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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 16 1989

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 018201-8. MCPB Data Call-In Notice. Review of Submitted Teratology Studies

Tox. Chem. No. 558
Project No. 9-0315

TO: Franklin I. Rubis, PM Team No. 50
Generic Chemical Support Branch (TS-767c)
Special Review and Reregistration Division

FROM: Pamela M. Hurley Ph.D., Toxicologist Pamela M. Hurley 1/6/89
Section I, Toxicology Branch I
Insecticide, Rodenticide Support
Health Effects Division (TS-769c)

THRU: Edwin R. Budd, Section Head Judith W. Hancwicz for EB 1/12/89
Section I, Toxicology Branch I
Insecticide, Rodenticide Support
Health Effects Division (TS-769c)

Record No(s). 234438

Background and Request:

Rhone-Poulenc AG Company submitted two teratology studies conducted on MCPB in response to a data call-in notice. The Toxicology Branch (TB-1) has been asked to review and comment on these two studies.

Response:

TB has reviewed the two teratology studies, one conducted on rats and one conducted on rabbits. Both studies have been classified as Core Guideline and are acceptable as fulfilling the requirements for teratology studies on MCPB.

In the rat study, MCPB was tested for possible teratogenic effects on CD rats at dose levels of 0.0, 25.0, 100.0 and 225.0 mg/kg/day. The NOEL for maternal toxicity was 25.0 mg/kg/day, based upon decreased body weights and body weight gains during the gestational period. The NOEL for developmental toxicity was also 25.0 mg/kg/day, based upon decreased mean body weights/litter, increases in unossified or poorly ossified sites and increases in several other skeletal variations. The LOEL for both maternal and developmental toxicity was 100.0 mg/kg/day.

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It should be noted that the Toxicology Branch (TB) normally prefers that statistical analyses be conducted on fetuses as well as litters. In this case, the results of the analyses would not have affected either the NOEL or the LOEL for the study. Therefore, they will not be required for this study. However, in future studies they should be conducted.

In the rabbit study, MCPB was tested for possible teratogenic effects in New Zealand white rabbits at dose levels of 0, 1.0, 5.0, and 20.0 mg/kg/day. The NOEL for maternal toxicity was 5.0 mg/kg/day and the LOEL was 20.0 mg/kg/day based upon maternal deaths, clinical signs of toxicity, decrease in body weight gain during treatment and change in color of the liver and kidneys. The NOEL for developmental toxicity was 20.0 mg/kg/day (HDT).

Reviewed By: Pamela Hu...
Section I, Tox. Branch, IRS (TS-...
Secondary Reviewer: Edwin Budd for EB
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DATA EVALUATION REPORT

STUDY TYPE: Teratology - rat (83-3)

TOX. CHEM. NO.: 558

ACCESSION NUMBER/MRID NO.: 408654-02

TEST MATERIAL: MCPB

SYNONYMS: None

REPORT NUMBER: 51-532

SPONSOR: Rhone-Poulenc Ag Company, Research Triangle Park, NC

TESTING FACILITY: Bushy Run Research Center, Export, PA

TITLE OF REPORT: Developmental Toxicity Evaluation of MCPB Administered by Gavage to CD (Sprague-Dawley) Rats

AUTHOR(S): Rochelle W. Tyl

REPORT ISSUED: September 13, 1988

CONCLUSION: MCPB was tested for possible teratogenic effects in CD rats at dose levels of 0, 25, 100 and 225 mg/kg/day. The maternal toxicity NOEL is 25.0 mg/kg/day, based upon decreased body weights and body weight gains during the gestational period. The developmental toxicity NOEL is also 25.0 mg/kg/day, based upon decreased mean body weights/litter for males and females combined, for males alone and for females alone, increases in unossified or poorly ossified sites and increases in several other skeletal variations. The LOEL for both maternal and developmental toxicity is 100.0 mg/kg/day.

Classification: Core Guideline

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A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: Butyric acid, 4(4-chloro-o-tolyl)oxy)-

Description: white, lumpy powder
Batch #(s), Other #(s): Lot # NPD X094 R July 87
Purity: 97.6%
Source: Rhone-Poulenc Ag Company
Vehicle (if applicable): Mazola corn oil

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female CD outbred albino rats

Age: 63 days (males), 56 days (females) upon arrival, after which they were quarantined for 2 weeks

Source(s): Charles River Breeding Laboratories, Inc., Kingston, NY

3. Procedure:

a. Dosing Formulations: The appropriate amount of the test material was weighed out and mixed with a weighed portion of corn oil. The dosing formulations were prepared once during the treatment phase of the study. Each female received a volume of 5.0 ml/kg, based upon the most recent body weight of the animal.

b. Concentration, Homogeneity and Stability Analyses:

Standard solutions of the test chemical in corn oil were prepared and analyzed for concentration of test material along with samples of the dosing solutions. All samples were analyzed with a Waters HPLC. A stability study was conducted in which samples were analyzed for MCPB content at 0, 7, 13, 14, and 21 days when stored at room temperature. A homogeneity study was conducted on 50 and 1.5 mg/ml dosing solutions. Each sample was analyzed from 3 regions of each solution (top, middle and bottom).

