

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-789

PHARMACOLOGY REVIEW(S)

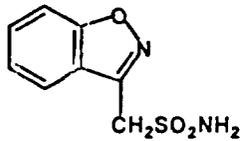
Review and Evaluation of Pharmacology and Toxicology
Original NDA Review

NDA: 20-789

Sponsor: Dainippon Pharmaceutical USA
Teaneck, NJ 07666

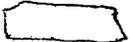
Drug: Generic Name: Zonisamide
 Code Name(s): AD-810, CI-912, PD-110,483
 Chemical Name: 1,2- benzisoxazole-3-methanesulfonamide
 Molecular Formula: C₈H₈N₂O₃S
 Mol. Wt.: 212.2

Structure:



APPEARS THIS WAY
ON ORIGINAL

Category: Antiepileptic

Related IND: 

APPEARS THIS WAY
ON ORIGINAL

Table of Contents

	Page
I. Pharmacology	3
II. ADME	4
III. Toxicology	9
IV. Carcinogenicity	18
V. Genetic Toxicity	24
VI. Reproductive Toxicity	26
VII. Summary and Evaluation	39
VIII. Recommendations	51
IX. Appendices	52

**APPEARS THIS WAY
ON ORIGINAL**

Note: Portions of this review were excerpted from the sponsor's submission.

I. PHARMACOLOGY

A) SUMMARY OF PHARMACOLOGY (RR 740-00910; Vol. 1.9)

Zonisamide (CI-912) was active against electroshock-induced maximal seizures (MES) in mice (poED50=19.6 mg/kg), rats, dogs, and rabbits, with PIs comparable to other agents (NT50/ED50=11.6 in mice), but was not active against PTZ-induced minimal seizures. Effective plasma concentrations in these species were 9.8, 10.8, 9.6, and 12.6 ug/ml, respectively, while 74, 96, 117, and 96 ug/ml were reported to be the respective neurotoxic plasma concentrations. Zonisamide was also showed activity against seizures induced by the potassium channel antagonist 4-aminopyridine in mice (poED50=41.5 mg/kg) and reduced the afterdischarge duration and raised the threshold for generalized convulsions in neocortical and hippocampal (but not amygdala) kindled rats (50-100 mg/kg po, 20-50 mg/kg iv). Zonisamide suppressed interictal spikes and secondarily generalized seizures induced by cortical freezing in cats and cortical application of tungstic acid gel in rats. Anti-MES activity was unchanged following administration of 20 mg/kg/day for 14 days, and there was no effect on hexobarbital-induced sleep times. Anticonvulsant activity and plasma concentrations in PB- or SKF-525A-pretreated rats were not different from those in untreated rats. The anticonvulsant effect of zonisamide against MES was not abolished by reserpine, as seen with acetazolamide, although efficacy was reduced.

Weak carbonic acid inhibition relative to acetazolamide (100-1000-fold less potent) was observed in rats *in vitro* and *ex vivo*. No effects on the spontaneous EEG or EEG arousal response were seen in cats and rats. Doses of 30 and 100 mg/kg iv produced slight increases in blood pressure in anesthetized cats, and a concentration of 10-4 g/ml reduced contractile force by 22% in isolated guinea pig atrium. In anesthetized rats, doses of 1-30 mg/kg iv had no effects on the HR or ECG; 100 mg/kg iv decreased HR by 10% through 30 min postdose without affecting ECG. Oral doses of 10-100 mg/kg increased urine volume and urine sodium and potassium concentrations in rats, and doses of 30 and 100 mg/kg decreased the volume and acidity of gastric secretions in rats.

B) MECHANISM OF ACTION STUDIES (RR-740-02008, -01607, -01703, -02477, PR27; Vol. 1.9)

Zonisamide blocked sustained repetitive firing in cultured mouse spinal cord neurons at a concentration of 3 ug/ml, which is within the range of unbound plasma concentrations (2-14 ug/ml) in rats that are protected from MES and also within the range of unbound plasma concentrations (1.5-5 ug/ml) in patients receiving the drug clinically. No changes in postsynaptic GABA responses were observed at relevant concentrations. In cultured rat cortical neurons, zonisamide dose-dependently reduced voltage-dependent transient inward T-type Ca^{2+} currents without affecting long-lasting L-type Ca^{2+} currents over the concentration range 1-500 uM. Zonisamide had no effect on the uptake of GABA into rat brain slices and did not significantly displace batrachotoxinin binding (site associated with activation of voltage-sensitive Na^{+} channels in CNS) in rat brain synaptosomes at relevant concentrations.

C) ROLE OF CARBONIC ANHYDRASE INHIBITORY ACTIVITY IN ANTICONVULSANT EFFECT (PR43, PR44; Vol. 1.35)

The 7-methylated derivative of zonisamide was found to have the same potency as the parent in inhibiting rat CA *in vitro* (IC50=1.6 x 10⁻⁵; relative potencies of compared to acetazolamide (AZA): 1/235 and 1/206, respectively), but it was inactive in the MES test at doses (up to 500 mg/kg po or 50 mg/kg iv) which resulted in brain concentrations (22.8 ug/g) about 2-fold the minimally effective brain concentration of zonisamide in rats (10.8 ug/g). These results indicate that the anticonvulsant effect of zonisamide is not primarily due to CA inhibition. The relative potencies of zonisamide to AZA in inhibiting CA in human erythrocytes and purified human CA I and CA II *in vitro* were found to be 1/150, 1/3, and 1/188, respectively. CA I, the low activity enzyme in erythrocytes, reportedly plays a limited role in CO₂ hydration in erythrocytes *in vivo* and in the anticonvulsant activity of AZA.

II. ADME

A) PHARMACOKINETICS IN RATS (PK Reference 21, published study, 1992, Vol 1.37)

Following bolus iv administration of a dose of 20 mg/kg to rats (N=4), the mean \pm SD values of elimination half-life ($t_{1/2}$), volume of distribution (Vd), and AUC were 9.2 ± 0.7 h, 1.10 ± 0.03 L/kg, and 245.6 ± 16.9 ug.h/ml, respectively. PK parameters following single oral doses of 10, 20, and 40 mg/kg or 9 daily oral doses of 20 mg/kg are shown in Table II.1. ZNS disappeared monoexponentially from plasma with $t_{1/2}$ s of about 9 h at all doses. Tmax was 2-4 h and increased with dose. PK parameters did not appear to change with repeated administration.

Table II.1

Pharmacokinetic Parameters^a of ZNS after Single or Multiple Oral Administration of ZNS in Rats

Dose (mg/kg)	Single			Multiple ^b
	10	20	40	20
k_e (h ⁻¹)	1.82 (0.52)	1.56 (0.31)	1.24 (0.33)	2.10 (0.35)
$t_{1/2}$ (h)	8.7 (0.4)	9.1 (0.6)	9.3 (1.5)	9.5 (1.2)
Vd/F (l/kg)	1.34 (0.07)	1.24 (0.11)	1.32 (0.09)	1.30 (0.09)
AUC (ug.h/ml)	101.0 (10.4)	215.6 (7.2)	412.8 (37.8)	210.8 (35.3)
Bioavailability ^c	0.82	0.88	0.84	—

a) Each value represents the mean (\pm S.D.) of 4 rats. b) Multiple: 20 mg/kg, for 9 d. c) Calculated from the normalized AUC values of ZNS after oral and intravenous administrations.

B) SINGLE AND MULTIPLE DOSE PHARMACOKINETICS IN DOGS (PK3 [redacted] RR-764-00089, conducted by [redacted] 1983, Vol. 1.33)

Pharmacokinetic parameters in plasma and blood were calculated from plasma level determinations (HPLC analysis) following single administration of 5 oral doses (10, 30, 100, 150, 200 mg/kg) and after 7 or 6 daily oral doses of 100 or 150 mg/kg, respectively (Table II.2). Absorption was variable, with Tmax ranging from 1-12 hr in individual dogs. Concentration was highest in RBCs, followed by whole blood and plasma. The concentration ratio between plasma and RBCs was linear over the plasma concentration range of 2.7 to 254 ug/ml. Disappearance from plasma was nonlinear at doses of 30 mg/kg or greater, and the disappearance from blood was nonlinear at doses of 100 mg/kg or greater. There was a linear correlation between dose and AUG in both plasma and blood over the dose range studied, however, although a positive Y intercept was observed. Explanations proposed by the sponsor for these findings were: 1) some drugs undergoing nonlinear pharmacokinetics due to tissue binding have been shown to have a linear relationship between AUC and dose, and 2) a decreased fraction absorbed as the dose is increased could compensate for the expected nonlinear AUC vs dose relationship if the drug is metabolized by a saturable process.

BEST POSSIBLE COPY

Table II.2

Mean Pharmacokinetic Data (N = 4) From Single and
Multiple Dose Administration of Zonisamide to Dogs

PLASMA					
Dose (mg/kg)	T _{max} (hr)	C _{max} (μg/mL)	AUC* (μg·hr/mL)	Vd/F (L/kg)	t _{1/2} † (hr)
<u>Single Dose</u>					
10	2.3 (55.9)‡	9.2 (15.0)	257 (18.6)	1.02 (12.9)	17.8 —
30	5.5 (18.2)	24.3 (5.8)	803 (11.6)	1.06 (13.2)	19 —
100	7 (49.5)	81.8 (21.1)	3377 (23.2)	1.08 (15.4)	— —
150	7.5 (23.1)	112.6 (15.5)	4792 (15.6)	1.16 (14.7)	— —
200	9.8 (29.5)	143.2 (2.0)	6011 (15.4)	1.08 (25.8)	— —
<u>Multiple Dose</u>					
100	7.5 (40.0)	172 (2.5)	3653 (4.7)	1.3 (28.3)	— —
150	5 (23.1)	237.7 (5.0)	5146 (7.8)	1.9 (30.4)	— —

WHOLE BLOOD					
Dose (mg/kg)	T _{max} (hr)	C _{max} (μg/mL)	AUC* (μg·hr/mL)	Vd/F (L/kg)	t _{1/2} † (hr)
<u>Single Dose</u>					
10	3.0 (38.5)‡	16.9 (40.2)	912 (9.8)	0.579 (9.1)	35.8 —
30	7.0 (49.5)	32.2 (6.4)	1473 (14.9)	0.859 (7.6)	28.6 —
100	6.3 (33.0)	105.3 (27.2)	4583 (24.6)	0.850 (14.3)	24.7 —
150	9.0 (27.2)	135.2 (13.2)	6119 (12.9)	0.903 (21.7)	24.0 —
200	9.0 (27.2)	189.2 (17.2)	8505 (5.7)	0.950 (24.4)	21.2 —
<u>Multiple Dose</u>					
100	7.0 (49.5)	206 (7.4)	4293 (4.5)	1.39 (13.7)	— —
150	4.5 (22.2)	279.7 (8.5)	5981 (7.1)	1.782 (40.7)	— —

* AUC is t = 0 to ∞ for single dose, t = 0 to 24 hours for multiple dose.

