adaptive response secondary to slightly increased hepatic thyroxin elimination. Plasma exposures of unbound parent drug at a NOAEL of 6 mg/kg in the 1-month study produced 2-18 fold the human exposure at MRHD of 20 mg.

Minimal to moderate acinar hypertrophy of the submandibular glands was noted from 25 mg/kg in the 1-month study (PH-28163A), in the high-dose males and from the mid-dose females (25 mg/kg) in the 3-month study, and in high-dose females (75 mg/kg) in the 6-month study. Two-year study also revealed the statistically significant increase in mid-dose males (15 mg/kg) and high-dose females (75 mg/kg M, 25 mg/kg F). Acinar hypertrophy of the parotid glands was seen from 25 mg/kg in the 1-, 3- and 24-month studies and at 75 mg/kg in the 6-month study. The AUC of unbound parent drug at a NOAEL of 6 mg/kg in the 1-month study corresponded to 2-18 fold the human exposure at MRHD of 20 mg.

Diffuse/periinsular hypertrophy in the exocrine pancreas were observed at high dose with slightly increased incidence and severity in the 1-, 3-, 6-month studies. The acinar hypertrophy was also found in high-dose males with slightly increased incidence in the 2-year study. 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). Sponsor stated the acinar atrophy was not correlated with the hypertrophy since the lesions associated with mild interstitial fibrosis, mononuclear cell infiltration, and pigment deposits were observed in isolated lobuli, clearly apart from the areas showing acinar hypertrophy. After a 4-week recovery period, only mild interstitial fibrosis with pigment deposits persisted, indicating no further progression after the end of the treatment.

In the adrenals, minimal to slight vesicular vacuolation of the zona glomerulosa cells was observed in the 1- and 6-month, and 2-year studies. In the 1-month study (PH-30893), vacuolation of small and large vesicles was observed from 40 mg/kg. In the 6-month study, the vacuolation (small vesicles) was increased in severity and incidence from low-dose males (3 mg/kg) and was evident at all treated groups in females. (M; 2/3/20/20, F; 0/6/20/19). Additionally, a large vesicular vacuolation of the zona glomerulosa cells was seen at high dose. In the 2-year study, 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 (75 mg/kg M, 25 mg/kg F), which was frequently accompanied by an increased vacuolation of the zona glomerulosa cells. Total plasma exposures of unbound drug (parent+M-1) at a NOAEL of 3 mg/kg in the 6-month study produced 2-12 fold the human exposure at the MRHD of 20 mg.

Increased incidence of basophilic tubules in the kidneys was noted in high-dose females in the 1-, 3- and 6-month studies in rats, and 2-year mouse study. The findings were generally associated with increased organ weights (non-reversible during the 4-week recovery period) and increased plasma urea or inorganic phosphate. Sponsor stated the toxicological significance questionable since a low grade of basophilic tubules is a known age-related finding in rats.

Since markedly increased hepatic enzyme activities (aminopyrine-N-demethylase, N-DEM; *p*-nitroanisole-O-demethylase, O-DEM) were noted in mid- or high dose rats and dogs with increased organ weights in the 2- and 4-week, and 3-month studies, CYP450-dependent monooxygenases (7-ethoxycoumarin deethylase, ECOD; 7-ethoxyresorufin deethylase, EROD; aldrin epoxidase, ALD) and phase II enzymes (EH, GST, GLUT) were evaluated in the subchronic studies in rats and dogs. Females who died in the high-dose groups in the 2-week, 3- and 6-month rat studies also showed hepatic congestion. Increased CYP450-dependent monooxygenases (EROD for 1A1 and ECOD) activities were observed from 3 mg/kg in females with statistical significance at high dose, and from mid dose (10 mg/kg) in males in the 4-week dog study (PH-

27983). In the 3-month rat study, statistically significant increase occurred in the activities of EH at high dose and GST in all treated males. ECOD, EROD and ALD were increased in high-dose females. The increase was reversible during the 4-week recovery period.

BAY 38-9456 was not genotoxic or mutagenic *in vitro* Ames test, V79 forward mutation assay, and mammalian chromosome aberration assay, or *in vivo* mouse micronucleus test.

Carcinogenic potential was evaluated in 2-year carcinogenicity bioassays in rats and mice. Rats were dosed by oral gavage, which gave up to 180-400 fold higher exposure than humans taking a 20 mg dose. Mice were dosed in the drinking water with exposures up to 21-37 fold higher than in humans. For neoplastic tumors, statistical significance was observed in adenocarcinomas of the pars distalis of the pituitary gland with negative trend and benign thymomas with positive trend in high-dose female rats. The benign thymoma findings were considered incidental since the incidence was within the historical range of 10%. In mice, histiocytic sarcomas were slightly higher in high-dose females (4/4/3/7), but were not considered to be treatment-related since the incidence was not statistically significant, was reduced in males (2/2/0/0) with significantly negative trend, and was close to historical control range from the RITA database in females (4-12%). A NOAEL for 2-year carcinogenicity studies was considered to be 200 ppm for mice and 3 mg/kg for rats. The total exposures of the unbound drug (parent+M-1) at the NOAELs produce 3- to 8-fold for mice and 8- to 10-fold for rats the human exposure at a maximum clinical dose of 20 mg (76 μg•hr/L for BAY 38-9456 & 41.1 μg•hr/L for M-1 at steady state $AUC_{0\text{-}24h}$ from #100196).

Reproductive toxicity studies were conducted in a Segment I fertility/early embryonic development study in rats, Segment II developmental toxicity study in rats and rabbits, and Segment III pre/postnatal development study in rats. In the fertility study, slightly reduced fertility index was seen in high-dose males (100 mg/kg), but the values were within the historical control range. Clinical signs of reddish discoloration of pinnae of the ears in all treated groups and salivation at high dose were observed. Treatment-related decreased body weight gain was noted from mid-dose females (25 mg/kg) during pre-mating/gestation and high-dose males during pre-mating period. Slightly decreased food consumption was noted during pre-mating from mid-dose females and high-dose males. In the rat development study, treatment-related severe maternal toxicity occurred at high dose (100 mg/kg). These included mortality, clinical signs (piloerection, sunken flanks, disturbance of gait, hypoactivity, light colored/reduced amount of feces), decreased food intake, increased water intake/urination, body weight loss, and pathological alterations of the stomach (filled with bedding material, reddish brown spots in gastric mucosa) and heart (myocardial fibrosis, edema, necrosis, diffuse inflammatory infiltration). One total resorption occurred possibly as a consequence of the severe maternal toxicity in the respective dam. Increased incidence of post-implantation loss and reduced litter size were observed in the remaining females. Reduced placental/fetal weights, retarded fetal ossification, and increased incidence of skeletal variations (asymmetric sternebrae) were found at high dose. Similar treatment-related effects were noted in high-dose F0 females in the pre/postnatal developmental study (Segment III) tested up to 60 mg/kg. The gestation index was marginally affected by a prenatal loss of a complete litter at high dose. Increased number of stillborn pups and mortality of f1 pups up to day 4 p.p. resulted in slightly decreased live birth index, litter size at birth/rearing period, and reduced rearing index. Body weight of f1 pups was decreased at high dose from birth to the end of the study, and was more pronounced during the rearing period and in males. Reduced lactation behavior at high dose was assumed because a few pups had no milk spots, and had clinical signs (pale/discolored skin) and reduced viability. Retarded physical development (eruption of incisors, development of fur) became evident from mid dose (8 mg/kg), and more pronounced at high dose (additional delay of pinnae detachment, eye opening, development normal gait, preputial separation). Decreased vertical activity occurred in all treated male groups and high-dose females on Day 22 p.p. The effects at low- and mid doses were equivocal since the ranges were within the historical controls and no effect was seen in a repeated test performed at 14 weeks. F1 generation displayed reduction of body weight with minor decrease of food intake in females at high dose. The numbers of pregnant females that delivered and litter size were marginally reduced in high-dose F1 rats, possibly as a consequence of the systemic effects. In a rabbit developmental toxicity study, females had reddening of the ears/hypoactivity at high dose (90 mg/kg), and decreased food intake/feces/urine from mid dose (18 mg/kg). Gestation rate was decreased by 1 total resorption at high dose possibly due to systemic toxicity. Post-implantation loss in the females with viable fetuses on Day 29 p.c. was increased (late resorption), and corresponding number of fetuses was decreased at high dose. Retarded ossification of single locations was noted in the high-dose fetuses. A NOAEL for the rat reprotoxicity studies was considered to be 100 mg/kg for F0 fertility in the Segment I, 18 mg/kg for maternal/developmental toxicity in the 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 the Segment III. Total AUC values of unbound drug (parent+M-1) for F0 fertility in the Segment I in a corresponding subacute toxicity study (PH-30893) was >400 times the AUC in humans at the MRHD of 20 mg. The exposure at the NOAEL of 18 mg/kg for maternal/developmental toxicity in the Segment II was equivalent to 100 fold the human exposure at 20 mg. The NOAEL doses of 8- and 1 mg/kg in the Segment III produce 4 fold for maternal toxicity/F1 fertility and 0.5 fold for F1 pre/postnatal development on a mg/m² basis, respectively. Based on the results of the rat teratogenicity study in the pregnant rat (Segment II), 8 mg/kg/day for maternal toxicity and 1 mg/kg/day for f1 pre/postnatal development are estimated to produce total AUC values for unbound drug (parent+M-1) of about 40 and 4 fold the human AUC at the MRHD of 20 mg, respectively. In the rabbit, a NOAEL was established at 3 mg/kg for maternal toxicity and 18 mg/kg for embryonic development, which produces 2- and 29 fold the unbound drug exposure (parent+M-1) at the MRHD of 20 mg, respectively.

Special toxicology studies were conducted to evaluate *in vitro* thyroidal enzyme interaction and *in vivo* comparative toxicity with sildenafil. Since repeated treatment of BAY 38-9456 altered thyroid hormones and increased thyroid activity in rats, interactions with key enzymes of thyroidal hormone synthesis and homeostasis were studied. Vardenafil HCl (BAY 38-9456) and sildenafil citrate (BAY 41-6484) did not affect thyroid peroxidase (TPO)-catalyzed guaiacol oxidation and iodine formation at 200-300 μM, indicating that the drugs neither inhibited TPO nor trapped iodinating species. The drugs neither affected iodithyronine deiodinase I-catalyzed phenolic ring deiodination of a substrate 3,3'5'-L-triiodothyronine nor iodothyronine deiodinase type II-catalyzed phenolic ring deiodination of T4. These results suggest that BAY 38-9456 did not interfere with key enzymes of thyroidal hormone synthesis and regulation. BAY 38-9456 may modify the effect of TSH on its target tissue as theophylline increases TSH through PDE inhibition by increasing the intracellular cAMP levels.

A 4-week comparative toxicity study was conducted in rats with 40- and 100 mg/kg BAY 38-9456 and 200 mg/kg BAY 41-6484 (corresponding to 142 mg/kg sildenafil free base). Sponsor stated that the BAY 41-6484 dose was selected since ~20 fold increase of hepatic GLUT activity was reported in rats. BAY 41-6484 increased liver enzyme activities (CYP450, N-DEM, O-DEM) accompanied by increased liver weight. Statistically significant increase in monooxygenases ECOD and ALD, and all Phase II enzymes (EH/GST/GLUT) were observed. BAY 38-9456 increased GST, EH and GLUT (females only) at 40- and 100 mg/kg. Significant induction of monooxygenases ECOD, EROD and ALD (females only) occurred at 100 mg/kg. ALD activity was reduced in male dosed with 40- and 100 mg/kg. The 6β-OHT substrate for CYP3A showed that the enzyme activity was increased dose-dependently parallel to 2β-OHT treated with BAY 38-9456 (2-5 fold) and BAY 41-6484 (3-9 fold) being more pronounced in females. Minimal to severe myocardial fibrosis was observed in females treated with 100 mg/kg BAY 38-9456. Increased incidence of basophilic tubules in kidneys correlated with increased organ weight and serum urea levels at 100 mg/kg in females. In addition, acinar hypertrophy in the pancreas (100 mg/kg only)/parotid glands/submandibular glands and minimal to slight vacuolation of the zona fasciculata (small vesicles)/zona glomerulosa in adrenal glands were observed from 40 mg/kg. In BAY 41-6484-treated rats, minimal to slight increase in vacuolation (small vesicles) of the zona fasciculata (males only) and the zona glomerulosa in the adrenals were noted. Sponsor concluded that the toxicological profiles of BAY 38-9456 and BAY 41-6484 were similar when taking into account the higher exposure of the animals treated with BAY 38-9456 in terms of pharmacological activity and relative to human exposure (see pp38).

