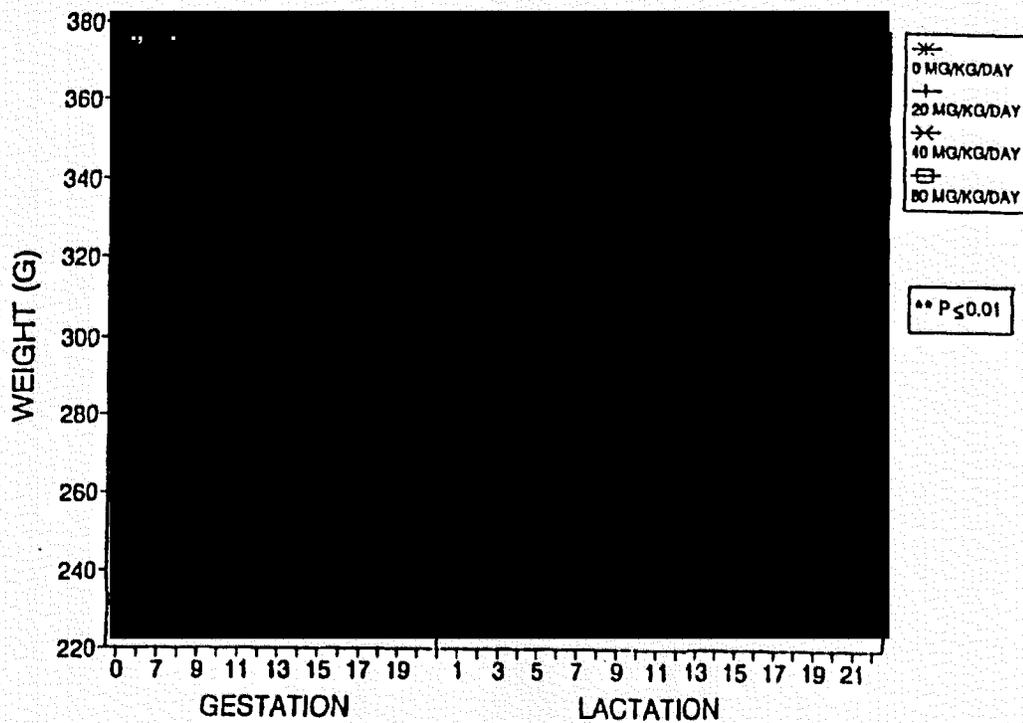


C. Segment II Teratology Study in Rats (TR7224-93-019)

a. Methods: Lot# (D)25064-MIC-8. Thirty-three to 35 pregnant CrI:SD(BR) VAP/Plus rats were given pioglitazone orally by gastric intubation at doses of 0, 20, 40 and 80 mg/kg/day on days 6 through 17 of presumed gestation. The rats from each dosage group were randomly selected for cesarean section on day 20 of gestation and the fetuses were obtained for morphological evaluation. Other F<sub>0</sub> dams in each group were permitted to deliver naturally. The development and reproductive function of the pups were assessed.

b. Results: No F<sub>0</sub> maternal deaths occurred and no clinical findings that are related to the treatment were observed. Dam body weight gains were significantly reduced on day 18 to 20 of gestation in the high dose group (See the fig. Below). These decreases in weight gain were, at least partly, the result of increased incidences in embryo deaths and reductions in gravid uterine weight in these groups. Biologically and statistically significant delays in the onset of parturition were found in the 40 and 80 mg/kg/day groups. While body weight gains were generally higher among the AD-4833(HCl) dosage groups during lactation, the numbers of pups in the 40 and 80 mg/kg/day groups were reduced.



Pioglitazone Effects on Body Weights in Fo Female Rats During Gestation and Lactation

Gross external, soft tissue and skeletal examinations of the F<sub>1</sub> fetuses did not reveal alterations attributable to AD-4833(HCl). However, the average F<sub>1</sub> fetal body weight was significantly reduced in the 80 mg/kg/day group. Administration of this drug at 40 and 80 mg/kg/day led to significant increases in the incidence of stillbirths and pup

deaths on postpartum day 1 and between postpartum days 2 and 4. Litter size was reduced throughout lactation and pup weight in the 80 mg/kg/day group was significantly reduced on postpartum day 1.