† Harmonic mean.

‡ Percent Relative Standard Deviation (%RSD).

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

B) ABSORPTION, DISTRIBUTION, AND EXCRETION IN RATS, DOGS, MONKEYS, AND HUMANS
 (PK2) [redacted] RR-764-00008, conducted by [redacted] (1977-'78, Vol. 1.33)

The distribution of 14C-labeled drug was studied in rats (N=3), dogs (N=2), and monkeys (N=2) after oral administration of a single dose 20 mg/kg. Maximum plasma radioactivity levels were reached about 3 hr after dosing in rats and dogs, and levels decreased exponentially thereafter (Table II.3). Rate of absorption appeared to be very different in the 2 monkeys (Tmax= 1 and 12 h), but AUC and t1/2 values were similar. ZNS is extensively taken up by erythrocytes, so Cmax and t1/2 values in RBCs were greater than those in plasma for all 3 species. Most of the radioactivity in plasma and erythrocytes was determined to be unchanged drug. Plasma protein binding in rats was 47.9% at 16 ug/ml in vitro and 46.2% at 14 ug/ml in vivo. In human serum, 60.4% was bound at 16 ug/ml in vitro. The percent bound to dog and monkey plasma protein in vitro was 40 and 35%, respectively. Tissue radioactivity levels in rats were maximal at 3 hr, with the highest levels found in the blood, liver, kidney, and adrenals (all about 2X plasma levels at 1-12 hr). Other tissue levels were similar to those in plasma (brain levels slightly higher than plasma). Maximal blood levels of radioactivity remained constant during repeated dosing of rats (seven consecutive daily doses of 20 mg/kg); and following the last dose, tissue levels and rate of decline were similar to those seen after a single dose. In rats given a single dose, radioactivity had been almost completely excreted in the urine (87%) and feces (16%) by 48 hr (Table II.4). Biliary excretion was found to account for about 20% of the dose, indicating that absorption was essentially complete. In dogs, most of the radioactivity had been excreted after 72 hr, and percentages of urinary (83%) and fecal (17%) excretion were similar to those in rats. In monkeys, most of the radioactivity was excreted in the urine (97%) over 96 hr. Following an oral dose of 200 mg (unlabeled) to humans, only about 34% had been recovered in the urine after 240 hr, 28% as unchanged drug and 6% as the glucuronide of the N-O cleaved metabolite.

BEST POSSIBLE COPY

Table II.3

Pharmacokinetic parameters of blood levels after oral administration of AD-810.

Species		Dose (mg/kg)	Tmax (h)	Cmax C24h (ug eq/ml)	Vd (l/kg)	T1/2 (h)	AUC (ug eq h/ml)	n
Rat	Plasma	20	3	14 2	1.0	8	221	3
	RBC		3	25 16		21		
Dog	Plasma	20	3	20 8	0.9	15	486	2
	RBC		6, 9	23 12		42		
Monkey	Plasma	20	1, 12	20 13	0.8	24	699	2
	RBC		1, 9	31 19		52		
Man	Plasma	4*)	4	3 2	1.6	68	202	2
	RBC		6, 8	26 23		130		

*) 200 mg/person.
 RBC: red blood cells.
 Tmax: Time of maximal plasma and RBC level (observed); Cmax: maximal plasma and RBC level (observed); C24h: plasma and RBC level at 24h; Vd: apparent distribution volume; T1/2: half-life; AUC: area under concentration-time curve.
 The values are means of n animals except for Tmax's of dog, monkey and human RBC and those of monkey plasma, which are individual values.

APPEARS THIS WAY
ON ORIGINAL

Table II.4

Urinary and fecal excretion of AD-810 after oral administration.

Animal Species		Dose (mg/kg)	Time interval (h)	Recovery (% of dose)	n
Rat	Urine	20	0- 24	77	3
			24- 96	10	
	Feces (Bile)			0- 24	14
			24- 96	2	
Dog	Urine	20	0- 24	50	2
			24- 72	33	
	Feces			0- 24	9
			24- 72	8	
Monkey	Urine	20	0- 24	36	2
			24- 96	61	
	Feces			0- 24	-
			24- 96	4	
Man	Urine	4 ¹⁾	0- 24	6	2
			AD-810	1	
			Metabolite ²⁾		
			24-216	22	
			AD-810	5	
			Metabolite ²⁾		

¹⁾ 200 mg/person.

²⁾ The conjugate of N-O cleaved compound.

- < 0.1.

n: Number of animals (subjects). Values are means of n animals (subjects). Values for animals are total radioactivity.

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

D) METABOLISM IN RATS, DOGS, MONKEYS, AND HUMANS (PK10 [redacted] RR-764-00022, conducted by [redacted] 1977-78, PK Reference 1, published [redacted] study, Vol. 1.37)

Urinary metabolites were elucidated by MS and NMR following administration of single oral doses of 20 mg/kg ZNS to rats, dogs, monkeys. The metabolites identified and the % of urinary radioactivity they represented in the various species are shown in Table II.5. In rats, unchanged drug accounted for most (32.2%) of the urinary radioactivity, followed by a carboxylic acid derivative (16.1%), an N-acetyl derivative (10.9%), and polar metabolites thought to contain the glucuronide conjugate of the parent drug (4%) and the sulfate conjugate of a ring-opened derivative (N-O cleaved compound) as well as other unidentified metabolites (34.2%). The urinary metabolite composition was not changed appreciably after an iv dose of 20 mg/kg or an oral dose of 200 mg/kg, but after repeated dosing for 14 days, the proportions containing UD (42.3%) and its glucuronide (7.2%) were significantly increased and the proportion containing the ring-opened derivative was decreased (22.2%) compared to those after a single dose. In dogs, UD was the main component in urine (28.7%), and the ring-opened derivative and glucuronide of the hydroxylated derivative were each present at levels of about 5%. In monkeys, UD made up 18% of urinary radioactivity, the glucuronide of the ring-opened derivative was 22%, and the acetylated derivative was about 10%. In humans given an oral dose of 200 mg, UD (28% of dose) and the glucuronide of the ring opened-derivative (6%) were the primary components identified in 216 hr urine, with only trace amounts of acetyl-ZNS detected. Proposed metabolic pathways for ZNS in various species are shown in Figure II.1, which incorporates more recent information and uses different metabolite designations (from Seino et al, Pharmacology Reference 1, Vol 1.34).

The anticonvulsant activity (MES test) of the ring-opened metabolite (2-SMAP) and the acetyl derivative was evaluated in mice (reported in PK Reference 20). Neither showed any activity after oral (100 and 300 mg) or iv (50 mg) administration.

Table II.5

Composition of urinary metabolites after administration of [¹⁴C]zonisamide in some animal species

Species	Administ-ration	Route	Dose	Urinary excretion	Metabolites						n
					UD	MI	MII*	MIII	MIV	MV*	
			mg/kg	% of dose	% of urinary radioactivity						
Rat	Single	PO	20	72.7±4.8	32.2±1.7	4.2±1.8	34.2±1.8	10.9±0.3	—	16.1±0.4	3
			200	67.3±5.4	28.3±0.2	—	38.4±2.4	12.9±0.8	—	16.8±3.5	3
		IV	20	78.1±1.7	27.7±2.3	—	41.4±1.6	10.1±0.4	—	18.1±0.8	3
	Consecutive	PO	20	81.5±0.7	42.3±1.2**	7.2±1.7	22.2±1.7**	9.0±0.5	—	15.0±0.4	3
Dog	Single	PO	20	49.5	28.7	1.1	4.5	—	5.9	1.4	2
Monkey	Single	PO	20	35.6	17.9	—	21.7	10.3	—	—	2

Values are means of n animals (±S.E.). Empty columns indicate no determinations. — : not detected n : Number of animals UD : Zonisamide, MI : Zonisamide glucuronide, MII : Conjugate of N-O cleaved compound, Rat MII is the fraction containing unknown metabolite(s). MIII : Acetylated-zonisamide, MIV : Glucuronide of hydroxylated-zonisamide, MV : Carboxylic acid. Dog MV is a glucuronide.

* : Previously identified by TLC/MS in rat urine".

** : p<0.01, compared with single administration

APPEARS THIS WAY
ON ORIGINAL

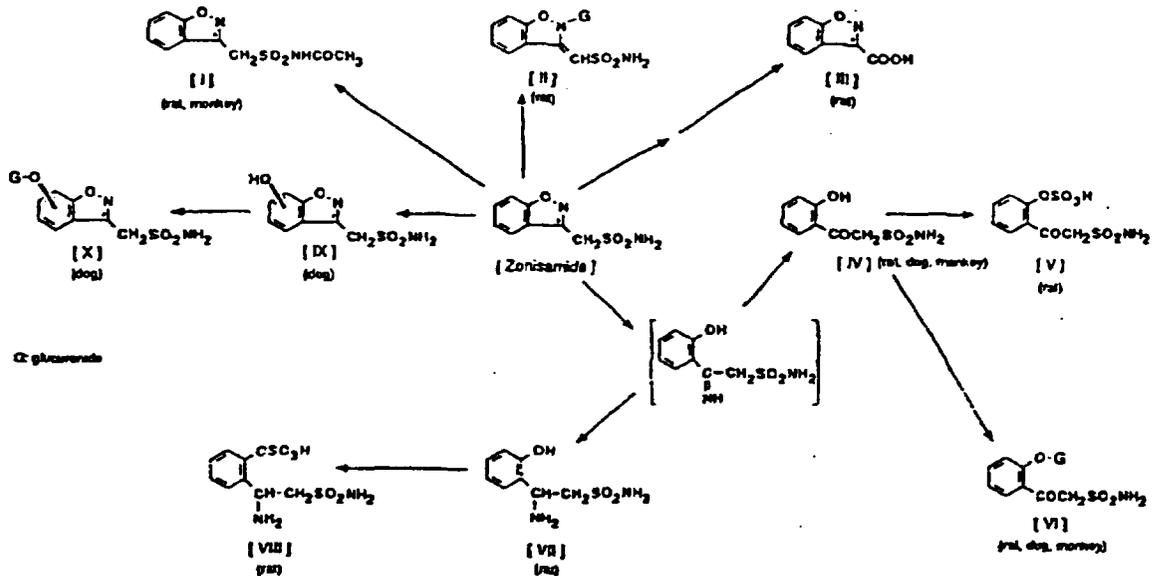


Figure II.1

Possible metabolic pathways of zonisamide.

