

D) TERATOGENICITY STUDY OF CI-912 IN RATS (RR 745-00468, Vol. 1.30; study conducted by [redacted] in 1978, pre-GLP)

1. Treatment

Mated female rats were given vehicle (0.5% tragacanth solution; N=30) or zonisamide (20, 40, 80, or 200 mg/kg; 22-23/grp) on gestation days 7 through 17 by oral gavage. A pair fed group (N=23) and two phenobarbital-treated groups (40 or 80 mg/kg; 22-23/grp) were also included (complete PB results not described in this review). According to the report, the LD was expected to produce plasma levels shown effective against MES in rats (10 ug/ml), while the HD was expected to produce maternal toxicity. On day 21, 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 1/2 of fetuses from each litter were examined for visceral (Nishimura) and 1/2 for skeletal (Dawson method) abnormalities.

Strain: Jcl:SD

Drug lot #: 10

2. F₀ Parameters

- a) There were no maternal deaths. Mild clinical signs of neurotoxicity were observed only in the HD group.
- b) Maternal BW gain was significantly decreased during treatment in the 40 (10% below C), 80 (27%), and 200 mg/kg (38%) zonisamide groups, while BW at term was significantly lower in the two highest dose groups. The group pair fed to the HD zonisamide group showed a similar decrease in BW gain (39%).
- c) No T-R gross abnormalities were found at maternal necropsy, but liver, kidney, spleen, and adrenal weights were increased in the HD ZNS group.

3. Maternal and Fetal Drug Levels

Mean maternal plasma ZNS concentrations measured 4 hr postdose on GD 17 were 64.3 and 110.7 in the 80 (N=5) and 200 mg/kg (N=4) groups. Mean whole fetal drug levels in these respective treatment groups (3 fetuses/dam) were 52.1 and 84.5 ug/g.

4. C-Section Data

- a) Embryo/fetal mortality was comparable across groups.
- b) Small but statistically significant decreases in fetal BW occurred in the 80 (mean 4.90 g; 4% below C) and 200 mg/kg (mean 4.79; 6% below C) ZNS groups. Mean fetal weight in the pair fed control group was only slightly lower than in C (4.98 vs 5.08 g).
- c) Fetal and litter incidences of total abnormalities were significantly increased at the HD of ZNS (30.1/77.3% affected vs 6.6/30 in C and 9.1/47.8 in PFC). This was primarily due to an increased incidence of "persistence of cords of thymic tissue", a common visceral variation in rats (fetal incidence 28.7% vs 3.3 in C and 7.1 in PFC). This defect was also increased in HD PB-exposed litters (16.7%). Incidences of ventricular septal defect were similar between ZNS HD (4.2%) and C (3.8%) groups, but were higher in these groups than in PFC (1.9%) and much lower than in the PB groups (13.7 and 54% at LD and HD, resp). Skeletal ossification (sternbrae, cervical, thoracic, and caudal vertebrae, phalanges, metatarsals) was retarded in the 80 and 200 mg/kg ZNS groups as well as in both PB groups.

E) EMBRYO-FETAL DEVELOPMENT STUDY IN RATS (RR 745-01027, Vol. 1.30; study conducted by [redacted] in 3/85-3/86; said to be in compliance with Japanese GLP but did not include periodic determinations of drug concentration in dosing solutions as per FDA GLP requirements)

1. Treatment

Zonisamide was administered by oral gavage to pregnant female rats (40/grp) on days 7 through 17 of gestation at doses of 0 (vehicle=0.5% tragacanth solution), 20, 60, or 200 mg/kg. Dose selection was based on the results of the previous teratogenicity study. Twenty-five females/group were killed and cesarean-sectioned on day 21 of gestation, while 15/group were allowed to deliver and raise their litters (culled to 8 on PND 0) to weaning. Offspring behavior was examined in 2/sex/litter and reproductive function was evaluated in 1/sex/litter.

Strain: Jcl:SD
Drug lot #: T85004

2. F₀ Female Parameters

- a) There were no maternal deaths. (1 C and 1 HD female were found not pregnant and were excluded from analysis.)
- b) Clinical signs of neurotoxicity (abnormal gait, decreased locomotor activity, decreased body tone) were observed in the HD group.
- c) Maternal BW gain was significantly decreased in the MD (23% below C between GD 7 and 18) and HD (57% below C) groups during gestation. Food consumption was also significantly decreased in these groups.
- d) In dams necropsied at term, the incidence of atrophied thymus was significantly increased in the HD group compared to C. Heart, thymus, pituitary, and thymus weights were significantly decreased in MD and HD group dams at term sacrifice, and liver and adrenal weights were significantly increased at the HD. No D-R gross findings or organ weights were found in the dams necropsied after weaning.

3. C-Section Data (1/2 of fetuses from each litter examined for visceral defects by Nishimura method and 1/2 examined for skeletal defects by Dawson method; individual data not provided in report)

- a) Embryo/fetal mortality was comparable across groups.
- b) Fetal BW was dose-dependently decreased, reaching statistical significance at the MD (mean 3% below C) and HD (13%). The number of retarded fetuses (<70% of mean control BW) was also significantly increased in the HD group compared to C (0, 0.8, 0.6, and 4.8% of fetuses in C, LD, MD, and HD groups).
- c) The incidences of total visceral defects (8.2, 9.3, 15.3, and 22.8% of fetuses examined in C, LD, MD, and HD litters, respectively), ventricular septal defects (0.5, 1.5, 2.8, and 4.4%, respectively) and persistence of cords of thymic tissue (3.8, 4.6, 9.6, and 16.1%, respectively) were dose-dependently increased in treated litters, reaching statistical significance at the HD (Table VI.4). No increase in external or skeletal defects was observed, but retarded skeletal ossification (parietal bone, sternbrae, cervical, thoracic, and caudal vertebrae, phalanges, metatarsals) was seen in the MD and HD groups.

4. Delivery Data

- a) Number of pups delivered, number of live-born pups, birth index, and postnatal viability were comparable in control and treatment groups. 1 dead MD pup had multiple external anomalies.
- b) BW was significantly decreased in HD females at birth (5% below C) and during the postweaning period (9% below C at 6 weeks).

- c) Attainment of several physical landmarks of development (tooth eruption, eye and ear opening, testis descent, vaginal opening) was delayed in all drug-treated groups, but the changes were small (1-3 days) and not always D-R.
- d) No adverse effects were observed in behavioral reflex development (righting, startle, pivoting, etc), spontaneous activity, Biel water maze performance, conditioned avoidance performance, estrous cyclicity, or mating and fertility.
- e) In animals autopsied at 6 weeks, the incidence of persistence of cords of thymic tissue was significantly increased in the HD group. Animals autopsied at later times (11-17 weeks) had lower (but still increased compared to C) incidences of this structural variant.

Table VI.4

External, visceral, and skeletal examinations of F₁ fetuses

Dose (mg/kg)	Vehicle control	20	60	200
External				
No. of fetuses examined	352	372	347	351
No. of fetuses with abnormalities (%)	0	0	1 (0.3)	0
Short tail	0	0	1 (0.3)	0
Visceral				
No. of fetuses examined	182	184	177	180
No. of fetuses with abnormalities (%)	13 (8.3)	18 (8.3)	37 (19.3)	41 (22.8)**
Persistence of cords of thymic tissue	7 (3.8)	9 (4.8)	17 (9.6)	29 (16.1)**
Ventricular septal defect	1 (0.5)	3 (1.5)	5 (2.8)	8 (4.4)*
Supernumerary coronary orifice	2 (1.1)	3 (1.5)	4 (2.3)	1 (0.6)
Quadricuspid pulmonary valve	1 (0.5)	0	0	3 (1.7)
Right subclavian artery arising from the aortic arch	1 (0.5)	0	1 (0.6)	1 (0.6)
Persistent right azygos vein	1 (0.5)	1 (0.5)	0	4 (2.2)
Dilatation of the renal pelvis	2 (1.1)	3 (1.5)	2 (1.1)	0
Dilatation of the ureter	2 (1.1)	0	0	1 (0.6)
Skeletal				
No. of fetuses examined	170	173	169	171
No. of fetuses with abnormalities (%)	0	3 (1.7)	0	5 (2.9)
Arrested fusion of the sternbrae	0	2 (1.1)	0	4 (2.3)
Fusion of costal cartilages	0	1 (0.6)	0	1 (0.6)
No. of fetuses with variations (%)				
Extra 14th ribs	2 (1.2)	6 (2.4)	2 (1.2)	0
Rudimentary 14th ribs	73 (42.9)	56 (31.5)	54 (32.0)	29 (17.0)**
Short 13th ribs	0	0	1 (0.6)	0
Dislocated sternbrae	0	1 (0.6)	0	0
Additional sternbrae	1 (0.6)	0	0	0
Inverted Y-shaped 6th sternbrae	1 (0.6)	1 (0.6)	0	2 (1.2)
7 lumbar vertebrae	4 (2.4)	5 (2.8)	2 (1.2)	3 (1.8)
Maturation of ossification				
Delayed ossification				
Parietal bone				
Suture grading ^a (Mean±S.E.)	2.0±0.02	2.9±0.03	2.9±0.05	2.7±0.09**
Sternbrae (%)	19 (11.2)	24 (13.5)	31 (19.5)	67 (39.2)**
Thoracic vertebrae (%)	5 (2.9)	10 (5.6)	2 (1.2)	15 (8.8)*
No. of ossification centers (Mean±S.E.)				
Cervical vertebrae	2.5±0.25	2.5±0.25	2.1±0.25	1.3±0.11**
Caudal vertebrae	6.6±0.11	6.3±0.14	6.2±0.11**	5.6±0.10**
Fore limb	2.0±0.00	2.0±0.02	2.0±0.00	2.0±0.01
Metacarpals				
Proximal phalanges	7.6±0.11	7.3±0.17	7.8±0.14	5.7±0.27**
Mid limb	2.5±0.11	2.6±0.10	2.4±0.10	2.4±0.08**
Metatarsals				
Proximal phalanges	2.3±0.42	2.2±0.43	1.8±0.26**	0.3±0.12**

*: One fetus with external abnormality is excluded from calculation *: Grading ranges 1-4
 *: Significantly different from control at $p < 0.05$
 **: Significantly different from control at $p < 0.01$

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F) TERATOGENICITY STUDY OF CI-912 IN DOGS (RR 745-00470, Vol. 1.30; study conducted by [redacted] in 1978, pre-GLP)

1. Treatment

Pregnant female beagle dogs (6-7/group) received 0 (lactose), 10, 30, or 60 mg/kg zonisamide orally in gel caps on gestation days 14 through 35. An additional 3 pregnant females were treated with 30 mg/kg on GDs 14-35 for fetal drug level determinations. C-sections were performed on day 55 of gestation, and fetuses were examined for abnormalities. Dose selection was based on the results of a subacute toxicity study in which 100 mg/kg produced vomiting, gastric follicle hyperplasia, and a reduction in AI-P activity, and an increase in absolute and relative liver weights was seen in all groups.

