

**SINGLE DOSE BIOAVAILABILITY IN MALE RATS** [REDACTED] (vol 1.77)

R6437. Animal studies (Bayer, Wuppertal July 1994 to May 1995)

PK analyses ([REDACTED] February 1995 to May 1995)

TREATMENT: Three male Wistar rats/time point were treated with 0.2 mg/kg i.v. or 0.02, 0.2, and 2 mg/kg p.o., by gavage in phosphate buffered saline, pH 7.4 (carcinogenicity study used dietary dosing). There was no statement about how plasma was transported/stored before analyses. Analyses were by [REDACTED]. After analyses, plasma was stored "below -18° C". Bay w 5679 levels were too low to measure. Clearance was 2.4 l/h kg; mean retention time 1.2 hours.

**BAY w 6228** [REDACTED]

	0.2 mg/kg i.v.	0.02 mg/kg p.o.	0.2 mg/kg p.o.	2 mg/kg p.o.
AUC (ng h/ml)	83	6.2	17	570
Cmax (ng/ml)		1.8	7.0	380
tmax (h)		1.0	0.8	0.8
t1/2 (h)	1.3	n.c.	1.5	1.4
f (%)*		74	21	68

Mean±SD (n=3/time point)

n.c. not calculated \*bioavailability

**ENTEROHEPATIC CIRCULATION IN MALE RATS** (vol 1.80)

21776(P) Bayer. July 1992.

Greater than 90% of the dose is excreted by the bile with 50% of the radioactivity excreted being reabsorbed.

**ELIMINATION** (vol 1.79)

Most of elimination is by biliary/fecal excretion in rats, mice, and dogs.

**EFFECT OF FOOD/FASTING ON PLASMA LEVELS** (vol 1.80)

19849(P) Bayer. November 1990.

The systemic availability [REDACTED] was 50% less in fed vs fasted rats.

**PLASMA PROTEIN BINDING** (vol 1.80)

20067(P) Bayer. March 1991.

Fraction unbound was 2.5% (rats and dogs) and 0.5% (man) over the range of 0.1 to 10 ug/ml. Drug was primarily bound to serum albumin.

24850(P). Bayer. March 1996.

Fraction unbound was 1.5% (mouse), 2.5% (rat), 2% (dog), and 0.6% (human).

**BIOTRANSFORMATION IN LIVER MICROSOMES IN VITRO (vol 1.79)**

24774(P). November 1995. Bayer.

Microsomes of male rats, female pigs, male micro pigs, male rabbits, female dogs, male mice, and male and female rhesus monkeys were used to isolate metabolites of BAY w 6228 using two [redacted] systems. Incubations were 5 ml total with 1 ml withdrawn at 0.5, 30, 60, 120, and 180 minutes (the incubation ingredients and level of drug were not specified).

"Microsomes do not represent the full metabolic capacity of the liver, since a variety of important metabolic reactions like phase II conjugations and -oxidation can not be observed. For BAY w 6228, this limits an *in vitro/in vivo* correlation, because *in vivo* glucuronidation is a major pathway in the dog and -oxidation predominates in rat and mouse."

Human (two 1<sup>0</sup> biotransformation rx): demethylation to M1 and hydroxylation to M23; M24 formed by combination of both reactions, detected to a "lesser extent" (p.97).

"In liver microsomes of all the animal species investigated here, demethylation to M1 is the major metabolic pathway."

**METABOLIC PATTERNS IN RAT (vol 1.78)**

24499(P). Two-four males received [<sup>14</sup>C]BAY w 6228 [1.7 mg/kg p.o., 2 mg/kg i.d., or 1.0 mg/kg i.v.] (plasma, liver, bile, urine); 3 females received 2 mg/kg i.d. (bile and urine). Plasma and liver were investigated 0.5, 4, and 24 hours postdose but the data were "impaired by the low contents of radioactivity". A proposed metabolic pathway was provided with structures of metabolites as obtained by [redacted] analyses. A repetition of the liver analysis showed that results could not be repeated, "probably due to remaining enzymatic activity in the stored liver samples (-20<sup>0</sup> C...).

**METABOLIC PATTERNS IN ONE FEMALE DOG (in plasma, urine and bile) (vol 1.78)**

22025(P). No information was provided as to who did the work, when it was done, or how tissue was handled after treatment. The metabolic pattern in bile was from one bile-cannulated dog after 2 mg/kg intraduodenally; the pattern in plasma and urine was from one dog from a PK study (2 mg/kg p.o.). "Due to the fact that the labelled drug used in this experiment did not have the recommended purity, it was not used for quantitative balancing of bile pattern." A proposed metabolic pathway was provided with structures of metabolites as obtained by [redacted] analyses in comparison with reference compounds.

**METABOLIC PATTERNS IN MALE MICE (vol 1.79)**

25010(P). Bayer. April 1996.

Male B6C3F1 mice were given 2 mg/kg radioactive BAY w 6228 by gavage and metabolite patterns examined by [redacted] in plasma, bile, and urine. Samples were collected at 0.5 and 4 hours postdose. M27 and M28 were the major metabolites but structures of 14 metabolites were identified.

Liver: 80% of metabolites identified (unknowns not listed)

Bile: 60% of metabolites identified (21 listed; known + unknown)

Urine: 50% of metabolites identified (14 listed; known + unknown)

**METABOLIC PATTERNS IN MALE HUMANS (vol 1.78)**

PH 25040. Bayer. May 1996.

Four male volunteers were given a single oral dose of 400 ug [<sup>14</sup>C]BAY w 6228 (specific activity not provided) as an aqueous solution. Plasma was analyzed 2 and 4 hours postdose; urine was collected up to 72 hours postdose and feces out to 120 hours. HPLC analyses were used. Radioactivity was too low in plasma to use [redacted] was used instead (120-240 dpm/plate). In plasma, M1 and M23 accounted for 90% of radioactivity; however, so little radioactivity was present that only major components could be detected. In urine and feces together, M23 (and its lactone) accounted for 23%, M24 for 7% and M1 and its lactone (M22) accounted for 32% of the dose. They state that M23 and M24 are unique to humans. Unchanged drug was 1-2% of excreted dose. M1 was the major metabolite in rats, dogs, and humans.

**BIOTRANSFORMATION IN HUMAN LIVER MICROSOMES (in vitro) (vol 1.78)**

PH 24571. Bayer. October 1995.

Oxidative Phase I metabolism during one hour incubation in vitro was investigated using [<sup>14</sup>C]BAY w 6228 and two different [redacted] systems. No information was provided as to who did the work, when it was done, or how tissue was obtained and incubated. There was demethylation to M1, hydroxylation to M23 with conversion of both to M24 (dihydroxylated).

**EFFECT ON LIVER DRUG METABOLIZING ENZYMES (vol 1.80)**

R6482. Bayer. June 1991. [redacted]

After one week dosing at doses up to 1 mg/kg (gavage in physiological saline), there was no effect on liver wt, cyt. P450, aniline hydroxylase, or aminopyrin N-demethylase in male Wistar rats. There were increases in all parameters (except aniline hydroxylase) with phenobarbital.

**STEREOCHEMICAL PURITY OF THE DRUG IN RAT AND DOG BILE (vol 1.80)**

22913(P). Bayer. February 1994.

Following intraduodenal administration, essentially all drug is excreted by bile in both species. Unchanged drug represents 7-8% (rat) and 23% (dog) bile. The lactone form (bay w 8877) represents 18% of the dose in dog bile vs <2% in rat bile. There was no conversion of the S,R configuration by either rat or dog bile of a 2 mg/kg radioactive dose.

**TOXICITY STUDIES****13-WEEK ORAL GAVAGE TOXICITY STUDY IN MONKEYS (vol 1.58)**

6520. March 1993. [redacted]

**TREATMENT:** Four groups of rhesus monkeys (3s/g; 3-4 years old from [redacted]) were given by oral gavage in 0.5% methylcellulose, 0, 10, 30, or 100 ug/kg/day for three months. Plasma was collected for drug levels.

**RESULTS:** "... a daily oral dose of 100 ug/kg BAY w6228 was the no adverse effect dose under the conditions of this study."

**1-YEAR ORAL GAVAGE TOXICITY STUDY IN MONKEYS (vol 1.58)**

6520. November 1993. [REDACTED]

**TREATMENT:** Four groups of wild-caught rhesus monkeys (4/s/g; 4-8 years old from [REDACTED]) were given by oral gavage in 0.5% methylcellulose, 0, 10, 30, or 100 ug/kg/day for one year.

**RESULTS:** A complete toxicological analysis was done but there were no findings in any parameter measured "... the non-toxic (NOEL) dosage level of BAY w6228 was greater than 100 ug/kg/day when administered orally to male and female rhesus monkeys for 52 weeks."

**58-WEEK ORAL TOXICITY STUDY IN DOGS (vol 1.55)**

T 0040367. Bayer AG, Wuppertal, Germany. July 1991.

Batch#: 509245 (97.4%)

**TREATMENT:** Three groups of beagle dogs (4/s/g; 4-5-months old) were given by gavage in tap water, 0, 100, and 300 ug/kg/day. *Treatment was 58 weeks.* At the end of the treatment period (week 59), 4 control males and 3 treated males (100 ug/kg) had a hemi-orchietomy and were given a 13-week recovery period to investigate reversibility of testicular findings in the remaining testis. The remaining testes was removed at week 72. Dogs were "usually fed 1-3 hours postdose". The high dose dogs (300 ug/kg) died or were killed after 2 weeks treatment; only the 100 ug/kg group survived. Ejaculation tests were performed weeks 51, 58, 72 (separate report).

**RESULTS****MORTALITY:**

100 ug/kg: one male died week 25 (no previous symptoms) but there was blood on the floor of the cage and a blood-smearred anus.

300 ug/kg: three died days 11-14; the remaining 5 were killed day 15.

**CLINICAL SIGNS:**

Gingival bleeding in one male at 100 ug/kg

Gingival bleeding in all 8 dogs at 300 ug/kg

Anal bleeding/blood-stained feces: one male at 100 ug/kg  
three males at 300/kg

**BODY WEIGHTS:** No effect in females; males at 100 ug/kg males had 10% reduced gain

**FOOD CONSUMPTION:** male that died at 100 ug/kg males had reduced intake before death

**OPHTHALMOLOGY:** Opacity of the anterior or posterior suture in week 58 in two females (100 ug/kg)

**HEMATOLOGY:**

At 300 ug/kg: reduced ery, Hb, PCV, thro; increased reticulocytes

Anisocytosis and poikilocytosis, polychromasia, hypochromasia

At 100 ug/kg: increased reticulocytes

**CLINICAL CHEMISTRY** (no statistics provided; maximum effects seen given below):

AST: increased 6x at 300 ug/kg; 2x at 100 ug/kg

ALT: increased 4x at 300 ug/kg; 8x at 100 ug/kg (week 27 peak)

LDH: increased 3x at 300ug/kg

CPK: increased 4x at 300ug/kg

-amylase: increased 2x at 300 ug/kg

Lipase: increased 5x at 300 ug/kg; 2x at 100 ug/kg

Cholesterol: decreased 50% at 300 ug/kg and 100ug/kg

**58-WEEK ORAL TOXICITY STUDY IN DOGS** (0,100,300 ug/kg/day; 4/s/g)**URINALYSIS:** "No toxicological effects"**PATHOLOGY****ORGAN WEIGHTS** (as % of BW; no statistics; 0, 100, and 300 ug/kg/day)

LUNG: increased at 300 ug/kg

LIVER: 35; 40, 42% (M) and 34; 41, 44% (F)

