CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number: 020740

PHARMACOLOGY REVIEW(S)

MAY 19 1997

NDA 20-740

May 12, 1997

Submitted: June 26, 1996

PHARMACOLOGY REVIEW OF NDA

DRUG: Cerivastatin, Baycol^{R} (Bay W6228)

CATEGORY: Lipid altering (Cholesterol lowering)

MECHANISM OF ACTION: HMG CoA reductase inhibitor (synthetic, chiral)

RELATED DRUGS (marketed): Lovastatin (NDA 19-643), Simvastatin (NDA 19-766), Pravastatin (NDA 19-898), Fluvastatin (NDA 20-261); Atorvastatin (NDA 20-702)

REVEIWERS RECOMMENDATION: Approval

APPEARS THIS WAY
ON ORIGINAL

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cc: NDA Arch
HFD-510
HFD-510/Barbehenn/Steigerwalt
HFD-900/Contrera
Cerevast.nda

5/16/97

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PREVIOUS MAJOR REVIEWS	Review Date
1-month dog (0,0.05,0.5,5 mkd; capsules)	(3/12/92)
3-month dog (capsule) 0, 0.01, 0.03, 0.1 ug/kg + 0.1 reversible	(4/17/91)
3-month dog (capsule) 0, 0.02, 0.1, 0.5 mkd + mevalonate	(4/17/92)
3-month minipig (0,30,400,4000/3000 ug/kg/day)	(11/16/92)
3-month rat (range-finding; 0,0.5, 2, 10, 50 ppm)	(10/21/91)
3-month mouse (range-finding; 0,1,5,50,150 ppm)	(10/21/91)
3-month rat (0 or 5 ppm diet)	(6/27/94)
6-month rat diet (0,0.1,0.5,2.5 ppm)	(9/24/92)
1-year dog gavage (0,8,25,70 ug/kg/day)	(9/24/92; 6/24/93; 11/16/92)
1-year dog gavage, in-life portion (0, 100,300 ug/kg/day)	(9/24/92)
Fertility (rats)	(6/24/93)
Dominant lethal mouse	(6/24/93)
Other Genotoxicity studies	(4/17/91)

ADME
ASSAY

ASSAY

CROSS-REACTIVITY OF METABOLITES IN
October 1995.

Standard: BAY w 6228 (MW 460; Batch R-24-6; purity 98%)
Metabolites:
BAY w 5679 (M1): MW 468; purity 91%
BAY w 8877 (M8): MW 442; purity 99%
BAY 17-5111 (M23): MW 498; purity 95%
BAY 19-3103 (M24): MW 484; purity 75%
RMA 0333-7 (M27): MW 444; isolated from mouse bile; purity not provided

Antiserum: polyclonal *rabbit* antiserum against BAY w 6228
Four expts were carried out: extraction from
and from *dog plasma* (two sets with different concentrations of drugs).

Metabolite	**% Cross-reactivity at ED50**	**Concentration dependence**
M1	5.8	moderately
M8	155	none
M23	12.7	moderately
M24	0.1	moderately
M27	57.1	slightly

"Cross-reactivity similar after spiking into either buffer or plasma" (for dog).

CROSS-VALIDATION OF

; Bayer (appendix 8); A&M (appendix 1)

TREATMENT: *Three male rats and three male dogs were given a single oral and i.v. dose of 0.1 mg/kg.* The rats were treated April 15 and 22, 1993 and the dogs were treated April 21 and 22, 1993. Blood samples were taken predose (and 12 times postdose out to 32 hours; rats) and predose (and 11 times to 28 hours; dogs). Plasma was stored below -15^0 C.

Different dates for each of the 6 separate studies (November & December 1993 and February, March, July, and August 1994)
July and August 1993

evaluated by Dr. Krol of Bayer, West Haven, CT) December 1993-February 1994. There was no methodology or protocol provided.

PK parameters were calculated using each methodology.