Drug lot #: 16

2. Effects on F0 Females:

- a) There were no maternal deaths.
- b) T-R vomiting occurred in 3 MD and 2 HD females but no other signs were reported.
- c) BW gain during treatment was dose-dependently decreased in all groups compared to C (0.7, 0.1, 0.2, and -0.7 kg gained between GDs 14 and 36 in C, LD, MD, and HD, respectively), and BW gain was significantly decreased throughout gestation in the HD group (34% below C).
- d) There were no D-R changes in blood biochemical parameters.
- e) Mean maternal plasma drug concentrations measured 4 hr postdose on GD 14 were 12.8, 25.3, and 43.8 ug/ml in LD, MD, and HD females, respectively. Mean plasma levels 24 hr after the first dose were 7.0, 15.3, and 25.8 ug/ml in the respective treatment groups. At 4 hr after the last dose on day 35, mean plasma levels were 11.6, 44.5, and 78.5 ug/ml in these same groups, indicating drug accumulation. The threshold plasma level for anti-MES activity in beagle dogs was 12.6 ug/ml.

3. Fetal Evaluation (brain, heart, and kidneys of all fetuses fixed and examined microscopically; skeletal exams on 1/2 of fetuses per litter by Dawson method)

- a) A significant increase in fetal mortality was observed in the HD group. Fetal mortality was 4.3, 8.1, 7.7, and 24.5% in the C, LD, MD, and HD groups, respectively.
- b) Fetal BWs were D-D decreased and the incidence of retarded fetuses increased (0, 15.8, 16.7, and 32.5% in C, LD, MD, and HD, respectively; ss at HD) at all doses.
- c) Incidences of external, visceral, and skeletal abnormalities were significantly increased in the HD group (Tables VI.5-VI.7). The fetal incidences of external abnormalities were 4/44, 0/57, 2/60, and 21/40 in C, LD, MD, and HD litters, respectively. All HD litters had affected fetuses. The specific abnormalities kinky, short, or rudimentary tail were significantly increased at the HD (50% of fetuses vs 9% in C). The fetal incidences of thoracic visceral abnormalities were 4/44, 1/57, 6/60, and 22/40 in C, LD, MD, and HD litters, respectively. All HD litters had affected fetuses. Among individual malformations, the incidence of ventricular septal defect (18/40 fetuses vs 0 in C) was significantly increased. A number of additional CV defects were increased in HD litters (see Table). Incidences of total abdominal visceral defects were increased in MD (slightly) and HD litters (significantly). The only head malformation (hydrocephaly) was found in a HD fetus. Among fetuses examined for skeletal defects, those in the HD group had significantly increased incidences of total skeletal malformations (24/38 fetuses, all litters affected) and fusion and/or deformity of the caudal vertebrae (20/38 fetuses). Incidences of

- skeletal variations were increased at the MD and HD(significant).
- d) Mean fetal drug levels 4 hr after the final dose on GD 35 in 3 MD litters were 40.1, 31.3, and 34.8 ug/ml, while the corresponding maternal plasma levels were 36.8, 37.1, and 41.1 ug/ml, indicating that fetal and maternal exposures were equivalent.

Table VI.5

External abnormalities of F₁ fetuses (Summary)

Dose (mg/kg)	Control		10		30		60	
	No.	%	No.	%	No.	%	No.	%
No. of litters examined	6		7		7		7	
No. of fetuses examined	44		57		60		40	
Litters with abnormalities	2	33.3	0	0	2	28.6	7	100*
Fetuses with abnormalities	4	9.1	0	0	2	3.3	21	52.5**
Kinky, short or rudimentary tail	4	9.1	0	0	1	1.7	20	50.0**
Edema	0	0	0	0	0	0	2	5.0
Bilateral cleft lip	0	0	0	0	0	0	1	2.5
Clubbed foot	0	0	0	0	1	1.7	0	0
Syndactyly of the 4th and 5th fingers of the right hand	0	0	0	0	0	0	1	2.5

*: Significantly different from control at $P < 0.05$.

** : Significantly different from control at $P < 0.01$.

Table VI.6

Visceral abnormalities of F₁ fetuses (Summary)

Dose (mg/kg)	Control		10		30		60	
	No.	%	No.	%	No.	%	No.	%
Head								
No. of litters examined	6		7		7		7	
No. of fetuses examined	25		31		32		23	
Hydrocephalus	0	0	0	0	0	0	1	4.3
Thorax								
No. of litters examined	6		7		7		7	
No. of fetuses examined	44		57		60		40	
Litters with abnormalities	3	50.0	1	14.3	4	57.1	6	85.7
Fetuses with abnormalities	4	9.1	1	1.8	6	10.0	22	55.0*
Ventricular septal defect	0	0	0	0	4	6.7	18	45.0**
Cardiomegaly	0	0	0	0	0	0	2	5.0
Thickening of the atrioventricular valve	0	0	0	0	0	0	2	5.0
Riding aorta	0	0	0	0	1	1.7	3	7.5
Partial transposition of the great arteries	0	0	0	0	0	0	3	7.5
Bicuspid pulmonary valve	0	0	0	0	0	0	1	2.5
Supernumerary coronary orifice	2	4.5	0	0	0	0	0	0
Coarctation or absence of the aorta	0	0	0	0	1	1.8	6	15.0
Hypoplastic aorta	0	0	0	0	0	0	3	7.5
Hyperplastic pulmonary artery	0	0	0	0	0	0	1	2.5

*: Significantly different from control at $P < 0.05$.

** : Significantly different from control at $P < 0.01$.

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Table VI.6 (continued)

Visceral abnormalities of F₁ fetuses (Summary)

Dose (mg/kg)	Control		10		30		60	
	No.	%	No.	%	No.	%	No.	%
Left common carotid artery arising from the aortic arch	1	2.3	0	0	2	3.3	6	15.0
Retoroesophageal right subclavian artery	0	0	1	1.8	0	0	0	0
Undescended thymus	1	2.3	0	0	0	0	0	0
Hypoplasia and/or dysplasia of the thymus	0	0	0	0	0	0	4	10.0
Hypoplasia of the lung	0	0	0	0	0	0	1	2.5
Abdomen								
No. of litters examined	6		7		7		7	
No. of fetuses examined	44		57		60		40	
Litters with abnormalities	0	0	1	14.3	1	14.3	5	71.4 ^a
Fetuses with abnormalities	0	0	1	1.8	2	3.3	8	20.0 ^a
Reddish-yellow, firm liver	0	0	0	0	0	0	1	2.5
Hypoplasia and/or dysplasia of the spleen	0	0	1	1.8	2	3.3	4	10.0
Umbilical hernia	0	0	0	0	1	1.7	2	5.0
Undescended testis	0	0	0	0	0	0	3	15.0

^a: Significantly different from control at P < 0.05.

Table VI.7

Skeletal examinations of F₁ fetuses

Dose (mg/kg)	Control		10		30		60	
	No.	%	No.	%	No.	%	No.	%
No. of litters examined	6		7		7		7	
No. of fetuses examined	25		26		29		38	
Skeletal abnormalities								
Litters with abnormalities	1	16.7	0	0	2	28.6	7	100 ^{aa}
Fetuses with abnormalities	3	12.0	0	0	2	6.9	24	63.2 ^{aa}
Fusion of the sternebrae	0	0	0	0	1	3.4	4	10.5
Sternochisis	0	0	0	0	0	0	1	2.6
Fusion and/or deformity of the caudal vertebrae	3	12.0	0	0	1	3.4	20	52.6 ^{aa}
Skeletal variation								
Litters with variations	1	16.7	1	14.3	4	57.1	6	85.7 ^a
Fetuses with variations	1	4.0	2	7.7	8	27.6	14	36.8 ^a
14th ribs	0	0	0	0	0	0	1	2.6
Incomplete fusion of the 8th sternebrae & lumbar vertebrae	0	0	2	7.7	3	10.3	2	5.3
Maturity of ossification	1	4.0	0	0	6	20.7	12	31.6
Naturity of ossification								
Poorly ossified or unossified 1st cervical vertebral centrum	0	0	0	0	3	10.3	3	7.9
No. of ossified caudal vertebrae ^a (Av. ±SE)	18.2±0.14		17.6±0.16 ^a		17.9±0.26		16.9±0.16 ^{aa}	

^a: The fetuses with abnormal tail are excluded from calculation of average number of ossified caudal vertebrae

^a: Significantly different from control at P < 0.05.

^{aa}: Significantly different from control at P < 0.01.

G) TERATOGENICITY STUDY IN MONKEYS (RR 745-00471, conducted by [redacted] in 1978-'79, pre-GLP but has compliance statement from [redacted] Vol. 1.31)

1. Treatment

Pregnant cynomolgus monkeys (4 C, 7 LD, 8 HD) were treated with 0 (0.5% tragacanth soln), 10, or 20 mg/kg orally (gavage) on days 21 through 45 of gestation and were cesarean-sectioned on day 100 of gestation. Blood samples were taken at 4 and 24 hr after dosing on days 21 and 45 for drug level determinations. Dose selection was based on the results of the dog study (above).

Drug lot #: 16

2. Maternal effects

- a) There were no maternal deaths.
- b) T-R vomiting was noted in both treatment groups.
- c) BW gain was decreased in all groups during treatment but was more pronounced in drug-treated animals (-0.10, -0.22, and -0.26 kg in C, LD, and HD, respectively). Food consumption was also decreased in both treatment groups.
- d) Vaginal bleeding as a result of abortion was observed in 1 LD (day 43) and 3 HD (days 45, 56, and 72, respectively) dams. Uterine bleeding was not observed in another HD dam with a dead fetus at the time of C-section.
- e) Maternal plasma drug levels were 5.2 and 13.2 ug/ml at 4 hr and 1.6 and 5.0 ug/ml at 24 hr after the first dose on days 21 in the LD and HD groups, respectively. After the last dose on day 45, mean plasma levels were 10.2 and 21.9 ug/ml at 4 hr and 4.0 and 8.7 ug/ml at 24 hr in the LD and HD group animals, respectively.

