The lung showed red discoloration in all rats including the control that died during the period of study. But the incidence was increased in the high dose groups. The red discoloration was microscopically found to be due to congestion often secondary to dosing accident. But, it is also possible that the higher incidence of red discoloration of the lung seen in the high dose rats might be either due to a higher mortality rate or secondary to cardiac involvement causing pulmonary congestion. The fluid seen with a slightly increased frequency in the thoracic, abdominal and pericardial cavities of treated rats was considered to be secondary to cardiac edema.

Ulceration of the foot was seen all groups including the control groups, but the incidence was slightly more in all treated groups of females and in the 8, 16 and 63 mg/kg/day males, of which reason was not clear. Other macroscopic changes were all considered to be of spontaneous or agonal origin.

Organ Weights: Organ weights that were recorded at necropsy indicate an elevation in cardiac weights in all treated groups except the low dose group animals. Additionally, the percentage ratio of heart weight over body weight was also increased in the two high doses as shown below. The increase in cardiac weight was attributed to the treatment-related hypertrophy seen microscopically.

Effects of Pioglitazone on Heart Weight in 2-Y Carcinogenicity Studies in Rats							
Group	Vehicle	Placebo	1 mg	4 mg	8 mg	16 mg	63 mg
Body Wt	630	619	683	685	653	660	581
Heart Wt	1.95	1.86	1.98	2.20*	2.38*	2.62*	2.60*
H/B x10	3.15	3.09	2.96	3.29	3.76	4.03*	4.47*

H/B(%) indicates heart weight/body weight x 1000. *P<0.05 compared to vehicle or placebo control. The unit of pioglitazone dose was in mg/kg/day.

Microscopic Findings: The changes involved the brown adipose tissue, femoral and sternal marrow, parotid salivary gland, glandular stomach, femoral and sternal bone, heart, urinary bladder, lung and foot. The fibrosis in the interscapular adipose tissue was pronounced in high dose group females, and was dose-dependent in both sexes. Fatty infiltration in bone marrow of femur and sternum and steatopathy were common in control groups as well as the drug-treated groups, but the incidences were apparently increased in the high dose groups. The changes seen in the bones were closure of the growth plate in the femur and increased width of the sternebrae.

The changes in the heart consisted of hypertrophy of the atrial musculature and thrombosis in the atrium, which were related to cardiac enlargement and distension. The hypertrophy was seen in all drug treated groups of males with increased incidence and severity with increasing dose. In females, the trend was similar, although the incidences were less pronounced in low dose groups. Thrombosis was seen in the control and treated groups of males, but showed a slightly higher incidence in the high dose group.

Test article-related lesions of the urinary bladder were epithelial hyperplasia and benign and malignant transitional cell tumors, which was related to macroscopic masses, nodules

and thickening of the urinary bladder. The nodular type was evident only in males in the 8 and 16 mg/kg/day groups, which were not confirmed in the high dose group as shown below. The combined incidence of all three types of hyperplasia was increased in males in the 8, 16 and 63 mg/kg/day groups. Benign and/or malignant transitional cell tumors were seen in the 4 mg/kg/day group and high dose groups of males.

Rat Urinary Bladder Lesions in 2-Y Carcinogenic Studies with Pioglitazone

Urinary Bladder Lesion	Incidence by Dose (mg/kg/day)						
	0[a]	0[b]	1	4	8	16	63
Males							
Hyperplasia, epithelial, papillary	3	0	1	2	1	1	1
Hyperplasia, epithelial, simple	1	3	0	2	7	10	7
Hyperplasia, epithelial, nodular	0	0	0	0	4	1	0
Carcinoma, transitional cell	0	0	0	2	3	5*	4
Transitional cell tumor, benign	0	0	0	0	4	2	2
Females							
Hyperplasia, epithelial, papillary	0	0	1	1	-	0	1
Hyperplasia, epithelial, simple	1	1	2	5	-	3	11
Transitional cell tumor, benign	0	0	1	1	-	1	0

[a]Vehicle, [b]Placebo. *Two of the 5 animals also had benign transitional cell tumors, but are not included in the totals for transitional cell tumors.