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c. Animal Assignment and Dose Levels:

Test Group	Dose Administered	Female
Contr.	0.0 mg/kg/day	25
1	25.0 mg/kg/day	25
2	100.0 mg/kg/day	25
3	225.0 mg/kg/day	25

d. Procedures for Studies Other Than Feeding and/or Additions. Changes in Feeding Study: Rats were mated 1:1 and each male was used only once during the study. The day a copulation plug was found was designated gestational day 0. The timed-pregnant dams were dosed daily by gavage with either MCPB in vehicle or with vehicle alone on gestational days 6-15. The dose levels were selected from a range-finding study.

e. Clinical Observations and Mortality: All females were examined daily for clinical signs of toxicity (twice daily during the dosing period). In addition, the animals were examined twice daily for mortality and morbidity.

f. Body Weight Determinations: All females were weighed on gestational days 0, 6, 9, 12, 15, 18, and 21.

g. Food and/or Water Consumption: Food consumption was measured on all females for the gestation day intervals 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-21.

h. Maternal Examinations: The females were sacrificed by carbon dioxide asphyxiation. The body cavities of the dams were opened by midline thoracolaparotomy. The following organs/tissues were grossly examined: the gravid uterus, ovaries (including corpora lutea), cervix, vagina, and abdominal and thoracic organs and cavities. In addition, the lumen and lining of the esophagus, stomach and trachea were examined for any indications of irritation from the dosing solutions or dosing errors. Ovarian corpora lutea of pregnancy were counted and maternal liver and uterine weights were determined. In addition, the report stated that, "the uteri were externally examined for signs of hemorrhage, removed from the abdominal cavity, and dissected longitudinally to

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expose their contents. All live and dead fetuses and resorption sites (early and late) were noted and recorded. Uteri from females that appeared nongravid were placed in a 10% ammonium sulfide solution for detection of early resorptions."

i. Fetal Examinations: Live fetuses were weighed and sexed, and all fetuses were examined for external malformations including cleft palate, and variations. One-half of the fetuses per litter were examined for thoracic and abdominal visceral abnormalities by a modification of the Staples method. The heads of these fetuses were removed and examined by a modification of Wilson's method. The remaining fetuses in each litter were prepared for skeletal examination and stained with alizarin red S. The decapitated fetuses were also processed for staining, but were not examined.

j. Statistical Analyses: Maternal body weights, organ weights, fetal weights and other quantitative continuous variables were compared using Levine's test for equal variances, analysis of variance, and t-test with Bonferroni probabilities for pairwise comparisons. Nonparametric data were statistically treated using the Kruskal-Wallis test followed by the Mann-Whitney U test when appropriate. Incidence data were compared using Fisher's Exact Test.

B. RESULTS:

1. Analyses of Dosing Solutions:

Concentration Analyses: The concentration analyses revealed that the dosing solutions ranged from 93.1 to 102.0 % of the target concentrations.

Homogeneity Analyses: The results of the homogeneity analyses indicated that the solutions were homogeneous. For the 50.0 mg/ml solution, the concentrations ranged from 94.8 to 95.2% of the nominal concentration and for the 1.5 mg/ml solution, the concentrations ranged from 102.7 to 104.7% of the nominal concentration.

Stability Analyses: The concentrations of MCPB in solution proved to be stable up to a period of 21 days. The results of the stability study were as follows: for the 50.0 mg/ml solution, the concentrations ranged from 93.4 to 99.8% of the nominal concentration over the 21 days with a concentration value of 96.6% of the

nominal value at 21 days; and for the 1.5 mg/ml solution, the concentrations ranged from 96.7 to 106.7% of the nominal value with a value of 100.7% at 21 days.

2. Maternal Examinations:

Clinical Observations and Mortality: There were no treatment-related deaths in any dose group. In addition, there were no abortions or early deliveries. Statistically significant increases in the incidence of alopecia were observed in dams at 225.0 mg/kg/day during and after the treatment period.

