findings identified by the sponsor achieved statistical significance (cf. Addendum B).

Conclusions: The study in rats was negative, i.e., no drug-related increases in neoplastic findings were observed in either males or females. The adequacy of dosing was demonstrated only by reductions in body weight in HD animals (10 and 19% in HDM and HDF, respectively) as compared to controls. Although this is adequate justification for limiting the high dose (if not due to unpalatability of the drug-diet admixture), it is unfortunate that other dose-limiting toxicities were not observed since the primary reason for limiting body weight effects is to avoid a loss in sensitivity due to the potential protective effects of body weight "restriction" on spontaneous tumor rates. Regarding the palatability issue, it is unlikely that the reduced body wts (compared to C grps) in HD animals was due to unpalatability of the diet. In the chronic (6-mo) study in rats dosing was by gavage and body wts were reduced in males at all doses (17, 31, and 35% at 10, 40, and 200 mg/kg, respectively); in females, however, body wt was slightly elevated at the LD (i.e., 10 mg/kg), but reduced by 6 and 9% at the MD and HD). Although body wt was not as affected in females, it is unlikely that there is a sex difference in palatability. Unfortunately, a different strain of rats was used in the 6-mo study (Sprague-Dawley instead of Long-Evans) and, therefore, the studies may not be entirely comparable.

REPRODUCTION

The following reproductive toxicity studies were conducted: Segment I study in Sprague-Dawley rats, Segment II studies in Sprague-Dawley rat and New Zealand White rabbit (3 studies), Segment III study in Sprague-Dawley rat. In addition, maternal toxicity was assessed in studies in Sprague-Dawley rat and New Zealand White rabbit. [Additional data were provided at the request of the Division; these are reviewed in Addendum A.]

Segment I: the effect of ziprasidone on mating and fertility was tested in Sprague-Dawley rats in two studies (one preliminary). In the preliminary study, ziprasidone was administered by gavage at doses of 0, 10, 40, and 160 mg/kg; included in this study was a grp in which treated (HD) males were mated with untreated females and a grp in which treated females (HD) were mated with untreated males. Females were dosed from 29 days prior to mating, throughout gestation and lactation. Males were dosed from 4 wks prior to mating through the mating period. In the definitive study, ziprasidone was administered at doses of 0, 5, 10, and 40 mg/kg (oral by gavage). In females, dosing was started 15 days prior to mating and continued throughout the lactation period. Males were dosed for 72 days prior to mating and during the mating period. Each male was mated with 2 females. One-half of the dams were delivered by Cesarean section and fetuses were examined. The remaining dams were allowed to deliver naturally and postnatal pup development was assessed.

In treated dams, there were no unscheduled deaths; however, drug-related effects were noted at all doses. Clinical signs consisted of dose-related sedation and chromodacryorrhea (>5 mg/kg). Body wt gain was reduced primarily at doses ≥40 mg/kg during gestation and, in the definitive study, body wt remained 7-10% lower than CF in HDF followed through the lactation period. Mating was delayed at doses >5 mg/kg in both studies. The pregnancy rate, however, was reduced only at 160 mg/kg. Drug-related effects on fertility may be due to effects on the female since the pregnancy rate was not affected when HDM (160 mg/kg) were mated with untreated females. Although estrus cycles were noted in both Segment I studies, the sponsor noted in the report for the definitive study that the data were too variable and no clear pattern could be determined. There was no discussion of the estrus data in the report of the preliminary study.] In the definitive study, there were no clear drug-related effects on the number of corpora lutea, implantation sites, resorptions, or live fetuses. However, the number of resorptions tended to be higher in MDF and HDF and the no. of live fetuses tended to be lower in HDF. Also, the ratio of males to females was reduced in HDF. [None of these differences on reproductive parameters achieved statistical significance.] No external or visceral findings were observed, but delayed or absent ossification of certain skeletal

components was increased at all doses. Fetal body wt was reduced at the MD and HD in the definitive study. The number of stillborn pups was increased in both studies at doses >5 mg/kg. Pup survival during the first four days of lactation was reduced at the HD (40 mg/kg) in the definitive study, and at all doses in the preliminary study. Pup body wt was reduced the HD in both studies. In the definitive study, this effect was noted throughout the lactation period in HD pups. Also, there were delays in a number of developmental milestones (e.g., negative geotaxis, eruption of incisors, grip strength, air righting) primarily at the HD in the definitive study; however, delays in air righting were noted at all doses in male pups. No effects were noted on the reproductive performance of F_1 animals.

Segment II: the teratogenic effects of ziprasidone were assessed in Segment I and Segment II studies in Sprague-Dawley rats and in 3 Segment II studies in New Zealand White rabbit.

In the Segment II study in rats, ziprasidone was administered at doses of 0, 10, 40, and 160 mg/kg from Day 6 through Day 17 of gestation. There were no unscheduled deaths. Clinical signs (prostration, piloerection, dyspnea) were observed in all MD and HD animals. Body weight was reduced throughout the dosing period in MDF and HDF (7 and 10% compared to CF). There were no drug-related effects on the number of viable fetuses, dead implants, or the male/female ratio. Fetal body weight was reduced (compared to CF) at all doses, although differences were statistically significant only at the MD and HD. There were no external or visceral findings in fetuses, however, delays in ossification of certain skeletal components were noted at all doses. In a separate maternal toxicity, ziprasidone was administered to Sprague-Dawley rats at doses of 0, 10, 40, and 160 mg/kg from Day 6 to Day 15 of gestation. Drug-related clinical signs (prostration, piloerection) were evident at all doses, with hunched posture and lacrimation observed in all MD and HD animals. Body weight loss was observed in MD and HDF and body weight gain was reduced in LDF during the first few days of dosing (i.e., Days 6-9 of gestation). Body weight gain was reduced in MDF and HDF during the rest of the dosing period, whereas in LDF, body wt gain tended to normalize. Catch-up growth occurred after the end of dosing in HDF. Even though clinical signs and body weight effects were consistent with maternal toxicity, none of the reproductive parameters measured (including viable litters, no. of fetuses, embryomortality rate, fetal body wt) was affected.

Three Segment II studies were conducted in New Zealand White rabbit. The original study was conducted at doses of 0, 10, 30, and 60 mg/kg (gavage) administered from Day 6 through Day 18 of gestation. This study was inadequate for several reasons: (1) only 1/2 of fetuses were examined for visceral and 1/2 for skeletal findings, (2) the number of does producing viable litters was unacceptably low in the MD and HD grps (i.e., 16, 17, 12, and 8 in CF, LDF, MDF, and HDF, respectively). This reduced number was the result of both drug-related and non drug-related effects, i.e., deaths in 2 HDF (reduced food intake, diarrhea), decreased pregnancy rate, decreased implantation rate, loss of entire litter (1 MDF, 2 HDF). These factors combined to result in only 41-42, 45-48, 33, and 22-25 pups examined for visceral or skeletal findings. The only possibly drug-related effects were (1) decreases in mean fetal body wt at the MD and HD and (2) increases in skeletal variants (primarily delays in ossification of certain skeletal components) at the HD. The sponsor was asked to repeat the study. The repeat study was conducted at doses of 0, 10 and 30 mg/kg (gavage) administered from Day 6 through Day 18 of gestation. In this study, 1 HDF was sacrificed moribund on Day 22 of gestation. Drug-related clinical signs (decreased activity, loose stool) were noted only at the HD. There were no body weight effects, nor any drug-related changes in any of the reproductive parameters measured (e.g., live/dead fetuses, fetal wt, placental wt, sex ratio). A total of 19 C, 20 LD, and 18 HD litters were examined, with an adequate number of fetuses for both visceral and skeletal examination. The only skeletal finding was reduced ossification of the pubis in HD pups. Upon visceral examination, ventricular septal defect (associated with other cardiac defects) was detected in 3 HD fetuses in 3 different litters. In addition, dilated renal pelvis was detected only in 6 HD fetuses in 3 different litters. Compared to the historical control data provided by the sponsor, the incidence of ventricular septal defects (2%

affected fetuses, 17% affected litters) is higher than those for the years 1991-1994. The closest HC incidence occurred in 1993 (0.34% of fetuses, 3% of litters). [HC data were not provided for dilated renal pelvis.]

In order to further study ziprasidone's effect on viscera, the sponsor conducted a third Segment II study in New Zealand White rabbits. In this repeat study, ziprasidone was administered at a single dose level (30 mg/kg; a C grp was included) from Day 6 through Day 18 of gestation. Fetuses were examined only for external and visceral findings. One HDF was sacrificed moribund on Day 27. Loose/soft stools was the only drug-related clinical sign. Body weight was not affected. There were no drug-related effects on reproductive parameters (including live/dead fetuses). Twenty-eight C litters and 26 DT litters were examined. There was no increase in ventricular septal defect in the DT grp (1 C and 1 DT fetus affected). However, ectopic kidney was detected in 3 HD fetuses from 3 different litters.

The only "definitive" Segment II study in rabbits was the first repeat. Unfortunately, this study was conducted only at 2 dose-levels.

One other study was conducted, a maternal toxicity study in rabbits. In this study, ziprasidone was administered at doses of 0, 10, 40, and 80 mg/kg (gavage) from Day 7 to Day 18 of gestation to only 7/grp. There were no unscheduled deaths and no clear drug-related clinical signs. Body weight was reduced (compared to C) at the MD and HD (maximum of 9% at Day 9 of gestation). By Day 28, body weights had normalized. The number of viable litters was low in all grps, including the C grp (3-4/grp). However, the embryomortality rate was increased in MDF and HDF. Fetal body wts were similar among grps. Fetuses were not otherwise examined.

The data from the maternal toxicity study (and the other Segment II studies) in rabbits suggest that higher doses could have been used to assess the embryofetal development of ziprasidone. In addition, the visceral findings at 30 mg/kg in two of the Segment II studies would suggest that this dose may be close to a threshold dose for adverse fetal effects. [Embryolethality was increased at 40 and 80 mg/kg in the maternal toxicity study.] Therefore, the sponsor should be asked to conduct a definitive Segment II studies using 3 dose-levels with the HD >30 mg/kg (doses of 40 and 80 mg/kg were well-tolerated in the maternal toxicity study).

Segment III: a peri- and postnatal development study was conducted in Sprague-Dawley rats at doses of 0, 5, 10, and 40 mg/kg (oral by gavage) from Day 6 of gestation through Day 21 of lactation. All dams were allowed to deliver naturally and pups were followed for attainment of development landmarks; reproductive ability was not assessed in F_1 pups.

There were no unscheduled deaths in treated dams. Clinical signs (i.e., sedation and chromodacryorrhea) were evident at all doses. Body weight was affected primarily in HDF. Body weight was lower during gestation and lactation in HDF as compared to CF (4-6 and 12%, respectively). There was an increase in the number of stillbirths and a concomitant decrease in the number of live pups at the MD and HD . [According to the sponsor, this effect was observed only at the HD. However, the sponsor's conclusion was based on evaluation only of litters that met certain criteria, i.e., ≥2 pups/sex/litter and the total number of pups in the litter had to≥7. When the excluded litters were included in the evaluation, the increase in stillbirths was noted at both MD and HD.] There was a decrease in pup survival to Day 4 of lactation in HDF. Thereafter, pup survival was similar among grps. Mean body wt was reduced throughout the lactation period in HD F_1 pups, and from Wk 1 postpartum on in MD F_1 pups. After weaning, body wt continued to be reduced in HD F_1 pups through Days 36-50 postpartum. Significant delays in eye opening and air righting were observed in HD F_1 pups. In addition, the % of pups failing the visual cliff test was increased in HDM-F_1 and in female

F_1 pups at all doses; however, these differences were not statistically significant. Effect on other developmental landmarks was not observed, except for a dose-related increase in motor activity in female F_1 pups. On the Functional Observation Battery, there was a slightly greater reactivity to tail pinch in HDM-F_1 pups and reduced body temperature in HDM-F_1 and at all doses in female F_1 pups. There were no gross pathology findings in either treated dams or the (untreated) F_1 pups.

GENOTOXICITY

The genotoxic potential of ziprasidone was tested in the following assays: Ames tests, mouse lymphoma (gene mutation), *in vitro* chromosomal aberration assays in human lymphocytes, *in vivo* chromosomal aberration assays in mouse. All but one of these studies were submitted with the original IND and have been reviewed previously (J.J.DeGeorge, Ph.D., P/T review, 10/5/89). One *in vitro* chromosomal aberration test in human lymphocytes was not previously reviewed. This review is based on an examination of the original data from all of the genotoxicity studies.

The Ames test was conducted in tester strains TA1535, TA1537, TA98, and TA100 both in the absence and presence of metabolic activation. For metabolic activation, separate assays were performed using the S9 fraction prepared from rat and mouse liver. An increase in revertants (2.4-2.8 fold) was consistently observed (three separate experiments) with TA1537 in the absence of metabolic activation. An increase in revertants was also observed with TA98 in the 3 separate experiments; however, the effect was smaller (1.4-1.9 fold). No increase in revertants was observed when mouse urine (instead of pure drug) was incubated with the tester strains.

These experiments were inadequate for two main reasons: (1) one of the tester strains sensitive to cross-linking mutagens, i.e., *E. coli* WP2uvrA, *E. coli* WP2uvrA(pKM101), or *S. typhimurium* TA102, was not included (cf. ICH guideline, Genotoxicity: A standard battery for genotoxicity testing of pharmaceuticals) and (2) there was no evidence of cytotoxicity or insolubility at the HC for any tester strain; therefore, higher concentrations should have been used.