3. Litter Effects (the following determinations were made only on live fetuses: sex, BW, head circumference, C-R length, tail length, arm, hand, leg, and foot lengths, organ weights, and limited skeletal evaluation)

- a) Fetal BWs were D-D increased in treated groups (avg: 100, 110, and 137 in C, LD, and HD, respectively). Head circumference and C-R length were also increased in litters born to drug-treated animals.
- b) No external, visceral, or skeletal abnormalities were reported for live fetuses. The 1 dead HD fetus was apparently not examined.

H) PRENATAL-POSTNATAL STUDY IN RATS (RR 745-01055, conducted by [redacted] in 1986, non-GLP, compliance statement from [redacted] Vol. 1.31)

1. Treatment

Pregnant female rats (20/group) were treated with 0 (vehicle), 10, 30, or 60 mg/kg orally (gavage) from gestation day 17 through postnatal day 20. The dams were allowed to deliver and rear offspring. F1 offspring were evaluated for survival, growth, physical and behavioral development, and reproductive performance. Dose selection was based on the results of the rat embryo/fetal development study (RR 745-01027, above) in which 200 mg/kg resulted in dystocia and neonatal death. The initial HD chosen for this study was 100 mg/kg, but in a dose range-finding study, 2/7 dams treated with this dose died on day 22 or 23 of gestation and most of the pups in 2 of the 5 remaining litters died by PND 1.

Strain: Sprague Dawley (Jcl:SD)

Drug lot #: T85010

2. Maternal Effects :

- a) No deaths and no clinical signs were reported.
- b) Maternal BW gain during the gestational treatment period was significantly decreased (-28%) by the HD. Food consumption was also decreased during gestation in HD dams.
- c) Duration of gestation was comparable among groups, and no abnormalities were observed at parturition (all dams gave birth to live pups). Nest-building behavior and pup retrieval were significantly decreased in HD dams, but these effects were only seen late in the lactation period when maternal behavior is normally decreasing.

4. Offspring Effects

- a) The number of perinatal deaths/pups delivered was increased (1.8, 1.8, 3, and 14% in C, LD, MD, and HD groups, respectively) and the number of liveborn pups/number of implantation sites was decreased (90.6, 93, 90.7, and 82.3%, respectively) in the HD group. Pup weights at birth were similar among groups, and no external abnormalities were reported.
- b) Viability during lactation was apparently not affected by treatment (lactation index: 66.7, 83.8, 81.9, and 69.5% in C, LD, MD, and HD groups, respectively), although the number of pups dying in the C group is suspicious. BWs were comparable among groups from birth to 10 weeks of age.
- c) Testes descent, a developmental landmark reflecting sexual maturation, appeared to be delayed slightly in HD pups compared to C, but there were no group differences for other preweaning landmarks (pinna unfolding, tooth eruption, ear opening, eye opening, vaginal opening).
- d) Most measures of reflex development indicated no group differences (surface righting, pivoting, air righting, rope climbing, visual placing, auditory startle, pain response, corneal reflex); however, the emergence of walking was delayed slightly (1 day) in MD and HD pups. There were no differences in spontaneous activity measured at 5 weeks.
- e) There was no apparent effect of treatment on offspring performance in a water-filled multiple T-maze test of learning and memory at 5 weeks of age. When the conditioned avoidance response was examined at 10 weeks, an apparent dose-dependent deficit was observed in treated males (total responses 33% below C at HD), although the differences did not reach statistical significance.
- f) Reproductive performance of F1 animals was comparable among groups.
- g) Visceral and skeletal examinations of F1 animals that died or were sacrificed postnatally revealed no apparent increase in abnormalities related to treatment.

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VII. SUMMARY AND EVALUATION

Pharmacology

Zonisamide (ZNS) was discovered when, during routine screening of synthetic 1,2-benzisoxazole derivatives, some of the sulfonamide analogs were found to be active against maximal electroshock seizures (MES). In initial screening tests, ZNS demonstrated an anticonvulsant profile similar to that of PHT or CBZ, in that it was effective against MES but not against PTZ-induced threshold seizures. Other studies revealed the ability to suppress spiking activity induced by cortical freezing in cats and tungstic acid gel application in rats, which are also suppressed by VPA but not by PHT or CBZ, suggesting a broader spectrum of activity for ZNS. The therapeutic index was relatively high in rodents, with antiepileptic activity manifested at plasma concentrations of about 10 ug/ml and toxicity observed at levels of 70 ug/ml or greater. Because ZNS has a sulfamoyl group in common with acetazolamide (AZA), it was initially thought that its mechanism of action would be related to inhibition of carbonic anhydrase (CA); however, it demonstrated relatively weak CA inhibition in vitro and ex vivo in rats, and evidence for a dissociation of the two effects was found in rat studies involving the methyl analog of ZNS. The methylene group between the 1,2-benzisoxazole and sulfamoyl groups is thought to be what differentiates it from AZA in this respect. Studies of the cellular mechanism of action indicated effects on Na⁺ and Ca²⁺ channels at pharmacologically relevant concentrations. ZNS blocked sustained repetitive firing in cultured mouse spinal cord neurons, as does PHT, 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. In addition, ZNS dose-dependently reduced voltage-dependent transient inward T-type Ca²⁺ currents in cultured rat cortical neurons. By blocking these channels, it is believed that ZNS may disrupt hypersynchronized neuronal firing and subsequent epileptic activity, thus limiting the spread of seizure discharges. No receptor binding studies were performed by the sponsor, but there have been reports in the literature of interactions with the GABA receptor complex (decreased binding of [3H]flunitrazepam and muscimol in rat brain synaptosomes). However, electrophysiological studies in cultured mouse spinal cord neurons found that ZNS had no effect on GABA- or glutamate-evoked postsynaptic responses.

ADME

PK parameters following single or multiple dose oral administration to rats are shown in Table II.1. ZNS was well absorbed and exhibited linear kinetics over this dose range (10-40 mg/kg). Pharmacokinetic parameters in dogs following single or repeated (6-7 days) oral administration are shown in Table II.2. Absorption was variable, with T_{max} ranging from 1-12 hr in individual dogs, and the decline of drug in the plasma and whole blood as a function of time was nonlinear at higher doses (30-100 mg/kg; slope of decline greater at low than at high concentrations), although there appeared to be a linear correlation between dose and AUC in both plasma and blood over the entire dose range. The cause for the nonlinear elimination in dogs was not determined in this study, but the possibilities of a saturable metabolic pathway or nonlinear uptake into tissues were discussed. Disproportionately high plasma drug levels were also seen at the HD (75 mg/kg po) in the 1-year dog toxicity study, and plasma concentrations never appeared to achieve steady-state (based on increasing C_{min}) over the course of the study (Table III.5).

After oral administration of a single dose of 14C-labeled drug (20 mg/kg), tissue radioactivity levels in rats were maximal at 3 hr, with the highest levels found in the blood, liver, kidneys, 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). Most of the radioactivity in plasma and erythrocytes was determined to be unchanged drug. Radioactivity had been almost completely excreted in the urine (87%) and feces (16%) by 48 hr (Table II.4). 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 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. Plasma protein binding in rats was 47.9% at 16 ug/ml in vitro and 46.2% at 14 ug/ml in vivo, and the percent bound to dog plasma protein in vitro was reported to be 40%.

In humans volunteers given single oral doses of 200 or 400 mg, peak plasma levels of 2.9 and 5.1 ug/ml, respectively, were seen at 5-6 hr. The apparent volume of distribution was 1.47 L/kg and plasma clearance was 15 ml/min after the 400 mg oral dose. With repeated administration of the maximum recommended dose of 200 mg BID to healthy volunteers (PK Study #11), the steady state plasma PK parameters were: C_{max}, 30.3 ug/ml; T_{max}, 2.1 hr; Cl/F, 10 ml/min; t_{1/2}, 68.6 hr; AUC(0-12), 339 ug.hr/ml. Binding to human plasma protein *in vitro* was reported to be 40% at plasma concentrations of 1-70 ug/ml. The therapeutic range of plasma levels in clinical trials was said to be 15-25 ug/ml, although adverse events have been associated with plasma concentrations >30 ug/ml. Following administration of radiolabeled ZNS (300 mg) to human volunteers, 62% of the dose was recovered in the urine over 9 days, while fecal excretion accounted for only 3%. All of the radioactivity in the plasma was determined to be unchanged drug, and approximately 15% of the total dose was recovered as unchanged drug (Biopharm review).

The disposition of ZNS may be complicated by saturable binding to erythrocytes. Because of their affinity for CA, various sulfonamides are known to concentrate in erythrocytes, and uptake of ZNS by RBCs was demonstrated in rat and human blood *in vitro* and in rats *in vivo*. The characteristics of ZNS uptake into RBCs were similar to those observed for other sulfonamide CA inhibitors, such as sulthiame and acetazolamide. Uptake was described by the summation of a linear process and a saturable process, and high and low affinity dissociation constants of 6 and 76 uM were calculated for ZNS in intact human erythrocytes; the high affinity (saturable) binding site is thought to represent CA, while binding to another protein(s) in RBCs is thought to account for the low affinity component. ZNS binds to erythrocytes with higher affinity than to plasma protein, which would account for the marked concentration of ZNS observed in RBCs in rats and humans. Because of the limited binding capacity of RBCs (about 450 umoles/liter), nonlinear pharmacokinetics would be expected over some concentration ranges. In one published human study (Wilensky et al, in *Metabolism of Antiepileptic Drugs*, edited by Levy et al, 1984), the median erythrocyte/plasma partition ratio varied from 18 at a plasma level of 0.14 ug/ml to 6 at a plasma level of 4.6 ug/ml. According to these authors, the high erythrocyte/plasma partition ratio and its concentration dependence would be expected to affect the drug's distribution between blood and tissues and result in a non-linear dose-plasma concentration relationship and/or variation in the volume of distribution at different doses. Published human studies (Wilensky et al, *Epilepsia* 26:212-220, 1984; Wagner et al, *Ther Drug Monit* 6:277-283, 1984) as well as PK studies conducted by the sponsor (see Biopharm review) have found evidence of nonlinear kinetic behavior at doses of 400-800 mg/day, with higher plasma levels achieved after chronic dosing than were predicted from single-dose studies. When administered in a multiple dose regimen to patients (200-600 mg bid), the steady-state plasma clearance of ZNS was less than 1/2 of that observed following administration of a single dose of 400 mg (Wagner et al, above). The sponsor reported decreases in plasma Vd/F following high single doses (800 mg) or after multiple therapeutic doses (300-400 mg/day), which was considered to reflect saturable binding to RBCs. Although the mechanism for the nonlinear behavior observed with ZNS is not certain, its occurrence in the therapeutic dose range, coupled with an apparently narrow therapeutic range, may indicate the need for careful dose individualization.