TESTES: 0.97; 0.95, 0.43%

PROSTATE: 0.94; 0.77, 0.17%

THYROID: 0.09; 0.10, 0.1% (M) and 0.06, 0.07, 0.12% (F)

PANCREAS: 2.5; 2.6, 4.4% (M) and 3.0; 2.8, 4.4% (F)

HISTOPATHOLOGY	(0,100,300 ug/kg/day; 4/s/g)	
	MALE 0 L HD	FEMALE 0 L HD
<b>LIVER:</b>		
Degeneration, necrosis	0 1 3	0 0 3
Hyperemia, congestion	0 1 2	0 0 1
Focal fibrosis	0 0 0	0 1 0
Mesenchymal activation	0 1 3	0 0 3
Focal faty change	0 0 2	0 2 0
<b>HEART</b>		
Hemorrhage	0 1 2	0 0 0
<b>LUNGS</b>		
Edema	0 1 2	
Focal fibrosis	1 3 0	0 1 0
Interstitial inflammation	1 4 3	0 1 4
Alveolar macrophages	1 3 4	0 3 4
<b>PAROTID</b>		
Round cell infiltration	0 2 0	0 1 0
<b>STOMACH</b>		
Hemorrhage	0 1 2	0 0 2
<b>INTESTINE</b>		
Hemorrhage (large intestine)	0 1 2	0 1 1
Necrosis lymph. tiss	0 0 2	0 0 0

**GALLBLADDER**

Hemorrhage	014	002
Edema	014	001
Cholecystitis/erosion	001	001

**PANCREAS**

Hemorrhage	010	000
Edema	014	002

**58-WEEK ORAL TOXICITY STUDY IN DOGS (0,100,300 ug/kg/day; 4/s/g)**

<b>HISTOPATHOLOGY</b>	<b>MALE 0 L HD</b>	<b>FEMALE 0 L HD</b>
<b>TONSILS</b>		
Hemorrhage	001	013
Necrosis lymph tiss	012	000
<b>THYMUS</b>		
Hemorrhage	021	001
Edema	003	002
Lymphocyte depletion	013	002
<b>MANDIB. LYPH NODE</b>		
Necroses	010	000
<b>MESENT. LYPH NODE</b>		
Necroses	012	000
<b>BRAIN</b>		
Vascular degeneration	010	000
Hemorrhage	010	000
<b>SPINAL CORD</b>		
Hemorrhag subarach	010	000
<b>SCIATIC NERVE</b>		
Vasculitis	010	000
<b>OPTIC NERVE</b>		
Vascul degeneration	010	
<b>URINARY BLADDER</b>		
Hemorrhage	010	
Edema	011	
<b>TESTES</b>		
Giant cells	020	
<b>UTERUS</b>		
Lymphatic follicles		002
Hemorrhage		001
<b>EPIDIDYMIDES</b>		
Hypo/aspermia	004	
<b>SKELETAL MUSCLE</b>		
Myopathy	012	002
<b>TONGUE</b>		
Myopathy	004	003

**PLASMA DRUG LEVELS:** (page 11 of review)**1-YEAR ORAL TOXICITY STUDY IN MALE DOGS** (vol 1.56)

T6055475. Feb. 1994-May 1995. Bayer AG, Wuppertal, Germany.

Batch#: 513240 manufactured Feb. 1993 with 2-yr expiration (p.224,5)

**TREATMENT:** Two groups of male beagle dogs (15/g; 12 to 16 months-old) were given 0 or 100 ug/kg by gavage in tap water for 12 months. At the end treatment, a hemiorchiectomy was done (left testis and epididymis fixed) and a 3 months recovery from drug begun. After the 3 months drug-free period, all dogs were killed and a necropsy and histopathology done on brain, optic nerves, testis (right), epididymis (right), prostate and organs with macroscopic findings.

The stability data for the drug was from a study done in January 1991 (batch# 507277) and showed a 10% decline in strength over 2 weeks in tap water. (p.226)

**RESULTS****CLINICAL SIGNS**

**MORTALITY:** one control (March 1995; thought to have died of viral infection) and two treated (March 1994 and January 1995; see below)

**PATHOLOGY****GROSS (killed in extremis):**

Control: hematoma of spleen but thought to have died of possible viral infection in brain (p.171)

Treated: Gallbladder and upper GI tract: dark red contents (Z 629)

GI tract: red or dark red contents (Z 659)

Lymphatic tissues: enlarged or reddened

Lungs: solid/firm, gray-red

Liver: solid/firm

**GROSS (killed termination of study):**

Lungs: discoloration or change in consistency; control: (n=2); treated (n=5)

Liver: distinct lobulation or discoloration (yellow or yellow/brown) (2 control; 5 tx)

**HISTOPATHOLOGY**

**LEFT TESTES** (hemiorchiectomy): "multifocal degeneration of the seminiferous epithelium" in one treated. "This finding is regarded to correspond to the lesions reported in the first 12 month dog study (T 1039793)...In that study, degeneration of the seminiferous epithelium was considered to be a treatment effect." (the right testis after 3 months recovery had no findings.)

**CNS** (killed in extremis): fibrinoid degeneration of vessel walls occurred in the choroid plexus, associated with granulocyte infiltration and local hemorrhage (2 dogs). One of these two also had vessel wall degeneration and granulocyte infiltration in the ciliary body of the eyes.

**LUNGS:** chronic pneumonia + focal fibrosis in 6 treated dogs.  
acute pneumonia in one control (moderate) and one treated (severe)

**1-YEAR ORAL TOXICITY STUDY IN MALE DOGS (0, 100 ug/kg; 15/g)****LIVERS**

"Disseminated degeneration and necrosis of liver cells with a remarkable generalized activation of Kupffer cells 'mesenchymal activation' occurred only in treated dogs killed in extremis. This lesion is well-known as an induced change and was also seen in previous studies with Bay w 6228." (Vol 1.56, p.170)

**FINDINGS AFTER DEATH OF TREATED DOGS:****Dog# Z 659:**

**Lungs:** chronic pneumonia

**Choroid plexus vessels:** fibrinoid degeneration

**Liver:** degeneration/necroses, mesenchymal activation

**GI tract (stomach, ileum cecum, colon, rectum, gallbladder):** mucosal erosions/hemorrhage  
(=cause of death)

**Spleen:** lymphocyte depletion

**Adrenal glands:** hemorrhage

**Skeletal muscle, tongue:** myopathy

**Dog# Z 629: (same as above for Z 659) plus:**

**Mesenteric lymph node and Peyers patches (ileum):** lymphocyte depletion

**Choroid plexus vessels and ciliary body:** fibrinoid degeneration

**DIETARY CARCINOGENICITY STUDY IN MICE (0, 1, 5, 25, 125 ppm) (vols 25-28)**

T3040234. July 1991- August 1993. Bayer AG, Wuppertal, Germany.

Lot#s: 507277 July to September 1991

509236 until January 1992

509269 until August 1992

511284 until November 1992

511239 until March 1993

513240 until August 1993

Lots were analyzed for purity *one year before date of first use* and ranged from 97 to 99%.

Storage: at RT with protection from light

Admixture: 1% Peanut oil (DAB 9 or 10) "to minimize dust formation"

The required quantity of drug was first dissolved in peanut oil and mixed feed. Mixtures were made up the week before needed (until week 69); mixtures then were made up for the week after next (weeks 70-104).

Stability data were derived from study T4037194 (p.17).

Analysis: day of mixing and again after storage "under animal room conditions" for 7 days.

**TREATMENT:** Five groups of B6C3F1 mice from [REDACTED] (60/s/g; 4-5 weeks old on arrival "as calculated from body weights") were given in the diet 0, 1, 5, 25, and 125 ppm (0.27, 1.3, 6.8, and 46 mg/kg/day for males and 0.47, 2.3, 12, and 65 mg/kg/day for females).

Ten/s/g were killed after one year (interim sacrifice). Mice arrived July 10; study started July 21, 1991. In week 79, mice were moved (in closed containers) to another "nearby" room.

Diet was Altromin 1321 meal available ad libitum. Intake was calculated weekly (until week 13) for the first 20/g and then every 4 weeks (weeks 14-104). Blood for hematology and clinical chemistry (one week apart) was taken weeks 27, 52, & 79, and 104 on 10/s/g. "As far as possible, the same mice were used." Blood was collected from the retro-orbital venous plexus. TK was done on first 5/s/g on blood collected between 10 am and 12 noon (p.4). Liver and muscle were collected at interim and terminal necropsy for TK (separate report). EM was done on liver from first 5/s/g at 0 and 125 ppm at terminal sacrifice.

### CARCINOGENICITY STUDY IN MICE

#### RESULTS

##### DRUG INTAKE:

Average over 24 months= 90% (1 ppm), 93% (5 ppm), 84% (25 ppm) or 83% (125 ppm).

CLINICAL SIGNS (incidence over 24 months in 60/s/g noticed sporadically wk 48 on):

Rough coat: 0, 1, 2, 2, 11 (males); 1, 0, 1, 4, 16 (females)

Poor general condition: 0, 0, 3, 3, 6 (males); 2, 2, 4, 2, 5 (females)

##### MORTALITY (% males dead, cumulative out of 50 mice/g)

Dose (ppm)	0	1	5	25	125
week 51	0	0	2	4	0
week 78	2	4	2	4	8
week 104	8	6	10	18	28

##### MORTALITY (% females dead, cumulative out of 50 mice/g)

Dose (ppm)	0	1	5	25	125
week 51	4	4	2	5	0
week 78	8	4	8	8	6
week 104	24	18	26	26	24

##### BODY WEIGHT GAIN:

Female: HD gained less from very beginning of study (7% less at end of study);

Male: HD gained less from very beginning of study (9% less at end of study). Control and other treated groups gained, plateaued, and then lost weight beginning about DW 60 and plateaued again about DW 80.

**CARCINOGENICITY STUDY IN MICE****FOOD CONSUMPTION and DRUG INTAKE (averaged over 2 years)**

DOSE (ppm)	Food Intake		Drug Intake			
	g/animal/day		g/mouse/day		mg/kg/day	
	male	female	male	female	male	female
0	7.2	10.5				
1	7.6	10.8	0.01	0.01	0.27	0.47
5	7.4	10.5	0.04	0.05	1.3	2.3
25	7.4	10.7	0.19	0.27	6.8	11.5
125	9.3	11.4	1.16	1.43	46	65

**WATER INTAKE ( ml/mouse/day; cumulative over 2 years):**

5.5; 5.7, 6.0, 5.6, 5.2 (males) and 6.2; 5.9, 5.9, 5.5, 5.0 (females)

**HEMATOLOGY (10/s/g):** statistically significant, but "no evidence of any toxicologically relevant changes" in differential or normal erythrocyte values.