The mouse lymphoma gene mutation assay was repeated three times. The first two assays conducted in the absence of metabolic activation were not used for assessment of mutagenic potential due to "incomplete solubility" of CP-88,059 at all of the concentrations used, i.e., 548-1064 and 271-532 µg/mL, and excessive cytotoxicity. [In neither of these assays were the relative cloning efficiency (68-70% of C) or total growth (18-26% of C) reduced to an unacceptable extent (cf. OECD guidelines).] In neither of these assays were there any increases in mutation frequency with CP-88,059. The third study (using concentrations of 111-399 µg/mL) was considered (by the sponsor) to be a valid demonstration of the lack of genotoxic potential even though "Incomplete compound solubility" was noted over the entire concentration range used and the relative cloning efficiency and total growth were reduced to a similar extent (i.e., 44 and 22% of C, respectively) as in the assays not used. There was an ≈2-fold increase in mutation frequency at the HC. At this concentration (399 µg/mL), the relative cloning efficiency was 44% and relative total growth was 22%, values not indicative of unacceptable cytotoxicity.

In the presence of metabolic activation, the data from the first study (at concentrations of 548-1064 µg/mL) was not considered by the sponsor to be a valid test of genotoxic potential due to excessive cytotoxicity (i.e., ≤50% total growth) and incomplete solubility of the drug. However, relative cloning efficiency and total growth of 55 and 26% are not indicative of excessive cytotoxicity. In this assay, >2-fold increases in mutation frequency were observed at concentrations of 798, 878, and 968 µg/mL. The results of two additional studies (410-798

and 149-532 µg/mL) were considered to be valid and to indicate a lack of genotoxic effects. However, in these assays, the presence of ppt was detected at all concentrations tested and, according to the sponsor, "Due to the insolubility of the compound in culture medium, no defined pattern of cytotoxicity was discerned in this test" and "...higher concentrations of compound produced lower cytotoxic effects...probably the result of the article precipitating out of the culture medium resulting in a lower effective exposure of the cells to the test compound". In addition, the relative total growth was similar in one of these "valid" assays to that obtained in the "invalid" assay (i.e., 26%). Greater than 2-fold increases in mutation frequency were obtained at concentrations of 601, 660, and 798 µg/mL (second study); no increases were noted at lower doses or at 724 µg/mL in the same study, or at the lower concentrations used in the third study. Relative cloning efficiency or total growth were not unacceptably reduced.

The primary problem with these studies (±S9) is the presence of test article precipitate at all concentrations tested in all assays both with and without metabolic activation. The lack of concentration-related increases in mutation frequency could well be due to incomplete solubility. It is difficult to understand the criteria by which the sponsor decided that assays were "valid" or "invalid" considering that ppt was always present and that reductions in total growth were similar in "valid" and "invalid" studies.

The sponsor conducted two separate *in vitro* chromosomal aberration assays in human lymphocytes. The first study was performed in 1988. Concentration-limiting cytotoxicity occurred in both the absence (24-hr incubation) and presence (1-hr incubation) of metabolic activation. Drug precipitate was detected at all concentrations. In the absence of metabolic activation, no increases in the no. of cells with chromosomal aberrations were noted at any of the concentrations tested (200, 300, 400 µg/mL). In the presence of metabolic activation, the no. of abnormal cells was increased at all concentrations tested (50, 100, 150 µg/mL), although the response was not concentration-dependent. [It should be noted that the incubation time of 1 hr used in the presence of metabolic activation is considered to short to constitute a valid test (cf. OECD guidelines).] The second study was conducted in 1995 using more current methodology. CP-88,059 was incubated for 3 and 24 hr (repeat studies conducted at the latter time point) in the absence of metabolic activation. At the 3-hr incubation time, there were no increases in the no. of abnormal cells at any of the concentrations tested (320-500 µg/mL); however, ppt was detected at all 3 concentrations. The MI was reduced only to 64-67% of C at the HC. In the first 24-hr test, MI was reduced to 52-56% of C at the HC. Increases in the no. of abnormal cells were noted at 205, 320, and 400 µg/mL (concentration range: 'g/mL), however, the effect was not concentration-related. In the repeat 24-hr test, the MI was reduced to ≈58% of C at the HC (range: µg/mL). No increases in the no. of abnormal cells were detected. Drug ppt was detected at all concentrations at both the 3 and 24-hr (including the repeat assay) sampling times. In the presence of metabolic activation (3-hr incubation, 2 studies), the MI was reduced to 68-79% [according to the table, 92% according to the text] of C at the HC (concentration range: µg/mL) in the first assay. Due to the lack of sufficient cytotoxicity, these cells were not scored for chromosomal aberrations. In the repeat study, the same concentrations were examined (i.e., 320-500 µg/mL). In this assay, the MI was reduced to 73% of the C at the HC. [This reduction in MI was insufficient to limit the HC.] No increases in the no. of abnormal cells were obtained at any of the concentrations tested. In both 3-hr replicate studies, drug ppt was detected at all concentrations scored.

The primary problems with these studies are two-fold: (1) the presence of drug ppt at all concentrations tested in all assays (including repeats) indicates a solubility problem as observed in the mouse lymphoma studies and (2) insufficient cytotoxicity (i.e., MI >50% of C) was obtained in the presence of metabolic activation to limit the HC. Also, in none of the assays were gaps noted.

Two *in vivo* chromosomal aberration assays were conducted in CD-1 mice (5/sex/grp). CP-

88,059 was administered as a single oral (gavage) dose of 30 mg/kg in the first study and of 200 mg/kg in the second study. [The sponsor noted in the report that the concentration of CP-88,059 in the dosing formulation was verified "mathematically" and not analytically, and that stability in the vehicle was not determined.] Sampling times were 6, 24, and 48 hr postdosing. The 30 mg/kg dose was not associated with any evidence of toxicity (no clinical signs or mortality); therefore, the dose was increased to 200 mg/kg for the repeat assay. At the higher dose, clinical signs were noted (reduced activity, decreased respiration, ataxia). Slight increases in % abnormal cells were noted in males at the 24-hr sampling time (<2-fold) and in females at the 6-hr sampling time (<2-fold). The primary problems with this assay were as follows: (1) only 50 metaphases per animal were analyzed (only 1/2 of the number recommended in the OECD guidelines) and (2) only one dose level was used; according to the OECD guidelines, if one dose level is used in this assay, it should be ≥2000 mg/kg. Otherwise, 3 dose levels should be used for the first sampling time (12-18 hr, not 6 hr as used in both assays).

CONCLUSIONS

PHARMACOLOGY

1. In *in vitro* receptor binding assays, ziprasidone exhibited high affinity for the D_2, D_3, $5HT_{2A}$, $5HT_{2C}$, $5HT_{1A}$, $5HT_{1D}$ and α_1 receptors (K_i's of 4.8, 7.2, 0.4, 1.3, 3.4, 2, and 10 nM, respectively). Ziprasidone exhibited an ≈12-fold greater affinity for the $5HT_{2A}$ receptor as compared to the D_2 receptor. Results of *in vitro* functional tests indicated that ziprasidone exhibited antagonist effects at the D_1, D_2, $5HT_{2A}$, $5HT_{2C}$, $5HT_{1D}$, and α_1-receptor, but agonist effects at the $5HT_{1A}$ receptor. Ziprasidone also inhibited reuptake of NE, 5HT, and DA in rat brain crude synaptosomal preparations, but was more potent at the NE and 5HT sites consistent with its *in vitro* binding affinity profile.

   Ziprasidone was activity in behavioral paradigms considered to have predictive validity for both clinical antipsychotic efficacy and side-effect liability. The ratio of ID_{50}/EC_{50}'s for side-effect liability:efficacy ranged from 1 to 12.5, depending upon the assay.

   Plasma metabolites, M10 (ziprasidone sulfoxide) and M 8 (ziprasidone sulfone), exhibited weak-to-no affinity for the D_2, D_3, D_4, or $5HT_{2A}$ receptors. The sponsor has concluded from these data that these metabolites have no pharmacological activity; however, the number of assays used to test these metabolites is far too limited to support this conclusion.

2. Original evaluation of the cardiovascular effects of ziprasidone in safety pharmacology studies was minimal. Only one, inadequate study was conducted in Mongrel dogs. Of 5 original dogs, 2 vomited after dosing and were removed from analysis; these animals were not replaced. Therefore, only 3 dogs were evaluated at only one acute dose level (6 mg/kg p.o.). Ziprasidone increased heart rate (≤60%), but had no effects on MAP or QA-interval. QT interval was not measured.

   Two additional *in vitro* studies were conducted, at the request of consultants from the Division of Cardio-Renal Drug Products: a canine Purkinjie fiber assay and an Kir channel assay. Final reports of these assays have not been submitted to the Division (HFD-120) for review. A summary of the results of the canine Purkinjie assay was provided in meeting briefing packages (letter dates: 2/16/98 and 3/20/98). The results of the Kir channel assay have not been submitted in any written form; they were presented briefly by the sponsor at a meeting held with the Division on 3/27/98. Reports of these studies have been requested by this reviewer.

   Cardiovascular effects of ziprasidone were tested in the 6-mo and 1-yr oral toxicity studies in Beagle dog. The sponsor reported no adverse effects on any parameter, including QT interval. ECG data were provided for the 6-mo study, but the interval between dosing and ECG measurement was not specified. In the 1-yr study, measurements were taken prior to dosing and at 2 hr postdosing (≈T_{max}, based on the data provided); however, data were not provided.

PK/ADME

The PK/ADME of ziprasidone was assessed primarily in CD-1 mice, rats (Sprague-Dawley, Long-Evans), and Beagle dog.

1. Parent compound accounted for 15-20, 50-60, and 28-34% of peak levels of total radioactivity and 8-9, 40-50, and 12-21% of total radioactivity AUC in CD-1 mouse,

Long-Evans rat, and Beagle dog, respectively. In humans, unchanged ziprasidone is the major circulating drug-related compound, accounting four 50-60% of total radioactivity (single 20 mg dose).

Parent compound and identified metabolites accounted for ≈80, 70-84, 70-83, and 58-70% of total circulating radioactivity following oral dosing in mouse, rat, dog, and human, respectively.

2. Absolute oral bioavailability was fairly low (25-36%) in all but female mouse (60-90%), indicating extensive first-pass metabolism. Estimates of clearance rate were well within the range of hepatic blood flow in mouse, rat, and dog. Estimates of V_d suggested moderate tissue distribution in all three species (0.7-2.4 L/kg).

   Tissue distribution studies in Sprague-Dawley rat and Long-Evans rats confirmed distribution of radioactivity into tissues. Highest levels of radioactivity were detected in liver, eye, and kidney; lowest levels were detected in brain. Long-term (6-mo to 1-yr) retention of radioactivity was demonstrated in eye (in pigmented rats) and testes (in both strains). Within eye, radioactivity was detected in areas with melanin pigment; however, the distribution of radioactivity was not entirely consistent with distribution of melanin. In *in vitro* studies, ziprasidone was found to bind to melanin via a hydrophobic interaction.

3. The primarily route of elimination is via the feces in the animals species tested and in humans. In bile-cannulated rats and dogs, biliary radioactivity accounted for 20-35 and 8-9% of dose radioactivity, respectively.

4. In general, the major metabolic pathways for ziprasidone were shared by mouse, rat, dog, and human: (1) cleavage of parent compound at ethyl side chain at the piperazinyl nitrogen, (2) oxidation on the benzisothiazole moiety (not at the sulfur atom), (3) oxidation of the sulfur atom, resulting in formation of the sulfoxide and sulfone metabolites, and (4) hydroxylation on the 3-position and subsequent oxidation of the benzisothiazole moiety. *In vitro* studies indicated involvement of CYP3A4 in the formation of the sulfoxide and sulfone metabolites. Ziprasidone inhibited CYP2D6 and CYP3A4 *in vitro* (K_i = 5.4 ± 4.8 and 64 ± 22 μM, respectively).

   While qualitatively similar among species in *in vitro* and *in vivo* assays, the metabolism of ziprasidone differed quantitatively among species. The most marked difference was that the ziprasidone sulfone was not detected in rat plasma (only in urine at ≤1% of dose radioactivity); in humans, this metabolite was identified as a major metabolite. The potential toxicity of the sulfone metabolite was assessed, however, since it was a major plasma metabolite in both CD-1 mouse and Beagle dog.

   It is unfortunate that no plasma metabolite was monitored in toxicity studies in any of the animal species tested or in human clinical trials because relative exposures to these metabolites cannot be compared.

5. ziprasidone was found to bind extensively (≥98%) to human albumin and α_1-acid glycoprotein *in vitro* and in monkey (cebus, cynomolgus) and human *in vivo*.

TOXICOLOGY

1. Acute toxicity studies were conducted in albino mice (p.o., i.p.) and Sprague-Dawley rats. None of the studies were definitive. Drug-related deaths occurred only in male mice via the i.p. route. Sedation was the primary clinical signs. No target organ for

toxicity was identified. The oral LD_{50} in mice and rats was >2000 mg/kg.