ZNS is extensively metabolized in all species examined. Metabolites identified by the sponsor in rats, dogs, monkeys and their proportions in urine are shown in Table II.5. 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). Recent studies by Stiff and Zemaitis (PK Reference 14) indicate that the *in vivo* phase I metabolism of ZNS in rats involves the P-450-mediated reductive cleavage of the N-O bond in the 1,2-benzisoxazole ring leading to the formation of the major metabolite, 2-(sulphamoyl)phenol (2-SMAP; metabolite IV in Fig II.1), and a minor metabolite, 2-[1-(amino)sulphamoyl]phenol (metabolite VII), which are then conjugated. A carboxylic acid derivative (metabolite III) and an N-acetyl derivative (metabolite I) were also identified in rats. Three unidentified metabolites accounted for 19% of urinary radioactivity. In dogs, the ring-opened derivative (metabolite IV) and a hydroxylated derivative (metabolite IX) as well as their glucuronide conjugates were identified by the sponsor. The glucuronide of the ring-opened derivative and the acetylated derivative were the major metabolites found by the sponsor in monkeys. When the anticonvulsant activity (MES test) of the ring-opened metabolite (2-SMAP) and the N-acetyl derivative was evaluated in mice (PKR20), neither showed any activity after oral or iv administration. Animal (rodent) studies indicated that metabolism is not subject to auto- or hetero-enzyme induction.

Urinary excretion studies indicate that a conjugate of ZNS, a glucuronide conjugate (metabolite VI in Fig II.1) of the ring-opened metabolite (metabolite IV), and N-acetylzonisamide (metabolite I) are formed in humans (Taylor et al, in *New Antiepileptic Drugs*, edited by Meldrum and Porter, 1986; Ito et al, *Arzneimittelforsch* 32:1581-1586,1982). After oral administration of 200 mg to volunteers, parent drug and the glucuronide conjugate of the N-O cleaved metabolite accounted for 28 and 6% of the administered dose after 9 days, with only trace amounts of N-acetylzonisamide reported (sponsors report PK 10). These data indicated that urinary excretion of unidentified metabolites and/or excretion via other routes also occurs. Other published studies have shown N-acetylzonisamide to account for about half of the unchanged drug, ie, to represent the major component, after oral administration of 300 mg to human volunteers (Woolf and Chang, *Pharm Res* 3(suppl):159S, 1986). It should be noted that significant percentages of administered radioactivity were also unaccounted for or unidentified in the experimental animal species, particularly dogs. In 21 human liver microsomal preparations, the major enzyme responsible for metabolizing ZNS to the ring-opened derivative (IV) was identified as a P450 3A species.

Toxicology

Acute

The acute lethal toxicity of ZNS in various species was determined and is shown in **Table III.1**. Similar clinical signs were observed in rats and mice following administration of zonisamide by various routes, including sedation, ataxia, loss of righting and corneal reflexes, hypothermia, respiratory depression, coma, and death. Oral administration of <300 mg/kg produced no effects on spontaneous behavior in rodents, but higher sublethal doses produced marked sedation within 1 hr that lasted for up to 3 days. Decreased abdominal tonus was observed in both species only after oral administration, and exophthalmus occurred only in mice. Dogs exhibited depression, emesis, staggered gait, ataxia, prone position, coma, respiratory depression, and loss of corneal reflex. 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. The absence of signs of hyperexcitability, as seen at high doses of other anticonvulsants such as DPT and CBZ, is noteworthy. With the exception of swelling and hemorrhage of the urinary bladder in rodents, pathology findings in dead animals appeared to be nonspecific.

Repeated Dose - Rat

In repeated dose oral toxicity studies in rats (20, 60, 200 or 600 mg/kg for 1 month; 10, 30, 100 or 300 mg/kg for 9 months; 2, 20, or 200 mg/kg for 12 months - all gavage), consistent findings were: decreased RBC parameters, neutrophilia and lymphopenia; increased urine volume and electrolyte (Na⁺) excretion; and clinical chemistry changes indicative of kidney and liver toxicity (small to moderate increases in BUN, bilirubin, cholesterol, inorganic phosphorus, total protein, ALP, ALT, and AST). With the exception of the increase in BUN, which occurred at doses as low as 2 mg/kg in the 1-year study, these changes were primarily seen at doses of 100 mg or greater. The cause of the slight anemia and other hematological effects was not determined, but these were not associated with any histopathological findings in the bone marrow or spleen. The elevated reticulocyte counts and serum bilirubin which accompanied the anemia in some or all of these studies could indicate hemolysis, however, which might result from the concentration of ZNS in erythrocytes. Although not seen in the above toxicity studies, the increased incidence of hemosiderin-laden macrophages in spleen in the rat carcinogenicity study also points to increased red cell destruction. The urinalysis changes were attributed to the drug's CA inhibitory action and the resultant effects on renal physiology, although there were no apparent effects on urine pH and HCO₃⁻ was not measured. In addition to the effects on urine output, low incidences of renal calculi were found in MHD and HD females in the 9 month study and in HD males (4/20) and females (1/20) in the 1 year study, and cell infiltration in the urinary bladder submucosa, which could indicate chronic irritation, was found only in HD males in the 1-year study. Elevated BUN was not accompanied by increases in creatinine in these studies (may indicate prerenal etiology, eg, secondary to diuresis), and while kidney weights tended to be increased at doses of 100 mg/kg or more, there were no clear histopathological correlates. Low incidences of regeneration of the renal tubules, focal fibrosis, calcification, and infiltration were found in treated females in the 1-month study (**Table III.2**). In the 1-year study, "yellow-

white dots" in the renal medulla were found in 2 MD and 1 HD females during gross examination, and an enlarged kidney was noted in 1 HD male with stones. The only apparent T-R microscopic findings in the kidneys, however, were an increased incidence of hyaline droplets in the renal tubule in HD males and an increase in the incidence of brown pigment deposition in the renal tubules of HD females (Table III.3). In the 2-year rat carcinogenicity study, incidences of hyperplasia of the pelvic epithelium and renal pelvic mineralization were increased somewhat in HD (80 mg/kg) rats compared to C (Table IV.8). Although increased liver weights accompanied the clinical chemistry changes noted above at doses of 100 mg/kg or more, there was no consistent histopathologic evidence of liver damage. In the 1-year study, bile duct proliferation appeared to be increased somewhat in treated animals (Table III.3), and the incidence of liver cell adenoma was increased slightly in HD males in the carcinogenicity study (Table IV.8).

There are no good toxicokinetic data for the rat studies. However, mean plasma drug levels determined 24 hr after the last dose in the 9-month study were 0.6, 3.5, 20.3, and 82.3 ug/ml in the C, LD, MLD, MHD, and HD groups, respectively, and brain levels at this time were 1.9, 5.3, 23.8, and 83.6 ug/g, respectively. Mean maternal plasma drug concentrations measured 4 hr postdose on GD 17 in a rat embryo-fetal development study were 64.3 and 110.7 ug/ml in dams receiving 80 and 200 mg/kg, respectively. Mean steady-state C_{max} values in volunteers given the proposed maximum daily dose of 400 mg for 35 days were 30.3 (BID) and 28.0 ug/ml (QD). These data would suggest that peak plasma levels at doses associated with toxicity in the chronic rat studies (ie, ≥ 100 mg/kg) were probably somewhat greater than those expected clinically.

Repeated-Dose - Dog

In an exploratory oral rising dose study 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. In a 2-month dog study (10, 30, or 100 mg/kg), D-R increases in liver weights were seen at all doses, but there were no clinical laboratory or pathology findings.

In the chronic oral dog study (10, 30, or 75 mg/kg in capsules for 1-year), there were no apparent electrocardiographic, ophthalmological, or hematological effects, but clinical chemistry analyses revealed mild but significant increases in alkaline phosphatase, ALT, and GGT activities and decreases in plasma albumin in HD dogs, and there were gross and microscopic findings in the urinary bladder and liver at the MD and HD. Congestion and/or mucosal thickening/nodularity of the urinary bladder was seen grossly in 3 HD males and 1 MD female (Table III.4). 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. Dark brown discoloration of the liver was noted macroscopically in all HD females, 3/5 HD males, and 1 MD male. There were no microscopic findings thought to correlate with the brown pigmentation; however, mild hepatocyte hypertrophy and vacuolization were also found in 2 of the HD dogs (1 M and 1 F) with dark brown discoloration as well as an additional HD male, and bile duct hyperplasia was found in 1 HD female and 1 MD male (no gross findings). 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. Only C and HD male livers were examined by EM and reversibility was not assessed. According to the sponsor, such findings, which may be referred to as concentric lamellar bodies, arrays, or whorls, have been reported with high doses of enzyme-inducing drugs such as PHB and hydantoins. But zonisamide does not appear to induce either its own metabolism or the metabolism of other drugs. It is not known whether the concentric membrane lamellae within hepatocytes were related to the discoloration of the liver noted grossly. There were said to be no other ultrastructural findings, such as abnormal pigment deposition, which might cause such discoloration. Hepatic discoloration has not been reported with other drugs causing similar membrane changes. The biochemical changes seen primarily in HD dogs of both sexes in this study (elevated liver enzymes and decreased plasma albumin) were probably related to the liver changes seen at necropsy, since plasma albumin is synthesized by the rough

endoplasmic reticulum in the liver, and alkaline phosphatase, ALT, and GGT are plasma markers for hepatic injury. At the HD, plasma drug concentrations increased over the course of the study, never appearing to achieve steady-state, and were disproportionately higher than LD and MD levels. Since the drug is extensively metabolized in the liver, this could also be a consequence of the hepatic effects observed.

Plasma levels 4 hr after dosing in the 2-month dog study 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 (combined sex means). Levels measured after the 14th dose were approximately 1.5 times those measured after the first dose but did not appear to increase thereafter. Plasma drug concentrations during the 1-year dog study are shown in Table III.5. Plasma levels 6 hr after dosing ranged from 6.3 to 11.8 ug/ml at the LD, 19.2 to 43.1 ug/ml at the MD, and 49.6 to 142.6 ug/ml at the HD. As noted above, HD plasma drug levels were disproportionately high and never appeared to achieve steady-state over the course of the study (C_{min} increased 35% between weeks 26 and 52). In a pharmacokinetic study in which dogs were given 7 daily oral doses of 100 mg/kg, C_{max} and AUC values of 172 ug/ml and 3653 ug.h/ml were determined (Table II.4). For comparison, the minimal effective plasma level in dogs against MES was said to be 12.6 ug/ml, and with repeated administration of the maximum recommended dose of 400 mg to human volunteers, the steady state plasma C_{max} was about 30 ug/ml and the AUC(0-12) was 339 ug.hr/ml.