E) EFFECT ON HEPATIC DRUG-METABOLIZING ENZYMES (PK9, [redacted] report no. BF93026A, 1987, Vol. 33)

Activities of aminopyridine demethylation and aniline hydroxylation in the presence of NADPH were measured in rat microsomes prepared from rats given 0 (vehicle), 10 or 100 mg/kg for 7 days. A phenobarbital-treated group served as the positive control. There was a significant elevation of aminopyridine-metabolizing activity in the PB group compared to C, but neither of the enzyme activities was significantly different in the ZNS groups. Thus, it was concluded that at these doses and for this duration, ZNS did not affect drug metabolizing enzymes in rat liver.

BEST POSSIBLE COPY

III. TOXICITY

A) ACUTE TOXICITY IN MICE, ADULT AND NEONATAL RATS, BEAGLE DOGS, AND MONKEYS (RR 745-00462; Vol. 1.10)

Clinical signs observed in rats and mice following administration of zonisamide by various routes were similar, including sedation, ataxia, loss of righting and corneal reflexes, hypothermia, respiratory depression, coma, and death. Decreased abdominal tonus was observed after oral administration only, in both species, and exophthalmus occurred only in mice. Clinical signs observed in neonatal rats (1 day, 1, 2, and 4 weeks of age) were similar at ages and doses, but the time of appearance was shorter than in adults; these included loss of righting, pallor, hypothermia, coma, and respiratory depression. Dogs exhibited depression, emesis, staggered gait, ataxia, prone position, coma, respiratory depression, and loss of corneal reflex. Three females died 32-48 hr after dosing. Sign began abating after 48 hr and surviving animals recovered by 4 days postdosing. Monkeys exhibited staggering gait, sedation, incoordination, nasal mucous discharge, and emesis within 4 hr postdosing, and later, prone position, loss of pain and corneal reflexes, hypothermia, and anorexia. These abated within 2-5 days after treatment. Edema and lung congestion were observed in rats and mice that died earlier after administration, while hemorrhages of the urinary bladder, stomach and lungs were found in those that died at least 8 hr after dosing. Pneumonia and hemorrhage of the stomach were seen in dogs that died.

APPEARS THIS WAY
ON ORIGINAL

Table III.1. Acute Toxicity of Zonisamide in Mice, Rats, Dogs, and Monkeys

Species	Sex	Age	LD ₅₀ values	(95% confidence limits) mg/kg	
			Oral	Intravenous	Subcutaneous
Rat	Male	Adult	2049(1700-2308)	705(665-748)	1128(981-1296)
	Female		1992(1724-2352)	672(628-719)	925(743-1150)
	Male	1 day	273(239-312)	---	---
	Female		270(229-318)	---	---
	Male	1 week	399(360-443)	---	---
	Female		420(370-476)	---	---
	Male	2 week	528(445-626)	---	---
	Female		541(465-629)	---	---
	Male	4 week	1738(1376-2195)	---	---
	Female		1858(1526-2262)	---	---
Mice	Male	Adult	1917(1674-2152)	854(758-963)	1195(1002-1425)
	Female		2134(1766-2608)	816(725-917)	1009(848-1200)
Dog	M & F	Adult	= 1000*		
Monkey	M & F	Adult	> 1000**		

* 1000 mg/kg killed 3/4 females but not males
** 1000 mg/kg was not lethal for monkeys

BEST POSSIBLE COPY

B) ONE-MONTH ORAL TOXICITY STUDY IN RATS (RR 745-00466, conducted by [redacted] in 1977; GLP compliance statement from [redacted] dated 2/82; Vol. 1.11)

1. Treatment

Rats (10/sex/group) were dosed with 0 (0.5% tragacanth soln) , 20, 60, 200 or 600 mg/kg by oral gavage six times per week for 9 months.

Strain: Sprague-Dawley (Jcl:SD)

Drug Lot #: not provided

2. Mortality and Clinical signs

One HD male (day 7) and 1 HD females (day 9) died during the study. Cause of death was not determined. Clinical signs attributed to treatment were noted in HD males and females and consisted of decreased locomotor activity, ataxia, and decreased abdominal muscle tone. These were observed from 2-3 after dosing during the initial days of dosing but gradually decreased and disappeared after about 2 weeks.

3. Body weight and Food consumption

BW gain and food consumption were decreased in both sexes at the MHD (final BWs 9 and 11% below C in males and females, respectively) and HD (final BWs 20 and 12% below C in males and females, respectively).

4. Hematology, Clinical chemistry, and Urinalysis (blood samples obtained at termination; urine collected at 1 and 4 weeks.)

Hemoglobin was decreased in treated males at all doses (7% below C at HD), although the changes were not clearly D-R. HCT and clotting times were also decreased in HD males. RBCs, HGB, and HCT were decreased in HD females (6-9%). Neutrophilia and lymphopenia in the differential count were seen in females from the 2 highest dose groups (55 and 19%, respectively, at HD). There were no changes in bone marrow parameters between the 200 mg/kg males and C.

Increased serum calcium and decreased glucose were found in HD males (9 and 4%, respectively). Urea nitrogen and chloride were increased in males (30 and 2%, respectively, at HD) and females (51 and 3%, respectively at HD) at 200 mg/kg or greater. Total bilirubin was increased in HD males (50%) and in MHD (38%) and HD (125%) females. Alkaline phosphatase was increased in HD males (42%) and in females from the 3 highest doses (88% at HD). SGOT was increased in HD males (41%) and SGPT was increased in HD males (111%) and females (79%).

Urine volume was increased at the 2 highest doses (2-4-fold at HD) and Na⁺ excretion and Na⁺/K⁺ ratios were increased at 60 mg/kg or greater (2-fold at HD).

5. Organ weights

Absolute liver weight was increased in HD females (20%), and relative weight was increased in MHD and HD males (10 and 15%, respectively) and females (25 and 38%, respectively). Absolute kidney weight was increased in HD females (13%), and relative weight was increased in HD males (27%) and in MHD (15%) and HD (31%) females. Absolute pituitary weight was decreased in MHD and HD females (21 and 40%, respectively), and relative weight was decreased in HD females (19%). Absolute thymus weight was decreased in HD males (33%) and females (38%). Absolute epididymal weight was decreased in HD males

C) NINE-MONTH ORAL TOXICITY STUDY IN RATS (RR 745-00467, conducted by [redacted] completed 6/78; GLP compliance statement from [redacted] dated 2/82; Vols. 1.13-1.14)

1. Treatment

Rats (15/sex/group) were dosed with 0 (0.5% tragacanth soln), 10, 30, 100 or 300 mg/kg by oral gavage six times per week for 9 months. After the treatment period, 5/sex/grp were allowed to recover for 6 weeks. Doses were said to be based on the results of previous subacute studies.

Strain: Sprague-Dawley (Jcl:SD)
Drug Lot #: not provided

2. Mortality and Clinical signs

One LD male (week 18) and 2 MHD females (weeks 6 and 37) died during the study. The LD death was attributed to dosing error, while both MHD females had bladder stones at necropsy, which were considered spontaneous by the sponsor. Sedation, decreased muscle tone, and ataxia were initially observed in HD animals of both sexes 3-4 hr after dosing, but these gradually diminished so that they were no longer observed from week 2 on.

3. Body weight and Food Consumption

BW gain was significantly decreased in MD (11 and 17% below C in M and F, respectively, at 9 mo) and HD rats (26 and 34% below C in M and F, respectively). Food consumption was also decreased in these groups. Partial BW recovery was seen following treatment.

4. Hematology, Clinical chemistry, and Urinalysis (blood samples obtained at termination and after recovery; urine collected at 1, 4, 13, and 39 weeks and after recovery)

Hemoglobin was decreased in HD males (5%) and in MHD (5%) and HD females (8%). An increase in % reticulocytes was seen in HD males (2-fold). Slight neutrophilia and lymphopenia were seen in the differential count for MHD and HD males. BUN was elevated in males at all doses (25% at HD) and in MHD and HD (25%) females; total bilirubin was increased in MHD and HD (40%) males and HD females (33%); and inorganic phosphorus and cholesterol were increased in MHD and HD females (24 and 30%, respectively, at HD). Urine output and Na⁺ and K⁺ excretion were increased at the MHD and HD in both sexes throughout the study (vol increased 4-fold in HD M and F at 9 mo); the Na⁺/K⁺ ratio was increased in MLD, MHD, and HD males and in MHD and HD females; Cl⁻ was increased in MHD and HD males and in HD females; and urea nitrogen and urinary protein were increased in HD females. All values returned toward normal after the recovery period.

5. Organ weights and Pathology (histopathological examinations were performed on all animals for all tissues except bone marrow, which was examined in C, MLD and HD males only)

Kidney and liver weights were increased at the MHD or greater in both sexes (relative kidney wts 40 and 30%, relative liver wts 40 and 50% above C in HD M and F, respectively). Values returned to normal during recovery.

There were no T-R gross or microscopic findings at terminal sacrifice; however, 2/5 HD recovery females had renal calculi compared to 0/5 C. There were some apparent D-R changes (none SS) in the bone marrow differential counts in treated animals compared to C (increase in megakaryocytes, lymphocytes, early neutrophils; decrease in rubriblasts, rubricytes, eosinophils, monocytes, transitionals, late neutrophils) but no clear indication of marrow toxicity (no difference in M/E ratios or total cell counts).