2. Subchronic oral toxicity studies were conducted in Long-Evans rats (2-wk, 1-mo; gavage) and Beagle dogs (2-wk, 1-mo).

   (a) In rat, the 2-wk study was inadequate (small "n", limited histopathology). In the 1-mo study, the primary drug-related effects involved the CNS (sedation) and reductions in body weight gain) at all doses (0, 10, 40, 160 mg/kg). No target organ for toxicity was identified. The mean plasma C_{max} (AUC data were not determined) for parent compound at the HD was ≈9-10 µg/mL. This is 25-30 times higher than the estimated plasma C_{max} in humans at the maximum recommended human dose (MRHD)(100 mg b.i.d.). [C_{max} of 300-350 ng/mL is estimated based on extrapolation from values obtained at 80 mg b.i.d.. This assumes linearity from 80 to 100 b.i.d., even though linearity in humans was demonstrated only to 80 mg b.i.d.]. Interspecies comparisons of plasma metabolite levels could not be made due to the lack of data in animals and humans.

   (b) In dog, the 2-wk study was inadequate (small "n", no terminal studies). In the 1-mo study, the only clear drug-related effect was sedation. Liver enzymes (ALT, AST) were elevated at 20 and 40 mg/kg (MD, HD, LD was 10 mg/kg; doses given b.i.d.); however, liver wt was not affected and there were no histopathological correlates. The mean plasma C_{max} (AUC data were not determined) for parent compound at the HD was ≈0.6 µg/mL (Day 23). This is ≈2 times higher than the estimated plasma C_{max} in humans at the MRHD (estimated C_{max} of 300-450 ng/mL at 100 mg b.i.d.). Interspecies comparisons of plasma metabolite levels could not be made due to the lack of data in animals and humans.

3. Chronic oral toxicity studies were conducted in Sprague-Dawley rats (6-mo; gavage) and Beagle dogs (6-mo, 1-yr).

   (a) In rat, the primary drug-related effects were sedation (noted at all doses, 10, 40, and 200 mg/kg), reductions in body wt (males: 17-35% compared to CM, all doses affected; females, 6-9% compared to CF at MD, HD) and microscopic changes in lung (pleuritis, multifocal granulomatous pneumonia), adrenal gland (multifocal cystic degeneration, telangiectasia, diffuse hypertrophy), and prostate (prostatitis) (MD, HD). The sponsor attributed none of these to drug; however, drug-related effects could not be ruled out due to dose-related increases in incidence and/or severity in all three organs. The mean plasma C_{max} (AUC data were not determined) for parent compound at the HD was 9-10 µg/mL (Days 89-173). This is 25-30 times the estimated plasma C_{max} in humans at the MRHD [cf. Conclusions #2(a)]. No NOEL identified in this study due to CNS and body wt effects at the LD.

   It should be noted that there were a number of unscheduled deaths (i.e., 11) in this study, 8 of which were stated to be due to either trauma during dosing or during recapture. The nature of the injuries attributed to trauma sustained during re-capture (i.e., skull fracture) would suggest a lack of adequately trained personnel.

   (b) In dog, drug-related clinical signs (e.g., decreased activity, tremors, hypersalivation, aggressive behavior, pacing/circling) were evident at all doses (6-mo: 0, 5, 10, 40, MD and HD given as 5 and 20 mg/kg b.i.d.; 1-yr: 0, 5, 10, 20

mg/kg, MD and HD given as 5 and 10 mg/kg b.i.d.). [One HDM was sacrificed after fracturing several teeth and the jaw during cage-biting in the 6-mo study.] Except for these and sporadic increases in ALT in individual animals, particularly HD animals), no other drug-related effects were observed in the 1-yr study. In the 6-mo study, additional drug-related effects consisted of decreases in body wt (relative to C grps, all doses), increases in alkaline phosphatase and ALT (HD), and microscopic effects in kidney (lipofuscin deposits in renal proximal tubules, MD and HD) and liver (intrahepatic cholestasis, HD). Therefore, in the 6-mo study, CNS and liver were clearly demonstrated to be target organs for toxicity. No NOEL was determined in either study due to CNS effects observed at all doses.

Comparisons between the systemic exposure to parent compound achieved in the chronic dog studies and that estimated in humans at the MRHD [cf. Conclusions #2(a)] were as follows:

(1) at the lower dose associated with renal and liver toxicity in the 6-mo study, plasma levels were ≈1 and 3 times the estimated human exposure at the MRHD for C_{max} and AUC, respectively.

(2) at the dose not associated with renal and liver toxicity in the 6-mo study, plasma levels were ≈0.5 and 0.3 times the estimated exposure in humans at the MRHD for C_{max} and AUC, respectively.

(3) at the HD used in the 1-yr study (a dose associated with marked CNS signs only), plasma levels were ≈1 and 0.9-1.7 times the estimated exposure in humans at the MRHD for C_{max} and AUC, respectively.

CARCINOGENICITY

Two-year carcinogenicity studies were conducted in Swiss CD-1 mice and Long-Evans rats. Ziprasidone was administered orally in the diet at doses of 0, 0, 50, 100, and 200 mg/kg in mice and 0, 0, 2, 6, and 12 mg/kg in rats.

1. The study in rats was negative, i.e., no drug-related increases in neoplastic findings were observed in either males or females. The adequacy of dosing was demonstrated only by reductions in body weight in HD animals (10 and 19% in HDM and HDF, respectively) as compared to controls. Although not entirely convincing, comparison of the body weight data from the 2-yr carcinogenicity (dietary) and 6-mo chronic toxicity (gavage) studies would suggest that body weight effects are not due to unpalatability of the drug-diet admixture.

   The doses used were 0.1, 0.3, and 0.6 times the maximum recommended human dose on a mg/m^2 basis. Plasma levels of parent compound (C_{max}, AUC) were ≈2-fold higher in females than in males. Comparison of systemic exposure achieved in rats to those estimated in humans at the MRHD [values of 300-350 ng/mL for C_{max} and 200 ng•hr/mL for AUC extrapolated from data in humans at 80 mg b.i.d. (MRHD = 100 mg b.i.d.); there are no data to support linearity from 80 to 100 mg b.i.d.] are as follows:

   C_{max} 0.3-0.4 and 0.7-0.8 times the estimated human exposure in male and female rats, respectively, at HD

AUC 1 and 2 times the estimated human exposure in male and female rats, respectively, at the HD

2. In mice, drug-related increases in the following tumors were noted in females: (1) adenoma of the pituitary gland, (2) adenoma and carcinoma combined of the pituitary gland, and (3) adenocarcinoma (incidental and fatal) of the mammary gland. Pair-wise comparisons indicated that increases in the incidences of these tumors were significant at all doses tested. No drug-related increases in tumors were noted in males. As in the rat study, the adequacy of dosing in males was demonstrated only by reductions in body weight in HDM (8%). In mice, no toxicity studies using gavage dosing are available in order to assess whether or not body wt effects could have been due to the unpalatability of the diet.

   The tumor pattern observed in female mice is consistent with prolactin-mediated effects. Ziprasidone was shown to increase serum prolactin in female, but not male, mice (1-mo dietary study) and to have no effect on serum prolactin in rats (5-wk dietary study) at the same doses used for carcinogenicity testing. It should be noted, however, that the toxicities observed in the 1-mo dietary study in mice were greater than those observed in the 2-yr study. Therefore, the two studies may not be entirely comparable.

REPRODUCTION

The reproductive toxicity of ziprasidone was tested in a Segment I study in Sprague-Dawley rats, Segment II studies in Sprague-Dawley rats and New Zealand White rabbits, and a Segment III study in Sprague-Dawley rats. Only one of the 3 Segment II studies in rabbit could be considered definitive (the original was clearly inadequate), and only two dose levels were evaluated in this study.

1. Ziprasidone produced delays in mating (i.e., increased time to successful mating) in rats at doses of 10 to 160 mg/kg. Impairment of fertility was observed only at 160 mg/kg. The effect on fertility appeared to be due to effects on the females since a decrease in pregnancy rate was not observed when treated males (160 mg/kg) were paired with untreated females.

2. Ziprasidone produced no clear evidence of teratogenicity when given to rats at doses of 5 to 160 mg/kg or rabbits at doses of 10 and 30 mg/kg. However, in the "definitive" rabbit study (only 2 dose levels were tested), ventricular septal defects (associated with other cardiac effects) were detected in 3 HD fetuses (in 3 affected litters) and dilated renal pelvis was detected in 6 HD fetuses (3 affected litters). In a follow-up study, no increases in ventricular septal defects were noted a the one dose-level tested (i.e., 30 mg/kg) (1 affected C and 1 affected DT fetus); however, ectopic kidney was detected in 3 HD fetuses (3 affected litters).

   Ziprasidone did produce adverse effects on fetuses, as evidenced by (1) decreases in fetal body wt (at doses of 10-160 mg/kg in rat fetuses and at 30 and 60 mg/kg in rabbit fetuses) and (2) increases in skeletal variants (primarily, but not exclusively, delays in ossification) in both rat and rabbit fetuses. Effect-doses in rat fetuses were 5 to 160 mg/kg and in rabbit fetuses were 30 and 60 mg/kg.

   Signs of maternal toxicity (primarily reduced body weight gain) were observed at 40 and 160 mg/kg in rat dams in the studies assessing embryofetal effects. In a separate study, maternal toxicity was noted at 40 and 160 mg/kg, but no adverse effects on fetuses were observed. Although some maternal toxicity was observed at 160 mg/kg, there were no drug-related deaths and it is not clear that the toxicities (i.e., body wt changes, clinical signs) were more severe at 160 than at 40 mg/kg. Therefore, higher

doses (i.e., >40 mg/kg) could probably have been used in the Segment II study in rats.

Clear signs of maternal toxicity were not evident in the "definitive" or repeat toxicity study in rabbit (at 10 and 30 mg/kg). In a separate study, equivocal maternal toxicity (defined only as a 9% decrease in body wt; there were no deaths or clear drug-related clinical signs) was observed at 40 and 80 mg/kg; however, as in rat, the effects were not obviously more severe at the higher dose. At 40 and 80 mg/kg, there was an increase in embryolethality; this finding was not observed at the lower doses tested in the Segment II studies.

The data from the Segment II studies and the maternal toxicity study in rabbits demonstrate that (1) higher doses could have been used to study the embryofetal effects of ziprasidone, (2) at the doses used (10 and 30 mg/kg), there was equivocal evidence of teratogenicity, (3) at doses higher than those used in the Segment II studies, embryolethality was detected. Therefore, the sponsor should be asked to conduct a repeat embryofetal development study in rabbits using three dose levels, with the HD >30 mg/kg (e.g., 40-80 mg/kg). Such a study is needed to more convincingly rule out possible teratogenic effects and to better delineate adverse effects on embryofetal development in rabbit.

3. In the mating and fertility and the peri/postnatal studies in rats, there were increases in still born pups and a decrease in postnatal survival (to Day 4) at doses of 10 to 160 mg/kg. Developmental delays were observed in rat pups at all doses tested (5 to 40 mg/kg).

GENOTOXICITY

The genotoxic potential of ziprasidone was tested in the following assays: Ames test, mouse lymphoma (gene mutation), *in vitro* chromosomal aberration assays in human lymphocytes, *in vivo* chromosomal aberration assays in mouse. However, none of the studies was adequate because of methodological limitations.

1. In the Ames test, the only positive finding was a reproducible increase in revertants in the absence of metabolic activation with tester strain, TA1537. The studies were inadequate because (1) one of the tester strains sensitive to cross-linking mutagens [e.g., TA102 or *E. coli* WP2uvrA or *E. coli* WP2uvrA(pKM101)] was not included and (2) there was no evidence of cytotoxicity or insolubility at the HC for any tester strain.

2. In the mouse lymphoma assay (3 replicate studies), increases in mutation frequency were observed at the HC (399 µg/mL) in the absence of metabolic activation, and at concentrations ≥ 600 µg/mL in the presence of metabolic activation. However, none of the studies conducted was adequate due to the presence of test article precipitate at all concentrations tested, both with and without metabolic activation. A repeat of this assay would not routinely be requested in this case since the sponsor has conducted *in vitro* chromosomal aberrations tests; however, considering the positive findings, it would seem reasonable to either repeat this assay after overcoming the solubility problems or to conduct another assay to assess the potential mutagenic potential in mammalian cells.

3. Two *in vitro* chromosomal aberration assays were performed using human lymphocytes. In the first study, there was an increase in the number of abnormal cells at all concentrations tested in the presence of metabolic activation and in the second study, there was an increase in the number of abnormal cells in the absence of metabolic activation. Neither effect was concentration-related. Both studies were inadequate because (1) drug precipitate was present at all concentrations tested and (2)

sufficiently high concentrations were not used in the presence of metabolic activation.

4. Two *in vivo* chromosomal aberration assays were conducted in CD-1 mice. The first study was repeated at a higher dose since no drug-related signs were evident at the dose used. The second study was inadequate because only one-half of the recommended number of metaphases was examined and only one dose level was used.

The genotoxic potential of ziprasidone has not been adequately tested. The sponsor should repeat the entire battery, taking care to solubilize the drug and to use sufficiently high drug concentrations.

RECOMMENDATIONS

From a pharmacology/toxicology standpoint, there is no objection to the approval of this NDA. However, there are certain issues that need to be addressed postmarketing.