In order to determine the relevance of the urinary bladder tumors, analyses of the calculi obtained in the rat carcinogenicity study revealed the primary components as calcium, magnesium, phosphate and protein. Pioglitazone and its metabolites were not present in the calculi, which suggests the drug and/or its metabolites are not responsible for the cause of the tumor. However, it has not been documented whether the drug or its metabolites facilitate the formation of the calculi.

A strong correlation was observed between the presence of calculi and proliferative lesions in the urothelium of the rats. Increased urinary pH, high osmolality and protein in rats might be favorable conditions for the formation of calculi. However, there was no clear evidence that the drug or its metabolites increased the pH, osmolality and amyloid deposits in rat bladder in drug dose-related manner. As a matter of fact, correlation between the incidence of of the tumor and calculi is rather poor, although there was a positive association between transitional cell carcinoma and all combined parameters such as calculi, dystrophic calcifications, mineralization and corpora Amylacea. It has not been documented that the drug or its metabolites alter all of the parameters in drug dose-related fashion.

Summary and Conclusions of Rat Carcinogenicity Studies

Pioglitazone treatment increased mortality at 16 and 63 mg/kg/day, particularly in male rats. Body weight was slightly increased at all doses. Erythrocyte counts were decreased in males at 16 and 63 mg/kg/day and in females at 63 mg/kg/day. Hemoglobin and hematocrit in females at 63 mg/kg/day were also reduced. Brown fat was enlarged at 4 mg/kg/day and higher. Absolute heart weight was also increased in males at 4 mg/kg/day and above and in females at 16 and 63 mg/kg/day. The urinary bladder was thickened in males at all doses, and increased numbers of masses or nodules in the urinary bladder of males at 4 mg/kg/day and above. Urinary bladder lesions included papillary, nodular and simple epithelial hyperplasias in males at 8 mg/kg/day and above and in females at 63 mg/kg/day, and benign and/or malignant transitional cell tumors in males at 4 mg/kg/day and above.

Histopathological evaluations revealed steatopathy of the brown fat (all doses), fatty infiltration of the bone marrow, the parotid salivary gland and the glandular stomach (males at all doses and females at 16 and 63 mg/kg/day), ossification of the femoral epiphyseal plate (4 mg/kg/day and above), increased width of the sternbrae (males at 4 mg/kg/day and above and females at 63 mg/kg/day), atrial muscle hypertrophy (males at all doses and females at 16 and 63 mg/kg/day), and atrial thrombosis (males at 63 mg/kg/day).

It has been confirmed that the presence of urinary bladder calculi in some of the animals with urinary bladder tumors. The sponsor performed additional evaluations, which suggested that the tumors might be specific to the rat, not in the mouse and dogs. And the tumors might be related to the formation of urinary calculi, which contributed to the formation of epithelial tissue hyperplasia. However, there has been no investigation to test the possibility that pioglitazone or its metabolites influence the formation of urinary calculi in drug dose-related manner. It is also difficult to correlate the calculi quantity and the prevalency of the tumors in each group. Although the drug or its metabolites might not produce transitional urinary bladder tumors directly, they may play a role in the tumorigenesis processes indirectly by facilitating calculi in the bladder.

8. OVERALL SUMMARY AND CONCLUSION

Pharmacology and Toxicology: Pharmacology and pharmacodynamic studies indicate that thiazolidinediones produced antidiabetic effects by activating peroxisomal proliferator activated receptor gamma (PPARγ). The precise underlying mechanism of the beneficial action of pioglitazone remains to be elucidated. Toxicological data indicate that the drug produced various toxicities such as left atrial thrombosis, hydrothorax, cardiohypertrophy and elevation of hepatic enzymes in different laboratory animals.

The fact that pioglitazone produced multiple toxicities might be related its ability to activate PPARγ, which alters gene expression that dictates multiple molecular and

cellular processes. Pioglitazone increased cardiac weight in drug-treatment duration as well as dose dependently. Experimental evidence indicates that its effect on cardiac weight might not be due to its direct actions on cardiovascular system, but as an adaptive resonses to its effects on hemodynamic and water distribution. But, we really do not know its primary active site(s) and how it initiates the chain of reactions, which compel the reviewer to take due precaution. In particular, our currently available information does not permit one to predict the life-long exposure effects to the drug on any organ systems. The reveiwer believes that we have to wait until we have some understanding of underlying fundamental mechanism(s) of such multiple toxic actions of the drug.