6. Plasma and Brain Drug Levels

Mean plasma drug levels determined by GC analysis 24 hr after the last dose were 0.6, 3.5, 20.3, and 82.3 ug/ml in the C, LD, MLD, MHD, and HD groups, respectively. Brain levels at this time were 1.9, 5.3, 23.8, and 83.6 ug/g, respectively. The proposed human daily dose is 400 mg, or 6.7 mg/kg based on 60 kg. Mean steady-state C_{max} values in volunteers given daily doses of 400 mg for 35 days were 30.3 (BID) and 28.0 ug/ml (QD).

D) ONE YEAR ORAL TOXICITY STUDY IN RATS [redacted] Study No. 86104C, conducted by [redacted] completed 4/91; no GLP statement; Vols. 1.15-1.16)

1. Treatment

Rats (20/sex/group) were dosed with 0 (0.5% tragacanth soln), 2, 20, or 200 mg/kg by oral gavage (2 ml/kg) for 1 year. Doses were said to be based on the results of the 9-month oral toxicity study (RR 745-00467).

Strain: Sprague-Dawley (Jcl:SD)

Drug Lot #: T86104

2. Mortality and Clinical signs

Two C females, 3 LD females, 1 MD female, and 2 HD males animals died or were euthanized during the study. The cause of death was thought to be dosing error in the C and LD rats, pituitary adenoma in the MD rat (#2213, week 51), and leukemia (#1307, week 29) and cystitis (#1306, week 34) in the 2 HD rats. None of these were considered T-R; however, the HD male with cystitis was found to have "many piece(s)" of calculi in the urinary bladder.

Flaccidity of the abdominal muscles was reported in HD males and females 0.5-3 hr after dosing during the first 9 days of treatment, and increased salivation was observed in this group prior to dosing throughout treatment.

3. Body Weight, Food and Water Consumption

BW gain was significantly decreased in HD males (20% below C) and females (40%) throughout dosing. At 52 weeks, BWs were significantly lower in HD males (16%) and females (22%) compared to C. Food consumption was sometimes significantly lower in this group, primarily during the first half of the study. Water consumption was significantly increased in HD animals (effect more pronounced in females).

4. Ophthalmological Examinations (slit-lamp and fundoscopic examinations performed on 10/sex/grp on wks 25 and 50)

There were no ophthalmological findings that appeared T-R.

5. Hematology, Clinical Chemistry, Urinalysis (hematology and blood biochemistry performed on all animals at termination; urinalysis performed on 6/sex/grp at 12, 24, and 51 wks)

RBCs, hemoglobin, hematocrit, and lymphocytes were decreased (5-10%) and neutrophils were increased (32 and 14%, respectively) in HD males and females. Reticulocytes tended to be increased in HD animals (ns).

Bilirubin was increased in HD males (33%) and females (36%), BUN was increased in females from all drug-treated groups (16-42%), albumin was increased in HD males (5%) and decreased in HD females (6%), chloride was increased in HD males and females, and

alkaline phosphatase was increased in HD females (75%).

Urine volume, protein, Na⁺, and the N⁺/K⁺ ratio were increased (1.5-2X) in HD males and females.

6. Organ Weights and Gross Pathology

Absolute and relative kidney weights were increased in HD males (9.5 and 30%, respectively) and females (3.9 and 30%, respectively). Relative liver weights were also increased in this group (24 and 31% in HD M and F, respectively). In addition, a decrease in the absolute weight of the spleen and increases in absolute and relative adrenal weights were observed in HD males, and an increase in the absolute and relative weight of the thymus was found in HD females.

Upon gross examination of the kidneys at terminal sacrifice, fine renal pelvic stones were found in 3/18 males (1315, 1318, 1320) and 1/20 females (2301) in the HD group, "yellow-white dots in the medulla" were found in 2 MD (2202 and 2209) and 1 HD (2306) females, and an enlarged kidney was noted in 1 of the HD males with stones (1315). The nephroliths found in 2 of the HD animals were determined to be composed of magnesium phosphate.

7. Histopathology (histopathological examinations were performed on all animals)

There were no clear histopathological correlates for the gross kidney findings. The only apparent T-R microscopic findings in the kidneys were an increased incidence of hyaline droplets in the renal tubule in HD males (5/18 vs 1/20 in C) and an increase in the incidence of brown pigment deposition in the renal tubules in HD females (16/20 vs 10/18 in C). In the liver, the incidence of bile duct proliferation appeared to be increased somewhat in treated animals (6/20 HD females vs 2/18 C). Cell infiltration in the urinary bladder submucosa was found only in HD males (2/18) and mammary gland fibroadenoma (2/20) was found only in HD females.

Table III.5. Histopathological Findings in Surviving Rats during 1-Year Oral Toxicity Study

Group	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
N	20	18	20	17	20	19	18	20
Kidney								
brown pigment in renal tubules	9	10	9	11	10	11	9	16
edema in renal papilla	0	0	0	0	0	0	0	1
cyst	0	0	0	0	0	1	0	1
hyaline droplets in renal tubules	1	0	0	0	1	0	5	0
Urinary bladder								
cell infiltration in submucosa	0	0	0	0	0	0	2	0
Liver								
microgranuloma	8	5	5	5	9	8	4	7
brown pigment in Kupffer cell	0	0	1	0	0	0	0	1
proliferation of bile ducts	2	2	2	4	4	2	2	6
Mammary gland								
fibroadenoma	/	0	/	0	/	0	/	2

E) ONE YEAR ORAL TOXICITY STUDY IN DOGS [redacted] Report No. 250-01357, conducted by [redacted] completed 3/84; GLP; Vols. 1.19-1.21)

1. Treatment

Beagle dogs (5/sex/group) were dosed with 0, 10, 30, or 75 mg/kg orally (capsules) for 1 year. Doses were said to be based on results of a 2-month study (RR 745-00464) and a toxicokinetics study (RR 764-00089). In the toxicokinetics study (see above), emesis, anorexia, depression, ataxia, prostration, and tremors were observed at oral doses greater than 100 mg/kg, with signs persisting for up to 24 hr postdose. In the 2-month study, in which dogs (3/sex/grp) received oral doses of 10, 30, or 100 mg/kg, emesis was observed at the HD during the first 2 weeks, and D-R increases in liver and lung weights were seen at all doses, but there were no clinical laboratory or pathology findings. Plasma levels 4 hr after dosing ranged from 4.9 to 8.8 ug/ml at the LD, 21.4 to 29.6 ug/ml at the MD, and 53.3 to 77.2 ug/ml at the HD. Levels measured after the 14th dose were approximately 1.5 times those measured after the first dose but did not appear to increase thereafter. The minimal effective plasma level in dogs against MES was said to be 12.6 ug/ml. In an exploratory oral rising dose study in dogs (RR 745-00532), in which dogs (1/sex) were given escalating doses of from 25 to 250 mg/kg, the drug was well tolerated at up to 100 mg/kg, but higher doses produced anorexia and CNS effects. At termination after 7 days, central discoloration of the cornea, increased R wave amplitude in the ECG, and increased bone marrow myeloid/erythroid ratios were noted in both dogs, and prominent medullary rays and cytoplasmic vacuolization of the distal tubules were seen in the kidneys of the female animal. Based on these results, 50-100 mg/kg was recommended as the MTD for subsequent studies.

Drug Lot #: X-43689

2. Mortality and Clinical signs

There were no deaths during the study. T-R signs included emaciation in 1 female from each drug-treated group and aggression in 2 HD and 1 MD males. These findings were made primarily during the last half of the study period.

3. Body weight

There were no statistically significant differences in BW or BW gain among groups; however, several individual HD animals showed significant BW loss early in the study, which prompted the sponsor to divide doses in half (3-4 hr apart) and supplement the diet with canned dog food starting with the 3rd week of treatment.

4. Ophthalmoscopic Examinations (performed pretest and at weeks 13, 26, and 52)

There were no ophthalmological changes that appeared to be related to treatment.

4. ECG and Heart rate (electrocardiography performed predose and 60 min postdose [following each dose in HD] at 0 [first dose], 6, 13, 26, and 52 weeks [last dose])

There were no apparent T-R changes in HR or ECG parameters during the study.

5. Hematology, Clinical Chemistry, Urinalysis (pretest, weeks 7, 13, 26, 39, and 52)

There were no apparent T-R changes in hematological parameters.

Alkaline phosphatase was dose-dependently increased in males (about 2-3-fold pretreatment and C values at HD) and females (about 2-fold at HD) from all dose groups throughout the study. GGT was increased in HD males at 52 weeks (50% above C) and ALT was increased in some individual HD dogs. Albumin was decreased in HD males and females (both about 20%) throughout much of the

study. BUN tended to be slightly increased in treated males, primarily MD and HD animals, compared to C and pretreatment values (up to 40%).

There were no T-R changes in urinalysis parameters.

6. Organ weights and Pathology (all tissues from all animals examined microscopically)

Absolute and relative liver weights were D-D increased in males (22 and 19%, respectively, at HD compared to C) and females (26 and 15%, respectively, at HD). Kidney weights were also increased somewhat in HD males (absolute: 8%; relative: 5%) and females (absolute: 15%; relative: 14%).

Significant gross and microscopic findings were noted in the liver and urinary bladder (Table 1):

Liver. Dark brown discoloration of the liver was noted macroscopically in all HD females, 3/5 HD males (#s 1918, 1919, 1920) and 1 MD male (#1921). There were no microscopic findings thought to correlate with the brown pigmentation, and no evidence of abnormal pigment deposition in the liver sections; however, mild hepatocyte hypertrophy and vacuolization were also found in 2 of the HD dogs (1 M - #1918 and 1 F - #1896) with dark brown discoloration as well as an additional HD male (#1917), and bile duct hyperplasia was found in 1 HD female (#1899) and 1 MD male (#1925; no gross findings). HD males #s 1918 and 1919 had among the highest AP and GGT values, and HD females #s 1896 and 1899 had elevated AP and/or ALT values at several points during the study. EM examination of liver tissue from all 5 HD males revealed concentric lamellae of paired smooth membranes within the cytoplasm of hepatocytes, which were devoid of ribosomes and occasionally seen to be continuous with the smooth endoplasmic reticulum. These were not seen in any of the 5 C males examined.

