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#s: 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 (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 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)

	Food Intake		**Drug Intake**			
DOSE (ppm)	**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, inflammtion 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 endoplasmatic 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)

APPEARS THIS WAY
ON ORIGINAL

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

Mice (NMRI 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*

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

*$P<0.05$

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.
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.
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 III) 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):

Day 6-18	Days 0-29
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.

F0: REPRODUCTIVE DATA	(0,	30,	150,	750 ug/kg)
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 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

APPEARS THIS WAY
ON ORIGINAL

PLACENTAL TRANSFER IN PREGNANT RATS (vol 1.79)
PH 24832. Bayer. February 1996.
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. Whole body autoradiography as well as direct counting of tissues 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;
unknown metabolites. The unbound fraction in dams was 2.3% vs 5.5% in fetuses.

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

(one rat/time point with levels measured

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

Nanomoles/ml (ϕ) in plasma after one year treatment

		Ubiquinone 50		Vitamin E	
dose (ug/kg)		**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 ±. 02	21 ± 3.2	18 ± 3

ϕMean±SD (n=4/s/g)

APPEARS THIS WAY
ON ORIGINAL

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 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)

		HEART		SKELETAL MUSCLE		LIVER	
dose (ug/kg)		**Male**	Female	**Male**	Female	**Male**	Female
control		**110 ± 38ϕ**	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

ϕ 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)

		KIDNEY		TESTIS
dose (ug/kg)		**Male**	Female	**Male**
control		**41 ± 8ϕ**	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

ϕ 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)

		HEART		MUSCLE		LIVER	
dose (ug/kg)		**Male**	Female	**Male**	Female	**Male**	Female
control		**25 ±13ϕ**	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

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

APPEARS THIS WAY
ON ORIGINAL

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

		KIDNEY		BRAIN		TESTIS
dose (ug/kg)		Male	Female	Male	Female	Male
control		107 ± 34ϕ	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

ϕ 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; 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 ½ 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.
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.
Dr. Günzel-Apel 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:
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 -20^0 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 ± SD)
37± 16 ng/ml (week 56)
150 ± 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. 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±6.7 vs 4.3±4.0; p=0.051)
time 4 (5.8±6.0 vs 3.0±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 Spermatoza: 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 diffuculty 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 relativey 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)

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. Bayer Yakuhin, 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 immunohistochemical assay in 20/g. These are *F344 rats* whereas *Wistar rats were used in the carcinogenicity study*.

Dose (mg/kg)	**GST-P positive foci**	
	no./cm^2	area/cm^2
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

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_{10}), "the most important ubiquinone in dog and in man", was measured in rats and dogs. Six-months of dietary treatment in **rats** (at a Cmax 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 Cmax 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 Cmax).

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 immunoreactive 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 .. *In the animal studies* was used to separate metabolites which along with the use
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 (-20^0 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 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 β-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 β-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 (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 -20^0 C because of continued enzymatic activity.

PK DATA: provided more reliable PK data (better reproducibility; less variability) than radioimmune assay "Compared to the 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 ı. Unfortunately, most of the PK data available was obtained only became available much later in drug development.

There was for the mouse and rat carcinogenicity studies. The PK study in pregnant *rabbits using 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).

COMPARISON OF SPECIES "Cmax"

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

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, inflammtion 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: centrolobular 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 0F 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**
Males (adenoma and multiple adenoma at terminal necropsy)					
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**
Females (adenoma and multiple adenoma at terminal necropsy)					
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) recommeded 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-Smironov and analysis of variance tests, only an indication of whether the particualr 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

	Test #1	Test #2
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 **Increase in dead implants per fertilized female** (Mating interval #)

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

Per Cent Pregnant (Mating interval #)

TEST #1	TEST #2
Control, 25, 50 mkd	**Control, 25, 50 mkd**
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 signigicance 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 $AUC_{(0\text{-}tn)}$ of the average fetus reached 54% of the $AUC_{(0\text{-}tn)}$ 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 71with 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 spermatoza (increases in per cent of eosin-stained spermatoza, 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.