Conclusions: The preclinical studies were conducted with adequate safety margins with respect to the exposure at relevant oral doses in the animals. The myocardial damage and/or periarteritis occurring in rats and dogs are probably due to the exaggerated pharmacological activity of BAY 38-9456 at hypotensive and tachycardiac doses.

General Toxicology Issues: Testicular atrophy/degeneration and arteritis are the major concerns for the PDE5 inhibitors. With sildenafil, most arteritis findings were seen in high-dose dogs. These included a focal coronary arteritis (1/2F) in the 10-day study, a coronary arteritis (1/3F) in the 1-month study, a periarteritis (2/4M) in the 6-month study and a disseminated arteritis (3/4M, 1/4F) in the 1-year study. Total exposures of the unbound drug were 42-49 fold greater than human exposure at 100 mg at the affected doses and 8-30 fold greater at the NOAELs. The findings were considered related to the vasodilatory properties of the drug. Testicular atrophy/degeneration was not remarkable.

With cialis, vasculitis was observed in dogs, mice and rats with increased incidence and severity, but the effects varied considerably between studies. Drug exposures at the affected doses were 6-9 times in mice, 2-33 times in rats and 1-54 times in dogs the human exposure at 20 mg. In dogs, effects included perivascular inflammation in the lungs, increased incidence of coronary arterial lesions and marked disseminated arteritis. The vasculitis findings were not seen in the 1-year dog study with doses giving exposures up to 33 times the human exposure. Instead, marked thrombocytopenia/neutropenia were seen in 1 mid- and 1 high-dose female with 14- and 18 fold the human exposure. The findings occurred without significant hemodynamic changes. Possible systemic symptoms of hypersensitivity such as myalgia, back pain or infection were the most frequently reported adverse human events associated with cialis. Irreversible testicular degeneration/atrophy was observed in mice and dogs with no/low safety margin compared to the human dose of 20 mg cialis. In men, there were no clinically significant effects on semen parameters up to 6 months at 20 mg.

With BAY 38-9456, myocardial leisions indicative of possible arteritis was observed at high doses in all rat studies. The myocardial damage was still present at the end of the recovery period, and resulted in deaths of high-dose female rats. High dose dogs also had focal fibrosis in the left ventricular wall or myocardial necrosis in the 1-month study. Subepicardial and pericardial edema in the atrium occurred from mid dose (10 mg/kg) with increased incidence and severity. Periartetitis/arteritis were observed in multiple cardiac locations at high dose in the 3-month dog study. The periarterial edema was found in 1 of each sex at high dose in the 1-year study, suggesting no aggravation over time. The effects were associated with hypotension and tachycardia, indicative of an exaggerated pharmacodynamic response of the drug, although the exact mechanism of action was not demonstrated by blocking with specific pharmacologic/physiologic antagonists. Frequency of arteritis/periarteritis in the testes, pancreas or tongue was decreased in high-dose male rats over the 2-year period. Exposures at NOAELs of the unbound parent drug for the arteritis findings corresponded to about 10 fold in the 1- and 3-month studies, and 130 fold in the 1-year study the human exposure at the MRHD of 20 mg. The effects occurred at exposures of 80-600 fold the human exposure at 20 mg. Oligospermia/debris in the epididymides was observed from mid dose (10 mg/kg) with increased incidence in the 3-month dog study. In the 3-month rat study, testes degeneration/tubular atrophy was found in one of each at mid- and high doses. The effects in the dog were seen at exposures of 85 fold with a safety margin of 11 fold at a NOAEL the human exposure at 20 mg. The incidence was not seen in the 6-month rat study at high dose, and occurred in one of each at mid- and high doses in the 1-year dog study, suggesting that the testicular atrophy was not progressive over time. In mice, statistically significant decrease in the incidence of diffuse tubular atrophy and Leydig cell atrophy in the testes as well as decreased aspermia, inflammation and epithelial vacuolation in the epididymides were seen at high dose compared to control group over 2 year period. Thus, the arteritis and testicular findings observed with BAY 38-9456 in animals are of equal severity with sildenafil.

X. APPENDIX/ATTACHMENTS:

Addendum/appendix listing:
Appendix I: IND Reviews
Appendix II: Exec CAC minutes for protocols and final studies
Appendix III: 2-Year carcinogenicity statistical review and evaluation from the agency

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Alexander W. Jordan
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Appendix I

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IND
Review #2

IND

February 3, 1999

Drug: BAY 38-9456, Phosphodiesterase type V inhibitor
Sponsor: Bayer Pharmaceuticals Division, Westhaven, CT
Contact: Dr. Richard Fanelli, 203-812-2010

Submission: Serial # 001, January 8, 1999
Information to the Sponsor: Yes (X) No ( )

Div. of Reproductive and Urologic Drug Products, HFD-580
Reviewer: Jeri El-Hage, Ph.D.

Review and Evaluation of Rodent Carcinogenicity Protocols
Review # 2

Oncogenicity Study in Mice

Protocol Design:
The study will be conducted according to OECD /GLP guidelines at the Institute of Toxicology, BAYER AG. CD-1 mice (50/sex/dose) will be administered 0, 40, 200 and 1000 ppm BAY-38-9456 orally via the drinking water. Doses are equivalent to the following mg/kg/day doses: Males: 0, 6.7, 36.6, and 150.7 mg/kg/day ; Females: 0, 10.1, 51.0, and 203.1 mg/kg/day. Animals will be observed for mortality and signs twice daily, body weight and water consumption recorded weekly, and food consumption measured every 4 weeks. Hematology and limited clinical chemistry parameters will be evaluated during weeks 26, 52, 78, and 104. Bayer will conduct a complete histopathologic evaluation for all dose groups.

Toxicokinetics: The sponsor plans to measure plasma concentrations at 23:00 p.m. from 5 animals per sex during weeks 1, 52 and 104. This toxicokinetic evaluation is inadequate. It is known that there is a major, biologically active metabolite, M1, in all species. The sponsor has not characterized the toxicokinetics of this metabolite in any species. In addition, the sponsor has provided only limited toxicokinetics data for the parent compound (measured in only one study for all species).
Recommendation: Cmax and AUC values for the parent compound and M1 metabolite should be provided for all dose levels during weeks 1, 52 and 104. It is also recommended that this data be obtained from groups of satellite animals for the 1 and 52 week sampling times.

Rational for Dose Selections:
A summary of the findings from a 14 week dose-finding study in CD-1 mice was provided (all referenced tables present in appendix I).
Mice (n = 10/sex/dose) were administered 0, 40, 200 and 1000 ppm BAY 38-9456 in the drinking water.
Mortality/Signs: none
Body weight: There were no drug-related effects on body weight or food consumption (tables 1, 2 and 3). Water consumption was decreased in high dose females during the first weeks of the study suggesting poor palatability of the high dose solution.
Hematology : Hematology findings included decreased mean leukocyte counts (30%) at all doses in treated females and decreased platelet counts (13%) in mid and high dose males (tables 5 and 6).

Blood chemistry: Serum glucose concentrations were increased (15%) in low and high dose mice of both sexes (table 8). BUN and bilirubin were slightly increased in high dose mice of both sexes (table 8).
Organ weights: Absolute and relative heart, liver, and spleen weights were increased in high dose male mice. The increased heart weights were secondary to the pharmacologic effects of the compound (reduced blood pressure) and the increased liver weights are most probably secondary to liver enzyme induction, as was observed in rats.
Histopathology: data were not provided but sponsor states there were no treatment-related observations.
Toxicokinetics: The available TK data for mice are summarized in the table below. The only available human pharmacokinetics data are single dose data from young male volunteers. The drug is indicated primarily for elderly men with sexual dysfunction. Data from mice, rats, and dogs suggest there is not significant accumulation with multiple dosing. The data in mice and dogs also demonstrate no significant sex differences in exposures. The sex differences in kinetics in rats, with significantly higher levels in females, are secondary to sexual dimorphism in CYP 3A4 in rats. The CYP 3A4/5 isozymes are primarily responsible for biotransformation in all species but do not display sex differences in humans.

Summary: There was no dose-limiting toxicity associated with the doses proposed for use in the 2-year mouse CA study. The dose selections were made based on multiples of the human AUC exposure. BAY 38-9456 tested negative for genotoxicity in the Ames test, in vitro chromosome aberrations assay in Chinese hamster lung V79 cells, and the mouse micronucleus assay. The metabolite profile of BAY 38-9456 is qualitatively and quantitatively similar in mice, rats, dogs and humans. (in vitro data only for mice). The degree of protein binding is also similar in mice, rats and humans (93-95% bound).

The AUC of parent drug, BAY 38-9456, in healthy young males receiving the highest proposed clinical dose of 40mg is 134 ng.hr/ml. **The doses to be used in the mouse carcinogenicity study should result in parent drug AUC exposures 1, 4-5 , and > 60 times the human AUC exposure with the highest therapeutic dose.** Therefore, the dose levels clearly meet the criteria of > 25 times the AUC with the highest therapeutic dose. The large multiple at the highest dose of 1000 ppm should allow for any differences in kinetics in the elderly.

The major concern I have regarding the use of the AUC multiple for dose selection is that parent drug is rapidly metabolized in all species. The M1 metabolite is the major metabolite (>25% of circulating radioactivity in all species). The M1 metabolite is biologically active with comparable potency to the parent compound at inhibiting PDE V. Toxicokinetics for the M1 metabolite have not been provided for any of the preclinical studies. The sponsor was informed at the pre-IND meeting that they should provide TK data for the M1 metabolite.

The preliminary metabolism data that we have available suggests that the required exposure multiples will still be met with total exposure data (parent + M1). Summaries of metabolism data in rats and dogs describe 25-50 % of the radioactivity attributable to parent and 25% attributable to M1. The M5 metabolite and unknown metabolites also represent a significant proportion of radioactivity (30%). Information regarding the proportions of parent drug and metabolites in mouse serum was not provided. Bayer did provide information on concentrations of the M1 metabolite in men. Values for both Cmax or AUC of parent drug were 3 –4 times greater than metabolite M1 concentrations in male volunteers.

Conclusions:.
The major problem with the information provided to support the dose selections is that it is known that the drug is rapidly metabolized to a major active M1 metabolite. Exposures to the drug must be represented by total exposure to the parent compound and M1 metabolite. The sponsor has not provided toxicokinetics for the M1 metabolite for any species despite being advised to do so in the pre-IND meeting (sildenafil kinetics are similar). In addition, submitted protocols suggest no intention to do so in the future. The preliminary metabolism data for the rat and dog and PK data in male volunteers suggest the sponsor will should still be able to meet the >30 times human AUC exposure multiple when parent and M1 are quantified.

There is one additional problem with the use of AUC multiples for dose selection. AUC was evaluated only during week 3 of the mouse dose-finding study. Plasma concentration data was also collected on study days 5 and 89, and demonstrated that plasma concentrations with the highest dose of 1000ppm were significantly lower on day 89 than on day 5. BAY 38-9456 induces liver enzymes and its own metabolism in the rat and increased liver weights in male mice. AUC data collected on day 21 may not adequately assess for this liver enzyme induction and a consequent increase in drug metabolism in mice. The day 22 AUC data may overestimate exposures in the carcinogenicity study.

Recommendation: Dose selections based on AUC exposure multiples are appropriate. The sponsor should be requested to collect toxicokinetics data (Cmax and AUC) for parent drug and the M1 metabolite in the mouse carcinogenicity study during weeks 1, 52 and 104. The approval of the doses should be qualified by a statement indicating that the use of AUC ratios is deemed appropriate , but total (parent plus M1) AUC exposures in mice must be greater than 25 times human total AUC exposures with the highest therapeutic dose of 40 mg. In addition, please be aware that we are concerned that use of AUC exposure data obtained on day 22 of the mouse dose-finding study may overestimate the actual total AUC exposures in chronically treated mice if significant enzyme induction occurs.