The target organs of the toxicities of pioglitazone are usually vital organs such as heart, liver and blood cell forming cells, although we have limited knowledge on the actions of the drug in other organs such as brain, bone marrow, and neural tissues. Particularly, the drug has very low margin of safety as the ratio of animal to clinical AUC values at the threshold doses for cardiac changes (Please see the table below) is usually around 10.

Species	Dose(mg/kg/day)	AUC(µg.hr.ml)	AUC Ratio*
Mouse	30(C)	119.3	11.9
Rat	4(Male)	70.4	7.0
Dog	3(Male)	9.4	0.9
Monkey	8.9(C)	74.7	7.4

*Indicates animal to human AUC ratio. Clinical AUC=10 µg.hr.ml after 30 mg dose; C and M stand for combined sexes and males, respectively.

Genotoxicity: Genotoxicity potential of pioglitazone appears to be negative in the tests of bacterial mutagenicity, mammalian cell forward mutation, chromosomal aberration, unscheduled DNA, mouse micronucleus tests. But the incidence of forward mutations at the thymidine kinase locus of mouse lymphoma L5178Y cells was increased significantly by pioglitazone metabolite MI in triplicate assays in the presence of S-9 mix. The results of assays with metabolite MVI in the absence of S-9 mix were equivocal but results in the presence of metabolitc activation were weakly positive. Since MI is not found in humans and the weight of evidence suggests pioglitazone is not genotoxic, its actions in reference to the mouse lymphoma assay were deleted from the label.

Carcinogenecity: Pioglitazone produced transitional epithelial carcinoma in rat bladder. The sponsor considers that was due to hyperproliferative effect induced by calcium or phosphate calculi which were found in rat bladders. However, there was no evidence that pioglitazone caused a dose-related change in the urine which would have induced differential development of calculi. In addition, it has not been documented that pioglitazone and its metabolites do not play clearly no role in the formation of calculi in the bladder.

Reprotoxicity: The drug was embryotoxic in rats at 40 mg/kg/day, as evidenced by increased postimplantation losses. Treatment-related fetotoxicity occurred at doses as low as 10 mg/kg/day since it reduced fetal body weights and crown-rump lengths.

Pioglitazone did delay F_1 development and delayed F_1 fertility as shown below. These findings should be documented in drug label with Pregnancy Category C.

Pioglitazone Effects on F1 Pup Weights in Segment I Oral Toxicity Study in Rats				
Postpartum Day	0 mg/kg/day	10 mg/kg/day	20 mg/kg/day	40 mg/kg/day
0	6.3	5.8*	5.2*	5.1*
1	7.0	6.6	5.6*	5.7*
4	10.7	10.0	8.3*	7.8*
7	15.5	14.7	12.4*	11.3*
14	31.0	30.1	26.1*	23.1*
21	51.0	46.7*	41.6*	34.8*

*Indicate P<0.05.

[redacted]

10. RECOMMENDATION:

Pharmacology recommends to approve pioglitazone(NDA21-073) for the proposed indication.

11. Review Code: AP

/S/ [redacted]

Herman M. Rhee, Ph.D.
Pharmacologist

cc: Original NDA, HFD-510, HFD-345
Ronald Steigerwalt/H. Rhee/J. Weber

See team leader memo to file

APPEARS THIS WAY ON ORIGINAL

/S/ [redacted]

6/30/99

COMMENTS ON PHARMACOLOGY & TOXICOLOGY LABEL SECTION

DRAFT LABELING

[redacted]

DRAFT LABELING

[redacted]

Left ventricle

IND [redacted]

REVIEW & EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA
(Original Submission)

I. GENERAL INFORMATION

Date of Submission: 9-20-89

Date of Receipt by CDER: 9-25-89

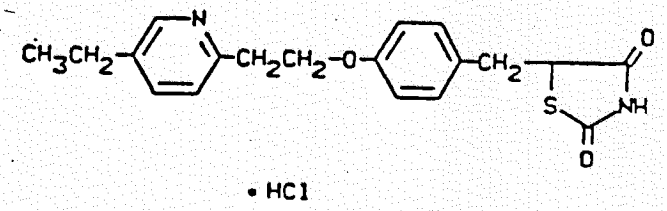
Date Assigned to Reviewer: 9-27-89

Sponsor: [redacted]