Table 1 Rat (Interim Kill at 1year)

Remarkable Histopathological Findings in Liver and Skeletal Muscle (Interim Kill)						
Dose (ppm)	0	0.1	0.5	2.5	5	500 (*)
Males						
Number of animals examined	10	10	10	10	8	9
LIVER						
periportal pleomorphism			1	8	8	7
clear cells (increased)			1	4	3	3
clear cell foci/focus				2	5	3
basophilic foci/focus	1		1	6	6	1
focal fatty change					1	1
focal vacuolation	1			2	3	
SKELETAL MUSCLE						
atrophy					1	8
fatty tissue replacement						5
Females						
Number of animals examined	10	10	10	9	10	10
LIVER						
periportal pleomorphism	1	1	1	9	10	10
clear cells (increased)				2	1	3
basophilic foci/focus	1		6	9	9	10
single cell necrosis				7	8	
focal fatty change			1	3	9	3
focal vacuolation				2	5	2
SKELETAL MUSCLE						
fibre necrosis					2	
atrophy					9	8
fatty tissue replacement				1	4	8

(*) Lovastatin

Table 2 Rat (terminal Kill at 2 years)

Remarkable Histopathological Findings (animals scheduled for terminal kill (n=50 §))										
sex	male					female				
dose (ppm)	0	0.1	0.5	2.5	500 (*)	0	0.1	0.5	2.5	500 (*)
Liver										
basophilic foci	C 1	5	18	33	28	C10	14	32	43	38
clear cell foci	A28	31	34	38	27	C11	4	16	28	23
mixed cell foci	C 1	0	2	8	5	C 1	3	11	10	12
vacuolated cell foci	B 1	5	7	9	9	C 1	1	3	11	8
pleomorphism	B 0	0	1	5	4	C12	5	13	49	41
bile duct hyperpl.	31	33	33	21	14	4	7	11	6	21
bile duct cysts	A 2	2	0	7	9	A 3	2	2	8	16
atrophy/red.glycog.	2	3	1	3	10	1	4	2	0	8
fatty change perip.	A26	22	36	34	25	1	8	3	2	3
fatty change focal	C 1	0	4	9	7	C 5	2	9	35	28
extramedul. haemato.			1	1		1	1	1	6	5
Skeletal muscle										
fibre necros./degen.	6	3	5	9	19	2	0	1	5	9
atrophy	0	0	0	2	6	0	1	1	0	6
fatty tiss. replace.	0	0	0	0	2	A 0	1	0	4	18
Tongue muscle										
fibre necros./degen.	0	0	0	2	13	0	0	0	0	6
Stomach (forestomach)										
hyperplasia	0	4	2	4	20	0	0	3	1	3
Mammary gland										
hyperplasia	0	0	1	1	0	24	17	23	15	4
degenerative lesions	0	0	1	0	0	10	11	9	6	1
Pituitary gland										
hyperplasia p.d.	10	13	7	17	12	12	13	6	7	8

(*) Lovastatin § number of organs examined see Table 18
Trend test BAY w 6228 groups: A = p<0.05; B = p<0.01; C = p<0.001
Comparison of groups: underlined = p<0.05;
double underlined = p<0.01

Table 14 MOUSE

Tumor Incidences
(animals scheduled for terminal kill)