Urinary bladder. Congestion and/or mucosal thickening/nodularity of the urinary bladder was seen grossly in 3 HD males (#1916, 1917, 1918) and 1 MD female (#1903). This urinary bladder congestion, which was said to be particularly prominent in the region of the urethral orifice, was confirmed histologically in animals with gross lesions as well as in an additional HD male (#1919).

Kidney. The incidence of interstitial medullary mineralization was increased in HD males compared to C.

Table III.4. Pathology Findings in 1-Year Dog Toxicity Study

Group	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
Kidney								
interstitial medullary mineralization	1	2	1	5	2	1	5	3
interstitial fibrosis	0	0	0	1	0	0	1	0
regenerative tubules	0	0	0	1	1	0	1	0
Liver								
dark brown	0	0	0	0	0	1	3	5
hepatocellular hypertrophy/vacuolization	0	0	1	0	0	0	2	1
bile duct hyperplasia	0	0	0	0	1	0	0	1
Urinary bladder								
mucosal congestion/thickening	1	0	0	0	0	1	3	0
submucosal congestion/edema	1	0	0	0	0	1	4	0

7. Plasma drug levels (samples collected immediately predose and at 6 hr postdose, which was estimated to be Tmax, during weeks 1, 13, 26, and 39, and 52)

At the HD, plasma concentrations never achieved steady-state (based on increasing Cmin) over the course of the study and were disproportionately higher than LD and MD levels. Since the drug is extensively metabolized in the liver, the increasing plasma concentrations during the study could be a result of hepatotoxicity at the HD. Concentrations were generally somewhat higher in males than in females. The t1/2 of zonisamide in humans and dogs was about 51 and 15 hr, respectively. The proposed human dose is 400 mg/day. Mean steady-state Cmax values in volunteers given daily doses of 400 mg for 35 days were 30.3 (BID) and 28.0 ug/ml (QD).

Table III.5. Mean Plasma Concentrations in Dogs During 1-Year Toxicity Study

Mean Plasma Concentrations of Zonisamide in Dogs Given Daily Oral Doses of 10, 30, and 75 mg/kg for 52 Weeks (µg/mL)														
Dose (mg/kg/day)	Week 1		Week 13		Week 26			Week 39			Week 52			
	0hr	6hr	0hr	6hr	**10hr	0hr	6hr	10hr	0hr	6hr	10hr	0hr	6hr	10hr
Male (%RSD*)														
10	0	7.1 (9.3)	7.3 (15.3)	12.3 (8.0)	-	6.6 (19.0)	11.8 (14.2)	-	8.5 (11.6)	13.8 (10.2)	-	8.4 (15.3)	14.0 (12.9)	-
30	0	19.1 (10.6)	26.0 (33.69)	43.7 (31.8)	-	22.1 (45.1)	37.5 (28.3)	-	30.6 (39.0)	46.4 (28.0)	-	31.5 (43.5)	48.0 (31.7)	-
75	0	50.1 (13.5)	93.7 (13.5)	126.1 (8.4)	121.0 (14.5)	80.4 (9.5)	114.1 (10.0)	109.0 (7.7)	106.7 (13.4)	143.3 (14.8)	131.9 (16.8)	112.9 (14.4)	153.5 (13.7)	140.0 (17.3)
Female (%RSD*)														
10	0	5.5 (17.1)	5.2 (37.9)	10.4 (20.2)	-	3.4 (29.0)	8.6 (12.6)	-	4.8 (18.4)	9.7 (10.2)	-	4.8 (22.2)	9.5 (16.4)	-
30	0	19.3 (13.1)	18.5 (27.5)	34.9 (18.7)	-	17.8 (39.8)	32.8 (20.2)	-	21.2 (32.9)	36.6 (21.1)	-	22.3 (22.8)	38.2 (17.1)	-
75	0	49.1 (15.3)	61.2 (16.6)	99.7 (15.9)	91.5 (19.5)	70.6 (24.2)	100.8 (14.4)	96.7 (24.5)	75.6 (29.4)	118.0 (21.4)	103.6 (20.0)	93.2 (42.2)	131.7 (33.7)	118.1 (35.9)
Combined Male and Female (%RSD*)														
10	0	6.3 (17.9)	6.3 (30.3)	11.4 (16.3)	-	5.0 (39.8)	10.2 (20.9)	-	6.7 (32.0)	11.8 (21.1)	-	6.6 (33.2)	11.7 (24.6)	-
30	0	19.2 (11.3)	2.22 (35.4)	39.3 (28.6)	-	19.9 (42.5)	35.1 (24.8)	-	25.9 (40.4)	41.5 (27.3)	-	26.9 (40.4)	48.1 (28.2)	-
75	0	49.6 (13.6)	77.5 (26.2)	112.9 (16.7)	106.3 (21.5)	75.5 (17.9)	107.5 (13.2)	102.8 (17.4)	91.2 (26.4)	130.7 (19.7)	117.8 (23.1)	103.0 (29.3)	142.6 (24.3)	129.0 (26.8)

*RSD = Percent Relative Standard Deviation

** The dosing regimen for the 75 mg/kg/day group was adjusted to equally divided doses (37.5 mg/kg) given 3-4 hours apart. The second six-hour postdose sample for this group was designated a 10-hour sample.

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

IV. CARCINOGENICITY

A) TWO YEAR CARCINOGENICITY STUDY IN MICE [redacted] Study No. 721, conducted by [redacted]
 [redacted] GLP compliance, completed 1/87, Vols. 1.22-1.25)

1. Treatment

Mice (50/sex/group) were given doses of 0, 20, 40, or 80 mg/kg in the diet for 104 weeks.
 Strain: B6C3F1

Drug Lot #: X-43793

Dose selection: Doses were selected on the basis of a 3-month dose range-finding study performed at H-IFT (RR-745-00782), in which doses of 50, 100, 200, 400, and 800 mg/kg were administered in the diet. None of these doses produced any significant systemic toxicity and there were no T-R deaths. BW gain suppression was 2, 8, 11, 2, and 17% in females and 0, 10, 11, 13, and 19% in males at 50, 100, 20, 400, and 800 mg/kg, respectively. There was no difference in food consumption among groups. No T-R gross or microscopic findings were reported. According to the sponsor, regression analysis using this data indicated that doses of 100 mg/kg or greater would result in greater than a 10% reduction in BW gain in the carcinogenicity study, so 80 was chosen as the HD.

2. Mortality

Study mortality rates were low and comparable among groups (Table IV.1).

Table IV.1: Mortality in 2-Year Mouse Carcinogenicity Study

Sex Group	Male				Female			
	1	2	3	4	1	2	3	4
Died during study	5	6	11	7	13	14	13	11
Survivors	45	44	39	43	37	36	37	39

3. Observed Signs

There were no treatment-related differences in clinical signs.

4. Body Weight and Food Consumption (ad libitum feeding)

BW gain over the course of the study was decreased in MD (9%) and HD (22%) males and in females from all treatment groups (12, 10, and 16% in LD, MD, and HD, respectively) compared to C (Table IV.2; Appendix 1). Mean BW was significantly lower in the HD group (both sexes) at 104 weeks (Table IV.3). The magnitude of the BW deficit was greater in males than in females. There were no T-R differences in food consumption.

Table IV.2: Body Weight Gain in 2-Year Mouse Carcinogenicity Study

Dose (mg/kg)	<u>Group Mean Weight Gain in Grams</u>							
	<u>Weeks 0-26</u>		<u>Weeks 26-52</u>		<u>Weeks 52-78</u>		<u>Weeks 78-104</u>	
	M	F	M	F	M	F	M	F
0	12.6	8.2	2.2	1.8	2.9	2.3	-2.7	0.2
20	10.7	7.5	2.5	1.6	3.4	2.2	-0.9	-0.3
40	9.8	7.3	1.7	1.4	2.7	2.0	-0.5	0.5
80	9.9	7.3	1.6	1.8	2.7	1.9	-2.5	-0.5

Table IV.3: Body Weights at Week 104 in Mouse Carcinogenicity Study

Group Means in Grams (% difference from C)

<u>Dose (mg/kg)</u>	<u>Males</u>	<u>Female</u>
0	38.4	32.0
20	38.8 (+1.0)	30.3 (-5.3)
40	37.0 (-3.6)	30.7 (-4.1)
80	35.1 (-8.6)***	30.1 (-5.9)**

** p<0.01 *** p<0.001

5. Ophthalmoscopy (performed at 6 month intervals)

Eye examinations did not reveal any evidence of a treatment effect.

6. Tissue Mass Observations

There was no indication that drug treatment had any effect on the type or incidence of tissue masses.

7. Hematology (only HB, MCH, MCHC, MCV, PCV, total RBC, and total WBC measured at terminal sacrifice)

HB and RBC values were slightly (ns) decreased in treatment group males and females.

8. Gross Pathology and Organ Weights (organ weights recorded for all animals)

Gross lesions were not reported. Absolute liver weights were significantly decreased in MD and HD males and in females from all treatment groups (20-30% below C). Relative liver weights were only significantly lower in MD males (24%) and females (16%).

9. Microscopic Pathology (complete microscopic examinations were performed on all animals)

There was no apparent treatment effect on incidences of neoplastic lesions (**Appendix 2**). However, according to the study report, development of ZNS was stopped by the original sponsor, prior to completion of data analysis, and there is no indication that the current sponsor analyzed the tumor data. A data tape was submitted with the NDA but has not yet been review by Biostatistics.

The incidence of foci of cellular alteration in the liver was slightly increased in HD males, but there was no D-R and the total number of animal affected was not increased. Malignant lymphoma appeared to be increased in individual organs in HD females, but when they were totaled there was no difference among groups.

Among non-neoplastic findings, thyroid follicular cysts were increased in treated males (2/48, 2/50, 5/48, and 7/49 in C, LD, MD, and HD, respectively) and females (2/49, 9/50, 12/50, and 10/50), and corpus hemorrhagicum of the ovary was increased in HD females (left: 1/49, 2/49, 1/50, and 5/50).

BEST POSSIBLE COPY

B) TWO YEAR CARCINOGENICITY STUDY IN RATS [redacted] Study No. 753, conducted by [redacted] completed 12/16/85; GLP compliance; Vols. 1.26-1.28)

1. Treatment

Rats (50/sex/group) were dosed with 0, 20, 40, or 80 mg/kg in the diet for 2 years.