Drug: Pioglitazone Hydrochloride [redacted]

Chemical Name and Structure:
5-(4- 2-(-ethyl-2-pyridinyl)ethoxy phenyl)-methyl)-2,4-thiazolidinedione HCl

[redacted]



Category: Oral-antidiabetic

Related INDs: I [redacted]

Proposed Clinical Studies: Initially a single dose tolerance and pharmacokinetic study will be conducted with male volunteers with placebo or pioglitazone hydrochloride [redacted] doses of 2, 6, 18, 30, 45, or 60 mg. The duration of the study for each individual volunteer is two weeks but the total study period will be approx. two months. A repeated dose tolerance and pharmacokinetic study will then be initiated with the same treatment regimen along with [redacted] 2, 6, 10, 15, or 20 mg TID for seven days. The duration of this study for each individual volunteer is three weeks and the estimated duration of the total study is four months. After completion of this study, a repeated dose efficacy study in otherwise healthy NIDDM (noninsulin dependent diabetes mellitus) volunteers will be undertaken.

II. PHARMACOLOGY

A. Pharmacology Studies Related to the Anti-Diabetic Potential:

1. Hypoglycemic Effects in Models of NIDDM:

Under this heading were reported the studies of dose-dependent hypoglycemic effects of [redacted] in laboratory animals (three strains of diabetic mice and Chinese hamsters) and of reducing triglycerides and free fatty acids in all animal species (including dogs) that were tested.

2. Improved Insulin Sensitivity Following Treatment:

Enhanced insulin sensitivity was shown *in vivo* in Wistar fatty rats in response to insulin injection. In euglycemic clamp studies, pioglitazone produced significant increase in insulin-stimulated glucose clearance and decrease in hepatic glucose production. Similarly there was an enhanced responsiveness of skeletal muscle to insulin in drug-treated KKA_y mice and in Zucker fatty rats. In beagle dogs, however, one week treatment with formulated drug at 0.30 mg/kg/d did not increase the response to a single intravenous injection of insulin (10 or 30 mU/kg).

3. Effects on Thyroid Hormones:

Reduction in plasma thyroid hormone (T_3 & T_4) levels occurred in ob/ob mice on a "cafeteria" diet alone and on drug plus control diet while this effect did not occur in animals given "cafeteria" diet plus drug (42 mg/kg/d) for 7 weeks. KKAy mice (a crossbred from the obese yellow mouse with the insulin resistant black KK mouse; they are also obese, hyperinsulinemic,, and insulin resistant like the ob/ob mouse but are more advantageous for screening since they remain hyperglycemic throughout their lifespan) treated with pioglitazone, however, showed no change or increases in thyroid hormone levels. Thus, the effects of pioglitazone on circulating thyroid hormone levels in mice are inconsistent and substantial variation occurs in the outcomes of different studies.

B. Cardiovascular and Respiratory Effects:

No cardiovascular or respiratory changes were observed with single doses of pioglitazone intravenously up to 3 mg/kg in dogs or orally up to 10 mg/kg of formulated drug in dogs and cats. No difference of effect was seen between vehicle control and drug on pressor responses to bilateral carotid occlusion, phenylephrine, tyramine, dimethyl-phenyl-piperazinium, or angiotensin II. No difference was seen on depressor responses to acetylcholine, isoproterenol, or histamine.

C. CNS Pharmacology:

50 mg/kg of [redacted] given via i.p. to mice had no effect upon amphetamine toxicity, apomorphine cage climbing response, hypoxic stress assay, Bicuculin T.E. protection test, or evoked responses to yohimbine, oxotremorine or apomorphine. Likewise, Pioglitazone 300 mg/kg as a solution in citric acid given orally to rats had no effect upon body temperature, skeletal muscle coordination, or pentobarbital sleeping time. It was also reported that pioglitazone 1 uM applied to rat brain membranes did not affect the equilibrium binding to receptors of etorphine, spiroperidol, flunitrazepam, QNB, dihydroalprenolol, prazosin, clonidine, or LSD. In summary, no CNS activity and no CNS receptor binding were noticed with single doses of drug well above the expected therapeutic level of 0.03 - 0.1 mg/kg.