Carcinogenicity

Mouse (BW figures and tumor incidence table attached in Appendices 1 and 2)

In the mouse (B6C3F1) carcinogenicity study (20, 40, or 80 mg/kg in the diet for 2 years), there were no treatment-related effects on survival, hematology parameters, clinical signs, ophthalmological findings, palpable masses, or histopathological findings. Small but statistically significant BW reductions in HD mice (mean BW 9 and 6% below C in M and F, respectively, at 104 weeks, Tables IV.2 and IV.3) were the only findings of any consequence in the study, making dose selection questionable. Doses were selected on the basis of a 3-month dose range-finding study (performed at the same lab and with the same mouse strain as the 2-year study) in which doses of 50, 100, 200, 400, and 800 mg/kg (in the diet) produced BW gain suppression of 2, 8, 11, 2, and 17% in females and 0, 10, 11, 13, and 19% in males, respectively, but no effect on food consumption. None of these doses produced any significant systemic toxicity and there were no T-R deaths. No T-R gross or microscopic findings were reported. According to the sponsor, regression analysis using the BW 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 mg/kg was chosen as the HD. However, as stated, this is an inappropriate criterion for estimating the MTD, which should produce about a 10% decrement in BW gain in the subchronic study but a 10% difference in final BW in the 2-year study. In addition, the BW changes in the 3-month study were really too inconsistent to form the basis for HD dose selection. The resulting HD effect was marginal, particularly in females. There are no toxicokinetic data in mice. It should be noted that, according to the study report submitted, development of ZNS was stopped by the original sponsor, Warner-Lambert, prior to completion of statistical data analysis, and there is no indication that the current sponsor analyzed the tumor incidence data. A data disc was submitted with the NDA, but has not yet been review by Biostatistics.

Rat (BW figures and tumor incidence table attached in Appendices 3 and 4)

The rat (Wistar) carcinogenicity study was conducted with the same doses used in the mouse study (20, 40, or 80 mg/kg in the diet for 2 years). There were no significant T-R differences in mortality rates, clinical signs, ocular changes, clinical pathology, or unalysis parameters; however, BW gain and food consumption were reduced by treatment, and terminal BWs were significantly lower in HD males (9%) and in MD (10%) and HD (16%) females compared to C (Tables IV.5 and IV.6). 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). The sponsor did not consider any of the gross or microscopic findings to be treatment-related; however, incidences of several non-neoplastic histopathological findings appeared to be increased in treated animals, including hemosiderin laden macrophages in the spleen, renal pelvic epithelial hyperplasia, mineralization of the renal pelvis, testicular mineralization and interstitial cell hyperplasia in males, and uterine

cystic endometrial hyperplasia in females (Table IV.7). There were no statistically significant increases in tumor incidence in the sponsor's (W-L) analysis (pairwise and trend test analysis, 1% significance level), but incidences of several tumor types were increased slightly in drug-treated groups, including testicular interstitial cell tumors, liver cell adenoma, osteosarcoma, and glioma in males, and uterine adenocarcinoma and thyroid C-cell adenoma in females (Table IV.8). (The combined incidences of thyroid C-cell hyperplasia and adenoma were similar between HD and C females).

Doses were said to be based on the results of only a 4-week dose range-finding study (Wistar rats) in which doses of 50, 100, 200, 300, and 600 mg/kg (diet) produced BW gain suppression of 3, 12, 29, 44, and 83% in males and 2, 23, 27, 47, and 103% in females, respectively. D-R suppression of food consumption also occurred in males (3 to 51%) and females (4 to 54%). No deaths, clinical signs, or clinical laboratory or pathological changes were reported in that study, although liver weights were increased in HD males and in females receiving 200 mg/kg or greater. Comparable reductions in BW gain and food consumption were seen in a 4-week oral gavage study (in S-D rats), indicating that these effects are at least partially independent of mode of administration (ie, weight gain suppression not due to extrinsic factors, such as poor palatability). Despite the short duration of the dose range-finding study, it appears the prospective dose selection was appropriate and that the definitive study can be considered acceptable based on the BW changes. There are no toxicokinetic data from this study, but in the dose range-finding study, mean plasma levels measured in samples collected at termination were 14.5, 28.7, 49.0, 69.0, and 141.4 ug/ml in groups receiving 50, 100, 200, 300, and 600 mg/kg, respectively (male and female combined, no sex difference seen). Although it is not known where on the concentration-time curve the samples were taken, these data indicate that plasma concentrations at the HD in the 2-year study may have been lower than those expected in humans at the maximum recommended dose (steady state C_{max}=30 ug/ml at 400 mg/day in volunteers; >40 ug/ml measured in patients receiving 400 mg/day during controlled trials).

Genotoxicity

Zonisamide was negative for genotoxicity in the Ames test, mouse lymphoma assay, sister chromatid exchange test, and in vitro (human lymphocytes) and in vivo (rat bone marrow) cytogenetics assays. However, in a forward mutation (HGPRT locus) assay in cultured V79 Chinese hamster lung cells, mutant frequencies were significantly increased compared to vehicle controls in the absence of metabolic activation (Table V.1). Because there was no dose-response trend, the sponsor considered the results negative; but it appeared that the failure to see a dose-response trend may have been due to the narrow dose range used (1000-1400 ug/ml) as well as the high response variability, so the study should probably be considered positive. Mutant frequencies were not significantly different from controls in the activation phase of this test.

Reproductive Toxicology

Fertility and Reproductive Performance

In an oral (gavage) male and female rat fertility study (20, 60, or 200 mg/kg prior to and during mating to GD 7 in females), reproductive (D-R decrease in numbers of corpora lutea, implantation, and live fetuses; Table VI.1) and maternal toxicity (decreased BW gain) were observed at all doses (both statistically significant at the MD and HD). Clinical signs and irregular estrus cycles were seen in HD females, but there were no effects on mating or fertility indices. There was no effect on reproductive organ weights. Neither histopathological evaluation of reproductive organs nor sperm analysis were performed. An earlier fertility study with dietary administration (25, 50, or 100 mg/kg prior to and during mating to GD 20 in females) also found decreases in corpora lutea, implantations, and live fetuses at all doses, although the effects were not clearly D-R, possibly because of the mode of administration. Developmental toxicity (persistent growth retardation at all dose) was also seen in this study. While it appears that ZNS has an adverse effect on the process of ovulation, additional studies would be required to identify the cause of changes in the apical reproductive endpoints evaluated in these studies, since they could reflect effects at a number of points in the reproductive process.

Embryo-Fetal Development - Mouse

In a mouse embryo-fetal development study (125, 250, or 500 mg/kg by gavage on GDs 6 to 15), decreased fetal weights, increased embryo-fetal death, and increased incidences of malformations and variations were found in treatment group litters, primarily at the HD, where effects on BW and malformation incidences reached statistical significance (Tables VI.2- VI.3). Abnormalities observed more frequently in HD fetuses included cleft palate, open eye, dilated brain ventricles, dilated renal pelvis, vertebral and rib malformations, cervical ribs, and decreased skeletal ossification. Incidences of craniofacial defects (cleft palate, open eye) and skeletal variations were dose-dependently increased at all doses (fetal/litter incidences of craniofacial defects: 1.4/17, 3.3/22, 3.5/33, and 7.3/46% in C, LD, MD, HD, respectively), however, so there was no clear no-effect level. Although craniofacial malformations are the most common spontaneously occurring defects in mice, the fact that their incidences can be increased by various teratogens is well documented. Incidences at the LD were above the historical control range in ICR mice. Significant maternal toxicity (decreased BW gain, clinical signs) was seen at the HD in this study.

Embryo-Fetal Development - Rat

In a rat embryo-fetal development study (20, 60, or 200 mg/kg by oral gavage on GDs 7 to 17), fetal BW was decreased, the number of retarded fetuses was increased, and incidences of total visceral defects (8.2, 9.3, 15.3, and 22.8% of fetuses examined in C, LD, MD, and HD litters, respectively), ventricular septal defects (0.5, 1.5, 2.8, and 4.4%, respectively) and persistence of cords of thymic tissue (3.8, 4.6, 9.6, and 16.1%, respectively) were dose-dependently increased in treated litters (Table VI.4). No increase in external or skeletal defects was observed, but retarded skeletal ossification (parietal bone, sternbrae, cervical, thoracic, and caudal vertebrae, phalanges, metatarsals) was also seen in exposed fetuses. Statistical significance was reached at the HD for all of these effects and at the MD for effects on fetal BW and ossification, but there was not a clear no effect dose. Incidences of quadricuspid pulmonary valve and persistent right azygos vein were increased only in HD fetuses. In litters allowed to deliver, BW was significantly decreased in HD females at birth as well as during the postweaning period (9% below C at 6 weeks), and the incidence of persistence of cords of thymic tissue remained significantly increased in the HD group when offspring were autopsied at 6 weeks. Animals autopsied at later times (11-17 weeks) had lower incidences of this structural variant. Evidence of maternal toxicity (decreased BW gain during gestation, clinical signs, decreased organ weights) was seen at the MD and HD in this study. The significance of persistent cords of thymic tissue is uncertain. This is a common finding, with average frequencies of 5-10% in the strain of rats used in this study, so its classification as a malformation is questionable. Thymic remnant is usually considered a variation, and its increased incidence probably reflects treatment-related developmental delay. Like increases in skeletal variations, T-R increases in the frequencies of such visceral anomalies can be an indication that the embryotoxic range of dosage is being approached. This finding would not be expected to have any long-term consequences, however. The dose-dependent increase in cardiovascular defects observed in ZNS-exposed fetuses is of greater concern (see dog results below). These are also a fairly common spontaneous finding in rats, but incidences in this study were increased compared to both concurrent and historical controls. The historical control range for VSD is about 0-3% of fetuses in Japanese studies using Jcl:SD rats during the period of the present study. A second rat embryo-fetal development study (20, 40, 80, or 200 mg/kg by gavage on GDs 7-17) found decreased fetal weights and increased incidences of abnormalities (persistence of cords of thymic tissue, retarded ossification) at 80 mg/kg or greater, however, the fetal incidences of VSD were similar between the HD (4.2%) and C (3.8%) groups. Mean maternal plasma drug concentrations measured 4 hr postdose on GD 17 were 64.3 and 110.7 ug/ml in dams receiving 80 and 200 mg/kg, respectively.

