the abdominal muscles and flattened posture were observed in the groups receiving 510 mg/kg and more. Hypotonia of the abdominal muscles was also observed beginning one day after dosing in the 260 and 360 mg/kg groups. Secretion from the Harderian gland was observed beginning one day after dosing in the 1000 mg/kg group and beginning two days after dosing in the 710 mg/kg group. Animals that survived to necropsy were free of these symptoms within six to 12 days.

Respective mortalities in the five dose groups were 0, 1, 1, 4, and 5 for the males, and 0, 0, 1, 4, and 5 for the females. Almost all deaths occurred between two and six days post-dosing. Body weight was decreased or body weight gain suppressed one or two days after dosing in all groups. However, body weight data collected seven and 14 days after dosing indicated normal body weight gain in all groups.

Necropsy of rats that died during the observation period revealed hyperemia of the lung and remnant test article in the peritoneal cavity. In addition, ascites (2-4 mL) and interlobar adhesion of the liver were observed. In rats that survived to necropsy, remnant test article was observed in the peritoneal cavity. Also, enlargement and interlobar adhesion of the liver were observed, and ascites (approx. 3 mL) was noted in a few rats. It was concluded that the intraperitoneal LD_{50} in rats was 558 mg/kg for males and 587 mg/kg for females.

C. Acute Oral Escalating Dose Toxicity Study (T-7)

a. Methods: Pioglitazone was administered orally to two male and two female Cynomolgus monkeys in escalating doses of 15, 60 and 240 mg/kg of the free base (3 to 5 days between doses). All animals received 240 mg/kg and were observed for 14 days. A similar group received the vehicle only.

b. Results: No treatment related effects on mortality, clinical signs, body weight, food consumption, body temperature, heart rate, hematology, and blood chemistry were seen at any dose. Therefore, the minimum lethal dose of AD-4833(HCl) in Cynomolgus monkeys was >240 mg/kg.

D. 13-Week Oral Toxicity Study in Mice(T-8)

a. Methods: Pioglitazone Lot# was INV.R3095. Ten Crl:CD-1 mice/sex/group were administered pioglitazone orally at doses of 0, 3.2, 10, 32, 100 and 320 mg/kg/day for 13 weeks.

b. Results: Treatment-related clinical signs included excessive salivation after dosing and mortality in the high-dose group mice. Four high-dose males and six high-dose females died as did four high-dose satellite mice (one male and three female). Body weight was slightly (under 5%) increased in the high-dose females, and food consumption was slightly(under 8%) increased in the males (32, 100 and 320 mg/kg/day) and females (10, 32, 100 and 320 mg/kg/day). Both the body weight and food consumption effects were considered pharmacological effects of the drug. Platelet counts, erythrocyte counts and

hematocrit and hemoglobin values were decreased in mice administered 320 mg/kg/day AD-4833(HCl). Mean platelet counts were also decreased in mice administered 100 mg/kg/day.

At necropsy, an increased fat:cell ratio was observed in the femoral bone marrow samples of all treated male groups and in females administered 32 mg/kg/day and above. Increased numbers of megakaryocytes were observed in the splenic red pulp of mice administered 100 and 320 mg/kg/day, as compared to controls.

Mean absolute heart weights were increased in high-dose mice(27%) and in males administered 100 mg/kg/day(13%). The increased heart weight did not have a correlative histopathologic change. Liver weight was increased in the high-dose group(130%); hepatocellular hypertrophy was observed in all AD-4833(HCl) treated groups. This change was primarily centrilobular and increased in both incidence and severity in a dose related manner.

E. 13-Week Oral Toxicity Study in Rats (#89-256)

a. Methods: Lot#R1940. Ten Sprague-Dawley (Jcl:SD) rats were given pioglitazone orally at doses of 0 (distilled water control), 0 (vehicle control), 30 (low), 100 (middle) and 300 (high) mg/kg as free base equivalents for 13 weeks.

b. Results: There were no treatment-related changes in body weight, food consumption, urinalysis and ophthalmology examination. No treatment-related deaths occurred, but one vehicle control female, one low-dose female, one mid-dose male and two high-dose males died due to dosing errors.

Hematology revealed statistically significant decreases in erythrocytes (in both sexes of the mid- and high-dose groups) and hematocrit (in mid- and high-dose females) as shown below. Hemoglobin concentrations (in mid- and high-dose females) was also reduced similarly. And statistically significant increases in mean corpuscular hemoglobin (in both sexes from the high-dose group) were noted. These changes were consistent with anemia. Plasma T_4 levels were decreased in all male treatment groups, but this was determined to be of no toxicological significance because the plasma T_3 level, which has greater physiological activity than T_4, remained unchanged.