D. G.I. Physiology:

Pioglitazone was administered orally (in intact rats) or intraduodenally (in pylorus ligated rats). Compared to animals treated with vehicle only, there were no differences in intestinal transit time, volume of gastric juice, or acidity of gastric juice and there was no protection against ethanol-enduced gastrric injury.

E. Other Screening Data:

The IND contains a number of other studies by Upjohn which seem to be the result from a general screening assays for drug such as platelet aggregation; interleukin-1 and -2; cytotoxicity to T-cells, etc. and the results were all found to be inactive.

III. METABOLISM

The metabolism studies of pioglitazone [redacted] were conducted with either the acetate [redacted] or the HCl formulation [redacted]

A. Absorption:

a. Rat: The bioavailability of [redacted] in 5% gum arabic suspension at 10 mg/kg was 53% relative to an oral solution containing citric acid. Plasma levels (AUC) increased linearly with increasing dose at the dosage range from 10 mg/kg to 100 mg/kg, while AUC at 300 mg/kg dose was markedly less than the value expected from the AUC at 100 mg/kg, suggesting that the oral absorption phase was close to saturation at doses higher than 100 mg/kg (Fig. 1). The AUCs of HCl forms [redacted] of drug administered at a dose of 10 mg/kg in 5% gum arabic were estimated to be 62% as compared with that of a solution of [redacted] in 0.3M citric acid and this analog did not show any apparent difference in availability over the range from 10 to 100 mg/kg. No absorption study was conducted via i.v. route due primarily to a very poor solubility of this drug.

b. Dog: Studies in beagle dog also showed that the addition of citric acid (up to 3 times the amount of the drug) enhanced drug bioavailability (72%; relative bioavail.= 56% compared to an oral solution formula = 39%) from tablets manufactured by either the wet granulation or direct compression methods while micronization of the drug had no or little effect on the bioavailability. The mean AUC, C_{max} (27.0 + 7.3 ug.hr/ml and 8.73 + 2.12 ug/ml, n = 8) for drug with citrate were 77% and 122% greater than the corresponding values of the drug without citrate (15.34 + 10.05 ug.hr/ml and 3.92 + 1.72 ug/ml, n = 8). The T_{max} for the former was, on an average, at one hour after administration while the latter was variable among dogs.

Another dog study where oral solutions of [redacted] were administered showed dose proportionality for the drug from 0.6 to 7 mg/kg (dose range from 10 to 100 mg). No apparent effect on T_{max} and $t_{1/2}$ was observed with increasing dose suggesting that the disposition of drug was independent of dose levels of up to 7 mg/kg (linear pharmacokinetics). The dose-normalized C_{max} and AUC values for [redacted] were within the range observed for [redacted].

Another report from the four-way crossover dose proportionality study in the dog using a [redacted] in citrate over the range of 5 to 75 mg/kg, the projected dose range to be used in toxicity studies, showed 1) the AUC values of drug increase proportionally over the dose range tested, 2) C_{max} show a similar trend toward dose proportionality, 3) other disposition profiles (T_{max}, k, $T_{1/2}$) were independent of dose, i.e. linear pharmacolinectics.

The terminal disposition phase of the parent drug was fairly uniform for the various formulations with a rate constant of 0.4-0.5 hr^{-1} ($T_{1/2}$ = 1.4 to 1.7 hr.). Three metabolites of unknown structure were identified. The parent and M-1 did not appear to accumulate while M-2 and M-3 showed some accumulation. Dosing 1 hr prior to feeding gave an absorption and disposition profile similar to a fasted animal while dosing either with food or 1 hr after feeding caused a reduction (approx. 30%) in absorption and prolonged the absorption phase (T_{max} increase from 0.8-1.2 hr to 2-4 hr).

c. Monkey: To determine the bioavailability of drug in monkeys, two female subjects received 10 mg/kg and two other females received 20 mg/kg of four different formulations, one each successive days for a total of 4 days. Assays of the serum levels of [redacted] disclosed that the drug in citric acid resulted in the highest serum levels in three of the four monkeys. In addition, a large degree of intersubject variability was present and no dose proportionality was observed when serum levels of low dose group were compared to those from high dose group. It was concluded from the findings that the monkey offers no improvement over the dog as the animal choice.