The following information should be relayed to the sponsor:

1. The genotoxicity battery (cf. ICH Guideline, Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals) should be repeated, addressing the methodological limitations of the original studies. The Ames test conducted was inadequate because (1) one of the following tester strains was not included: TA102, *E. coli* WP2uvrA, or *E. coli* WP2uvrA(pKM101) and (2) there was no evidence of cytotoxicity or insolubility at the high concentration for any tester strain tested. The mouse lymphoma gene mutation assay and the *in vitro* chromosomal aberration assays in human lymphocytes were inadequate due to the presence of precipitate at all concentrations tested. In addition, in the *in vitro* chromosomal aberration assays, higher drug concentrations should have been used in the presence of metabolic activation (the Mitotic Index was >50% of control at the high concentration tested). The *in vivo* chromosomal aberration assays were inadequate because (1) only one-half of the recommended number of metaphases per animal was analyzed and (2) only one dose level was used and that dose was not ≥2000 mg/kg (cf. OECD guidelines).

2. Full reports for the *in vitro* studies conducted to assess the potential cardiotoxicity of ziprasidone [i.e., canine Purkinje fiber assay and the rapidly activating delayed rectifier potassium channel (I_{Kr})] should be submitted for review. Please make sure to include (1) methodology and data for all negative and positive controls and (2) drug solubility data for both of these assays.

Lois M. Freed, Ph.D.

NDA orig (#20-825)
div file
HFD-120
/G.Fitzgerald Rec'd 5/7/98
/L.M.Freed See T.L. memo for comments 5/8/98
/S.Hardeman

5 pages redacted from this section of the approval package consisted of draft labeling

ADDENDUM A: sponsor's response to Division's request for information on reproductive studies (12/22/97). Requests are bolded (as provided in the sponsor's response); a review of the data follow the response to each request.

1. **A complete summary table for ALL skeletal findings in the following studies:**

   Study no. 94-720-30 (Segment II study in rabbits)
   Study no. 92-720-20 (Segment I study in rats)
   Study no. 91094/95 (Segment II study in rats)
   Study no. 91096/97 (Segment II study in rabbits)

Sponsor's comments: the requested data have been submitted.

Reviewer comments

Study no. 94-720-30

For each skeletal finding, the sponsor provided the following: (1) no. and % of affected fetuses, (2) the litter incidence, and (3) mean litter %. This latter parameter was not the % of affected litters (this was not provided); it may be the mean % of affected fetuses per litter.

The only finding which appeared drug-related was reduced ossification of the pubis. The numbers (%) of affected fetuses for this finding were as follows: 2/163 (1.23), 1/153 (0.65), and 6/142 (4.23%) in CF, LDF, and HDF, respectively; litter incidences were 1/19, 1/20, and 5/18, respectively, and the mean litter % were 2.1, 0.5, and 4.6%, respectively.

Study no. 94-720-20

The skeletal data were expressed in the same ways as for Study no. 94-720-30. However, all findings were not included in the summary table; metacarpal and metatarsal data were missing. Selected skeletal findings are summarized in the following table:

FINDING	PARAMETER	CF	LDF	MDF	HDF
HEAD hyoid absent ossification	no. affected fetuses (%) litter incidence (%)	10/113 (9) 7/16 (44)	24/118 (20) 11/16 (69)	28/129 (22) 10/18 (56)	21/98 (21) 5/15 (33)
STERNEBRA(E) absent ossification	no. affected fetuses (%) litter incidence (%)	42/115 (37) 13/16 (80)	44/123 (36) 13/16 (80)	52/133 (39) 17/18 (94)	58/98 (60) 14/15 (93)
reduced ossification	no. affected fetuses (%) litter incidence (%)	63/115 (55) 15/16 (94)	86/123 (70) 16/16 (100)	83/133 (62) 17/18 (94)	68/98 (70) 13/15 (87)
misshapened	no. affected fetuses (%) litter incidence (%)	0 0	0 0	1/133 (<1) 1/18 (<1)	1/98 (1) 1/15 (<1)
RIBS 14th pair, rudimentary	no. affected fetuses (%) litter incidence (%)	4/115 (3) 3/16 (19)	2/123 (2) 2/16 (12)	4/133 (3) 3/18 (17)	5/98 (5) 3/15 (20)
14th pair rudimentary, one-side	no. affected fetuses (%) litter incidence (%)	3/115 (3) 3/16 (19)	9/123 (7) 5/16 (30)	6/113 (4) 4/18 (22)	4/98 (4) 1/15 (<1)
VERTEBRA(E) cervical centra present	no. affected fetuses (%) litter incidence (%)	44/115 (38) 12/16 (75)	64/123 (52) 15/16 (94)	32/133 (24) 12/18 (67)	35/98 (36) 13/15 (87)
lumbar arch(es) reduced ossification	no. affected fetuses (%) litter incidence (%)	0 0	0 0	1/132 (1) 1/18 (<1)	1/98 (1) 1/15 (<1)
sacral arch(es) absent ossification	no. affected fetuses (%) litter incidence (%)	0 0	7/123 (6) 4/16 (25)	16/133 (12) 8/18 (44)	7/98 (7) 3/15 (20)
sacral arch(es) reduced ossification	no. affected fetuses (%) litter incidence (%)	24/115 (21) 11/16 (69)	44/123 (36) 11/16 (69)	54/133 (41) 15/18 (83)	46/98 (47) 14/15 (93)
LOWER LIMB pubis reduced ossification	no. affected fetuses (%) litter incidence (%)	1/115 (<1) 1/16 (<1)	1/123 (<1) 1/16 (<1)	7/133 (5) 4/18 (22)	8/98 (8) 6/15 (40)
pubis absent ossification	no. affected fetuses (%) litter incidence (%)	0 0	0 0	4/133 (3) 1/18 (<1)	1/98 (1) 1/15 (<1)
ischium reduced ossification	no. affected fetuses (%) litter incidence (%)	0 0	0 0	2/133 (2) 1/18 (<1)	0 0
ilium reduced ossification	no. affected fetuses (%) litter incidence (%)	0 0	0 0	0 0	2/98 (2) 1/15 (<1)

Study no. 91094/95

A summary of the skeletal findings were provided, as requested. Data were expressed in terms of no. (%) of affected fetuses and litters. The sponsor also noted the following:

(1) the dictionary included in the software used to generate the summary tables has been modified from the original; "H" (i.e., hypoplastic) in the current tables refers to "R" (rudimentary) in the original tables.

(2) in the current summary tables, the degree of ossification (fetus, litter) is expressed as

"normal" (= maximum score of 18) or "delayed" (= score <18) ossification.

(3) in the current summary tables, the term "sternebra(e) misshapen" replaces the terms, "sternebra(e) dumbbell-shaped", sternebra(e) asymmetrical", and sternebra(e) asymmetrically dumbbell-shaped", used in the original report.

Selected skeletal findings are summarized in the following table:

FINDING	PARAMETER	CF	LDF	MDF	HDF
5TH METACARPUS, L absent ossification	no. affected fetuses (%) litter incidence (%)	56/157 (36) 18/20 (90)	49/149 (33) 16/18 (89)	58/145 (40) 14/19 (74)	74/140 (53) 17/18 (94)
5TH METACARPUS, R absent ossification	no. affected fetuses (%) litter incidence (%)	51/157 (32) 16/20 (80)	47/149 (32) 16/18 (89)	56/145 (39) 13/19 (68)	72/140 (51) 16/18 (89)
PUBIC BONES, L rudimentary	no. affected fetuses (%) litter incidence (%)	3/157 (2) 2/20 (10)	3/149 (2) 2/18 (12)	5/145 (3) 3/19 (16)	4/140 (3) 3/18 (17)
PUBIC BONES, R rudimentary,	no. affected fetuses (%) litter incidence (%)	2/157 (1) 2/20 (10)	3/149 (2) 2/18 (11)	5/145 (3) 2/1 (11)	3/140 (2) 3/18 (17)
SACRAL/CAUDAL VERTEBRA(E) slight delay ossification	no. affected fetuses (%) litter incidence (%)	22/157 (14) 10/20 (50)	26/149 (17) 8/18 (44)	35/145 (24) 10/19 (53)	32/140 (23) 11/18 (61)
STERNEBRA(E) misshapen	no. affected fetuses (%) litter incidence (%)	10/157 (6) 9/20 (45)	14/149 (9) 9/18 (50)	11/145 (8) 10/19 (53)	14/140 (10) 8/18 (44)
THORACIC bod(ies) misshapen	no. affected fetuses (%) litter incidence (%)	15/157 (10) 9/20 (45)	21/149 (14) 12/18 (67)	14/145 (10) 10/19 (53)	23/140 (16) 11/18 (61)
bod(ies) rudimentary	no. affected fetuses (%) litter incidence (%)	1/157 (<1) 1/20 (5)	0/149 (0) 0/18 (0)	0/145 (0) 0/19 (0)	2/140 (1) 2/18 (11)

Study no. 91096/97

Skeletal findings were summarized, with notes regarding terminology the same as for Study no. 91094/95. In addition, the sponsor noted that pregnant females that died during the study were included for calculation of mean no. of corpora lutea and implantation sites only.

Selected skeletal findings are summarized in the following table:

FINDING	PARAMETER	CF	LDF	MDF	HDF
ASTRAGALUS, R/L absent	no. affected fetuses (%) litter incidence (%)	0/41 (0) 0/15 (0)	0/45 (0) 0/17 (0)	0/33 (0) 0/12 (0)	1/25 (4) 1/8 (12)
STERNEBRA(E) delayed ossification	no. affected fetuses (%) litter incidence (%)	1/41 (2) 1/15 (7)	6/45 (13) 5/17 (29)	2/33 (6) 2/12 (17)	2/25 (8) 2/8 (25)
misshapen	no. affected fetuses (%) litter incidence (%)	1/41 (2) 1/15 (7)	2/45 (4) 2/17 (12)	1/33 (3) 1/12 (8)	4/25 (16) 4/8 (50)
divided	no. affected fetuses (%) litter incidence (%)	1/41 (2) 1/15 (7)	0/45 (0) 0/17 (0)	0/33 (0) 0/12 (0)	1/25 (4) 1/8 (12)
fused	no. affected fetuses (%) litter incidence (%)	1/41 (2) 1/15 (7)	1/45 (2) 1/17 (6)	2/33 (6) 2/12 (17)	4/25 (16) 4/8 (50)
rudimentary	no. affected fetuses (%) litter incidence (%)	0/41 (0) 0/15 (0)	0/45 (0) 0/17 (0)	1/33 (3) 1/12 (8)	1/25 (4) 1/18 (12)
supernumerary	no. affected fetuses (%) litter incidence (%)	3/41 (7) 2/15 (13)	1/45 (2) 1/17 (6)	1/33 (3) 1/12 (8)	2/25 (8) 2/8 (25)
RIBS 13th right rib only	no. affected fetuses (%) litter incidence (%)	2/41 (5) 2/15 (13)	3/45 (7) 3/17 (18)	2/33 (6) 2/12 (17)	2/25 (8) 2/8 (25)
PUBIC BONES, R/L rudimentary	no. affected fetuses (%) litter incidence (%)	1/41(2) 1/15 (7)	1/45 (2) 1/17 (6)	1/33 (3) 1/12 (8)	2/25 (8) 2/8 (25)

2. **A summary table and individual line listings for gross pathology findings in F_0 dams for the Segment III study in rats (Study No. 93-720-28) should either be provided or the location of the information in the report should be provided.**

Sponsor's comments: a table of individual findings was prepared (Attachment V). No drug-related effects were noted, as stated in the original report.

Reviewer comments: according to the sponsor's table, no gross findings (not just no drug-related findings) were observed in any animal.

3. **Data documenting the stability of ziprasidone in suspension (0.5% methylcellulose) for 7 days at room temperature. Also, data documenting the concentration and homogeneity of drug suspensions for all reproductive studies.**

Sponsor's comments: 24-stability data for the 60 mg/mL are provided in Attachment VI. When this concentration was used, suspensions were made fresh daily. Concentration data for the following studies are provided in Attachment VI:

Study No. 941094/95: Teratology study in Sprague-Dawley rats
Study No. 91096/97: Teratology study in New-Zealand White rabbits
Study No. 94-720-30: Teratology study in New-Zealand White rabbits
Study No. 95-720-34: Teratology study in New-Zealand White rabbits
Study No. 92-720-17: Fertility study in Sprague-Dawley rats
Study No. 92-720-20: Fertility study in Sprague-Dawley rats

Reviewer comments: 8-day stability data were provided for concentrations of 5 and 10 mg/mL (solutions, 0.5% methylcellulose; lot no. 23,638-38-1F). The data document stability of these two concentrations at rm temperature either protected or exposed to light for 8 days. The 60 mg/mL suspension was stable for 24 hr when stored at rm temperature and exposed to light. Concentrations of 1 and 20 mg/mL were tested for stability at rm temperature, exposed to light. The data documented stability of these two concentrations (in 0.5% methylcellulose) for 1 wk under these conditions.

Verification of drug concentrations were provided for the studies listed. Combining all data for all studies listed, the achieved concentrations were 82-118% of intended. In addition, the sponsor provided homogeneity data for Study No's. 941094/95 and 91096/97. In these studies. the achieved concentrations (top, middle, bottom samples) for all dose levels ranged from % of intended.