Oncogenicity Study in Rats

Protocol Design:
The study will be conducted according to OECD and GLP regulations at the Institute of Toxicology, BAYER-AG, Wuppertal, Germany. Wistar rats (n= 50/sex/dose) will be administered 0, 3, 15 and 75 mg/kg/day BAY 38-9456, orally by gavage, daily for 2 years. Clincal condition and signs will be checked twice daily. Body weight, food and water consumption will be monitored weekly. Opthalmologic exams will be performed on control and high dose animals pre-dose and at study termination. Hematology and clinical chemistry evaluations will be performed on 10 rats/sex/dose during weeks 26, 52, 78 and 104. A complete histopathologic evaluation will be performed for all dose groups.

Toxicokinetics: The sponsor plans to measure plasma concentrations at 23:00 p.m. from 5 animals/sex/group during weeks 1, 52 and 104. As stated for the mouse study, this toxicokinetic evaluation is inadequate.
It is known that there is a major, biologically active metabolite, M1, in all species. The sponsor has not characterized the toxicokinetics of this metabolite in any species. In addition, the sponsor has provided only limited toxicokinetics data for the parent compound (measured in only one study for each species). In addition, if the sponsor plans to use exposure multiples as the criteria for dose selection, total drug exposures (parent + M1 metabolite) must be confirmed in the carcinogenicity study.
Recommendation: Cmax and AUC values for the parent compound and M1 metabolite should be provided for all dose levels during weeks 1, 52 and 104. It is also recommended that this data be obtained from groups of satellite animals for the 1 and 52 week sampling times.

Rationale for Dose Selection:
1. **4-Week Oral Toxicity in Rats:** Wistar rats (10/sex/dose) were administered 0, 6, 25 and 100 mg/kg/day BAY 38-9456 orally by gavage for 4 weeks. (referenced data are present in Appendix III).

Mortality: There was no drug-related mortality.
Signs: Flushing was dose-related in incidence and duration and was observed at all dose levels.
Body weight: There were no drug-related detrimental effects on body weight or food consumption (figures 1-4). There was a slight increase in body weight gain in HD female rats. High dose animals of both sexes also displayed increases in water consumption.
Hematology: Mild increase in erythrocytes, hemoglobin and hematocrit were observed in high dose female rats (table 1). There were no drug-related effects on white blood cell counts in either sex (table 2).
Serum Chemistry: Mild increases in mean BUN and total bilirubin values were observed in mid and high dose male rats (table 3).
Organ weights: Absolute and relative heart, liver and kidney weights were increased in high dose females. Liver weights were also increased in high dose males. Relative brain weights were decreased in high dose females (tables 6 and 7).
Gross pathology: No drug-related findings.
Histopathology:
Heart-Minimal to slight focal fibrosis of the ventricular myocardium was observed in 3/10 high dose female rats. The atria were unaffected. There were no dose-related mycocardial lesions in males (1 MD male with myocardial necrosis).
Thyroid- colloidal vacuolization was increased in treated males (5/8/5/10 in C, LD, MD, HD)
Follicular cell hypertrophy was increased in treated females (2/2/4/8 in C, LD, MD, HD)

Toxicokinetics: See table 8. Drug concentrations (Cmax) and exposures (AUC) increased greater than dose proportionally. Drug exposures in females rats were significantly greater than in males, presumably because of the sexual dimorphism in CYP 3A4 in rats. This enzyme is known to be primarily responsible for the biotransformation of BAY 38-9456. The AUC_{0-21} for parent drug in young men receiving the highest proposed therapeutic dose of 40 mg = 134 ng.hr/mi. Therefore, male rats in this study were exposed to approximately 1, 9 and 70 times the highest therapeutic exposure. The relative total drug exposures are unknown since the M1 metabolite was not quantified.

2. **13-Week Oral Toxicity in Rats (only study summary provided)**
   Wistar rats (10/sex/dose) were administered 0, 1, 5, 25 and 125 mg/kg/day BAY 38-9456 orally by gavage , daily for 13 weeks.
   Mortality: 2 HD males and 3 HD females. The sponsor states deaths are drug-related but histopathology were not provided to distinguish the cause of death (gavage accidents vs cardiovascular collapse). Death attributable to cardiovascular collapse does not seem likely since the sponsor states there were no clinical signs (tremor, prostration, seizure, labored breathing). Based on the minimal to mild severity of the myocardial lesions in HD females treated with 100 mg/kg/day for 4 weeks the cardiac lesions would not be expected to be fatal. The only histopathology noted in the study was myocardial necrosis in 0/0/1/9 females and 0/0/0/2 males. It is not stated whether the males with myocardial necrosis were the premature decedents. The timing of the deaths during the study was not described.
   Signs: none
   Body weight/food consumption (tables 1 and 3): unremarkable
   Water consumption (table 4): increased 12% in HD males and 40% in HD females
   Hematology (tables 5 and 6): hemoglobin and hematocrit were mildly increased in high dose females during weeks 5 and 13 . High dose females had significantly increased (2-fold) white blood cell counts (neutrophils, lymphocytes, monocytes, basophils, leukocytes).
   Blood chemistry (tables 7 and 8): BUN increased (25-40%) in high dose rats of both sexes. Phosphorus was increased (20-40%) in high dose rats of both sexes. The increased phosphorus levels were still observed after a 4 week recovery period.
   Organ weights (tables 14 and 15): Absolute and relative heart and liver weights were increased in high dose female rats.
   Histopathology: Data were not provided. The only pathology discussed was the observation of mycardial necrosis as described above . 0/0/1/9 females; 0/0/0/2 males (C/LD/MD/HD) .
   Toxicokinetics (tables 16, 17 and 18): Plasma concentrations of BAY 38-9456 were measured 30 minutes (Tmax) and 24 hours after dosing on study days 1 and 98. The 0.5 hour measurement probably provides a good approximation of Cmax . AUC data were not provided. Data are not available regarding the toxicokinetics of the M1 metabolite in rats. The Cmax values for the 100 mg/kg/day dose on day 28 and the 125 mg/kg/day dose on day 98 are very comparable.

Comparison of Cmax values from 4 and 13 Week Toxicity Studies in Rats

Dose, mg/kg	Day 28, Males	Day 28, Females	Day 98/Males	Day 98/Females
1	N/A	N/A	5	97
5/6*	111	656	139	784
25	849	5395	1038	5130
100/125*	5560	11714	5544	12322

* Day 28 values for 6, 25, and 100 mg/kg, day 98 values for 1, 5, 25, and 125 mg/kg.

Summary and Conclusions: Based on the presence of mortality in high dose animals the sponsor concluded that 125 mg/kg/day exceeds the MTD. Without the histopathology data it is impossible to confirm that the cause of death but the fact that they were all in the high dose group supports a compound-related etiology. The sponsor has selected doses of 3, 15 and 75 mg/kg/day orally by gavage for the rat carcinogenicity study. These doses can be justified based upon adequate multiples of human AUC. The AUC data for parent drug from the 28 day study are summarized in the table below.

AUC_{0-7} (ng.hr/ml) of BAY 38-9456 in Wistar Rats on Day 28

Species/sex	6 mg/kg	25 mg/kg	100 mg/kg	75 mg/kg*	Multiple of Human AUC_{0-21}
Rat, Male	160	1511	19516	4500 (?)	1, 10, 130 X
Rat, Female	1621	12362	44834		10, 82, 300 X

* AUC value for male rats receiving 75 mg/kg/day was extrapolated by the sponsor. This value looks like an underestimate since 4-fold increases in dose produced 10-fold increases in AUC in male rats. A more appropriate estimate would appear to be an AUC of 15000 ng.hr/ml in male rats for a 75 mg/kg/day dose.

I do not agree with the sponsors choice of doses for the following reasons. First, in the 3 month dose finding study we had drug-related deaths in 2/10 HD males and 3/10 HD females. Although we do not have AUC data for the 3 month study, the plasma concentration data (see Cmax data above) suggest that the exposures obtained in the 1 month study with 100mg/kg were roughly comparable to those in the 3 month study with 125 mg/kg. This would be consistent with liver enzyme induction by BAY 38-9456.
Myocardial necrosis was observed in 3/10 HD females (100 mkd) in the one month study. In the 3 month study, myocardial necrosis was observed in 1 MD, 9 HD females and 2 HD males. Therefore, with longer duration of dosing we begin to observe myocardial lesions at lower AUC exposures of 12,000 – 20, 000 ng.hr/ml and Cmax values of > 5000 ng/ml (achieved in HD males and MD females). Since the rat studies suggest a lowering of the threshold dose for myocardial damage with increasing duration of dosing, I would expect that 75 mg/kg/day will exceed the MTD in both sexes in a two year study.

Recommendations:

1. **Male rats should receive doses of 6, 20 and 60 mg/kg/day.** This should produce AUC exposures to parent drug which are multiples of 1, 8-10, and 50 times the human AUC with the highest therapeutic dose of 40 mg. It is possible that the 60 mg/kg/day dose may still prove too high,but the mid-dose will then represent a 10-fold multiple of human exposures. The 50 times multiple will also provide a cushion if there are significant differences in drug metabolism in women or the elderly, or if the total exposures (parent + M1) vary from AUC comparisons for parent only.

2. **Due to the demonstrated sex differences in metabolism and drug exposures the doses should be significantly reduced for female rats. I would recommend female rats receive doses of 2, 6 and 20 mg/kg/day.** These doses should provide AUC exposures to parent drug which are exposure multiples of 1-2, 10-11, and > 60 times the human AUC with 40 mg.

3. Cmax and AUC values for the parent compound and M1 metabolite should be provided for all dose levels during weeks 1, 52 and 104. It is also recommended that this data be obtained from groups of satellite animals for the 1 and 52 week sampling times.

cc: IND \HFD-580 IND
HFD-580/ Colangelo/ElHage/Jordan
— #2

IND. —

AUG 21 2000

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: BAY 38-9456, Erectile dysfunction, Phosphodiesterase Inhibitor
Reviewer Name: Jeri El-Hage, Ph.D./ Karen Davis-Bruno Ph.D.
Division Name: Reproductive and Urologic Drug Products
HFD#: 580
Review Completion Date: 8/10/00

Review number: 3
IND:
Serial number/date/type of submission: # 018, 5-25-99 (6 vol.); #025, 7-15-99 (4 vol.) ; # 033, 9-20-99 (1 vol.) ; #035 (4 vol.) ; #046, 2-17-00 (3 vol.); #048, 3-2-00 (1 vol.)
Information to sponsor: Yes (X) No ( )
Sponsor (or agent): Bayer Pharmaceutical Division, West Haven, CT 06516
Manufacturer for drug substance: Bayer AG, Wuppertal, Germany

Drug: Code Name: BAY 38-9456
Chemical Name:

Molecular Formula: Molecular Weight:

Relevant INDs/NDAs/DMFs: NDA 20-895 Viagra;
IND for IC 351; IND for BAY 38-9456;
IND for BMS-341400 all PDE V Inhibitors.
Drug Class: Phosphodiesterase type V inhibitor

Indication: Erectile dysfunction
Route of administration: Oral
Introduction and drug history: BAY 38-9456 has been administered to >600 humans in single and multiple dose studies. In repeated doses at 40 mg, one case of LFT elevation (serious adverse event) was observed in a patient with a maximum AST of 142, four days after disconinuation followed by return to normal. On Day 14, given 80 mg/day some patients developed severe myalgias and back pain attributed to study medication. Currently study doses up to 20 mg for 6 months are being tested in healthy volunteers to elderly males with BPH.