B. Tissue Distribution:
Distribution in body tissues is wide in the rat with highest concentrations in fat depots followed by other organs, liver and adrenal glands. No tissue distribution studies was made in dog or monkey.

C. Metabolism:
No detectable intact pioglitazone was found in urine or bile of rats and of dogs. The major metabolite in bile in both species was pioglitazone carboxylic acid and its taurine conjugate (rat only) followed by the monohydroxy isomers.

D. Excretion:
Excretion of radioactivity is almost complete via bile and recoveries of radioactive materials were very good both in rats (99%) and in dogs (96%). Almost all drug-related materials is cleared from systemic circulation within 24 hr after a single IV or PO dose.

IV. TOXICOLOGY

Most toxicology studies submitted in this application were conducted by the sponsor in compliance with the GLP requirements (CFR Title 21, Chapter 1, Parts 58, 312, 314) and compliance statements (CFR Title 21, Chapter 1, Sub-chapter D, Parts 312, 314) were included in the respective study reports. Toxicity studies of 4- and 13-week in rats and 4-week in dogs were conducted by [redacted].

As was the case in metabolism studies, the toxicity studies of pioglitazone [redacted] were also performed with either the acetate [redacted] or the HCl formulation [redacted]

A. Acute Toxicity:

Due to an extremely poor solubility of [redacted] death occurred from both i.v. (dose range from 18 to 54 mg/kg) and oral dosing (337 to 6596 mg/kg) of the [redacted] in citric acid and the vehicle control group alone prior to the presence of any acute toxic signs of drug. Signs of ataxia were however noted in most of the surviving animals from both groups due to acidosis from the excessive citric acid vehicle.

Gross and histological examination confirmed that the animals died from gastric or gastrointestinal bloat as a result of excessive growth of bacteria which used citrate and produced excessive gas. Pathological evaluation also revealed no differences between animals received citrate alone and those with drug in citrate. Although the acute toxicity of [redacted] in rats and mice could not be defined in this study, the estimated LD_{50} in CF1 mice via i.v. was between 22.5 and 27 mg/kg whereas in SD rats was between 27 and 45 mg/kg.

B. Repeated-Dose Toxicity:

1. Rat(Sprague-Dawley; Jcl:SD)

a. Four-Week Oral Toxicity (conducted [redacted] suspended in water containing 5% gum arabic, was dosed orally (gastric intubation) to rats (10/s/gp) once daily at doses of 0 (control), 100 (L), 300 (M), 1000 (H) mg/kg. From all parameters observed, there was an increased heart weight in the males from M-, H-dose groups and an increased amount of brown adipose tissue in the interscapular and mediastinal spaces of all dose groups. No other abnormalities were present in this study. It was thus concluded that oral treatment of U-72,107E at a dose as high as 1000 mg/kg/d for 4 consecutive weeks did not cause any adverse toxic effect in rats.

b. Thirteen-Week Oral Toxicity (conducted by [redacted]) in a citrate-based [redacted] powder suspended in distilled water, was dosed orally to groups of 10 male and 10 female SD rats once daily at free base equivalent U-72,107 doses of 0 (distilled water), 0 (Vehicle), 30 (L), 100 (M), and 300 (H) mg/kg. The results showed that

there were no treatment-related changes in BW, food intake, urinalysis and ophthalmological exams. Five animals died from suffocation during the study due to technical dosing errors. Hematological changes of anemia were noted from drug treatment in terms of a decreased RBC in both sexes of M- and H-dose groups, decreased Hct and Hb in female M- and H-dose gps, and an increased MCH in both sexes from H-dose gp.

Organ weight changes were also noted which included an increased heart wt in both sexes and an increased liver wts in females from M- and H-dose gps. There were dose-dependent increases in the amount of adipose tissue in interscapular and mediastinal spaces in all drug treated gps. Histologically, there was no significant lesion. The only lesion possibly related to drug treatment was splenic extramedullary hematopoiesis (SEH) which was considered by the sponsor to be most likely a compensatory response secondary to anemia.

