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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:

Benzoic acid, 2-(isocyanatosulfonyl methyl)-, methyl ester
CAS # 83056-32-0

This letter is to inform you of the results of several toxicity studies (eye irritation study, acute oral study, 28-day oral study, reproduction study, and two mutagenicity studies) with the above referenced test substance.

Eye Irritation Study:

In the eye irritation study, 0.1 mL of test substance was administered to 1 eye of 6 New Zealand White rabbits. The eyes remained unwashed following treatment. The conjunctiva, iris, and cornea of each treated eye were evaluated and scored according to a numerical scale approximately 1, 24, 48, and 72 hours and 7, 14, and 21 days following administration of the test substance.

Severe conjunctival redness (score of 3) was observed in the treated eye of 4 rabbits, severe conjunctival chemosis (score of 4) was observed in the treated eye of 2 rabbits, and severe corneal opacity (score of 4) was observed in the treated eye of 1 rabbit. Corneal ulceration was observed in the treated eye of all 6 rabbits. Myosis was observed in the treated eye of all rabbits and the treated eye of 2 rabbits had reduced pupillary reflex. Neovascularization was observed on the cornea of the treated eye of 5 rabbits. Total necrosis of the treated eye was observed in 2 rabbits. The conjunctiva of the treated eye of one of these rabbits was gray in color. The cornea of the treated eye of one rabbit exhibited a deformity of the cornea. Complete reversibility of ocular effects did not occur in the treated eye of 4 rabbits. At 21 days (study termination), total necrosis, conjunctival chemosis (score of 2), and conjunctival redness (score of 1) were still present in the treated eye of one rabbit. Corneal opacity (score of 2) and circumcorneal injection of the iris were still present in the treated eye of another rabbit. Another rabbit exhibited corneal opacity (score of 2), corneal neovascularization, deformity of the cornea, corneal ulceration, and circumcorneal injection and congestion of the iris in the treated eye at 21 days. A fourth rabbit exhibited corneal opacity (score of 2), corneal neovascularization, and circumcorneal injection of the iris at 21 days.

Acute Oral Study:

The test substance was administered to SD rats (5/sex) once at the dose level of 2536 mg/kg (corresponding to 2080 mg/kg of ai) by gavage. Control rats received purified water. Examinations for mortality and clinical signs were performed 15 min, and 1, 2, and 4 h after administration and daily thereafter for 14 days.

One animal died on day 2 and the LD50 was > 2536 mg/kg (corresponding to 2080 mg/kg of ai). The dose was selected based on a preliminary study where 2 rats/sex/group were dosed at 589, 1178 and 2356 mg/kg and incidences of mortality were 0, 1 and 1, respectively. Following clinical signs were noted: Coma – 3 rats on day 1; lethargy – 4 rats on day 1 and 4 rats on day 2; prostration – 5 rats on day 1 and 2 rats on day 2; subdued behavior – 9 rats on day 1 and 1 rat on day 2.

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28-Day Subchronic Oral Study:

The test substance was administered by gavage to SD rats (5/sex/group) daily for 4 weeks at 0, 0, 50, 150 or 600 mg/kg/day. Since xylene was the vehicle for the test substance, two controls (untreated and xylene treated) were used. Full clinical examination, body weight and food consumption were collected weekly. Clinical and anatomic pathology were performed at termination.

Two intermediate dose females and four high dose males died during the study. Salivation was noted in all the animals in all the treated groups (including xylene control) immediately after dosing, but ceased soon after. It was probably related to the taste of xylene. Subdued behavior was noted only in the test substance treated animals. Subdued behavior was sporadic (1 or 2 animals in the 1st or 2nd weekly interval) in the low dose, 1 and 3 males in week 1 and 2, respectively, and 3 females in week 1 and 1 female thereafter in intermediate dose, and all males in all weekly intervals and all females in week 1 and 2, and 2 or 3 females in week 3 and 4. Body weight and food consumption were lower in all the treated groups.

Mean packed cell volume in groups 2, 3, and 4 was slightly higher than controls, and mean total white blood cell count of group 2, 3, and 4 females and group 3 and 4 males was slightly lower than controls. The surviving group 5 male had slightly lower hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration than both controls. Mean polymorphonuclear neutrophils of group 4 males were slightly higher than controls. The same trend was seen in all treated females (including vehicles) and in group 2 and 5 males. Chloride in group 3, 4, and 5 males, calcium and total cholesterol in group 2, 3, and 4 males and group 2, 3, 4, and 5 females, inorganic phosphorus in group 2 and 3 males, protein in group 2 females and blood urea nitrogen and total bilirubin in group 4 males, and alanine amino transferase in group 4 males and females and group 5 females were higher than controls. Organ weight changes observed in treated groups (including xylene treated group) principally in relative values were probably due to the decrease of body weights of the surviving animals. The main macroscopic finding was thickening of the non glandular portion of the stomach in the majority of the animals from all treated groups (including xylene treated group) and raised areas in the stomachs of 4 group 5 females. Hepatocellular hypertrophy was noted in groups 2 and 5 males and females and in group 4 males. Lymphoid atrophy of the white pulp of the spleen was noted in 3 group 5 males. Acanthosis and hyperkeratosis of the non glandular portion of the stomach were noted in all treated groups. In addition, 2 group 5 animals showed erosion of the squamous mucosa.

Treatment with the test substance for 28 days caused marked toxicity at the dose levels of 150 and 600 mg/kg/day, characterized by mortality, subdued behavior, and clinical and anatomical pathology changes. The no effect level (NOEL) was 50 mg/kg/day.