In the F<sub>1</sub> generation, no deaths occurred and clinical observations were not remarkable. Mean body weights in the 80 mg/kg/day F<sub>1</sub> males were significantly reduced from day 1 pre-mating to cohabitation, and mean body weight gain in the 80 mg/kg/day F<sub>1</sub> females was significantly reduced between days 0 and 6 of gestation. Differences between groups in sexual maturation, mating and fertility in the F<sub>1</sub> generation were not apparent. Gross external examination of the F<sub>2</sub> fetuses did not reveal alterations attributable to AD-4833(HCl) administration to the F<sub>0</sub> maternal animals. Cesarean section and litter parameters in the F<sub>1</sub> dams on gestation day 20 were comparable in all groups.

c. Conclusion: The basis of the results from this study, particularly the delays in parturition found in the 40 and 80 mg/kg/day groups, the maternal no-observable-adverse-effect-level in F<sub>0</sub> females for AD-4833(HCl) was 20 mg/kg/day. The NOAEL for developmental toxicity was also judged to be 20 mg/kg/day, based on the reductions in embryo viability, litter size and pup survival in the 40 and 80 mg/kg/day groups; fetal and pup (postpartum day 1) weight in the 80 mg/kg/day group were also reduced. The NOAEL for the F<sub>1</sub> generation rats was 40 mg/kg/day based on reduced body weights (pre-mating period) for males and reduced body weight gains (gestation period) for females in the 80 mg/kg/day group.

#### D. Range-Finding Segment II Study in Rabbits (TR#7224-92-078)

a. Methods: Lot#INV.R2895. Five Dutch-belted pregnant rabbits/group were given pioglitazone orally at doses of 0, 5, 40, 100 or 400 mg/kg/day on gestation days 6 through 18. All does were sacrificed on gestation day 28. Plasma samples were collected from two does per group on gestation days 6, 12 and 18.

b. Results: Mortalities occurred in the control and two highest dose groups. One control rabbit died on gestation day 15, one 100 mg/kg/day rabbit died on gestation day 8 due to a dosing error, and all high-dose rabbits died within one to three days of dosing. Thus, the sponsor reduced the top dose from 400 to 160 mg/kg/day in subsequent segment II study as presented under item E.

At necropsy, one control animal had pitted kidneys and the control animal that died had tan fluid in the intestines, the 100 mg/kg/day doe that died exhibited fluid in the thoracic cavities and discolored lung lobes, and two high-dose does exhibited reddened stomach mucosa and distended stomachs. Cesarean section revealed no effect on the numbers of resorptions, corpora lutea, implantations, and live or dead fetuses. No malformations occurred.

Plasma concentrations of AD-4833, M-II, M-III and M-IV increased with increasing dose, but in a less than proportional manner. Concentrations of the parent drug decreased from gestation day 6 to day 12, with a further decrease on day 18. An increase in

metabolite levels was not noted during this time. It appears that the NOAEL for maternal and developmental toxicity might be near 40 mg/kg/ day.

### E. Segment II Teratology Study in Rabbits (TR7224-93-021)

a. Methods: Lot# INV.R3286. Fifteen to 18 Dutch-belted female rabbit were given pioglitazone orally by gastric intubation on days 6 through 18 of gestation at doses of 0, 40, 80 and 160 mg/kg/day. The does were sacrificed on gestation day 28 for cesarean section examinations.

b. Results: Drug administration resulted in slight maternal toxicity in the mid- and high-dose groups as evidenced by increased incidences of toxicological signs (slight weight loss, scant feces and not eating), which occurred mostly during the treatment period. Some of the findings are documented in a table below. One doe in the high-dose group (doe 73) died on gestation day 26; the cause of death could not be determined for this animal.

The drug was slightly embryotoxic (increased post-implantation loss) at 160 mg/kg/day, a dose that also produced maternal toxicity as shown in a table below. AD-4833 and four of its metabolites were found in circulating sera following oral administration. The levels of AD-4833 increased with dose, as did the four quantitated metabolites and the total quantifiable drug-related material. Although the levels of AD-4833 and three of its metabolites (M-II, M-III, and M-IV) decreased on day 13 of dosing (day 18 of gestation), relative to the first day of dosing, the serum level of the other quantitated metabolite (M-V) increased; thus, the average total steady-state concentration of the analytes was approximately the same on both days.

Observations or Major Findings	Pioglitazone(free base) Dose in mg/kg/day			
	0	40	80	160
Body Weight(g) on Gestation Day 15	3293	3244	3283	3073
Weight Change(%) from Control@	0	-2	0	-6
Not Eating(Mean # of Days)	2.0	1.8	2.9	7.1
Scant Feces(Mean # of Days)	2.7	1.0	4.8	5.8
No. of Does Inseminated	19	19	19	19
No. of Litters	17	17	16	11
Mean No. of Implantations per Doe	7.2	6.8	7.6	6.8
Mean No. of Live Fetuses	6.6	5.9	6.4	4.6
Mean No. of Early Resorptions per Doe	0.4	0.7	1.0	1.5
Mean No. of Early and Late Resorption	0.6	0.9	1.2	2.2*
Postimplantational Loss(%)	8.9	13.1	15.5	32.5*

\*Statistically significantly different from the control group,  $p < 0.05$ . @The data were based on findings on gestation day 15.

c. Conclusion: The segment II teratology study indicates that female Dutch-belted rabbits dosed orally on days 6 to 18 of gestation had mild signs of maternal toxicity at 80

and 160 mg/kg/day. The no-observed-adverse-effect-level for maternal toxicity was 40 mg/kg/day. Slight embryotoxicity was observed at 160 mg/kg/day, a dose that also produced maternal toxicity. Thus, the doses were appropriate and experimental procedures were carried out under valid conditions.