Pioglitazone Effect on Hematological Parameters in SD Rat after 13-Week Treatment				
Dose(mg/kg/ Day)	Male Rat		Female Rats	
	Erythrocytes@	Hematocrit(%)	Erythrocytes@	Hematocrit(%)
Control	940	42.6	851	40.9
Placebo	947	43.1	840	40.5
30 mg	938	42.6	820	39.9
100 mg	883*	41.9	770*	38.0*
300 mg	868*	41.0	731*	36.9*

Erythrocyte counts should be multiplied by 10,000. Each value represents mean of 8 to 10 animals.
*$P<0.05$

Organ weight changes considered to be related to AD-4833(HCl) treatment included a statistically significant increase in heart weight in males and females treated with 100 and 300 mg/kg/day and a statistically significant increase in liver weight in females treated with 100 and 300 mg/kg/day as shown below. Histopathology did not reveal any abnormalities in the liver or hearts.

Pioglitazone Effect on Absolute Organ Weight in SD Rat after 13-Week Treatment				
Dose(mg/kg/ Day)	Male Rat		Female Rats	
	Heart Weight	Liver Weight	Heart Weight	Liver Weight
Control	1.35	13.0	0.76	6.33
Placebo	1.27	13.11	0.73	6.64
30 mg	1.41	12.18	0.83	6.26
100 mg	1.70*	12.38	0.97*	7.22*
300 mg	1.57*	11.82	1.02*	7.94*

*Weights were expressed in mean gram of 8 to 10 animals.

AD-4833(HCl)-related, dose-dependent increases in the amount of adipose tissue in interscapular and mediastinal spaces were found in all AD-4833(HCl)-treated groups. As previously mentioned, the increased subcutaneous adipose tissue in the inguinal-lumbar region was considered a pharmacological effect of AD-4833(HCl). No other gross abnormality was considered to be treatment-related. The only lesion related to AD-4833(HCl) treatment was splenic extramedullary hematopoiesis (SEH). This lesion was present in none of the 20 males or 20 females in both control groups, in 2 males and 1 female of the low-dose group, in 6 males and 3 females of the middle-dose group and in 8 males and 5 females of the high-dose group. The incidence of SEH in the low-dose group was considered by the authors to be well within the range of normal variation based on the historical control data, but because SEH was absent in the control group of this study, a potential effect on hematopoietic function at 30 mg/kg could not be ignored.

c. Conclusion: Anemia with reduced erythrocytes, hematocrit and hemoglobin concentration and splenic extramedullary hematopoiesis were present in rats after 13 weeks of oral administration of AD-4833(HCl) at doses of 100 and 300 mg/kg. The toxicological no-effect dose might be near 30 mg/kg/day.

F. Twenty Six-Week Oral Toxicity Study in Rat(Study#1182)

a. Methods: Lot#R2843. Ten Jcl:SD rats/sex/group were administered pioglitazone orally at doses of 10, 30 and 100 mg/kg/day for 26 weeks. Satellite groups of five rats/sex/dose were also included and treated with the same three AD-4833(HCl) doses as the main study animals. Plasma samples were collected during weeks 13 and 26, and parent compound and M-III and M-IV were measured.

b. Results: No treatment-related changes were seen in mortality, clinical signs, ophthalmoscopy, urinalysis or necropsy. Body weight gain tended to be increased in males in the 10 and 30 mg/kg/day groups from an early stage in the dosing period, but not in the 100 mg/kg group. This increased weight gain was considered to be due to the pharmacological action of the test compound.

Absolute and relative heart weights in both sexes of high-dose rats were increased as shown below. However, there was no change in liver weights and no histopathological changes were noted. Extramedullary hematopoiesis in the spleen and hypocellularity of the femoral and/or sternal bone marrow were observed during histopathological evaluation of the 100 mg/kg/day rats.

Pioglitazone Effect on Absolute Organ Weight in SD Rat after 26-Week Treatment				
Dose(mg/kg/ Day)	Male Rat		Female Rats	
	Heart Weight	Liver Weight	Heart Weight	Liver Weight
Control	1.46	15.80	0.93	7.78
Placebo	1.45	15.22	0.93	8.41
10 mg	1.69*	14.21	1.00	7.55
30 mg	1.71*	13.71	1.05	8.03
100 mg	1.75*	13.68	1.24*	9.10

*P<0.05 and weights were expressed in mean gram of 9 to 10 animals.