BAY w 6228

it does not make sense to find an identical value with both

picking up as well as parent drug (see p.49) while the

only parent. Furthermore, these findings do not agree with the other studies in the NDA where

In line with higher values for

METHOD VALIDATION (BAY 17-5111 & BAY 19-3103; M23 & M24) (vol 1.77)

An

for rat and dog plasma. The Plasma samples of 17-5111 were stable for one month at -18° C; 19-3103 was stable for at least two months at this temperature.

METHOD VALIDATION (BAY 5679 & BAY 8877) (vol 1.77)
R6369.
A . was
developed for BAY 5679 for rat, dog, and human plasma.
Storage stability was not known.

BAY 8877 converted so rapidly to BAY w 6228 (open acid) in aqueous solution and plasma even at -18° C that it was impossible to validate a method for this metabolite.

PLASMA DRUG LEVELS IN MOUSE CARCINOGENICITY STUDY (vol 1.83; p.53)

CA study done July 1991-Sept. 1993 (Bayer, Wuppertal);
was August 1995. *PK done October-December 1995*

TREATMENT: Blood from 10/s/g sampled days 382, 547, and 718 about 8:00 am (region of Cmax). Samples were stored at Bayer until they were shipped to
Samples were
stored below -15° C.

MOUSE CMAX FROM CARCINOGENICITY STUDY (ng/ml)

	day 382ϕ		day 547		day 718	
DOSE	male	female	male	female	male	female
0 ppm	<0.47*	<0.47*	<0.47*	<0.47*	<0.47*	<0.47*
1	<0.47*	<0.47*	<0.47*	<0.47*	<0.47*	<0.47*
5	0.74*	<0.47*	<0.47*	0.91	<0.47*	0.49
25	1.9±1.6	1.3±1.5	2.2±1.4	3.5±1.9	4.6±1.3	5.6±1.4
125	11±1.6	12 n.c.∘	12±1.8	15±1.5	31 ±1.6	22±2.7

ϕmeans and geometric SD (ng/ml for n= 7-10/s/g)
*median
∘ n=4
n.c.= not calculated

[The human Cmax at 300 ug/day is 4 ng/ml.]

PLASMA DRUG LEVELS IN MOUSE CARCINOGENICITY STUDY (vol 1.83; p.22)
PH 24969. *CA study done July 1991-Sept. 1993* (Bayer, Wuppertal, Germany);
PK study done February 1993-May 1994
(Mouse CA study)
Submission of 4/22/97: Sample transfer to was Oct. 1992, Feb. 1993, & Aug. 1993.

TREATMENT: Blood from 10/s/g sampled days 382, 547, and 718 between 7:45 and 8:45 am (region of Cmax), plasma prepared and stored frozen at Bayer. Mice were not always the same ones sampled each time. Samples were sent to Individual plasma concentrations were determined and geometric means and SD calculated. The limit of detection was "about 1 ng/ml".

MOUSE CMAX FROM CARCINOGENICITY STUDY(ng/ml)ϕ

	day 382		day 547		day 718	
DOSE	male	female	male	female	male	female
0 ppm	<1	<1	<1	<1	<1	<1
1	2.1±1.4	1.3±1.8	2.4±1.2	2.8±1.5	1.8±1.6	A
5	12±2.0	7.5±1.4	12±1.6	13±1.3	7.7±1.3	9.5±1.3
25	50±1.9	33±1.4	63±1.4	44±1.4	43±1.5	42±1.2
125	170±1.3	95±1.4	210±1.8	120±1.4	180±1.5	160±1.7

ϕmean and geometric SD (ng/ml for n= 7-10/s/g) with blood collected at 8:00 am.
A: 5 of 10 values above limit of quantitation (1 ng/ml): 1, 1.4, 6.3, 1.6, 1.3 ng/ml

TIME COURSE OF DRUG LEVELS IN MICE (DIETARY STUDY) (vol 1.83, p.1)
T5040858. March 1992. Bayer. Batch#: 509236
The same doses and mouse strain (B6C3F1 as used in the CA study were used with the to track plasma level over time (the level of cross-reactivity has not been determined). The mice **(6-weeks old)** were **on drug for six days** before plasma analysis. Plasma was collected beginning at 8:00 am at 3 hourly intervals from 3/s/g. The minimum was at 2 pm but levels, in general, did not vary much with time (levels rose to a plateau at between 5 and 8 pm at which level they were maintained until the following morning at 8 am). AUC was calculated by multiplying Cmax by 24 hours (sub. 4/22/97).