**CLINICAL CHEMISTRY (significant changes,  $p < 0.05$ ; 10/s/g):**

**AST:** Increased 1.7 to 2x at 25 and 125 ppm at 53 and 105 weeks (males) and 1.7x (at 125 ppm females DW 105 only)

**ALT:** Increased 1.7 to 2x (females at 125 ppm; at 53 and 105 weeks)

**ALKP:** Increased at 25 and 125 ppm (males and females at 27, 53, and 105 weeks)

**Glutamic dehydrogenase:** Increased at 25 and 125 ppm (males and females at 105 weeks)

**Cholesterol:** Decreased at 5 to 25 ppm and above (males and females most times)

**TG:** Decreased at 25 ppm (males and females)

**Urea:** increased at 25 ppm (males & females)

**Protein and albumin:** decreased at 125 ppm (females)

**PATHOLOGY (\* $p < 0.05$ ; \*\* $p < 0.01$ )****ORGAN WTS (interim kill; 10/s/g; % BW)**

**LIVER:** 5.0; 4.6, 5.0, 4.9, 5.4\* (Male); 4.9; 5.3\*, 5/2\*, 5.4\*, 6.5\*\* (Female)

**SPLEEN:** 0.20, 0.20, 0.22, 0.24\*\*, 0.24\*\* (Male)

**ORGAN WTS (terminal kill; 50/s/g; % BW in 0, L, M, HD; \* $p < 0.05$ ; \*\* $p < 0.01$ )**

**LIVER:** 5.0; 4.9, 5.3, 6.9\*\*, 6.5\*\* (Male); 5.0; 5.1, 5.0, 5.3\*, 7.1\*\* (Female)

**SPLEEN:** 0.26, 0.27, 0.26, 0.32\*, 0.27 (Male) 0.77, 0.75, 0.72, 0.68, 0.47\* (Female)

**KIDNEY:** 2.1, 2.1, 2.1\*, 2.0\*\*, 1.9\*\* (Male); 1.5, 1.5, 1.5, 1.4\*, 1.4\*\* (Female)

**TESTES:** 0.62, 0.62, 0.60, 0.61, 0.64\*

**CARCINOGENICITY STUDY IN MICE****GROSS (INTERIM KILL)**

**GROSS (terminal kill; 50/s/g)**

Dose (ppm)	Male					Female				
	0	1	5	25	125	0	1	5	25	125
<b>LIVER</b>										
Areas on surface	0	0	5	4	10	1	3	2	3	6
Nodules	7	12	15	28	32	0	1	4	8	19
HAIR reddish color	0	0	0	0	10	0	0	0	0	16
BODY thin	1	1	0	3	5	1	4	1	2	4

(p.633: skin, rather than hair, "discoloration")

**HISTOPATHOLOGY (NON-NEOPLASTIC) (Interim kill; n=10/s/g)**

Dose (ppm)	Male					Female				
	0	1	5	25	125	0	1	5	25	125
<b>LIVER</b>										
periportal karyomegaly	0	0	0	2	6	1	0	0	0	1
clear cell focus	0	0	0	0	4	0	1	4	8	19
fatty infiltration										
peripheral	0	0	0	4	1	0	0	0	2	5
centrilobular	4	4	0	0	0	0	0	1	0	0
<b>KIDNEY loss of autophagic vacuole</b>	0	0	0	0	8	*	*	*	*	*

\*no data provided for female mic

**CARCINOGENICITY STUDY IN MICE**

APPEARS THIS WAY ON ORIGINAL

**HISTOPATHOLOGY (NON-NEOPLASTIC) (terminal kill/moribund; n=48-50/s/g)**

Dose (ppm)	Male					Female				
	0	1	5	25	125	0	1	5	25	125
<b>LIVER</b>										
eosinophilic cell foci	2	1	0	4	5	0	0	1	0	10
basophilic foci	4	2	2	9*	16	0	2	3	1	2
clear cell focus	1	1	1	1	5	1	0	0	0	1
necrosis	2	1	4	11*	9*	7	6	6	5	5
fat, panlobular	3	1	1	4	7	5	0	3	3	1
<b>KIDNEY</b> loss of autophagic vacuole	0	0	0	3	28*	0	0	0	0	0
<b>LYMPH NODES</b> adipose tissue	2	2	3	7	16*	4	6	6	10	27*
<b>FORESTOMACH</b>										
hyperplasia	0	0	2	5	10*	1	3	2	4	9*
hyperkeratosis	1	1	2	1	7	2	3	4	4	9
<b>SKELETAL MUSCLE</b>										
mineral deposits	0	0	0	1	7*	1	0	0	0	1
sarcolysis	0	0	1	2	8*	1	0	0	1	1

\*p<0.003; liver necrosis average grades were 3 (moderate) for 25 ppm and 2 (mild) for 125 ppm.  
p<0.005

Mouse HPLC PK data for this study (p.5) at two years are <0.1, <0.1, 1, and 6-8x the human Cmax at a 300 ug/day dose.

**ELECTRON MICROSCOPY LIVER (periportal region; 0 & 125 ppm) (vol 1.28; p.1396)**

"slight increase of the smooth and rough endoplasmic reticulum and mitochondria in hepatocytes" with "many vesicle-like and cisterna-like expansions". The sponsor stated that the effect was similar to what was seen with lovastatin but the quantitative difference, "lower intensity of the findings, was presumed

APPEARS THIS WAY ON ORIGINAL

**CARCINOGENICITY STUDY IN MICE**  
**HISTOPATHOLOGY (NEOPLASTIC) (terminal kill; n=48-50/s/g)**

Dose (ppm)	Male					Female				
	0	1	5	25	125	0	1	5	25	125
No. mice	50	50	48	49	46	49	49	49	50	49
<b>LIVER</b>										
Adenoma	7	7	8	18	24	0	3	2	7	21
Carcinoma	0	2	8	13	9	0	0	0	2	2
Tumors total	7	9	16	31	33	0	3	2	9	23

**p values in male mice (carcinoma and multiple carcinoma at terminal necropsy)**

Males	controls	1 ppm	5 ppm	25 ppm	125 ppm
pairwise	-	-	0.003	0.0002	<0.0001
trend test	-	0.25	0.001	<0.0001	<0.0001
<b>Males (adenoma and multiple adenoma at terminal necropsy)</b>					
pairwise	-	-	-	0.008	0.0001
trend test	-	-	0.444	0.004	<0.0001

**p values in female mice (carcinoma and multiple carcinoma at terminal necropsy)**

Females	controls	1 ppm	5 ppm	25 ppm	125 ppm
pairwise	-	-	-	0.253	0.247
trend test	-	-	-	0.064	0.023
<b>Females (adenoma and multiple adenoma at terminal necropsy)</b>					
pairwise	-	-	-	0.007	<0.0001
trend test	-	-	0.206	0.005	<0.0001

**DIETARY CARCINOGENICITY STUDY IN RATS (0,0.1,0.5,2.5 ppm) (vol 36-43)**

PH 23558 (T4039903). May 1991- May 1993. Bayer AG, Wuppertal, Germany.

Lot#: 507277 May to September 1991

509236 until January 1992

509269 until August 1992

511284 until November 1992

511239 until March 1993

513240 until May 1993

Lots were analyzed for purity *one year before date of first use* and ranged from 97 to 99%.  
Storage: at RT in a dessicator with protection from light

Auxiliary: 1% Peanut oil (DAB 9 or 10) "to minimize dust formation"

Diet mixture: The required quantity of drug was first dissolved in peanut oil and mixed into rest of feed. Mixtures were made up the week before needed (until week 78); mixtures then were made up for the week after next (weeks 79-104). "Drug was stable for 1-3 weeks."

**TREATMENT:** Five groups of Wistar rats from [REDACTED] (50/s/g; 4-5 weeks old on arrival) were given in the diet containing 1% peanut oil, 0, 0.1, 0.5, and 2.5 ppm (0, 6, 30, 160, 370 ug/kg/day (males) and 8, 40, 200, and 490 ug/kg/day (females) (p.10).

*Additional groups of 10/s/g were treated for 12 months for interim analysis. Twenty males and 20 females received 5 ppm for 12 months to test the MTD (these had no hematology or clinical chemistry exams). A satellite group received 1500 ppm lovastatin (50/s/g + 10/s/g interim kill) which was dropped to 500 ppm in DW 4 (30 mkd for males and 43 mkd for females).*

They were housed in Makrolon cages with wood granulate bedding. In week 61, the whole study was moved (with the rats in closed containers) to another building "nearby".

Diet was Altromin 1321 meal available ad libitum. Intake was calculated weekly (until week 13) for the first 20/g and then every 4 weeks (week 14-104). Blood for hematology and clinical chemistry (one week apart) was taken weeks 27/28, 52/53, (& 79 differential) and 104 on 10/s/g. "As far as possible, the same rats were used." Blood was collected from the retro-orbital venous plexus. TK was done on blood collected between 10 am and 12 noon. EM was done on liver from the first 5 rats/s scheduled for interim kill in the 0, 2.5, and 5 ppm and lovastatin groups and the first 5 rats/s scheduled for terminal kills in the 0, 0.5, and 2.5 ppm and lovastatin groups.

## CARCINOGENICITY STUDY IN RATS (vol 1.38)

### RESULTS

**CLINICAL SIGNS** (incidence over first 3 months provided in Table 1; 50/s/g):

**0, 0.1, 0.5, 2.5 ppm:** no clinical signs listed

**5 ppm (males):** rough coat (4); loss of wt (5); poor condition (3); weakness in hindquarters (4)

**5 ppm (females):** " (4); " (12); " (3); " (3)

Lovastatin: similar findings to those with 5 ppm Bay w 6228 (but higher incidence)

**MORTALITY:** No drug effect except at 5 ppm where 60% of males and 50% of females died (out of 20/g) after 6 months treatment. 30% of lovastatin rats died during this time period.

**BODY WEIGHT:** The 5 ppm and lovastatin groups did not gain weight for the first 2 weeks; after that, the gain paralleled the other groups (p.52) and "by week 51 the males were 5% and females 7% lighter than controls" (p.51).

**DRUG INTAKE** calculated from mg food consumed (p.56):

**FOOD CONSUMPTION and DRUG INTAKE (averaged over 2 years except 5 ppm=1 yr)**

DOSE (ppm)	Food Intake		Drug Intake			
	g/animal/day		mg/rat/day		ug/kg/day	
	male	female	male	female	male	female
0	21	17				
0.1	21	17	2	2	6	8
0.5	20	16.7	10	8	30	40
2.5	20	16.9	50	42	160	200
0	21	17.6				
5	20	17.2	100	86	370	490

**WATER INTAKE:** "increased 12% at 5 ppm"

**HEMATOLOGY** (main study with doses up to 2.5 ppm): no effects up to 2.5 ppm

**CLINICAL CHEMISTRY** (significant findings of main study up to 2.5 pm; 10/s/g):

ALKP: increased 40% at 2.5 ppm females wk 28

GLDH: decreased 90% at 2.5 ppm & lovastatin (males/females wks 28, 53, 78, 104)

LDH: decreased 40% sporadically at 2.5 ppm and lovastatin

Cl: increased 2-4% in all treated males wks 53, 78, 104; females week 104

TG: decreased males/females at most times usually at 0.5 ppm

**CARCINOGENICITY STUDY IN RATS** (vol 1.38)

**URINALYSIS** (significant findings):

VOL: decreased all treated males wk 27, 51

**OPHTHALMOLOGY:** no drug effects on cornea or lens turbidity (most rats had lens turbidity listed as "all degrees"; most males had cornea turbidity, "all degrees")

**ORGAN WEIGHTS** (interim and terminal kills):

no toxicologically significant effects on brain, adrenal, heart, lung, liver, spleen, kidney, testes

**NECROPSY** (interim kill; 10/s/g):

	Males	Females
	0, 0.1, 0.5, 2.5, 5 Bay; 500 lova [gps 1-6]	
Liver (areas discolored*):	0, 0, 0, 1, 2; 5	0, 0, 0, 0, 2; 4
Skeletal muscle (regression):	0, 0, 0, 0, 1; 9	0, 0, 0, 0, 1; 9
Eyes (discolored):	0, 0, 0, 0, 2; 1	0, 0, 0, 0, 5; 0
Stomach (areas):	0, 0, 0, 0, 3; 0	0, 0, 0, 1, 4; 0
Intestine (changes content)	0, 0, 0, 0, 7; 1	0, 0, 0, 0, 2; 0
Lungs (discoloration)	0, 0, 0, 0, 8; 0	0, 0, 0, 0, 2; 0