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Studies reviewed within this submission:
Serial # 018
1. 13 Week Oral Toxicity in Wistar Rats (Study T5067057)
2. 13-Week Oral Toxicity Study in Beagle Dogs (study PH-28660)
3. Chromosomal Aberration Assay (HPRT) in V79 Cells (study T3059810)
4. Toxicokinetics for 3 Month Rat Study (T5067057)
5. Toxicokinetics for 3 Month Dog Study (T5064221)
6. PK of Parent Drug/Radioactivity in Female Dogs (Studies 8001106, 0001135, 1001154)
7. ADME of Radioactivity After Single Administration in Wistar Rats (study28563)
8. In vitro Metabolism of BAY 38-9456 in Dog, Human , Monkey, Mouse , Rabbit and Rat Liver Microsomes (PH-28510)
9. Identification of CYP Isozymes Involved in the In vitro Metabolism of BAY 38-9456

Serial # 025
1. Developmental Toxicity in Rabbits (study PH 28625)

2. Single Dose Pharmacokinetics of BAY 38-9456 in Mice (study P 5011887)
3. Plasma PK BAY 38-9456 & BAY 44-5576 Via Oral Dosing Rat (14 Wk) (PH28519)
4. Plasma Concentrations in Mice After Single Oral Dose BAY 38-9456 (PH28657)

Serial # 033
1. Fertility and Early Embryonic Development in Rats (study T2060592)
2. Developmental Toxicity Raf (T2061375)

Serial # 035
1. Plasma and Blood Binding Invitro (PH-28322)
2. Autoradiography in rats after single iv and po administration (PH-28710)
3. Distribution to Tissues of Male Rats after single oral administration (PH-28903)
4. Pharmacokinetics of Metabolite M1 (BAY 44-5576) in Female Dogs (PH-28912)
5. Toxicokinetics of BAY 38-9456 and M1 in Pregnant Rats (PH-28960)
6. Developmental Toxicity Study in Rats (PH-29093)
7. Toxicokinetics of BAY 38-9456 and M1 in Pregnant Rabbits (PH- 29153)

Serial # 046
1. 6-Month Oral Toxicity in Wistar Rats (study PH-29532)
2. 12-Month Oral Toxicity in Beagle Dogs (study T3067488)
3. Effects of BAY 38-9456 and Metabolites on human CYP 450 Isoforms (study PH-29340)
4. Plasma Protein Binding of BAY 44-5576 (M1 Metabolite) (study PH-29430)
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6. ——— of BAY 38-9456 & M1 in Species (PH29249)
7. Inhibition of CYP Isoforms by M1 and M4 (PH-29340)
8. In vitro Plasma Binding (PH-29430)
9. Whole Body Autoradiography in Pregnant Rat IV/PO Dose (PH-29438)

Studies not reviewed within this submission: Radiosynthesis of labeled reference compounds for metabolites M1, M5, M6, M7 (PH-28493).

PHARMACOLOGY: see review #1

PHARMACOKINETICS/TOXICOKINETICS:

Comment: All PK/TK data are presented by the sponsor as geometric means. In most cases this does not change the mean value significantly, however, this calculation significantly reduces the standard deviations making it impossible to assess individual variability. (Plasma drug concentrations vary up to 10-fold for individual animals within a given dose group). The sponsor will be advised to present data in the conventional manner as arithmetic means in future submission and in the NDA.

Toxicokinetic studies are included in the Toxicology Section:

14-Week Oral Rat (T5067057) see pg. 10
3- Month Oral Dog (T5064221) see pg. 14
27-Week Rat (PH29532) see pg. 17
52-Week Dog (T3067488) see pg. 20

Single Oral PK Male Mice (PH-28657)

	BAY 38-9456			BAY 44-5576		
	1 mg/kg	3 mg/kg	10 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
AUC (µg h/l)	Not calculated	44.8	409	Not calculated	Not calculated	176
Cmax (µg/l)	9.31	71.8	520	3.05	21.6	148
Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5

The sponsor notes that the AUCs are a "rough estimate only" since the plasma concentrations at 7 and 24h after administration were <LOQ µg/l).

PK of [^{14}C] Bay 38-9456 in Female Beagle Dogs (studies I 8001106, I0001135, and I001154)

Female beagle dogs (n = 3) were administered single intravenous infusions of 0.3 and 1 mg/kg and oral doses of 0.3, 1 and 3 mg/kg [14C] BAY 38-9456. Pharmacokinetics parameters of parent drug and radioactivity are presented in the table below. Total plasma clearance = 2.6 L/hr.kg and the volume of distribution was 5.2 L /kg. The AUC of radioactivity was 3-8 fold higher than the level of parent drug. The terminal elimination half-life of radioactivity was 49 hours after iv dosing and 40 hours after oral dosing. The free fraction of [14C]-BAY 2 hours postdose was 14-19%. The AUC exposures to radioactivity were 3 – 9 times the parent compound.

Pharmacokinetics of Metabolite M1 in Female Beagle Dogs (PH-28912)

Female beagle dogs were administered 1 mg/kg, iv as a 15 minute infusion or 3 mg/kg, po and pharmacokinetics parameters of BAY 44-5576 (M1) were calculated. Data are summarized in the table below with results of studies above.

	BAY 38-9456		BAY 44-5576		Radioactivity (ng.eq)	
Parameter	1mg/kg, iv	1 mg/kg, po	1 mg iv	3 mg po	1 mg/kg, iv	1 mg/kg, po
Cmax, ng/ml	325	32		59	422	66-162
Tmax, hours	1.1 – 1.5	1.1		1.3	1.1	1.1
AUC $_{0-24}$, ng.hr/ml	390	102	421	356	1211	615-901
Half-life, hours	1.75	1.7	3.6	3.7	49.4	40
Clearance, L/hr.kg	2.6		2.4		n.c.	
V_D, L/kg	5.24		8.5		n.c.	
Oral bioavailability		28%		30%		76%
Biliary/fecal excretion					95%	94%
Urinary excretion					5%	4%

n.c.- not calculated

Absorption, Plasma Concentrations, and Excretion of Substance –Associated Radioactivity in Wistar Rats After Single Oral or Intravenous Administrations (study PH 28563):

Male rats were administered single oral or iv doses of 1 mg/kg 14C-BAY 38-9456.
PK data are summarized in the table below. Excretion of radioactivity was 3% urinary and 97% fecal regardless of dose route(iv and po).

Pharmacokinetics of Radioactivity

PK Parameter	1 mg/kg, po	1 mg/kg, iv
Cmax, ng.eq/ml	100	
AUC ng.eq.h/ml	240	2,300
T ½, hrs	7.5	6.6
Tmax, minutes	10-20	

Plasma PK of BAY 38-9456 and BAY 44-5576(M1) in Rats Administered via the Drinking Water (PH-28519)

BAY 38-9456 was orally administered to Wistar rats at doses of 0, 8, 40 and 200 mg/kg via the drinking water for 14 weeks. AUC exposures to parent drug and M1 increased over-proportionally with increasing dose.
Accumulation of parent drug was observed with multiple dosing in males (3-fold) and females (2-fold). Blood samples were collected every 3 hours on day 22/23 for determination of PK parameters. The data are summarized in the table below.
Kinetics data were not provided for the M1 metabolite.

Kinetics of BAY 38-9456 in Rats After Oral Dosing via the Drinking Water

PK Parameter	8 mg/kg/day		40 mg/kg/day		200 mg/kg/day	
	Male	Female	Male	Female	Male	Female
AUC_{0-21} ng.hr/ml	n.c.	935	816	5,463	11,830	61,743
Cmax, ng/ml	29.8	98.4	88	636	1591	6614
Tmax, hours	12	21	21	15		15

n.c. = not calculated

	BAY 38-9456 (µg/l)				BAY 44-5576 (µg/l)	
	Day 5		Day 89		Day 89	
Dose (mg/kg)	Male	Female	Male	Female	Male	Female
8	4.12	94.5	6.06	59.9*	10.6	18.2
40	110	662	188	527*	173	183
200	815	1923	2802*	4113	1551	1406

Exposure to BAY 38-9456 was lower in males than females while M1 was similar. Accumulation ratios of BAY 38-9456 was 147-344% in males and 63-214% females.

Toxicokinetics of BAY 38-9456 and 44-5576 in Pregnant Rats (PH-28960)

Toxicokinetics parameters were calculated from plasma samples collected from pregnant rats in the embryotoxicity study T2061375. Rats were dosed from day 6 – 17 postcoitum with 0, 3, 18 and 100 mg/kg/day. Blood samples were collected on day 16 at 0.5, 2, 7, and 24 hours after dosing. Data (geometric means) are summarized in the table below taken directly from the submission (vol 15.1, p.170).

Plasma Concentrations 0.5 hours Postdose (Cmax)

Dose of 38-9456, mg/kg	Parameter	Maternal Plasma, Ng/ml	Fetal tissue Ng/g	Ratio (%) [Fetal]/[Maternal plasma]
3	38-9456(parent)	510	52	10%
18		2307	456	20%
100		4387	4281	98%
3	44-5576 (M1)	75	3	4%
18		344	36	10%
100		571	548	96%

Maternal AUC (0-24)

Dose of 38-9456, mg/kg	Parameter	Ng.hr/ml
3	38-9456(parent)	1716
18		9501
100		58713
3	44-5576 (M1)	313
18		3171
100		14726

Protein Binding: *In vitro* protein binding of ^{14}C-BAY 38-9456 to plasma of various species was investigated via ultrafiltration. Drug concentrations of 0.1, 1, and 10 ug/mL were evaluated and binding remained constant over the concentration range. Data are summarized below. Drug was extensively bound in all species and binding was comparable with 5-7% free drug in all species except dogs. Binding was less extensive in dogs with 12-13% of drug unbound.

Species	Gender	Free Fraction BAY 38-9456
CD 1 mouse	Male	6 – 7 %
NMRJ Mouse	Male	6 - 7 %
Rat	Male Female	5 – 6% 5%
Rabbit	Female	3 – 4%
Dog	Male Female	12 – 13% 12 – 13%
Human	Male Female	5 – 7% 5%

Distribution:

Whole body autoradiography in rats (PH-28710)

Wistar rats were administered single doses of ^{14}C-BAY 38-9456 orally (3 mg/kg) or intravenously (1 mg/kg) and killed at selected times up to 7 days postdose. Drug was also administered to one male pigmented rat killed 24 hours postdose. Drug was concentrated and retained in pigmented tissues such as the eye and meninges in the hooded rat.

IV dosing with 5 minute and 2 hour sacrifices:

At 5 minutes, high blood concentrations and heterogeneous distribution within organs indicated drug and metabolites were still in distribution phase. The same pattern of distribution was observed after iv and po dosing as described below.

At 2 hours post-dose, iv and 2, 4, 8 hours po:

Bile ducts and small intestine (extra high) > Harderian gland, kidney, liver, spleen, pharyngeal and large intestinal mucosa (high) > adrenal, aorta, bone marrow, brown adipose, choroid plexus, piutitary, lung, lymph nodes, pancreas, prostate, salivary glands, skeletal muscle, thymus (medium) > BLOOD, cartilage, meninges, lens, skin, spinal nerves, testes, white adipose (low) . > brain, spinal cord.

At 24 hours after oral dosing: large intestine, bile ducts (high) > pituitary, liver, preputial gland (medium) > adrenal, brown adipose, lens, submandibular gland, kidney, lung, spleen, stomach, intestinal mucosa, testes(low) > blood, brain, bone, muscle, pancreas, prostate, skin (background levels).

At 48, 72, and 168 hours post dose: levels were still high in bile ducts, large intestine > medium in pituitary, liver, small intestine> low in adrenals, brown adipose, lens, submandibular gland, kidney, lung, spleen, mucosa , stomach and testes.

Whole Body Autoradiography of ^{14}C-BAY 38-9456 in Pregnant Rat After Single IV/PO (PH-29438)

Pregnant albino Wistar rats were dosed Gestation Day 19 at 1 mg/kg and sacrificed up to 24 h post dose. The highest radioactivity was in GI, bile ducts, bladder> adrenal cortex, liver, lacrimal glands> salivary gland, kidney, spleen, lung> adrenal medulla, heart, skeletal muscle, brown adipose, thyroid, aorta, hypophysis, pineal, mammary, amnion, placentas, ovaries. There was low/no detectable radioactivity in maternal brain, spine, amniotic fluid, cartilage, bone and most fetal organs. Penetration across maternal/fetal BBB was low. Penetration of the placenta was low with concentrations analogous to maternal organs (except cartilage). Maximum fetal concentrations were observed in the GI, liver, lungs, heart, adrenal cortex, eye, skin and cartilage. Low fetal levels were observed in kidney, brown adipose, skeletal muscle, blood, brain and spine. No residual radioactivity was found in fetuses one day post oral dosing. The relatively high radioactivity in mammary gland suggests secretion of drug/metabolites into milk.

Tissue Distribution of ^{14}C-BAY38-9456 in Male Wistar Rats (PH-28903)

Male Wistar rats administered 1 mg ^{14}C-38-9456, po and tissue concentrations of radioactivity were measured. Data are summarized in the table below. Concentrations of radioactivity were greater than plasma for most tissues (see organ /plasma ratios). Concentrations in liver were 43 times plasma and concentrations in spleen, kidneys, adipose, adrenals and lungs were 5 – 10 times higher than plasma. Tissue drug half-lives were less than or equal to plasma half-life for most tissues with the notable exceptions of liver, kidney and testes.