Reproduction Study:

The objective of the study was to investigate the effects of the test substance on gonadal function, the estrous cycle, mating behavior, conception, pregnancy, parturition, lactation and the growth and development of the offspring when administered orally by gavage to the parental generation. Male Crl:WI(Glx/BRL/Han)BR rats were treated daily at 0, 25, 50, or 100 mg/kg/day of the test substance in corn oil for 10 weeks before pairing with females that had been treated with the same doses for 2 weeks. Dosing continued throughout paring, gestation and lactation.

There was no dose related toxicity as shown by the clinical observation, body weight, food intake and necropsy finding of the parental animals. All males survived to the end of the study. Four females died or were euthanized during or post lactation period. All high dose males had excess salivation immediately after dosing at the end of the first week and at the start of the second week of treatment. At the end of the second week this condition was seen in all animals from the low, intermediate and high dose groups together with a paddling movement of the forelimbs associated with wiping the face. These effects continued in the intermediate and high dose males for the remainder of the study, but the incidences became less from week 5 with only a few animals affected on most occasions. Effects in low dose males were seen only on day 13 of the treatment. Salivation also occurred in the first three weeks of treatment immediately after dosing of the intermediate and high doses in a few females. Paddling was noted only occasionally. The excess salivation was considered to be a local reaction rather than adverse systemic toxicity. The paddling movement of the limbs was a natural grooming response associated with the excess salivation around the mouth and face.

None of the reproductive or developmental parameters were affected at any of the dose levels.

Mutagenicity Studies:

The test substance was evaluated for genotoxicity in the *in vitro* chromosome aberration test with human peripheral blood lymphocytes (HPBL) in the absence or presence of an exogenous metabolic activation system (Aroclor-induced rat liver S9), and in the *in vivo* micronucleus (MN) test in rat bone marrow cells.

The results from the *in vitro* chromosome aberration assay are positive, and the results form the *in vivo* MN assay are equivocal.

In vitro chromosome aberration

The chromosome aberration assay was used to evaluate the clastogenic (chromosome breaking) potential of the test substance. Cytogenetic analyses were conducted from cultures treated without S9 at 508, 1020, and 1530 µg/mL (3 hr treatment) or at 1020 and 1530 µg/mL (continuous treatment); and from cultures treated with S9 for 3 hours at 1020, 1530, and 2290 µg/mL. Severe cytotoxicity was observed at dose levels ≥2030 µg/mL without S9, and at ≥3500 µg/mL with S9. The top doses scored for chromosomal aberrations had acceptable mitotic indices. The test substance was concluded to cause statistically significant increases in clastogenic activity in the absence of S9 metabolic activation at the highest dose level scored, 1530 µg/mL

In vivo micronucleus assay

The *in vivo* micronucleus assay was used to evaluate the test substance's ability to induce micronucleated polychromatic reticulocytes (i.e., aneugenic or clastogenic alterations by the test substance) in bone marrow cells following oral intubation (gavage) in Sprague-Dawley rats.

A preliminary toxicity study was conducted where groups of 3 males and 3 females were administered the test substance as a single dose by oral intubation of vehicle (xylene) control, an absolute negative (water) control , 197, 351, 627, 1120, and 2000 mg/kg body weight, or positive control. Transient clinical signs included subdued behavior (at ≥627 mg/kg), ataxia (at 627 mg/kg), lethargy (at 2000 mg/kg), ptosis and watering (at ≥1120 mg/kg), and respiratory distress (at 2000 mg/kg). Neurological signs were observed on days 1 and 2.

Dose levels for the main study were selected based on the results from the preliminary toxicity study. Groups of animals were administered a single dose by oral intubation of vehicle (xylene) control, an absolute negative (water) control , 627, 1120 and 2000 mg of test substance/kg body weight, or positive control. Each group contained 10 male and 10 female rats, sacrificed at two time points (5 animals/sex/time points).

The vehicle control, xylene, was dosed at 2 mL/kg xylene. The test substance was administered at a dose volume of 10 mL/kg body weight. Cyclophosphamide (CP), the positive control, was administered at a concentration of 60 mg/kg body weight and a dose volume of 10 mL/kg.

Approximately 24 and 48 hours after dose administration, bone marrow samples were collected for the microscopic micronucleus evaluation. The animals were weighed prior to treatment and sacrifice, and were observed for clinical signs and mortality/moribundity daily.

No significant changes in weight were recorded. Transient clinical signs included subdued behavior (at ≥627 mg/kg), respiratory distress (at 627 mg/kg and 2000 mg/kg), chromodacryorrhea and staggering gait (at 1120 mg/kg), piloerection (at ≥1120 mg/kg), and lateral decubitus, hypothermia, ptosis, and watering (at 2000 mg/kg). One male at 2000 mg/kg died on Day 2. The clinical singes were considered to occur in a dose-responsive manner.

Micronuclei were analyzed in the polychromatic erythrocytes (MNPCE) and the frequency of PCE/normochromatic erythrocytes (NCE) was used as a measure of bone marrow

A statistically significant increase (4.56-fold higher than the concurrent vehicle control) in the mean number of MNPCE was observed in the bone marrow of females at 2000 mg/kg at the 48-hr time point. No statistically significant increases in the mean number of MNPCE were observed in the males at 2000 mg/kg. When both sexes were combined, a statistically significant increase (2.73-fold) in the mean number of MNPCE was observed at 2000 mg/kg, when compared to the concurrent negative control. The vehicle, absolute negative and positive control groups exhibited a response consistent with the historical control data. No effect was observed for the vehicle

control. A statistically significant decrease in the PCE/NCE ratio was observed at 1120 and 2000 mg/kg at the 48-hr sacrifice time point only.

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, Ph.D.
Director - Regulatory Affairs

AMK/SSA/CC/EMD: clp
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