AUC_{0-24} (6-day study in 6-week old mice

Dose (ppm)	Sex	AUC 0-24h (ng h/ml)
1	male female	85 87
5	male female	380 330
25	male female	1,500 1,400
125	male female	5,900 5,400

PLASMA DRUG LEVELS IN RAT CARCINOGENICITY STUDY (vol 1.83; p.155)
PH-24562. *Institute of PK, Bayer (days 6 & 237)* *(days 349, 552, and 723).*
TREATMENT: Blood from 5/s/g was sampled days 6, 237, 349, 552, and 723 between 10 and 11:30 am (region of Cmax) and analyzed The carcinogenicity study was performed between 1991 and 1993 and plasma samples analyzed 1993. Samples were stored "frozen" until analysis.

ANALYTICAL METHOD (p.160): "*The validation data from the investigated QCs at Bayer showed a higher inaccuracy compared to the method validation*. This might be due to an error during preparation of the QCs. On the other hand, if the bias is due to a true inaccuracy during the analysis of the study samples, the sample results may be inaccurate to a similar extent as the QC samples." "Comparison Bayer and in sufficiently high agreement; do not support the hypothesis of a relevant inaccuracy of the samples measured at Bayer."

PLASMA DRUG LEVELS IN RAT CARCINOGENICITY STUDY (vol 1.83; p.155)

"...some containers were empty especially those of the dose groups 0 and 1 ppm of the weeks 52, 78, and 102." (But there was no collection on these days.) "The samples of day 382 and day 547 are labeled identically, day 718 are marked with date 8/7/93 or 9/7/93.." (p.83; vol 1.83).

"*The validation data from the investigated QCs at Bayer showed a higher inaccuracy compared to the method validation.*

"It has not been possible to ascertain definitely the extent of the cross-reactivity of the antibodies used in this test with BAY w 6228 metabolites." (p.161)

RAT CMAX in carcinogenicity study **(ng/ml)ϕ**

	day 237		day 349		day 718	
DOSE	male	female	male	female	male	female
0 ppm	1.4°	1.1° 1.5°	<0.25	<0.25	<0.5	0.57°
0.1	n.d.	n. d.	no data	no data	no data	no data
0.5	1.5±2.0	2.6±1.4	0.33±1.8	1.3±1.5	1.1±2.4	2.8±1.2
2.5	3.5±1.5	14±1.6	2.7± 1.5	7.9 ±1.5	5.0± 1.9	17± 2.0
5	8.5± 1.7	30 ±1.4	5.6± 1.3	26±1.5	n.s.	n.s.

ϕMeans and geometric SD for 5/s/g.
All samples were pooled plasma (unless otherwise specified)
° values from individual rats (where detectable levels)
n.s. no sample; rats died/were sacrificed before
n.d. not detectable (only 1 or 2 samples out of 5 could be read and were at the limit of detection)
Limit of detection: days 6 and 237 (~1 ng/ml; Bayer analyses)
day 349 (~0.25 ng/ml;
day 723 (~0.5 ng/ml;

	day 6	
DOSE	male	female
0 ppm	<1	<1
0.1	<1	<1
0.5	<1	<1
2.5	12	12
5	25	40

FROM RAT CA STUDY week 104 (vol 1.77, pp.43-45)

Dose (ppm)	Males (ng/ml)	Females (ng/ml)
0.1	n.c.	n.c.
0.5	n.c.	n.c.
2.5	0.90±2.3	5.0±2.2

APPEARS THIS WAY ON ORIGINAL

n.c. not calculated because below level of quantitation
Mean±SD for 10/s/g
Human Cmax= 4 ng/ml at the 300 ug/day dose.

