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DATA EVALUATION REPORT

STUDY TYPE: 83-4. Reproductive and Fertility Effects: Range-Finding Study (Rats)

TOX CHEM. NO.: 116A
MRID NO.: 406609-07

TEST MATERIAL: Bronopol, pharmaceutical grade, while crystals, purity (a.i.): 99.9%, batch no.: 845309, readily soluble in water, and stable in aqueous solutions at pH 4.0 and lower.

SYNONYMS: Bronopol-Boots

STUDY NUMBER(S): TX 86009

SPONSOR: The Boots Company, PLC, Nottingham, England.

TESTING FACILITIY: International Research and Development Corporation (IRDC), Mattawan, Michigan.

TITLE OF REPORT: Bronopol: Range-Finding Reproduction Study in Rats.

AUTHOR(S): A.M. Beswick

REPORT ISSUED: February 6, 1986

CONCLUSIONS:

Systemic NOEL: < 25 mg/kg (Decreased body weight gain; males; LDT; not determined for females)

Reproductive NOEL: ≥ 200 mg/kg (HDT)

Developmental NOEL: 25 mg/kg
LEL: 50 mg/kg (Decreased number of pups born/dam)*

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*The mean numbers of pups born in the 50 and 200 mg/kg groups were lower (by 20.8% in each case) than in the control group, but this finding was not observed in the 25 and 100 mg/kg groups. Because the mean numbers of implantations were similar for all groups and the dose-response relationship was lacking, it is not clear whether this finding was treatment-related.

The anticipated doses of Bronopol were 25, 50, 100 and 200 mg/kg of body weight/day for the males and females, but the actual (achieved) mean doses for the males were 17.2, 31.8, 64.3 and 113.3 mg/kg/day and for the females, 30.9, 52.7, 95.7 and 193.7 mg/kg/day. The lower than intended doses resulted from the decreased consumption of drinking water in which Bronopol was administered.

Based on the results of this study, nominal doses of 25, 70 and 100 mg/kg/day of Bronopol were selected for the 2-generation rat reproduction study (No. 510-013).

Classification: Core-Supplementary (Range-Finding Study)

EXPERIMENTAL PROCEDURES

The purpose of this study was to determine dose levels for the two-generation rat reproduction study, conducted by IRDC (No. 510-013; see separate review). This study was started on 6/25/85 and terminated on 8/20/85. It involved a parental group F_0 (5 males and 5 females/dose level) and one litter identified as F_1 litter or F_1 pups. Bronopol was administered in drinking (tap) water during the premating (14 days), mating (up to 10 days), gestation and lactation (3 days) periods, at which time all of the animals on the study were sacrificed. The pH of water was adjusted to 4.0 with hydrochloric acid in order to ensure the stability of Bronopol. The target concentrations of Bronopol used were 0.025, 0.05, 0.1 and 0.2%, corresponding to 25, 50, 100 and 200 mg/kg/day, respectively (anticipated dose levels). The controls received tap water adjust to pH 4.0. Dose concentrations were based on the results of six-week and two-year drinking water studies reported by the sponsor. Dosing solutions were prepared twice weekly (Monday and Thursday) and were dispensed in glass water bottles. Remaining solutions were stored in amber bottles at room temperature (< 25° C). Samples of dosing solutions (drinking water) were analyzed for concentration of Bronopol prior to study initiation and on study weeks 1 and 4. The rats (Charles River COBS® CD® strain were:

1. Obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan.
2. Acclimated for 20 days.
3. Seventy-six days old and weighed 332-364 g (males) and 205-237g (females) at the initiation of the study.
4. Assigned to groups randomly, on a weight basis, by the computer-generated system and the groups were evaluated for homogeneity using Bartlett's test.
5. Fed basal laboratory diet of Purina Certified Rodent Chow #5002.
6. Housed individually, except during mating, in suspended wire-mesh cages, at temperature of 24±0.5° C, humidity of 51±5.5%, and 12-hour light/dark cycle.

7. Identified by cage and group, and individually by a Monel® metal ear tag bearing the animal number.

The following parameters were examined for the F_0 rats:

1. Reproductive: Copulatory interval, male and female fertility indices, gestation length, and number of implantation sites.

2. Appearance, Behavior and Mortality (Animals were observed twice daily).

3. Body Weights. Weekly, from the initiation of treatment until sacrifice.

4. Food Consumption. Weekly, except during mating.

5. Water and Bronopol consumption. Weekly, except during mating.

6. Necropsy. All animals on the study were necropsied. The uteri from females that appeared nonpregnant were opened and placed in 10% ammonium sulfide solution for detection of implantations.