4. **There seems to be a discrepancy in the number of dams included in the body wt (during gestation) summary tables for the Segment I study in rats. The numbers given are 32, 31, 34, and 32 for CF, LDF, MDF, and HDF, respectively. However, according to the individual table, the number of females with viable litters (on which the summary table is based) are 33, 36, 35, and 33, respectively. Could this apparent discrepancy be clarified?**

Sponsor's comments: the "n's" listed in the body weight summary table for Study No. 97-720-20 were checked and found to be correct. The following "exclusion" table was submitted by the sponsor:

Dose group	No. dams reported in Summary Table 3	No. dams excluded	Findings for exclusion - dam #
Control	32	8	NG – #s 94, 164, 168, 171, 173, 176 No S+ - # 92 No viable fetuses – # 93
5.0 mg/kg	31	9	NG – #s 101, 120, 186, 198 No S+ – #s 106, 113, 181, 195 Missed S+ sign – # 110
10.0 mg/kg	34	6	NG – #s 204, 207, 210, 214, 217 No S+ - # 211
40.0 mg/kg	32	8	NG – #s 142, 155, 229, 230, 231, 232, 239, No S+ - # 226

NG = non gravid
No S+ = no sperm positive sign in vaginal lavage
Missed S+ sign = dam delivered litter early; S+ sign recorded after day of impregnation

Reviewer comments: after a comparison of the excluded dams, it was clear that the reason for the discrepancy was that dams were excluded from body weight data, even if they had delivered viable pups, if they were designated as "No S+" or "Missed S+ sign".

ADDENDUM B: Statistical Review and Evaulation: Review of Carcinogenicity Data (Roswitha E. Kelly, Mathematical Statistician, HFD-710, 1/18/98)

Minutes of Exe-CAC Meeting, 4/28/98

APPEARS THIS WAY
ON ORIGINAL

Statistical Review and Evaluation

Review of Carcinogenicity Data

JAN 1 6

IND#: [ ]

APPLICANT: Pfizer

NAME OF DRUG: Ziprasidone Hydrochloride Oral

DOCUMENTS REVIEWED: Volumes 41.1-41.9 Dated Feb. 1, 1996.

PHARMACOLOGY REVIEWER: L. Freed, Ph.D.

I. Background

Dr. Freed (HFD-120) requested from the Division of Biometrics I a statistical review of the rat and mouse studies data as well as an evaluation of the sponsor's report.

II. The Rat Study

II.1 Sponsor's Findings

In this study 250 male and female rats were given the drug (preblended with lactose) in the feed at concentrations of 0, 0, 2, 6, and 12 mg/kg/day for about two years. The two identical control groups received only the lactose in the feed. There were also three dosed satellite groups of 4 animals each which were used for plasma determinations.

The drug did not affect survival negatively. There was actually a numeric increase in survival for the high dose males and the high and intermediate dose females.

Keratoacanthomas of the skin and adnexa showed a statistically significant trend ($p=0.0079$) among the males when the controls were combined. The significance was lost after Bonferroni adjustment ($p=0.1185$). The trend including only the first

control group was significant at p=0.0091 but not including the second control group (p=0.0234). For the female rats there were no statistically significant tumor trends with dose.

Mean body weights of the treated animals were generally lower than control group 1. For the first year the differential reached about 9 percent for the high dose males and about 7 percent for the high dose females. This differential subsided towards the end of the study for the males but increased further for the females, reaching almost 19 percent by week 97.

II.2 Reviewer's Findings

This study appeared to be well controlled and executed. This reviewer confirmed that the sponsor's findings that there was no negative effect of the drug on survival of either the male or female rats (Tables 1-4, Figures 1-2). There was actually a statistically significant increase ($p \leq 0.03$) in survival with dose among the male rats. Minor differences in tabulations did not affect the results. Specifically, in this reviewer's tabulations six female animals an one male rat were classified as terminal sacrifice (past day 722) whereas the sponsor had classified them as moribund (one femalein control group 1) and found dead (one female in control group 1, four femalesin the medium dose group, and one male in the medium dose group).

The sponsor computed tumor trends only for tumors appearing in at leave five animals. As this reviewer used exact permutation trend tests and not asymptotic theory, such a restriction was not necessary. For the female rats this reviewer found that none of the tumor trend statistics (increasing trend with dose) reached the statistical level of significance necessary for common ($\alpha = 0.005$) or rare ($\alpha = 0.025$) tumors (Table 5). For the male rats benign keratoacanthoma of the skin and adnexa had a p-value of 0.0289 based on the permutation trend test (Table 6). The sponsor's Cochran-Armitage test for linear trend had an associated p-value of 0.0079 which became non-significant after a Bonferroni correction for multiplicity of testing. This reviewer considers a Bonferroni correction too liberal in general, however, in this case the p-value associated with the permutation trend test also did not pass the criterion of significance for rare tumors. Therefore, this finding is considered statistically non-significant. As there is generally an interest in the p-value of the High dose versus the control comparison for tumors with significant trends, this reviewer also performed this calculation for keratoacanthoma of the skin among the male rats. The associated p-value was 0.0592, again not statistically significant. Certain tumors (see Appendix) were combined as suggested by the pharmacologist. None of these groupings, however, reached statistical significance.

As there were no statistically significant tumor trends among either female or male rats, the validity of the two study arms need to be evaluated. For this, two questions need to be answered (Haseman, Statistical Issues in the Design, Analysis and Interpretation of Animal Carcinogenicity Studies, Environmental Health Perspectives, Vol 58, pp 385-392, 1984):

(i) Were enough animals exposed for a sufficient length of time to allow for late developing tumors?

(ii) Were the dose levels high enough to pose a reasonable tumor challenge in the animals?

The following are some rules of thumb as suggested by experts in the field: Haseman (Issues in Carcinogenicity Testing: Dose Selection, Fundamental and Applied Toxicology, Vol 5, pp 66-78, 1985) had found that on the average, approximately 50 % of the animals in the high dose group survived the two-year study. In a personal communication with Dr. Karl Lin of HFD-715, he suggested that 50 % survival of the usual 50 initial animals in the high dose group between weeks 80-90 would be considered as a sufficient number and adequate exposure. Chu, Cueto, and Ward (Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays, Journal of Toxicology and Environmental Health, Vol 8, pp 251-280, 1981) proposed that "To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50 % survival at one year". From these sources, it appears that the proportions of survival at weeks 52, 80-90, and at two years are of interest in determining the adequacy of exposure and number of animals at risk.

In determining the adequacy of the chosen dose levels, it is generally accepted that the high dose should be close to the MTD. Chu, Cueto, and Ward (1981) suggest:

(i) "A dose is considered adequate if there is a detectable weight loss of up to 10 % in a dosed group relative to the controls."

(ii) "The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."

(iii) "In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

In another paper, Bart, Chu, and Tarone (Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity, Journal of the National Cancer

Institute 62, 957-974, 1979), stated that the mean body weight curves over the entire study period should be taken into consideration with the survival curves, when adequacy of dose levels is to be examined. In particular, "Usually, the comparison should be limited to the early weeks of a study when no or little mortality has yet occurred in any of the groups. Here a depression of the mean weight in the treated groups is an indication that the treatment has been tested on levels at or approaching the MTD."

Between 48 and 60 percent of the treated females and between 32 and 50 percent of the treated males survived to terminal sacrifice after 103 weeks of treatment. As the highest survival occurred among the high dose animals, sufficient numbers of animals were exposed for a sufficient length of time to manifest late developing tumors. The body weight gain of either sex was suppressed with respect to their controls. The high dose females manifested decreased mean body weight when compared to control group 1 of up to 10 percent for the first 14 months and further decreases to up to 19 percent till 23 months (Table 7). For the high dose males a body weight differential of about -10 % compared to control group 1 was maintained for most of the study. These findings indicated that the high dose was probably close to the MTD, establishing the validity of this study.

III. The Mouse Study

III.1 Sponsor's Findings

The drug was administered to 250 male and 250 female CD-1 mice for close to 24 months at doses of 0, 0, 50, 100, and 200 mg/kg/day. The three dose groups all received 50 mg/kg for two weeks, then the mid and high dose animals were increased to 100 mg/kg for another two weeks, after which the high dose received 200 mg/kg for the remainder of the study.

Quartiles and median and mean survival times were presented as well as survival plots. The sponsor found no statistically significant differences in the mean survival times between groups in either sex.

Neoplastic findings with incidence greater than four were analyzed using Peto's methods for fatal and incidental tumors. The sponsor observed statistically highly significant increases in the incidence rates of adenocarcinoma of the mammary gland ($p=0.0000$) and adenoma of the pituitary ($p=0.0000$) among the female mice. Additionally, there was a statistically significant increase in bronchoalveolar adenomas ($p=0.04$) among these animals, which did not remain significant when

combined with carcinomas. The male mice were found free of treatment related pathology.

For the mid and high dose female mice there were body weight losses compared to the controls during the last three months of the study. The sponsor also reported that mean body weight and mean body weight gain of the mid and high dose males were statistically significantly lower than the combined controls.

III.2 Reviewer's Findings

This reviewer verified the sponsor's findings and concurs that there were no statistically significant negative dose responses of the drug on survival of the female or male mice (Tables 8-11 and Figures 3-4). There was one low dose male mouse that had died prior to its terminal sacrifice which the sponsor classified as a death and this reviewer as a termianl sacrifice.

For the female mice there were highly statistically significant trends in the tumor incidence rates of adenoma, and adenoma and carcinoma combined of the pituitary, pars distalis, and of adenocarcinoma (incidental and fatal combined) of the mammary glands (Table 12). The frequency distributions were as follows:

	Ctrl 1	Ctrl 2	Low	Medium	High
Pituitary/Adenoma	0	0	5	10	15
Pituitary/Ade+Carc	0	0	5	10	17
Mammary/Adenocarc	1	2	9	5	21

The pairwise comparisons of controls versus high dose animals were also highly significant for these tumors. The tumor groupings as suggested in the Appendix did not produce any further statistically significant trends. The sponsor's finding of bronchoalveolar adenomas with a p=0.04 is not considered to be statistically significant. Among the male mice no statistically significant trends in tumor incidence rates were observed (Table 13). Again, the specified groups of tumors did not produce any statistically signicant trends.

Evaluating the validity of the male arm, it is clear that there was a sufficient number of high dose animals (60 percent) exposed to the compound for a sufficient length of time (103 weeks). From the sponsor's Table 2 it became clear that the high dose animals had a reduced average weight when compared to the controls early on (see table below). This differential reached almost 10 percent at day 372, suggesting that the high dose was close to the MTD.

Mean Body Weights (g), Male Mice

Dose	Day 8	Day92	Day 176	Day 204	Day316	Day 372	Day 540	Day 708
0	30.43	36.27	37.93	38.01	39.39	39.19	38.38	38.54
200	28.29	33.19	34.70	34.80	35.97	35.42	35.11	34.90
% Diff.	- 7.0	- 8.5	- 8.5	- 8.4	- 8.7	- 9.6	- 8.5	- 9.4

IV. Summary

In the rat study it was found that the drug did not have any negative effect on the survival of either sex. Also, neither sex showed a statistically significant increased linear trend in tumor incidence rates with dose. Investigating the validity of each study arm it was found that there were a sufficient number of high dose animals surviving a sufficient length of time to manifest any late developing tumors. Both high dose sexes experienced decreased weight gain of up to 10 percent during the first year when compared to their controls, suggesting that the high dose was close to the MTD.

In the mouse study, again, there was no negative effect on the survival of either sex by the drug. For the female mice there were highly statistically significant trends in adenomas, and adenomas and carcinomas combined of the pituitary, and in adenocarcinoma of the mammary glands. The tests for signficant differences between controls and high dose animals were also highly statistically significant. The male mice showed no statistically significant increases in tumor incidence rates. It was found, however, that there were a sufficient number of high dose males surviving to the end of the study. As the average body weight was suppressed to about 10 percent by the end of the first year one can conclude that the high dose was probably close to the MTD and the study appeared valid.

In either study groupings of certain tumors as listed in the Appendix did not produce any additional statistically significant results.

/S/

Roswitha E. Kelly
Mathematical Statistician

Concur:

/S/

Todd Sahlroot, Ph. D.
Team Leader

/S/ 1/16/98

George Chi, Ph.D.
Director, DB I

cc:Archival IND[ ]Ziprasidone HCL, Pfizer CARCINOGENICITY
HFD-120/Division File
HFD-120/Dr. Freed
HFD-120/Dr.Fitzgerald
HFD-120/Mr. Hardeman, CSO
HFD-344/Dr. Barton
HFD-710/Chron.
HFD-710/Dr. Chi
HFD-710/Dr. Sahlroot
HFD-710/Ms. Kelly
HFD-700/Dr. Fairweather

This review consists of 7 pages of text, 13 tables, 4 figures, and 1 Appendix.

