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September 16, 2009

Via Federal Express

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency, ICC Building
1201 Constitution Ave., NW
Washington, DC 20004

Dear 8(e) Coordinator:

8EHQ-06-16436/8EHQ-06-16478

This letter is to inform you of the results of a 90-day oral toxicity study in rats with the above referenced test substance. This test substance is subject to a Consent Order, PMN P-08-509.

The test substance was administered orally by gavage once daily for a minimum of 90 consecutive days to 3 groups (Groups 2-4) of Crl:CD(SD) rats. Dosage levels were 0.1, 10 and 100 mg/kg/day for males and 10, 100 and 1000 mg/kg/day for females. A concurrent control group (Group 1) received the vehicle on a comparable regimen. The dose volume was 10 mL/kg for all groups. Each group consisted of 10 animals/sex (Main Study). Additional animals (10/sex/group) were added in Groups 1 and 4 to assess the reversibility of effects (Recovery Group). Body weights, food consumption, and clinical observations were evaluated. Functional observational battery and locomotor activity data were recorded and ophthalmic examinations were performed for all animals. Following a minimum of 90 days of dose administration, surviving Main Study animals were euthanized; the surviving Recovery Group animals in the control and high-dose groups were euthanized following a 29/30-day recovery period. Clinical pathology and gross and microscopic pathology endpoints were evaluated.

There were 3 test substance-related deaths in the 1000 mg/kg/day group females. One of these rats was euthanized *in extremis* on study day 8 due to the clinical observation of impaired use of the hind limbs and forelimbs. Two of these rats were found dead on study day 21 or 37.

Most of the 1000 mg/kg/day females exhibited intermittent wet clear material around the mouth at the time of dosing and approximately 1-2 hours post-dosing. Two 100 mg/kg/day males exhibited wet clear material around the mouth on 2 days at the time of dosing. One 100 mg/kg/day male vocalized during handling on one occasion.

There were no adverse or test substance-related effects on body weights or food consumption.

Test substance-related organ weight changes consisted of higher kidney and liver weights. All kidney weight parameters (absolute, relative to body and brain weight) were minimally increased in the high dose male and female groups (100 mg/kg/day males; 1000 mg/kg/day females). In the 1000 mg/kg/day females these changes were associated with evidence of diuresis (increased urine volume and decreased urine osmolality) and microscopic changes in the kidneys–mostly in early death animals. In the 100 mg/kg/day males there were no clinical pathology or microscopic changes suggestive of kidney injury. Minimal kidney weight increases (statistically significant) were also present in the recovery group males but not females. Kidney weight relative to body weight was also increased in the 10 mg/kg/day male and female groups and the 100 mg/kg/day female group. However, at these dose levels, there were no changes in other kidney weight parameters (absolute kidney weight and kidney weight relative to brain weight), and no correlative changes in clinical chemistry, urinalysis, or histopathology suggestive of renal

toxicity. Thus, the statistically significant changes in kidney weight relative to body weight in the 10 mg/kg/day male and female groups, and the 100 mg/kg/day female group, were not considered to be adverse.

All liver weight parameters (absolute, relative to body and brain weight) were increased in the 10 and 100 mg/kg/day male groups and in the 1000 mg/kg/day female group. Liver weight changes correlated with microscopic hepatocellular hypertrophy, but they were not associated with degeneration or necrosis in the liver or with changes in clinical chemistries suggestive of liver toxicity. Therefore, these liver weight increases were not considered to be adverse. In 100 mg/kg/day males, liver weight changes were reversible except for liver weight relative to body weight, which was mostly, but not completely reversible. Changes in liver weight in females showed partial recovery but were not completely reversible following the 4-week recovery period.