The following parameters were examined for the F_1 pups:

1. Litter size and number of stillbirths and live births.

2. Survival and behavior abnormalities, daily until sacrifice on lactation day 3.

3. Individual body weights and external examination on lactation day 3.

4. Necropsy of intact dead pups.

RESULTS

Bronopol: Concentration in Dosing Solutions (Drinking Water)

The concentrations of Bronopol in dosing solutions ranged from 90 to 108% of target levels.

F_0 Rats (Parental Data)

Appearance, Behavior and Mortality

Two 200 mg/kg male rats appeared thin during study week 2 and one was sacrificed. Prior to sacrifice, the animal was moribund, had labored breathing and reduced righting reflex, and

was cool to touch. Nothing unusual was observed in the appearance and behavior of the remaining rats on the study and there were no other mortalities.

Body Weights

For the males, body weights were reported weekly during the entire study (8 weeks). For the females, body weights were reported only during the premating period (first 2 weeks of study). It was stated by the testing laboratory that further evaluation of weight changes were inappropriate because the animals became pregnant at different times and, consequently, were not all at the same stage of gestation when they were weighed.

All treated males gained less weight than did the controls but the reduction was dose-unrelated. During the 8 weeks of study, the group mean body weight gains for the 0 (control), 25, 50, 100 and 200 mg/kg males were 174, 149, 142, 146 and 128 g, respectively. During the 2 weeks of study, the group mean body weight gains for the females were 9, 24, 22,18 and 16 g, respectively.

Food Consumption

Food intake was reported as g/rat/day and g/kg/day, during the entire study for the males and during the premating period only for the females. During the first week of study, the 200 mg/kg males consumed 34.1% and females 19.2% less food than did the controls, but thereafter the food consumption (g/kg/day; mean values) of the treated and untreated rats in all groups was similar.

Water Consumption

Water consumption was reported as g/rat/day and g/kg/day, during the entire study for males and during the premating period only for the females. Throughout the study, the water consumption of male rats in all treated groups was reduced relative to that of the control group. The greatest reduction was observed in the high-dose group, 56.7% during the first week and 20.2-32.1% during the remaining 7 weeks. Relative to the control values, decreases in the water consumption in the 25, 50 and 100 mg/kg male groups were 2.4-22.6, 14.4-30.4 and 9.8-26.7%, respectively. In the females, the reduction of water consumption in the 25, 50, 100 and 200 mg/kg groups, during the first week was 6.6, 16.0, 28.8 and 39.6%, respectively, relative to the control value. During the second week, the highest reduction in water consumption (27.1%) was observed only in the 100 mg/kg group.

Bronopol Consumption

The anticipated doses of Bronopol were 25, 50, 100 and 200 mg/kg of body weight/day for the males and females, but the actual (achieved) mean doses for the males were 17.2, 31.8, 64.3 and 113.3 mg/kg/day and for the females, 30.9, 52.7, 95.7 and 193.7 mg/kg/day. Since, for the females, consumption of drinking water in which Bronopol was administered was reported only for 2 weeks (premating period) because further evaluation of water intake was not appropriate, it is ambiguous why Bronopol consumption was reported as "mean compound consumption during the entire treatment period." The lower than intended doses of Bronopol resulted from the decreased consumption of drinking water.

Necropsy Findings

One 200 mg/kg male rat, sacrificed in extremis during study week 2, had ulcers and thickened mucosa in the non-glandular stomach, and blood-stained gastrointestinal contents. These findings were regarded as treatment-unrelated. Nothing remarkable was observed in the remaining rats, in this and other groups, at the termination of the study.

Histopathology was not performed and organs were not weighed.

Reproductive Parameters

Nothing remarkable was observed in any group.

Litter (Developmental) Parameters

The mean numbers of pups born in the 50 and 200 mg/kg groups were lower (by 20.8% in each case) than in the control group, but this finding was not observed in the 25 and 100 mg/kg groups. Because the mean numbers of implantations were similar for all groups and the dose-response relationship was lacking, it is not clear whether this finding was treatment-related.

COMMENTS

This study is clearly reported and there are no obvious factual or typographical errors. Because it is a range-finding study, meeting the November 7, 1989 Acceptance Criteria does not apply and the study is classified as Core-Supplementary. This study was inspected 13 times (Quality Assurance Inspections) during its course and the preparation of the report (6/25/85-1/22/86).

Body weights, food intake, and water and Bronopol consumption were reported during the entire study (8 weeks) for

the males, but only during the premating period (2 weeks) for the females. Since the control females gained less weight than the treated females during the premating period, the effects of Bronopol on weight gain cannot be meaningfully evaluated.