\*"pale discoloration and tiny beige areas" (vol 1.39; p.254)

"May include necrosis or hemorrhage" (submission of 3/7/97)

**NECROPSY (terminal kill; 50/s/g; 0, 0.1, 0.5, 2.5, 500 lova):**

	Males	Females
Liver (areas discolored)*:	2, 5, 4, 8, 8	1, 2, 1, 18, 10
(cysts)	1, 1, 1, 5, 7	3, 1, 3, 8, 13
(surface changes)	0, 0, 0, 0, 0	0, 1, 1, 4, 3
Skeletal muscle (regression):	0, 0, 0, 0, 10	0, 0, 0, 1, 14
Testes (smaller)	8, 1, 0, 3, 11	
(consistency change)	6, 7, 5, 4, 13	

\*"pale discoloration and tiny beige areas" (vol 1.39; p.254)

"May include necrosis or hemorrhage" (submission of 3/7/97)

**HISTOPATHOLOGY (Liver and Skeletal Muscle):**

Interim (see table 1; appendix)

Terminal (see table 2; appendix)

**RAT CA STUDY (Histopathology findings in 5 ppm group treated for one year)**

**FINDINGS IN RATS THAT DIED:**

Heart: necrosis, auricular thrombosis, inflammation of atria, inflammation/fibrosis ventricle, vacuoles

Lungs: congestion, edema, alveolar macrophages increased

Liver: centrilobular and non-zonal necrosis, hepatocellular atrophy

Tongue: muscle fiber necrosis

Skeletal muscle: fiber necrosis, atrophy, fatty tissue replacement

Pancreas: acinar hypertrophy, islet cell hyperplasia

Kidney: dilated tubule/collecting duct, congestion

Lymphoid organs: atrophy, lymphoid depletion, lymphocytic necrosis

Salivary glands: acinar atrophy

Lungs: edema, congestion

Testes, Epididymis, Prostate, Seminal Vesicles: "juvenile"\*

Epididymes: oligospermia, spermatid giant cells

\* maturation retarded at the time of death (subm. 4/22/97); however, sperm in epididymes implies sexual maturity as does the age (5-7 weeks old= sexual maturity in rats and rats were 5-6 weeks old at the start of study and 7-8 weeks old when killed).

**LIVER (EM FINDINGS):** Enlarged hepatocytes with swollen mitochondria; increased rough and smooth endoplasmic reticulum at 2.5 ppm (and lovastatin). "Taken together, these morphological and biochemical changes are signs of liver damage, which were evident at 0.5 ppm BAY w 228 and above..." (p.105).

**NEOPLASTIC FINDINGS (terminal necropsy):** none drug-related (vol 1.38, pp.94-97)

**DOMINANT LETHAL TEST (POSITIVE CONTROL)** (Submission: 10/3/94)  
Study 21907. BAYER. November 11, 1991.

Mice (NMRI strain from Winkelmann, Borcheln; ages 8 to 12 weeks according to body weights)  
Males (20/g) were given a single oral dose of 40 mg/kg or 80 mg/kg cyclophosphamide.  
Males were mated for 6 periods (4 days each).

There were no effects on clinical signs, food consumption, appearance, or mortality.  
Cyclophosphamide had no effect on fertilization rates or pre-implantation losses. Post-implantation losses were statistically significant at the 80 mg/kg dose during the second mating period and total test.

**DEAD IMPLANTS (PER FERTILIZED FEMALE) (Positive control)**

Mating period	control	40 mg/kg	80 mg/kg
1	1.06	1.17	1.71
2	0.57	0.94	2.76*
3	1.57	1.75	2.39
4	1.63	1.40	2.00
5	0.93	0.67	1.38
6	0.75	0.93	0.88

**Cerivastatin: test #1**

Mating period	control	25 mg/kg	50 mg/kg
1	1.00	0.95	0.86
2	1.20	0.66	1.07
3	0.78	0.80	1.68*
4	0.95	1.05	0.69
5	1.02	1.02	0.85
6	0.74	0.69	1.16
7	1.07	0.81	1.81*

APPEARS THIS WAY ON ORIGINAL

**Cerivastatin: test #2**

Mating period	control	25 mg/kg	50 mg/kg
1	0.86	1.00	0.90
2	1.05	1.15	0.87
3	0.87	0.89	0.95
4	0.75	1.02	0.76
5	0.74	0.71	0.74
6	0.58	0.77	0.98
7	1.07	1.40	1.04

\*P&lt;0.05

**DOMINANT LETHAL TEST**(cerivastatin)

Study #1: February 1991; 3/51 males died; mice were 8 to 12 weeks old; batch 518801

Study #2: May 1995; no adverse effects; mice were 4 to 12 weeks old; batch 513312

**DEVELOPMENTAL GAVAGE TOXICITY (SEGMENT II) IN RATS** (vol 1.64)

MTDO285. ██████████ May 1991.

Batch#: 518832 (prepared in d.water)

**TREATMENT:** Four groups of female CD rats (55/g; 10 weeks old) were mated with 15 week-old males. Pregnant females were given, by oral gavage in d.water, 0, 32, 160, or 720 ug/kg/day on days 6 to 15 of gestation. On day 16, 10/g were killed for blood and tissue samples; on day 20 of gestation, 30/g were killed and on day 21 of lactation, 15 females were killed.

On day 4 of lactation, each litter was reduced to 8 (4/s). Randomly selected neonates were subjected to sensory and reflex tests. One male and 1 female from 15 litters/g were retained for a 70-day growth and development period after which they were mated to determine fertility.

**RESULTS (F0)****MORTALITY:** one "treatment-related" (died day 20 gestation; ataxic, chromodacryorrhea)**CLINICAL SIGNS:** no changes due to drug**BODY WEIGHT/FOOD CONSUMPTION:** no drug effects**ORGAN WTS (% of BW):** No effect on liver or heart on day 16**(F0)****GROSS PATHOLOGY:** One HD had reddened margins of eyelids, bloody vagina, gastric mucosa dotted with pinpoint to pinhead bloody areas, small intestine contents liquid**CLINICAL PATHOLOGY****AST, ALT, ALKP, GGT:** no effects**Cholesterol:** increased 40% in HD**TG:** decreased 40% (at 160 and 720 ug/kg)**F0: HISTOPATHOLOGY:** "no treatment-related findings"

**F0: REPRODUCTIVE EFFICACY:**

No. Pregnant/40: 37, 35, 38, 37  
 No. Corpora lutea: 16.2; 17.0, 16.5, 17.5  
 Litter size: 14.3; 14.6, 14.4, 15.1  
 Wt male: 4.0; 4.1, 4.1, 4.0  
 Wt female: 3.8; 3.8, 3.9, 3.8  
 % postimplantation loss: 8.3; 6.1, 5.7, 5.7

**DEVELOPMENTAL GAVAGE TOXICITY (SEGMENT II) IN RATS**  
 0, 32, 160, or 720 ug/kg/day on days 6 to 15 of gestation

**F1: EXTERNAL (litter basis but not broken down into malformations vs variations)**

No. Litters= 27 24 30 28 (all findings in one fetus in the HD litter)  
 Cleft lip: 0 0 0 1  
 Agnathia 0 0 0 1  
 Microstomia 0 0 0 1  
 Cleft palate 0 0 0 1

**F1: VISCERAL FINDINGS**

	No. Litters= 25	23	27	27
Major vessels innominate, short	0	0	0	1

**SKELETAL FINDINGS**

	No. Litters= 26	25	27	27
Vertebrae: lumbar center incompletely ossified	0	5	6	9** (p<0.01)
Skull: bone unossified, fused, abnormal, malpositioned, extra abnormal bone, sutures fused	0	0	0	1

**F1 NEONATES (necropsy findings for those surviving)**

No. Litters=	15	15	14	13
Anophthalmia	0	0	0	1
Optic nerve(s) missing	0	0	0	1

**Historical control data from 15 studies given on fetal basis (vol 1.66, pp 430-451).**

Submission of 4/22/97: In 15 of the "most current" Segment II studies from this lab, anophthalmia was seen in one control group/25(?) control groups/study implying that 14 studies x 25 control litters/study had no findings; it was not listed in the table of external findings.

Missing optic nerves was also not listed and no data was supplied..

Finding	% of fetuses in 15 studies
skull bone unossified	0.13
skull bones abnormal	0.08
Skull bones malpositioned	0.04
Cleft palate	0.01

**F1** (weight and developmental indices: reflex, sensory, learning, activity tests):  
no drug effects but data are *very* variable and no S.D. are provided.

**DEVELOPMENTAL GAVAGE TOXICITY (SEGMENT II) IN RATS**  
0, 32, 160, or 720 ug/kg/day on days 6 to 15 of gestation

**F1 MATING/GESTATION**

No. Litters= 13, 13, 15, 12

No effect on copulation, fertility, gestation index

Litter size: 15.8; 14, 13.8, 13.8

% preimplantation loss : 1.7; 2.5, 12.9, 10.5

% postimplantation loss: 5.9; 7.2, 5.4, 3.8

External findings: appear in control and MD except shared implantation site in 1/12 HD litters

**PERI- AND POSTNATAL GAVAGE (SEGMENT III) STUDY IN RATS** (vol 1.67)

MTD0295. Toxicology, Miles Inc., Elkhart, IN. May 1991.

Lot#: 518832

**TREATMENT:** Four groups of pregnant Charles River Sprague-Dawley CD rats (30/g; 13 weeks of age) were given, by oral gavage in d.water, 0, 32, 100, or 320 ug/kg/day (from day 15 to day 21 of lactation). The dosing solutions were stored in a refrigerator (7° C) and stirred during administration. On day 4 of lactation, each litter was culled to 4/s (culled neonates were sacrificed and necropsied). Randomly selected neonates were subjected to a battery of sensory and reflex tests. On day 21 of lactation, all dams were sacrificed and necropsied.

One/s of neonates from 15 litters/g were retained for a 70-day growth & development period after which the F1 generation was mated.