Microscopic findings in two of the 1000 mg/kg/day females that died (nos. 7315 and 7318) were similar, suggesting that these deaths were test substance related. Female no. 7315 (found dead on study day 21) had microscopic lesions of renal tubular necrosis, renal papillary necrosis, hepatocellular hypertrophy, and lymphoid depletion in multiple tissues. Female no. 7318 (found dead on study day 37) had microscopic lesions of renal papillary necrosis, hyperplasia of the transitional epithelium of the urinary bladder, coagulation necrosis of portions of an adrenal gland, hepatocellular hypertrophy, and lymphoid depletion in multiple tissues. Lymphoid and adrenal changes in these animals were likely secondary to agonal stress.

The other early death female (euthanized *in extremis* on study day 8) had gross lesions of red areas in the stomach, urinary bladder, and thymus. Microscopic findings included necrosis, hemorrhage, and focal thrombosis of the spinal cord, thrombosis and myocardial fiber degeneration of the heart, necrosis in the glandular stomach, and hemorrhage in the lung, thymus, and urinary bladder. These pathology findings in this animal were not observed in other animals in this group and thus, the relationship of this early death to treatment is uncertain. In addition to the microscopic changes observed in the 1000 mg/kg/day group females found dead or euthanized *in extremis*, one 1000 mg/kg/day group female (animal no. 7279) had minimal renal tubular necrosis and regeneration at the study week 13 primary necropsy.

Minimal hepatocellular hypertrophy was observed in the livers of some primary necropsy animals in the 10 and 100 mg/kg/day male groups and in the 100 and 1000 mg/kg/day female groups. Hepatocellular hypertrophy was associated with increased eosinophilic granularity of the hepatocyte cytoplasm consistent with peroxisome proliferation. Hypertrophy was not associated with microscopic changes indicative of liver injury (such as degeneration or necrosis) or with changes in clinical chemistry indicative of liver injury. Therefore, these changes were not considered to be adverse.

Test substance-related hematology changes in red cell mass parameters (red cell counts, hemoglobin, hematocrit,) were present the high dose male (100 mg/kg/day) and female (1000 mg/kg/day) groups at the end of the exposure period. Decreases in these parameters were approximately 11 - 13% below controls in males and 18 - 28% below controls in females. In addition, individual values for these erythrocyte parameters in several animals at these dose levels were below laboratory reference interval. The decreases in red cell mass parameters were associated with an increase in the absolute reticulocyte count in both sexes and, in females, changes in red cell indices, including an increase in mean cell volume (MCV) and mean cell hemoglobin (MCH), and a decrease in mean cell hemoglobin concentration (MCHC). The changes in reticulocyte counts and red cell indices indicate a regenerative response to the decreases in red cell mass. Based on the magnitude of the decreases in the mean erythrocyte parameters, as well as the presence of anemia in some animals—as indicated by decreases in red cell mass parameters below reference intervals–the erythrocyte changes in high dose males and females were considered to be test substance-related and adverse.

Consistent with their regenerative nature, the red cell changes in high dose males and females, showed recovery following the approximately 4-week recovery period. In females, recovery was complete, as values for some red cell mass parameters were statistically increased (along with a decrease in reticulocytes and an equivocal increase in MCV) compared to controls. In males, recovery was present but was not complete, as slight decreases (about 5% below controls) in red cell mass parameters were still present at the end of the recovery period. In addition, absolute reticulocyte counts remained minimally elevated in this group. Based on the regenerative response noted in the high dose recovery group males, complete recovery would be expected with increased recovery time

Statistically significant decreases in erythrocyte parameters were also present in the 10 mg/kg/day male group. At this dose level, the decreases were minimal (approximately 7% below controls), and values for individual animals

were within laboratory reference intervals (except hematocrit in two males were 0.2 percentage points below the reference interval). Consistent with the minimal nature of the erythrocyte changes at this dose, there were no statistically significant changes in absolute reticulocyte counts. Based on the minimal nature of the effects on red cell parameters, the lack of an increase in reticulocyte counts–suggesting a lack of an erythropoietic stimulus–and the absence of anemia in individual animals, the erythrocyte effects in the 10 mg/kg/day male group were not considered to be adverse.