Strain: Wistar-Cri:(W)BR

Drug Lot #: X-43793

Dose Selection: Doses were said to be based on the results of a 4-week dose range-finding study (RR-745-00665) in which doses of 50, 100, 200, 300, and 600 mg/kg (dietary admixture) were evaluated in Wistar rats. Body weight gain suppression in males and females at these doses was 3, 12, 29, 44, and 83% and 2, 23, 27, 47, and 103%, respectively; suppression of food consumption was 3, 10, 15, 26, and 51% and 4, 13, 16, 30, and 51%, respectively. No deaths, clinical signs, or clinical laboratory or pathological changes were reported in that study, but liver weights were increased in HD males and in females receiving 200 mg/kg or greater. Mean plasma levels measured in samples collected at termination were 14.5, 28.7, 49.0, 69.0, and 141.4 ug/ml in the respective groups (male and female combined, no sex difference seen). The study report recommended that the maximum dose for long-term studies not exceed 80 mg/kg. In a 4-week oral gavage study in Sprague-Dawley rats (RR-745-00665), doses of 20, 60, 200, and 600 mg/kg decreased BW gain by 4, 2, 21, and 47% in males and 8, 15, 30, and 36% in females, respectively, and decreased food consumption by 0, 0, 14, and 38% in males and 0, 2, 14, and 36% in females, respectively. This indicates that the effects on BW and food consumption are at least partially independent of mode of administration.

2. Mortality

There was no T-R effect on mortality. The 2 year mortality rates were 48, 44, 38, and 40% for males and 32, 26, 26, and 30% for females in C, LD, MD, and HD groups, respectively.

Table IV.4: Mortality in 2-Year Rat Carcinogenicity Study

Sex Group	Male				Female			
	1	2	3	4	1	2	3	4
Died during study	24	22	19	20	16	13	13	15
Survivors	26	28	31	30	34	37	37	35

3. Observed Signs

There were no T-R differences in clinical signs.

4. Body Weight and Food Consumption (ad libitum feeding)

Overall BW gain was 5, 2, and 11% lower in males and 12, 15, and 22% lower in females at the LD, MD, and HD, respectively, compared to C; and terminal BWs were significantly lower in HD males (9%) and in MD (10%) and HD (16%) females (Tables IV.5 and IV.6; Appendix 3). Food consumption was similarly reduced in treated groups.

BEST POSSIBLE COPY

Table IV.5: Body Weight Gain in 2-Year Rat Carcinogenicity Study

Dose (mg/kg)	<u>Group Mean Weight Gain in Grams</u>							
	<u>Weeks 0-26</u>		<u>Weeks 26-52</u>		<u>Weeks 52-78</u>		<u>Weeks 78-104</u>	
	M	F	M	F	M	F	M	F
0	431	190	49	51	52	63	-64	16
20	418	174	56	33	37	85	-65	-11
40	401	161	53	44	52	50	-46	18
80	370	158	39	34	47	46	-40	11

Table IV.6: Body Weights at Week 104 in Rat Carcinogenicity Study

Dose (mg/kg)	<u>Group Means in Grams (% difference from C)</u>	
	<u>Males</u>	<u>Female</u>
0	644	460
20	620 (-3.7)	421 (-8.5)
40	632 (-1.9)	412 (-10.4)*
80	587 (-8.9)*	386 (-16.1)**

* p<0.05 ** p<0.01

5. Ophthalmologic Examinations (pretest and at 6 month intervals)

No T-R ocular changes were reported.

6. Tissue Mass Observations

No drug-related changes in number of palpable masses.

7. Clinical Pathology (conducted at 52 weeks on 10/sex/grp; WBCs and RBC also measured at week 105)

HGB was slightly decreased (2-3%; ns) and WBC were increased (37 and 31%, respectively; ss in M) at 52 weeks in HD males and females. Absolute neutrophils were D-D increased in males (60% at HD) and neutrophils and monocytes were increased in HD females (49 and 150%, respectively). There were no significant group differences in RBCs or WBCs at moribund or terminal sacrifices. Serum creatinine was significantly increased (11%) in HD females at 52 weeks. SGOT was slightly increased in HD males (16%) and females (22%) and alkaline phosphatase was increased (25%) in HD females. In addition, serum globulin was decreased and the albumin/globulin ratio was increased (20%) in HD females. There were no urinalysis changes.

8. Gross Pathology and Organ Weights (complete necropsy performed on all rats, but only heart, liver, spleen, kidneys, brain, and gonads weighed.)

Incidences of tan area(s) in the lung (M and F) and enlarged pituitary (M) were dose-dependently increased at necropsy.

Absolute spleen weights were decreased at all doses in males (20, 23, 32% below C in LD, MD, and HD, respectively; all ss) and at the MD and HD in females (25 and 33%, respectively; ss at HD). Relative spleen weights were decreased in males at all doses (16, 19, and 25%, respectively; ss at MD and HD) and in MD and HD females (18 and 21%,

respectively; ns). Absolute kidney weights were D-D decreased in females (8-14%; ss at all doses). Relative kidney weights were also D-D decreased in males (5% at HD), although statistical significance was not reached.

9. Microscopic Pathology (complete microscopic examinations were performed on all animals from all groups.

a) Non-neoplastic (Table IV.7)

Incidences of a number of non-neoplastic histopathological findings appeared to be increased in treated animals: lung histiocytosis in females (17, 26, 27, and 28 in C, LD, MD, and HD, respectively; 50/grp), hemosiderin laden macrophages in the spleen in females (4, 9, 15, and 18), renal pelvic epithelial hyperplasia in HD males (3, 1, 2, and 6) and females (19, 20, 20, and 27), mineralization of the renal pelvis in HD females (10, 12, 13, and 18), testicular mineralization (1, 6, 8, and 8) and interstitial cell hyperplasia (4, 2, 9, and 9) in males, adrenal cytoplasmic vacuolization in males (29, 36, 37, 38), and uterine cystic endometrial hyperplasia in HD females (1, 1, 1, 4).

b) Neoplastic (Table IV.8, Appendix 4)

In the sponsors statistical analysis (pairwise and trend test analysis, 1% significance level), there were no significant increases in either percent tumor bearing animals or mortality-adjusted tumor incidence (Biostatistics review not yet available). The mortality-adjusted incidence rates for all tumors were 84.7, 90.3, 82.0, and 79.2% for males and 91.8, 91.8, 87.3, and 94% for females in the C, LD, MD, and HD groups, respectively.

Although not statistically significant, incidences of the following tumor types were increased somewhat in drug-treated groups: testicular interstitial cell tumors (3, 5, 8, and 7 in C, LD, MD, and HD, respectively; 50/grp), liver cell adenoma (0, 1, 0, and 3), osteosarcoma (0, 0, 0, and 2), and glioma (1, 0, 0, and 3) in males and uterine adenocarcinoma (2, 3, 5, 5) and thyroid C-cell adenoma (4, 4, 4, 9) in females.

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

Table IV.7: Non-Neoplastic Histopathology Findings in 2-Year Rat Carcinogenicity Study

Group	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
N	50	50	50	50	50	50	50	50
Kidney hyperplasia, pelvic epithelium mineralization, renal pelvis	3 0	19 10	1 1	20 12	2 0	20 13	6 1	27 18
Pituitary hyperplasia	1	2	3	5	4	4	4	4
Spleen hemosiderin laden macrophages	5	4	9	9	8	15	5	18
Testes interstitial cell hyperplasia mineralization	4 1	/ /	2 6	/ /	9 8	/ /	9 8	/ /
Thyroid C-cell hyperplasia	6	16	13	29	17	26	6	11
Uterus cystic endometrial hyperplasia (bilateral)	/	1	/	1	/	1	/	4

Table IV.8: Neoplastic Histopathology Findings in 2-Year Rat Carcinogenicity Study

Group	C		LD		MD		HD	
	M	F	M	F	M	F	M	F
N	50	50	50	50	50	50	50	50
Liver hepatocellular adenoma hepatocellular carcinoma	0 1	2 0	1 2	3 0	0 2	1 1	3 0	2 0
Testes interstitial cell tumor	3	/	5	/	8	/	7	/
Thyroid follicular adenoma follicular carcinoma C-cell adenoma	3 1 3	1 2 4	7 0 3	0 1 4	3 1 5	2 0 4	1 0 1	0 2 9
Uterus adenocarcinoma	/	2	/	3	/	5	/	5

BEST POSSIBLE COPY

V. GENOTOXICITY

- A) Ames Test (RR-745-00463, conducted by [redacted] in 1980; [redacted] GLP compliance statement; Vol. 1.31)

When zonisamide (Lot no. 20) was evaluated in tester strains TA1535, TA1537, TA1538, TA100, TA98, and WP2 uvrA at doses ranging from 5 to 5000 ug/plate (no toxicity seen at highest concentration) with and without S-9, no significant increases in the number of revertant colonies were produced. Appropriate control responses were observed.

- B) Ames Test (RR 745-00473, conducted by [redacted] in 1980; QA statement only; Vol. 1.31)

No significant increases in the number of revertant colonies were produced when zonisamide (lot not given) was evaluated in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA100, TA98 at concentrations of from 1 to 10000 ug/plate (no toxicity observed) in the presence and absence of S-9 mix (incubation at 37C for 48 hr).

- C) Mouse Lymphoma Assay (RR-726/2, conducted by [redacted] in 1994; GLP, Vol. 1.31)

L5178Y TK +/- mouse lymphoma cells (heterozygous at the thymidine kinase locus) were treated with zonisamide at concentrations of 132.5, 265, 530, 1060, 2120 ug/ml (no toxicity in preliminary testing at up to 2120 ug/ml, which is equivalent to 10 mM, considered limit concentration), in duplicate, together with vehicle control and positive control. There was no significant increase in mutant frequency with zonisamide, in the presence or absence of metabolic activation, while appropriate control results were obtained.

- D) Forward Mutation and Sister Chromatid Exchange in V79 Chinese Hamster Lung Cells (RR-745-00792, conducted by [redacted] in 1984; GLP, Vol. 1.32)

The potential of zonisamide (Lot X43689) to induce a forward mutation (HGPRT locus) or sister chromatid exchange in cultured V79 Chinese hamster lung cells was evaluated at concentrations of from 1000-1400 ug/ml (800 and 900 ug/ml included in SCE activation phase), with and without metabolic activation (3 hr exposure). Precipitation of drug in the culture medium occurred at the highest concentration or greater.