Body Weight Determinations: Statistically significant decreases in mean body weight were observed in the highest dose dams when compared to control animals from day 9 through day 21 of the gestation period. These decreases ranged from 95.5 to 90.3% of the control values, the percentages generally decreasing and then leveling off after gestational day 15 (the day treatment stopped). In addition, at scheduled sacrifice, there were significant decreases in absolute and corrected body weight (body weight at sacrifice minus gravid uterine weight) in these animals. Mean body weight gains were significantly less than controls for this dose group from days 6-9, 9-12 and 12-15, and for days 6-15 (treatment period). The percentages ranged from a negative number the first 3 days to 51% of control body weight gains from days 9-12, to 43% from days 12-15. The overall decrease in mean body weight gain in the high dose dams during the treatment period (days 6-15) was 29.7% of the control values. For the entire gestation period (days 0-21), the overall decrease in bodyweight gain in the high dose dams was 79.9% of the controls.

The mean body weight of the dams in the 100.0 mg/kg/day dose group was statistically significantly decreased when compared to controls at gestational day 15 (95% of the control value). The mean body weight gains for this group were statistically significantly reduced for gestational days 6-9 and 6-15 when compared to the controls (9 and 66%, respectively). The mean body weight gain for days 15-18 were statistically significantly increased for this dose group (120% of the control value).

From these data, it appears that MCPB was affecting body weights and body weight gains in the dams in both the 225.0 and 100.0 mg/kg/day dose groups. See Table A for details.

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Food and/or Water Consumption: Food consumption was significantly decreased at all dose levels for the gestation day intervals 6-9, 9-12, and 6-15. In addition, food consumption for the gestation day interval 12-15 was significantly reduced at the 225.0 mg/kg/day dose level. All these values were less than 90% of the controls.

Gross Examinations: No treatment-related differences between the treated and control animals.

Organ Weights: Mean maternal relative liver weights were significantly increased at 225.0 mg/kg/day, but there were no significant treatment-related effects on mean absolute liver weights. In addition, there were significant decreases in mean gravid uterine weights at this dose level when compared to controls.

Gestational Parameters: There were no differences between the treated and control animals in the number of animals that were pregnant. The numbers were 23/25, 25/25, 21/25, and 23/25 for the controls, low dose, mid-dose and high dose animals. All dams had one or more viable fetuses. There were no significant differences between the treated and control groups in the mean number of corpora lutea, total implants, non-viable implants, early resorptions, late resorptions or dead fetuses. In addition, there were no differences in the the mean percent preimplantation loss, the mean percent live fetuses and the mean sex ratio (% male fetuses). There was a statistically significant increase in the mean number of viable implants in the 100.0 mg/kg/day group. There were statistically significant decreases in mean fetal body weights/litter for all fetuses, male fetuses and female fetuses at the highest dose level (approximately 81% of the control values). In addition, there was a statistically significant decrease in the mean body weight of the male fetuses at the 100.0 mg/kg/day dose level when compared to the control value (approximately 95% of the control value). The authors state that "this finding may be confounded by the slight (but statistically significant) increase in live litter size at 100.0 mg/kg/day." See Table 1 for details.

3. Fetal Examinations:

Gross Examinations: No significant differences were observed between the treated and control groups. See Table 2 for details.

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Visceral Examinations: A few visceral malformations and a number of visceral variations were observed in all groups, including controls. No significant differences were observed between the treated and control groups. See Table 3 for details.

Skeletal Examinations: A few skeletal malformations were observed in one fetus in the highest dose group and a number of skeletal variations were observed in all groups, including controls. Statistically significant differences were observed in the ossification of a variety of sites in the highest dose group when compared to controls. These included both increases and decreases in either unossified or poorly ossified sites or other skeletal variations. In two cases (cervical centrum #5 and some metatarsals (hindlimb - unossified), there was a statistically significant increase in the 100.0 mg/kg/day group as well. These increases and decreases appeared both in the number of fetuses affected and in the number of litters affected, but were only statistically significant in the number of litters affected. See Table 4 for details.

4. Quality Assurance Measures: A signed quality assurance statement was provided.

C. DISCUSSION: The maternal toxicity NOEL is 25.0 mg/kg/day, based upon decreased body weights and body weight gains during the gestational period. The developmental toxicity NOEL is also 25.0 mg/kg/day, based upon decreased mean body weights/litter for males and females combined, for males alone and for females alone, increases in unossified or poorly ossified sites and increases in several other skeletal variations. The LOEL for both maternal and developmental toxicity is 100.0 mg/kg/day. This appears to be a well conducted study and is classified as Core Guideline. It should be noted that the Toxicology Branch (TB) normally prefers that statistical analyses be conducted on fetuses as well as litters. In this case, the results of the analyses would not have affected either the NOEL or the LOEL for the study. Therefore, they will not be required for this study. However, in future studies they should be conducted.