TIME COURSE OF DRUG LEVELS IN RATS (10-DAY, DIETARY STUDY)
#22284. Bayer (toxicology)
Batch#: 507277 (for study T3039957); 2.5 ppm; Males only (April 1991)
Batch#: 509269 (for study T3041215); 0.1, 0.5 ppm in Males & Females (December 1992)
Wistar rats (200-220 g females and 260-300 g males; no ages specified) were on drug for ten days before plasma analysis. Plasma was collected beginning at 8:00 am at 3 hourly intervals from 3/s/g). The same doses and rat strain as used in the CA study. Plasma levels were fairly flat across the 24 hours; AUC calculated by multiplying Cmax by 24 hours (submission of 4/22/97). Doses were **6, 34, and 170 ug/kg** (0.1, 0.5, and 2.5 ppm in diet). (p.199).

AUC_{0-24} (10-day study in young rats

dose (ppm)	**sex**	**AUC 0-24h (ng h/ml)**	**Cmax (ng/ml)**
0.1	male female	3.5 5.2	0.2
0.5	male female	17 20	2
2.5	male female	75 not done	5

APPEARS THIS WAY ON ORIGINAL

PLASMA DRUG LEVELS IN 6-MONTH DIETARY RAT STUDY (vol 1.83; p.228)
T0039909. Bayer Rat study= February to August 1991; PK study= February 1992
Time of sampling: 10:00-11:30 am (Cmax). Wistar rats.
Limit of Detection (LOD): 1 ng/ml

RAT CMAX (ng/ml) ϕ

	day 86		day 169	
DOSE	male	female	male	female
0 ppm	<1	<1	<1	[A]
0.1	<1	<1	[B]	[A]
0.5	<1	<1	[C]	1.3±1.8
2.5	2.0	9.7	2.2± 1.7	7.7 ±1.3
2.5+mev	1.6	5.0	1.9± 1.4	4.1±1.4

ϕMeans and geometric SD for 5/s/g. All samples were pooled plasma from one value/group.
[A] 1/5 above LOD (1.5 ng/ml); [B] 1/5 > LOD (2.0 ng/ml); [C] 1/5> LOD (1.2 ng/ml)

PK FROM ONE YEAR MALE DOG STUDY
PH 24279. (T6055475; p.26 review)
Animal study: Bayer, Wuppertal (*Feb. 1994-May 1995; sampling 2/, 6/, & 8/94; 2/95*)
PK study: (*August 1994 to June 1995*)

Parameter (RIA)	**100 ug/kg (males)**
AUC_{0-t} (ng h/ml)	1,100 ±1.5
Cmax (ng/ml)	130 ± 1.2
tmax (h)	2.3 ± 2.3
t1/2 (h)	7.4 ± 1.3

PK FROM LOW DOSE ONE YEAR DOG STUDY
T 1039793.
Animal study (Bayer, April 1991 to April 1992)
PK Analyses: May-Oct. 1992 **0, 8, and 25 ug/kg** doses and **Bayer: 70 ug/kg**
There was no information about how plasma was stored or shipped. The values 24 hours postdose were about 10% of those at one hour postdose.

CONCENTRATIONS 1 HOUR POSTDOSE·

	day 1		day 351	
DOSE (ug/kg/day)	Male	Female	Male	Female
ϕGeometric 8	8	6	6	6
25	23	17	24	15
70	38	52	35	27

· ng/ml (mean value); fasted; 4-6/s/g; gavage in water

APPEARS THIS WAY
ON ORIGINAL

PK FROM HIGH DOSE ONE YEAR DOG STUDY (vol 1.84)
22575 (T0040367). Batch#: 509245
Bay w 6228 at **0, 100, and 300 ug/kg/day** for 58 weeks in male and female dogs (at Wuppertal) from July 1991 to September 1992. The PK study was done at . (October 1992 to February 1993) using an Blood samples were obtained immediately before as well as 1 and 24 hours after dosing (except at 407th day, pre-dose, 0.5, 1, 4, 7, 24 hours postdose).

