Review By: David G. Van Ormer, Ph.D. DVO 07-08-88
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Section III, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Teratogenicity - Mouse TOX Chem No.: 501B

Test Material: Arsenic Acid (75%) MRID No.: 406462-02

Formula: H_3AsO_4, 75% (aqueous)

Laboratory Study No.: WIL-75028

Sponsor: Pennwalt Corporation, Agrichemicals Division

Testing Facility: WIL Research Laboratories, Inc.

Title of Report: A Teratology Study in Mice with Arsenic Acid (75%)

Author: Mark D. Nemec, B.S.

Report Issued: June 1, 1988

Summary and Conclusions:

Pregnant Crl:CD-1(ICR)BR mice (25 per dose group) received single daily gavage of aqueous Arsenic Acid (75%) from day 6 through 15 of gestation. Dosages were 0, 10, 32, and 64 mg/kg/day. Controls received deionized water. Body weights were recorded at six periods. Cesarean section was on day 18. Fetuses were weighed, sexed, and examined for external, skeletal, and soft tissue malformations and variations. At top dose, two dams died. Signs included lethargy, decreased urination and defecation, soft stool or mucoid feces, brown urogenital matting, and red material around the eyes. Necropsy showed bilateral reddening of cortico-medullary junction (kidneys), and red areas in the stomach. At mid and (especially) top dose, the dams showed weight loss and an elevated incidence of total litter resorption.

NOEL, maternal toxicity = 10 mg/kg/day (LDT)
LEL, maternal toxicity = 32 mg/kg/day (total litter resorption and reduced body weight)

NOEL, developmental toxicity = 32 mg/kg/day
LEL, developmental toxicity = 64 mg/kg/day (HDT)
(reduced mean viable fetuses and fetal weights, elevated postimplantation loss, exencephaly, and omphalocele)

The top-dose litter incidences of the malformations exencephaly and omphalocele were elevated above historical control litter incidences.

Classification: Minimum Data

A. Materials:

1. Test Compound - Arsenic Acid (75%); a clear, green aqueous liquid. Batch No. 8619. Manufacturer not specified. Analysis by graphite furnace AA (with Zeeman correction) showed that the experimental analytical values for the diluted dosing solutions were within 6 percent of target concentrations. Contaminants were not reported. Stock solution and dosing solutions were prepared fresh daily, according to the Report. Deionized water was the vehicle.

2. Test Animals - Virgin female mice, Crl:CD®-1(ICR)BR, 72 days old when received from Charles River Breeding Laboratories, Inc., Kingston, NY. The body weights ranged from 25.3 to 33.5 g on day 0 of gestation, at approximately 13 weeks of age.

B. Study Design:

1. Breeding and Dosage Assignment - During a 22-day acclimation period, the females were observed at least twice daily for appearance and behavior. Animals judged to be in good health and with a minimum body weight of 20 g were mated with an untreated male of the same strain and source. The day on which evidence of mating was confirmed was termed day 0 of gestation. Females were then returned to individual suspended wire-mesh cages, and consecutively assigned in a block design to dose groups containing 25 mice each by a randomization procedure. Identification was by a combination ear punch and toe clip. The Report states that mean body weights on gestation day 0 were statistically comparable between dose groups.

   Test material was administered orally by gavage once daily for 10 consecutive days, gestation day 6 through 15. The dose levels were 0, 10, 32, and 64 mg/kg/day at a dosage volume of 5 mL/kg. Controls received 5 mL/kg deionized water on a comparable regimen. Dosages were calculated on the most recent body weights.

2. Diet, Drinking Water, and Maintenance - The basal diet was Purina® Certified Rodent Chow® #5002. Appropriate analyses are provided by the manufacturer. Tap water (from on-site wells) was analyzed for contaminants twice yearly. Diet and water were provided ad libitum. According to the Report, the determined contaminant levels were not expected to interfere with the Study.

   Environmental conditions included a temperature range of 69 to 75 °F and a relative humidity at 42 to 78 percent.