#### F. Segment III Peri-Postnatal Study I in Rats (TR7224-93-012)

a. Methods: Lot# INV.R2895. Pioglitazone was given orally by gastric intubation at dosage levels of 0, 10, 20 and 40 mg/kg/day to groups of 24 female Crl:CD[BR] (Sprague-Dawley) rats. The rats arrived timed-pregnant (gestation day 0) and were treated on gestation day 15 through postpartum day 21 (weaning); they were killed after F<sub>1</sub> weaning. F<sub>1</sub> rats were evaluated for functional development and reproductive ability.

b. Results: In adult F<sub>0</sub> female Crl:CD[BR] rats the test article did not produce overt clinical toxicity or gross lesions (F<sub>0</sub> dams and F<sub>1</sub> pups). However, F<sub>0</sub> maternal body weight gain during gestation and lactation was increased in all dose groups. The increases in body weight gain occurred, with or without corresponding increases in food consumption. Maternal exposure to AD-4833(HCl) at all dose levels resulted in reduced pup body weight throughout lactation and maturation, which led to delays in anatomical and functional (behavioral) development. Also, there was a slight decrease in pup survival over the first 24 hours postpartum in the high-dose group only. Because administration of AD-4833(HCl) at the three dose levels tested resulted in a general increase in F<sub>0</sub> maternal body weight (gestation and postpartum), increased food consumption during gestation and decreased postpartum food consumption and adversely affected F<sub>1</sub> body weight (decreased) and functional (behavioral) development (delayed), a no-observed adverse effect level in the rat for F<sub>0</sub> maternal and F<sub>1</sub> developmental toxicity could not be established. However, the dose selection was valid since the mid and high doses increased F<sub>0</sub> cardiac weight significantly (P<0.01) from the control by 116% and 115%, respectively.

c. Conclusion: AD-4833 treatment of pregnant Sprague-Dawley rats from gestation day 15 through postpartum day 21 at levels of 0, 10, 20 and 40 mg/kg/day resulted in maternal effects (body weight gain with and without increased food consumption). Pups from dams of all dose groups had reduced body weight and delayed functional (behavioral) development. Pups from dams in the high-dose group had slightly decreased survival for the first 24 hours postpartum. AD-4833(HCl) treatment did not adversely affect F<sub>0</sub> or F<sub>1</sub> reproductive performance. A NOAEL for F<sub>0</sub> maternal and F<sub>1</sub> developmental toxicity could not be established in this study. It is possible that AD-4833(HCl) affected milk production and/or the makeup of the milk.

#### G. Segment III Peri-Postnatal Study II in Rats (Protocol#520-002)

a. Methods: Lot# INV R4209. Pioglitazone was administered orally to Crl:CDBR VAF/Plus female rats (n = 25/group) at doses of 0, 0.3, 1, 3, and 10 mg/kg/day.

Treatment for the  $F_0$  females began on gestation day 15 and continued through postpartum day 22. Adult males were not treated. On day 4 postpartum, each litter was culled to eight pups. Weaning occurred on day 22 postpartum at which time 25 males and 25 females from each treatment group were selected. On day 90, these  $F_1$  animals were mated. The males were sacrificed immediately following the cohabitation period and the females were sacrificed on gestation day 20.

b. Results: For the  $F_0$  females, no deaths occurred and no treatment effects were reported for clinical signs, body weight, body weight gain, gross lesions, and absolute and relative (to body weight) heart weights. Absolute and relative food consumption was statistically significantly increased on gestation days 15 to 20 in the  $F_0$  females treated with 3 and 10 mg/kg/day. High-dose dams experienced an increased percentage of dams with stillborn pups. An increased average number of stillborn pups per litter and a statistically significant increase in the percentage of still born pups, although these effects were not determined to be treatment-related for several reasons. In the high-dose group, pup weight was decreased throughout lactation reaching statistical significance on days 14 and 22 postpartum. No effects were noted on duration of gestation, gestation index, duration of delivery per pup, or the average number of implantation sites per delivered litter. The live birth index, sex ratio, weaning index, surviving pups per litter, percent male pups and mean live litter size were also not affected.

Clinical and necropsy findings in the pups were not related to AD-4833(HCl) treatment and no effect was noted on reflex or physical development (surface righting, pinna unfolding, eye opening, acoustic startle, air righting, pupil constriction).