Plasma samples collected during weeks 13 and 26 were analyzed for AD-4833, M-III and M-IV. The relative amounts of the parent drug and metabolites in the plasma were: AD-4833 > M-IV > M-III. All analytes produced less than dose proportional increases in Cmax and AUC values. Females attained higher plasma levels of the parent drug and M-IV than males during both collection times. No effect of repeated dosing on Cmax or AUC values was noted for AD-4833 or M-IV. As the dose increased, the AD-4833 Tmax increased, from 0.5 hours at 10 mg/kg/day to 4 to 8 hours at 100 mg/kg/day. Higher plasma levels of M-IV were measured than M-III. M-III exhibited similar pharmacokinetics as AD-4833 and M-IV, with one exception. Repeated dosing resulted in lower Cmax and AUC values in males.

c. Conclusion: Increased heart weight (without accompanying pathology), hypocellularity in the femoral and/or sternal bone marrow and decreased erythrocyte counts (male only) were observed in the 100 mg/kg group. Based on these results, it is concluded that the non-toxic dosage level (or NOAEL) of AD-4833(HCl) was 30 mg/kg/day.

G. One-Year Oral Toxicity Study in Rats (TR#7226-94-007)

a. Methods: Two lots (INVR2728 and INVR2895) were used in the study. Thirty Crl:CD(BR) rats/sex/groups were administered pioglitazone orally via gastric intubation at doses of 4, 16, 63 and 160 mg/kg/day (3.6, 14.5, 57.1 and 145.1 mg/kg/day as AD-4833 free-base equivalents) for a year. 15 rats/sex/group were killed at the completion of the one-year dosing phase. The remaining rats (up to 15/sex/group) were killed after a four-month recovery period. An additional twelve rats/sex/group underwent the same treatment and plasma was collected on days 43, 183 and 363 for toxicokinetic analyses.

b. Results:

Mortality: Three control and 48 AD-4833(HCl) treated rats died prior to the one-year sacrifice. Drug-related early death due to apparent heart dysfunction occurred in the 63 and 160 mg/kg/day males (8 and 18 deaths, respectively) and in the 160 mg/kg/day females (10 deaths) as shown below. In addition, one control and six treated rats were sacrificed during the recovery phase. The cardiac-related deaths were delayed in onset with first mortality on Day 114 for the 160 mg/kg/day males, Day 154 for the 160 mg/kg/day females and Day 255 for the 63 mg/kg/day females.

Pioglitazone Effect on Mortality due to Cardiac Failure in 1-Year Rat Study						
Death due to	Sex	0 mg	4 mg	16 mg	63 mg	160 mg
Cardiac Failure	Male	0	0	0	8	18
	Female	0	0	0	0	10
Total Death	Male	2	2	3	9	20
	Female	1	0	1	0	13

*The drug dose was in mg/kg/day and each value indicates number of cardiac death.

Cardiovascular Effects: AD-4833(HCl) administered to rats at 63 and 160 mg/kg day caused early increases in body weight followed by delayed decreases (onset around the fourth month of dosing), clinical signs suggestive of cardiopulmonary dysfunction and decreased elasticity of the lumbar skin (females only). Statistically significantly increased absolute and relative (to brain weight) heart weights as well as volume were observed in all treated male groups and in female groups administered 63 and 160 mg/kg/day at the one-year necropsy as shown in a table below. This was considered the primary toxicological effect of the drug, since the changes were not reversed, particularly in male rats after 4-month recovery period. Males were more sensitive to cardiac dysfunction, and cardiomyopathy was present at doses as low as 16 mg/kg/day in males and 64 mg/kg/day in females.

Pioglitazone Effects on Cardiac Measurements in SD Rats in One Year Studies										
Sex	Males					Females				
Dose	0	4	16	63	160	0	4	06	63	160
N	15	16	15	15	15	15	15	15	15	15
Length	18.93	20.40*	21.62*	22.30*	22.00*	16.68	16.98	17.19	18.44*	18.10*
DVW	12.15	12.83*	13.27*	13.63*	13.63*	10.96	10.96	11.34	12.16*	12.42*
Lateral	13.73	14.51*	15.57*	15.83*	16.03*	11.76	11.83	12.27*	13.23*	13.64*
Volume	3166	3814*	4483*	4839*	4819*	2152	2208	2408*	2976*	3080*
Weight	1.61	1.91*	2.22*	2.46*	2.55*	1.18	1.19	1.26	1.58*	1.66*

The unit of dose was in mg/kg/day and N indicates animal numbers. DVW stands for dorso-ventral width in mm and cardiac volume was in mm^3 . The heart weight was in gm. *P<0.05.