(On four occasions, the Report states that the recorded temperature was 64, 67, and 66 °F (twice). The animals were subjected to a 12-hour light/12-hour dark cycle, and there were approximately 10 fresh-air changes per hour).

3. Statistics - All analyses utilized two-tailed tests at a minimum significance level of 5 percent, comparing each treated group to vehicle control. Tests applied to specific parameters are as follows:

   a. Chi-square (with Yates' correction) - fetal sex ratios.

   b. Fisher's Exact Test - malformations and variations, by fetuses and litters.

   c. Mann-Whitney U-Test - early and late resorptions, dead fetuses, and postimplantation losses.

   d. One-Way ANOVA and Dunnett's Test - corpora lutea, total implantations, viable fetuses, fetal body weights, maternal body weights, and weight gains.

4. Quality Assurance - Ten QA inspections were conducted during the study. The Report states there were no known "significant deviations from the Good Laboratory Practice regulations which affected the quality or integrity of the study."

C. Methods:

1. Maternal Observations - The animals were observed for moribundity and signs at least three times daily during the week (twice daily on weekends). In addition, a 1-hour postdose observation was recorded for each animal after each dosing. Animals not surviving were necropsied before term. (Data on implantation sites and corpora lutea were recorded.) Three females delivered on gestation day 18 before sacrifice: two from the 10 mg/kg/day dose group and one from the 64 mg/kg/day dose group. One-half the fetuses from two of these litters (one litter from the 10 mg/kg/day dose group and the litter from the 64 mg/kg/day dose group) were processed for soft tissue examination. Skeletal examination of the other half of the fetuses from these two litters was not completed due to an error in fixation. The findings for the delivered fetuses are presented separately in Appendix B of the Report, and do not affect the Study conclusions. The Report states that these data are not included in the Report tabulations or statistical analyses.

Maternal body weights were recorded individually on gestation days 0, 6, 9, 12, 15, and 18. Mean body weight changes were calculated for these corresponding intervals, and for days 6 to 15 and 0 to 18.

2. Cesarian-Section Data - After sacrifice by carbon dioxide inhalation on gestation day 18, the contents of the abdominal and thoracic cavities were examined. Corpora lutea were counted (using low power magnification when necessary). The number and location of viable and nonviable fetuses, early and late resorptions and the total number of implantation sites were recorded. Implantation sites were numbered consecutively from the left distal uterine horn to the right distal horn. Maternal tissues were preserved for possible histopathological examination, which was not considered necessary based on subsequent macroscopic findings. In the absence of macroscopic implantation, uteri were examined for early implantation loss by the "ammonium sulfide" method of Salewski.

3. Fetal Examination - External examination included, but was not limited to, the eyes and palate. Crown-rump lengths of late resorptions were recorded and the tissues discarded. Half the fetuses of each dam were placed in Bouin's fixative prior to soft tissue examination by the Wilson sectioning technique. Fetal kidneys were graded for renal papillae development by a method described by Woo and Hoar. The other half of the fetuses were eviscerated and then fixed in 95% isopropyl alcohol, macerated in potassium hydroxide, and stained with Alizarin Red S. Skeletal examination was under low power magnification.

   Laboratory historical control data are appended to the Report.

D. Results:

1. Maternal Data - Two dams at top dose died "after receiving one or two doses." Signs in one of the mice included lethargy, lowered temperature, soft stool, decreased urination, and brown urogenital matting. The other female did not exhibit significant signs. Three additional mice delivered on the scheduled day of Cesarean section, two at low dose and one from the top- dose group.

   The Report states that signs were minimal in top-dose animals surviving to scheduled sacrifice. Five of the animals had at least one of the following findings: dried brown matting, reddening or yellow staining of the

anogenital area, dried red material around the eyes or nose, soft stool, decreased defecation, or mucoid feces. Three of these top-dose females also had total litter resorption. One of the mid-dose females showed red discharge in the urogenital area, associated with total litter resorption. There was one litter at low dose and six litters at top dose with total resorptions vs. zero in control and one in historical control.