Sex		Male					Female				
Dose ppm		0	1	5	25	125	0	1	5	25	125
BRAIN							49	49	49	50	49
meningeoma	m						2				
LUNGS		50	50	48	49	46	49	49	49	50	49
adenoma	b	2	1	3	4		1			1	1
adenoma multiple	b		1								
carcinoma	m	2		1	2	1	1				
carcinoma multiple	m	1	1								
SPINAL CORD		50	50	49	49	48	49	49	49	50	49
osteosarcoma	m							1			
SPLEEN		50	50	48	48	47	49	49	49	50	49
hemangioma	b		1		1		1				
hemangiosarcoma	m		2				1	1			
LIVER		50	50	50	49	48	49	49	49	50	49
hemangioma	b	1								2	
hemangiosarcoma	m			1			1				
hemangiosarcoma multiple	m			1							
adenoma	b	C7	7	8	14a	19c	C	3a	2	7b	16c
adenoma multiple	b	C			4a	5c	C				5a
carcinoma	m	*	2	8b	11c	9c				2	1
carcinoma multiple	m				2		+				1
hepatocellular tumors total	b/m	7	9	16a	31b	33b		3a	2	9b	23b
STOMACH		50	50	49	48	48	49	49	49	50	49
papilloma	b			1		1				1	
squamous cell carcinoma	m				1						
DUODENUM		50	50	49	49	48	49	49	49	50	50
adenoma	b				1						

Peto trend test: C: $p \leq 0.001$; *: $p = 0.06$; +: $p = 0.03$
Group comparison vs. control: §: $p = 0.02$; a: $p \leq 0.05$;
b: $p \leq 0.001$; c: $p \leq 0.001$

Exec CAC Meeting
April 29, 1997

CAC members:
Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph F. Contrera, Ph.D., HFD-900, Member
Jim Farrelly, Ph.D., HFD-530, Rotating Member
Ronald Steigerwalt, Ph.D., HFD-510, Division Team Leader
Lillian Patrician, MS, MBA, HFD-024, Project Manager

NDA 20-740 [Barbehenn/Steigerwalt - HFD-510]
Cerevastatin (Bayer Corporation)
The maximum human dose will be 300 micrograms per day.

Rodent Carcinogenicity Studies
MOUSE study: There was lethality at 200 ppm in a dose-range-finding study. Therefore, a high dose of 125 ppm (MTD) was used in a 2-year mouse (B6C3F1) study with dietary administration of control, 1, 5, 25, and 125 ppm.

The CAC concluded:
1) The label should state at which doses there was a statistically significant increase in the combined analyses of hepatic adenomas and carcinomas. In the case where analysis was done on carcinomas and adenomas uncombined, there were, for males, significant increases in tumor incidences beginning at 5 ppm (carcinomas) and 25 ppm (adenomas); for females, there was an increase in adenomas at 25 ppm. Tumor incidence was significant by both statistical tests used (trend test and pairwise comparisons to control).

2) The combined tumor incidence exceeded the control range indicating the apparent effect was not due to an abnormally low control incidence of hepatic tumors. Thus, the concurrent control data should be used for statistical comparisons and determination of no effect levels. The doses administered should be estimated based on mg/kg.

RAT study: In a 2-year rat (Wistar) study of dietary administration of control, 0.1, 0.5, 2.5, and 5 ppm, an MTD of 2.5 ppm was established (5 ppm was lethal, causing mortality of 50% of females and 60% of males within the first 6 months of treatment). There were no drug-related tumors; however, the plasma exposure was only 1 - 2.5 times that of humans at the high dose.

The CAC concurs.
It was suggested that other drugs in the class be reevaluated by the division so that a decision based on any new data or new approaches to the evaluation, can be made with regard to revising the labels.

5/7/97

Joseph DeGeorge, PhD
Chair, CAC

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: May 28, 1997

FROM: Expert Mathematical Statistician (Applications in Pharmacology and Toxicology)
Division of Biometrics II, HFD-715
Office of Epidemiology and Biostatistics

TO: File (NDA 20-740)

SUBJECT: Additional Comments on Results of Tests for Linear Trend in Liver Hepatocellular Carcinoma in Male Mice

(1). Background Information and Comments on the Discrepancy in P-values of Tests for Linear Trend in Baycol (NDA-20-740) Carcinogenicity Studies

(2). Historical Control Data of Spontaneous Background Rates of Liver Hepatocellular Carcinoma in B6C3F1 Mice

