CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020740/S002

PHARMACOLOGY REVIEW(S)

NDA 20-740/S002 **Review Completed: April 27, 1999**

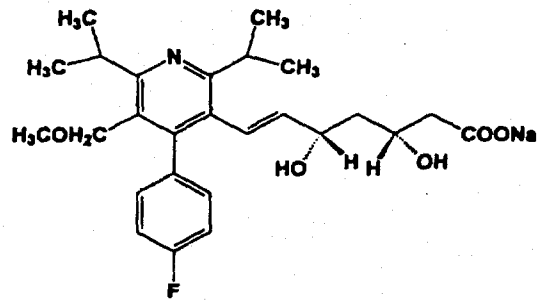
Sponsor: Bayer Corporation; 400 Morgan Lane; West Haven, CT 06516-4175

Date Submitted: April 22, 1999 **Date Received: April 23, 1999**

PHARMACOLOGY REVIEW OF INITIAL IND SUBMISSION
Supplement to NDA 20-740 #002 BM (April 22,1999)

DRUG: Baycol (cerevistatin sodium tablets), 0.4 mg dose. BAY 6228

STRUCTURAL FORMULA:



CATEGORY: Lipid Lowering, "Statin"

INDICATION: Hypercholesterolemia

RELATED IND: NDA 20-740 is approved for currently marketed doses of 0.2 and 0.3 mg tablets. 0.05 and 0.1 mg tablets were approved but never marketed.

CLINICAL STATUS: Approved drug, efficacy supplement to market 0.4 mg tablet. Sponsor indicates that 0.5 mg was approved, but not marketed.

ANTICIPATED SPECIAL RISKS: Extensive listing is provided in the labeling.

BACKGROUND/SUPPLEMENT CONTENTS: Label changes were requested from the sponsor to base animal: human dose multiples on AUC of parent + metabolites on March 25, 1999. The sponsor has submitted an updated label. The issue of AUC has not been resolved. The sponsor indicates that this is still being addressed. The sponsor mentions that In a telecon on March 10, 1999, that I said the "current labeling could remain as is, but that we should try to get the requested information as soon as possible". I believe that this statement was misinterpereted, since the Cmax/free is still part of the label. What I intended to convey is that the label could base multiples on Cmax as in the current package label (as listed in the PDR), not that Cmax/free is acceptable. If AUC data are unavailable, comparisons should be recalculated for the new dosage based on Cmax.

TO BE COMMUNICATED TO SPONSOR:

In your submission of April 22, 1999, reference is made to a telecon on March 25, 1999 that Dr. Steigerwalt said the "current labeling could remain as is, but that we should try to get the requested information as soon as possible". This statement may have been misinterpereted. What Dr. Steigerwalt intended to convey was that the label could base multiples on Cmax as in the current package label (as listed in the PDR), not that Cmax/free is acceptable. If AUC data (parent + metabolites) are unavailable, comparisons should be recalculated for the new dosage based on Cmax.

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader

cc: IND Arch
HFD510
HFD510/Steigerwalt/Simoneau
Review code: IR
Filename: 20740.002bm.doc

NDA 20-740/S002 **Review Completed: March 23, 1999**
Sponsor: Bayer Corporation; 400 Morgan Lane; West Haven, CT 06516-4175
Date Submitted: July 16, 1998
Date Received: July 17, 1998
DRUG: Baycol (cerevistatin sodium tablets), 0.4 mg dose. BAY 6228
CATEGORY: Lipid Lowering, HMG CoA Reductase inhibitor, "Statin"

	TABLE OF CONTENTS	Page #
1.	Background/Supplement Proposal	2
2.	Report #PH-25491: Activity Of Human Metabolites of Cerivastatin	3
3.	Summary of Preclinical "Safety Pharmacology"	3
4.	Study R6691: Liver Medium-Term Bioassay of BAY w 6228	4
5.	Study PH25201: Cytotoxicity on Primary and Permanent Muscle Cell Cultures in an *In Vitro* Model	5
6.	Study PH25699: In Vitro Effects of BAY W 6228 and Related Compounds on Primary and Permanent Neuronal Cell Cultures	6
7.	Study R6570: A Preliminary Repeated Dose Toxicity Study of BAY17-5111 Administered IV to Rats for 2 Weeks (SBL95-41)	7
8.	Study R6571: A Preliminary Repeated Dose Toxicity Study of BAY17-5111 Administered IV to Beagles for 2 Weeks (SBL95-45)	9
9.	Study R6863: A Repeated Dose Toxicity Study of BAYw6228 Administered IV to Beagles for 4 Weeks (SBL95-83)	10
10.	Report: R6830 (SBL95-77): Determination of Rat Plasma Concentration of BAYw6228 Applied By Oral And IV Administration as a Single Dose	12
11.	Report PH-27272 (T3061673): Plasma Concentrations of BAYw6228 After 4 Times Daily vs Dosing to Beagle Dogs for 4 Weeks	13
12.	Report R6736 ([redacted]008/96):Plasma and Liver Concentrations of the Metabolite Bay 17-5111 (M23) After Oral And IV Administration to Male SD Rats	14
13.	Report PH25144: [redacted]C]BAYw6228: Metabolic Pattern in Rat Plasma and Liver Tissue	14
14.	Report PH25276: Metabolism of Cerivastatin by Human Liver Microsomes *In Vitro*. Characterization of Primary Metabolic Pathways and of Cytochrome P450 Isozymes Involved	15
15.	Report PH27187: Cytochrome P450 Isozymes Involved in the Metabolism of Cerivastatin in Humans	16
16.	Study PH-27176: Determination of the Inhibitory Potency of Cerivastatin and Its Pharmacologically Active Metabolites M-1 (BAYw5679) and M-23 (Bay 17-5111) Towards Human Cytochrome P-450 Isozymes	17
17.	Study PH 27579: Exposure in a 4 Week Mouse Study Mimicking the Conditions of a Cancerogenicity Study (T9061994)	18
18.	Labeling Review	19
19.	Conclusions	20
20.	**Recommendation/TO BE COMMUNICATED TO SPONSOR**	22
21.	APPENDIX (copy of original NDA review)	23

/S/ [redacted]

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader

3/23/99

cc: IND Arch
HFD510
HFD510/Steigerwalt/Simoneau
Review Code: AP
Filename: 20740.002.doc

[redacted]APPEARS THIS WAY ON ORIGINAL

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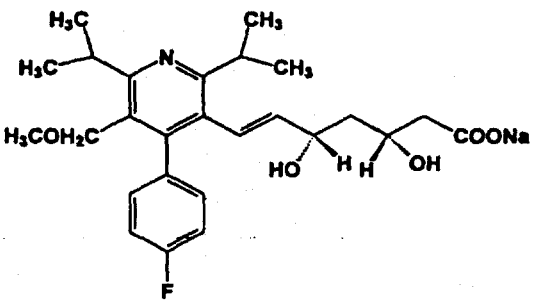

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CLINICAL STATUS: Approved drug, efficacy supplement to market 0.4 mg tablet. Sponsor indicates that 0.5 mg was approved, but not marketed.

ANTICIPATED SPECIAL RISKS: Extensive listing is provided in the labeling.

BACKGROUND/SUPPLEMENT CONTENTS: A number of published references regarding the mechanism of action of Cerevistatin were provided. Most of these do not add significantly to what was already described in Dr. Barbehenn's review of the original NDA. Of particular interest for this review, however, are the pharmacokinetic studies that were submitted. The increased dose will change relative safety margins. The sponsor proposes to base estimations of relative exposure on Cmax,free rather than Cmax upon which comparisons were previously based. These studies are considered most relevant and are reviewed herein. Some of these pharmacokinetic studies have apparently been published while others have not. Several intravenous studies were performed to characterize the toxicity of the metabolites found in humans, but not normally seen with oral dosing in animals. A number of references provide evidence of additional benefits that may be possible with statin treatment (e.g. direct cardiovascular/anti-atherosclerosis effects). Since the sponsor is not pursuing additional indications in this submission, these studies are not extensively reviewed here.

REVIEW OF REPORT #PH-25491:
ACTIVITY OF HUMAN METABOLITES OF CERIVASTATIN
(NO SPONSOR TITLE)

NOTE: No GLP statement or QA. Study dated October 1, 1996.
PURPOSE: To determine the activity of the primary human cerivastatin metabolites.

EXPERIMENTAL DESIGN: Rico rats were fed 15 days with cholestyramine (40g/kg food). Livers were homogenized and microsomal membranes were isolated. Membranes were incubated with [^{14}C]HMGCoA and metabolite. (Metabolites were synthesized by the sponsor) Reaction product ([^{14}C]-mavanolactone) was isolated by [redacted]

RESULTS: All metabolites showed strong inhibition of rat HMGCoA-Reductase on the same order as the parent compound.