Table 1:

Number of Animals 10:07 Wednesday, January 7, 1998 1

Species: Rat

Sex: Female

	Treatment Group					
	CTRL1	CTRL2	LOW	MED	HIGH	Total
	Count	Count	Count	Count	Count	Count
Time Interval						
0-52	2	2	1	2	1	8
53-78	7	6	2	1	3	19
79-91	8	9	6	9	7	39
92-103	8	9	17	6	9	49
104-105	25	24	24	32	30	135
Total	50	50	50	50	50	250

Table 2:

Dose-Mortality Trend Tests

10:07 Wednesday, January 7, 199

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Rat
Sex: Female

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	2.42	0.1201
	Depart from Trend	0.26	0.9681
	Homogeneity	2.67	0.6141
Kruskal-Wallis	Dose-Mortality Trend	2.59	0.1073
	Depart from Trend	0.78	0.8535
	Homogeneity	3.38	0.4968

Table 3

Number of Animals 10:07 Wednesday, January 7, 1998
Species: Rat
Sex: Male

	Treatment Group					
	CTRL1	CTRL2	LOW	MED	HIGH	Total
	Count	Count	Count	Count	Count	Count
Time Interval						
0-52	2	1	.	4	1	8
53-78	9	8	13	5	6	41
79-91	16	14	5	9	7	51
92-103	9	6	16	13	11	55
104-104	14	21	16	19	25	95
Total	50	50	50	50	50	250

Table 4 >

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Rat
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	4.80	0.0285
	Depart from Trend	2.66	0.4472
	Homogeneity	7.46	0.1135
Kruskal-Wallis	Dose-Mortality Trend	4.98	0.0257
	Depart from Trend	1.99	0.5739
	Homogeneity	6.97	0.1374

Table 5;

Test for Positive Dose-Response (Tumor) Linear Trend

Species: Rat 10:07 Wednesday, January 7, 1998

Sex: Female

Sorted by: Organ Name

..gan Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
2	Adrenal	10	B-CORTICAL ADENOMA	0.9921	0.9872	0.9880
2	Adrenal	11	B-PHEOCHROMOCYTOMA	0.5500	0.5377	0.5556
4	Brain	382	M-ASTROCYTOMA	0.6371	0.7010	0.7373
4	Brain	245	M-HISTOCYTIC SARCOMA	1.0000	0.8312	0.8569
4	Brain	295	M-NEUROFIBROSARCOMA	1.0000	0.8312	0.8569
4	Brain	380	M-OLIGODENDROGLIOMA	0.6530	0.6459	0.6875
5	Cervix	347	B-GRANULAR CELL TUMOR	1.0000	0.9133	0.9247
5	Cervix	281	B-LEIOMYOMA	0.2169	0.0466	0.0581
5	Cervix	371	M-LEIOMYOSARCOMA	1.0000	0.7974	0.8282
5	Cervix	362	M-SARCOMA, N.O.S.	0.6530	0.6459	0.6875
12	Jejunum	355	M-ADENOCARCINOMA	0.6371	0.7010	0.7373
13	Kidney	129	B-LIPOMA	1.0000	0.9248	0.9334
13	Kidney	119	B-RENAL TUBULE ADENOMA	1.0000	0.8312	0.8569
13	Kidney	180	M-LIPOSARCOMA	0.4593	0.3686	0.4099
13	Kidney	120	M-RENAL MESENCHYMAL TUMOR	0.2222	0.0516	0.0640
13	Kidney	338	M-TRANSITIONAL CELL CARCI	0.2169	0.0466	0.0581
14	Liver	181	B-HEPATOCELLULAR ADENOMA	0.4827	0.4547	0.4767
17	Lymphoreticular	387	M-EPITHELIAL THYMOMA	0.6217	0.6778	0.7160
	Lymphoreticular	377	M-LYMPHO-RETICULAR NEOPLA	0.1795	0.0341	0.0434
	Lymphoreticular	193	M-LYMPHOBLASTIC LYMPHOMA	1.0000	0.8159	0.8436
17	Lymphoreticular	220	M-MALIGNANT FIBROUS HISTI	0.6293	0.6364	0.6599
17	Lymphoreticular	321	M-PLASMA CELL LYMPHOMA	1.0000	0.8274	0.8535
17	Lymphoreticular	310	M-SMALL LYMPHOCYTIC LYMPH	0.1837	0.0276	0.0357
17	Lymphoreticular	211	M-THYMIC SQUAMOUS CELL CA	1.0000	0.8174	0.8449
18	Mesenteric node	353	M-HEMANGIOSARCOMA	0.6371	0.7010	0.7373
19	Mouth	341	M-SQUAMOUS CELL CARCINOMA	0.2099	0.0446	0.0560
20	Ovaries	204	B-CYSTADENOMA	1.0000	0.8053	0.8346
20	Ovaries	330	B-THECAL/GRANULOSA CELL T	0.0353	0.0045	0.0056
20	Ovaries	297	M-CYSTADENOCARCINOMA	0.6811	0.6390	0.6676
20	Ovaries	240	M-LEIOMYOSARCOMA	0.6413	0.6868	0.7243
21	Pancreas	112	B-ACINAR ADENOMA	0.6865	0.7390	0.7639
21	Pancreas	71	B-ISLET CELL ADENOMA	0.9873	0.9761	0.9779
21	Pancreas	165	M-CARCINOMA, ISLET CELL	0.7095	0.6709	0.6981
22	Pituitary	8	B-ADENOMA, PARS DISTALIS	0.9652	0.9620	0.9630
23	Salivary gland	284	B-ADENOMA [PAROTID GLAND]	1.0000	0.8312	0.8569
25	Skin and adnexa	351	B-ADENOMA [CLITORAL GLAND	0.6371	0.7010	0.7373
25	Skin and adnexa	195	B-ADENOMA, MAMMARY GLAND	0.7664	0.7669	0.7835
25	Skin and adnexa	146	B-FIBROADENOMA, MAMMARY G	0.2739	0.2659	0.2707
25	Skin and adnexa	59	B-FIBROMA, SUBCUTANEOUS	0.4360	0.3048	0.3322
25	Skin and adnexa	280	B-KERATO-ACANTHOMA	1.0000	0.8312	0.8569
	Skin and adnexa	296	B-LIPOMA	0.8700	0.8542	0.8710
	Skin and adnexa	217	B-SQUAMOUS CELL PAPILLOMA	0.1837	0.0276	0.0357
25	Skin and adnexa	298	M-ADENOCARCINOMA, MAMMARY	0.3678	0.3548	0.3699
25	Skin and adnexa	354	M-MELANOMA, MALIGNANT	0.6371	0.7010	0.7373
30	Thymus	209	M-CARCINOMA, SQUAMOUS CEL	0.4135	0.2828	0.3095
30	Thymus	227	M-THYMOMA, EPITHELIAL PRE	0.6217	0.6778	0.7160

Table 5 con'd

	Thymus	294	M-THYMOMA, LYMPHOCYTIC PR	0.1916	0.1546	0.1680
	Thyroid	277	B-ADENOMA (PARATHYROID)	1.0000	0.8312	0.8569
31	Thyroid	43	B-C-CELL ADENOMA	0.6525	0.6436	0.6511
31	Thyroid	58	B-FOLLICULAR CELL ADENOMA	0.5827	0.5664	0.5838
31	Thyroid	154	M-C-CELL CARCINOMA	0.9920	0.9851	0.9861
34	Uterus	274	B-FIBROMA	1.0000	0.9133	0.9247
34	Uterus	152	M-ANGIOSARCOMA	1.0000	0.9063	0.9185
34	Uterus	137	M-LEIOMYOSARCOMA	1.0000	0.8123	0.8404
35	Zymbal's gland	188	M-CARCINOMA	1.0000	0.8159	0.8436

Table 6:

Test for Positive Dose-Response (Tumor) Linear Trend

Species: Rat

Sex: Male

Sorted by: Organ Name

10:07 Wednesday, January 7, 1998

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
1	Abdomen	269	B-LIPOMA	1.0000	0.8324	0.8569
1	Abdomen	54	M-FIBROSARCOMA	1.0000	0.8149	0.8421
2	Adrenal	10	B-CORTICAL ADENOMA	0.8601	0.8485	0.8546
2	Adrenal	11	B-PHEOCHROMOCYTOMA	0.5451	0.5362	0.5451
2	Adrenal	285	M-CORTICAL CARCINOMA	0.8781	0.8617	0.8773
2	Adrenal	381	M-PHEOCHROMOCYTOMA, MALIG	0.4632	0.3944	0.4344
5	Bone, unspecdif	315	M-OSTEOSARCOMA	0.2800	0.0753	0.0908
6	Brain	307	B-GRANULAR CELL TUMOR	0.2043	0.0411	0.0516
6	Brain	245	M-HISTOCYTIC SARCOMA	1.0000	0.8420	0.8654
13	Heart	66	M-ATRIOCAVAL MESOTHELIOMA	1.0000	0.8189	0.8455
13	Heart	289	M-ENDOCARDIAL SARCOMA	0.6974	0.6819	0.7086
14	Kidney	129	B-LIPOMA	1.0000	0.7525	0.7881
14	Kidney	119	B-RENAL TUBULE ADENOMA	1.0000	0.8429	0.8688
14	Kidney	180	M-LIPOSARCOMA	0.2000	0.0411	0.0522
14	Kidney	120	M-RENAL MESENCHYMAL TUMOR	0.5985	0.5141	0.5388
14	Kidney	293	M-RENAL TUBULE CARCINOMA	1.0000	0.8324	0.8569
15	Liver	181	B-HEPATOCELLULAR ADENOMA	0.6159	0.6039	0.6187
15	Liver	290	M-HEPATOCELLULAR CARCINOM	0.4578	0.3286	0.3566
	Lymphoreticular	193	M-LYMPHOBLASTIC LYMPHOMA	1.0000	0.8310	0.8565
	Lymphoreticular	220	M-MALIGNANT FIBROUS HISTI	0.5167	0.5076	0.5246
8	Lymphoreticular	91	M-MYELOGENOUS LEUKEMIA	0.8361	0.8181	0.8336
8	Lymphoreticular	310	M-SMALL LYMPHOCYTIC LYMPH	0.0529	0.0109	0.0133
8	Lymphoreticular	255	M-STEM CELL LEUKEMIA	0.4016	0.3319	0.3725
9	Mesenteric node	118	M-SARCOMA, UNDIFFERENTIAT	1.0000	0.8189	0.8455
0	Mouth	259	M-OSTEOSARCOMA	0.4917	0.4119	0.4529
1	Pancreas	112	B-ACINAR ADENOMA	0.7223	0.7110	0.7208
1	Pancreas	71	B-ISLET CELL ADENOMA	0.1848	0.1696	0.1767
1	Pancreas	165	M-CARCINOMA, ISLET CELL	0.7730	0.7648	0.7767
2	Pituitary	8	B-ADENOMA, PARS DISTALIS	0.9998	0.9996	0.9996
3	Prostate	270	M-ADENOCARCINOMA	1.0000	0.8405	0.8647
4	Salivary gland	176	M-CYSTIC SARCOMA	1.0000	0.8306	0.8561
7	Skin and adnexa	195	B-ADENOMA, MAMMARY GLAND	1.0000	0.8324	0.8569
7	Skin and adnexa	46	B-DYSKERATOMA, WARTY	0.6954	0.6902	0.7134
7	Skin and adnexa	146	B-FIBROADENOMA, MAMMARY G	0.5936	0.6664	0.7054
7	Skin and adnexa	59	B-FIBROMA, SUBCUTANEOUS	0.7622	0.7495	0.7592
7	Skin and adnexa	182	B-FIBROUS HISTIOCYTOMA	1.0000	0.8189	0.8455
7	Skin and adnexa	264	B-HAIR FOLLICLE TUMOR	0.3594	0.2930	0.3112
7	Skin and adnexa	280	B-KERATO-ACANTHOMA	0.0289	0.0181	0.0201
7	Skin and adnexa	296	B-LIPOMA	0.6316	0.7100	0.7441
7	Skin and adnexa	217	B-SQUAMOUS CELL PAPILLOMA	0.4399	0.3892	0.4153
	Skin and adnexa	298	M-ADENOCARCINOMA, MAMMARY	0.0526	0.0114	0.0139
	Skin and adnexa	359	M-FIBROSARCOMA	0.5141	0.4860	0.5078
3	Soft tissue	109	B-FIBROMA	1.0000	0.8141	0.8412
3	Soft tissue	287	B-LIPOMA	1.0000	0.8324	0.8569
3	Soft tissue	229	M-SCHWANNOMA, MALIGNANT	0.5991	0.6678	0.7067
9	Spleen	348	M-HISTIOCYTIC SARCOMA	0.6316	0.7100	0.7441

Table 6' cont'd

30	Stomach	236	M-LEIOMYOSARCOMA	0.6666	0.7091	0.7445
31	Testes	267	B-INTERSTITIAL CELL ADENO	0.4195	0.3870	0.4060
32	Thymus	227	M-THYMOMA, EPITHELIAL PRE	0.5854	0.6073	0.6531
33	Thyroid	277	B-ADENOMA (PARATHYROID)	0.6316	0.7100	0.7441
33	Thyroid	43	B-C-CELL ADENOMA	0.7595	0.7503	0.7574
33	Thyroid	58	B-FOLLICULAR CELL ADENOMA	0.8529	0.8450	0.8487
33	Thyroid	154	M-C-CELL CARCINOMA	0.9364	0.9215	0.9278
33	Thyroid	379	M-FOLLICULAR CELL CARCINO	0.6316	0.7100	0.7441
36	Zymbal's gland	188	M-CARCINOMA	0.0758	0.0590	0.0655

Table 7

Summary of Mean Body Weight Changes In Long Evans Rats
Treated In Feed for Two Years with CP-88,059-1
Study No. 92-720-21