PLASMA LEVELS ONE HOUR POSTDOSE

dose (ug/kg)	**day 1**	**day 113**	**day 354**	**day 407**	**Day 407 (100 ug/kg dose)**
Males 0 100 300	 0.4± 2.4ϕ 75± 1.8 430± 1.2	 0 69 ± 1.6 n.s.	 0 94± 1.3 ns	 0 64± 1.3 ns	AUC (ng h/ml) = 900 Cmax (ng/ml) = 92 t1/2 (h) = 7
Females 0 100 300	 0.8± 1.9 85 ± 1.6 420± 1.5	 0 73± 2.2 ns	 0 73 ± 2.3 ns	 0 130± 1.4 ns	AUC (ng h/ml) = 900 Cmax (ng/ml) = 140 t1/2 (h) = 5

ϕ ng/ml ± geometric SD

RABBIT PLASMA DRUG LEVELS) (vol 1.83)
R 6368. March 1995. Bayer (treatment);
New Zealand White Rabbits (3/g; sex not stated) were given **30, 100, or 300 ppm in the diet** along with 0.5% cholesterol. On day 5/6 of feeding, six blood samples were taken over 24 hours and plasma analyzed (INDIVIDUAL VALUES):

Dose (ppm)	**Cmax Bay w 6228 (ng/ml)**	**Cmax Bay w 5679 (ng/ml)**
	3 rabbits/g	
30	**22, 28, 10**	3.9, 4.4, 1.8
100	**43, 17, 17**	4.2, 4.2, 7.7
300	**38, 290,110**	48,160,58

PREGNANT HIMALYAN RABBITS (GAVAGE DOSING (vols 1.53& 1.83)
T6040660. Bayer. Batch#: 509236
Toxicity study was done November 1991 and PK analysis was done August 1993.
Himalyan rabbits (CHBB:HM) were given orally by gavage in water, **0, 30, 150, or 750 ug/kg/day days 6-18 of gestation ("under identical conditions to the teratology study"** where no PK was done). Sampling was at 0, 1, 4, 7, and 24 hours postdose days 6 and 18 using RIA.

Bay w 6228 LEVELS IN PREGNANT RABBITS

	AUC 0-24 (ng hr/ml)		Cmax (ng/ml)	
dose (ug/kg)	day 6	day 18	day 6	day 18
30	no data 9 35	6 7 7	<0.5 3.4 1.6	2.5 4.1 4.0
150	29 16 45	32 14 16	12 4 5	11 6 4
750	160 150 (1,100?)	69 120 (2,500?)	60 60 (450?)	18 30 (540?)

3-MONTH MONKEY GAVAGE STUDY (DAY 90 (vol 1.86)
There were no measurable levels in liver, femoral muscle, testis, or lens when these tissues were sampled 24 to 30 hours after the last dose.

PLASMA LEVELS

Dose (ug/kg)	**Cmax (ng/ml)**
10	1.5±1.7
30	7.3± 2.0
100	18 ± 1.8

means±geometric SD (1 hr postdose) in 3 male and 3 female Rhesus monkeys.
Study performed by

1-YEAR MONKEY GAVAGE STUDY (vol 1.87 & 3/20/97)

dose (ug/kg)	Cmax (ng/ml)		AUC (ng h/ml)	
	day 90	**day 363**	**day 90**	**day 363**
10	2.4	1.5	2.8	2.1
30	5.1	3.1	7.4	5.4
100	36	17	36	22

geometric mean

HUMAN PK DATA

300 ug/day dose to healthy male volunteers
Cmax: 3.9 ± 1.3 ng/ml
AUC: 15± 1.4 ng h/ml
Tmax: 2.5±1.4 h
T1/2: 2.7± 1.3
MRT 5.3± 1.2 h

300/day to patients for 7 days (sub. 3/6/97)
3.9 ng/ml
21 ng h/ml

Cmax: 7 ng/ml (300 ug/day)