Tissue Concentrations of Radioactivity in Male Wistar Rats

Tissue	AUC_{0-24}, mg.eq.h/kg	T ½, hours	Organ Ratio Plasma
Plasma	0.2	6.7	1.0
Blood	0.14	5.4	0.7
Spleen	1.3	2.9	4.5
Liver	5.88	51	43
Kidneys	1.65	71	9.8
Adipose (renal)	2.74	1.3	6.3
Adrenal	1.58	1.7	10
Testes	0.173	33	0.2
Epididymes	0.4	4.7	0.5
Skeletal muscle	0.2	1.9	1
Bone	0.2	1.9	1
Heart	0.36	4.3	2.3
Lungs	1.59	3.3	6.5
Brain	0.02	1.5	0.1
Thyroid	0.6	2.5	3.4
Submandibular gland	0.5	4.0	3.0
Preputial gland	0.7	5.9	2.2
Harderian gland	3.2	7.5	4.9
Eyes	0.15	2.3	0.6
Aorta	0.5	2.3	2.8
Bone marrow	0.7	1.6	4.0
Skin	0.3	15.6	0.8

Note: Organ/plasma ratios were calculated based on Cmax tissues/Cmax plasma and do not correct for differences in tissue half-lives.

Plasma Binding of ^{14}C-BAY 44-5576 (M1) In Vitro in Rat, Dog, Mouse, Rabbit, Man (PH-29430)

Radiolabled parent was stable in plasma for at least 0.5 h. Gender differences were not observed. The sponsor suggests that pH differences may influence the species differences observed since this has been observed previously with the parent, BAY 38-9456. Increased pH results in decreased free fractions. Serum albumin contributes to the total binding but not exclusively (free fraction 25%). α_1-acid glycoprotein is not involved (unbound fraction = 63%).

Plasma Species	Fraction Unbound (%)	Fraction Bound (Calculated %)
Dog	15	85
Rabbit	11	89
Mouse	10	90
Rat	7-9	91-93
Human	4-6	94-96

Metabolism:

<u>*In vitro* Comparison of Phase I Metabolism in Dogs, Human, Monkey, Rabbit, and Rat Liver Microsomes (PH 28510)</u> Bay 38-9456 is metabolized by cytochrome P-450 of all species with the primary metabolite in all species being the M-1 desmethyl derivative. Species comparison of major metabolites formed in incubations with liver microsomes

Metabolite	Human	Wistar Rat	Beagle Dog	Rabbit	CD1 mouse	Rhesus Monkey
M-8	12.75	1.8	5.2	5.4	6.5	4.0
M-5	16	7.8	6.5	2.5	16	9.2
M-4	9.1	1.4	4.0	3.4	6.0	2.9
M-7	8.8	3.1	1.2	1	6.1	7.5
M-2	4.6	2.4	20.9	3.6	9.1	3.4
M-1	23.7	53.4	25.9	30.8	13.1	31.6
M-9	3.0	---	2.5	0.8	2.9	2.6
Parent	8.0	19.1	24.6	49.0	14.3	23.3

<u>Identification of Human CYP Isozymes Involved in In Vitro Metabolism (PH-28562)</u>

CYP 3A4/5 were identified as the decisive enzymes for the metabolism of BAY 38-9456 in man. Formation of the M-1 and M-4 metabolites correlated exclusively with CYP 3A4/5 activity. CYP 2C9 contributed to a minor extent to the formation of M-1.

<u>Inhibition of Human CYP Isozymes By BAY 38-9456, M1 (BAY 44-5576) and M4 (BAY 44-5578)</u> Formation of both M1 and M4 are catalyzed by CYP3A4. Therefore Ki values toward CYP3A4 were 1.4 µM and 20.6 µM for M1 and M4 respectively. M1 also inhibited the turnover of standard substrates for CYP2C9 and 2D6 with Ki values of 80 and 25 µM respectively and M4 had similar Ki values of 45 µM for each isoform. M4 inhibits CYP2C19 with a Ki=10 µM. A 80 mg BAY 38-9456 oral dose to healthy humans resulted in Cmax for M1 and M4 of 0.06 µM and 0.03 µM respectively. Since the Ki =1.4 µM for M1 and CYP3A there is a potential for interactions drug-drug interactions with this isoform.

<u>BAY 38-9456 and M1 (BAY 44-5576) Determination in Plasma of Rat, Mice, Dog By</u> — This method (M1107) has an extraction efficiency of 60-70% and 70-80% for M1 and BAY 38-9456 respectively. The LOQ was — µg/l with a linear range of — µg/l for both analytes by injection of 0.020 ml of crude plasma from rats, mice and dog. The precision of the assay was — % for parent and — % for M1 with an accuracy of — % for BAY 38-9456 and — for M1 irrespective of species. The advantage of this method is the speed since sample preparation involving protein precipitation can be avoided. The sponsor cross validated this method with the — assay (M1099) previously used with real study samples, yielding similar accuracy and precision between assays.

TOXICOLOGY: Summaries of the 14-week rat and mouse subchronic toxicity studies were submitted to support the dose selections for the rodent carcinogenicity studies (see review #2). The final reports for the 14-week rat and dog studies are reviewed below. Data from the 14-week mouse study is still pending.

<u>Subchronic (14-Week) Oral Toxicity in Wistar Rats</u>

Study No: T5067057
Amendment # 018, Vol #1, and page #1:
Conducting laboratory and location: Institute of Toxicology, Bayer AG, Wuppertal, Germany
Date of study initiation: June 26, 1998
GLP compliance: Yes
QA- Report Yes (X) No ()

Methods:
Dosing:
- species/strain: Wistar rats
- #/sex/group or time point: 10/sex/dose
- age: 5-6 weeks at study initiation
- weight: males: 178-218g; females 131-166 g
- satellite groups used for toxicokinetics or recovery: 10/sex/dose treated with 0 or 125 mg/kg/day for 14 weeks with a 4 week treatment free recovery period.
- 5/sex/dose treated for 14 weeks for toxicokinetics evaluations.
- dosage groups in administered units: 0, 1, 5, 25, and 125 mg/kg/day
- route, form, volume,: gavage in 0.5% Tylose

Drug: lot# 503836 % purity (base): 83%
Formulation/vehicle: formulations in 0.5% Tylose stable for 11 days. Drug was quantified for analysis by —— —— (1.3 – 130.5 ug/ml concentration range). Dose validation was performed during study months 1, 2, and 3. Concentrations of dosing solutions were within 4% of nominal dose.
Basis for dose selections: 4- Week subacute study with 0, 6, 25 and 100 mg/kg/day in 10/sex/dose. Myocardial fibrosis was observed in 3/10 HD females. Thyroid follicular cell hypertrophy was observed in HD males and females. Mild increases in liver weights and liver enzymes observed in HD rats. . NOAEL = 25 mkd.
Observations and times:
- Clinical signs: twice daily weekdays, once on weekends
- Body weights: weekly
- Food /water consumption: weekly
- Ophthalmoscopy: pretreatment, termination, end of recovery for control and high dose animals only
- EKG: not performed
- Hematology: weeks 6, 14 (main) and 18 (recovery)
- Clinical chemistry: weeks 6, 14 (main) and 18 (recovery). Also evaluated T3, T4, TSH
- Urinalysis: weeks 6, 14 (main) and 18 (recovery)
- Organs weighed: brain, heart, liver, spleen, thymus, kidneys, adrenals, ovaries and testes
- Gross pathology /Histopathology: see table
- Toxicokinetics: days ½ and 98/99 , blood collected by retro-orbital sampling 0, 5, and 24 hours post-dose
- Other: Liver enzymes were quantified in 5 rats/sex/dose as follows: aminopyrine-N-demethylase, p-nitroanisole-0-demethylase,
- p 450 monooxygenases : 7-ethoxycoumarin-deethylase (ECOD), 7-ethoxyresorufin-deethylase (EROD), aldrin-epoxidase (ALD)
- Phase II enzymes: epoxide hydrolase (EH), glutathione S-transferase(GS-T), UDP-glucuronyl-transferase (GLU-T).

Results:
- Mortality: Four drug-related deaths at 125 mg/kg/day – 1M # 48 and 3 F # 91, 93, 136 secondary to myocardial lesions . Two LD females (#76, 77) died during blood collection . One HD M (#116) died due to gavage accident.
- Clinical signs: Sponsor states no clinical signs but data not provided.
- Body weights: There were no significant drug-related effects on body weight or body weight gain in either sex

Final Body weights (g) Week 14

Sex	Controls	1 mg/kg	5 mg/kg	25 mg/kg	125 mg/kg
Males	408	360	378	390	364 (↓ 10%)
Females	226	230	227	216	217

- Food consumption: unremarkable
- Water consumption: High dose males drank 13% and females 41% more water than controls (no effect with lower doses).
- Ophthalmoscopy: No drug-related findings.
- Electrocardiography: not performed
- Hematology: No significant changes with doses ≤ 25 mg/kg.
- RBC parameters- Hemoglobin , hematocrit and mean corpuscular hemoglobin (MCH) were mildly increased (5%) in HD males and females during weeks 6 and 14.

- WBC parameters- Leukocyte, neutrophil, lymphocyte, basophil and atypical WBC counts were increased (2-fold) in high dose females during week 14. Elevations in these parameters were also observed in HD females during week 6 but were not statistically significant. Changes in RBC and WBC parameters observed in HD females were reversed after a 4- week drug free recovery.
- **Clinical chemistry**:
- Liver enzymes: Mild (20%) decreases in ASAT, ALAT, and alkaline phosphatase were observed in high dose (125 mg/kg) rats of both sexes during week 6. More severe decreases (50%) in LDH were observed in HD animals during week 6. Decreases were no longer significant by week 14.
- Urea levels were increased in HD rats of both sexes weeks 6 and 14 (6.5-7mmol/L at baseline, to 8 mmol/L at week 6, to 9 mmol/L at week 14.)
- Thyroid hormones: T3 mildly increased (20%) during week 6 in rats of both sexes dosed with ≥25 mg/kg/day. No changes in T4 or TSH.
  Chloride –decreased from 102 to 97 mmol/L in HD rats of both sexes week 14.
- Inorganic Phosphate increased in HD rats of both sexes , 25% at week 6 and 45-48% at week 14, not entirely reversed at the end of a 4- week recovery period.

- **Urinalysis**: Urine volume increased (50%) in HD rats of both sexes most probably secondary to increased water consumption..

- **Organ Weights**:
- Adrenal – increased absolute and relative wts (20 %) in HD males.
  Kidney – increased relative weights (13-14%) in HD males and MD, HD females, still present after recovery period.
  Testes- relative weights increased 19% in HD males.
  Heart – absolute and relative weights were increased (32%) in HD female rats.
  Wts were increased 13% in HD females after recovery.
  Liver – increased relative weights in HD males (125%) and MD, HD females (14% in MD, 44% in HD). After the recovery period, relative liver weights were increased 15% in HD males and 13% in HD females indicating some reversibility.

Histopathology: Findings are presented as number of animals affected (n=10/s/d)

Tissue/ Finding	Control		1		5		25		125	
	M	F	M	F	M	F	M	F	M	F
Heart, Myocardial fibrosis								1	2	9
Mononuclear infiltration									3	
Recovery – myocardial fibrosis									2	8
Parotid glands, acinar hypertrophy	1					1	2	8	10	10
Submandibular glands acinar hypertrophy								4	10	10
Pancreas, acinar atrophy			2		1				5	
Mononuclear cell infiltration	1				3		2		6	
Interstitial fibrosis Main study									6	
Recovery									6 R	
Brown pigment (hemorrhage)	1	1	1	2	2	3	1	2	3 8R	
Periinsular acinar hypertrophy	1		1		3		1		5	8
Eyes Retinal atrophy									1	1
Degeneration/optic nerve									1	1
Testes Degeneration/germinal cells									1	
Tubular atrophy							1			

Lesions of myocardial fibrosis were located in the septum and left ventricle. Lesions were of minimal severity in affected males and mild to moderate severity in HD females.
Acinar hyperplasia in the salivary glands was of minimal severity in controls and mild to moderate severity in BAY treated rats.
Findings in the pancreas were graded minimal except in HD males that displayed mild findings.
Retinal atrophy and optic nerve degeneration were observed in 3 HD animals (1 M with retinal atrophy, 1 M with optic nerve degeneration, 1 F with both findings). The retinal degeneration was described as atrophy of the second and third neuron with multifocal thinning or missing tissue.