Embryo-Fetal Development - Dog

An early (78) study in beagle dogs (10, 30, 60 mg/kg orally in capsules on GDs 14 to 35; 6-7 dogs/grp) found increased fetal mortality, decreased fetal weights, and increased incidences of external, visceral, and skeletal malformations and skeletal variations among ZNS-exposed offspring (Tables VI.5-VI.7). These effects were marked (and statistically significant) at the HD; malformations increased in frequency at the HD included

ventricular septal defect, various aortic anomalies, valvular defects, transposition of the great vessels, hypoplasia/dysplasia of the thymus and spleen, tail anomalies, and fusion an/or deformity of the caudal vertebrae. Incidences of cardiovascular malformations (particularly VSD) and skeletal variations also appeared to be dose-dependently increased at the MD; and apparent effects on growth and viability were seen at all doses, although the LD and MD effects could have been secondary to increased litter sizes. Maternal BW gain was significantly decreased throughout gestation in the HD group (34% below C). Mean maternal plasma drug concentrations measured 4 hr postdose were 12.8, 25.3, and 43.8 ug/ml on GD 14 and 11.6, 44.5, and 78.5 ug/ml on GD 35 in LD, MD, and HD females, respectively. Fetal drug levels measured in 3 MD litters at 4 hr after dosing showed that fetal and maternal exposures were equivalent. The threshold plasma level for anti-MES activity in beagle dogs was 12.6 ug/ml, and the human therapeutic range is reportedly 15-25 ug/ml. Dogs are no longer commonly used in teratology studies (seasonal breeders, fiscal deterrent), and there is little information available on the occurrence of spontaneous malformations in this species. But despite this and the relatively small Ns, there is little question about the teratogenicity of ZNS in this study because of the high frequencies of specific malformations (18/40 HD fetuses had VSD vs 0/44 in C). The apparent sensitivity of dogs to the teratogenic effects of ZNS could be intrinsic or related to differences in placental transfer, pharmacokinetics, or metabolism. Dogs seem to metabolize the drug differently than rats or monkeys (see Table II.5, Fig II.1), but the data are incomplete and certainly inadequate to assess the comparability of metabolism between dogs and humans.

Embryo-Fetal Development - Monkey

In a monkey embryo-fetal development study (10 or 20 mg/kg by oral gavage on GD 21-45; 4C, 7 LD, 8HD), dose-related embryo-fetal death (abortion, death at C-section) was observed at both doses (1/7 LD and 4/8 HD fetuses lost), but no malformations were reported (only limited fetal evaluations were performed). Intrauterine death has been shown to be somewhat more prevalent than malformations in monkeys (and probably humans) at embryotoxic dose levels, but it is also possible that malformations were masked by embryoletality. Maternal BW gain was D-D decreased during treatment and T-R vomiting was seen at both doses. Maternal plasma drug levels were 5.2 and 13.2 ug/ml at 4 hr and 1.6 and 5.0 ug/ml at 24 hr after the first dose on days 21 in the LD and HD groups, respectively. After the last dose on day 45, mean plasma levels were 10.7 and 21.9 ug/ml at 4 hr (ie, at or below human therapeutic levels) and 4.0 and 8.7 ug/ml at 24 hr in the LD and HD group animals, respectively. There was no clear relationship between maternal plasma levels and embryofetal death in individual animals, but levels were not highly variable.

Pre- and Postnatal Development

In a pre-/postnatal study in rats (10, 30, or 60 mg/kg by oral gavage from GD 17 through PND 20), the number of perinatal deaths was increased among HD offspring and testes descent and emergence of walking were slightly delayed. There were no apparent effects on learning or reproductive performance in exposed offspring, however. Maternal BW gain was decreased during the gestational treatment period in HD dams.

Conclusions

The animal toxicology data indicate that ZNS has the potential to induce hematological, renal, and hepatic effects, based on clinical pathology findings, increased organ weights, and gross and ultrastructural (dogs) pathological changes, at doses (LOEL: 100 mg/kg in rats, 30 mg/kg in dogs) somewhat higher (~2.5X) than those used clinically (MRDD = 400 mg/day) on a mg/m² basis. The limited toxicokinetic data suggest that plasma drug levels associated with these doses in animals may be closer to those expected in humans (peak levels of 19.2 to 43.1 ug/ml measured in dogs at 30 mg/kg; therapeutic range in clinical trials said to be 15-25 ug/ml). However, evidence of toxicity was not pronounced even at the highest doses administered in the chronic rat (300 mg/kg) and dog (75 mg/kg) studies. The significance of liver findings in the 1-year dog study is unclear, though, and may warrant further investigation. Ultrastructural changes similar to those described in ZNS-treated dogs (concentric lamellar bodies in the cytoplasm of hepatocytes) have been observed in various species after administration of drugs that increase smooth endoplasmic reticulum formation and induce mixed function oxidase activity, such as phenobarbital and thiohydantoin, but are also reportedly

produced by hepatotoxic concentrations of such agents as polybrominated biphenyl, ethanol, and furosemide (Herdson et al, Lab Invest 13:1032-37,1964; Massey et al, Toxicology 43:149-60, 1987). ZNS did not exhibit hepatic enzyme inducing effects in mice or rats, and toxicokinetic data indicate that ZNS did not induce its own elimination in dogs. In fact, plasma drug levels tended to increase over the course of the 1-year dog study at the HD of 75 mg/kg. The apparent failure to reach steady-state at this dose was considered a possible consequence of the hepatic effects of treatment. Although it has not been determined that the liver ultrastructural changes are a species-specific effect of ZNS, since the liver was not examined at this level in rats, there appear to be significant species differences in metabolism (see Table II.5 and Fig II.1), which could play a role in these effects. Due to the gaps in the cross-species metabolism data, however, additional work would be needed to assess human relevance from this standpoint. Only C and HD male livers were examined by EM in the dog study, so a no effect level was not established (all HD males affected); and reversibility was not assessed. The relationship between ultrastructural changes and the discoloration of the liver noted grossly (≥ 30 mg/kg), which was not associated with microscopic evidence of abnormal pigment deposition, is uncertain. Because they seem to be unusual findings, the liver changes in dogs should probably be described in a Preclinical Toxicology section of the label.

Several of the rat findings (urinalysis changes, calculus formation, possibly 1 BUN) presumably resulted from the carbonic anhydrase inhibitory action of ZNS, although such a mechanism has not been established. These effects were not marked compared to those seen with other CA inhibitors, however, even weak ones like topiramate. There was no evidence of metabolic acidosis, urinary pH was not affected, the incidence of calculus formation was low, and none of the gi, renal, and urinary tract histopathological changes associated with administration of other CA inhibitors (including topiramate) to rodents were seen with ZNS. Small increases in the incidence of kidney stones have been reported in patients receiving ZNS in clinical trials, however (proposed label). While it has been shown to inhibit CA in vitro and in vivo, ZNS was generally much less potent than acetazolamide (~10-1000-fold, depending on the species, CA isozyme, and assay system employed). But because ZNS reaches the brain better than AZA, relative effects there may be greater than predicted in vitro (Hammond et al, Gen Pharmac 18:3,303-7,1987). CNS side effects qualitatively more similar to those associated with sulfonamides than with standard AEDs like DPH and CBZ have been reported in clinical trials with ZNS, with impairment of speech and higher mental function occurring at doses below those causing ataxia (Wilensky et al, Epilepsia 26:212-220,1985). Cognitive dysfunction has also been reported clinically with topiramate. Such effects would not be apparent in preclinical studies without specific neurotoxicological (behavioral) testing.

Although slight anemia and other hematological changes were seen in rats, there was no histopathological evidence of bone marrow suppression. Small T-R variations in hematological values are common in preclinical toxicity studies and are often attributed to physiological changes associated with exposure to high doses of drugs, eg, alterations in fluid and electrolyte balance or in food consumption and utilization. However, there was some indication of increased red cell destruction in the chronic rat studies (elevated serum bilirubin, reticulocytosis, increased incidence of hemosiderin-laden macrophages in spleen), which might result from the concentration of ZNS in erythrocytes due to CA binding. Serious hematologic toxicity has been reported with therapeutic use of acetazolamide and other CA inhibitors, as with sulfonamide use in general; however, the dyscrasias associated with these drugs are thought to usually represent idiosyncratic reactions for which preclinical studies have poor predictive value.

Testing in rodents did not reveal any evidence of carcinogenic potential, but the HDs used in the 2-year studies were marginal, particularly in mice (1- and 2-fold the MRHD on a mg/m² basis in mice and rats, respectively). Genotoxicity test results were generally negative (Ames test, mouse lymphoma assay, sister chromatid exchange test, human lymphocyte cytogenetics assay, and rat bone marrow cytogenetics), although an equivocal increase in mutant frequencies was seen in a forward mutation (HGPRT locus) assay in cultured V79 Chinese hamster lung cells in the absence of metabolic activation (treatment means significantly different from controls but no dose response trend).

Of greatest concern among the preclinical findings are the results of reproductive/developmental toxicity testing. ZNS demonstrated teratogenicity and/or embryoletality in all species tested, including mice, rats,

dogs, and monkeys; and in all species, effective doses in animals (LOELs: 125, 20, 30, and 10 mg/kg, respectively) were similar to or lower than (0.5 - 2.5-fold) human therapeutic doses (MRHD: 400 mg/day) on a mg/m² basis. Peak plasma ZNS levels measured in pregnant dogs receiving teratogenic doses (25 and 44 ug/ml) and in monkeys receiving embryo-lethal doses (5 and 13 ug/ml) were similar to or below the highest levels measured in humans at the MRHD (~40 ug/ml). Effects on reproductive performance were also found in rats at clinically relevant doses. A variety of external, visceral, and skeletal malformations was produced in rodents and dogs following exposure to the drug during organogenesis, but cardiovascular defects were prominent in both rats and dogs, particularly the latter (cardiovascular malformations were found in about 1/2 of fetuses exposed to the high dose in dogs). Limb reduction defects typically associated with prenatal exposure to CA inhibitors in rodents were not seen, however. Although no malformations were reported in monkeys, embryo-lethality (abortion, fetal death at C-section) was observed at both doses tested (50% at HD). Intrauterine death has been shown to be somewhat more prevalent than malformations in monkeys at embryotoxic ranges of dosage (Wilson, Fed Proc 30:104-9, 1971). Alternatively, teratogenicity may have been masked by embryo-lethality in this species. Retrospective studies have shown that concordance between developmental toxicity in laboratory animals and humans is strongest when there are positive data from more than one test species, although even in this case the results cannot be used to extrapolate specific types of effects across species; and quantitatively, humans tend to be more sensitive to developmental toxicants than the most sensitive test species (Rogers and Kavlock, Developmental Toxicity, in Hayes (ed): Principles and Methods of Toxicology, 1994). The ZNS findings clearly indicate a significant potential for teratogenicity under the conditions of therapeutic use, and this should be conveyed in the labeling.