The cardiac enlargement was correlated with histologic multifocal or diffuse myocardial hypertrophy. In addition, myocardial cells had increased cytoplasm and plump oval nuclei. At one year, all external dimensions of the heart had increased by approximately the same amount indicating that the increased weight was directly reflected in an externally measured increase in size. Cross-sectioning revealed increased muscle area in the left and right ventricles and increased size of both ventricles. Recovery animals

showed evenly proportional decreases in both ventricular muscles indicating at least partial recovery from the hypertrophy.

Changes indicating cardiac dysfunction included thoracic cavity fluid, bilateral atrial hypertrophy and increased lung weight. No recovery deaths were attributed to cardiac dysfunction. At the recovery necropsy, evidence of heart dysfunction was nearly reversed but some cardiac hypertrophy and cardiomyopathy (a secondary effect) were present. Recovery was considered complete in all treated females and in the low-dose males.

Hematological Effects: AD-4833(HCl) administered at 16, 63 and 160 mg/kg/day caused statistically significant([792, 756 and 738] x 10^4 /mm^3 , respectively, vs 834 in control) decreases in red blood cell count with a slight, reactive reticulocytosis, slightly decreased platelet counts at all dose levels and other secondary hematologic changes. All hematologic changes were reversed by the end of the four-month recovery phase.

Other Effects: The incidence of early closure of the femoral and/or tibial physeal plate was higher in all AD-4833(HCl) treated male groups than in the control. Decreased bone in the sternum was more prevalent in all AD-4833(HCl) treated groups than in the control group. Irreversible broadening of the sternum (osteodysplasia) was evident in all AD-4833(HCl) treated male groups and in female groups administered 63 and 160 mg/kg/day AD-4833(HCl). Pituitary weight and the incidence of pituitary adenoma of the pars distalis were increased in high-dose females at the end of the dosing phase. By the end of the study, the overall incidence of pituitary adenoma was similar for the control and the high-dose females. There was no effect on absolute weight in liver, but the ratio of liver to body weight was reduced by 10 to 20% from the control. Other changes included sporadically decreased in kidney, spleen and testes weight.

Pharmacokinetic Effects: Plasma samples were analyzed for AD-4833, M-II, M-III and M-IV on days 43, 183 and 363. The relative amounts of the parent drug and metabolites in the plasma were: AD-4833 > M-IV ≈ M-III > M-II. Less than dose proportional increases in Cmax and AUC values were reported for each of the four analytes. For parent drug, M-III and M-IV, females produced higher Cmax and AUC values than males, but for M-II the opposite effect was observed. Increasing duration resulted in increased Cmax and AUC values for the four analytes in the female rats; this effect was slight for M-II.

c. Summary: AD-4833(HCl) administered orally to rats for one year caused death due to cardiac dysfunction at doses of 63 and 160 mg/kg/day. Evidence of cardiac dysfunction was present at the one-year sacrifice in rats at doses of 16 mg/kg/day or above and cardiac hypertrophy was present in all treated male groups and females treated with 63 and 160 mg/kg/day. Reversible decreases in red blood cell count and adipose tissue changes were also recorded. Because evidence of toxicity occurred at all doses and the drug-related changes were not entirely reversible following a four-month recovery phase, a NOAEL was not determined.

H. 4-Week Oral Toxicity Study in Dog (Study#814/SU)

a. Methods: Three beagle dogs/sex/group received pioglitazone orally in capsules containing 0, 1, 3, or 10 mg AD-4833/kg/day as free base equivalents for 4 weeks.

b. Results: AD-4833(HCl) did not affect body weight, food or water consumption, body temperature, ophthalmoscopy, urine volume, urinalysis, gross pathology, organ weights or histopathology. Some animals in all groups experienced vomiting and soft feces. An increase in heart rate occurred in the low- and high-dose females, however, the values were within normal accepted ranges of variability. Mild extramedullary hematopoiesis was noted in one high-dose female following histopathology, but this effect was considered incidental because concomitant anemia did not occur. A dose-dependent increase in plasma AD-4833 levels was reported. It was concluded that no toxicity occurred in the dogs at doses up to 10 mg/kg/day.

I. 12-Week Oral Toxicity Study (#7220/90/043) in Beagle Dogs

a. Methods: Lot# R1895. Three to 5 beagle dogs/sex/group were administered pioglitazone orally at doses of 0, 1, 3 and 10 mg/kg/day as AD-4833 free base equivalents (1.12, 3.37 and 11.24 mg AD-4833(HCl)/kg/day for 3 months.

b. Results: No effect on body weight, ophthalmic appearance, or water consumption was noted during the dosing period. Food consumption during the dosing period was not statistically analyzed, since the amount of food was limited to 300 grams daily. Ophthalmic examinations and food and water consumption measurements were not performed during the recovery phase, since no treatment-related effects during dosing were observed.