TEST AGENT	IC50 (nm)
Cerivastatin	0.72±0.16
M1	1.05±0.15
M23	0.70±0.15
M24	0.72±0.16

CONCLUSION: Human metabolites show similar activity to parent compound in assays of HMGCoA-Reductase activity.

SUMMARY OF PRECLINICAL "SAFETY PHARMACOLOGY"

Two short reports on "safety pharmacology" were performed by the sponsor and presented in this NDA. They were performed by standard methods and are summarized below:

STUDY	SPECIES	DOSE/ ROUTE	RESULTS
Effect on acetylcholine-induced ileal spasms	In vitro Guinea pig ileum	In vitro	BAY 17-5111 did not affect acetylcholine-induced ileal contractions at doses up to 10^{-6}M
Acute IV tox in mice	mice	IV	Approximate LLD in mice for IV administration was 125-250 mg/kg. All animals died within 3 minutes at the HD of 500 mg/kg.

REVIEW OF STUDY R6691:
LIVER MEDIUM-TERM BIOASSAY OF BAY W 6228

NOTE: Performed by [redacted] (Sponsor: Bayer Yakuhin, Ltd, Osaka, JAPAN. Dated September 30, 1996 (completed 5/28/96). Note: this study is similar to some studies reviewed by Dr. Barbehenn under the initial NDA. Lot # 513312.

PURPOSE: To evaluate the hepatocarcinogenic potential of BAY w 6228 in a liver medium-term bioassay system, using DEN as the initiator. This model was studied as a way to further examine the finding of liver tumors detected in the two year mouse bioassay which were not noted in the rat bioassay. This is a medium-term assay to determine the potential of a test agent to promote the formation of preneoplastic lesions.

EXPERIMENTAL DESIGN: 20 Male rats/group were given a single injection of 200 mg/kg N-nitrosodiethylamine (DEN) i.p. 2 weeks later, daily oral doses of test agent were given for 6 weeks as shown in the accompanying table. In addition, two additional groups used mevalonic acid to determine if it was possible to "block" the effect of cerevistatin. Dose selection for BAY w6228 was based on previous toxicology studies that indicated liver effects and body weight loss at 0.3 mg/kg.

"Initiation" with 200 mg/kg DEN i.p.
Week 2: begin dosing with test agent for 6 weeks
Week 3: 2/3 partial hepatectomy
Endpoint: glutathione S-transferase placental form positive foci/liver were quantitated at the end of week 8.

DOSE	GROUP
1. VEHICLE CONTROL	SALINE
2. BAY w 6228	0.03 (mg/kg)
3. BAY w 6228	0.1 (mg/kg)
4. BAY w 6228	0.3 (mg/kg)
5."MAL" + BAY w 6228	dl-mevalonic acid lactone (MAL) + 0.3 mg/kg BAY w6228
6. Mevalonic control	dl-mevalonic acid lactone (MAL)
POSITIVE CONTROL	Sodium phenobarbital 500 ppm dietary

RESULTS

MORTALITY: Two rats in group 1 and one rat each in group 5 and 6 were found dead after partial hepatectomy (day22). Additionally, 2 rats of group 4 and one rat of group 6 were found dead on days 25, 27 and 51, respectively. Deaths were attributed to the surgical procedure.

BODY WEIGHTS: Mean body weights were not significantly different between groups. However, there were markedly reduced body weights in three rats which died after partial hepatectomy. Sodium phenobarbital group had elevated body weights.

CLINICAL CHEMISTRY: Significant elevations of GOT values were apparent in BAY w 6228-treated animals at all doses. This did not clearly increase with dose, but was not noted in vehicle control, MAL control or sodium phenobarbital control.

PATHOLOGY: There was no evidence of effect on liver weight of test article treated groups. There was an increase in liver weight in sodium phenobarbital treated animals. The numbers and areas of GST-P positive foci were similar to vehicle controls. Treatment with MAL also had

no effect (there was no measurable effect of MAL at blocking the effect of BAY w 6228 since there was no effect to be blocked). A significant positive finding for increases in liver in the sodium phenobarbital groups indicates that the assay system was working as expected. The positive finding for liver tumors in the 2 year bioassays was found in mice but not in rats.

DOSE GROUP	GST-P POSITIVE FOCI	
	#/cm^2	Area/cm^2
1. VEHICLE CONTROL	3.31	0.279
2. BAY w 6228 0.3 mg/kg	2.756	0.211
3. BAY w 6228 0.1 mg/kg	3.027	0.211
4. BAY w 62280.3 mg/kg	2.629	0.253
5."MAL" + BAY w 6228	2.961	0.231
6. Mevalonic control	30587	0.279
POSITIVE CONTROL	5.967**	0.686**

**Significantly different at $p<0.01$

COMMENTS: There were no findings of liver tumors in rats in the 2-year bioassay. The sponsor has performed similar studies under the NDA that were previously reviewed by Dr. Barbehenn. Although this study does not indicate that BAY w 6228 induces liver tumors in rats, it does not eliminate the positive finding noted in mice. Therefore, the labeling regarding this issue should remain as it is.

REVIEW OF STUDY PH25201: CYTOTOXICITY ON PRIMARY AND PERMANENT MUSCLE CELL CULTURES IN AN *IN VITRO* MODEL

NOTE: Study dated 6/24/96.

PURPOSE: To examine the direct cytotoxic effects of BAYw6228 *in vitro* in different muscle cell lines. This is to address the *in vivo* findings of degenerative changes in skeletal muscle in rats at 5 mg/kg. (This effect could be antagonized by the administration of mevalonic acid lactone indicating that the effect was due to the HMG CoA reductase activity of the BAYw6228.

EXPERIMENTAL DESIGN: Cell lines: Rat skeletal myoblasts (l6), rat heart myoblasts (H9c2), rat smooth myoblasts (A10) obtained from [redacted] Primary rat heart myoblasts from heart and skeletal muscle were obtained from rat fetus of Wistar rats. Vehicle was DMSO (10 mg/ml) Cells were plated into 96-well plates at $1x10^4$ cells/well for 24h. BAYw6228 was added for 72 h. Cytotoxicity was measured by viability staining with Neutral red. The following endpoints were measured: Mitochondrial dehydrogenase activity, cell proliferation, F-actin content, actin, myosin, protein binding.

RESULTS

1. Cell viability: Concentrations of 0.001-10 µg/ml were tested. 0.1 µg/ml decreased viability of heart myoblasts (primary and permanent) by 20-40% and smooth muscle cells by 50%. Higher concentrations intensified this effect. Skeletal muscle cells were affected at higher concentrations. Similar findings were determined by Neutral red and Mitochondrial dehydrogenase measurements of cytotoxicity.

2. Cell proliferation: Proliferation was inhibited at 0.1 µg/ml, particularly in primary heart cells. However, no effect on skeletal myoblasts was observed at this level. At doses of 1-10 µg/ml, smooth muscle and skeletal myoblasts were affected.
3. Cytoskeletal protein contents. F-actin was decreased at 0.1 µg/ml in primary heart cells and smooth myoblasts and less in skeletal myoblasts. Other cells were not significantly affected. Actin was decreased at 0.1 µg/ml in primary heart cells and slightly in other cells tested. Skeletal cell lines were least sensitive. Effects on myosin were less evident compared to the effects on actin. All cells were affected at high doses of 10 µg/ml.
4. Protein binding: BAYw6228 was only moderately bound in the incubation media (60%). The free and unbound fraction was independent of drug concentration between 1 and 11µg/ml.

COMMENTS

Direct cytotoxic effects of BAYw6228 on heart, skeletal and smooth muscle cells were observed. *In vitro*, the skeletal muscle cells appeared to be less sensitive than the other cell types. Permanent smooth muscle myoblasts were most sensitive. The most sensitive parameter in this study appeared to be the measurement of F-actin content. The sponsor draws conclusions regarding the effective concentrations *in vitro* and the concentrations observed clinically. This reviewer does not agree with this assessment since studies *in vivo* already indicate that the observed myotoxicity was reversible by mevalonic acid lactone, indicating that the effect observed *in vivo* was due to drug treatment. This occurred at levels obtainable *in vivo*. It is the relative exposure in animals and humans that should be considered in the safety evaluation relative to the effects on muscle.

REVIEW OF STUDY PH25699: IN VITRO EFFECTS OF BAY W 6228 AND RELATED COMPOUNDS ON PRIMARY AND PERMANENT NEURONOAL CELL CULTURES

NOTE: Study dated 12/2/96.