**RESULTS (F0)**

**MORTALITY:** One non-tx related at 32 ug/kg and one "treatment-related" at 320 ug/kg

**CLINICAL SIGNS (30/s/g):**

Arched back	0	1	0	5
Hypoactive	0	1	0	6
Dried, reddish substance around eyes	0	0	0	1
Weakened body tone	0	0	0	1
Dyspnea	0	0	0	1

**BODY WEIGHT:** no effects (gestation); HD decreased gain during lactation (p<0.01)

FOOD CONSUMPTION: no effects (gestation); no data (lactation)

**CLINICAL CHEMISTRY (F0)**

AST, ALT, ALP, GGT, TG: no effects

Cholesterol: increased HD ( $p < 0.05$ )

**ORGAN WEIGHTS (heart and liver as %BW):**

liver increased 20% HD ( $p < 0.01$ )

**PERI- AND POSTNATAL GAVAGE (SEGMENT III) STUDY IN RATS**

0, 32, 100, or 320 ug/kg/day (from day 15 to day 21 of lactation)

**F0: NECROPSY:** no significant drug-related findings

**F0: HISTOPATHOLOGY (25/g)**

(Control and HD for liver, kidney, uterus, bladder; stomach and muscle=all groups):

	0	32	100	320 ug/kg/day
LIVER (hypertrophy hepatocyte)	1	nd	nd	3
STOMACH (acanthosis)	1	0	1	7
SKELETAL MUSCLE				
Degeneration	0	0	0	6
Granuloma	0	0	0	4
Calcification of muscle fibers	0	0	0	3

nd= no data

**F1: REPRODUCTIVE EFFICIENCY**

No effects on fertility, litter size, no. implantations, birth index

Total Dead pups (stillborns + deaths; %) 1 2 2 11\*

One HD litter of 20 lost day 2 (cannibalization & maternal neglect)

One HD litter of 16 lost day 5 when dam died

**F1: CLINICAL FINDINGS:** none drug-related

**NECROPSY FINDINGS:**

Neonates that died: none drug-related

Neonates that lived: none drug-related

Weight and developmental indices: no drug effects but no S.D. provided

Reflex, sensory, learning, and activity tests: no drug effects but no S.D. provided

WEIGHT GAIN (g for 15/s/g; day 0-70): 320±11, 332±14, 370±16, 390±11 (mean±SE; males)  
160±5, 170±6, 160±6, 160±3 (Mean±SE; females)

FOOD CONSUMPTION: no effects at any time during growth or gestation

MALE or FEMALE REPRODUCTIVE PERFORMANCE: no effects

**F1: NECROPSY**

TESTES: bilateral, soft to touch 1 HD  
pinkish discoloration 1 HD

**PERI- AND POSTNATAL GAVAGE (SEGMENT IID STUDY IN RATS**

**0, 32, 100, or 320 ug/kg/day (from day 15 to day 21 of lactation)**

**F1: REPRODUCTIVE EFFICACY**

% Pregnant: 100, 100, 100, 93  
No. Corpora lutea: 16.2; 17.0, 16.5, 17.5  
Litter size: 14.4; 14.3, 13.7, 14.2  
Wt male: 3.8; 3.8, 3.7, 3.8  
Wt female: 3.6; 3.5, 3.6, 3.5  
% postimplantation loss: 9.0; 12, 4.5, 4.9  
% preimplantation loss: 3.4; 9.0, 5.8, 11.6

**F2: EXTERNAL FINDINGS: none drug-related**

**RANGE-FINDING FOR RABBIT TERATOLOGY STUDY (vol 1.66)**

T6040660. Bayer, Wuppertal, Germany. November 1991.

Batch#: 509236

TREATMENT: Rabbits (strain CHBB:HM; Himalyan) arrived in September and October 1991. Doses used were 0, 30, 150, and 750 ug/kg/day by gavage in d.water on (days 6 to 18 post coitus in 3/g). Rabbits were killed on day 19 gestation. Blood was sampled for TK analysis on days 6 and 18 (see ADME section).

**RESULTS**

MORTALITY: none

CLINICAL SIGNS: none, little, soft, or small fecal pellets present as a f(dose)

BODY WEIGHT GAIN: +80; +103, -5, -144 g (days 6-18 of gestation)

FOOD INTAKE: reduced at M and HD

WATER INTAKE: reduced HD

GROSS PATHOLOGY: liver punctate with 1 mm discolored spots (1/3 HD)

**HISTOPATHOLOGY**

Liver (single cell necrosis): 2/3 HD

Skeletal muscle (fibre necrosis): 1/3 HD

**TERATOLOGY STUDY IN RABBITS (vol 1.66)**

T40407049. Bayer, Wuppertal, Germany. February 1992.

Batch#: 509236

TREATMENT: Himalayan rabbits (2-2.8 kg, strain CHBB:HM) arrived in July, September, and October 1991 and January 1992. Doses used were 0, 30, 150, and 750 ug/kg/day by gavage in d.water on (days 6 to 18 post coitus in 15/g). The historical control data for malformations (p.188) is from 1985 to 1990 (one litter in 1991).

**F0: RESULTS**

**MORTALITY:** one HD (day 18) ruffled fur; bloody excretion days 17 and 18

**CLINICAL SIGNS:** one HD dragged hind limbs day 17

**BODY WEIGHT GAIN (0,L,M,HD):**

<u>Day 6-18</u>	<u>Days 0-29</u>
82; 41, 53, 40 g	270; 240, 220, 270 g

**FOOD CONSUMPTION** (calculated as g/animal/day rather than g/kg/day): scattered statistical significance at L and MD

#### GROSS PATHOLOGY

Female that died: reddened margins of eyelids, bloody areas on gastric mucosa, intestine filled with small amount liquid content.

<b>F0: REPRODUCTIVE DATA</b>	<u>(0,</u>	<u>30,</u>	<u>150,</u>	<u>750 ug/kg)</u>
Inseminated:	13	15	15	13
Corpora lutea:	9.0	9.0	8.1*	8.2
Implantations:	7.3	7.4	6.9	7.0
Animals with viable fetuses:	13	15	15	13
Male:female:	1:0.70	1:0.69	1:0.66	1:0.93

#### PLACENTAS

coarse-grained: one LD

partly necrotic: one MD

#### VARIATIONS

No summary table

**MALFORMATIONS:** none of the skeletal effects were treatment-related (vol 1.66, p.27)

#### SECRETION OF BAY W 6228 INTO THE MILK (vol 1.80)

24304(P). Bayer. July 1992

Four lactating rats (day 8 of lactation) were given a single dose of 2 mg/kg of [<sup>14</sup>C]Bay w 6228 with milk collected up to 48 hours postdose. Between 4 and 24 hours the ratio of milk to plasma ranged between 1.2 and 1.7.

	Arithmetic mean	
Milk	AUC (ug h/ml)	4.4
	Cmax (ug/ml)	0.2
	tmax (h)	6
	MRT (h)	22
	t1/2 (h)	14
Plasma	AUC (ug h/ml)	3.7
	Cmax (ug/ml)	2.8
	tmax (h)	4
	MRT (h)	22
	t1/2 (h)	15
milk/plasma	AUC	1.3
	Cmax	1.3

### PLACENTAL TRANSFER IN PREGNANT RATS (vol 1.79)

PH 24832. Bayer. February 1996.

[<sup>14</sup>C]BAY w 6228 was given to pregnant Wistar rats about 3 days before delivery (day 19 of pregnancy) at single doses of 2 mg/kg (po; n=4) or 1 mg/kg (i.v.; n=2). Rats were killed 5 minutes and 2 hours (i.v.) or 1, 3, 7, and 24 hours (po) postdose. [redacted] as well as [redacted] was done.

Oral dosing: Liver, gi tract, and kidneys of dams and fetuses had the most counts at all times postdose. About 40-60% of liver counts were parent drug; 8% was lactone and rest were unknown metabolites. The unbound fraction in dams was 2.3% vs 5.5% in fetuses.

### TISSUE LEVELS RADIOACTIVITY (after one 2 mg/kg dose to pregnant rats)

(one rat/time point with levels measured by autoradiography)

Tissue	1 h postdose	3 h	7 h	24 h
Liver	v.high (d)	ext high (d)	ext high (d) high (f)	ext. high (d) very high (f)
Kidney	m.high (d)		high (d) low (f)	low (d) low (f)
Cranial nerves		med (d)		med (d)
Urine		high (d)	v.high (d)	low (d)
Bronchia		m.high (d)	v.high (d)	high (d)
Adrenal gland		m.high (f)	med (d) low (f)	low (d) low (f)
Pancreas			med (d)	low (d)
Myocardium			med (d) low (f)	low (d) low (f)
Adipose tissue			med (f) low (d)	
Skin			med (f) low (d)	

**UBIQUINONE AND VITAMIN E LEVELS IN DOG PLASMA** (vol 1.70)

21592 (P). Bayer. July 1992.

Plasma samples from a 1-year dog toxicity study were analyzed for ubiquinone and vitamin E levels after 16, 28, and 52 weeks of exposure to Bay w 6228 using an [REDACTED] assay with [REDACTED] detection.

Nanomoles/ml ( ) in plasma after one year treatment

dose (ug/kg)	Ubiquinone 50		Vitamin E	
	Male	Female	Male	Female
control	0.28 ± 0.05	0.28 ± 0.1	26 ± 5.4	28 ± 13
8	0.21 ± 0.03	0.21 ± 0.05	22 ± 6.7	27 ± 5
25	0.18 ± 0.04	0.17 ± 0.03	26 ± 3.2	21 ± 2
70	0.23 ± 0.02	0.14 ± 0.01	25 ± 5.0	24 ± 4
control + mevalonic	0.37 ± 0.07	0.32 ± 0.07	29 ± 0.5	40 ± 16
70 + mev	0.16 ± 0.01	0.12 ± 0.02	21 ± 3.2	18 ± 3

Mean±SD (n=4/s/g)

**UBIQUINONE AND VITAMIN E LEVELS IN DOG TISSUES** (vol 1.70)

T1 039 793. Bayer.

Tissue samples were collected at necropsy from the 1-year dog toxicity study, stored at -20°C, and analyzed by [REDACTED] assay for ubiquinone 50 (coenzyme Q) and vitamin E. Heart, thigh muscle, liver, kidneys, brain, and testes were analyzed after 24 weeks (2/s/g) and 54 weeks (4/s/g).

Millimoles of Ubiquinone 50 /gram tissue after one year treatment with bay w 6228 (dogs)

dose (ug/kg)	HEART		SKELETAL MUSCLE		LIVER	
	Male	Female	Male	Female	Male	Female
control	110 ± 38	180 ± 66	64 ± 2.7	59 ± 30	24 ± 6	21 ± 4
8	74 ± 22	72 ± 10	47 ± 3.7	22 ± 7	30 ± 6	14 ± 4
25	52 ± 21	47 ± 14	61 ± 16	14 ± 6	21 ± 5	11 ± 3
70	32 ± 8	41 ± 4	33 ± 10	11 ± 1.7	9 ± 3	5 ± 1
control + mevalonic	120 ± 1.7	130 ± 11	73 ± 18	33 ± 3.4	26 ± 3	27 ± 12
70 + mev	37 ± 1.4	48 ± 26	40 ± 7	18 ± 13	17 ± 3	21 ± 2

Mean±SD (n=4/s/g for main study; 2/s/g for drug + mevalonic)

**Millimoles of Ubiquinone 50 /gram tissue after one year (dogs)**

dose (ug/kg)	KIDNEY		TESTIS
	Male	Female	Male
control	41 ± 8	51 ± 10	3.2 ± 0.1
8	49 ± 10	31 ± 6	3.1 ± 0.5
25	35 ± 11	24 ± 6	2.6 ± 0.5
70	23 ± 3	24 ± 5	2.5 ± 0.4
control + mevalonic	54 ± 19	62 ± 7	3.1 ± 0.2
70 + mev	64 ± 24	44 ± 3	2.8 ± 0.1

Mean±SD (n=4/s/g for main study; 2/s/g for drug + mevalonic)

**Millimoles of Vitamin E/gram tissue after one year treatment with bay w 6228 (dogs)**

dose (ug/kg)	HEART		MUSCLE		LIVER	
	Male	Female	Male	Female	Male	Female
control	25 ± 13	31 ± 2.5	31 ± 10	57 ± 30	39 ± 4.6	37 ± 13
8	30 ± 8.4	34 ± 5	26 ± 3	32 ± 26	45 ± 15	47 ± 8.7
25	34 ± 12	23 ± 5	73 ± 37	15 ± 15	41 ± 13	34 ± 8.9
70	20 ± 7	20 ± 14	40 ± 10	17 ± 8	16 ± 7	22 ± 7.2
control + mevalonic	20 ± 2.5	45 ± 19	62 ± 44	31 ± 4	31 ± 5.5	63 ± 12
70 + mev	20 ± 7.1	27 ± 12	32 ± 4.6	4.3 ± 6.1	32 ± 1.5	51 ± 5.3

Mean±SD (n=4/s/g for main study; 2/s/g for drug + mevalonic)

**Millimoles of Vitamin E/gram tissue after one year (dogs)**

dose (ug/kg)	KIDNEY		BRAIN		TESTIS
	Male	Female	Male	Female	Male
control	107 ± 34	150 ± 34	43 ± 4.0	50 ± 8.1	42 ± 13
8	140 ± 62	160 ± 66	44 ± 9.6	49 ± 3.4	62 ± 15
25	160 ± 50	120 ± 30	54 ± 12	44 ± 6.0	45 ± 12
70	95 ± 29	260 ± 110	53 ± 11	45 ± 3.6	39 ± 7.7
control + mevalonic	110 ± 8.9	160 ± 3	33 ± 1.3	53 ± 3.9	43 ± 7.4
70 + mev	130 ± 46	170 ± 33	51 ± 3.6	49 ± 5.6	72 ± 13

Mean±SD (n=4/s/g for main study; 2/s/g for drug + mevalonic)

**STUDIES ON METABOLITE BAY 17-5111** (vols 1.22, 1.70-1.74 & 1.80; required by Japan)  
SBL 95-55 (vol 1.70):

I.V. dosing in mouse (250, 500, and 1000 mg/kg): deaths at 500 and 1000 mg/kg. At 250 mg/kg, a decrease in spontaneous activity and bradypnea up to 3 hours postdose with no abnormalities on necropsy. Estimated toxicity to be 1/2 to 1/4 of parent drug.