The pathology report suggests only the tissues from affected organs (heart, salivary glands, pancreas, kidney, thyroid) were examined in recovery animals.

- Toxicokinetics:. Plasma samples were collected at 0.5 and 24 hours after administration on day 1 and 98/99. Plasma concentrations of parent and BAY 44-5576 (major active metabolite) were measured by [redacted] Data are presented in the table below taken directly from the submission.

Mean Plasma Concentrations of BAY 38-9456 (ng/mL) in Wistar Rats (n=5)

Dose, mg/kg	Day 1, 0.5 hr[a]	Day 1, 24 hrs	Day 98, 0.5 hr	Day 98, 24 hrs
Males 0				4.3 (?!)[c]
1	8	1.3	5	BLQ
5	95	1.3	139	3.4
25	907	BLQ	1038	3.5
125	3235	1.3	5544	2.8
Females 0				3.6[c]
1	60	BLQ	97	4.7
5	435	0.9	784	BLQ
25	3289	2.6	5130	1.8
125	10708	893 (?)	12322	5.8

a. Time postdose
b. BLQ –below limit of quantification ([redacted] ng/ml)
c. BAY 38-9456 was measurable in 5/5 control rats on day 98 with concentrations greater than any of the treated group males. NOTE: BAY 38-9456 was measured in samples from control rats as follows: 2/20 samples day 1 (1M, 1F), 3/10 samples day 2 (3/5 M), 10/10 samples on day 99. The sponsor explains the findings as contamination of samples.

Plasma Concentrations at 24 h

	BAY 44-5576 (ng/ml)			
	@0.5 h		@ 24 h	
Dose, mg/kg	Day 98 Male	Day 98 Female	Day 98 Male	Day 98 Female
0			< 1	< 1
1	11	3	< 1	< 1
5	141	187	< 1	< 1
25	1233	1113	1	1
125	4381	2425	7.7	1.6

Exposures to parent drug were higher (3 – 20X) in females than in males receiving comparable doses. Although not directly demonstrated for BAY, the compound is most probably metabolized by 3A4 which displays a sexual dimorphism in rats. (Viagra and IC 351 both are metabolized primarily by 3A4 and also display sex dependent metabolism/exposures in rats).

A slight accumulation of BAY 38-9456 was observed (< 2-fold) with multiple dosing.

- Other: Liver metabolizing enzyme activity was measured . No changes in triglyceride or P450 content were measured. The activities of N and 0-demethylase were mildly increased (20%). Aldrin epoxidase was increased (50%) in HD females and epoxide hydrolase was increased (50%) in HD rats of both sexes.

Key Study Findings:

Drug-related deaths occurred in 4 high dose rats (125 mg/kg/day -1M, 3 F) secondary to myocardial lesions. BAY 38-9456 had no significant effects on clinical signs, body weight, food consumption, or ophthalmologic evaluations. Water consumption was increased significantly in high dose rats (13% in males, 41% in females). No changes in hematologic parameters were observed in low and mid dose rats (1, 5, and 25 mg/kg/day). High dose rats had mildly increased (5%) hemoglobin, hematocrit and mean corpuscular volume after 6 and 14 weeks of dosing. High dose females also had 2-fold increased in WBC parameters (leukocytes, lymphocytes, basophils, atypical WBC). Changes in RBC and WBC parameters reversed after the 4-week drug free recovery period. Decreases in liver enzymes (ALT, AST, AlkP, LDH) were observed in high dose rats during week 6 but not significant by week 14. Progressive increases in urea levels were observed in HD rats of both sexes . Inorganic phosphate levels increased in HD rats of both sexes (25% at 6 weeks, 45% at 14 weeks, not entirely reversed after 4-week recovery). Urine volume increased in HD rats of both sexes most probably secondary to increased water consumption. Adrenal, kidney, liver, and testes weights were increased in HD male rats. Kidney, heart, and liver weights were increased in HD female rats. Non-reversible myocardial fibrosis was observed in high dose rats (2M, 9F main study; 2M, 8F recovery) resulting in early deaths in 4 rats. Female rats were more severely affected since drug exposures were higher in females (Cmax for 125 mg/kg/d in males = Cmax for 25 mg/kg/day in females). Myocardial lesions were not observed with 25 mg/kg or lower in males and 5 mg/kg or lower in females (Cmax plasma concentrations < 1000 ng/ml; 16-40 times human Cmax with the highest proposed therapeutic dose of 20 mg). Acinar atrophy of the pancreas, parotid, and submandibular glands was observed with increased frequency at doses ≥ 25 mg/kg/day. Retinal atrophy and/or degeneration of the optic nerve were observed in 3 high dose rats (2M, 1F). Seminiferous tubule degeneration/atrophy were observed in 1 male at 25 and 1 M at 125 mg/kg/day. Testicular degeneration has been an observed toxicity with all other PDE V inhibitors, although observations have been primarily in the dog.

13-Week Oral Toxicity Study in Beagle Dogs

Study No: PH-28660
Amendment # 018, Vol 7.3 and page: 1
Conducting laboratory and location: Dept of Toxicology-Pharma, BAYER AG. Wuppertal. Germany
Date of study initiation: June 22, 1998
GLP compliance: Yes
QA- Report Yes (X) No ()

Methods:

Dosing:

- species/strain: beagle dog
- #/sex/group or time point: 4/sex/dose
- age: 20-24 weeks (5-6 months)
- weight: 6.8 – 10.1 kg
- satellite groups used for toxicokinetics or recovery: Additional groups of 2/sex were administered 0 or 30 mg/kg/day for 13 weeks and were observed for a 4-week drug free recovery period prior to termination.
- dosage groups in administered units: 0, 1, 3, 10, and 30 mg/kg/day
- route, form, volume: orally by gavage in 0.5% tylose
- Drug: BAY 38-94567 lot#: 503846 % purity: 83.9% free base

Formulation/vehicle: 0.5% tylose, 5 ml/kg
Basis for dose selections: 4 week dog study. .

Observations and times:

- Clinical signs: twice daily weekdays, once on weekends
- Body weights: weekly
- Food /water consumption: daily
- Ophthalmoscopy: week –3, 8, 13, 18
- EKG/blood pressure: week –2, day 3, weeks 2, 6, 13 predose and 2 hours postdose
- Hematology: weeks –3, -1, 2, 6, 13, and 17 (recovery only)
- Clinical chemistry: : weeks –3, -1, 2, 6, 13, and 17 (recovery only)
- Urinalysis: weeks –3, 3, 6, 13 and 17.
- Organs weighed: brain, heart, liver, lungs, spleen, adrenals, kidneys, pancreas, thyroid, pituitary, testes, prostate gland, uterus, thymus and ovaries.
- Gross pathology:
- Histopathology: see table below for tissues examined.
- Toxicokinetics: Samples were collected porior to dosing and 2 hours postdose on day 1 and during week 13 for measurement of BAY 38-9456 and the major active metabolite, BAY 44-5576.

Results:

- Mortality: One high dose female (recovery) was killed during week 7 after misdosing.
- Clinical signs: No drug-related effects on reflexes or body temperature.

Slight increase in frequency of mushy or liquid feces in dogs receiving BAY 38-9456 at concentrations ≥ 3 mg/kg/day.
Dose-dependent increase in the observation of reddened gums and eyes in BAY- treated dogs at doses ≥ 3 mg/kg/day.

- Body weight/Food consumption: unremarkable
- Ophthalmoscopy: Grey clouding/ discoloration of the cornea was observed in 2 treated males. 1 @ 3 mg/kg weeks 8 and 13; 1 @ 30 mg/kg week 13.

- Electrocardiography: Dose-dependent decreases in systolic, diastolic and mean blood pressures and increases in heart rate were observed at doses ≥ 10 mg/kg/day. Changes were mild to moderate in dogs dosed with 10 mg/kg/day and moderate to marked in dogs dosed with 30 mg/kg/day.

Percent Change from Control Values

Parameter	1 mg/kg/d	3 mg/kg/day	10 mg/kg/d	30 mg/kg/d
Systolic BP, % decrease			6 –17%	28 – 38%
Diastolic BP, % decrease			10 – 35%	37 – 44%
Heart Rate, % increase			21 – 41%	61 - 75%

Control mean SBP = 160- 180 mmHg, control mean DBP = 90-103 mmHg, control mean HR = 100- 140 bpm.

- Hematology: unremarkable
- Clinical chemistry: unremarkable
- Liver enzyme activity: BAY 38-9456 had no effect on N• or 0-demethylase activity , P450 or triglyceride concentrations.
- Urinalysis: drug treatment had no effect on urine volume or specific gravity.
- Significant blood in urine was observed more frequently in treated dogs. This finding was grade 1 (10 erythrocytes) in control dogs and low dose females but was graded 2 , 3, and 4 in other treated dogs (> 25, 80 and 250 erythrocytes , respectively). Affected dogs with grade of finding in parentheses are presented below. Overall incidence of dogs with significant (grade 3 or 4) blood in urine was 0 C, 2 LD, 2 LMD, 1 HMD, 4 HD.

Animals with Observations of Blood in Urine

Dose, mg/kg/day	N	Affected males	Affected females
0	6/sex	767 (2), 745 (1)	756 (1)
1	4/sex	757 (3), 775 (4)	724(3), 742 (1), 764 (1)
3	4/sex	747(1); 765, 769, 771 (2)	730 (3), 744 (4)
10	4/sex	759, 761 (2); 743 (3)	
30	6/sex	741, 749 (3); 729 (4)	732(4)

- Organ Weights:

Heart- mild (10- 15%) increase in absolute and relative heart weights at 10 and 30 mg/kg in males and 30 mg/kg in females.

Liver – slight (10%) increase in absolute and relative weights in males at ≥ 10 mg/kg and high dose females.

Thymus- slight decrease in absolute and relative weight in high dose males
(19, 20, 17, 18, 15.5 g in control, 1, 3, 10, 30 mg/kg males, respectively)

Uterus- increased absolute and relative weight at doses ≥ 10 mg/kg;
(3.8, 4.8, 4.8, 5.8, 5.6 g in control, 1, 3, 10, and 30 mg/kg, respectively).

- Gross pathology: no drug-related findings except in HD female with lung discoloration and foamy contents secondary to gavage error.

- **Histopathology:** It is not clear if all tissues were examined in recovery animals or only the hearts in which findings were acknowledge in main study animals. (data presented for heart only and no findings in any other tissues. Therefore, data are presented for main study animals only (n = 4/sex/dose).
- **Heart:** The sponsor acknowledges the observation of mild to moderate periarteritis and arteritis in 3 HD males and 1 HD females. Since this finding was observed only in dogs receiving the highest dose, it most probably represents effects secondary to the drug-induced hypotension and tachycardia observed with this high dose
- **Kidney:** The sponsor also acknowledged the observation of minimal to mild karyomegaly in the proximal tubular epithelium of six dogs (3M, 3F) . Bayer states the finding of karyomegaly is observed in their colony and is regarded as familial. However, observations were all in treated dogs (1 LD, 1 LMD, 2 HMD, 2 HD) and historical control incidences were not provided.
- Numerous other findings were observed with increased frequency in treated dogs and were not acknowledged. These findings include:
- **Liver:** A drug-related increase in cytoplasmic inclusions was observed (2 C, 3 LD, 4 LMD, 5, HMD, 5 HD). Leukostasis and mononuclear infiltration were also observed with increased frequency in drug-treated dogs, although no clear dose-relationship was apparent.