PURPOSE: To investigate the toxicity of HMG CoA reductase inhibitors (BAYw6228, lovastatin and pravastatin) on the CNS and neuronal cell cultures from rats and humans. Cell lines: astrocytes and cortical neuronal cell cultures of rat, human glioma (GO-G-IJKT) and neuroblastoma (Kelly) cell lines. Endpoints evaluated included cytotoxicity, enzyme activity assays, neurotransmitters, cytoskeleton elements and cell specific targets.

RESULTS

BayW6228 and lovastatin were cytotoxic in primary astrocyte and neuronal cell cultures of rat at concentrations >0.1-1 µg/ml. Comparing IC_{50} for this response, BAYw6228 was 10X more cytotoxic than lovastatin. However, there appeared to be variances in the specific cell types afffected (lovastatin appeared to be more toxic to neurons than to astrocytes). BAYw6228 appeared to interfere with the catecholaminergic system. The human cell lines were more sensitive than the rat cell cultures. Pravastatin was less active than the other two compounds.
Neutral red assay: There was no interference with cell membrane integrity below the cytotoxic concentration at 0.01 µg/ml and above.
Acetyl cholinesterase activity (primary cells only): no effect.
Choline acetyltransferase activity: (primary cells only): CHAT was reduced by BAYw6228 at doses below cytotoxic range in astrocytes (NOEC 0.01µg/ml); lovastatin appeared more potent against cortical cell culture neurons.

ELISA's for GFAP, NSE, MAP-2, Neurofilaments, GAD, GS, DNA, A2B5, Serotonin, Dopamine, GABA, TH: The amino acid neurotransmitter systems appeared to be more sensitive than the catecholaminergic transmitter systems. Amino acid transmitter systems were decreased in the cytotoxic range after treatment with BAYw6228 and lovastatin. Microtubule associated protein-2 was reduced at drug levels below the cytotoxic concentration for BAYw6228 and pravastatin in primary cell cultures of rat and in human permanent cell lines.

COMMENTS

The present study indicates that there is the possibility of direct neurotoxicity of BAYw6288. In the intial NDA, clinical evidence of neurotoxicity occurred in up to 37% of dogs given 180 mg/kd/day lovastatin for 11 or more days. This was not observed at 60 mg/kg. The CNS of dogs affected exhibited endothelial degeneration and hemorrhagic encephalopathy. The nerve degeneration was interpreted as due to ischemic effects. Although animals had lower levels of vitamin E, the neurological effects were not reversed by oral supplementation. A Wallerian-like degeneration was observed in the optic nerve. In addition, the present studies also demonstrate that effects on microtubules and transmitter systems were detected at doses below the cytotoxic range. These studies were designed to help determine if the toxicity was direct or vascular. Toxicity ranking was BAYw6228>lovastatin>pravastatin. The sensitivity of cell types to BAYw6228 was glia>neuronal cells, with human cells being more sensitive than rat cells. Similar comments were made regarding clinical exposure and the effective doses *in vitro* as were made for muscle cells. However, as noted in the muscle cell study, the neurotoxicity occurred at levels obtainable *in vivo* and it is the relative exposure in animals and humans that should be considered in the safety evaluation relative to the effects on neurons.

REVIEW OF STUDY R6570: A PRELIMINARY REPEATED DOSE TOXICITY STUDY OF BAY17-5111 ADMINISTERED IV TO RATS FOR 2 WEEKS (SBL95-41)

NOTE: Study dated 5/6/96. Testing facility was [redacted] In [redacted] No GLP or QA statements were provided.

PURPOSE: To evaluate the toxicity of the BAYw6228 metabolite, BAY 17-5111, and determine dose levels for future toxicity studies.

EXPERIMENTAL DESIGN: 4 Crj:CD (SD) rats/sex/group received BAY 17-5111 IV at 0, 0.6, 3.0 and 15.0 mg/kg and 1.0 mg/kg BAYw6228 for 2 weeks.

RESULTS

MORTALITY: None in metabolite groups. One BAYw6228 female died on day 13, Necropsy revealed a dark red liver, enlargement of adrenals and "red eye gum", as well as elevated adrenal, heart, lung and liver weights and decreased thymus weight. Cause of death was not stated.

OBSERVED EFFECTS: No treatment-related effects. Clinical signs in the BAYw6228 animal that died included emaciation, decreases in food and water consumption and spontaneous activity after day 12.

BODY WEIGHT: Decrease in body weight in one male and female in the HD group on day 13. Mean weights were slightly, but not significantly decreased in HD males. Body weight gain was decreased non-significantly in MD and HD animals of both sexes, particularly in one high dose male that didn't gain as much weight as other animals of the group.

FOOD CONSUMPTION: No treatment-related effects.

VITAL SIGNS: No data.

OPHTHALMIC EXAMINATION: No treatment-related effects.

HEMATOLOGY: Signs of anemia in one HD female and low Hct and Hb was noted in one MD female. In BAYw6228, there was an increase in segmented neutrophilic leukocyte count and ratio, low lymphocyte count and ratio.

COAGULATION: No treatment-related effects.

BONE MARROW: No data.

BLOOD CHEMISTRY: Elevated ASAT, ALAT was noted in HD males (3X and 5X, respectively). ALAT was also elevated in MD females and HD females (significantly in MD, not HD). ALP was elevated non significantly, but with a dose-related trend in males. An elevated β-globulin ratio was noted in HD females. Albumin was reduced in all male groups. For BAYw6228, elevated ASAT in males and females, elevated ALAT in females. In females, there was elevated LDH, CPK, α_2- and β–globulin ratios and low albumin concentration and ratio.

URINALYSIS: No treatment-related effects. There was a trend to decreased total excretion of potassium, sodium and chloride in all BAY17-551 treated groups. This did not occur clearly in females and was not clearly dose-related in males. The significance of this finding which was not mentioned by the sponsor is unclear.

ORGAN WEIGHTS: No treatment-related effects. In BAYw6228: elevated relative spleen weights.

GROSS PATHOLOGY: No treatment-related effects.

HISTOPATHOLOGY: Slight single cell necrosis of hepatocytes and microgranulomas were seen from 1 male in MD and HD groups. In BAYw6228: mononuclear cell infiltration of the portal area and slight single cell necrosis of hepatocytes.

DISCUSSION

Based on the results of this study, Doses of 0.4, 1.5, and 6.0 mg/kg BAY 17-5111 were chosen for a 4-week IV study in rats.

REVIEW OF STUDY R6571: A PRELIMINARY REPEATED DOSE TOXICITY STUDY OF BAY17-5111 ADMINISTERED IV TO BEAGLES FOR 2 WEEKS (SBL95-45)

NOTE: Study dated 5/6/96. Testing facility was [redacted] In [redacted]. No GLP or QA statements were provided.

PURPOSE: To evaluate the toxicity of the BAYw6228 metabolite, BAY 17-5111, and determine dose levels for future toxicity studies.

EXPERIMENTAL DESIGN: 2 beagles/sex/group received BAY 17-5111 IV at 0, 0.04, 0.2 and 1.0 mg/kg and 0.1 mg/kg BAYw6228 for 2 weeks.

RESULTS

MORTALITY: None.

OBSERVED EFFECTS: No treatment-related effects.

BODY WEIGHT: No treatment-related effects.

FOOD CONSUMPTION: No treatment-related effects.

VITAL SIGNS: No data.

OPHTHALMIC EXAMINATION: No treatment-related effects.

HEMATOLOGY: No treatment-related effects.

COAGULATION: No treatment-related effects.

BONE MARROW: No data.

BLOOD CHEMISTRY: Slightly elevated ALAT in one each of the HD males and females and one MD female. There was a slight decrease in total cholesterol and phospholipid in the HD male. Similar findings were seen with BAYw6228.

URINALYSIS: No treatment-related effects.

ORGAN WEIGHTS: No treatment-related effects.

GROSS PATHOLOGY: No treatment-related effects.

HISTOPATHOLOGY: No treatment-related effects.

DISCUSSION

Based on the results of this study, Doses of 0.04, 0.2, and 1.0 mg/kg BAY 17-5111 were chosen for a 4-week IV study in beagles.

REVIEW OF STUDY R6863: A REPEATED DOSE TOXICITY STUDY OF BAYw6228 ADMINISTERED IV TO BEAGLES FOR 4 WEEKS (SBL95-83)

NOTE: Performed by [redacted] Study dated June 20, 1997. GLP statement [redacted] provided. QAU statement provided. Lot No. 513312. 97.3% purity.

PURPOSE: To examine the toxicity of BAYw6228 when administered intravenously to beagles daily for 4 weeks. It is expected that the IV route may generate human metabolites not normally seen in dogs. This study was to characterize the toxicities of metabolites

EXPERIMENTAL DESIGN: 3/sex/group 11-15 month old beagles were administered physiological saline (control) or 0.3 mg/kg BAYw6228 at 5 ml/kg/day.