Hematological changes observed in the combined sexes and attributed to AD-4833(HCl) treatment included a statistically significant reduction in hematocrit($P<0.05$) and hemoglobin concentration on Days 72 and 90 and in erythrocyte count on Day 90 in Group 4 animals. These parameters returned to normal during the recovery phase. Statistically significant serum chemistry changes which were related to AD-4833(HCl) treatment were observed in Group 4 males or in the sexes combined during the dosing period. These included a decrease in serum albumin on Days 56, 72, 84 and 90, a decrease in total protein on Days 14, 56 and 90, and an increase in alanine aminotransferase on Days 28, 42, 56, 72, 84 and 90 as shown in a table below. There was also an increase in lactate dehydrogenase on Days 42, 72, and 84, and an increase in chloride on Days 28, 42, 56 and 90.

Pioglitazone Effects on Alanine Aminotransferase Level in 12-Week Dog Studies										
Sex	Males					Females				
Day@	28	42	56	72	90	28	42	56	72	90
0 mg#	31	27	29	32	31	21	21	21	24	25
1 mg	25	27	27	29	31	30	30	31	31	31
3 mg	29	34	36	37	35	26	24	26	28	30
10 mg	48	50	49	57	60	28	31	34	33	36

ALT unit was in U/L and @ and # indicate days after the drug treatment and pioglitazone dose in mg/kg/day, respectively.

Treatment with AD-4833(HCl) resulted in a small amount of clear fluid observed grossly in the pericardium of one female and one male dog treated with 10 mg/kg/day and sacrificed at dosing termination; this was not observed in any recovery dog. A statistically significant increase in heart weight was noted in Group 4 animals sacrificed at dosing termination. The increase in heart weight was also noted in Group 4 recovery animals; however, no statistical analysis of recovery animals was performed due to the small number of animals. No treatment-related microscopic lesions were noted in any animal, including those in the recovery group.

J. 26-Week Oral Toxicity Study in Beagle Dogs(Study#1183/CH)

a. Methods: Pioglitazone used for this study was citrate-based granulated powder(Lot# R2843), which was provided by Upjohn company. Four beagle dogs (4/sex/group) were administered pioglitazone daily at doses of 0, 1, 3 and 10 mg/kg/day (0, 0.89, 2.67 and 8.90 mg/kg/day as free base equivalents) for 6 months.

b. Results:
Mortality and Clinical Signs: There was no drug-induced death, although a control female dog was sacrificed because of moribund situation. No treatment-related changes were seen in clinical signs, body weight, food consumption, body temperature, heart rate, urine output, water intake, electrocardiography, ophthalmoscopy, urinalysis, plasma T_4 and T_3 levels or the blood coagulation test. No treatment-related changes were observed in any examination in the 1 or 3 mg/kg/day groups.

Slight pale discoloration of the oral mucosa occurred in two high-dose males from weeks 5 to 9. In the 10 mg/kg/day group, one female exhibited decreased erythrocyte count during week 26 (37.8% decrease in comparison to the value during week 14). ALT levels were not affected significantly by the drug, but spleen weights in males were reduced significantly as shown below. A statistically significant increase in platelets and leukocytes was also reported in the high-dose females. Increased heart weight with hydropericardium was observed in one 10 mg/kg/day male; however, no abnormalities were seen in electrocardiography or histopathology. Hydropericardium was also observed in animals receiving more than 10 mg/kg/day in a three-month oral toxicity study.

Pioglitazone Effect on ALT and Spleen Weight in 26-Week Dog Study					
Parameters	Sex	0 mg	1 mg	3 mg	10 mg
ALT	Male	24	31	24	27
	Female	25	23	26	27
Spleen	Male	31	18*	24	20*
	Female	21	24	23	23

@Drug dose was in mg/kg/day. The units of ALT and spleen weight were in U/L and in gram, respectively.

Interstitial fatty infiltration in the parathyroid gland occurred in one male and three females in the 10 mg/kg/day group; however, the toxicological significance of this change was not clear since no histologic changes occurred in the parenchymal cells.