Plating efficiency was unaffected at the solubility limit in both phases of the mutation assay. Mutant frequencies ranged from 1-23 mutants/10⁶ clonable cells in nonactivation phase (Table V.1) and were significantly increased (p < 0.0008) compared to vehicle controls (4 mutants/10⁶). There was no dose-response trend (greatest increases seen at highest and lowest concentrations), but this may have been due to the narrow dose range and the high variability. Mutant frequencies ranged from 0-20 mutants/10⁶ clonable cells in the activation phase and were not significantly different from solvent controls (14 mutants/10⁶). Appropriate responses were seen with both positive controls in the mutation assay (829 mutants/10⁶ for EMS and 650 mutants/10⁶ for BP).

In the SCE assay, drug means ranged from 0.15 to 0.224 SCE per chromosome without activation and from 0.174 to 0.228 with activation, and in neither case were they significantly higher than the negative control rate (0.186 and 0.228, respectively).

Table V.1

SUMMARY STATISTICS FOR EACH TREATMENT GROUP
 IN VITRO MUTATION ASSAY OF C1-912 IN CHINESE HAMSTER CELLS, STUDY 8766
 ACTIVATION: S9

TREATMENT GROUP	NO. PLATES	MEAN COUNT	STD ERROR	COEFF. REL. VAR.	MEAN COUNT	STD ERROR	COEFF. REL. VAR.	PLATING EFFICIENCY (%)	MUTANT FREQUENCY	APPROX. STD. ERROR OF MUTANT FREQUENCY
SOLV. CTRL.	1	65.2	3.01	0.046	0.9	0.23	0.259	65.20	13.80	3.635
1000 UG/ML	0	69.2	2.20	0.032	0.0	0.00	-0.000	69.20	0.00	0.000
1100 UG/ML	5	63.6	0.61	0.076	1.2	0.25	0.208	60.60	13.80	4.363
1200 UG/ML	6	70.6	2.52	0.046	0.0	0.22	0.553	76.60	5.22	2.896
1300 UG/ML	7	69.0	3.70	0.053	0.7	0.21	0.305	69.00	10.09	3.122
1400 UG/ML	8	77.2	5.36	0.069	0.6	0.31	0.509	77.20	7.77	3.998
POS. CTRL.	9	64.0	3.26	0.049	41.0	1.07	0.003	64.00	649.70	42.360

CONTRAST	Z-SCORE	P-VALUE
POSITIVE CONTROL - SOLVENT CONTROL	16.96	<.0001*
DOSE RESPONSE TREND	0.61	0.2725
TREATMENTS - SOLVENT CONTROL	-1.33	0.9090

DEACTIVATION: S9-

TREATMENT GROUP	NO. PLATES	MEAN COUNT	STD ERROR	COEFF. REL. VAR.	MEAN COUNT	STD ERROR	COEFF. REL. VAR.	PLATING EFFICIENCY (%)	MUTANT FREQUENCY	APPROX. STD. ERROR OF MUTANT FREQUENCY
SOLV. CTRL.	11	81.2	5.21	0.064	0.3	0.15	0.509	87.20	3.69	1.896
1000 UG/ML	14	86.0	2.17	0.025	2.0	0.52	0.250	86.00	23.26	6.033
1100 UG/ML	15	78.0	2.15	0.027	0.6	0.22	0.369	78.00	7.61	2.076
1200 UG/ML	16	84.0	2.63	0.031	0.8	0.16	0.400	84.00	4.76	1.950
1300 UG/ML	17	88.0	2.41	0.025	0.1	0.10	1.000	88.00	1.67	1.672
1400 UG/ML	18	78.0	5.23	0.067	1.8	0.44	0.246	78.00	22.96	5.045
POS. CTRL.	19	66.0	3.35	0.050	55.0	2.01	0.036	66.00	629.30	51.300

CONTRAST	Z-SCORE	P-VALUE
POSITIVE CONTROL - SOLVENT CONTROL	16.06	<.0001*
DOSE RESPONSE TREND	-0.39	0.6932
TREATMENTS - SOLVENT CONTROL	3.95	0.0006*

* P<.05 (POSITIVE CONTROL) OR P<.025 (TEST COMPOUND COMPARISONS).
 * BASED ON FIVE PLATES, EACH INITIALLY HAVING 100 CELLS PER PLATE (INDIVIDUALLY) WITH FINAL COUNTS BASED ON TOTAL PLATE AREA.
 * BASED ON FIVE PLATES, EACH WITH 100% CELLS (NORMAL).
 * MUTANTS PER 10⁶ SURVIVING CELLS (ASSUMING OBSERVED PLATING EFFICIENCY).

E) Rat Bone Marrow - In Vivo Cytogenetics (RR-745-01386, conducted by [redacted] in 1988, GLP; Vol. 1.32)

The clastogenic activity of zonisamide (Lot X43759) was evaluated at 3 sacrifice times (8, 24, and 48 hr) after oral administration of 3 doses (20, 700, and 900 mg/kg) to rats (4/sex/grp at each sacrifice time). The HD was lowered from 1400 mg/kg, which was expected to be the MTD, after unexpected lethality occurred at that dose; 900 mg/kg was considered the LD01. There were small increases in number and % of cells with aberrations and number of aberrations/cell in drug-treated groups compared to controls at all of the sacrifice times, but none of the differences reached statistical significance. The mitotic index was significantly reduced for treated female but not males at 48 hr, indicating toxicity. Significant increases in % of cells with aberrations and mean number of aberrations/cells and decreases in mitotic index were found in positive controls (TMP) compared to vehicle controls.

F) Human Lymphocytes - In Vitro Cytogenetics (RR-726/1, conducted by [redacted] in 1994, GLP; Vol. 1.32)

Zonisamide (Batch no. 422) was evaluated for clastogenicity in human lymphocytes at concentrations of 265, 530, and 1060 ug/ml for 20 hr without S9; at 530, 1060, and 2120 ug/ml for 20 hr with activation; at 795 ug/ml for 44 hr without S9; and at 2120 ug/ml for 44 hr with S9 (dose selection based on toxicity, using mitotic index). Small increases in the frequency of cells with aberrations were seen in some treated groups, but there were no statistically significant differences. A significant increase in polyploid cells was seen after 44 hr at 2120 ug/ml in the presence of S9.

BEST POSSIBLE COPY

VI. REPRODUCTIVE TOXICITY

A) FERTILITY AND REPRODUCTION STUDY OF CI-912 IN RATS (RR 745-00843; conducted by [redacted] in '83-84; GLP; Vol 29)

1. Treatment

Male and female rats (24/sex/grp) received 0, 25, 50, or 100 mg/kg in the diet prior to (60 days for males, 14 days for females) and throughout mating (1:1 cohabitation, 10 day maximum) and until sacrifice. Males were sacrificed after 15 weeks of treatment, while females were either sacrificed on day 20 of gestation (1/2) or after delivery and weaning of offspring.

Strain: Charles River COBS CD
Drug lot #: RxX-43793

2. Fo Data

- a) There were no deaths or T-R clinical signs.
- b) Small to moderate, D-R decreases in BW gain were seen in the MD and HD groups during premating (M and F), postmating (M), gestation, and the first week of lactation (F). At the MD and HD, rats weighed an average of 4 and 10% less than C, respectively, over the course of the study.
- c) There were no T-R effects on estrous cycles, copulatory intervals, fertility indices (male and female), gestation length, parturition, or maternal behavior.
- d) Neither histopathologic evaluation of reproductive organs nor sperm analysis were performed.

3. Term Sacrifice Parameters

- a) Corpora lutea were decreased somewhat (6-8%) in all treated groups, but there was no D-R.
- b) Mean fetal weights were decreased in MD (6%) and HD (9%) litters (statistically significant at HD).
- c) There was no effect on malformation incidence, but the total incidence of developmental variations was slightly increased at the HD.

4. Delivery and Offspring Developmental Parameters

- a) Decreases in liveborn pups in the MD (19%, SS) and HD (15%, NS) groups were not considered T-R by the sponsor because of the lack of a D-R. There were no group differences in stillbirth or pup survival. No effect on malformation incidence was apparent.
- b) Offspring BWs were decreased in all treatment groups at birth (6% at HD) and during lactation, reaching statistical significance on PNDs 7 (HD), 14 (HD), and 21 (MD and HD). The weight reduction persisted postweaning (until day 35) in the HD group.
- c) Eye opening was delayed (1-2 days) in all treatment groups, but there were no other differences in preweaning development.
- d) Activity was reduced in HD offspring on PND 35 (11% compared to C), but there were no differences in postweaning tests of auditory response or rotorod performance.
- e) There were no group differences in offspring reproductive function.

B) REPRODUCTION STUDY OF CI-912 IN RATS (RR 745-00993, conducted by [redacted] in '85, GLP; Vol. 29)

1. Treatment

Male and female rats (25/sex/grp) were dosed with 0 (vehicle), 20, 60, or 200 mg/kg orally by gavage for 63 (males) or 14 (females) days prior to mating and throughout mating. Treatment continued for 100 or 101 days in males or up to day 7 of gestation in females. Females underwent C-section on day 21 of gestation and fetuses were examined for external, visceral (1/2, Nishimura method), and skeletal (1/2, Dawson method) abnormalities.

Strain: Sprague-Dawley (Jcl:SD)

Drug lot #: T83013

Dose selection was said to be based on the results of a 1-month oral (gavage) toxicity study in rats (RR 745-00466) in which the HD of 600 mg/kg produced clinical signs (decreased abdominal muscle tone, ataxia), BW gain suppression, anemia, diuresis, clinical chemistry changes (anemia, diuresis and increased bilirubin, BUN, ALP, GPT, and GOT), and organ weight changes (increased kidney, liver, and adrenal weights and decreased spleen and thymus weights) and 200 mg/kg produced BW gain suppression, diuresis, increased ALP and bilirubin values, and increased liver and kidney weights in females.