Labeling

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LABELING

2 pages
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LABELING

VIII. RECOMMENDATIONS

The NDA is approvable with respect to the pharmacology/toxicology portion. Recommendations concerning the proposed labeling are made in the Summary and Evaluation section of the review.

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cc:
NDA (20-789)
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/S/ 2/5/98

/S/
E. Fisher, Ph.D.

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Appendix 1

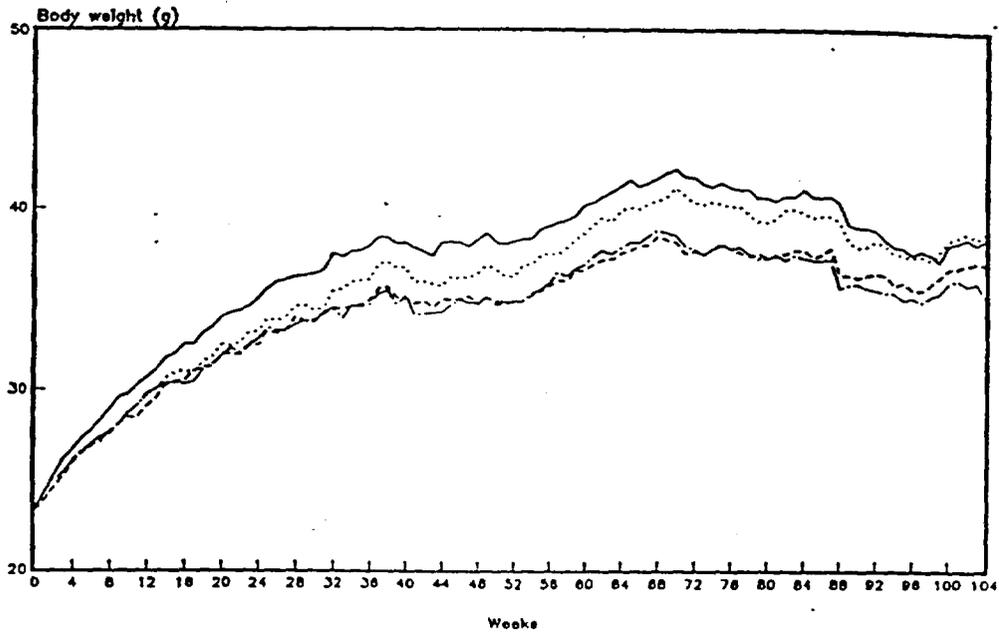
Carcinogenicity study in the mouse - Mean body weight - Males

GROUP 1
(0 mg/kg)

GROUP 2
(20 mg/kg)

GROUP 3
(40 mg/kg)

GROUP 4
(80 mg/kg)



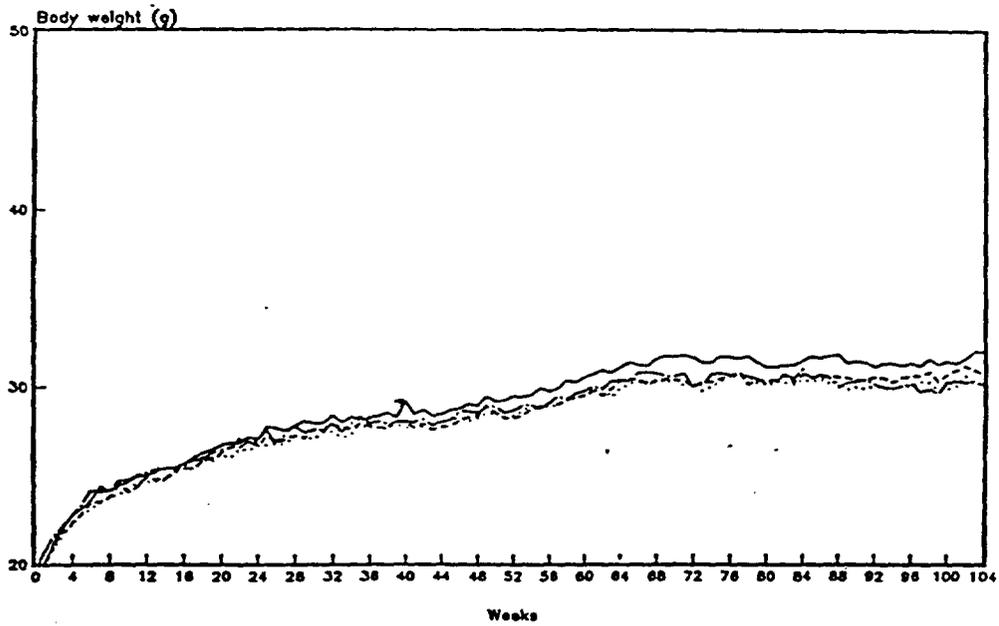
Carcinogenicity study in the mouse - Mean body weight - Females

GROUP 1
(0 mg/kg)

GROUP 2
(20 mg/kg)

GROUP 3
(40 mg/kg)

GROUP 4
(80 mg/kg)



Appendix 2

Carcinogenesis Bioassay in Mice Summary of Primary Neoplasms
(RR-745-01269)

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
LUNG								
Adenoma	8	4	4	2	4	1	3	2
Carcinoma	1	2	1	1	1		1	1
HARDERIAN GLAND								
Cystadenoma	5	3	4	1	2	3	3	1
Cystadenomacarcinoma					1			1
LIVER								
Adenoma	8		4	2	4		6	2
Carcinoma	5	2	7		6		4	1
Hemangioma	1			1				
Hemangiosarcoma						1	1	
PITUITARY								
Adenoma		2	1	7	1	4		3
ADRENAL								
Adenoma	5	1	7		2		2	1
Carcinoma		1						
Pheochromocytoma				1		1		
Unilateral ganglioneuroma		1						
LYMPHORETICULAR								
Histiocytic lymphoma					1		1	1
Mixed lymphoma	4	8	2	4		4	2	8
Granulocytic leukemia						1		1
Lymphocytic Lymphoma	1	5		4	1	8	1	6
Reticulum cell sarcoma		2		2				1
EPIDIDYMIDES								
Leiomyosarcoma			1					
TESTIS								
Leydig cell tumor	1		1		1			
UTERUS								
Endometrial Polyp		2		2		1		
Endometrial Sarcoma						2		1

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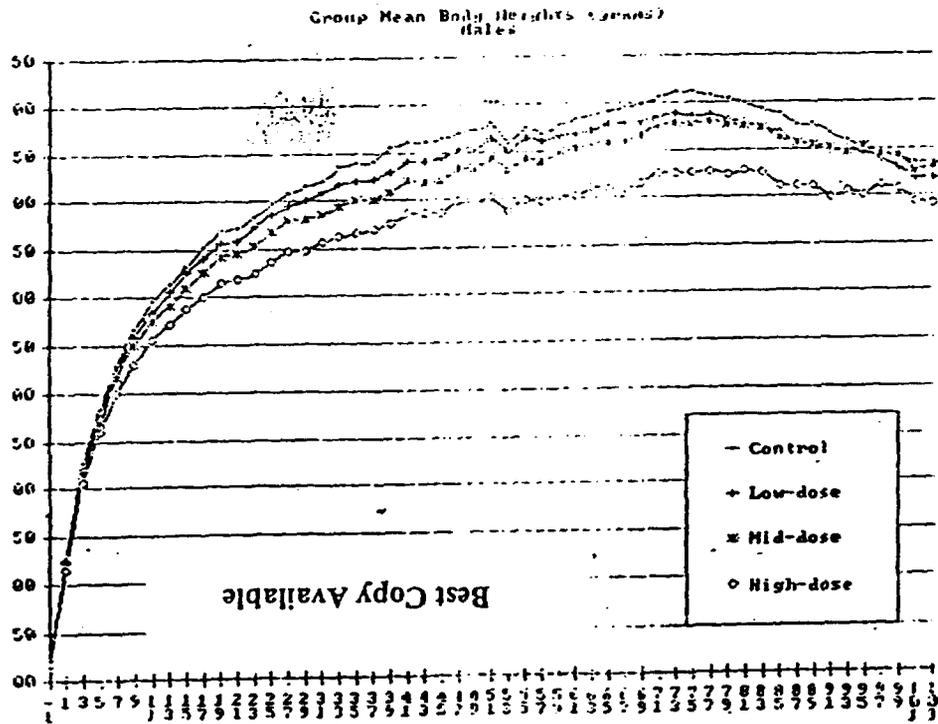
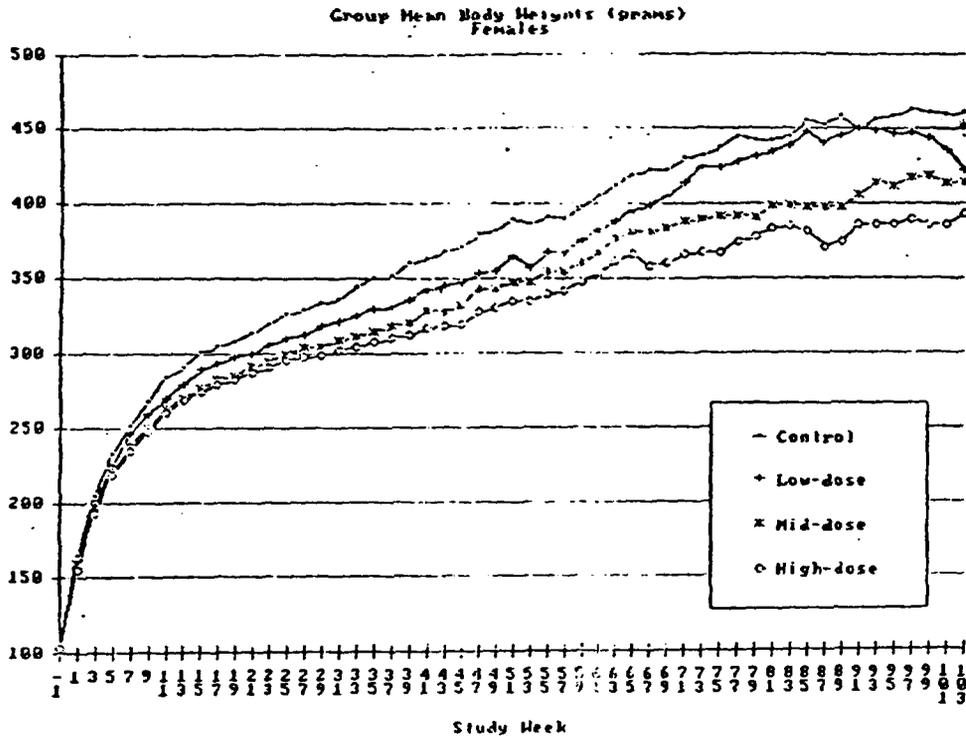
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Carcinogenesis Bioassay in Mice Summary of Primary Neoplasms
(RR-745-01269)
(Continued)