MCPB toxicology review

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Secondary Reviewer: Edwin Budd for EB
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DATA EVALUATION REPORT

STUDY TYPE: Teratology - rabbits (83-3)

TOX. CHEM. NO.: 558

ACCESSION NUMBER/MRID NO.: 408654-01

TEST MATERIAL: MCPB

SYNONYMS: None

REPORT NUMBER: 51-547

SPONSOR: Rhone-Poulenc Ag Company, Research Triangle Park, NC 27709

TESTING FACILITY: Bushy Run Research Center, R.D. #4, Mellon Road, Export, PA 15632

TITLE OF REPORT: Developmental Toxicity Evaluation of MCPB Administered by Gavage to New Zealand White Rabbits

AUTHOR(S): R.W. Tyl and T.L. Neeper-Bradley

REPORT ISSUED: 09/12/88

CONCLUSION: Timed-pregnant rabbits were fed MCPB by gavage on gestational days 6-18. The NOEL for maternal toxicity was 5.0 mg/kg/day and the LOEL was 20.0 mg/kg/day (maternal deaths, clinical signs of toxicity, decrease in body weight gain during treatment, change in color of liver and kidneys). No malformations or variations were observed in the fetuses. The NOEL for developmental toxicity was 20.0 mg/kg/day (HDT).

Classification: Core Guideline

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A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: Butyric acid, 4(4-chloro-o-tolyl)oxy)-

Description: white, lumpy powder
Batch #(s), Other #(s): Lot # NPD X094R, Sample # 50-410
Purity: 97.6%
Source: Rhone-Poulenc Ag Company
Vehicle (if applicable): Mazola® corn oil

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female New Zealand white rabbits

Age: 6 months
Weight(s): 2.5-3.5 kg
Source(s): Hazelton Dutchland Laboratories, Inc., Denver, PA (females), Bushy Run Research Center breeding colony (males)

3. Procedure:

a. Dosing Formulations: The appropriate amount of the test material was weighed out and mixed with a weighed portion of corn oil. Each animal received a volume of 2.0 ml/kg bodyweight of the mixture. The formulations were prepared twice during the study.

b. Concentration, Homogeneity and Stability Analyses: Standard solutions of the test chemical in corn oil were prepared and analyzed for concentration of test chemical along with samples of the dosing solutions. All samples were analyzed with a Waters HPLC. A stability study was conducted in which samples were analyzed for MCPB content at 0, 7, 13, 14, and 21 days. A homogeneity study was conducted on 50, 1.5, and 0.5 mg/ml dosing solutions. Samples were analyzed from 3 regions of each solution.

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c. Animal Assignment and Dose Levels:

Test Group	Dose Administered	Female
Contr.	0.0 mg/kg/day	20
1	1.0 mg/kg/day	20
2	5.0 mg/kg/day	20
3	20.0 mg/kg/day	20

d. Procedures for Studies Other Than Feeding: The females were naturally bred to "proven" fertile males. The day the mating was observed was designated gestational day 0. The timed-pregnant does were dosed with the test material by gavage at the assigned dose levels on gestation days 6 - 18. The dose levels were selected from range-finding studies.

e. Clinical Observations and Mortality: Examined daily after mating to gestation day 6, twice daily during treatment period, once daily after treatment period to necropsy for clinical signs of toxicity; twice daily throughout the study for mortality and moribund status.

f. Body Weight Determinations: Does weighed on gestation days 0, 6, 9, 12, 15, 18, 24, and 29.

g. Food and/or Water Consumption: Measured daily on gestation days 0-29.

h. Maternal Examinations: All surviving females were sacrificed on gestation day 29 and the following organs were grossly examined: gravid uterus, ovaries (including corpora lutea), cervix, vagina, abdominal and thoracic organs, and cavities. Ovarian corpora lutea of pregnancy were counted and maternal liver weight was determined. The uterus was ligated at the cervical end, externally examined for signs of hemorrhage, removed and weighed, and dissected. All live and dead fetuses and resorption sites (early and late) were noted and recorded. Uteri from apparently nongravid females were preserved for future confirmation of pregnancy status.

i. Fetal Examinations: All fetuses were euthanized, weighed and examined for external malformations and variations. Live fetuses were further examined for thoracic and abdominal visceral