(1). Background Information and Comments on the Discrepancy in P-values of Tests for Linear Trend in Baycol (NDA-20-740) Carcinogenicity Studies

Dr. Baldeo Taneja of Division of Biometrics II has written a draft statistical review and evaluation report on the two carcinogenicity studies of Baycol included in NDA 20-740. I have read the report. The draft report included results of Dr. Taneja's statistical analyses using the standard procedures and computer programs developed and routinely used by statistical reviewers in the Center. The draft report seems fine. A copy of the draft report was also given to Dr. Elizabeth Barbehenn of HFD-510, the reviewing pharmacologist of this NDA, for her comments. Dr. Barbehenn pointed out two discrepancies in the p-values of two tests for linear trend performed by Dr. Taneja and the sponsor.

The p-values showing discrepancies in FDA statistical reviewer's and the sponsor's results were those from the tests for linear trend in liver hepatocellular carcinomas in male and female mice. The p-values of the tests for linear trend in hepatocellular carcinomas in male mice was 0.074 in Dr. Taneja report, and was less than 0.0001 in the sponsor's report. The corresponding p-values of the tests for linear trend in hepatocellular carcinomas in female mice were 0.034 (by Dr. Taneja) and 0.023 (by the sponsor).

The overall liver hepatocellular carcinoma rates for individual treatment groups for both males and females were as follows:

For male mice

In Dr. Taneja's report

	Dose Group (In ppm)				
	0	1	5	25	125
With tumor	0	2	8	13	9
Without Tumor	50	48	42	37	41
Total	50	50	50	50	50

In the sponsor's report

	Dose Group (In ppm)				
	0	1	5	25	125
With tumor	0	2	8	13	9
Without Tumor	50	48	42	36	39
Total	50	50	50	49	48

For female mice

In Dr. Taneja's report

	Dose Group (In ppm)				
	0	1	5	25	125
With tumor	0	0	0	2	2
Without Tumor	50	50	50	48	48
Total	50	50	50	50	50

In the sponsor's report

	Dose Group (In ppm)				
	0	1	5	25	125
With tumor	0	0	0	2	2
Without Tumor	49	49	49	48	47
Total	49	49	49	50	49

Since the concern is the large discrepancy in p-values in the tests for linear trend in the tumor type in male mice, my comments are on the test of this tumor type in the sex.

There are factors which could cause the discrepancy. Those factors include the weights, the time intervals, the statistical procedures used in the analyses. Since the sponsor's report did not state clearly the exact statistical procedures they used, several computer runs were done, before contacting the sponsor for further information, to figure out the possible causes.

Our findings were that the time intervals were not a main factor for the discrepancy; and that the only possible explanation for the large discrepancy was the uses of different sets of weights by the sponsors and FDA. If the serial numbers 1, 2, 3, 4, and 5 were used as weights, then the p-values from our computer runs were closed to those of the sponsor. So it was assumed that the

sponsor must have used the serial numbers as weights in their analyses.

To make sure that our guessing is correct, we contacted the sponsor for further information through a series of telephone conferences, arranged by Ms. Margaret Simoneau, CSO of HFD-510, with the sponsor's statisticians, pharmacologists, and regulatory affairs people of Bayer in Germany. The sponsor has submitted new information we requested.

However, to our surprise, after the telephone conferences and submissions of new information, we learned that the sponsor reanalyzing the data using the same statistical procedures and the same weights (the actual doses) we used and came up with a p-value of 0.0729732 (close to the p-value of 0.074 by Dr. Taneja). The slightly difference in p-values was due to the small differences in numbers of animals at risks as can be seen from the above tables.

The sponsor was next asked in a telephone conference why the p-value on page 1392 of their earlier report, <0.0001, was so different from that of their 5/27/97 report, 0.072732. The sponsor's explanations for the discrepancy of their earlier and current p-values were that the early and current sets statistical analyses were done by separate statisticians; that the person who did the early analyses was not available for answering the question; and that the earlier analyses were simply 2x5 contingency table analyses using chi-square tests.