Plasma samples were collected on days 81 and 179 and were analyzed for AD-4833, M-III and M-IV. The relative amounts of the parent drug and metabolites in the plasma were: AD-4833 > M-IV > M-III. For the females, greater than dose proportional increases in AD-4833 Cmax and AUC values occurred at 3 mg/kg/day and less than dose proportional increases occurred at 10 mg/kg/day. Less than dose proportional increases occurred in the males at all doses. No gender or duration effects were evident for the parent drug. M-IV was formed in greater amounts than M-III. M-III Cmax and AUC values increased in a greater than dose proportional manner for the females and a less than dose proportional manner for the males. Females had higher levels than males and increasing duration produced a slight increase in Cmax and AUC values in both sexes. For M-IV, the increases in Cmax and AUC values followed the same pattern as for M-III, but no gender or duration effects occurred.

Decreased erythrocyte count, increased spleen weight in one female and increased heart weight with hydropericardium in one male were observed in the 10 mg/kg/day group. Based on these results, it is concluded that the non-toxic dosage level of AD-4833 was 3 mg/kg/day.

K. 52-Week Oral Toxicity Study in Dogs (TR#7226-93-002). This study was conducted by [redacted] under GLP standards.

a. Methods: Pioglitazone Lot# was R2763-R2874. Six beagle dogs/sex/group were given pioglitazone orally at doses of 0, 1, 3 or 10 mg/kg/day for one year. Following the dosing phase, 2 dogs/sex/group were maintained without drug exposure for a four-month reversibility phase.

b. Results: Clinical Signs: General clinical observations were conducted following the morning dosing and again in late afternoon. Clinical signs related to a toxicological or pharmacological effect were not apparent during this study.

Body Weight and Food Consumption: During the first week of the study, 92% of the study animals exhibited weight loss ranging from 50 to 650 grams. Group mean total body weight changes indicate that the test compound did not have any noticeable effect

on food or water consumption. A consistent body weight decrease was observed in only the mid-dose (3 mg/kg/day) females during the dosing phase(Please see the table below).

Pioglitazone Effect on Body Weight in 52-Week Oral Toxicity Study In Female Dogs				
Treated Week	O mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
00	9.0	8.8	8.1	9.2
10	9.0	8.4	7.7	6.7
20	9.2	8.5	8.0	8.7
30	10.0	9.1	8.4*	9.0
36	9.9	9.1	8.2*	8.9
40	9.9	9.1	8.4*	9.0
46	9.9	9.3	8.3*	9.2

Indicate P<0.05 compared to the control.

Hematology and Blood Chemistry: Hematology parameters demonstrated evidence of a regenerative anemia in the mid-dose (3 mg/kg/day) males and high-dose males and females. Serum chemistry assays revealed a biologically significant increase in alanine aminotransferase (ALT) levels in the high-dose males from week 3 to the end of dosing as shown below. By the end of the reversibility phase, all of the hematologic parameters had returned to control values and the ALT levels in the high-dose males had decreased to near normal levels.

Pioglitazone Effect on ALT Level in 52-Week Oral Toxicity Study In Dogs					
Week	Sex	O mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
3	M	26.8	28.2	31.3	39.1*
	F	27.1	23.5	25.3	28.9
28	M	31.0	29.4	34.1	59.7*
	F	27.0	28.6	25.9	34.0
52	M	32.1	31.8	44.5	80.4*
	F	25.8	31.4	28.8	37.1

Indicate P<0.05 compared to the control.

Plasma bilirubin levels were documented in a table below, which were not altered in all groups. However, the level in females of the high dose group at the week of 55 appeared high. The value was not analyzed statistically by Dunnett's test since there were only two dogs, whose blood level was returned subsequently during the recovery period. Thus, there was no apprarent association with other changes such as ALT.

Pioglitazone Effects on Total Plasma Bilirubin Level in the High Dose (10 mg/kg/day) Group of Beagle Dogs in 52-Week Toxicity Studies*										
Week@	0	3	7	25	38	46	52	55	59	68
Male	0.3	0.1	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.1
Female	0.2	0.1	0.1	0.1	0.2	0.3	0.3	0.5	0.2	0.2
N	6	6	6	6	6	6	6	2	2	2

Total bilirubin levels were in mg/dL and @indicates weeks after pioglitazone treatment.

Electrocardiography: There were no meaningful treatment related differences in heart rates, PR, QRS or QT intervals, rhythm or amplitude associated with test article. ST segment depression was noted in all dose groups at a level of 0.1 mv or less, but the ST segment depression above 0.15 mv was seen only in some treated animals in all dose groups. It was not clear whether the ST segment depression was associated with compound administration because there were no pretest electrocardiogram.