**ANALYSES OF DOG LENS (1-year dog toxicity study) (1.54)**

Part of dog study T 1039793. March 27, 1992. [REDACTED]  
92-0/2-54.

Eyes were trimmed, weighed, and deep-frozen on a freezing stage and separated with a trepan into a central cylinder and equatorial ring. Three lenses each from control group I (0) and groups II-IV (8, 25, and 70 ug/kg/day) and 2 lenses from groups I (mevalonic acid) and V (mevalonic acid + 70 ug/kg BAY) were used. Enzyme activities were measured: aldolase, LDH, PFK, GAPDH as well as crystallines (on polyacrylamide gels) and some lipids. ATP, ADP and AMP were to be measured but, unfortunately, were not as they are good indicators of tissue status.

There were no differences detected that were statistically significant although the sponsor stated that there was a "marked increase" in GAPDH (60% at the high dose on a "volume basis"); however, by specific activity (units/mg protein), the SD appeared to overlap.

**ASSESSMENT OF TESTOSTERONE LEVELS (1-year dog toxicity study) (vol 1.54)**  
November 1992. [REDACTED] for

[REDACTED] looked at the testosterone data from males in the 1-year study above and concluded that levels were similar to those in the literature.

**ENDOCRINE MONITORING IN 1-YEAR DOG STUDY** (vol 1.56)

T0040367. Animal study and blood collection and shipment (Bayer, Wuppertal)

Hormone assays: [REDACTED]

**TREATMENT:** This study was part of study T 0040367 (p.23 of review). Initial blood samples were taken July 1991 (week 0). After 58 weeks, males had a hemiorchiectomy. Blood was collected weeks 0, 1, 2, 5, 12, 19, 25, 32, 38, 51, 57, and 70. GNRH challenge tests were done at weeks 56 and 71. Samples were shipped on dry ice and stored at -20° C until analysis. Estradiol, testosterone, and LH were analyzed by RIA (sheep and rabbit antiserum). Four randomly selected dogs/s/g were tested, but no list was provided as to which these were.

**RESULTS:** Most of the data were in the form of lists of individual data points. There were two tables of GNRH stimulation data for weeks 56 and 71 (with data as figures for 4 male controls, 3 male treated, 2 female controls, and 2 female treated vs 4/s/g analyzed).

**ENDOCRINE MONITORING IN 1-YEAR DOG STUDY** (continued)

No drug effects were seen between controls and treated in estradiol or testosterone levels over time, but there is no information as to time of day sampled, and there were wide changes over time. Estradiol concentrations in female dogs were stated to be "below proestrus levels at the

time points investigated suggestive of a lack of ovarian cyclicity". *In fact, estradiol levels were almost identical in male and female dogs!*

LH and testosterone levels after GNRH stimulation were provided for weeks 56 and 71 only and the variability was so great that no conclusions were possible.

LH in Control Male Dogs (mean  $\pm$  SD)

37  $\pm$  16 ng/ml (week 56)

150  $\pm$  180 ng/ml (week 71)

#### ANDROLOGICAL STATUS OF MALE BEAGLES (vol 1.56)

Part of study T6055475 (1-year dog treatment in 12-16 month-old males at 0 and 100 ug/kg/day; 15/g; followed by hemiorchiectomy and 3 months recovery period). Animal study was done at Bayer, Wuppertal. [REDACTED], [REDACTED] analyzed testes and semen. Following a baseline of two measurements 3 weeks apart (=time 0; February and March of 1994), semen was collected every 13 weeks (=times 1 to 4) plus a fifth collection at the end of the recovery period (=time 5). There was no information on collection or storage of semen.

#### **RESULTS (0 vs 100 ug/kg/day; 15/g):**

**TESTICULAR MEASUREMENTS:** no effects on length, width, volume

**EJACULATE PARAMETERS:**

Volume: The treated group was smaller than control at time 5 (9.0 $\pm$ 6.7 vs 4.3 $\pm$ 4.0; p=0.051)  
time 4 (5.8 $\pm$ 6.0 vs 3.0 $\pm$ 2.9)

Total sperm: no significant effects

Per Cent Progressively Motile Sperm: Higher in controls at times 4 (p=0.006) and 5 (p=0.035) even excluding two treated dogs that had such a very small ejaculate volume that they could not be used.

Per Cent of Eosin-Stained Spermatozoa: Higher in treated at all times except baseline (values were significant at times 1, 2, and 4 but "within normal range").

Acrosome or Head Alterations (%): Higher in treated at time 4 (p=0.02)

Neck Alterations (%): no significant findings

Mid piece alterations (%): Higher in treated at all times (p ranges from 0.002 to 0.023)

End piece alterations (%): Higher in treated but not significant (SD too large)

#### ANDROLOGICAL STATUS OF MALE BEAGLES (continued)

##### **LIBIDO AND COPULATORY BEHAVIOR**

Semen collection was completed without prompting or difficulty in 7 controls and on the first stimulation in the remaining 8. On the other hand, four treated dogs had to be stimulated on some occasions two or more times before semen collection was possible, and this was often accompanied by a relatively short duration of ejaculation (<5minutes). (p.294)

The investigator hypothesized that the small ejaculate volume was the result of the short duration of ejaculation in treated dogs. "The significantly lower percentage of progressively motile spermatozoa observed in the treated group at time 4 coincides with the increase of

morphologically altered spermatozoa." *"The significantly higher percentages of morphologically altered spermatozoa found during the treatment period in the treated group are attached to medication influences. The increase is attributed to persisting cytoplasmatic droplets located in the midpiece region...indicating disturbances of epididymal sperm maturation...temporarily impaired by medication. The significant increases of acrosome and head alterations are thought to be another short-term medication effect."* (pp.295-296)

#### **ANALYSIS OF TUMOR RISK TO HUMANS (vol 1.74)**

The argument is that BAY w 6228 is non-genotoxic and that "liver tumors seen in mice are the result of hepatotoxicity associated only with the high doses used." However, these "high" doses are only high when calculated on the mg/kg basis. *On a Cmax basis*, the highest mouse dose resulted in an exposure that was 3x the human Cmax at the 300 ug/day dose; the mid-high mouse dose exposure was 1/4 to 1/2 that of humans (both mouse doses were carcinogenic).

#### **EFFECT OF DEN PRETREATMENT ON MOUSE LIVER NEOPLASMS (vol 1.70)**

March 1991.

Male B6C3F1 mice were divided into 9 groups that received either no treatment or were treated for 10 weeks with diethylnitrosamine (DEN) i.p. at doses ranging from 100 to 400 umoles/kg body weight followed by 4 weeks of no treatment (NT) followed by 24 weeks of NT, Bay w 6228 (0.5 mg/kg by gavage in water), or 2-acetylaminofluorene (AAF; 400 mg/kg in the diet). At weeks 20, 26, and 32, 5/s/g were killed; at week 38 the remaining mice were killed (14-27/g).

**RESULTS:** The problem with the study was that DEN alone caused hepatocellular adenomas in 80-100% of the mice; thus, there was no way to see if adding Bay w 6228 caused an increase. Furthermore, there were only 14 to 27 mice left for the final necropsy at week 38, not enough animals to power the study.

#### **EFFECT OF DEN PRETREATMENT ON RAT LIVER NEOPLASMS (2/27/97 to IND)**

9602. [redacted] of [redacted] submitted to Bayer Yakuin, Ltd, Osaka, Japan. Completed May 1996. Lot: 513312

**TREATMENT:** Male F344 rats (6-weeks of age) were given a single injection of DEN (200 mg/kg, ip) and 2 weeks later began receiving orally, by gavage, daily 0, 30, 100, or 300 ug/kg. Three weeks after DEN, rats had partial hepatectomy. All surviving were killed week 8. The positive control was sodium phenobarbital (spb; 500 ppm in the diet). Numbers and areas of glutathione S-transferase activity were quantitated/cm liver by [redacted] in 20/g. These are F344 rats whereas Wistar rats were used in the carcinogenicity study.

APPEARS THIS WAY ON ORIGINAL

Dose (mg/kg)	GST-P positive foci	
	no./cm <sup>2</sup>	area/cm <sup>2</sup>
0.0	3.3±1.7	0.28±0.15
30	2.8± 1.6	0.21± 0.18
100	3.0± 1.8	0.21± 0.13
300	2.6± 1.8	0.25± 0.19
spb*	6.0± 2.8	0.69± 0.43

\*spb: sodium phenobarbital

#### INFLUENCE OF BAY W 6228 ON PLATELET AGGREGATION (sub. 3/20/97)

PH 25214. Wuppertal. February -March 1996

**TREATMENT:** As part of T 4060341, four male beagle dogs were treated for two weeks with 200 ug/kg/day. Platelet-rich plasma was obtained one hour *before dosing on day 1 and one hour after dosing on days 1, 3, 5, 8, 10, and 12*. Platelet aggregation was determined by a turbidometric method in an [REDACTED] at 37<sup>0</sup> C. Collagen was added to induce platelet aggregation. All values were in reference to 0 hours on day 1 (=100%) after 30 ug/ml collagen addition.

**RESULTS:** Platelet aggregation in dogs was decreased by Bay w 6228 after 1 day and after 5 days the response was "completely reduced or only weak".

#### PLATELET AGGREGATION IN DOGS (%) (2-weeks at 200 ug/kg/day)

collagen (ug/ml)	day 1		day 3	day 5	day 8	day 10	day 12	day 15
	0 h	1h	1h	1h	1h	1h	1h	1h
30	100	111	94	74	47	21	7	11
8	115	104	77	78	0	0	-	2
3	100	112	0	0	0	-	-	-
1	109	0	0	-	-	-	-	-
0.6	0	-	-	-	-	-	-	-

**SUMMARY AND EVALUATION:** Cerivastatin will be the 6th HMG CoA reductase inhibitor to be approved for the purpose of lowering cholesterol. The drug functions by inhibiting HMG CoA reductase, an enzyme that catalyzes the early rate limiting step in the cholesterol synthetic pathway. Cholesterol is not the only important product of the pathway: ubiquinone (a coenzyme of the mitochondrial respiratory chain), dolichol (an intermediate important in adding oligosaccharide chains to proteins), and farnsyl pyrophosphate (adds farnsyl groups to proteins to facilitate membrane interactions) are also important end-products. Fat soluble vitamin levels can be effected since they are transported in LDL particles which decrease in number.