TISSUE AFTER ORAL DOSE IN MALE RATS (vol 1.75)

PH 25044. Bayer, Germany

study# 34-022: TREATMENT (*March to June 1995*): Fasted male Wistar rats from Harlan-Winkelmann, Borchen, FRG, approx 8 weeks old, were given a **single dose of 1 mg/kg** BAY w 6228 by oral gavage in 0.9% saline (p.122). Tissues were dissected, weighed, combusted, and counted by LSC.

study# I 3000427: TREATMENT (*February to May 1992; Wuppertal, Germany*): Fasted male Wistar rats from Harlan-Winkelmann, Borchen, FRG, approx 8 weeks-old, were given **22 daily doses of 1 mg/kg** BAY w 6228 by oral gavage in 0.9% saline (three lots of drug were used).

EXCRETION

Single dose: Urine 0.5% and Feces 97% (at 48 hours postdose)
Multiple doses: Urine 0.4% and Feces 150% (at 72 hours after last dose)

RAT PLASMA LEVELS

	AUC (ug h/ml)	Cmax (ug/ml)	tmax (h)	t1/2 (h)
First dose	0.51±1.3	0.085± 1.1	1.2± 1.5	3.4 ±1.4
Last dose	1.6 ±1.3	0.17± 1.3	0.4± 2.9	6.2 ±1.2

Mean ± SD (5/g)

RAT TISSUE LEVELS AFTER ONE ORAL DOSE OF 1 MG/KG

Tissue	AUC (ug h/ml)	Cmax (ug/ml)	tmax (h)	t1/2 (h)
Plasma	1.8	0.1	0.5	58
Blood cells	1.6	0.018	0.5	280
Kidneys	4.8	0.227	0.5	63
Liver	137	17.2	0.5	65
Bone (femur)	2.2	0.014	0.5	589

Mean ± SD (5/g)

RAT TISSUE LEVELS AFTER 22 ORAL DOSES OF 1 MG/KG BAY w 6228

Tissue	AUC (ug h/ml)	Cmax (ug/ml)	tmax (h)	t1/2 (h)
Plasma	5.7	0.288	0.5	50
Blood cells	6.67	0.0845	0.5	176
Kidneys	19.9	0.842	0.5	102
Liver	252	19.2	0.5	79
Bone (femur)	4.07	3.48	0.5	118
Spleen	12.7	0.16	0.5	323
Adrenal	43.4	0.455	0.5	223

Mean ± SD (5/g)

RATIO TISSUE LEVELS (22 DOSES VS 1 DOSE)

Tissue	AUC	Cmax
Plasma	2.9	2
Blood cells	0.8	4.7
Kidneys	1.9	3.7
Liver	1.1	1.1
Bone (femur)	0.79	8
Vitreous body	3.5	7
Seminal vesicle	4.1	8.5
Testes	3.7	3.8

TISSUE AND BLOOD LEVELS IN RATS, MICE, AND DOGS (vol 1.77)

PH 24977. Animal studies (Bayer, Wuppertal)

Animal Studies:

1-year dog study at 100 ug/kg (T0040367; ended August 1992; tissue sampling *Sept. 1992*)
1-week dog study at 100 ug/kg (T5055393 (PK study); ended December *1993)*
2-year rat CA study at 2.5 ppm (T4039903; ended June *1993*)
2-year mouse CA study at 125 ppm (T 3040234; ended at *August 1993*)

February 1994 to January 1995 (measuring unchanged BAY w 6228)

Samples were taken 24-28 hours after the last dose (1-year dog, 2-year rat and 2-year mouse studies); blood, liver, and muscle were stored below -15° C. Stability in samples was not provided here, but in another study (24499P), the sponsor stated that they couldn't repeat the results on liver metabolites when samples were stored at -20^0 C because of continued enzymatic activity under these conditions.