Mean Body Weight Percent difference from Control I

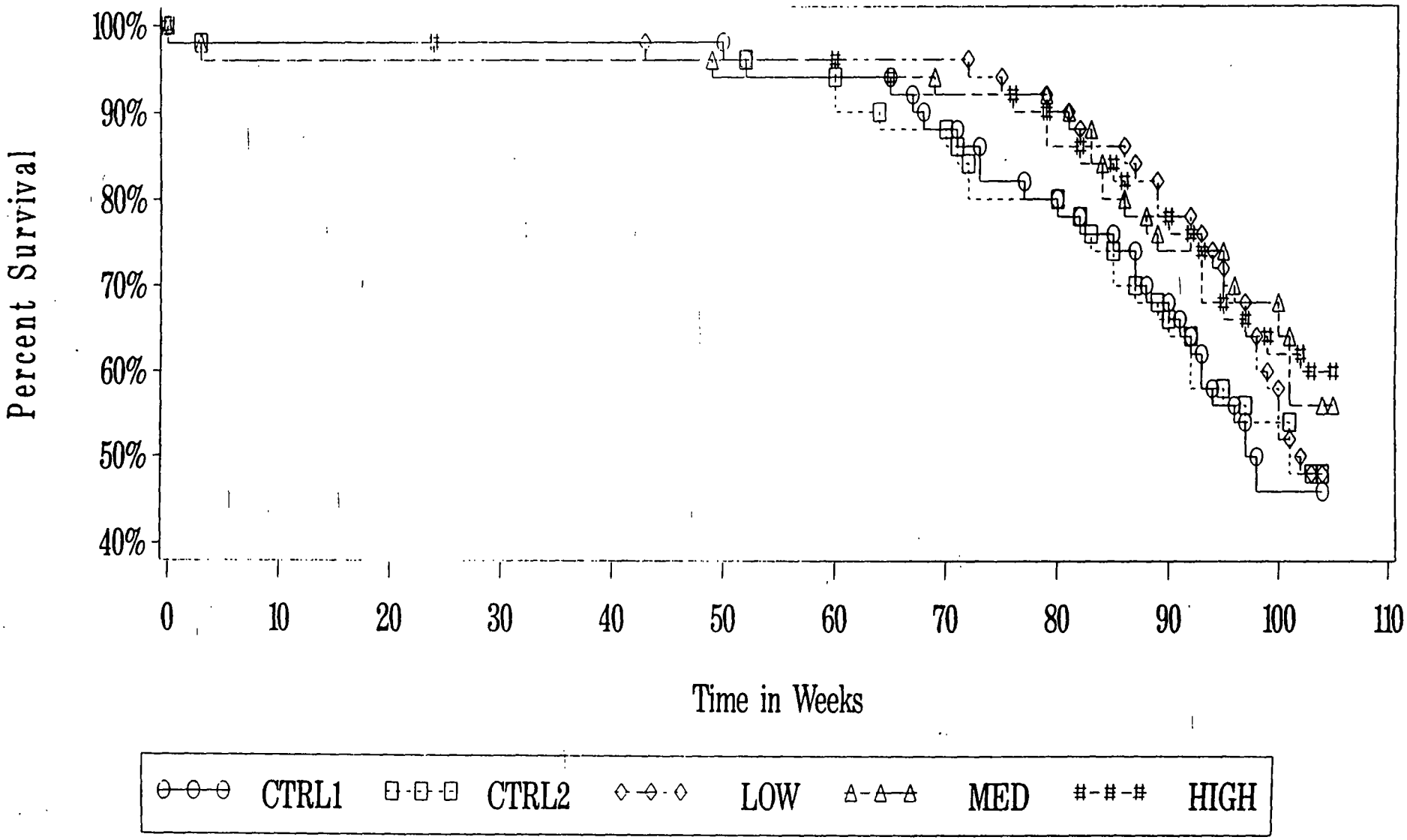
DOSE MG/KG	SEX	Day of Test 8	92	176	204	316	372	428	540	624	680	708
0*	M	0	0	0	0	0	0	0	0	0	0	0
0	M	+1.6	+3.9	+3.8	+3.9	+3.9	+3.9	+4.3	+5.5	+6.3	+12.1	+13.1
2	M	-0.4	-0.5	-0.5	-0.7	-0.5	0	-0.8	+0.7	+5.3	+2.4	+6.6
6	M	-0.3	-3.5	-4.8	-4.5	-4.3	-4.1	-4.9	-3.9	+0.6	+4.7	+7.5
12	M	-1.4	-6.3	-8.3	-8.7	-9.0	-9.0	-9.6	-9.2	-3.5	-2.2	-1.9
0*	F	0	0	0	0	0	0	0	0	0	0	0
0	F	+0.1	-2.5	-1.5	-0.6	-1.2	-0.5	-1.0	-3.7	-2.7	-2.4	+2.6
2	F	-3.8	-6.1	-5.3	-6.5	-3.3	-3.9	-3.6	-6.4	-9.4	-15.5	-8.8
6	F	-0.8	-5.8	-3.1	-2.5	-2.3	-2.5	-3.1	-6.6	-5.9	-11.2	-6.9
12	F	-2.6	-5.2	-4.9	-4.9	-5.7	-7.1	-9.8	-13.7	-16.3	-18.7	-13.6

*Control-1 group.

Kaplan–Meier Survival Function

Species: Rat

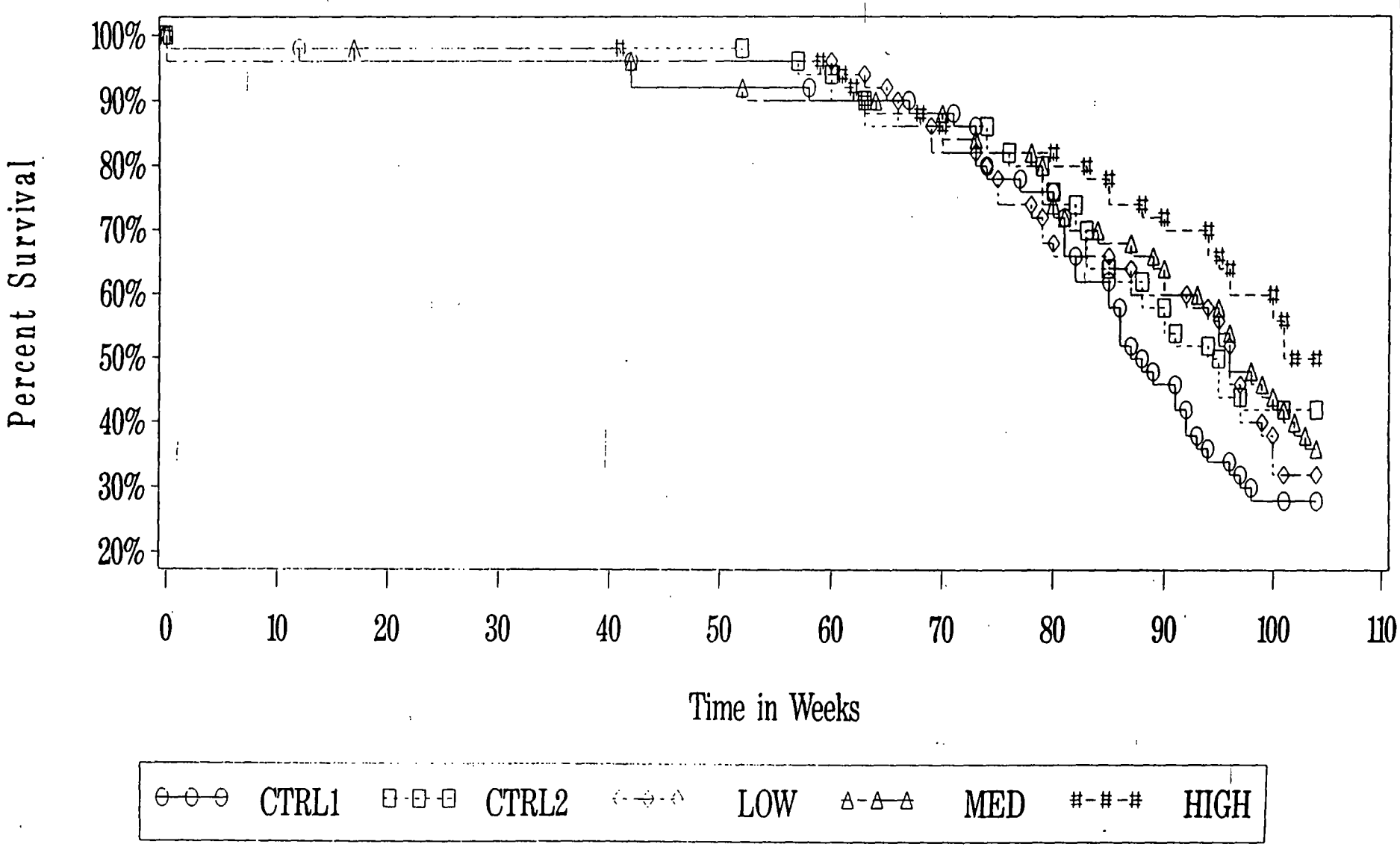
Sex: Female



Kaplan–Meier Survival Function

Species: Rat

Sex: Male



Species: Mouse
Sex: Female

	Treatment Group					
	CTRL1	CTRL2	LOW	MED	HIGH	Total
	Count	Count	Count	Count	Count	Count
Time Interval						
0-52	2	.	3	4	2	11
53-78	13	8	11	16	11	59
79-91	10	9	10	9	8	46
92-103	4	11	5	6	9	35
104-104	21	22	21	15	20	99
Total	50	50	50	50	50	250

APPEARS THIS WAY
ON ORIGINAL

Source: C:\fem1.dat

Table 7:

Dose-Mortality Trend Tests

15:13 Tuesday, December 16, 1997

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Female

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	0.47	0.4923
	Depart from Trend	4.38	0.2230
	Homogeneity	4.85	0.3026
Kruskal-Wallis	Dose-Mortality Trend	0.51	0.4750
	Depart from Trend	6.21	0.1020
	Homogeneity	6.72	0.1517

Source: C:\fem1.dat

Number of Animals
Species: Mouse
Sex: Male

	Treatment Group					
	CTRL1	CTRL2	LOW	MED	HIGH	Total
Time Interval	Count	Count	Count	Count	Count	Count
0-52	2	.	1	2	.	5
53-78	9	7	7	4	1	28
79-91	12	9	11	5	13	50
92-103	3	5	7	14	6	35
104-104	24	29	24	25	30	132
Total	50	50	50	50	50	250

Source: C:\mal1.dat

Table 11:

Dose-Mortality Trend Tests

17:22 Tuesday, January 6, 1998

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	1.43	0.2313
	Depart from Trend	2.22	0.5279
	Homogeneity	3.65	0.4550
Kruskal-Wallis	Dose-Mortality Trend	2.46	0.1168
	Depart from Trend	2.60	0.4568
	Homogeneity	5.06	0.2809

Source: C:\mal1.dat

Test for Positive Dose-Response (Tumor) Linear Trend

Species: Mouse 15:13 Tuesday, December 16, 1997

Sex: Female

Sorted by: Organ Name

Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
1	Abdomen	312	M-FIBROSARCOMA	1.0000	0.8116	0.8133
2	Adrenal	67	B-ADENOMA, SUBCAPSULAR CE	0.8138	0.7799	0.7811
2	Adrenal	315	M-CORTICAL CARCINOMA	1.0000	0.8150	0.8167
6	Brain	191	M-SARCOMA, MENINGEAL	0.5748	0.5952	0.5977
10	Cranium	270	M-OSTEOSARCOMA	0.2033	0.0419	0.0425
11	Duodenum	415	M-ADENOCARCINOMA	0.3535	0.3277	0.3301
14	Harderian gland	55	B-ADENOMA	0.6998	0.6729	0.6737
14	Harderian gland	135	M-ADENOCARCINOMA	0.2020	0.0388	0.0393
19	Liver	108	B-HAEMANGIOMA	0.6278	0.6191	0.6207
19	Liver	161	B-HEPATOCELLULAR ADENOMA	0.0928	0.0472	0.0475
19	Liver	52	M-HEPATOCELLULAR CARCINOM	1.0000	0.8924	0.8932
20	Lungs	31	B-BRONCHIOLAR-ALVEOLAR AD	0.0432	0.0335	0.0335
20	Lungs	132	M-BRONCHIOLAR-ALVEOLAR CA	0.8314	0.8109	0.8114
22	Lymphoreticular	427	M-HISTIOCYTIC SARCOMA	0.8508	0.8263	0.8268
22	Lymphoreticular	40	M-LYMPHOBLASTIC LYMPHOMA	0.9310	0.9229	0.9230
25	Ovaries	221	B-CYSTADENOMA	0.9202	0.8794	0.8801
25	Ovaries	247	B-DYSGERMINOMA	0.2571	0.0634	0.0641
25	Ovaries	416	B-LEIOMYOMA	1.0000	0.8086	0.8104
	Ovaries	232	B-SEX CORD/STROMAL TUMOUR	0.8989	0.8746	0.8750
	Ovaries	397	B-TUBULOSTROMAL ADENOMA	0.5797	0.5391	0.5403
28	Pituitary	240	B-ADENOMA, PARS DISTALIS	0.0000	0.0000	0.0000
28	Pituitary	374	B-ADENOMA, PARS INTERMEDI	0.0464	0.0280	0.0282
28	Pituitary	267	M-CARCINOMA, PARS DISTALI	0.0479	0.0089	0.0090
30	Skeletal muscle	289	B-HAEMANGIOMA	0.3722	0.3361	0.3385
31	Skin and adnexa	292	B-ADENOMA, MAMMARY GLAND	0.4028	0.2832	0.2847
31	Skin and adnexa	170	B-HAEMANGIOMA	1.0000	0.9016	0.9024
31	Skin and adnexa	404	B-KERATO-ACANTHOMA	0.2020	0.0388	0.0393
31	Skin and adnexa	109	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.8171	0.8189
31	Skin and adnexa	249	M-ADENOACANTHOMA, MAMMARY	0.2571	0.0634	0.0641
31	Skin and adnexa	237	M-ADENOCARCINOMA, MAMMARY	0.0000	0.0000	0.0000
32	Soft tissues	334	M-FIBROUS HISTIOCYTOMA, M	0.5715	0.5996	0.6021
33	Spleen	425	M-HAEMANGIOSARCOMA	0.5656	0.5844	0.5870
34	Stomach	159	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.8467	0.8484
35	Thymus	205	M-THYMOMA, EPITHELIAL PRE	0.7044	0.6576	0.6593
39	Uterus	218	B-ENDOMETRIAL STROMAL POL	0.9997	0.9941	0.9942
39	Uterus	332	B-HAEMANGIOMA	0.8454	0.8049	0.8063
39	Uterus	307	B-LEIOMYOMA	0.9968	0.9879	0.9880
39	Uterus	400	M-CARCINOMA	0.2020	0.0388	0.0393
39	Uterus	319	M-LEIOMYOSARCOMA	1.0000	0.8126	0.8144
39	Uterus	423	M-SARCOMA, N.O.S.	0.5656	0.5844	0.5870
	Uterus	219	M-STROMAL CELL SARCOMA	1.0000	0.9808	0.9809