RESULTS

MORTALITY: 2 females died on day 19 and 17. 2 males were sacrificed moribund on days 19 and 26. This left only a single surviving male and female in the treated group. (males #8, 9; females #10,11).

OBSERVED EFFECTS: In animals that died or were sacrificed, the following were observed: bloody or tarry stool, hematemesis, slight or moderate decrease in spontaneous activity, sitting, lateral or prone position, anorexia, gasping. Tremors, head shaking, diarrhea, soft stools, salivation or vomiting with accompanying decrease in food consumption and body weight. In surviving animals, vomiting was observed 3 times in the female, no abnormalities were noted in the clinical signs of the surviving male.

BODY WEIGHT: Decrease in body weight was noted at death or sacrifice (related to anorexia). In the single surviving male and female, no treatment-related changes were noted.

FOOD CONSUMPTION: In animals that died or were sacrificed, decreased food consumption was observed. In animals that died, there was a refusal to feed prior to death. There was no change in food consumption in the surviving animals.

WATER CONSUMPTION: No treatment-related changes were observed.

VITAL SIGNS: No changes observed in ECG.

OPHTHALMIC EXAMINATION: No treatment-related changes were observed.

HEMATOLOGY: High leukocyte count in one male and one female (sacrificed or died) during week 2, as well as the two remaining males and in one female (survivor) at week 4. In the affected animals, high segmented neutrophil count and ratio and low lymphocyte count and ratio were also noted in some animals. Due to the variability and small number of animals, it was difficult to clearly attribute this the treatment.

COAGULATION: Prolongation of APTT in one treated male at week 4.

BONE MARROW: No data.

BLOOD CHEMISTRY: Elevated ASAT, ALAT, LDH, CPK ALP and uric acid as well as decreased glucose, albumin and cholesterol were noted in the animals that died or were sacrificed moribund. In surviving animals, there was also high ASAT, ALAT and CPK. Low total cholesterol and phospholipid were expected pharmacological effects.

URINALYSIS: Males: Moderate positive reaction in bilirubin and marked positive reaction in occult blood. Decreased urine volume, low Na, K and Cl concentrations and low total excretion were noted at 4 weeks in one sacrificed male. No changes were noted in the other males.
Females: Slight positive reaction in bilirubin in one female that died (this was noted prior to dosing, however). Marked positive reaction in occult blood noted prior the initiation of dosing of all controls and one treated animal was considered to be due to estrous. There were no statistically significant changes in Na, K and Cl.

ORGAN WEIGHTS: Increases in lung (n=3) and spleen weights (n=2) were observed in dead or sacrificed animals. One animal had increases in pituitary, ovary and adrenal weights. In surviving animals, one female had increased lung and spleen weights.

GROSS PATHOLOGY: In dead or sacrificed animals, slight to marked dark red mucosa of stomach body, small intestine, large intestine was observed. Slight to moderate dark red color was noted in mesenteric lymph node of one male and one female. Slight or moderate dark red color was noted in the lung of three animals. Also, white foamy fluid was noted in the trachea of one male and one female. Moderate dark reddening was noted in the thymus of 2 females. Edmatous thickening of the gastric wall and slight dark red mucosa of the gallbladder in one female were also noted. Slight enlargement of the mesenteric lymph node was observed in one female. Moderate dark reddening of the liver was noted in the other female.

There were no abnormal gross changes in the surviving animals at the end of the test period.

HISTOPATHOLOGY: In dead or sacrificed animals (#8-11), local degeneration of myocardial fiber in 2 animals, diffuse degeneration of muscle fiber (skeletal muscle and tongue) of all animals, the esophagus of 3 animals and the skeletal muscle of 2 animals were noted. Mononuclear cell infiltration was noted in the heart. There were also congestion or congestion and hemorrhage in mucosa or mucosa to serosa in the gastrointestinal tract, kidney liver and gallbladder. Congestion and edema were also noted in the lung with inflammatory cell infiltration in the alveolus, perivascular mononuclear cell infiltration, focal giant cell proliferation, inflammatory cell infiltration in the bronchiole and proliferation of bronchiolar epithelium. Necrosis of the cortex of the thymus, congestion and hemorrhage in the adrenal, decrease in glycogen in hepatocytes and blood absorption in mesenteric lymph node and lymph nodule of the rectum. There was diffuse regeneration of the tongue in 2 animals and in skeletal muscle in 1 animal. Interestingly, in the surviving animals, the only treatment-related change was diffuse regeneration of the muscle fiber in the tongue of the female. Other findings were considered incidental. However, it is difficult to determine a complete toxicological profile in this study due to the low number of animals.

TOXICOKINETICS: AUC indicated no gender differences with repeated administration, however, this is based on one surviving male and female. AUC = 1453.67 and 1458.7 ng•h/ml for males and females, respectively after the first dose. The AUC in this study was approximately 3X that in the previous study (4 week IV in beagles with 0.1 mg/kg), which is in

agreement with the dose ratio of 0.1 and 0.3 mg/kg. There appeared to be no accumulation with repeated dose (data available for 1 male and 1 female only). The values were similar at the end of the study (one animal of each sex surviving 1445.0 and 1591 ng•h/ml for male and female respectively). $T_{1/2}$ was approximately 2 h.

DISCUSSION

Although this is a very limited study (only one dose was used), when taken with results from previous IV studies (SBL 95-63), the results indicate that that the NOAEL for IV Bay 6228 is approximately 0.1 mg/kg/day. The next dose tested was 0.3 mg/kg/day in this study proved fatal to most of the animals. Thus, there is a small margin of safety between doses that have relatively little effect and those that are fatal. The major human metabolite, while nearly equally biologically active to the parent, appears to be less toxic. This is curious, since most of the toxicity of these drugs is attributed to biological action. This study is insufficient to provide a complete toxicological profile due to the single dose group used and small number of test animal survivors. However, when comparing the previous study performed at 0.1 mg/kg/day, it could be estimated that a NOAEL would be 0.1 mg/kg/day.

PHARMACOKINETICS SUMMARY

REPORT: R6830 (SBL95-77): DETERMINATION OF RAT PLASMA CONCENTRATION OF BAYw6228 APPLIED BY ORAL AND IV ADMINISTRATION AS A SINGLE DOSE

NOTE: Testing facility: [redacted] Study dated 5/26/97. No GLP or QA statement provided.

EXPERIMENTAL DESIGN: Wistar rats, vehicle = PBS. 5 males/group.
Oral doses: 0.1, 0.2, 0.5,1.0 and 2/0 mg/kg: collection times: 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8 and 24 h after dosing.
IV dose: 0.5 mg/kg: collection time 2, 5, 15 and 30 min and 1, 2, 4, 6, 8 and 24 h after dosing.

RESULTS: (Arithmetic means)

Oral PK				
DOSE (mg/kg)	AUC (µg•h/L)	C_{MAX} (µg/L)	T_{MAX} (h)	$T_{1/2}$ (h)
0.1	15.3	3.81	0.60	6.29
0.2	29.0	9.16	0.40	5.80
0.5	104	35.3	0.40	4.84
1.0	208	110	0.70	5.45
2.0	444	292	0.55	11.3

IV PK	
PARAMETER	0.5 mg/kg DOSE
AUC (µg•h/L)	430
MRT	2.9
$T_{1/2}$ (h)	7.62
Vss	3.27
CL (l/h/kg)	1.18

SUMMARY:

1. AUC appeared to be dose proportional.
2. Cmax appeared to increase in a greater than dose proportional manner.
3. Cmax was generally reached by 0.6 h with oral dosing.
4. Absolute bioavailability ranged from 16.3-25.9%.

REPORT PH-27272 (T3061673): PLASMA CONCENTRATIONS OF BAYw6228 AFTER 4 TIMES DAILY VS DOSING TO BEAGLE DOGS FOR 4 WEEKS

NOTE: Testing facility: Sponsor. Study dated 3/11/98. Signed GLP and QA statement provided.

EXPERIMENTAL DESIGN: Oral administration to beagles for 4 weeks. QID dosing of 6.25, 17.5 and 50 µg/kg compared to OD dosing of 200 µg/kg. Samples were taken on days 1, 4 (only for 50 µg/kg qid and 200 µg/kg od) and 24 days at the dosing interval of 0-6 h for qid and 0-24 for od.