It was concluded that a toxicological NOEL of U-72,107 free base equiv. was 30 mg/kg, although the sponsor did suspect that a potential effect on hematopoiesis of [redacted] was present at 30 mg/kg free base equiv. of U-72,107.

c. Thirteen-Week Oral Toxicity [redacted]

Two 13-week toxicity studies were conducted to investigate the potential toxicity of [redacted] in SD rats. [redacted], suspended in water having 5% gum arabic, was dosed orally to groups of 5 male and 5 female rats once daily at doses 0, 30, 100 and 300 mg/kg. No treatment effect was found in BW, food cons, organ wt, and ophthalmoscopic, hematological, serum biochemical, and histological exams. A dose-dependent increase in brown adipose tissue in interscapular and mediastinal spaces and in the subcutis was observed from the drug treated groups. It was concluded that oral treatment with [redacted] at dose as high as 300 mg/kg/d for 13 consecutive weeks did not cause any adverse toxic effect in rats.

2. Rabbit (NZW; Preliminary Eye Irritation Study)

[redacted] powder was sprinkled in the eyes of 1 male rabbit at a single dose of 100 mg per eye or at a dose of 20 mg per eye per day for 5 days. The right eye was rinsed with sterile water 30 seconds after each dosing while the left eye remained unrinsed. The rabbit was observed for 14 days. Slight conjunctival redness was noted in both eyes at 1 hr after dosing. Both eyes appeared normal for the remainder of the study. It was concluded that [redacted] was practically nonirritating to rabbit eyes.

Similarly, a preliminary dermal irritation study where [redacted] powder was miostened with sterile water and applied to two skin sites, intact and abraded, of 1 male rabbit at a single dose of 500 mg per site or at a dose of 100 mg per site per day for 5 consecutive days. No irritation occurred at either the intact or the abraded site.

I [redacted]

3. Dog (Beagle)

a. Oral Range-Finding Study:

[redacted] in gelatin capsules was administered to beagle dogs (1/sex) at escalating doses, starting at 4 mg/kg/d and doubling daily to a maximum of 64 mg/kg/d. This dose was maintained for 10 days for a total of 14 days drug exposure. Observations included daily clinical exams as well as pre- and postdose hematology and serum chemistry determinations. Organ wts and complete gross observations were recorded at necropsy, and tissue specimens were taken for histologic exam. It was concluded from results that the drug was well tolerated at 64 mg/kg. Serum chemistry and histologic findings suggest the heart and liver were the target organs for this compound.

b. Two-Week Oral Toxicity Study:

4/s/dose were orally treated with [redacted] formulated in a citrate-based granulation and encapsulated in gelatin capsules for 14 days. The dogs received 75 mg/kg free base equivalents of [redacted] twice daily for a total dosage of 150 mg/kg. Clinical signs were unremarkable except for repeated episodes of vomiting after dosing when animals had been fasted prior to dosing. No gross or histological lesion was found to be directly attributed to [redacted] treatment, probably because of lack of drug absorption. It was concluded that drug administered a daily total dosage of 150 mg/kg had a potential to cause borderline anemia in dogs after 14 consecutive days of treatment.

c. Four-Week Oral Toxicity [redacted]

3/sex/d at free base equivalent U-72,107 doses of 0, 1, 3, and 10 mg/kg. No treatment-related changes were observed in any parameters measured and it was thus concluded [redacted] free base equivalent as high as 10 mg/kg/d did not cause any adverse effect in dogs.

d. One-Month Oral Toxicity:

4/s/gp (4 gps) dosed orally with encapsulated [redacted] daily for 30-days at 0, 15, 45, and 150 mg/kg/d. No treatment-related changes were noted except for a signif. changes in serum albumin in 150 mg/kg groups. Since there had been no hepatic enzyme abnormalities or loss of BW, the exact pathogenesis of this observation could not be determined. No histologic lesions were found related to treatments; however, signif. decreases in splenic weight (both absolute and relative to BW) were noted in the females from all 3 treatment groups.

e. 90-Day Oral Toxicity:

o Dosage

2/s/dose (2 gps) of 0, 10, 100 mg/kg/d. via oral with encapsulated U-72,107E.

o Results

1. Observed signs

All dogs appeared normal throughout the dosing period.

2. Mortality

None