- **Gallbladder:** An increased frequency of vacuolization was observed in the gallbladder of male dogs.
- **Eyes:** An increased vacuolization of the lens was observed in drug-treated males
  (2, 3, 3, 4, 4 in C, LD, LMD, HMD, HD, respectively).
- **Aorta:** Intramural fiber disorganization was observed with increased frequency in drug treated dogs.
- **Mesenteric lymph nodes:** hemorrhage was observed more frequently in drug-treated dogs.
- **Pancreas:** Apoptotic bodies and pancreatic atrophy were observed more frequently in treated dogs. (Note: increased apoptosis in testicular germinal epithelium has been observed with IC 351).
- **Testes/Epididymes:** Focal degeneration of the testes and oligospermia and debris in the epididymes were observed with a dose -related increase in frequency and severity. This finding has also been observed in dogs treated with other PDE V inhibitors (sildenafil and IC 351). With other PDE V inhibitors, the finding is cummulative with NOELs decreasing with more chronic dosing.
- **Hemorrhage:** Focal hemorrhages were observed with increased frequency in tissues from treated dogs including the lungs, mesenteric lymph nodes, tonsils, spleen, and brain stem. These are the same target tissues for drug-related vasculitis observed with the other PDE V inhibitors (sildenafil, IC 351). **It is important to note that hemorrhages in the lymph nodes, spleen, and brain stem were observed at all dose levels, even with the two lowest doses which did not produce significant hemodynamic alterations . Thus, the focal hemorrhages can not be dismissed as secondary to monitorable hemodynamic alterations.**

Histopathology Findings in Dogs Killed at 13 Weeks (n = 4/s/dose)

Tissue/Finding	**Controls**		**1 mg/kg**		**3 mg/kg**		**10 mg/kg**		**30 mg/kg**	
	M	F	M	F	M	F	M	F	M	F
Liver										
Cytoplamic inclusions	2		1	2	2	2	3	2	2	3
Leukostasis					1		1		1	
Mononuclear infiltration			1	2	2	1		1		1
Gallbladder										
Vacuolization	1	2		1	3	2			3	2
Heart [a]										
Periarteritis/arteritis-(ventricular wall)									3	1
Mononuclear infiltration	1	3	4	1	1	2	2	2	3	1
Arterial intramural edema (right atrium)					1	1		2	1	3
Heart- papillary muscle										
Fibrosis						1				
Necrosis							1			
Aorta- intramural fiber disorganization			2	1	2	2	2		2	1
Eyes										
Vacuolization, lens[b]	2	2	3	3	3	2	4	3	4	1
Lungs- Inflammatory cell infiltration	1	1	2	1	3	2	1	3		1
Pneumonia/hemorrhage		1		1	1	1		1		3

Histopathology Findings in Dogs Killed at 13 Weeks (n = 4/s/dose) Continued

Tissue/Finding	Controls M	Controls F	1 mg/kg M	1 mg/kg F	3 mg/kg M	3 mg/kg F	10 mg/kg M	10 mg/kg F	30 mg/kg M	30 mg/kg F
Kidney										
Karyomegaly			1			1	1	1	1	1
Mesenteric lymph node										
Hemorrhage			1	1	2	1	2	1	1	2
Pancreas										
Apoptotic bodies	2	2	4	4	3	2	3	2	4	3
Hemorrhage		1		1			1			
Atrophy			2	1			1	1		1
Stomach Erosion									1	
Inflammation			4		1					
Perivasculitis			1							
Submandibular gland Inflammation			1	1	1	1	3			1
Tonsils- Focal hemorrhage								1	2	
Spleen- focal hemorrhage		1	1	1		1				
Testes[c]-focal degeneration			1		1		2			
Giant cells			1		1		3		1	
Epididymides-										
Oligospermia							1		1	
Debris	1		1		1		2		2	
Brain stem- hemorrhage			1	1		1				1

a. Findings in heart were observed in ventricles, atria, septum and papillary muscle.
b. Lens vacuolization was grade 1 in controls grade 2 in treated dogs.
c. For testicular findings males with giant cells were not the same animals as with degeneration.

-Toxicokinetics: Plasma samples were collected at 0.5, 2, 4, 7, and 24 hours postdose on study days 1 and 88 for determination of BAY 38-9456 and BAY 44-5576 by —— AUC is calculated over the 0-7 hour interval since the only later measurement was at 24 hours and plasma concentrations were below the limit of quantification in all but the 30 mg/kg dose group at this timepoint. The plasma half-life of parent compound is 1-2 hours at all dose levels. The half-life of the M1 metabolite appears to be approximately the same, i.e, 1-2 hours.

- Exposures to BAY 38-9456 and its major metabolite were comparable in males and females and did not display significant accumulation with multiple dosing.

Pharmacokinetics of BAY 38-9456 and BAY 44-5576 (M1) in the Dog

Bay 38-9456, D88	**1 mg/kg/day**		**3 mg/kg**		**10 mg/kg**		**30 mg/kg**	
	M	F	M	F	M	F	M	F
AUC $_{0-7}$, ng.h/ml	67	115	361	422	2892	2965	11650	6178
Cmax, ng/ml	29	50	175	209	1506	1038	3437	2117
Tmax, hrs	1	.8	.7	.5	.5	1.4	.7	.7
Multiple of Human AUC with 20 mg[a]	¼		1 X		10 X		23 X	
Bay 44-5576, D 88	**1 mg/kg/day**		**3 mg/kg**		**10 mg/kg**		**30 mg/kg**	
	M	F	M	F	M	F	M	F
AUC $_{0-7}$, ng.h/ml	58	59	220	247	1498	1565	5328	3495
Cmax, ng/ml	17	17	61	58	316	333	1054	668
Tmax, hrs	1	1.2	.7	.7	2	2.4	4	3.9
AUC M1/Parent	86%	52%	61%	59%	52%	53%	31%	32%

D 88 = PK parameters on day 88 of dosing.

a. Exposure multiples were calculated based on PK data for BAY 38-9456 only obtained in healthy volunteers dosed with a single dose of 20 mg, the highest proposed therapeutic dose (study Impact 94). Mean AUC in volunteers dosed with 20 mg = 265 ng.hr/ml.

Key Study Findings: There was no drug-related mortality, or effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis in dogs treated with BAY 38-9456 for 3 months. Dogs receiving doses ≥ 3 mg/kg/day had loose stools and reddened eyes and gums. Ophthalmic exams revealed corneal clouding and discoloration in 1 male at 3 mg/kg and 1 male at 30 mg/kg. Significant dose- dependent decreases in blood pressure and increases in heart rate were observed at doses ≥ 10 mg/kg/day (> 10 times human exposures). Urinalysis revealed an increased frequency of significant blood in the urine in treated dogs . Heart and liver weights were mildly increased in HMD and HD male and HD female dogs. Thymic weights were decreased in HD male dogs. Histopathologic findings were observed with increased frequency in BAY treated dogs as follows:

1) **Heart:** minimal to mild periarteritis /arteritis and intramural edema were observed at multiple locations in the hearts of mid and high dose dogs (right and left atria and ventricles, papillary muscle). May be secondary to hemodynamic changes since it was observed in dogs dosed with 10 and 30 mg/kg/day in association with significant hemodynamic changes, but not in dogs dosed with 1 or 3 mg/kg/day in which hemodynamic effects were absent.
2) **Kidney:** karyomegaly was observed in the renal proximal tubules of treated dogs. BMS states it is a familial finding in their colony, but historical rates were not provided and the finding was only observed in treated dogs.
3) **Liver:** a dose-related increase in the incidence but not the severity of cytoplamic inclusions was observed (all minimal to mild in severity).
4) **Gallbladder:** increased vacuolization.
5) **Eyes:** increased incidence of vacuolization of the lens in treated dogs : 33%, 75%, 63%, 87%, and 55 % at 0, 1, 3, 10, 30 mg/kg/day, respectively . Severity was minimal at lower doses and mild in HD dogs.
6) **Focal hemorrhages:** were observed more frequently in the lungs, mesenteric lymph nodes, tonsils, spleen, and brain stem of treated dogs, even at the two lowest dose levels which did not produce significant hemodynamic changes.
7) **Aorta** – intramural fiber disorganization observed at all doses.
8) **Pancreas**- increased apoptosis and atrophy at all doses.
9) **Testes**- focal degeneration of seminiferous tubule germinal epithelium.

The pathologic findings are of concern since they were observed even at the two lowest dose levels that produce drug exposures equal to or less than therapeutic. However, two factors must be considered. First, the toxicities were observed with daily dosing while clinical use is "as needed". Also, protein binding was less extensive in dogs than other species, therefore, free drug concentrations are twice as high in dogs as in other species.

Chronic (27-Week) Oral Toxicity in Wistar Rats

Study No: T9068186; Report PH 29532
Amendment # 046, Vol #17.1, and page #: 2
Conducting laboratory and location: Bayer AG Institute of Toxicology, Wuppertal, Germany
Date of study initiation: March 19, 1999
GLP compliance: Yes
QA- Report Yes (X) No ()
Methods:
Dosing:

- species/strain: Rat, Wistar
- #/sex/group or time point: 20/sex/dose
- age: 6-7 weeks at study initiation
- weight: 185 – 209 g males; 139 – 166 g females
- dosage groups in administered units: 0, 3, 15 and 75 mg/kg by gavage

Drug : BAY 38-9456, lot # 503985, and % purity: not specified

Formulation/vehicle: 0.5% Tylose
Analysis of the dose formulations were performed 4 times during the study and confirmed the concentrations were 94-101% of nominal.
Observations and times:

- Clinical signs: twice daily weekdays, once daily weekends.
- Body weights: weekly
- Food consumption: weekly
- Ophthalmoscopy: pretreatment and termination
- EKG: not performed
- Hematology: weeks 14 and 26
- Clinical chemistry: weeks 14 and 26
- Urinalysis: weeks 13 and 25
- Organ weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes
- Gross pathology:
- Histopathology: see table for tissues collected. Larynx, pharynx, urethra, ureters ,and Zymbals gland were not examined.
- Toxicokinetics: Blood samples were collected via retro-orbital sinus sampling as follows: Day 1 – at 30 minutes and 24 hours after dosing. Day 182 at 0.5, 1, 2, 4, 7, and 24 hours after dosing.

Results:

- **Mortality: one control female died during blood sampling in week 25. One HD female (#143) died spontaneously on day 5 and was observed to have myocardial damage.**
- Clinical signs: no drug-related clinical signs
- Body weights: Drug treatment had no effect on body weight at doses up to 15 mg/kg. High dose rats (75 mg/kg) displayed depression of body weight gain. GAIN was decreased 8.7% in males and 6.7% in females by the end of the study (week 26).
- Food consumption: no drug effect.
- Water consumption: Water consumption was increased by 25% throughout the study in high dose female rats.
- Ophthalmoscopy: unremarkable
- Electrocardiography: not performed
- Hematology:

Hemoglobin and MCHC (mean corpuscular hemoglobin concentration) – increased in high dose rats of both sexes. Mean corpuscular hemoglobin (MCH) was also increased in males at all dose levels during week 14. The individual values remained within the historical control range for Wistar rats, but since these parameters were elevated in previous studies it was concluded the effects are probably compound-related.
WBC : High dose rats of both sexes had increased leukocyte and lymphocyte counts (25-30%) during weeks 14 and 26.

- Clinical chemistry: No effects at doses up to 15 mg/kg/day.

High dose rats of both sexes had mild decreases in ASAT and ALAT during weeks 14 and 26 and mild increases in plasma urea at both timepoints.
Inorganic phosphate levels were mildly increased in high dose females at Wk 14 and 26.

Plasma urea concentrations (mmol/L)

Dose, mg/kg/day	Week 14		Week 26	
	Males	Females	Males	Females
Controls	6.9	6.7	6.5	6.5
3	7.1	6.6	6.3	6.2
15	6.9	7.3	6.7	6.8
75	8.4**	9.7**	8.0**	9.8**

** P < 0.01 from control values.

- Urinalysis: Increased urine volume was observed in high dose rats of both sexes at both timepoints. This increase is probably secondary to increased water consumption in these groups.
- Organ Weights:
  - Heart- dose-dependent increases in heart wt in mid and high dose rats (+10, 15%)
  - Kidney – increased wts in all treated males (+10-14%) and MD, HD females (+ 7, 19%)
  - Liver – increased wts in HD males (+15%) and MD, HD females (+13, 37%)
- Gross pathology:
- Histopathology: Results are summarized in the table below (n = 20/sex/dose).

Adrenal – vacuolization of the zona glomerulosa was observed in all mid and high dose rats. The severity of the pathology also increased slightly from minimal in low dose to mild in mid and high dose rats.
Heart: myocardial fibrosis observed in 5/20 high dose females

Kidney: increased frequency of basophilic cortical tubules and tubular mineralization in drug-treated female rats.
Pancreas/salivary glands- acinar hypertropy was observed with increased frequency in high dose animals of both sexes.