Test substance-related and statistically significant changes in several clinical chemistry parameters were present in male rats administered 10 mg/kg/day and above and in females administered 100 mg/kg/day and above. Most changes were consistent with PPARα activation. In a previous 28-day study in rats, the test substance was shown to be a peroxisome proliferator based in increases in liver beta oxidation and microscopic changes in the liver. All changes were reversible following the approximately 4-week recovery period.

Test substance-related decreases (variable statistical significance) in cholesterol were present in male rats administered 10 or 100 mg/kg/day and in females administered 100 or 1000 mg/kg/day. Decreases were minimal, as values for most animals in the affected groups were within laboratory reference intervals. There are no known adverse effects associated with minimal decreases in cholesterol. As such, these changes were considered to be test substance related but non-adverse. Effects on cholesterol were reversible in both males and females as there were no statistically significant changes in cholesterol in the high dose recovery groups.

Higher albumin (males only) and lower globulin levels, as well as associated increases in albumin/globulin ratio, were present in the 10 and 100 mg/kg/day male groups and the 1000 mg/kg/day female group. Lower total protein (due to lower globulin) was also present in the 1000 mg/kg/day female group. Individual values for these protein parameters were outside laboratory reference intervals in several animals, especially in the high dose male and female groups. All serum protein changes were reversible, as mean values were similar to controls following the 4-week recovery period. The biological significance of the changes in total protein is uncertain. The pattern of change in serum proteins–decreased globulin and increased albumin–is consistent with the known anti-inflammatory properties of $PPAR_{\alpha}$ agonist.

Urea nitrogen was minimally increased in the 100 mg/kg/day group males. This increase was likely of non-renal origin as it was not associated with changes in creatinine, urinalysis parameters, or renal histopathology. As with serum protein changes, the pattern of changes in urea nitrogen is consistent with those reported for other peroxisome proliferators. Changes in urea nitrogen were reversible in males, as there were no statistically significant changes in these parameters following the approximately 4-week recovery period.

Alkaline phosphatase was minimally increased in the 10 and 100 mg/kg/day male groups and in the 1000 mg/kg/day female group. Alkaline phosphatase may be increased in association with cholestatic liver disease, however, in this study, other markers of cholestatic liver injury were not increased (bilirubin and glutamyl transferase were actually decreased in the 1000 mg/kg/day females), and there were no effects on other enzymes indicative of hepatocellular injury (ALT, AST, SDH). Additionally, there was no histopathological evidence of liver cytotoxicity. Therefore, these minimal increases in alkaline phosphatase were the result of extra hepatic factors and were likely due to induction of liver microsomal enzymes. In the previous 28-day study in rats, the test material was an inducer of total P450 enzyme. As such, these increases were not considered to be adverse. The increases in alkaline phosphatase were reversible following the approximately 4 week recovery period.

Serum phosphorus was minimally increased in the 10 and 100 mg/kg/day male groups and in the 1000 mg/kg/day female group. Values in individual animals in these groups were all within laboratory reference intervals, and the mean values in the affected male groups were actually lower than the historical mean. In addition, there were no changes in serum calcium. Based on these considerations, the changes in phosphorus were considered to be test substance-related but non-adverse. The changes in serum phosphorus were reversible in both males and females following the approximately 4- week recovery period.

Total bilirubin and glutamyl transferase were decreased in the 1000 mg/kg/day female group. Total bilirubin was also decreased in the 100 mg/kg/day female group. These changes were considered to be test substance related but non-adverse based on the direction of change (decreased rather than increased). The changes in both parameters were reversible following the approximately 4-week recovery period.

Statistically significant increase in urine volume and lower urine osmolality (not statistically significant) suggestive of diuresis were noted in the 1000 mg/kg/day group females at study week 13 as compared to the control group.

This information is submitted in accordance with current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act or, where it is not clear that reporting criteria have been met, it is submitted as a precautionary measure and because it is information in which EPA may have an interest.

Sincerely,

A. Michael Kaplan

A. Michael Kaplan, Ph.D.
Director - Regulatory Affairs

AMK/CC: clp
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