Based on the information we have received either through telephone conferences and reports submitted through faxes, it is concluded that the sponsor's earlier p-value of less than 0.0001 is not correct since the statistical procedure they used to obtain the p-value is not correct. The null hypothesis tested in the procedure is simply that there is no association between tumor occurrence and dose.

It seems difficult to interpret the tumor incidences 0, 2, 8, 13, 9 as not significant. However, it is important to adjust the possible differences in mortality among the treatment groups while comparing tumor incidence rates. Also the actual doses were used as weights in the analyses. Under this set of weights, the medium dose is five times of the low dose, the high dose is five times of the medium dose, and the maximum dose is five times of the high dose. It took a five-fold increase in dose each time to increase the tumor rates from 2 in the low group to 8 in the medium group, to 13 in the high group, and to 9 (a drop) in the maximum group. It can be interpreted that if there exists a

positive linear trend in tumor rates, then the tumors in the high dose group and the maximum dose group should be much higher than those actually observed.

The interpretation will be different if the serial numbers 1, 2, 3, 4, and 5 were used because it only took one-fold increase in dose (i.e., much stronger evidence of carcinogenic effect) to produce such increases in tumor rates observed.

(2). Historical Control Data of Spontaneous Background Rates of Liver Hepatocellular Carcinoma in B6C3F1 Mice

With regard to the spontaneous background rate of liver hepatocellular carcinoma, I mentioned in the conference that my impression of background rate for male was around 3% not 20% (10/50) mentioned by the sponsor based on their historical data of 14 studies (4/50, 7/50/, 8/50, 5/50, 3/50, 11/50, 6/50, 5/50, 10/50, 7/50, 7/50, 5/50, 5/50, 7/50) for B6C3F1 mice. I have checked my records of data compiled by Charles River Lab and NTP.

Based on Charles River Lab database, the background spontaneous rates of liver hepatocellular carcinoma for both B6C3F1 male, and female mice are 171/1294=13.2% with a rage 4.2%-24.6%, and 31/1273=2.4% with a range 0%-6.3%, respectively. Therefore my impression of around 3% was for female not for male mice.

Based on the sponsor's data of the 14 studies, the average spontaneous rate actually is 12.857% (90/700), which is almost the same as the rate based on Charles River Lab database.

Based on a little bit old NTP database published in the paper "Use of Historical Control Data in Carcinogenicity Studies in Rodents" by Haseman et al. in Toxicological Pathology, pp 126-135, the rates for male, and female B6C3F1 mice are 21.3% (498/2343), and 4.1% (101/2486), respectively. In his 10/22/1991 letter to Dr. Bob Temple, Haseman gave more updated rates but combining both adenoma and carcinoma. The combined rates reported in the letter are 32.6% and 13.7% for male and female B6C3F1 mice. Again, the two rates are combinations of both liver adenomas and carcinomas for male and female mice.

Based on the available historical control data information, the sponsor's historical data are reasonable and their argument that the tumor rate of 0% in control group of the Baycol study was unusually low is valid. If the control group of the Baycol study had an average rate of around 12% or 13%, then the trend in the rates of 12, 2, 8, 13, 9 tumor bearing animals would not show a significant result. Of course, one may argue that the tumor rates

for the four treated groups may also be lower than usual too.

Therefore, if the Baycol mouse carcinogenicity study was conducted correctly, the historical control data provide another justification for concluding that the positive linear trend in liver hepatocellular carcinoma in male mice in the Baycol Carcinogenicity study is not significant based on statistical test and historical control information.

Karl K. Lin

Karl K. Lin, Ph.D.

cc: HFD-510/EBarbehenn
HFD-510/RSteigerwalt
HFD-510/MSimoneau
HFD-700/WFairweather
HFD-715/ENevius
HFD-715/DMarticello
HFD-715/BTaneja
HFD-715/KLin
HFD-715/Division File Chron

APPEARS THIS WAY
ON ORIGINAL