Histopathologic Findings in Wistar Rats Dosed with BAY 38-9456 for 6 Months

Tissue/ Finding	Males				Females			
Dose, mg/kg→	0	3	15	75	0	3	15	75
Adrenal, zona glomerulosa								
Small vesicle vacuolization	2	3	20	20		6	20	19
Large vesicle vacuolization				5				19
Heart								
Myocardial fibrosis	1	1	2	2	1	1	0	5
Kidney								
Basophilic cortical tubules	14	13	17	14	2	3	6	11
Mineralization		1			2	5	7	6
Pancreas								
Acinar hypertrophy				11				6
Acinar atrophy/fibrosis	3	4	10	6				
Pigment	6	8	9	11		1	2	
Parotid								
Acinar hypertrophy				20				19
Submandibular gland								
Acinar hypertrophy								19
Thyroid								
Colloid alteration	9	11	14	10	2	2	3	8
Testes								
Tubular atrophy	1	2	2					

- Toxicokinetics: Kinetics of parent drug, BAY 44-5576 (M1), and BAY 44-5578 (M4) were evaluated. Concentrations of M4 in rats were very low (< 2% of parent concentrations) and therefore M4 is a minor metabolite in the rat. M1 concentrations were comparable to parent drug in males and 14-24% of parent exposures in female rats. Toxicokinetics data for BAY 38-9456 and BAY 44-5576 (M1) are summarized in the table below. Tmax was observed at 30 minutes at the two lower doses and 1 hour in high dose rats. Exposures to parent compound were gender specific, being significantly higher in female rats. Moderate accumulation of parent drug was observed with multiple dosing (Cmax values on day 182 were 2-5 times higher than on day 1. AUC values were not determined on day 1 to permit exposure comparisions).

Toxicokinetics of BAY 38-9456 and 44-5576(M1) on Day 182

Parameter Dose→	3mg/kg/day		15 mg/kg/day		75 mg/kg/day	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
BAY 38-9456						
AUC_{0-24}, ng.h/ml	113	1430	1866	10895	16510	48441
Cmax, ng/ml	79	581	1205	5026	4402	9370
Multiple of Human AUC with 20 mg[a]	½ - 1		7X		62 X	
BAY 44-5578	**3mg/kg/day**		**15 mg/kg/day**		**75 mg/kg/day**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
AUC_{0-24}, ng.h/ml	111	204	1762	2574	17857	11202
Cmax, ng/ml	84	104	1081	598	2730	1485

a. Exposure multiples were calculated based on PK data for BAY 38-9456 only obtained in healthy volunteers dosed with a single dose of 20 mg, the highest proposed therapeutic dose (study Impact 94). Mean AUC in volunteers dosed with 20 mg = 265 ng.hr/ml.

- Hormone Analysis (week 26): Plasma concentrations of T3, T4, and TSH were not influenced by treatment.

Key Study Findings: One high dose female died on day 5 with pathologic evidence of myocardial damage (75 mg/kg/day in female rats produces AUC exposures >180 times human exposure). Minimal increases in hemoglobin and mean corpuscular hemoglobin concentration. Mild increases in leukocyte and lymphocyte counts were observed in HD rats of both sexes. Dose-related increases in heart, kidney, and liver weights were observed in mid (10%) and high dose (15 –30%) rats of both sexes. High dose animals of both sexes had histopathologic findings of vacuolization of the adrenal zona glomerulosa and acinar hypertrophy in the pancreas and salivary glands. High dose females also had an increased incidence of myocardial fibrosis (5/20 affected). The mid dose of 15 mg/kg/day (> 7 times human exposure) was the NOAEL in this 6 month study.

52-Week Chronic Toxicity Study in Beagle Dogs

Study No: T3067488
Amendment # 046, Vol # 17.2, and page #: 1
Conducting laboratory and location: Institute of Toxicology, Bayer AG, Wppertal, Germany
Date of study initiation: November 2, 1998
GLP compliance: Yes QA- Report Yes () No (X) Draft Report

Methods:
Dosing:

- species/strain: Dog, beagle
- #/sex/group or time point: 4/sex/dose
- age: 21-25 weeks old - weight: 6.6 to 9 kg
- dosage groups in administered units: 0, 3, 10 and 30 mg/kg/day
- route : oral by gavage in 0.5% aqueous tylose

Drug: BAY 38-9456, lot# 503891 and 503985, purity: 84% free base
Observations and times:

- Clinical signs: daily. Reflexes and body temperature weeks 6, 13, 26, 39, 52.
- Body weights: weekly
- Food consumption: daily
- Ophthalmoscopy: pretreatment and weeks 6. 13, 26, 39, and 52.
- EKG/blood pressure: monitored 2 hours after dosing in weeks 6, 13, 26, 39 and 52.
- Hematology: prestudy and weeks, 6, 13, 20, 26, 39 and 52
- Clinical chemistry: prestudy and weeks, 6, 13, 20, 26, 39 and 52
- Urinalysis: prestudy and weeks, 6, 13, 20, 26, 39 and 52
- Organ weighed: brain, heart, liver, lungs, spleen, adrenals, kidney, pancreas, thyroid, pituitary, testes, prostate, uterus, thymus, ovaries, epididymes, gall bladder.
- Histopathology: see table
- Toxicokinetics: On day 1, and in week 15 and 50 blood samples were collected prior to treatment and at 0.5, 2, 4, 7, and 24 hours for measurement of BAY 38-9456 and metabolites.

Results:

- Mortality: None
- Clinical signs: Drug treatment had no effect on reflexes or body temperature. At doses ≥ 10 mg/kg/day dogs had increased heart rate (chest palpations), reddened gums and eyes, and inceased salivation on almost a daily basis.
- Body weights: No significant effects in treated males. HD females had decreased body weight gain compared to other dose groups (gain ranged from 5.3 to 5.9 kg for all groups but was only 4.65 kg for HD females).
- Food consumption: unremarkable
- Ophthalmoscopy : unremarkable
- Electrocardiography: Report states "no toxicologically relevant changes", no data provided.
- **Blood pressure:** No significant changes in blood pressure were observed in low dose dogs. Significant, dose-related decreases (> 20 mmHg) in systolic and diastolic pressures were observed two hours after dosing in dogs of both sexes throughout the study at doses ≥ 10 mg/kg . Data are summarized in the table below.

Mean Decreases (mmHg) in Blood Pressure 2 hours After Administration of BAY

Dose, mg/kg/day		Week 13		Week 26		Week 52	
		Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
10	Males	20	21	15	27	21	27
	Females	27	27	44	32	14	23
30	Males	64	40	28	25	72	49
	Females	62	48	74	53	68	53

- **Heart Rate**: No significant changes in heart rate were observed in low dose dogs (3 mg/kg). Significant, dose-related increases in heart rate were observed two hours after dosing in dogs of both sexes treated with 10 or 30 mg/kg/day. Heart rates were increased 25-40% with 10 mg/kg and 50-80% with 30 mg/kg/day. Data are summarized in the table below.

Mean Increases in Heart Rate (bpm) 2 Hours After BAY 38-9456 Administration

Dose/Sex		Week 13	Week 26	Week 39	Week 52
10 mg/kg	Males	44	38	42	29
	Females	41	40	56	14
30 mg/kg	Males	92	81	63	70
	Females	73	115	67	87

- Hematology/urinalysis/organ weights: unremarkable
- Clinical chemistry: Unremarkable with the exception that one mid dose male (E113) and one high dose female dog (E102) had 4-5 fold increases in creatinine kinase during the study. The increase in CK was observed during week 20 in MD dog E113 (52, 59 U/L in weeks 6, 13 and 259, 165, 153 U/L in weeks 21, 21, 26 , respectively). The increase in HD dog E102 was observed during week 6, the earliest measurement time (92 baseline, 417 U/L week 6 , 135 U/L week 7).
- Gross pathology: Red areas (hemorrhage) were observed in the intestinal mucosa of all dose groups. There was no histopathologic correlate for these findings.
- Histopathology: Pathologic findings were infrequent and generally mild in severity. Periarterial edema was observed in the hearts of 2/8 high dose dogs, but is most probably secondary to the marked effects of the high dose on blood pressure and heart rate (see table above).
- Mucosal lymphoid follicles in the gallbladder and cytoplasmic inclusions in the liver were observed with increased frequency in BAY-treated female dogs.
- Testicular tubular atrophy was observed in one mid dose and tubular degeneration in one high dose dog. This is most probably a true drug-related toxicity since testicular degeneration has been observed in dogs treated chronically (> 6 months) with other PDE V inhibitors.

Histopathologic Findings in Dogs Treated with BAY 38-9456 Daily for One Year

Tissue /Finding	Controls		3 mg/kg/d		10 mg/kg/d		30 mg/kg/d	
	Male	Female	Male	Female	Male	Female	Male	Female
Gallbladder, mucosal lymphoid follicles	1	1	1	1	2	2	2	3
Heart, periarterial edema							1	1
Kidney, mononuclear cell infiltration	0	0	2	1	1	1	0	1
Tubular hyperplasia					1			
Liver, cytoplasmic inclusions[a]	1	0	0	4	1	3	1	2
Ovaries, Antral follicles		1		1		2		4
Spinal cord, arteritis							1	
Epididymides Sperm debris								
Sperm retention					1 (marked)		1 (mild)	
Testes, tubular degeneration							1 (moderate)	
Tubular atrophy					1			

a. Liver inclusions were dose-related in severity, minimal severity in low dose to moderate severity in high dose females.

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- Toxicokinetics: Pharmacokinetics data for parent , 38-9456 and the major M1 metabolite, 44-5576 are presented in the table below. Values for the M4 metabolite 44-5578 were also provided but are not included because they were 1/20 of parent drug concentrations and 1/10 of M1 concentrations, so therefore, represent a very small percent of compound related drug exposures.

Pharmacokinetics of BAY 38-9456 and BAY 44-5576 (M1) in Dogs Treated for 50 Weeks

Dose of BAY 38-9456 Mg/kg		BAY 38-9456 AUC $_{0-24}$, ng.hr/mL	BAY 44-5576 AUC $_{0-24}$, ng.hr/mL	BAY 38-9456 Cmax, ng/ml	BAY 44-5576 Cmax, ng/ml
3	Male	755 (2.5 X)	352	207	56
	Female	672	336	150	55
10	Male	5361 (19 X)	2949	1680	352
	Female	4808	2313	1384	309
30	Male	20948 (75 X)	10763	4380	1017
	Female	16033	9989	3909	1007

Accumulation ratios revealed 2- fold increases in M1 and 3- fold increases in parent drug concentrations by week 50 with multiple dosing with the highest dose of 30 mg/kg/day.
Administration of 3, 10 and 30 mg/kg/day produced drug AUC exposures in male dogs that were 2.5, 19, and 75 times greater than human therapeutic AUC exposures.

Key Study Findings: The observation of testicular toxicity in the 3 and 12 month dog studies combined with the fact that this toxicity is also observed in dogs treated with all other PDE V inhibitors, strongly suggest that the finding is drug-related. The testicular toxicity did not appear to be progressive since fewer animals were affected in the one year study than the 3 month study, and only 1 mid and 1 high dose dog were affected in the one year study (AUC exposures in male dogs on mid dose ≥ 10 times human exposures).
The testicular toxicity observed in BAY- treated dogs appears less severe that with other PDE V inhibitors since it occurred less frequently and only at significant multiples of the human therapeutic exposures. Therefore, it was concluded that sperm analysis was not warranted in the clinical trial with BAY 38-9456.

Histopathology Inventory for IND #

Study	3 month	3 month	6 month	12 Month
Species	RAT	DOG	RAT	DOG
Adrenals	X	X	X	X
Aorta	X	X	X	X
Bone (femur)	X	X		X
Brain	X	X	X	X
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X	X
Esophagus	X	X	X	X
Eye	X	X	X	
Fallopian tube	X	X	X	X
Gall bladder		X	X	X
Gross lesions	X	X	X	X
Harderian gland	X		X	X
Heart	X	X	X	X
Hyphophysis	X			
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X	X	X	X
Lachrymal gland			X	X
Larynx	X	X	X	X
Liver	X	X	X	X
Lungs	X	X	X	X
Lymph nodes, cervical	X			
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X