Organ Weights: There was a trend towards increased heart weights in the mid- and high-dose males and high-dose females at the one-year necropsy(Please see table below), a trend that was still evident in the high-dose males at the end of the four-month reversibility phase. Electrocardiograms and gross and microscopic tissue evaluations failed to demonstrate any significant myocardial changes. There were not any other morphologic treatment-related tissue changes noted at either the one-year or recovery necropsies.

Pioglitazone Effect on Organ Weights at Terminal in 52-Week Oral Dog Toxicity Study					
Tissues	Sex	O mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Heart	M	92.2	85.2	95.7	107.5*
	F	71.5	72.6	67.0	77.7*
Liver	M	321.9	266.0	274.3	316.5*
	F	254.1	245.6	248.6	253.2*

*Indicate Dunnett's test was not applicable due to limited degrees of freedom and the unit of organ weight is in grams.

Plasma samples collected during months 3, 6, 9 and 12 were analyzed for AD-4833, M-II, M-III and M-IV. The relative amounts of the parent drug and metabolites in the plasma were: M-IV > AD-4833 > M-III > M-II. Virtually none of the dogs produced measurable levels of M-II. For the remaining three analytes (AD-4833, M-III and M-IV), less than dose proportional increases in AUC values were reported, except for a single instance of a greater than dose proportional increase for M-III in both sexes at 6 months. No consistent gender effects were noted, but increasing duration tended to produce increasing AUC values at 9 and 12 months.

c. Conclusion: when beagle dogs were given AD-4833 orally via gelatin capsules daily one year at dose levels of 0, 1, 3, and 10 mg/kg/day, evidence of a regenerative anemia, elevated serum ALT levels, and increased heart weights were observed at the mid- and/or high-doses. By the end of the four-month reversibility phase, the hematologic values had returned to normal and the ALT levels had lowered. However, the myocardial weights were still increased, especially in the high-dose males.

L. 52-Week Oral Toxicity Study in Monkey(Study#28-05). This study was performed by [redacted] under Japanese MHW GLP guidelines.

a. Methods: Pioglitazone Lot# was not specified. Four Cynomolgus monkeys/sex/group were given pioglitazone orally (nasogastric) at doses of 0, 1, 2, 8, and 32 mg/kg/day for 52 weeks. Three animals per sex were added to the control and high-dose groups and allowed to recover from treatment for 16 weeks. Blood samples were collected during weeks 26 and 52 and the plasma drug levels were determined.

b. Results: No mortalities occurred in any group and no treatment-related effects were noted on clinical signs, electrocardiography, ophthalmology, hematology, biochemistry, urinalysis, gross pathology, organ weights, or histopathology (via light or electron microscopy). In the biochemistry screen, a tendency towards a decrease in serum triglycerides was observed in the beginning of the dosing period for each treatment group. A statistically significant increase in food consumption occurred in females at all doses. The increase was noted at various times during weeks 11 through 14. An increase in body weight was measured in males exposed to 8 and 32 mg/kg/day and females exposed to all doses. No abnormalities occurred in any of the recovery animals.

Electron microscopy was performed on heart samples taken from the left ventricular wall of one control male and two high-dose males at the end of the dosing period along with one control male and two high-dose males at the end of the recovery period. Very slight lipofuscin granules were observed in the cardiac muscle cells of both groups of high-dose males as well as in the recovery control male.

Plasma data collected during weeks 1, 26 and 52 were analyzed for AD-4833, M-II, M-III and M-IV. The relative amounts of the parent drug and metabolites in the plasma were: AD-4833 > M-IV > M-III > M-II. Cmax and AUC values for AD-4833 and its three metabolites increased with increasing dose. However, the increase was not proportional to the dose. No sex differences were noted for AD-4833, but females had slightly higher M-II Cmax and AUC values than and males had higher M-III and M-IV AUC values than females. Increasing dose duration decreased Cmax and/or AUC values for the parent drug, M-II, M-III, and M-IV.

It appears that the doses that were selected for the monkey might not be toxicologically meaningful doses since the doses did not produce any toxic effects in male or female monkeys at doses as high as 32 mg/kg/day. The dose represents approximately 20 times of recommended human exposure, based on a body surface area comparison. In addition, Cynomolgus monkeys appear to be resistent species to toxicity induced by thiazolidinediones such as troglitazone or rosiglitazone.