Source: C:\fem1.dat

Table 15:

Test for Positive Dose-Response (Tumor) Linear Trend

Species: Mouse 17:22 Tuesday, January 6, 1998

Sex: Male

Sorted by: Organ Name

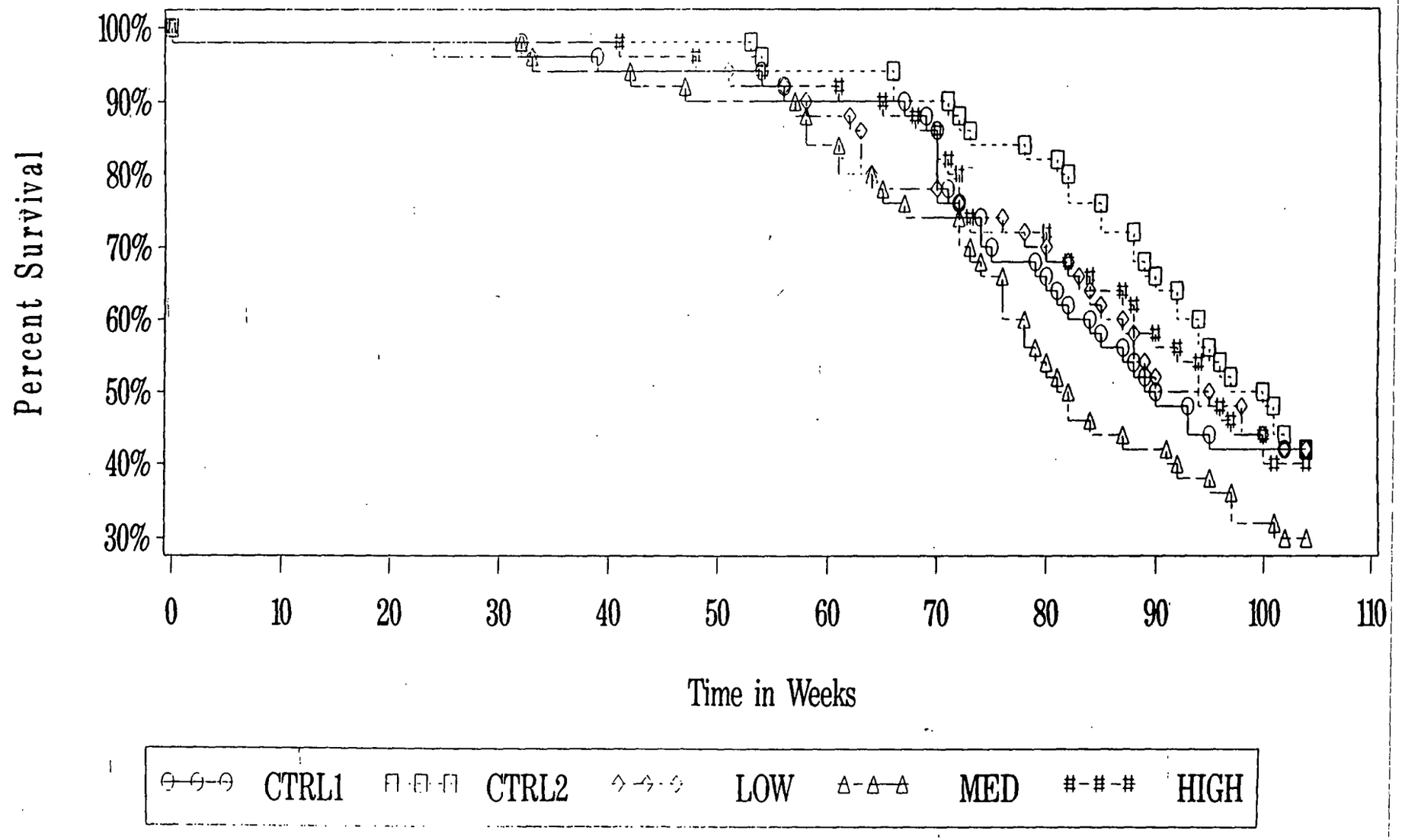
Organ Code	Organ Name	Tumor Code	Tumor Name	Exact-P	Asymp-P	AsyCor-P
2	Adrenal	67	B-ADENOMA, SUBCAPSULAR CE	0.9862	0.9693	0.9694
2	Adrenal	391	B-CORTICAL ADENOMA	0.4167	0.3665	0.3689
7	Brain	191	M-SARCOMA, MENINGEAL	0.4481	0.3748	0.3773
14	Harderian gland	55	B-ADENOMA	0.0527	0.0430	0.0433
14	Harderian gland	135	M-ADENOCARCINOMA	0.3688	0.2973	0.2987
17	Jejunum	365	B-PAPILLOMA	1.0000	0.8278	0.8295
18	Kidney	79	B-RENAL TUBULE ADENOMA	1.0000	0.9035	0.9043
19	Liver	108	B-HAEMANGIOMA	0.9775	0.9461	0.9464
19	Liver	161	B-HEPATOCELLULAR ADENOMA	0.3864	0.3633	0.3637
19	Liver	52	M-HEPATOCELLULAR CARCINOM	0.9978	0.9894	0.9894
20	Lungs	31	B-BRONCHIOLAR-ALVEOLAR AD	0.7094	0.6944	0.6945
20	Lungs	132	M-BRONCHIOLAR-ALVEOLAR CA	0.2888	0.2641	0.2652
22	Lymphoreticular	427	M-HISTIOCYTIC SARCOMA	0.7746	0.7485	0.7492
22	Lymphoreticular	40	M-LYMPHOBLASTIC LYMPHOMA	0.7489	0.7281	0.7290
22	Lymphoreticular	377	M-PLASMA CELL LYMPHOMA	1.0000	0.8278	0.8295
25	Pancreas	390	B-ISLET CELL ADENOMA	0.4167	0.3665	0.3689
27	Pituitary	374	B-ADENOMA, PARS INTERMEDI	0.6935	0.6661	0.6678
30	Seminal vesicle	75	M-LEIOMYOSARCOMA	1.0000	0.9019	0.9032
	Skin and adnexa	170	B-HAEMANGIOMA	0.6461	0.6434	0.6458
35	Skin and adnexa	109	B-SQUAMOUS CELL PAPILLOMA	1.0000	0.7589	0.7621
35	Stomach	159	B-SQUAMOUS CELL PAPILLOMA	0.4286	0.3696	0.3735
36	Testes	80	B-INTERSTITIAL CELL ADENO	0.3451	0.3151	0.3158
38	Thymus	193	M-SARCOMA	0.1714	0.0380	0.0387
39	Thyroid	146	B-FOLLICULAR CELL ADENOMA	0.1311	0.0863	0.0869
39	Thyroid	173	M-FOLLICULAR CELL CARCINO	0.6218	0.6288	0.6313

Source: C:\mal1.dat

Kaplan–Meier Survival Function

Species: Mouse

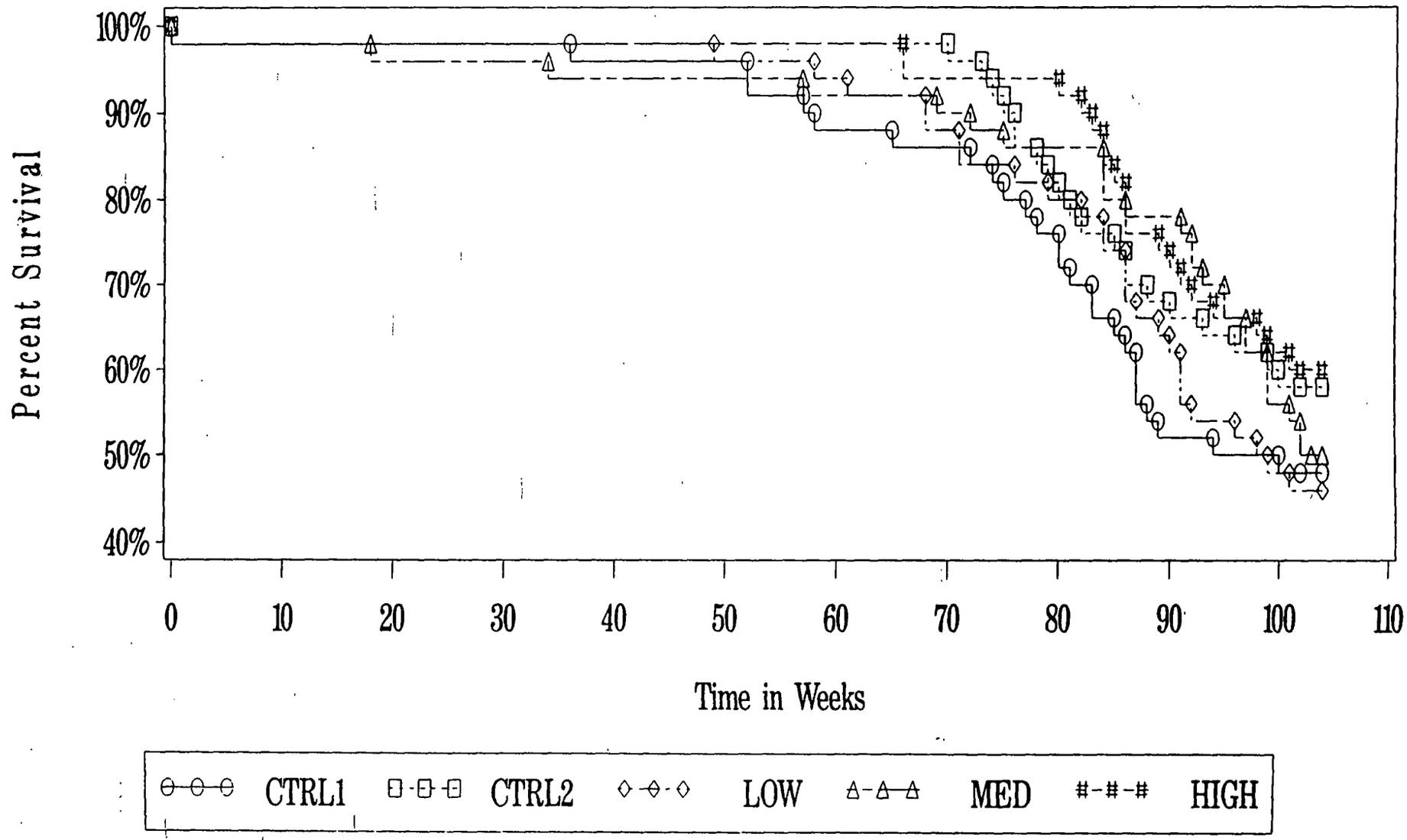
Sex: Female



Kaplan–Meier Survival Function

Species: Mouse

Sex: Male



APPENDIX

The following combinations of tumors were also tested for statistically significant trends with dose:

Mammary Gland:	**Adenomas, adenofibromas, fibroadenomas.**
Skin:	**Keratoacanthomas, squamous cell papillomas.**
Lung:	**Bronchilar alveolar adenomas, - carcinomas.**
Liver:	**Hepatocellular adenomas, - carcinomas.**
Thyroid:	**Follicular cell adenomas, - carcinomas, C-cell adenomas, - carcinomas.**
Vascular endothelium:	**Hemangiomas, hemangiosarcomas.**
Mouse Only: Adrenal:	**Subcapsular cell adenoma, cortical carcinoma.**