#### UBIQUINONE LEVELS (rats and dogs)

Ubiquinone 50 (CoQ<sub>10</sub>), "the most important ubiquinone in dog and in man", was measured in rats and dogs. Six-months of dietary treatment in rats (at a C<sub>max</sub> equal to that of humans at 300 ug/day) caused a 30-40% decrease in *heart muscle* ubiquinone. Dogs had decreased levels of ubiquinone in *heart and skeletal muscle, liver, and kidney* (males and females) after one year of treatment at 100 ug/kg/day with a C<sub>max</sub> approximately 12 times that of humans (based on PK from a 1-week study). Kidney ubiquinone, but not that of other tissues, could be kept from dropping by the addition of mevalonic acid. *Plasma levels* of ubiquinone decreased at all doses of cerivastatin as early as 16 weeks (the first measurement) but not as dramatically as tissue levels. In another one-year dog study (doses of 0, 8, 25, and 70 ug/kg/day), plasma ubiquinone levels declined after 16 weeks of treatment at the lowest dose tested (8 ug/kg; about 1x the human C<sub>max</sub>).

#### VITAMIN E LEVELS (dogs)

In a 1-year study (at 0 & 100 ug/kg/day), plasma vitamin E showed no measurable decline, but standard deviations were large. Vitamin E levels were not decreased in heart, skeletal muscle (except high dose females), liver (except high dose males), kidney, or brain; the lack of effect may have been due, in part, to the large standard deviations (see p.47). In another 1-year dog study (0,8,25,70 ug/kg/day), plasma vitamin E decreased as a function of dose (June 24, 1993 review).

#### ADME

The drug was metabolized in the liver and excreted by the bile and feces in all species. Bioavailability was high, about 70% in both rats and dogs when given as gavage doses. Greater than 90% of the dose was excreted by the bile with 50% of the radioactivity excreted being reabsorbed (in rats). In plasma, cerivastatin was primarily bound to serum albumin; the fraction unbound was 2.5% (rats and dogs) and 0.5% (man) over a drug range of 0.1 to 10 ug/ml. The systemic availability (measuring [redacted] material) was 50% less in fed vs fasted rats.

Metabolism (in vivo): An elucidation of the pathways of metabolism was attempted with male mice, rats, one male dog, and four male human volunteers: liver, plasma, bile, feces, and urine were analyzed from most species. There were technical problems with most of the studies: in *humans*, the absolute amount of radioactivity in plasma was very low, plasma was obtained at only two and four hours postdose, and there was a problem of cleanly separating metabolites on the [redacted] plates. *In the animal studies*, [redacted] was used to separate metabolites which along with the use of higher amounts of radioactivity, meant a better ability to separate and identify peaks. However, problems remained: a repetition of the liver analysis showed that results could not be repeated, "probably due to remaining enzymatic activity in the stored liver samples (-20° C)" (rats). Only one dog was examined; the sponsor stated that "Due to the fact that the labelled drug used in this experiment did not have the recommended purity, it was not used for quantitative balancing of bile pattern". Thus, *in vivo* metabolic pathways are tentative in all three species.

Metabolism (in vitro): Microsomes of male rats, female pigs, male micro-pigs, male rabbits, female dogs, male mice, and male and female rhesus monkeys were used to isolate metabolites of

BAY w 6228 using two [REDACTED] systems. Aliquots of microsomal mixtures were withdrawn at times up to three hours. The sponsor stated that "Microsomes do not represent the full metabolic capacity of the liver, since a variety of important metabolic reactions like phase II conjugations and -oxidation can not be observed. For cerivastatin, this limits an *in vitro/in vivo* correlation, because *in vivo* glucuronidation is a major pathway in the dog and -oxidation predominates in rat and mouse."

However, the sponsor stated that "In liver microsomes of all the animal species investigated here, demethylation to M1 is the major metabolic pathway." Humans had two primary biotransformation reactions: demethylation to M1 and hydroxylation to M23; M24 was formed by combination of both reactions and detected to a "lesser extent". There were four metabolites identified for humans and about a dozen for mice, rats, and dogs (but see above on reliability of metabolite measurements). "A significant amount of radioactivity (metabolites) was excreted in the urine (24% of dose)" with about 60% of these metabolites identified (submission of 3/20/97).

**TISSUE RADIOACTIVITY (Accumulation in rat tissues):**

AUC was increased when comparing 1 vs 22 doses: 2- fold (kidneys), 3-fold (plasma), and 4-fold (vitreous body, testes, seminal vesicles). Cmax was increased 2-fold (plasma), 4- to 5-fold (blood cells, kidneys, testes) and 7- to 9-fold (bone, vitreous body, and seminal vesicle). These levels could in reality be even higher since stability in samples was not provided, and in another study, the sponsor stated that they could not repeat the results on liver metabolites in samples stored at -20<sup>0</sup> C because of continued enzymatic activity.

PK DATA: [REDACTED] provided more reliable PK data (better reproducibility; less variability) than [REDACTED]. "Compared to the [REDACTED] assay, the bioassay results showed higher variation between -68% and +158% for unchanged BAY w 6228" (3/20/97 annual report). There were also *differences between labs*: the sponsor pointed out, that in the case of the rat carcinogenicity study, Bayer's [REDACTED] values were 2-3x higher than those of [REDACTED]. Unfortunately, most of the PK data available was obtained using the [REDACTED] since the [REDACTED] method only became available much later in drug development.

There was [REDACTED] data for the mouse and rat carcinogenicity studies. The PK study in pregnant rabbits using [REDACTED] employed a different strain and a different formulation from that used in the teratogenicity study. Three male dogs were analyzed after 1 week of gavage treatment (vehicle?) and three female dogs were analyzed after 1 dose (but they were dosed with capsules containing phosphate buffered saline instead of gavage in tap water as done in the toxicity studies)

APPEARS THIS WAY ON ORIGINAL  
[REDACTED]

**COMPARISON OF SPECIES "Cmax"**

Species	Sampling (gender)	Dose	Blood (ng/ml)	Ratio to man
<b>Dog (PK study)</b>	<b>1 week (male)</b>	<b>100 ug/kg</b>	<b>46±1.3</b>	<b>12x</b>
<b>Rat (CA study)</b>	<b>week 104 (male) (female)</b>	<b>2.5 ppm</b>	<b>0.9±2.3 5.0±2.2</b>	<b>0.2x 1x</b>
<b>Mouse (CA study)</b>	<b>week 52: (male &amp; female) week 104: (male) week 104: (female)</b>	<b>125 ppm</b>	<b>11±1.6 35±2.4 23±1.9</b>	<b>3x 9x 6x</b>
<b>Human (male)</b>	<b>1 week</b>	<b>300 ug</b>	<b>4±1.3</b>	<b>1</b>

HPLC method of analysis; Mean±SD (ng/ml or ng/g)

[3 males (dogs); 10/s/g (rats); 5 to 10/s/g (mice)]

These are all the highest doses tested in that particular study. Dogs were 1-year old and dosed similarly to those in the 1-year study.

**MOUSE TARGET ORGANS OF TOXICITY (at 3-8x human Cmax; see p. 33):**

Liver: eosinophilic, basophilic, and clear cell foci, necrosis (M/F)

Lymph nodes: adipose tissue (M/F)

Forestomach: hyperplasia (M/F)

Skeletal muscle: mineral deposits, sarcolysis (M)

Kidney: loss of autophagic vacuole (M)

Vacuoles are found in males only (dependent on testosterone levels); "absence considered secondary, possibly resulting from altered steroid metabolism" (not seen with other statins)

**RAT TARGET ORGANS OF TOXICITY (rats dying in 1-year study @ 5 ppm)**

Heart: necrosis, auricular thrombosis, inflammation of atria, fibrosis ventricle, vacuoles

Tongue: muscle fiber necrosis

Skeletal muscle: fiber necrosis and degeneration

Muscles of head, neck, around spinal cord, sternum, skin: cellular reaction to fiber necrosis

Lungs: congestion, alveolar macrophages increased

Liver: centrilobular and non-zonal necrosis, hepatocellular atrophy

Pancreas: acinar hypertrophy, islet cell hyperplasia

Kidney: dilated tubule/collecting duct, congestion

Lymphoid organs: atrophy, lymphoid depletion, lymphocytic necrosis

Salivary glands: acinar atrophy

**RAT TARGET ORGANS OF TOXICITY (rats dying in 1-year study @ 5 ppm)**

Tongue: muscle fiber necrosis

Lungs: edema, congestion

Testes, Epididymis, Prostate, Seminal Vesicles: "juvenile" in 12/20 males

Epididymis: oligospermia, spermatid giant cells

Forestomach: hyperplasia

**RAT TARGET ORGANS OF TOXICITY (rats necropsied after 1-year; see table 1 & below):**

Kidney: basophilic tubules, proteinaceous casts

**RAT TARGET ORGANS (rats necropsied after 2-years; see table 2 & below):**

Pancreas: acinar granulation acc (M)

Epididymis: intraepithelial globules (M)

Thymus: epithelial hyperplasia (M)

Lungs: interstitial pneumonia (F)

Mesenteric lymph node: histiocytosis (all treated females)

**DOG TARGET ORGANS OF TOXICITY**

(dogs at terminal necropsy or dying in 1-year study at 100 & 300 ug/kg):

Liver: degeneration, necrosis, focal fatty change, mesenchymal activation, parenchymal reaction

GI tract including gallbladder: mucosal erosions, hemorrhage

Pancreas: hemorrhage, edema

Lymphatic system: hemorrhage, edema, necrosis, lymphocyte depletion

Skeletal musculature: degeneration and necrosis

Tongue: myopathy

Heart: hemorrhage

Lungs: interstitial inflammation, focal fibrosis, edema

Eyes: cataracts

Testes: atrophy and vacuolization of the germinal epithelium, spermatidic giant cells

Epididymis: hypospermia, aspermia

CNS: microscopic multifocal hemorrhages;

Walls of blood vessels of choroid plexus, sciatic nerve, optic nerve, and ciliary body of eye:  
fibrinoid degeneration & reactive inflammation

(no clinical signs of CNS effects and no gross pathological changes in the brain)

**DOG TOXICITY:** "The two dogs killed in extremis showed the well-known lesions characteristic for the compound class with respect to liver, GI tract, including gallbladder, musculature and/or lymphatic tissue. In addition, there were degenerative changes of blood vessel walls in the choroid plexus (both dogs) or in the ciliary body of the eyes (one). This lesion (choroid plexus) was also present in one male of the second chronic study. *Vascular changes including fibrinoid wall degeneration and hemorrhage have been reported as induced lesions after statins. However, to our knowledge, they have so far not been observed in the choroid plexus and the ciliary body, both of neuroectodermal origin known for their close relationship in anatomy and physiology, respond to toxic injuries similarly. Fibrinoid vessel wall degeneration of the choroid plexus and the ciliary body are considered to be treatment-related in this study.*"

"Myopathy was *less prominent in the periphery (thigh) vs trunk (diaphragm, head, neck, tongue, eye muscles)* which might have caused dysphageia and aspiration of ingesta increasing risk of respiratory tract infection accounting for the chronic pneumonia (severe in one dog)."

Hemorrhage was observed microscopically at lethal doses in dogs along with a prolongation of PTT "indicating an influence on the pathways in blood coagulation". In a further study, there was "completely reduced or only weak" platelet coagulation observed in a 2-week coagulation study in dogs treated with 200 ug/kg/day; effects were seen as early as day 1 (p. 52).