In the  $F_1$  offspring, no treatment-related deaths, clinical signs, necropsy findings or changes in sexual maturation were reported. No effects were noted on terminal body weight, absolute individual testes or epididymides weights and the ratio of these organ weights to body weight in the  $F_1$  males. High-dose males and females exhibited a statistically significant reduction in body weight up to postweaning days 50 and 15, respectively. In addition, body weight gain was statistically significantly reduced for the high-dose  $F_1$  males through postweaning day 29. The body weight effect corresponded to a statistically significant reduction in absolute food consumption in the high-dose  $F_1$  males up to postweaning day 22. A related finding was the statistically significant increase in relative food consumption in high-dose  $F_1$  males between 22 and 71 days postweaning and in high-dose  $F_1$  females between 15 and 22 days postweaning.

Behavioral examinations conducted in the  $F_1$  postweaning rats revealed no adverse treatment effect on learning, short-term retention, long-term retention or response inhibition. Additionally, mating and fertility parameters (average number of days of cohabitation, number of rats mated, fertility index, or number of pregnancies per rats that cohabited) in the  $F_1$  offspring were not affected by maternal doses of AD-4833(HCl) as high as 10 mg/kg/day.

Of 25  $F_1$  females mated per group, the pregnancy incidences were 24, 25, 23, 24 and 25 at respective  $F_0$  maternal doses of 0, 0.3, 1, 3 and 10 mg/kg/day. Cesarean section did not

reveal any treatment-related changes in litter averages for corpora lutea, implantations, live litter sizes, resorptions, fetal body weights, fetal sex ratios or the number of dams with resorptions. No gross external fetal alterations were observed.

c. Conclusion: F<sub>0</sub> maternal NOEL might be 1 mg/kg/day (based on increased food consumption at the higher doses). In the offspring, reduced body weight occurred at 10 mg/kg/day during the lactation and postweaning periods. However, it is not clear whether this effect arose secondary to the therapeutic effects of pioglitazone or whether it was directly related to AD-4833(HCl) toxicological actions. Because of this, a NOEL was not unequivocally identified for the offspring, but the value was at least 3 mg/kg/day. In addition, it appears that the top dose might be too low to evaluate the peri-Postnatal actions of the drug since the dose did not produce any notable toxic signs in F<sub>0</sub>.

#### H. Overall Summary of Pioglitazone Effects on Reproductive System

In rats, pioglitazone had no remarkable effects on parental fertility at the top dose of 40 mg/kg/day, although the dose increased cardiac weight by 15%. The dose represents a multiple of approximately 9 times of the maximum recommended human oral dose of 45 mg based on mg/m<sup>2</sup>. But, the drug was embryotoxic at the dose since it increased postimplantation losses and reduced fetal body weights.

The teratologic effects of pioglitazone were evaluated in rabbits at doses of 40, 80 and 160 mg/kg/day. Two high doses produced some toxic signs such as not eating and weight losses in does. The top dose (9 multiples of the maximum recommended human dose) increased postimplantation loss as the case of rat studies. Pioglitazone effects on peri-postnatal development were studied in rat at doses of 10, 20 and 40 mg/kg/day. There was no overt clinical toxicity in F<sub>0</sub> dams, except a slight increase in body weight. Maternal drug exposure also resulted in reduced pup body weight, which led to delays in physical and behavioral development.

### 6. GENETIC TOXICITY STUDIES

#### A. Bacterial Mutagenicity Studies (Study#45/MH)

a. Methods: Pioglitazone was dissolved in DMSO and tested in the plate incorporation assay with a 20-minute liquid preincubation step using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2uvrA at doses of 156, 313, 625, 1250, 2500 and 5000 µg/plate with or without metabolic activation. Pioglitazone was also tested for induction of reverse mutations in the plate incorporation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at doses of 250, 500, 1000 and 2000 µg/plate.

b. Results: These two tests showed no evidence of bacterial mutagenicity, either in the presence or absence of rat liver S9 metabolic activation system. The positive controls

showed expected levels of mutagenicity. In the bacterial mutagenicity assay using *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WPuvrA, Metabolites M-I and M-IV showed no mutagenic activity either in the presence or absence of S9 mix. The mutagenicity of Metabolite-V and Metabolite-VI was examined in an Ames test. *S. typhimurium* strains TA98, TA100, TA1535 and TA37 and *E. coli* strain WP2uvrA were used with or without metabolic activation, of which results found that the metabolites were not mutagenic at concentrations of 250, 500, 1000 and 2000 µg/plate.