RESULTS:

1. There was no evidence of gender effects.
2. AUC and Cmax increased roughly dose proportionally with qid dosing.
3. Tmax was observed at 2h after administration. QID: Residual concentrations at the end of the dosing interval were 48-73% of the maximum concentration indicating a very low peak-trough fluctuation. In contrast, OD dosing had large fluctuations as might be expected. Trough concentrations were only 1-2% of Cmax.
4. A moderate accumulation was observed with QID dosing. Both AUC and Cmax increased 30-100% between days 1-24. No accumulation was observed with OD dosing.
5. The AUC_{0-6} of the first dose of 50 µg/kg equalled nearly ¼ of the AUC_{0-24} for 200 µg/kg which would be expected from dose linearity.
6. Cmax after qid dosing was nearly ¼ of the Cmax of od dosing of 200 µg/kg.
7. The main difference between the two dosing intervals was observed at the end of the respective dosing intervals. The sponsor suggests that the higher trough concentrations with qid dosing may be explain the more severe toxicity observed. However, there were no toxicity findings presented in this report to confirm this. There was approximately an 8-fold difference in trough values between qid and od dosing. If it is indeed the elevated trough levels that resulted in increased toxicity, Cmax may not be a good parameter upon which to base safety multiple calculations.

DAY 1				
DOSE (µg/kg)	6.25	17.5	50	200
AUC (µg•h/l)	19.1	46.4	120	975
AUC_{NORM} (kg•h/l•10^{-3})	3056	2654	2404	4875
C_{MAX} (µg/l)	4.98	12.5	27.3	198
$C_{MAXNORM}$ (kg/l•10^{-3})	797	713	545	988
T_{MAX} (h)	1.78	2.00	2.52	1.59
C(6h)/C_{MAX} (%)	48.0	47.8	63.2	n.c.
C(24h)/C_{MAX} (%)	n.c.	n.c.	n.c.	1.39
DAY 24				
DOSE (µg/kg)	6.25	17.5	50	200
AUC (µg•h/l)	34.4	68.5	no samples	1016
AUC_{NORM} (kg•h/l•10^{-3})	5505	3915		5082
C_{MAX} (µg/l)	7.23	15.7		226
$C_{MAXNORM}$ (kg/l•10^{-3})	1157	900		1128
T_{MAX} (h)	2.00	3.46		1.68
C(6h)/C_{MAX} (%)	74.8	73.3		n.c.
C(24h)/C_{MAX} (%)	n.c.	n.c.		1.25

REPORT R6736 (A&M008/96):PLASMA AND LIVER CONCENTRATIONS OF THE METABOLITE BAY 17-5111 (M23) AFTER ORAL AND IV ADMINISTRATION TO MALE SD RATS

NOTE: Study dated 12/9/96. Test facility: [redacted]

PURPOSE: To examine the PK of the major human metabolite, BAY17-5111 in plasma and liver of SD rats.

EXPERIMENTAL DESIGN: 3 rats/sex/group were administered BAY17-5111 as follows:
Oral: 0.5 and 5.0 mg/kg. Plasma and liver samples were taken at 1, 4, 7 and 24 h.
Intravenous: 0.03, 0.1, and 0.5 mg/kg. Plasma and liver samples were taken at 0.25, 2, 7 and 24 h.

RESULTS:

1. Most of the plasma samples were below the LOQ. For oral administration only the high dose could be evaluated for PK parameters.
2. For liver, PK could be measured in all dose groups.
3. C_{max} was reached by the first sampling time (iv, 15 min; oral, 1 h)
4. Both C_{max} and AUC in the liver were dose proportional with the exception of the AUC after IV administration was 1.7X over-proportional at the high dose
5. Liver concentrations were several-fold higher compared to plasma (ratios of liver to plasma ranged between 60-130 IV and 600-900 after oral).
6. Although initial concentrations were high, t1/2 was very short after iv administration (<1h). After oral administration, rapid elimination was observed between 1-4 h with a plateau from 4-7 h.
7. The results indicate a very high first pass extraction by the liver after oral administration.
8. Due to the low numbers of animals and few sampling times, the values presented by the sponsor can only be assumed to be rough estimates. Therefore, specific values are not presented in this review.

REPORT PH25144: [^{14}C]BAYw6228: METABOLIC PATTERN IN RAT PLASMA AND LIVER TISSUE

NOTE: Study Dated 6/5/96. Performed at [redacted] No QA or GLP statements provided.

PURPOSE: To investigate the metabolic pattern in plasma and liver tissue after a single oral administration of radiolabeled BAYw6228 to male Wistar rats.

EXPERIMENTAL DESIGN: 1 mg/kg of [redacted]C]BAYw6228 was administered to male rats. Samples were taken at 0.5, 1, 3, 7 and 24 h after dosing. Plasma samples were prepurified and concentrated by a [redacted] method. Recoveries ranged from 51.3% at 24 h to 100% at 0.5 h. Liver samples were extracted with water/acetonitrile and analyzed as plasma samples were by [redacted]

RESULTS:

Metabolic pattern in plasma and liver showed similar time-dependent changes. The relative amount of unchanged drug decreased with time whereas metabolites **M21** and **M30** increased. **M21** is formed by demethylation, single β-oxidation, dehydration and hydrogenation and is the major metabolite in rats, particularly at the later time points. It increased from 2.5 % to 46.2 % of the radioactivity found in plasma from 0.5 to 24 h. **M30** is the methylated congener of **M21** and was in trace amounts at 0.5 h and increased to approximately 4% after 3 h. The demethylated drug, **M1**, was found in small amounts in both liver and plasma (~4.9% and 0.9% at 0.5 and 7 h). There were 2 unknown metabolites that were detected at 2-4% at all time points between 0.5 and 7 h. In previous studies where metabolism was examined by [redacted] methods, the [redacted] method did not pick up large amounts of the corresponding lactone M8.

REPORT PH25276: METABOLISM OF CERIVASTATIN BY HUMAN LIVER MICROSOMES *IN VITRO*. CHARACTERIZATION OF PRIMARY METABOLIC PATHWAYS AND OF CYTOCHROME P450 ISOZYMES INVOLVED

NOTE: Study dated 7/19/96. Study performed by sponsor. No GLP or QA statements provided.

PURPOSE: To examine the *in vitro* metabolism of BAYw6228 using human liver microsomes.

EXPERIMENTAL DESIGN: Microsomes were added so that ratios of 1:5 to 1:8 (CYP: substrate) were achieved on a molar basis. After 3 min of preincubation at 37°C, the reaction was started by addition of 1 µl of labeled Cerivastatin in methanol. The reaction mixture was incubated with shaking and samples taken at 0.5, 5, 10,20 and 60 min after start of incubation. Reaction was stopped by adding 1 ml ice-cold acetonitrile. Supernatants were concentrated and examined by [redacted]

In addition, [[redacted]C] Cerivastatin was incubated with microsomes from 11 human B lymphoblastoid AHH-1 cell lines which stably express single CYP isozymes.
CYP 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2D6Met, 2D6Val, and 2E1 did not appreciably metabolize cerivastatin.
CYP 3A4 was found to produce a 2% turnover within 2 h. The product of this reaction was the demethylated metabolite M1. Specific inhibition experiments were performed to determine that metabolism was considerably blocked by TAO, a specific inhibitor of CYP3A enzymes.

RESULTS:
To major biotransformation reactions characterize the metabolism of cerivastatin in human liver microsomes: demethylation of the benzylic methylether leads to metaboite M1, while hydroxylation of one methyl group in the 6'-isopropyl moiety produces metabolite M-23. M-24 is the result of both of these reactions in tandem. Lactonization of M1 and M23 to M22 and M31, respectively was attributed to "sample work-up", but was specifically addressed in this study.

The inhibition experiment with TAO indicated that both metaboic pathways, hydrolylation and demethylation were equally affected and that cytochrome P4503A plays a major role in cerivastatin metabolism.

REPORT PH27187: CYTOCHROME P450 ISOZYMES INVOLVED IN THE METABOLISM OF CERIVASTATIN IN HUMANS

NOTE: Study dated 3/3/98. Performed by sponsor. No GLP or QA statements provided.

PURPOSE: To identify the CYP450 isozymes involved in the formation of human metabolites. Two major metabolites have been identified in humans: M1 is the result of demethylation of the benzylic methyl ether and M-23 which is the result of stereoselective hydroxylation of a methyl group on the 6-isopropyl group. M-24 is the result of both reactions. **It is important to note that metabolites M-1, -23 and -24 are of similar HMG-CoA reductase inhibitory activity as the parent compound.**

EXPERIMENTAL DESIGN: Human liver microsomes from 16 different samples were used to attempt to quantify differences in metabolic pathways. Microsomal preparations were characterized for the activities of CYP 1A2, 2A6, 2C19, 2D6, 2E1 and testosterone 6β hydroxylase. In addition, CYP 2C8 and 2C9 were examined. This characterization permitted correlation analysis for the metabolism of cerivastatin.

