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November 5, 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:

1,3,5-Triazine-2-amine-4-methoxy-N, 6-dimethyl-  
CAS # 5248-39-5

This letter is to inform you of the results of an *in vivo* mouse micronucleus assay, an acute oral study in rats, and a subchronic oral study in rats with the above referenced test substance. This test substance is subject to a Consent Order, PMN P-85-933.

Mouse Micronucleus Assay:

This study evaluated the clastogenicity of the test substance *in vivo* by examining micronuclei in the polychromatic erythrocyte (PCE) of mouse bone marrow. A range-finder study was conducted to select dose for main study. CD-1 mice of 4-5 weeks old were used in the study. The test substance did not induce clastogenic effect in the mice treated up to 250 mg/kg/day.

In the range-finder study the test substance was administered once daily by gavage, in 0.5% methylcellulose for two consecutive days to groups of 3 male and 3 female mice at 62.5, 125, 250, 300, 350, 500 or 600 mg/kg/day. Observations were made over a 2 day period following the first dose and signs of toxicity recorded. At least one death occurred at  $\geq 300$ mg/kg/day. Clinical signs noted in the 300 to 600 groups included lethargy, eye closure, abnormal gait, piloerection, excessive grooming, prostrate, and sporadic convulsions. No clinical signs were observed in any animal dosed at 62.5 or 125 mg/kg/day. At 250 mg/kg/day, clinical signs were limited to lethargy in two animals and therefore, doses up to 250 mg/kg/day were selected for the main study. Since no difference in toxicity between males and females, male mice were used for the main study.

In the main study, the test substance was administered once daily by gavage, in 0.5% methylcellulose for two consecutive days to groups of 6 male mice at 62.5, 125, or 250 mg/kg/day. The negative (vehicle) control in the study was 0.5% methylcellulose also administered orally by gavage once daily on two consecutive days. Cyclophosphamide (CPA), the positive control, was dissolved in saline and administered orally by gavage as a single dose at 40 mg/kg to a group of 6 male mice which were killed after 24 hours. No clinical signs were observed in any animal dosed at 62.5 or 125 mg/kg/day. At 250 mg/kg/day, 2/6 animals showed lethargy.

Acute Oral Toxicity Study:

The acute oral toxicity of the test substance was investigated in four groups of 5 male and 5 female CD rats at dosages in the range 202 – 567 mg/kg. The test substance was administered by oral gavage, at a volume-dosage of 20 mL/kg in 0.5% w/v aqueous methylcellulose. The animals were observed during a subsequent 14-day period and any signs of reaction to treatment were recorded. All decedents and surviving animals killed on Day 15 were subject to necropsy.

**CONTAINS NO CBI**

The mortality distribution was as follows: A) 202 mg/kg – all survived, B) 285 mg/kg – all survived, C) 402 mg/kg – 3 males and 1 female died, D) 567 mg/kg – all died. The majority of deaths occurred during the first overnight period, with single male animals treated at 402 mg/kg dying on Days 2 or 3.

The principal signs of reaction to treatment comprised lethargy, decreased motor activity, hunched posture, ataxia, breathing irregularities, pigmented orbital secretion, staining of the snout and closed eyes. All surviving rats were fully recovered by Day 4 and remained normal throughout the remainder of the observation period.

The following effects were observed during the study:

A) 202 mg/kg dose for animals (male & female) surviving Day 15: 1) decreased motor activity in all rats, 2) hunched posture in all rats, 3) piloerection in all rats, 4) pupils constricted in 2 female rats, 5) eyes closed in 3 female rats. All effects were observed on the day of dosing and no signs thereafter.

B) 285 mg/kg dose for all rats (male & female) surviving Day 15: 1) lethargy in all females only on the day of dosing, 2) decreased motor activity, 3) hunched posture, 4) ataxia, 5) hyperpnoea, 6) salivation on the day of dosing, 7) eyes closed on the day of dosing. All other effects, except where noted, were observed on the day of dosing, and Days 1 & 2 and no signs thereafter.

C) 402 mg/kg dose for all animals (2 males & 4 females) surviving Day 15: 1) lethargy on the day of dosing, and no signs thereafter, 2) decreased motor activity on the day of dosing, Days 1 & 2 and no signs thereafter, 3) hunched posture on the day of dosing, Days 1 & 2 and no signs thereafter, 4) ataxia on the day of dosing, Days 1 & 2 and no signs thereafter, 5) hyperpnoea on the day of dosing, Days 1 & 2 and no signs thereafter, 6) salivation on the day of dosing, no signs thereafter, 7) eyes closed on the day of dosing, and no signs thereafter.

D) 567 mg/kg dose for animals (male & female) on the day of dosing: 1) lethargy in all rats, 2) decreased motor activity in all rats, 3) hunched posture in all rats, 4) ataxia in all rats, 5) hyperpnoea in all rats, 6) salivation in 4 male rats, 7) piloerection in all female rats, 8) eyes closed in all rats. All the above-mentioned clinical signs were observed on the day and the day after dosing in animals that were dead.

Under the conditions of this study, the combined acute medium lethal dosage ( $LD_{50}$ ) was 410 mg/kg.

#### Subchronic Oral Toxicity Study:

Groups of 5 male and 5 female CD rats received the test substance by oral gavage at dosages of 8, 40 or 200 mg/kg/day for 29 consecutive days. The test substance was administered as a suspension in 0.5% w/v methylcellulose in distilled water at a volume-dosage of 5mL/kg bodyweight. A similarly constituted control group of animals received the vehicle alone at the same volume-dosage as treated animals. There were two deaths during the study. A female receiving 8 mg/kg/day was killed *in extremis* on Day 23, and a male receiving 40 mg/kg/day was found dead on Day 28. The cause of death was not considered to be an effect of the test substance.

Transient post-dosing salivation was observed throughout the treatment period amongst all animals treated at 200 mg/kg/day. This sign was observed occasionally in animals treated at 40 mg/kg/day and on one occasion in one animal treated at 8 mg/kg/day. Irritability, and subsequent lethargy and unsteady stance were observed after dosing from Day 22 onwards, in rats treated at 200 mg/kg/day. Piloerection was also observed amongst these animals at this time. Apart from irritability on one occasion in rats treated at 40 mg/kg/day, these signs were not observed at lower dosages.

There were no differences in food consumption that could be unequivocally ascribed to an effect of treatment with the test substance. The bodyweight gains of male rats receiving 40 mg/kg/day and male and female rats receiving 200 mg/kg/day were lower than those of the control animals. Food utilization was less efficient in male rats receiving 200 mg/kg/day than in the male controls. A similar effect was recorded for females receiving this dosage during Week 1 only.

Platelet numbers were lower in male and female rats receiving 200 mg/kg/day and males receiving 40 mg/kg/day, when compared to those of the control animals. Females treated at 200 mg/kg/day also had higher white blood cell numbers compared to the control females. Higher aspartate amino-transferase (females only) and alanine amino-transferase activities, higher total protein concentration (males only) and lower glucose (males only), sodium and potassium concentrations were recorded for rats treated with 200 mg/kg/day, when compared to control animals.

Similar differences were also apparent for the glucose (males only) and potassium concentrations of rats treated at 40 mg/kg/day.

Myocardial degeneration with or without fibrosis was observed at the ventricular apex of the majority of rats treated at 200 mg/kg/day.

The NOEL for this study was considered to be 8 mg/kg/day.

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 handwritten signature in cursive script that reads "A. Michael Kaplan". The signature is written in black ink and extends across the width of the text area.

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

AMK/SSA: clp  
(302) 366-5260