2. F₀ Data

- a) One HD female died, apparently due to dosing error. Abnormal gait and decreased locomotor activity were seen in HD males and females, primarily during the pre-mating period.
- b) A D-R decrease in BW gain occurred throughout treatment in males (9, 13, and 21% in LD, MD, and HD groups, respectively; significant at MD and HD). In females, BW gain was decreased in all treatment groups during the pre-mating and gestational treatment periods (16, 19, and 22% at LD, MD, and HD, respectively, during GD 0-8; significant at all doses).
- c) A significant increase in females with irregular estrus cycles was seen at the HD (5 HD females showed continuous metestrus II or diestrus for 4 to 10 days), but there were no effects on mating or fertility indices.
- d) No abnormal findings were reported at necropsy, but T-R increases in liver, kidney, and adrenal weights were seen in MD and HD males.

3. C-Section Data

- a) Numbers of corpora lutea, implantations, and live fetuses were dose-dependently decreased (about 10% at HD) in all treatment groups (statistically significant at MD and HD; Table VI.1).
- b) Incidences of malformations and variations were similar among groups, but ossification was slightly delayed in MD and HD fetuses.

Table VI.1

Cesarean section data in F₀ dams

Dose (mg/kg)	Vehicle control	20	60	200
No. of dams examined	25	22	24	23
No. of corpora lutea (Mean±S.E.)	409 16.4±0.23	358 16.2±0.38	371 15.5±0.35 ^a	329 14.3±0.34 ^{**}
No. of implantations (Mean±S.E.)	391 15.6±0.26	325 14.8±0.54	348 14.5±0.36 ^a	313 13.6±0.38 ^{**}
Implantations/Corpora lutea (1)	95.6	91.3	93.8	95.1
No. of fetal deaths				
Placental remnants and resorptions	23	17	23	24
Dead fetuses	0	1	1	0
Total	23	18	24	24
Fetal deaths/Implantations (1)	5.9	5.5	6.9	7.7
No. of live fetuses (Mean±S.E.)	368 14.7±0.46	307 14.0±0.57	324 13.5±0.40	289 12.6±0.51 ^{**}
Sex distribution (1) ^a	52.7	50.2	50.9	55.4
Fetal weights (g)(Mean±S.E.)	5.23±0.056	5.20±0.041	5.17±0.049	5.22±0.048
No. of retarded fetuses (1)	1(0.3)	0	2(0.6)	2(0.7)
No. of fetuses with external abnormalities (1)	1(0.3) ^b	0	0	0

a: (No. of male fetuses/No. of live fetuses) x 100
b: Short trunk, atretic anus and rudimentary tail
a: Significantly different from control at P < 0.05
**: Significantly different from control at P < 0.01

C) TERATOGENICITY STUDY OF CI-912 IN MICE (RR 745-00469, Vol. 1.29; study conducted by [redacted] in 1977, pre-GLP; contains 1982 [redacted] QA statement indicating no major deficiencies in the laboratory or report)

1. Treatment

Mated female mice (26-30/grp) were given 0 (0.5% tragacanth solution), 125, 250, or 500 mg/kg on gestation days 6 through 15 by oral gavage. On day 18, C-sections were performed on all dams; numbers of live, dead, and resorbed fetuses were recorded; live fetuses were weighed, sexed, and examined for external abnormalities; and fetuses from each litter were examined for visceral (1/2, Nishimura) or skeletal (1/2, Dawson) defects.

Strain: Jcl:ICR

Drug lot #: 10

2. Effects on the Dam

- No maternal deaths occurred.
- Neurotoxicological signs (sedation, ataxia) were seen at the MD (slight) and HD.
- BW gain during dosing was dose-dependently decreased in treated dams (statistically significant only at HD: 19% below C).
- At autopsy, involution of the thymus was found in MD and HD group dams.

3. Fetal Evaluations

- The number of fetal deaths and the % of fetal deaths/implantations were increased (about 2X) in HD litters (Table VI.2).

- b) Mean fetal BW was decreased (16% below C; statistically significant) and the incidence of retarded fetuses was increased (4X) in the HD group (Table VI.2).
- c) Malformation incidences were markedly increased in the HD group (Tables VI.3). Specific abnormalities found more frequently in HD fetuses included cleft palate, open eye, dilatation of the cerebral ventricles, dilatation of the renal pelvis, and vertebral and rib malformations. Fetal/litter incidences of craniofacial defects (cleft palate and open eye) appeared to be dose-dependently increased in all treatment groups (1.4/17, 3.3/22, 3.5/33, and 7.3/46% in C, LD, MD, HD, respectively), although only the HD effect was statistically significant.
- d) Incidences of skeletal variations were increased and ossification was delayed in drug-exposed litters, reaching statistical significance at the MD and HD (Table VI.3).

Table VI.2

Caesarean section data in mice

Dose (mg/kg)	Vehicle control	125	250	500
No. of dams examined	24	23	24	25
No. of implantations (Mean ± S.E.)	306 12.8 ± 0.34	289 12.6 ± 0.33	303 12.6 ± 0.34	345 13.3 ± 0.37
No. of fetal deaths				
Placental remnants and resorptions	10	10	10	16
Dead fetuses	4	4	4	13
Total	14	14	14	29
Fetal deaths/implantations (%)	4.6	4.8	4.6	8.4
No. of live fetuses (Mean ± S.E.)	292 12.2 ± 0.37	275 12.0 ± 0.37	289 12.0 ± 0.39	316 12.2 ± 0.39
Sex distribution (%) ^a	45.9	51.0	53.3	50.6
Fetal body weight (g) (Mean ± S.E.)	1.39 ± 0.017	1.35 ± 0.019	1.34 ± 0.017	1.16 ± 0.026**
No. of retarded fetuses (%)	5 (1.7)	4 (1.5)	0*	26 (8.2)

^a : (No. of male fetuses/No. of live fetuses) × 100

* : Significantly different from control at $p < 0.05$

** : Significantly different from control at $p < 0.01$

Table VI.3

External, visceral and skeletal examinations of mouse fetuses

Dose (mg/kg)	Vehicle control	125	250	500
External				
No. of fetuses examined	292	275	289	316
No. of fetuses with abnormalities	5 (1.7)	9 (3.3)	10 (3.5)	24 (7.6)*
Cleft palate	1 (0.3)	5 (1.8)	4 (1.4)	11 (3.5)
Open eye	3 (1.0)	4 (1.5)	6 (2.1)	12 (3.8)
Umbilical hernia	1 (0.3)	0	0	1 (0.3)

Table VI.3 continued

Visceral				
No. of fetuses examined	155	141	152	153
Head				
No. of fetuses with abnormalities (%)	2 (1.3)	6 (4.3)	2 (1.3)	46 (25.2)**
Dilatation of the cerebral ventricles	2 (1.3)	6 (4.3)	2 (1.3)	46 (25.2)**
Thorax				
No. of fetuses with abnormalities (%)	4 (2.6)	—	—	3 (1.6)
Ventricular septal defect	2 (1.3)	—	—	1 (0.6)
Supernumerary coronary orifice	1 (0.6)	—	—	0
Riding aorta	1 (0.6)	—	—	0
Corrected transposition of the great arteries	0	—	—	1 (0.6)
Persistent right azygos vein	0	—	—	1 (0.6)
Abdomen				
No. of fetuses with abnormalities (%)	0	2 (1.4)	1 (0.7)	6 (3.7)*
Dilatation of the renal pelvis	0	2 (1.4)	1 (0.7)	6 (3.7)*
Skeletal				
No. of fetuses examined	137	134	137	153
No. of fetuses with abnormalities (%)	0	0	0	8 (5.2)
Fusion of vertebrae	0	0	0	5 (3.3)
Fusion of ribs	0	0	0	3 (2.0)
Deformity of vertebrae and ribs	0	0	0	2 (1.3)
Absence of 13th ribs	0	0	0	2 (1.3)
Absence of 2nd sternebra	0	0	0	1 (0.7)
No. of fetuses with skeletal variations (%)				
Cervical ribs	10 (7.3)	17 (12.7)	20 (14.6)	34 (22.2)
14th ribs	39 (28.5)	35 (26.1)	27 (19.7)	28 (18.3)
Additional sternebra	21 (15.3)	8 (6.0)	4 (2.9)*	5 (3.3)*
Dislocated sternebrae	0	6 (4.5)*	2 (1.5)	6 (3.9)*
5 lumbar vertebrae	1 (0.7)	3 (2.2)	0	1 (0.7)
7 lumbar vertebrae	0	1 (0.7)	0	1 (0.7)
Maturation of ossification				
Delayed ossification				
Parietal bone				
Suture grading* (Mean ± S.E.)	2.9 ± 0.07	2.8 ± 0.06	2.7 ± 0.10	2.5 ± 0.09**
Supraoccipital bone (%)	0	6 (4.5)*	4 (2.9)	29 (19.0)**
Sternae (%)	14 (10.2)	22 (16.4)	23 (16.8)	39 (25.5)*
No. of ossification centers (Mean ± S.E.)				
Cervical vertebrae	6.9 ± 0.04	7.0 ± 0.03	6.9 ± 0.03	6.4 ± 0.25*
Caudal vertebrae	9.7 ± 0.23	9.4 ± 0.30	8.3 ± 0.24**	6.8 ± 0.30**
Fore limb (Unilateral)				
Proximal phalanges	3.9 ± 0.03	3.9 ± 0.06*	3.7 ± 0.10	3.3 ± 0.19**
Middle phalanges	1.5 ± 0.22	1.4 ± 0.20	1.2 ± 0.18	0.6 ± 0.17*
Distal phalanges	3.3 ± 0.25	3.1 ± 0.30	2.4 ± 0.31*	2.6 ± 0.31
Hind limb (Unilateral)				
Metatarsals	5.0 ± 0.01	5.0 ± 0.02	5.0 ± 0.02	4.9 ± 0.06*
Proximal phalanges	4.6 ± 0.09	4.6 ± 0.08	4.4 ± 0.10	3.6 ± 0.26**
Middle phalanges	0.7 ± 0.21	0.7 ± 0.18	0.4 ± 0.12	0.2 ± 0.12*
Distal phalanges	4.7 ± 0.09	4.7 ± 0.13	4.2 ± 0.23*	4.1 ± 0.23*

* : Grading ranges 1-4

* : Significantly different from control at $p < 0.05$ ** : Significantly different from control at $p < 0.01$