**B. Mammalian Cell Forward Mutation (CHO/HPRT and AS52/XPRT)** In this study the sponsor changed pioglitazone lot#(A)1185-TGS-65 to Lot#11885-TGS-65 due to inadvertent error( Document#PN661,T-62)

a. Methods: Dose levels for the definitive mutation assays were chosen on the basis of range-finding experiments using 0.167, 0.500, 1.67, 5.00, 16.7, 50.0, 167, and 5000 µg/mL with and without metabolic (S9) activation. The limit of solubility of pioglitazone was exceeded above 167 µg/mL, and cytotoxicity was noted in both cell lines in the absence of S9. Based on the results of this cytotoxicity prescreen, doses of 25.0, 50.0, 75.0, 100, 200, 300, 400 and 500 µg/mL with S9, and 25.0, 50.0, 75.0, 100, 125, 150, 175 and 200 µg/mL without S9 were evaluated in the mutation assay in each cell line.

b. Results: There were no dose-dependent or statistically significant increases in the average mutant frequencies of either cell line due to treatment with pioglitazone. This conclusion was true whether S9 was used or not. The positive, vehicle and untreated controls performed as expected. Therefore, AD-4833 was judged to be nonmutagenic in both the CHO/HPRT and the AS52/XPRT mammalian cell forward gene mutation assays.

**C. Mammalian Forward Mutation (L5178Y/TK) Assays (T-63)** This study was conducted by [REDACTED]

a. Methods: Pioglitazone was evaluated for its ability to induce forward mutations at the thymidine kinase (TK) locus in cultured mouse lymphoma L5178Y cells. Based upon the results of cytotoxicity prescreen, two independent assays were performed. In the first experiments concentrations of 25, 50, 100 and 200 µg/mL, and in the second experiment concentrations of 25, 50, 100, 200 and 300 µg/mL were evaluated in the presence and absence of S9.

b. Results: In mouse lymphoma assays, metabolite M-I produced a positive response in the presence of metabolic activation and an equivocal response in the absence of metabolic activation. Metabolite M-IV was considered negative in the assay at the limits of solubility, although an increase in mutant frequencies was seen in the presence of precipitated drug. Metabolite M-V was considered negative in this assay. Some of the positive responses occurred at concentrations that were also highly toxic to the cells and, thus, the positive responses must be viewed with caution. M-VI also produced a positive

response in the presence of metabolic activation and an equivocal response in the absence of metabolic activation.

#### D. In vitro Cytogenetic Test (T-68, T-69, T-70)

a. Methods: Chinese hamster lung cells (CHL) were exposed in vitro to AD-4833(HCl) for 24 or 48 hours in the absence of a rat liver S9 metabolic activation system and for 6 hours with or without S9. Treated metaphase cells were analyzed for chromosome aberrations at three successive doses set at two-fold intervals: from 0.02 to 0.313 mM in the 24- and 48-hour treatment groups and from 0.313 to 5 mM in the 6-hour treatment groups with or without S9. Dosage levels were determined on the basis of a previously conducted range-finding study.

b. Results: The frequency of cells with chromosomal aberrations did not increase significantly compared to the negative control at any dose tested in any treatment group, which suggests pioglitazone might not be mutagenic in this cytogenetic test. In a cytogenetics assay, however, metabolite M-I produced an increase in mutant frequencies.

#### E. Unscheduled DNA Synthesis (UDS) Assay (T-71)

a. Methods: Pioglitazone was evaluated in an unscheduled DNA (UDS) assay, which measures DNA repair following chemically-induced DNA damage. Two experiments were conducted, each using rat primary hepatocyte cultures prepared by collagenase perfusion of the liver from a male Fischer-344 rat. For each dose, triplicate cultures were treated in the presence of [<sup>3</sup>H]-thymidine for 19-20 hours. Treatment conditions were 24- and 48-hour treatment without S9 mix, which were followed by 6-hour pulse treatment with/out S9 mix. The cultures were fixed, washed, mounted on microscope slides and evaluated for UDS by autoradiography. In the preliminary experiment, cultures were treated with 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50 and 100 µg/mL AD-4833. Slides were evaluated for UDS at 1, 5, 10, 25, 50 and 100 µg/mL. In the replicate experiment, cultures were treated at 1, 5, 10, 25, 50 and 100 µg/mL and the slides were evaluated for UDS.

b. Results: Pioglitazone did not induce a significant increase in UDS over the solvent control. In each experiment, positive, solvent and untreated controls were used and each performed as expected. AD-4833 was not a genotoxic agent under the conditions of this in vitro rat hepatocyte DNA repair assay.

#### F. Micronucleus Test (T-72)

a. Methods: Pioglitazone was evaluated to determine its ability to induce the formation of micronucleated polychromatic erythrocytes in the bone marrow of treated animals. It was administered via a single intraperitoneal injection to CD-1 mice in a range-finding study involving two males and two females per group at doses of 250, 500, 750, 1000, 1250, 2500 and 5000 mg/kg of body weight. On the basis of the range-finding study, the doses chosen for the definitive micronucleus test were 1250, 2500 and 5000 mg/kg. Five

males and five females were treated for each dose group. Slides were prepared from the bone marrow cells collected at 30, 48 and 72 hours post-dose and scored blind. One thousand polychromatic erythrocytes per animal were scored for the presence of micronuclei.

b. Results: There were no statistically significant increases in the number of micronuclei in polychromatic erythrocytes at any dose due to treatment with AD-4833. The positive and negative controls performed as expected. Therefore, AD-4833 was judged to be nonmutagenic under the conditions of the assay.