5. REPRODUCTIVE TOXICITY STUDIES

A. Segment I Reproduction Study I in Rats (TR#7224-98-026)

a. Methods: Pioglitazone Lot#INV.R2728. Forty-two Crl:CD(BR) SD rats/sex/group were given pioglitazone[AD-4833(HCl)] orally by gastric intubation at doses of 0, 10, 20 and 40 mg/kg/day. The drug was administered from 7 and 14 days, respectively, prior to mating until sacrifice, in order to determine the possible adverse effects of this drug on fertility and general reproductive performance, as well as any effects on F_1 offspring development behavior and fertility.

b. Results:

Clinical Observations and Survival: The drug did not result in clinical effects suggestive of marked toxicity and did not affect survivability, estrus, spermatogenesis, copulation, fertility or gestation length in the parental generation. One male in the mid-dose group died prior to mating (the cause of death was most likely a dosing accident); in the high-dose group, one dam died and two were sacrificed in a moribund condition as the result of dystocia. F_0 male and female body weight gains and food consumption prior to mating were increased in all three treatment groups. The increased body weight gain, with increased food consumption, was most likely the result of increased adipose tissue, a pharmacological effect of AD-4833(HCl). Mean relative spleen weight of F_0 males was decreased in the AD-4833(HCl)-treated groups.

Histopathology: The placentas of treated dams sacrificed on gestation day 20 were enlarged. Histopathologic evaluation of the placentas revealed apparent differences in the morphology of the placenta between animals given control article and those given drug, but most were related to the pharmacological properties of the drug and were not considered to be of biologic importance. Necrosis in the cellular component of the basal zone from placentas of dams in the 40 mg/kg/day dose group was the single finding considered to be of toxicological importance and related to AD-4833 (HCl) treatment. Hematology changes included decreased hemoglobin concentrations (males and females) and hematocrits and increased mean cell volume (males only). AD-4833(HCl) treatment also affected serum chemistry parameters as indicated by decreased creatinine kinase and increased alkaline phosphatase, as well as increased cholesterol and decreased total protein (males only).

Fetotoxicity and Teratogenicity: The drug was embryotoxic at 40 mg/kg/day, as evidenced by increased postimplantation losses. Treatment-related fetotoxicity also occurred at doses as low as 10 mg/kg/day, as evidenced by reduced fetal body weights and crown-rump lengths, and increased incidences of visceral and skeletal variations. Gross, visceral or skeletal malformations indicative of teratogenicity in fetuses from dams treated with AD-4833(HCl) were not evident. However, the ability to deliver live newborns was adversely affected in the 40 mg/kg/day dose group, with three dams experiencing dystocia.

F_1 pup survivability was adversely affected in the mid-and high-dose groups during the early postpartum period. Survival rates and clinical observations were comparable in all groups, from weaning to maturation, in animals selected for the F_1 fertility evaluation. Delayed development continued through maturation in the mid- and high-dose groups, as evidenced by continued reductions in mean body weight. The F_1 animals in the high-dose group required three breeding periods to achieve fertility rates comparable to that of the control group. Finally, the means for F_1 gestation length, and the numbers of live, dead and abnormal F_2 pups, as well as mean F_2 pup weights on postpartum day 0, were not adversely affected by AD-4833(HCl) treatment.

c. Conclusion: Segment I fertility and general reproductive performance study with male and female Sprague-Dawley rats given AD-4833(HCl) throughout gametogenesis, mating and gestation as well as during the postpartum period (females only) at doses of 0, 10, 20 and 40 mg/kg/day (AD-4833 free base equivalents), did not determine a no-observed-adverse-effect level (NOAEL) for parental (F_0) toxicity. Other than the dystocia seen in the high-dose group, the parental effects observed in all three groups were consistent with the pharmacological actions of this drug. Teratogenicity was not evident in this study. However, pioglitazone did delay F_1 development as measured in body weight changes and the F_1 fertility was also delayed.

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

*Indicate $P<0.05$.

B. Range-Finding Segment II Study I in Rats (TR#7224-91-068)

a. Methods: Six pregnant Crl:CD[BR] rats/group received twice daily oral doses of 0, 50, 100, 200 or 400 mg/kg of pioglitazone on gestation days 6 through 20

b. Results: Decreased weight gain occurred in all treatment groups along with red vaginal discharge. The incidence of rales was increased at 400 mg/kg. Several maternal hematology parameters were increased including mean corpuscular volume, platelets, and lymphocytes at 400 mg/kg. AD-4833 was embryotoxic and fetotoxic with resorptions occurring in two and six rats at 200 and 400 mg/kg/day and fetal deaths occurring in one, two and two rats at 50, 100, and 200 mg/kg/day, respectively. In addition, treatment decreased the mean number of live pups per dam, increased the mean number of dead pups per dam, and decreased live pup weight at 50 to 200 mg/kg. Based on these data, a no-observed-adverse-effect-level likely to be below 50 mg/kg.