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abnormalities by modification of the Staples technique. They were sexed internally. One-half of the fetuses in each litter were then decapitated and their heads were fixed in Bouin's solution for examination of craniofacial structures by modification of Wilson's, van Juisingha's and Bennett's methods. All fetuses in each litter (50% intact and 50% decapitated) were eviscerated, processed for skeletal staining with alizarin red S, and examined for skeletal malformations and variations.

j. Statistical Analyses: The following statistical tests were employed for this study: Levene's test for equal variances, analysis of variance, and t-tests with Bonferroni probabilities for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances, followed when necessary, by the separate variance t-test. Nonparametric data were statistically treated using the Kruskal-Wallis test followed by the Mann-Whitney U test when appropriate. Incidence data were compared using Fisher's Exact Test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

B. RESULTS:

1. Analyses of Dosing Solutions

Concentration Analyses: The analyses indicate that the dosing formulations were within 91.4-106.4% of the target doses.

Homogeneity Analyses: For 0.5 mg/ml, the percent of the nominal concentration found from the top, middle and bottom of the mixing container ranged from 104.0-110.0 percent; for 1.5 mg/ml the range was 102.7-104.7 percent; and for 50.0 mg/ml the range was 94.8-95.2 percent.

Stability Analyses: For 0.5 mg/ml, the percent of the nominal concentration (mean value of 3 samples analyzed) found from day 0 to 21 ranged from 100.0 to 107.3; for 1.5 mg/ml, the percent ranged from 96.7 to 106.7; and for 50.0 mg/ml, the percent ranged from 93.4 to 99.8.

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2. Maternal Examinations

Clinical Observations and Mortality: Three does in the highest dose level and one animal in the control group died during the study. All of these four were pregnant. One animal each in the lowest and mid-dose groups and 2 animals in the highest dose group aborted. One doe from the mid-dose group was removed from the study due to a dosing accident. Therefore, 19 controls, 19 low dose, 18 mid-dose, and 15 high dose anim were examined at laparotomy. Although not in a stat ically significant number of does, clinical signs of toxicity were observed in some animals at the highest dose level. These signs included hypoactivity, paresis, paralysis and ataxia.

Body Weight Determinations: Statistically significant differences in body weights were not observed in any of the treatment groups either during or after the treatment period. Body weight gains were reduced in the highest dose group when compared to controls during gestation days 9-12, 12-15 and 15-18. These values were statistically significant only for the 12-15 gestation day period. During the post-dosing period, females in the highest dose group gained statistically significantly more weight than the control group (days 18-29). See attached table for details.

Food Consumption: No treatment-related changes were observed.

Gross Examinations: Statistically significant increases in color change in the liver and kidneys were observed in does at terminal sacrifice at the top dose level. No other dose-related effects were observed.

Organ Weights: No treatment-related differences in organ weights were observed.

Gestational Parameters: Of the does that were examined at laparotomy, 18/19 controls, 19/19 low dose, 17/18 mid-dose and 15/15 high dose animals were pregnant. One control doe had non-viable implants; and 17/18, 19/19, 17/17 and 15/15 does had viable implants in the control, low dose, mid-dose and high dose groups, respectively. No treatment-related differences were observed in any other gestational parameters including

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number of corpora lutea/dam, total implants, percent preimplantation loss, viable implants, non-viable implants, early resorptions, late resorptions, dead fetuses, percent live fetuses, sex ratio and fetal body weights/litter (all, male, and female) (see attached table).

3. Fetal Examinations

Gross Examinations: No treatment-related differences were observed. Three fetuses in two litters in the highest dose group had lowset ears, but the incidence was not statistically significant. One normal size fetus and two small fetuses in the highest dose group had dome-shaped heads but these were also not significant (see attached tables).

Visceral Examinations: No treatment-related differences were observed (see attached tables).

Skeletal Examinations: No treatment-related differences were observed (see attached tables).

Malformations (summary): No significant treatment-related differences were observed in any group when compared to controls.

Variations (summary): No significant treatment-related differences were observed in any group when compared to controls.

4. Quality Assurance Measures: A signed good laboratory practice statement and a signed quality assurance inspection summary was provided.

C. DISCUSSION: This appears to be a well conducted study. The NOEL for maternal toxicity was 5.0 mg/kg/day and the LOEL was 20.0 mg/kg/day (maternal deaths, clinical signs of toxicity, decrease in body weight gain during treatment, change in color of liver and kidneys). No malformations or variations were observed in the fetuses. The NOEL for developmental toxicity was 20.0 mg/kg/day (highest dose level tested). There were a few high dose fetuses that had lowset ears, but the number of these was not statistically significant. The report also stated that these were observed in one of the range-finding studies but again were not statistically significant.

MCPB toxicology review

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