## 7. CARCINOGENICITY STUDIES

A. 104-Week Oral (Gavage) Carcinogenicity Studies of pioglitazone in Mice(Study#295-153). [REDACTED] in Japan under GLP conducted this study.

a. Methods: Male and female Crl:CD-1.VAF/plus mice(60/sex/group) were given either vehicle, placebo(suspension) or pioglitazone(Upjohn preparation) at doses of 3, 10, 30 or 100 mg/kg/day for 104 weeks. An additional satellite group (20/sex/group) was also included for plasma analysis at 6, 12, 18 and 24 months.

The doses were selected in this study based on available data obtained from a 13-week oral gavage study in mice. In the study pioglitazone granulated formulation were administered at doses of 0, 3.2 10, 32, 100 and 320 mg/kg/day. There were increased heart weight in males at 100 mg/kg/day and in females at 320 mg/kg/day. Additionally 4 males out of 10 and 6 females out of 10, in the 320 mg/kg/day group died or were euthanized in extremis. Based on these data, the highest dose was set at 100 mg/kg/day and lower doses were selected using a geometric factor of 3.

b. Results: Mortality:

In males mortality was increased in the high dose group(See the table below), near the end of the study. Losses in the female in the high dose group also exceeded those in other groups, although % mortality in the vehicle group was about the same to the top dose group. It is not clear the reason why the mortality appeared low in the placebo and 10 mg/kg/day groups in males.

Sex	Vehicle	Placebo	3 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
Male	56.7	45.0	58.3	45.0	53.3	71.7
Female	68.3	63.3	60.0	48.3	56.7	65.0

Values are % of cumulative mortality at Week 104 and the drug dose was in mg/kg/day.

Clinical Signs: The test article was associated with an increase in the percentage of male animals that had body surface staining from Week 14 in the high dose group. The staining was apparent till the terminal of the study. Females did not develop the incidence

of such body surface staining. The test article had no apparent effect on the incidence, location or total numbers of palpable masses.

**Body Weights:** The mean body weights of the male group were 26-27 g, while those of the female group were 21-22 g. In the treated male groups, small but significantly decreased mean body weights, compared with vehicle and/or placebo group. However, there were no clear drug dose-dependent changes in the parameter as shown below.

Pioglitazone Dose	Male Group			Female Group		
	Mean*	A	B	Mean*	A	B
Vehicle	36	NA	-2.7	33	NA	0.0
Placebo	37	2.8	NA	33	0	NA
3 mg/kg/day	36	0	-2.7	34	3.0	3.0
10 mg/kg/day	34	-5.6	-8.1	32	-3.0	-3.0
30 mg/kg/day	36	0	-2.7	34	3.0	3.0
100 mg/kg/day	36	0	-2.7	32	-3.0	-3.0

\*indicate mean body weight in grams in Week 104. A and B indicate % difference from vehicle and placebo, respectively. NA stands for not applicable.

**Food Consumption:** Pioglitazone was associated with significantly decreased mean food consumption in the high dose group females. In males, the parameter was not clearly affected by the test article and the effect of the drug on the parameter appeared not significant since there were no drug dose-dependency. In addition, the data are scattered widely due to biological variation as shown below.

Pioglitazone Dose	Male Group			Female Group		
	Mean*	A	B	Mean*	A	B
Vehicle	144.0	NA	1.2	175.7	NA	1.2
Placebo	142.3	0.07	NA	173.6	-1.2	NA
3 mg/kg/day	144.1	0.07	1.3	168.9	-3.9	-2.9
10 mg/kg/day	145.8	1.3	2.5	174.4	-0.7	0.5
30 mg/kg/day	144.3	0.2	1.4	174.8	-0.5	0.7
100 mg/kg/day	143.0	-0.7	0.5	168.2	-4.3	-3.1

\*indicate mean food consumption in grams/kg/day in Week 104. A and B indicate % difference from vehicle and placebo, respectively. NA stands for not applicable

**Hematology:** There were no apparent test article-related alterations in hematological parameters.

**Organ Weights:** There were significant increases in the mean absolute heart weights in the 30 and 100 mg/kg/day males and females. The mean relative heart/body weight ratios were also increased in these groups, which appeared to be test article-related.

Microscopic Pathology: Test article-related microscopic changes were observed in the brown adipose tissue of all treated groups of male and female mice, which were characterized by cytoplasmic vacuolization and/or fibrosis in both sexes. The brown adipose tissue lesion of vacuolization was diagnosed as steatopathy. The incidence of pertinent brown fat lesions is shown below.