Mean maternal body weights were somewhat reduced at mid dose, relative to control (52.7 vs. 56.2 g), and very much reduced at top dose (46.7 g; p = 0.01, two-tailed Dunnett).

For the two deaths at top dose, both females were gravid with normally developing implantations. Necropsy findings for both dams included bilateral reddening of the cortico-medullary junction in the kidneys, and red areas in the stomach and/or stomach mucosa. One of the dams showed dilated meningeal blood vessels. Of the three females delivering on gestation day 18, the one at top dose exhibited an enlarged spleen.

2. Fetal Data - At top dose the "mean viable fetuses" was 6.6 vs. 12.3 in control and the mean postimplantation loss was 5.4 vs. 0.8 in control (both significant at 0.01 level, see Table 5 attached). Mean fetal weight at top dose also was significantly (at 0.01 level) reduced from control. At mid dose, the mean postimplantation loss was elevated relative to concurrent control, but was the same value as mean historical control: 1.1 vs. 0.8 in concurrent control and 1.1 in historical control (0.8 to 1.3). Also, at mid dose, the mean fetal weight was only somewhat less than control: 1.23 vs. 1.30 g, with the historical control range at 1.21 to 1.33 g (mean at 1.29 g).

The pattern of fetal external and skeletal malformations is shown in the attached Tables 6 and 7 from the Report. Exencephaly (with or without open eyelid) occurred in one fetus (0.4%) at low dose and in two fetuses (1.4%) at top dose vs. zero in control and 0.0 to 0.4 percent in historical control (1885 fetuses examined externally). Thoraco-gastroschisis occurred in two fetuses at mid dose vs. zero at all other doses, including control (historical control not listed). Omphalocele occurred in one fetus at top dose (0.7%) vs. zero at all other doses, including control (historical control ranged from 0.0 to 0.3%). At low dose there was one fetus with facial cleft and one fetus exhibiting microphthalmia and/or anophthalmia, with zero at all other doses (for both malformations); historical control was not listed. One fetus at

tions); historical control was not listed. One fetus at each dose (including control) showed cleft palate (historical control not listed). At mid dose, two fetuses showed sternoschisis and one showed rib anomaly (0.8%), neither of which occurred at other doses. Rib anomaly has a range of 0.0 to 0.6 in historical control, in which sternoschisis is not listed. Other malformations (carpal and/or tarsal flexure and sternebrae malaligned) both are without dose relationship.

Fetal variations (percent) are tabulated in the attached Table 9 from the Report. Incidence of "25 presacral vertebrae" is elevated at all doses relative to control: 0.7, 5.2, 5.3, and 2.7 percent in the control group and ascending dose groups, respectively, with historical control at 0.0 to 4.1 percent. (The litter incidences for this variation are 4.0, 10.0, 21.7, and 12.5%, respectively.) The incidence of 14th full rib(s) is elevated on a litter basis at 12.0, 30.0, 17.4, and 6.3 percent in control and ascending doses, with the historical control range at 8.0 to 30.4 percent. Likewise, the incidence of 7th sternebra has a similar pattern, with a litter incidence of 25 percent at low dose vs. 12.0 percent in control and 0.0 to 20.0 percent in historical control.

NOEL, maternal toxicity = 10 mg/kg/day (LDT)
LEL, maternal toxicity = 64 mg/kg/day (HDT)
resorption and reduced body weight).
Effects at 64 mg/kg/day (HDT)--death, reduced body weight, lethargy, lowered temperature, decreased urination and defecation, soft stool or mucoid feces, brown urogenital matting, red material around eyes or nose. Total litter resorption. Bilateral reddening of cortico-medullary junction (kidneys), red areas in stomach, dilated meningeal blood vessels, enlarged spleen.