"From previous toxicity studies in dogs, testes are known to represent a target organ for Bay w 6228." There was multifocal degeneration of the seminiferous epithelium observed bilaterally in all treated groups (first study T 1039793) but poorly reproducible in second study (T 0040367). In this 3rd study, one dog again had findings "identical to that reported in the first chronic study, present in the left testis but absent in the right one" (hemiorchiectomy) implying reversibility after 3 months.

"Dog was considered the most similar species to human with respect to disposition, systemic exposure and metabolism of cerivastatin." (vol. 1.2, p.56).

#### CARCINOGENICITY FINDINGS

##### MOUSE LIVER TUMORS (sponsor's analysis)

##### p values in male mice

##### (carcinoma and multiple carcinoma at terminal necropsy)

Males	controls	1 ppm	5 ppm	25 ppm	125 ppm
pairwise	-	-	0.003	0.0002	<0.0001
trend test	-	0.25	0.001	<0.0001	<0.0001
<b>Males (adenoma and multiple adenoma at terminal necropsy)</b>					
pairwise	-	-	-	0.008	0.0001
trend test	-	-	0.444	0.004	<0.0001

##### p values in female mice

APPEARS THIS WAY ON ORIGINAL

**(carcinoma and multiple carcinoma at terminal necropsy)**

Females	controls	1 ppm	5 ppm	25 ppm	125 ppm
pairwise	-	-	-	0.253	0.247
trend test	-	-	-	0.064	0.023
<b>Females (adenoma and multiple adenoma at terminal necropsy)</b>					
pairwise	-	-	-	0.007	<0.0001
trend test	-	-	0.206	0.005	<0.0001

Mouse Cmax (as multiple of human Cmax) ranged from 0.1x (at 5 ppm), to 0.5 to 1x (at 25 ppm) and 3-8x (at 125 ppm). The mouse carcinogenicity study appeared to be at the MTD: A 4-week range-finding study found lethality at 200 ppm and above.

**RAT CARCINOGENICITY STUDY:** There were no treatment-related neoplastic findings. The rat carcinogenicity study appeared to be at the MTD as demonstrated by lethality at 5 ppm (the highest dose used in the carcinogenicity study) with 2.5 ppm the next dose down. The rat Cmax (at the 2.5 ppm dose as a multiple of the human Cmax at the 300 ug/day dose) was 0.2x (for females) and 1x (for males); (sponsor stated that AUC was 1-2 times human at 0.3mg dose).

**RAT LIVER EM:** Cells were swollen, glycogen was not distributed as in controls, and the number of nuclei was increased. There was swelling of mitochondria with a condensed inner membrane and few, mainly peripheral, cristae. Histopathology showed pleomorphism of periportal hepatocytes (variation in size, shape, and staining properties). The nuclei were often hypertrophic and pleomorphic, the cytoplasm more eosinophilic.

**MOUSE LIVER EM:** The periportal region of liver at 125 ppm in carcinogenicity study showed "increase of smooth and rough endoplasmic reticulum and the mitochondria had many vesicle-like and cisterna-like expansions." There were no comments on nuclei.

**GENOTOXICITY:**

The following *in vitro* tests were negative: the Ames test, the forward mutation test in CHO/HGPRT, the UDS test in primary rat hepatocytes, the cell chromosome test in CHO cells, and the spindle inhibiting test in human lymphocytes. Two *in vivo* tests were done: the micronucleus test in the mouse was negative.

The dominant lethal test in the male mouse produced statistically significant increases in lethality at mating periods 3 and 7 at the 50 mg/kg dose level (first test) which the sponsor dismissed as due to environmental factors. Dr. Virginia Dunkel (CFSAN) recommended that the test be repeated. The "repeat" test appeared to be positive period 6. However, there was less toxicity and a lot more scatter of the data the second time, which may have been due to the differences in ages of mice (*ages varied between 4 and 12 weeks-old* at the start of the second study *vs 8 to 12 weeks-old* for the first study; this, in spite of the fact that the protocol submitted February 2, 1995 stated that mice would be 8 to 12 weeks old); a different batch of drug was also

used. Dr. Ching Ju Sheu (Genotoxicity, CFSAN) agreed with our concerns on ages ("4-week old mice are too young"). Mice appear to have been dosed based on initial body weight (only final body weights are provided for the first test and no body weight data was provided for the second test).

The requested statistical analysis for the second test (submission of 4/22/97) provided no numbers for the Kolmogorov-Smirnov and analysis of variance tests, only an indication of whether the particular test had been done and whether it was "significant".

The only positive control data for the dominant lethal test that has been submitted was from a study done in November 1991 (positive control data is supposed to be generated yearly (40CFR 798.5450); the two studies with cerivastatin were done in 1991 and 1995). The positive control was positive only at the higher of the two doses tested and at one time point, which does not make it appear very sensitive.

#### COMPARISON OF DOMINANT LETHAL TESTS ON BAY W6228

	<u>Test #1</u>	<u>Test #2</u>
Date of study	Feb. 1991	May 1995
Age mice (weeks)	8-12	4-12
Batch drug	518801	513312
Signs of toxicity	3/55 males died @ 50 mkd "Compound related symptoms apathy, reduced motility, roughened fur, sunken flanks"	0/60 died @ 50 mkd; "Single males showed apathy"
Conclusions	<u>Increase in dead implants per fertilized female</u> (Mating interval #)	

TEST #1	TEST #2
<u>Control, 25, 50 mkd</u>	<u>Control, 25, 50 mkd</u>
0.78, 0.80, 1.68 (#3)	0.58, 0.77, 0.98 (#6)
0.74, 0.69, 1.16 (#6)	0.77, 1.07, 1.07 (#11)
1.1, 0.80, 1.81 (#7)	

#### Per Cent Pregnant (Mating interval #)

TEST #1	TEST #2
<u>Control, 25, 50 mkd</u>	<u>Control, 25, 50 mkd</u>
90; 80, 74 (period #1)	84; 84, 80 (#1)
84; 58, 58 (period #2)	88; 80, 62 (#2)
92; 82, 63 ( " #3)	90; 76, 80 (#3)
86; 82, 73 ( " #4)	88; 90, 92 (#4)

## REPRODUCTIVE TOXICITY

### Fertility

**Rats (male and female; Segment I):** There was focal degeneration of the germinal epithelium in the testes at 300 ug/kg. There was a decrease in live births and an increased incidence of skeletal findings at the high dose (300 ug/kg). In the first mating of the F1 generation, there was a decrease in the number that littered, and an increase in the number of F2 that died (100 ug/kg).

**Dogs:** Lower percentage of progressively motile spermatozoa and higher percentage of morphologically altered spermatozoa attributed to disturbances of epididymal sperm maturation.

**Developmental toxicity in rats (Segment II):** There was an increased incidence of incomplete ossification of the lumbar center of the vertebrae in all treated F1 litters, with statistical significance at the high dose of 720 ug/kg/day (none of controls had this finding). There was also one litter at 720 ug/kg with a finding of multiple skull malformations including "skull bones malpositioned", seen in one out of 411 historical control litters (0.2%). The F2 generation at the high dose (720 ug/kg) had one litter with anophthalmia and one with the optic nerve missing: anophthalmia had been seen in one out of 411 historical control litters (0.2%) and "missing optic nerve" was not listed as an observed finding in historical control litters.

**Peri-natal and post-natal toxicity in rat (Segment III):** There was an increase in total dead pups (stillborns and deaths) at 320 ug/kg/day.

**Cerivastatin is secreted in the milk and crosses the placenta in rats.** Moderate to high concentrations were seen in both dam and fetus at 3 and 7 hours postdose: intestinal contents, liver, adrenals, kidneys, cranial nerves, skin, and adipose tissue (maternal levels) (see table p. 45). The  $AUC_{(0-m)}$  of the average fetus reached 54% of the  $AUC_{(0-m)}$  for maternal plasma (4 ng h/ml). Liver levels were 120x higher (dams) and 6x higher (fetuses) than respective plasma levels.

### HORMONE LEVELS:

Estradiol and corticosterone increased in rats at 200 ug/kg (6-month study) (p.22 NCSummary). Estradiol and testosterone were measured in a 1-year dog study (0,100,300 ug/kg/day) but the variability was so great that data were not useful and some data did not make sense: estradiol in female dogs was below proestrus levels and levels in males were almost identical to females. LH in control males varied 4-fold between weeks 56 and 71 with a coefficient of variation of 120% at week 71. (There were no drug effects in humans on levels of TSH and cortisol; data on steroid hormone levels was not available.)

### ANDROLOGICAL STATUS OF MALE DOGS

In a 1-year study at 100 ug/kg, there were transient *decreases* in ejaculate volume and percent of progressively motile sperm as well as *increases* in morphologically altered spermatozoa (increases in per cent of eosin-stained spermatozoa, acrosome or head alterations, and mid piece alterations). The sponsor's expert stated that "The significantly higher percentages of morphologically altered spermatozoa found during the treatment period in the treated group are attached to medication

influences. The increase is attributed to persisting cytoplasmatic droplets located in the midpiece region...indicating disturbances of epididymal sperm maturation...temporarily impaired by medication. The significant increases of acrosome and head alterations are thought to be another short-term medication effect." (There was loss of autophagic vacuoles in the kidneys, which are dependent on testosterone in male mice, an effect thought to be due to altered steroid metabolism.)

**SAFETY:** Although, due to its high affinity for HMG CoA reductase, cerivastatin doses are the lowest of any of the statins (the recommended high dose is 300 ug/day vs 80 mg/day for lovastatin, atorvastatin, and fluvastatin, and 40 mg/day for simvastatin and pravastatin), human efficacy is not as impressive: LDL-C lowering, after 6 months, was 19% (@100 ug/day), 26% (@200 ug/day), and 29% (@300 ug/day). As a result, a clinical study with cerivastatin using doses up to 800 ug/day is in progress. With the sharp increase in adverse reactions seen preclinically as the dose rises, there is concern that increasing the dose in people has the potential for serious toxicity (see p.23 for histopath findings in dogs and p.36 for histopath findings in rats that died). The major toxicities seen in both species were degeneration and necrosis of liver, muscle fiber necrosis, and atrophy of lymphoid organs, while in dogs, there were also degenerative changes in nerves, vascular degeneration, hemorrhage, and reduced platelet aggregation.

The difference between the dose where there was no mortality to the dose where there was mortality, was small: for rats, this margin was 2-fold ( 200 ug/kg vs 400 ug/kg) and for dogs, 1.4-fold (70 ug/kg vs 100 ug/kg).

For a drug that is to be used to prevent heart disease, it was discouraging to see the adverse effects on the heart: in rats, necrosis, auricular thrombosis, inflammation of atria, inflammation and fibrosis of the ventricles and in dogs, hemorrhage and necrosis. A potential mechanism postulated in the literature had to do with lowered levels of ubiquinone (a respiratory coenzyme) in tissues. Folkers et al. (PNAS, 87, p.8931, 1990) studied cardiac patients on lovastatin and found decreased ubiquinone levels correlated with impaired cardiac function and suggested adding ubiquinone along with the lovastatin as a preventive measure.

**CONCLUSION:** Cerivastatin presents a similar spectrum of toxicities to those seen with the class of "statins" as a whole. Although the margin of safety in the dog and rat models is not large, nevertheless, at the current clinical doses and with careful monitoring, cerivastatin would appear to provide a benefit similar to those in the class already approved.

APPEARS THIS WAY ON ORIGINAL