Dose	Vehicle		Placebo		3 mg*		10 mg		30 mg		100 mg	
	M	F	M	F	M	F	M	F	M	F	M	F
Mice	60	60	60	60	60	60	60	60	60	60	60	60
Steatopathy	12	6	21	7	34	30	46	35	50	43	55	58
Fibrosis			2		3	1	3	1	15	9	20	27

\*The unit of pioglitazone dose was in mg/kg/day.

Femoral and sternal bone marrows of treated animals were infiltrated with fats, which consisted of varying degrees of vacuolization within the marrow cavity that displaced or compressed the existing marrow. The vacuoles were spherical, clear and were the approximate size of normal fat vacuoles observed in inactive bone marrow. The incidence of fatty infiltration in the marrow is shown below.

Dose	Vehicle		Placebo		3 mg*		10 mg		30 mg		100 mg	
	M	F	M	F	M	F	M	F	M	F	M	F
Mice	60	60	60	60	60	60	60	60	60	60	60	60
Femur	1	5	2	6	12	9	27	17	31	22	43	37
Sternum					1		12	5	21	14	40	32

\*The unit of pioglitazone dose was in mg/kg/day.

The incidence of myocardial lesions appeared to be increased by the test article because there was a significant increase in mean heart weights of male and female mice in the mid and high dose groups as shown below. Microscopically, the increased weight was correlated with multifocal inflammatory cell infiltration, multifocal myocardial fibrosis and perivascular inflammatory cell infiltration around the coronary arteries.

Dose	Vehicle		Placebo		3 mg*		10 mg		30 mg		100 mg	
	M	F	M	F	M	F	M	F	M	F	M	F
Mice	60	60	60	60	60	60	60	60	60	60	60	60
Heart	11	6	9	7	25	15	19	18	25	19	20	11

\*The unit of pioglitazone dose was in mg/kg/day.

In liver, pigment deposition and hepatocellular hypertrophy were noted, which consisted of a fine, granular, brown pigment located in the cytoplasm of some centrilobular hepatocytes. Brown pigment was observed in both males and females. The

hepatocellular hypertrophy or enlargement of hepatocytes was observed in treated males from the mid and high dose group males and females from the high dose group as documented below.

Dose	Vehicle		Placebo		3 mg*		10 mg		30 mg		100 mg	
	M	F	M	F	M	F	M	F	M	F	M	F
Mice	60	60	60	60	60	60	60	60	60	60	60	60
Pigment*							1		1	2	14	6
Hypertrop*									2		9	2

\*The unit of pioglitazone dose was in mg/kg/day. \* indicate brown pigmentation and hepatocellular hypertrophy.

In the lungs, an increased incidence of congestion and foreign material was noted in the high dose animals, particularly in the females. In both sexes, congestion was only seen in died on study animals, which might be agonal change or due to gavage injury.

Neoplastic Lesions: Various tissues of control and treated groups had usual types of mouse tumors. Peto analysis of neoplastic lesions present in this study revealed 2 tumor types that were significant ( $P < 0.05$ ). In males, benign pheochromocytoma of the adrenal medulla and in females, leiomyosarcoma of the uterine cervix were statistically significant by both the Peto test and the Cochran Armitage trend test. The incidence of the two tumors is illustrated in a table below.

Dose	Vehicle		Placebo		3 mg*		10 mg		30 mg		100 mg	
	M	F	M	F	M	F	M	F	M	F	M	F
Mice	60	60	60	60	60	60	60	60	60	60	60	60
Pheochro*	0		0		0		0		2		1	
Leiomyos*		0		0		0		0		1		1

\*The unit of pioglitazone dose was in mg/kg/day. \* indicate pheochromocytoma and leiomyosarcoma, respectively.

Summary and Conclusions: Various toxicological responses to pioglitazone were manifested during the 2-year Carcinogenicity studies in mice. These effects included: increased mortality (100 mg/kg/day); increased mean absolute heart weight and relative (to body weight) heart weight (30 and 100 mg/kg/day); enlarged brown adipose tissue (10 mg/kg/day and higher); increased body staining in males (30 and 100 mg/kg/day); and decreased mean food consumption in females (100 mg/kg/day). Histopathological evaluation revealed brown tissue steatopathy, fatty infiltration of bone marrow, and cardiomyopathy at all doses, and hepatocellular hypertrophy in males at 30 and 100 mg/kg/day. Cardiomyopathy consisted of multifocal inflammatory cell infiltration, multifocal myocardial fibrosis and perivascular inflammatory cell infiltration. For mice, the no-observed-effect level (NOEL) was 3 mg/kg/day. Statistically significant increase in the incidence of two tumors was noted. They were benign pheochromocytoma of the

adrenal medulla in males and, in females, leiomyosarcoma of the uterine cervix. The findings were based on statistical analysis of paired points.