[[redacted]C]Cerivastatin was incubated with each of the characterized human liver microsome samples and formation of M-1 and -23 and total turnover was determined. Correlation values were determined from standard substrates representing distinct CYP 450 isozymes.

Based upon the findings with the initial incubations, the activities of CYP 2C8, 3A4, 2C9 and 2A6 which showed correlation with cerivastatin metabolism were further examined using incubations with human liver microsomes and isozyme-selective inhibitors.

Inhibitors used were: Furafylline, 7.8-benzoflavone, diethyldithiocarbamate, coumarin, quercetin, taxol, S-mephenytoin, Tranylcypromine, Quinidine, 4-methylpyrazole, ketoconazole, tricetyloleandomycin.

Additional experiments were performed with recombinant lymphoblastoid cell lines expressing specific CYP enzymes.

RESULTS:

In previous experiments, incubation with human liver microsomes led primarily to the formation of M-1 and M-23 which varied in relative amounts depending upon the donor. Only one microsome sample was able to form M-24 to appreciable amounts. The results from the current study indicate the following:

1. No correlation was found between cerivastatin metabolite formation and CYP 1A2, 2C19, 2D6, or 2E1.
2. In the initial studies, there was correlation between the rate of M-1 formation and CYP 2C8 and 3A4 which suggested the participation of both enzymes in the demethylation reaction.
3. In the initial studies, there was a good correlation (R^2 = 0.95) between the rate of M-23 formation and CYP 2C8. In addition, CYP 2C9 and 2A6 appeared to be involved in the hydroxylation reaction (R^2 =0.57) to M-23, but to a lesser extent than 2C8.
4. Incubation with the inhibitor, tricetyloleandomycine indicated that CYP 3A4 participates in metabolism.

5. Incubation with quinidine did not affect metabolism, indicating that 2D6 was not likely to be involved in metabolism.
6. The following results were obtained with an expanded list of enzyme inhibitors. These results indicated the involvement of CYP 2C8 and 3A4 in the metabolism of cerivastatin:

INHIBITOR	ENZYME INHIBITED	RESPONSE*
Furafylline	1A2	–
7.8-benzoflavone,	1A2	–
diethyldithiocarbamate	2A6, 2E1, 2C8, 2C9	+ M23 > M1
coumarin	2A6	–
quercetin	2C8	+ M23 > M1
taxol	3A4, 2C8	+ M1 & M3
S-mephenytoin	2C9	–
Tranylcypromine	2A6	–
Quinidine	2D6	–
4-methylpyrazole,	2E1	–
sulfaphenazole	2C9	–
1-aminobenzotriazole	nonspecific	+
ketoconazole	3A4	+ M1 >> M3
tricetyloleandomycin	3A4	+ M1, –M3

*(+ indicates positive for inhibition, – indicates no effect)

7. Further experiments with microsomes from recombinant CYP expressing cell lines indicated that 2C8 was involved in formation of both M-1 and M-23. A CYP3A4-transfected cell line only produced M-1. No other cell lines expressing other CYP enzymes had any effect.

CONCLUSIONS:
Based on the studies outlined above, **the primary metabolic enzyme appears to be CYP 2C8, with CYP 3A4 playing a less important role in the formation of M-1.** These enzymes also catalyzed the formation of a secondary metabolite, M-24. The sponsor suggests that the dual metabolic pathways mediated by different enzymes decreases the probability and severity of the likelihood of drug-drug interactions. In vivo studies with CYP 3A4 inhibitors (erythromycin) to healthy subjects resulted in a 1.2-fold increase in systemic cerivastatin exposure.

STUDY PH-27176: DETERMINATION OF THE INHIBITORY POTENCY OF CERIVASTATIN AND ITS PHARMACOLOGICALLY ACTIVE METABOLITES M-1 (BAYw5679) AND M-23 (BAY 17-5111) TOWARDS HUMAN CYTOCHROME P-450 ISOZYMES

NOTE: Study dated 2/11/98. No QA or GLP statements provided. Study performed by sponsor.

PURPOSE: To examine the inhibitory potency of cerivastatin and its pharmacologically active metabolites towards human cytochrome P450 enzymes.

EXPERIMENTAL DESIGN: Recombinant CYP isozymes (1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) were incubated with standard probes in the absence and presence of potential inhibitors to compare the extent of formation of respective metabolites.

No effect was defined as either no changes in turnover of the reaction or the decrease in formation of the metabolite was not higher than calculated, if a K_i value of 100 µM is assumed.

RESULTS:
Screening studies indicated that cerivastatin, M-1 and M-23 do not affect CYP 1A2, 2A6, 2C19, 2D6, or 2E1. For the enzymes previously shown to be involved in cerivastatin metabolism.

For the enzymes determined to be involved in cerivastatin metabolism, the method of Dixon was used to determine apparent inhibition constants:

Enzyme	Ki (μM)
CERIVASTATIN	
2C8	13.6
2C9	104.9
3A4	285.7
M-1	
2C8	15.0
2C9	–
3A4	151.9
M-23	
2C8	26
2C9	–
3A4	–

CONCLUSION: The sponsor suggests that the inhibition constants of cerivastatin and its metabolites are >2000-fold the plasma concentration in humans after a dose of 400 μg which would indicate that it is not likely that cerivsatatin or its metabolites would inhibit affect CYP 1A2, 2A6, 2C19, 2C9, 2D6, 2E1, 3A4 or 2C8 to a clinically meaningful extent.

STUDY PH 27579: EXPOSURE IN A 4 WEEK MOUSE STUDY MIMICKING THE CONDITIONS OF A CANCEROGENICITY STUDY (t9061994)

NOTE: Study dated June 24, 1998. Signed GLP and QA statements provided.

PURPOSE: To determine the plasma levels of Cerivastatin in mice under the conditions used in the dietary carcinogenicity study.

EXPERIMENTAL DESIGN: B6C3F1 mice (4/control group, 27/dose group) were dosed 1, 5, 25 and 125 ppm Cerivastatin in the diet for 1 month. Blood samples were collected on days 2 or 3 and week 4. Samples were analyzed by [redacted]

RESULTS: Control and LD groups were below the LOQ. In the 5 ppm group, most values were below LLQ, so AUC was not calculated.

5 ppm	Day 2/3		Week 4	
	Male	Female	Male	Female
Dose (mg/kg)	1.65	2.22	2.18	2.5
AUC (0-24) (μg•h/l)	n.c	n.c.	15.9	21.6
Cmax (μg/l)	<0.49	1.02	1.03	1.54
25 ppm	Day 2/3		Week 4	
	Male	Female	Male	Female
Dose (mg/kg)	8.09	11.0	11.2	13.2
AUC (0-24) (μg•h/l)	57.8	81.4	115	122
Cmax (μg/l)	3.44	4.97	7.5	9.21
125 ppm	Day 2/3		Week 4	
	Male	Female	Male	Female
Dose (mg/kg)	39.1	53.9	53.9	56.6
AUC (0-24) (μg•h/l)	423	297	672	577
Cmax (μg/l)	24.4	20.3	48.2	34.4

1. There was no gender difference evident.
2. A dose-related, roughly dose-proportional increase of C_{max} and AUC_{0-24} was noted at both measurements.
3. Ratio of C_{max} to C_{min} was roughly 3 at both time periods measured. C_{max} was noted at night between 11:00pm and 5:00 am.
4. There was a moderate increase in exposure between the day 2-3 measurements and the week 4 measurements in the MD and HD groups. This was accompanied by a slight increase in food intake. It was not possible to determine this for the 5 ppm and 1 ppm groups due to the lack of measurable plasma levels.

LABELING REVIEW

The key issue regarding preclinical pharmacology labeling issues in this submission arises from the change from comparing various toxicities on the basis of C_{max} as done during the initial NDA approval to the basis of $C_{max,free}$. Overall, this enhances the multiples determined of toxicities noted in animals relative to human exposure due to the fact that there is slightly higher plasma protein binding of drug in humans compared to animals. There is no consistency in expression of multiples of the human exposure in the preclinical sections of the labels for any of the approved statins (expression ranges from AUC for Lipitor, to mg/m^2 for Zocor, Lescol and Pravocol, and even varies within a label for carcinogencity studies vs pregnancy category descriptions for the same product). Therefore, it seems reasonable that the re-expression in terms of $C_{max,free}$ could be acceptable since it is generally considered that it is free drug that is the active moiety. The sponsor has not provided any preclinical data to support this contention, however. Instead, in some of the studies provided, there is indication that some human metabolites are equally potent compared to the parent compound in the activity of inhibition of HMG CoA Reductase. In one study, the sponsor suggests that the metabolite is less toxic than the parent compound. Since much of the toxicity appears to be reversible by administration of mevalonate, suggesting toxicity is due to biological activity, it is unclear how an active metabolite should be less toxic. In a dog study where QID and OD daily dosing are compared, the plasma levels appear to be more stable (and higher) with QID dosing compared to OD dosing. However, the sponsor remarks, without showing data, that the elevated trough levels might be responsible for the greater toxicity in the QID group. If this is true, C_{max} may not be the best basis for comparison of exposure levels. These calculations based on $C_{max,free}$, which essentially double the estimated "safety margins" should not be used as a basis to compare the relative safety of the various statins.