COMPARISON OF BLOOD AND TISSUE LEVELS

Species Gender	Sampling (h postdose)	Dose	Liver	Muscle	Blood	Testes	Lens
Dog* male	**1 h, day 7**	**100**	360±1.3	14±1.3	**46±1.3**	6.5±1.3	0.9±1.2
male	24 h, day 7	**ug/kg**	9.4±1.5	0.5±1.7	0.9±1.6	0.9±1.5	0.6±2.0
Dog female (gavage in tap water; fasted)	24-28 h, wk 59	**100 ug/kg**	5±2.5	<0.5	**1±3**		
Rat male	week 104	**2.5 ppm**	350±1.3	n.c.	**0.9±2.3**		
female			1100±1.4	1.8±1.8	**5.0±2.2**		
Mouse male	week 104	**125 ppm**	110±1.3	28± 2.3	**35±2.4**		
female			100±1.7	39± 2.5	**23±1.9**		

Mean±SD [ng/g or ng/ml for 3/s/g (dogs), 10/s/g (rats), 5-10/s/g (mice)]

ADME IN MALE B6C3F1 MICE (vol 1.75)

PH 24993. Bayer, Wuppertal, Germany.

TREATMENT: Groups of male mice (intact or bile duct-cannulated; 26-32 g) were given single oral doses of BAY w 6228 (2 mg/kg in phosphate buffered saline). Qualitative analysis was done using whole body (50 um sagittal whole body sections placed on film) at 0.5 to 24 hours post dose. In some mice, selected tissues were homogenized and freeze-dried before counting (quantitative analysis).

Excretion: Urine: 3.6%;
Feces: 90%
Bile: 92% (in bile-cannulated mice)
Total: 98% of counts excreted in 24 hours

Liver (peak at 0.5 hour) = 5.6 ug/ml

Per cent of dose at 30 minutes postdose (peak concentrations)

Liver	26
Skin	4
Body (excludinggastrointestinal tract)	50
Gastrointestinal tract	55
Total Counts Excreted	108%

SINGLE DOSE BIOAVAILABILITY IN FEMALE DOGS

R6386P. Animal studies (Bayer, Wuppertal September 1994 to October 1994)
PK analyses

TREATMENT: Three female beagle dogs were treated with a single dose in a cross-over design with 0.03 mg/kg i.v. or 0.01, 0.03, and 0.1 mg/kg p.o., all in phosphate buffered saline, pH 7.4 (vs gavage in tap water for toxicity studies to fasted dogs). The limits of quantitation were 0.5 to 89 ng/ml. Plasma was stored "below -18^0 C". There was no statement about how plasma was prepared or transported before analyses. Analyses were

	0.03 mg/kg i.v.	**0.01 mg/kg p.o.**	**0.03 mg/kg p.o.**	**0.1 mg/kg p.o.**
AUC (ng h/ml)	314 ±1.3	70 ± 1.2	180 ± 1.1	700 ± 1.2
Cmax (ng/ml)	no data	7.1 ± 1.6	18 ± 1.1	89 ± 1.5
t1/2 (h)	5 ± 1.2	5.5 ± 1.6	6.1 ± 1.5	4.6 ± 1.7
f (%)*		67	56	67

Geometric mean±SD (n=3)
*bioavailability

SINGLE DOSE BIOAVAILABILITY IN MALE RATS

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

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 After analyses, plasma was stored "below -18^0 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

	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 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 (immunoreactive material) 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)
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 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 β-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 β-oxidation predominates in rat and mouse."
Human (two 1^0 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]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 . **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...).**

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
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 (counting samples as a function of time of elution) 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 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. were used.

In plasma, M1 and M23 accounted however, so
present that only major components could be detected. In urine and feces together, M23 accounted for 23%, M24 for 7% and M1 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 BAY w 6228 and 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.
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.

There was no conversion of the S,R configuration by either rat or dog bile of a 2 mg/kg radioactive dose.