B. 104-Week Oral (Gavage) Carcinogenicity Study of Pioglitazone in Rats(Study#295-152). [REDACTED], in Japan conducted the study under GLP condition.

a. Methods: Male and female Sprague-Dawley (CrI:CD) rats(60/sex/group) were given orally either vehicle, placebo or pioglitazone at doses of 1, 4, 8 (male), 16 or 63 mg/kg/day for 104 weeks. An additional satellite group (20/sex/group) was also included for plasma analysis at 6, 12, 18 and 24 months for analysis of drug metabolite concentrations.

Selection of Dose and Dose Justification in Rats: The dosage levels utilized in this study were selected on the basis of a previous one year oral gavage study in which rats were treated with pioglitazone granulated formulation at 0, 4, 16, 63 and 160 mg/kg/day. There were increased heart weights in males at 4 mg/kg or higher and in females at 63 and 160 mg/kg or higher. In addition, there were findings of thoracic cavity fluid, bilateral atrial hypertrophy and increased lung weights in males at 16 mg/kg or higher and in females at 63 and 160 mg/kg. Mortality was noted in males at 63 and 160 mg/kg and in females at the top dose. On the basis of the data above, the highest dosage level was set at 63 mg/kg/day. Lower doses were selected using a geometric factor of 4.

Results: Mortality: There was a decrease in survival in the high dose group animals of both sexes. But, the percentage of deaths in females in the high dose group did not reach statistical significance and there was no apparent drug dose-response relationship in the parameter as shown below.

Dose	Control	Placebo	1 mg	4 mg	8 mg	16 mg	63 mg
Male	45	60	40	60	62	63	92
Female	45	52	38	45		65	60

\*The unit of the drug dose was in mg/kg/day and mortality was expressed in % death out of 60 animals in each cell.

Clinical Signs: The test article was associated with an increase in the percentage of rats that had body surface staining in the 66 through 104 week intervals in 8 and 63 mg/kg/day male groups. The incidence of rales was noted in the high dose group females during week 66 through 104 weeks. An increased incidence of plantar ulcers in all treated females and in the 8, 16 and 63 mg/kg/day males were also noted. All of the other clinical signs found were commonly observed in aging caged rats, which might not be related to the drug treatment.

Body Weights: In almost all animals, pioglitazone increased mean body weights prior to week 70 for both sexes. Toward the end of the studies the parameter was reduced in all

the animals in the high dose group as shown below. However, the reduction in body weight was under 10% of initial body weight for both sexes.

Dose(mg/ kg/day)	Male Rat Body Weight			Female Rat Body Weight		
	Grams	A	B	Grams	A	B
Vehicle	630	NA	-0.2	447	NA	-0.2
Placebo	631	0.2	NA	448	0.2	NA
1	678	7.6	7.4	457	2.2	2.0
4	668	6.0	5.9	455	1.8	1.6
8	640	1.6	1.4	NA	NA	NA
16	658	4.4	4.3	491	9.8	9.6
63	591	-6.2	-6.3	441	-1.3	-1.6

\*NA stands for not applicable and A and B indicate % difference from the vehicle and placebo, respectively.

#### Food Consumption:

Pioglitazone elevated mean weekly food consumption values in the first weeks of study in most of the treated groups except the 1 mg/kg/day group males, which was also observed sporadically in later weeks.

Dose(mg/ kg/day)	Male Rat			Female Rat		
	g/kg/day	A	B	g/kg/day	A	B
Vehicle	47.5	NA	0.8	58.9	NA	1.2
Placebo	47.1	-0.8	NA	58.2	-1.2	NA
1	46.3	-2.5	-1.7	58.3	-1.0	0.2
4	47.0	-1.1	-0.2	58.5	-0.7	0.5
8	46.9	-1.3	-0.4	NA	NA	NA
16	46.8	-1.5	-0.6	59.0	0.2	1.4
63	46.8	-1.5	-0.6	60.4	2.5	3.8

\*NA stands for not applicable and A and B indicate % difference from the vehicle and placebo, respectively

Macroscopic Findings: The brown adipose tissue showed enlargement with nodule formation at a dose of 4 mg/kg/day or higher. Cardiac tissues showed enlargement, distention or dilatation in some of mid dose groups and more animals in the high dose group males. In females, the incidence was somewhat lower.

In the urinary bladder there were nodules and occurrence of masses and thickening, which related to the drug treatment. The masses in bladder were more frequently observed in males than the females. Nodules were seen in the urinary bladder of males from the 4 and 16 mg/kg/day groups and in one female at the low dose group. Transitional cell tumors were noted in the mid and high dose group animals as described under microscopic findings.