According to the original review of this NDA, the metabolic pathways in various species seem to differ. In rats, findings could not be repeated. In this supplement, metabolism in rats in vitro suggest that M21 is the major metabolite in rats. Human data appear to be limited to males. M1 is the major metabolite in rats, dogs and humans.

Human metabolic pattern: M1, M23 ~ 90% of plasma radioactivity M23 and M24 are metabolites that are unique to humans. In urine and feces, M23 and M24 accounted for 7% of dose and M1 accounted for 32 % of dose. Unchanged drug was 1-2% of dose.

Given the fact that at least some of the metabolites exhibit activity and there are variations in metabolism between species, it would seem that relative multiples should be based upon AUC

of parent + metabolite. This was discussed in an internal meeting on March 10, 1999 with the FDA review team and agreed that the appropriate comparison would be based on AUC of parent + metabolites. This reviewer contacted William Maguire of Bayer on March 10, 1999 and communicated this preference to him and requested that the multiples based upon AUC of parent + metabolites in the final form of the label.

CONCLUSIONS

1. ACTIVITY OF METABOLITES: In a study with rat liver microsomes, Human metabolites show similar activity to parent compound in assays of HMGCoA-Reductase activity.
2. INTERMEDIATE DURATION CARCINOGENICITY DETERMINATION: There were no findings of liver tumors in rats in the 2-year bioassay. There were positive findings for liver tumors in the mouse bioassay. The sponsor has performed alternative carcinogenicity studies to determine the potential for induction of liver tumors in rats. Although the study provided does not indicate that BAY w 6228 induces liver tumors in rats, it does not eliminate the positive finding noted in mice. Therefore, the labeling regarding this issue should remain as it is.
3. *IN VITRO* TISSUE TOXICITY (MUSCLE): Direct cytotoxic effects of BAYw6228 on heart, skeletal and smooth muscle cells were observed. *In vitro*, the skeletal muscle cells appeared to be less sensitive than the other cell types. Permanent smooth muscle myoblasts were most sensitive. The most sensitive parameter in this study appeared to be the measurement of F-actin content. The sponsor draws conclusions regarding the effective concentrations *in vitro* and the concentrations observed clinically. This reviewer does not agree with this assessment since studies *in vivo* already indicate that the observed myotoxicity was reversible by mevalonic acid lactone, indicating that the effect observed *in vivo* was due to drug treatment. This occurred at levels obtainable *in vivo* and it is the relative exposure in animals and humans that should be considered in the safety evaluation relative to the effects on muscle.
4. *IN VITRO* TISSUE TOXICITY (NEURONS): The study presented indicates that there is the possibility of direct neurotoxicity of BAYw6288. In the intial NDA, clinical evidence of neurotoxicity occurred in up to 37% of dogs given 180 mg/kd/day lovastatin for 11 or more days. This was not observed at 60 mg/kg. The CNS of dogs affected exhibited endothelial degeneration and hemorrhagic encephalopathy. The nerve degeneration was interpreted as due to ischemic effects. Although animals had lower levels of vitamin E, the neurological effects were not reversed by oral supplementation. A Wallerian-like degeneration was observed in the optic nerve. In addition, the present studies also demonstrate that effects on microtubules and transmitter systems were detected at doses below the cytotoxic range. These studies were designed to help determine if the toxicity was direct or vascular. Toxicity ranking was BAYw6228>lovastatin>pravastatin. The sensitivity of cell types to BAYw6228 was glia>neuronal cells, with human cells being more sensitive than rat cells. Similar comments were made regarding clinical exposure and the effective doses *in vitro* as were made for muscle cells. However, as noted in the muscle cell study, the neurotoxicity occurred at levels obtainable *in vivo* and it is the relative exposure in animals and humans that should be considered in the safety evaluation relative to the effects on neurons. This neurotoxicity appears to be a direct of the drug on neurons rather than vascular effect.
5. IV TOXICITY IN ONE MONTH DOG STUDIES: **The NOAEL for IV BAY 6228 was approximately 0.1 mg/kg/day.** The next dose tested was 0.3 mg/kg/day in this study proved fatal to most of the animals. Thus, **there is a small margin of safety between**

doses that have relatively little effect and those that are fatal. The major human metabolite, while nearly equally biologically active to the parent, appears to be less toxic according to a comment by the sponsor. Data were not provided to support this, however. This is curious, since most of the toxicity of these drugs is attributed to biological action. This study is insufficient to provide a complete toxicological profile due to the single dose group used and small number of test animal survivors. However, when comparing the previous study performed at 0.1 mg/kg/day, it could be estimated that a NOAEL would be 0.1 mg/kg/day, indicating a small safety margin between NOAEL and lethal doses at 0.3 mg/kg.

6. SINGLE DOSE PK IN RATS:
    a. AUC appeared to be dose proportional.
    b. C_{max} appeared to increase in a greater than dose proportional manner.
    c. C_{max} was reached by ~ 0.6 h with oral dosing.
    d. Absolute bioavailability ranged from 16.3-25.9%.
7. ONE MONTH PK IN DOGS:
    a. There was no evidence of gender effect.
    b. AUC and C_{max} increased roughly dose proportionally with qid dosing.
    c. T_{max} was observed at 2h after administration. QID: Residual concentrations at the end of the dosing interval were 48-73% of the maximum concentration indicating a very low peak-trough fluctuation. In contrast, OD dosing had large fluctuations as might be expected. Trough concentrations were only 1-2% of C_{max} with OD dosing.
    d. A moderate accumulation was observed with QID dosing. Both AUC and C_{max} increased 30-100% between days 1-24. No accumulation was observed with OD dosing.
    e. The AUC_{0-6} of the first dose of 50 µg/kg equaled nearly ¼ of the AUC_{0-24} for 200 µg/kg which would be expected from dose linearity.
    f. C_{max} after qid dosing was nearly ¼ of the C_{max} of od dosing of 200 µg/kg.
    g. The main difference between the two dosing intervals was observed at the end of the respective dosing intervals. The sponsor suggests that the higher trough concentrations with qid dosing may be explain the more severe toxicity observed. However, there were no toxicity findings provided in this report to confirm this. There was approximately an 8-fold difference in trough values between qid and od dosing. If it is indeed the elevated trough levels that resulted in increased toxicity, C_{max} may not be a good parameter upon which to base safety multiple calculations.

8. METABOLISM BY HUMAN LIVER MICROSOMES: Based on the studies outlined above, **the primary metabolic enzyme appears to be CYP 2C8, with CYP 3A4 playing a less important role in the formation of M-1.** These enzymes also catalyzed the formation of a secondary metabolite, M-24. The sponsor suggests that the dual metabolic pathways mediated by different enzymes decreases the probability and severity of the likelihood of drug-drug interactions. In vivo studies with CYP 3A4 inhibitors (erythromycin) to healthy subjects resulted in a 1.2-fold increase in systemic cerivastatin exposure.
9. ACTIVITY OF BAYW6228 AND METABOLITES ON RECOMBINANT P450 ENZYMES: The sponsor suggests that the inhibition constants of cerivastatin and its metabolites are >2000-fold the plasma concentration in humans after a dose of 400 µg which would indicate that it is not likely that cerivsatatin or its metabolites would inhibit affect CYP 1A2, 2A6, 2C19, 2C9, 2D6, 2E1, 3A4 or 2C8.

RECOMMENDATION

Pharmacology recommends approval of NDA 20-740 supplement 002 pending the appropriate modification of the label to reflect animal and human exposure comparisons based on AUC of parent + metabolites.

TO BE COMMUNICATED TO SPONSOR

Given the fact that at least some of the metabolites of Baycol exhibit biological activity and there are variations in metabolism between species, the label should include relative multiples (human vs animal) based upon total AUC of parent + metabolite rather than Cmax,free. This is to reiterate the points conveyed in a telecon between Dr. Ronald Steigerwalt of the FDA and William Maguire of Bayer on March 10, 1999.