NOEL, developmental toxicity = 32 mg/kg/day
LEL, developmental toxicity = 64 mg/kg/day (HDT)
(reduced mean viable fetuses and fetal weights, elevated postimplantation loss, exencephaly, and omphalocele)

There was an insufficient percentage of surviving dams at top dose, but TB determines that the overall data of the study are adequate to form valid conclusions.

The top-dose litter incidences of the malformations exencephaly and omphalocele were elevated above historical control litter incidences.

Arsenic Acid Review

Page _____ is not included in this copy.

Pages 8 through 11 are not included in this copy.

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_____ Identity of product inert impurities.

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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✓ FIFRA registration data.

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Appendix

Range-Finding Study, MRID No. 405588-01, for Mouse Teratology Study with Arsenic Acid (75%) (MRID No. 406462-02)

Eighteen dose groups, each containing five bred female mice, Crl:CD-1(ICR)BR, received gavage administration once daily during gestation days 6 through 15. Dosage (after preliminary toxicity testing) was set at levels 0, 1, 3, 6, 9, 12, 18, 24, (2 groups), 32, 40, 48, 56, 64, 72, 80, 88, 96 and 104 mg/kg/day. Three vehicle control groups (5 bred females each) were dosed with deionized water on a comparable regimen.

At the level of 72 mg/kg/day or higher 15 females died or were sacrificed moribund. One female at 64 mg/kg/day aborted one late resorption and one placenta on gestation day 13. Clinical signs at the level of 80 mg/kg/day or higher included soft stool, decreased defecation, anogenital staining, and red vaginal discharge. Prior to death animals occasionally exhibited lethargy, ataxia, tremors and lowered temperature. Maternal body weight losses were apparent at dose levels above 72 mg/kg/day, but the body weight data are not easily interpreted.

Tabulation of fetal data shows that mean postimplantation loss was increased at the 80 mg/kg/day dose level (compared to both control and historical control), although only two females could be evaluated. Two gravid females, one each at the 88- and 96- mg/kg/day levels had 6 and one resorption(s), respectively. The Report states that there was no adverse effect on intra-uterine survival at doses up to 80 mg/kg/day. There was no apparent effect on mean viable fetuses.

For the animals that died or were sacrificed moribund the prevalent necropsy findings were dark red adrenals, dark red areas in the stomach, and a dark red cortico-medullary junction in the kidneys.

The study concludes that the administration of the test material to pregnant mice during organogenesis produced maternal toxicity at a level of 64 mg/kg/day. A dosage of 56 mg/kg/day was stated as a "no-effect" level for both maternal and embryotoxicity.

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

SEP 16 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: TB Project No. 8-0895; EPA ID No. 58475-C
Arsenic Acid: Teratogenicity Studies [redacted],
Dermal Sensitization Study

Tox Chem No. 501B

FROM: David G. Van Ormer, Ph.D.
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and

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Reviews are attached for the Subject Studies, including two range-finding studies for the teratogenicity studies.

The data are adequate.

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DATA EVALUATION REPORT

Study Type: Dermal Sensitization-Guinea Pig

TOX CHEM No.: 501B
MRID No.: 406462-01

Test Material: Arsenic Acid (75%)

Formula: H_3AsO_4

Study No.: HLA 80206225

Sponsor: Pennwalt Corporation

Testing Facility: Hazleton Laboratories of America

Title of Report: Dermal Sensitization Study of Arsenic Acid 75% in Guinea Pigs (Closed Patch Technique)

Author: Steven M. Glaza

Report Issued: June 2, 1988

Summary and Conclusions:

Ten male albino guinea pigs received 6-hour dermal contact with 75% arsenic acid (test material) once a week for 3 weeks (induction phase). Dosing volume was 0.4 mL (under an adhesive pad) and induction concentrations were 10 and 25 percent test material. Positive control was 0.3% DNCB. The challenge dose (1% test material, 0.1% positive control) was applied 2 weeks post induction phase. Ten naive controls were also challenged.