TOXICICTY STUDIES
13-WEEK ORAL GAVAGE TOXICITY STUDY IN MONKEYS (vol 1.58)
6520. March 1993.
TREATMENT: Four groups of rhesus monkeys (3s/g; 3-4 years old from 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.
TREATMENT: Four groups of wild-caught rhesus monkeys (4/s/g; 4-8 years old from) 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-orchiectomy 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-smeared 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
β-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)

(0,100,300 ug/kg/day; 4/s/g)

	MALE	FEMALE
HISTOPATHOLOGY	0 L HD	0 L HD
LIVER:		
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
HEART		
Hemorrhage	0 1 2	0 0 0
LUNGS		
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
PAROTID		
Round cell infiltration	0 2 0	0 1 0
STOMACH		
Hemorrhage	0 1 2	0 0 2
INTESTINE		
Hemorrhage (large intestine)	0 1 2	0 1 1
Necrosis lymph. tiss	0 0 2	0 0 0
GALLBALDDER		
Hemorrhage	0 1 4	0 0 2
Edema	0 1 4	0 0 1
Cholecystitis/erosion	0 0 1	0 0 1
PANCREAS		
Hemorrhage	0 1 0	0 0 0
Edema	0 1 4	0 0 2

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

	MALE	FEMALE
HISTOPATHOLOGY	0 L HD	0 L HD
TONSILS		
Hemorrhage	0 0 1	0 1 3
Necrosis lymph tiss	0 1 2	0 0 0
THYMUS		
Hemorrhage	0 2 1	0 0 1
Edema	0 0 3	0 0 2
Lymphocyte depletion	0 1 3	0 0 2
MANDIB. LYPH NODE		
Necroses	0 1 0	0 0 0
MESENT. LYPH NODE		
Necroses	0 1 2	0 0 0
BRAIN		
Vascular degeneration	0 1 0	0 0 0
Hemorrhage	0 1 0	0 0 0
SPINAL CORD		
Hemorrhag subarach	0 1 0	0 0 0
SCIATIC NERVE		
Vasculitis	0 1 0	0 0 0
OPTIC NERVE		
Vascul degeneration	0 1 0	
URINARY BLADDER		
Hemorrhage	0 1 0	
Edema	0 1 1	
TESTES		
Giant cells	0 2 0	
UTERUS		
Lymphatic follicles		0 0 2
Hemorrhage		0 0 1
EPIDIDYMIDES		
Hypo/aspermia	0 0 4	
SKELETAL MUSCLE		
Myopathy	0 1 2	0 0 2
TONGUE		
Myopathy	0 0 4	0 0 3

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 **hemiorchietcomy** 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), epidydimis (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, gallbaldder): 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 (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)

	Food Intake		**Drug Intake**			
DOSE (ppm)	**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)

	Male					Female				
Dose (ppm)	**0**	**1**	**5**	**25**	**125**	**0**	**1**	**5**	**25**	**125**
LIVER										
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)

	Male					Female				
Dose (ppm)	**0**	**1**	**5**	**25**	**125**	**0**	**1**	**5**	**25**	**125**
LIVER										
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
KIDNEY loss of autophagic vacuole	0	0	0	0	8	*	*	*	*	*

*no data provided for female mice

CARCINOGENICITY STUDY IN MICE

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

	Male					Female				
Dose (ppm)	**0**	**1**	**5**	**25**	**125**	**0**	**1**	**5**	**25**	**125**
LIVER										
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
KIDNEY loss of autophagic vacuole	0	0	0	3	28*	0	0	0	0	0
LYMPH NODES adipose tissue	2	2	3	7	16*	4	6	6	10	27*
FORESTOMACH										
hyperplasia	0	0	2	5	10*	1	3	2	4	9*
hyperkeratosis	1	1	2	1	7	2	3	4	4	9
SKELETAL MUSCLE										
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 MICROSCOPSY 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 presumably due to the low dose used in this chronic study."

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

	Male					Female				
Dose (ppm)	**0**	**1**	**5**	**25**	**125**	**0**	**1**	**5**	**25**	**125**
No. mice	*50*	*50*	*48*	*49*	*46*	*49*	*49*	*49*	*50*	*49*
LIVER										
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**
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**