/S/ [redacted]

Ronald W. Steigerwalt, Ph.D.
Pharmacology Team Leader

3/23/99

[redacted] APPEARS THIS WAY ON ORIGINAL

APPENDIX

NOTE: THE APPENDIX CONTAINS A COPY OF THE ORIGINAL NDA REVIEW. TO ACCOMMODATE SUBMISSION TO THE NEW ELECTRONIC FILING SYSTEM, A NUMBER OF FORMATTING CHANGES WERE INCORPORATED TO THIS COPY. NO CONTENT WAS ALTERED. THE PAGE NUMBERS LISTED IN THE TABLE OF CONTENTS OF THE ORIGINAL REVIEW, HOWEVER, WILL NOT CORRESPOND TO THE ACTUAL PAGE NUMBERS ON THIS COPY OF THE REVIEW.

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

/S/ [redacted]

Elizabeth Barbehenn, Ph.D.

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

CONTENTS

	Page
ADME	
Asssays (RIA and HPLC)	3
Mice (p5); Rats (p7); Dogs (p10); Rabbits (p12): Monkeys (p13); Humans (p14)	
TOXICITY STUDIES	
3-MONTH MONKEY (0, 10, 30, 100 ug/kg/day;	22
1-YEAR MONKEY (0, 10, 30, 100 ug/kg/day;	22
1-YEAR DOG (0,100, 300 ug/kg/day; 4/s/g; gavage)	22
1-YEAR MALE DOG (0,100 ug/kg/day; 15/g; gavage)	25
2-YEAR MOUSE CARCINOGENICITY diet (0,1, 5, 25, 125 ppm; 50/s/g)	27
2-YEAR RAT CARCINOGENICITY diet (0,0.1, 0.5, 2.5 ppm; 50/s/g)	33
GENOTOXICITY (Dominant Lethal)	36
REPRODUCTIVE TOXICITY	
TERATOLOGY (RATS) (0,32,160,720 ug/kg; 30 & 15/g)	38
PERINATAL-POSTNATAL (RATS) (0,32,100,320 ug/kg; 30/g)	40
TERATOLOGY (RABBITS) (0,30,150,750 ug/kg; 15/g)	42
SPECIAL STUDIES	
Secretion of radioactivity in milk; excretion across placenta	44
Ubiquinone and vitamin E in plasma & tissues of dog	46
Dog: lens, testicualar toxicity studies	49
SUMMARY AND EVALUATION	53
CONCLUSIONS	63

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)

[redacted]

CROSS-REACTIVITY OF METABOLITES IN [redacted]
R6535. [redacted] 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 [redacted] (two sets with different concentrations of drugs) 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 [redacted] AND BIOASSAY (animal plasma) (vol 1.76)
PH 24234. [redacted] (appendices 2-7); Bayer (appendix 8); [redacted] (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.

Transfer to [redacted] was August 1995. *PK done October-December 1995* ([redacted]

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 [redacted] laboratory and analyzed by [redacted] with [redacted] after [redacted] irradiation. Samples were stored below -15° C.

MOUSE CMAX ([redacted] ASSAY) FROM CARCINOGENICITY STUDY (ng/ml)

	day 382		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

means 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. [redacted] assay. *CA study done July 1991-Sept. 1993* (Bayer, Wuppertal, Germany); *PK study done February 1993-May 1994* ([redacted]
T 3040234 (Mouse CA study)
Submission of 4/22/97: Sample transfer to [redacted] 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 [redacted] and analyzed by [redacted] Individual plasma concentrations were determined and geometric means and SD calculated. The limit of detection was "about 1 ng/ml".

MOUSE CMAX ([redacted] ASSAY) FROM CARCINOGENICITY STUDY(ng/ml)

	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

mean 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 from [redacted]) as used in the CA study were used with the [redacted] method 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 by [redacted] assay)

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

[redacted] APPEARS THIS WAY ON ORIGINAL

PLASMA DRUG LEVELS IN RAT CARCINOGENICITY STUDY (vol 1.83; p.155)
PH-24562. *Institute of PK, Bayer (days 6 & 237) and* [redacted]
(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 by [redacted] The carcinogenicity study was performed between 1991 and 1993 and plasma samples analyzed 1993. Samples were stored "frozen" until analysis.

RIA 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 [redacted] 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 ([redacted] ASSAY) (ng/ml)

	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.

Means 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; [redacted] analyses)
day 723 (0.5 ng/ml; [redacted] analyses)

[redacted] APPEARS THIS WAY ON ORIGINAL

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

[redacted] **DATA 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

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) and [redacted] (PK). (vol 1.83, p.183)
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).

[redacted] APPEARS THIS WAY ON ORIGINAL

AUC_{0-24} (10-day study in young rats by [redacted] assay)

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

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.
Assay: [redacted] Limit of Detection (LOD): 1 ng/ml

RAT CMAX ([redacted] assay) (ng/ml)

	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

Means 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 [redacted] (vol 1.85)
PH 24279. (T6055475; p.26 review)
Animal study: Bayer, Wuppertal *(Feb. 1994-May 1995; sampling 2/, 6/, & 8/94; 2/95)*
PK study: [redacted] *(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 [redacted] (vol 1.85)

T 1039793.
Animal study (Bayer, April 1991 to April 1992)
PK Analyses: May-Oct. 1992 [redacted]: **0, 8, and 25 ug/kg** doses and **Bayer: 70 ug__g**
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
Geometric 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

PK FROM HIGH DOSE ONE YEAR DOG STUDY [redacted] (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 [redacted] (October 1992 to February 1993) using an [redacted] assay. 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					AUC (ng h/ml)
0	0.4± 2.4	0	0	0	= 900
100	75± 1.8	69 ± 1.6	94± 1.3	64± 1.3	Cmax (ng/ml)
300	430± 1.2	n.s.	ns	ns	= 92
					t1/2 (h) = 7
Females					AUC (ng h/ml)
0	0.8± 1.9	0	0	0	= 900
100	85 ± 1.6	73± 2.2	73 ± 2.3	130± 1.4	Cmax (ng/ml)
300	420± 1.5	ns	ns	ns	= 140
					t1/2 (h) = 5

ng/ml ± geometric SD

[redacted] APPEARS THIS WAY ON ORIGINAL

RABBIT PLASMA DRUG LEVELS (DIET & [redacted] (vol 1.83)
R 6368. March 1995. Bayer (treatment); [redacted] (PK analysis)
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 by [redacted] (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 &[redacted] (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 [redacted]

Bay w 6228 LEVELS [redacted] 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?)

[redacted] APPEARS THIS WAY ON ORIGINAL

3-MONTH MONKEY GAVAGE STUDY (DAY 90; [redacted] (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 [redacted] PK analysis by [redacted]

1-YEAR MONKEY GAVAGE STUDY [redacted] (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 [redacted]

HUMAN PK DATA ([redacted] ASSAY)

<u>300 ug/day dose to healthy male volunteers</u>
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

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

[redacted] ASSAY
Cmax: 7 ng/ml (300 ug/day)

TISSUE RADIOACTIVITY AFTER ORAL DOSE IN MALE RATS (vol 1.75)
PH 25044. Bayer, Germany [redacted]
study# 34-022: TREATMENT (*March to June 1995*; [redacted]: Fasted male Wistar rats from [redacted] approx 8 weeks old, were given a single dose of 1 mg/kg [C]BAY w 6228 by oral gavage in 0.9% saline (p.122) . Tissues were dissected, weighed, combusted, and counted by [redacted]

study# I 3000427: TREATMENT (*February to May 1992; Wuppertal, Germany*): Fasted male Wistar rats from [redacted] approx 8 weeks-old, were given 22 daily

doses of 1 mg/kg [[redacted]C]BAY w 6228 by oral gavage in 0.9% saline (three lots of drug were used).

EXCRETION (radioactivity):
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 (RADIOACTIVITY)

	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 [^{14}C] 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 [redacted] (vol 1.77)
PH 24977. Animal studies (Bayer, Wuppertal); [redacted]
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*)

HPLC: *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° C because of continued enzymatic activity under these conditions.

COMPARISON OF BLOOD AND TISSUE LEVELS [redacted] method of analysis)

Species Gender	Sampling (h postdose)	Dose	Liver	Muscle	Blood	Testes	Lens
Dog* male	**1 h, day 7**	**100 ug/kg**	360±1.3	14±1.3	**46±1.3**	6.5±1.3	0.9±1.2
male	24 h, day 7		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 [C] BAY w 6228 (2 mg/kg in phosphate buffered saline). Qualitative analysis was done using 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 ((vol 1.77)

R6386P. Animal studies (Bayer, Wuppertal September 1994 to October 1994)
PK analyses March 1995 to April 1995)

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 by

	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