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
<u>Ovary</u>								
Teratoma		1						
Luteoma		1						
Mixed tumor								1
<u>Vagina</u>								
Leiomyosarcoma		1						
<u>Jejunum</u>								
Adenocarcinoma							1	
<u>Colon</u>								
Leiomyoma				1				
<u>Rectum</u>								
Liposarcoma			1					
<u>Mesenteric Lymph Node</u>								
Hemangioma				1	1		1	
Histiocytoma					1			
<u>Spleen</u>								
Hemangioma							1	
<u>Mammary Gland</u>								
Adenoma				1				
Adenocarcinoma		2		2				1
<u>Skin</u>								
Sebaceous adenoma				1		2		
Basal cell tumor		1						
Sarcoma			1					
Adenocarcinoma								1
<u>Vertebral Column</u>								
Sarcoma			1					

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Appendix 4 Two-Year Carcinogenicity Study of CI-912 in Rats Summary of Primary Neoplasms (RR-745-01064)

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
THYMUS								
Thymoma	1	1	1				1	
JEJUNUM								
Adenocarcinoma	1						1	
Leiomyosarcoma					1			
CAECUM								
Adenomatous polyp					2		2	
MESENTERIC LYMPH NODE								
Hemangioma	1			1	1	2	1	
Angioma						2		
Hemangiosarcoma				1				
LIVER								
Hepatocellular adenoma		2	1	3		1	3	2
Hepatocellular carcinoma	1		2		2	1		
Cholangiocarcinoma					1			1
Hemangioma					1			
SPLEEN								
Hemangioma			1	1				
Hemangiosarcoma	1		1					
PANCREAS								
Islet cell adenoma	1	1		4	2		1	2
Acinar cell adenoma	2		1					
Islet cell carcinoma		2		1				
Acinar cell carcinoma				1				
KIDNEYS								
Adenoma	1							1

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Two-Year Carcinogenicity Study of CI-912 in Rats Summary of Primary
Neoplasms (RR-745-01064)
(Continued)

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
ADRENALS								
Cortical adenoma	2	2			1	1	1	
Cortical adenocarcinoma		1						
Benign pheochromocytoma	8	1	8	1	7		1	1
Malignant pheochromocytoma	1		1		2			
Ganglioneuroma				1				
PITUITARY								
Adenoma	13	34	19	27	18	32	17	30
BRAIN								
Glioma	1			1			3	1
Granular cell tumor	1		1		1	1	1	1
SKIN								
Skin								
Papilloma				1				
Squamous cell carcinoma			2		1		1	
SUBCUTANEOUS TISSUE								
Fibroma	6	1	2		1	1	1	1
Fibrosarcoma	1	1				1	1	
Sarcoma		1	1		2			
Histiocytic sarcoma	1						1	
Hemangioma	1							
Hemangiosarcoma	1				1			
Hemangiopericytoma	1							
Lipoma			1	2	2			1
MAMMARY GLAND								
Fibroadenoma	2	14	2	9	1	12		14
Adenoma		2		1		1		
Adenocarcinoma		2		1		2		1
Papillary cystadenoma						1		
Papillary adenocarcinoma						3		1
Carcinoma				1				

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**Two-Year Carcinogenicity Study of CI-912 in Rats Summary of Primary
Neoplasms (RR-745-01064)
(Continued)**

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
OPTIC NERVES								
Meningeal sarcoma			1					
THYROIDS								
Follicular adenoma	3	1	7		3	2	1	
Follicular carcinoma	1	2		1	1			2
C-cell adenoma	3	4	3	4	5	4	1	9
Medullary carcinoma						1	1	
PARATHYROIDS								
Adenoma	1		1		2	3	1	
TESTES								
Benign interstitial cell tumor	3		5		8		7	
OVARIES								
Benign granulosa cell tumor						1		
Benign granulosa theca cell tumor		1						
Malignant granulosa theca cell tumor		1						
Undifferentiated sarcoma				1				
Adenocarcinoma		1						
UTERUS								
Adenocarcinoma		2		3		5		5
Squamous cell carcinoma				1				
Sarcoma		1						
Polyp		5		3		2		5
CERVIX								
Adenocarcinoma				1				1
Squamous cell carcinoma				1				
Sarcoma		2		3		1		
Polyp		1						

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Appendix 4 continued

**Two-Year Carcinogenicity Study of CI-912 in Rats Summary of Primary
Neoplasms (RR-745-01064)
(Continued)**

	GROUP I 0 mg/kg		GROUP II 20 mg/kg		GROUP III 40 mg/kg		GROUP IV 80 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Number of Animals	50	50	50	50	50	50	50	50
<u>YAGINA</u>								
Sarcoma		1		1				1
Squamous cell carcinoma						1		
Polyp		1						
<u>MUSCLE</u>								
Rhabdomyosarcoma	1							
Hemangiosarcoma								1
<u>NASAL REGION</u>								
Osteosarcoma							1	
Squamous cell carcinoma					1			
<u>RIGHT HIND LIMB</u>								
Osteosarcoma							1	
<u>LYMPH NODES</u>								
Hemangioma					2			

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Executive CAC
1/27/98

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Ken Hastings, Ph.D., HFD-590, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader
Ed Fisher, Ph.D., HFD-120, Presenting Reviewer

Author of Draft: Ed Fisher

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 20-789
Drug Name: Zonisamide
Sponsor: Dainippon

Mouse Carcinogenicity Study

In the mouse (B6C3F1) carcinogenicity study (20, 40, or 80 mg/kg in the diet for 2 years), there were no treatment-related effects on survival, hematology parameters, clinical signs, ophthalmological findings, palpable masses, or histopathological findings. Decreased BW gain in MD and HD males and all treated females (sustained over most of study) and terminal BW deficits in HD mice (mean BW 9 and 6% below C in M and F, respectively, at 104 weeks) were the only findings of any consequence in the study. Doses were selected on the basis of a 3-month dose range-finding study (performed at the same lab and with the same mouse strain as the 2-year study) in which doses of 50, 100, 200, 400, and 800 mg/kg (in the diet) produced BW gain suppression of 2, 8, 11, 2, and 17% in females and 0, 10, 11, 13, and 19% in males, respectively, but no effect on food consumption. None of these doses produced any significant systemic toxicity and there were no T-R deaths. No T-R gross or microscopic findings were reported. There were no toxicokinetic data in mice. According to the sponsor, regression analysis using the BW data from the 13-week study indicated that "a dose of 100 mg/kg or higher might be expected to result in body weight gain suppression in excess of 10% in the tumorigenicity assay," so 80 mg/kg was chosen as the HD. The reviewer questioned the dose selection because, as stated, this is an inappropriate criterion for estimating the MTD, which should produce no more than a 10% decrement in BW gain in the subchronic study but would be expected to produce about a 10% difference in final BW in the 2-year study; and because the BW changes in the 3-month study were thought to be too inconsistent to form the basis for HD dose selection. The resulting HD effect was considered marginal in females.

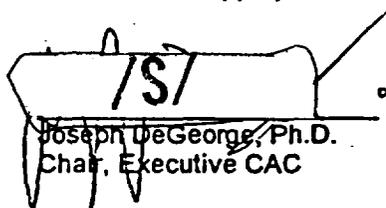
Rat Carcinogenicity Study or Rat Dose Selection

The rat (Wistar) carcinogenicity study was conducted with the same doses used in the mouse study (20, 40, or 80 mg/kg in the diet for 2 years). There were no significant T-R differences in mortality rates, clinical signs, ocular changes, clinical pathology, or urinalysis parameters; however, there was a sustained effect on BW gain in HD males and MD and HD females that resulted in terminal BWs in these groups that were 9%, 10%, and 16% below C, respectively. Parallel reductions in food consumption were seen. Relative spleen weights were decreased in males at all doses and in MD and HD females. The sponsor did not consider any of the gross or microscopic findings to be treatment-related, although incidences of several non-neoplastic histopathological findings appeared to be increased in treated animals, including hemosiderin laden macrophages in the spleen, renal pelvic epithelial hyperplasia, mineralization of the renal pelvis, testicular mineralization and interstitial cell hyperplasia in males, and uterine cystic endometrial hyperplasia in females. There were no statistically significant increases in tumor incidence in the sponsor's (W-L) analysis (pairwise and trend test analysis, 1% significance level), and while incidences of several tumor types were increased slightly in drug-treated groups, including testicular interstitial cell tumors, liver cell adenoma, osteosarcoma, and glioma in males, and uterine adenocarcinoma and thyroid C-cell adenoma in females, none of these changes were considered meaningful.

Doses were based on the results of a 4-week dose range-finding study (Wistar rats) in which doses of 50, 100, 200, 300, and 600 mg/kg (diet) produced BW gain suppression of 3, 12, 29, 44, and 83% in males and 2, 23, 27, 47, and 103% in females, respectively. D-R suppression of food consumption also occurred in males (3 to 51%) and females (4 to 54%). No deaths, clinical signs, or clinical laboratory or pathological changes were reported in that study, although liver weights were increased in HD males and in females receiving 200 mg/kg or greater. There are no toxicokinetic data from this study, but in the dose range-finding study, mean plasma levels measured in samples collected at termination were 14.5, 28.7, 49.0, 69.0, and 141.4 ug/ml in groups receiving 50, 100, 200, 300, and 600 mg/kg, respectively (male and female combined, no sex difference seen). Although it is not know where on the concentration-time curve the samples were taken, these date indicate that plasma concentrations at the HD in the 2-year study may have been lower than those expected in humans at the maximum recommended dose (Cmax=30 ug/ml at 400 mg/day). Despite the short duration of the dose range-finding study, the reviewer considered the dose selection appropriate and the definitive study acceptable based on the BW effects.

Executive CAC Recommendations and Conclusions:

The CAC agreed that there was no evidence of tumorigenicity in either study. While it was agreed that the HD was marginal in female mice, it was considered to be within 1/2-1/3 of what would have been recommended based on the dose range-finding study; so the mouse study was deemed acceptable. Palatability concerns were raised for the rat study (ie, that weight gain suppression may have been secondary to decreased food consumption), but additional data showed that reductions in BW gain and food consumption were comparable between a 4-week oral gavage study and the 4-week dietary dose range-finding study, indicating that these effects are independent of mode of administration. It was therefore concluded that the prospective dose selection was appropriate and that the rat study was acceptable.

 /S/ 2/6/18
Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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