Three animals succumbed to induction dosing following signs of hypoactivity and ataxia. Buehler scores during induction averaged 0.3 to 1.7 (individual high of 3.0--necrotic areas) with positive controls at 2.0 to 2.2. The challenge score of test animals (and naive controls) was uniformly zero. Challenge dosing of positive control produced necrotic reactions. Body weight changes and gross necropsy (other than test-site irritation) were unremarkable.

Not a dermal sensitizer (closed patch technique) in guinea pigs at a challenge dose of 1.0% aqueous.

Classification: Guideline

A. Materials:

1. Test Material - Aqueous 75% arsenic acid, Batch No. 8619, a light green liquid, stored at room temperature.

2. Test Animals - The male albino guinea pigs, Dunkin Hartley strain (350 to 524 g), were divided into a test group of 10 animals, a naive control group of 10 animals, and a positive control group of 4 animals. Housing was individually in screen-bottom cages in a temperature- and humidity-controlled room. Identification was by numbered ear tag.

B. Study Design and Methods:

1. Dose-Range Phase - An initial application of test material to four animals at concentrations of 25, 50, and 75 percent in deionized water (each animal receiving two concentrations) resulted in death or moribund sacrifice to all four animals. Results of a second dose-finding study (using concentrations of 0.1, 1, 10, and 25 percent (w/v) in deionized water) determined the selection of 10 and 25% (w/v) of the (75%) test material for the induction phase, and 1.0 percent (w/v), the highest nonirritating concentration for the challenge application.

2. Induction Phase - The hair was removed (electric clippers) from the back and flanks of each animal just prior to each application. A quantity of 0.4 mL of test material at the appropriate concentration (25% for the first two induction doses and 10% for the third induction dose) was placed on an adhesive pad (Hilltop Chamber, 25 mm diameter), which was then placed on the test site along the anterior left flank. The patch was covered with dental dam and secured with Elastoplast tape. Positive control (0.3% 2,4-dinitrochlorobenzene, DNCB, in 80% ethanol) was applied in the same manner to the four positive controls. All animals received one 6-hour application each week for three successive weeks, constituting the induction phase. Due to the high level of irritation, the third induction dose (reduced from 25 to 10% w/v) was applied to a site slightly posterior to the site of the first two doses.

3. Challenge Phase - Two weeks after the third induction dosing, a challenge dose of 1.0 percent w/v test material was administered as described above, but on the opposite flank of each animal. The positive control was similarly applied at 0.1 percent in acetone. At this time, the 10 naive (previously untreated) control animals received challenge application of test material (1.0%) in the same manner as for the test group.

4. Scoring and Observations - The application sites were given Buehler scores for erythema and edema at 24 and 48 hours after each of the three induction applications and after the challenge application. At 3 hours prior to the 24-hour examination, the test sites were depilated with Neet depilatory (20 minutes), which was then washed off with water.

   The animals were observed for general behavior and appearance once daily, and body weights were recorded weekly from initiation to termination. Gross necropsy was performed on all animals dying on test. A challenge grade of 1 or greater was taken as evidence of sensitization, provided grades of less than 1 are observed on the naive controls.

C. Results:

Three animals died during the study: one on the day after the second induction dose (Day 9) and two on Day 10. Signs were hypoactivity and ataxia, appearing at the second dosing. The Report states that all surviving animals

appeared normal from Day 11 through termination. In addition, the body weight gains were normal, and gross necropsy of the animals that died revealed no lesions (other than test-site irritation).

During induction phase, the test material Buehler scores averaged 0.3 to 1.7, with the highest three individual scores at a value of 3.0 (necrotic areas). Positive control animals showed an average of 2.0 to 2.2 during induction. For all naive control animals and all surviving test animals the dermal reaction score after challenge was zero. Scores of positive controls at challenge were all 3.0 (necrotic areas), except for one score at 2.0.

When tested by the closed patch technique in guinea pigs, the test material at a challenge dose of 1.0 percent is not a dermal sensitizer.