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DATA EVALUATION REPORT

Study Type: Teratogenicity - Rabbit

TOX Chem No.: 501B

Test Material: Arsenic Acid (75%)

MRID No.: 405588-0

Formula: H_3AsO_4, 75% (aqueous)

MRID 405588-02

Laboratory Project No.: WIL-75026

Sponsor: Pennwalt Corporation, Agrichemicals Division

Testing Facility: WIL Research Laboratories, Inc.

Title of Report: A Teratology Study in Rabbits with Arsenic Aci (75%)

Author: Mark D. Nemec, B.S.

Report Issued: January 15, 1988

Conclusions:

Artificially inseminated New Zealand White rabbits (20 pe dose group) received single daily gavage of aqueous Arsenic Aci (75%) from days 6 through 18 of gestation. Dosages were 0, 0.25 1, and 4 mg/kg/day. Controls received deionized water. Bod weights were recorded at six periods. Cesarian section was c day 29. Fetuses were weighed, sexed, and examined for external skeletal, and soft tissue anomalies.

Seven dams died or were sacrificed in extremis at top dose Other maternal effects at top dose included reduced body weight prostration, ataxia, decreased defecation and urination, mucoi feces, pale and soft kidneys and livers, dark red areas i stomach, and dark red lungs.

Fetal data showed increased mean postimplantation loss a top dose (1.8 versus 0.5 in control); and reduced mean viable fetuses at top dose: 4.9 vs. 6.7 in control.

NOEL, maternal toxicity = 1 mg/kg/day
LOEL, maternal toxicity = 4 mg/kg/day (HDT) (death, reduced body weight gain, prostration/ataxia, decreased defecation and urination, mucoid feces, pale and soft kidneys and liver, dark red areas in stomach, and dark red lungs).

NOEL, developmental toxicity = 1 mg/kg/day
LEL, developmental toxicity = 4 mg/kg/day (increased postimplantation loss, and decreased viable fetuses).

Reviewer's Comments - There were insufficient surviving dams at high dose, but TB determines that the overall data of the Study are adequate to form valid conclusions.

A developmental malformation in one fetus (absence of both kidney and ureter) was reported at top dose, the same dose at which there was extreme maternal toxicity (7/20 deaths). However, this observation is not determined to be a treatment effect, in view of the absence of a supporting pattern of fetal effects.

Classification: Minimum Data

A. Materials:

1. Test Compound - Arsenic Acid (75%); a clear, green aqueous liquid. Batch No. 8619. Analysis by graphite furnace AA (with Zeeman correction) showed an average of 105% theoretical for the diluted dosing solutions. Contaminants were not reported. Deionized water was the vehicle. Stock solution and dosing solutions were prepared fresh daily.

2. Test Animals - Female New Zealand White rabbits, 4 1/2 months old at receipt from Hazleton Research Products, Inc., Denver, PA. Weights ranged from 2478 to 4266 g.

B. Study Design:

1. Animal Assignment and Dosage - Assignment was based on body weight stratification in a block design, and included only animals judged by the Study Director to be in good health. Each of the four dose groups (including vehicle control) contained 20 females. Body weight at day 0 of gestation ranged from 3.192 to 4.969 kg. The Report states that mean body weights of control and treated groups were not statistically different on gestation day 0. The test material was administered by daily gastric intubation for 13 consecutive days, starting on gestation day 6, and including day 18. Dosages were based on the most recent body weights, and are tabulated in the Report as follows:

Group Number	Test Substance	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females
1	Deionized Water	0	0	1	20
2	Arsenic Acid	0.25	0.25	1	20
3	Arsenic Acid	1	1	1	20
4	Arsenic Acid	4	4	1	20

2. Diet, Drinking Water, and Maintenance - The basal diet was Purina Certified Rabbit Chow #5322, for which analysis is 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 study, the reported and determined contaminant levels were not expected to interfere with the study.

The acclimation period extended 10 weeks prior to initiation. During this period the animals were observed twice daily, and body weights were recorded (during

acclimation) every other week. Identification was by plastic ear tag. The animals were housed individually in stainless-steel, wire-bottom cages suspended above cage-boards.

Environmental conditions included temperature at 67 to 72 ^{o}F and relative humidity at 40 to 88 percent. 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 Quality Assurance 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. Insemination - The day of artificial insemination and intravenous injection of human chorionic gonadotropin was designated gestation day 0. Diluted semen contained at least 3 million motile sperm/mL. An equal number of females in each group (either one or two females) were inseminated by the same male.

2. Maternal Observations - From days 0 through 29 of gestation all rabbits were examined daily for appearance, behavior, moribundity, and mortality. For all deaths or sacrificed deaths, a gross necropsy was performed. Females aborting were necropsied the same day. The number of corpora lutea and the number and location of implantation sites were recorded. Tissues were preserved for possible histopathological examination only when

prompted by gross findings. Data were not recorded for fetuses associated with abortions. Maternal body weights were individually recorded on gestation days 0, 6, 12, 18, 24, and 29.

3. Cesarean-Section Data - On gestation day 29 all surviving females were sacrificed by intravenous injection of T-61 Euthanasia solution. Any abnormalities in the abdominal and thoracic cavities and contents were noted. The corpora lutea on each ovary were counted, and the number and location of viable and nonviable fetuses, early, and late resorptions, and total implantation sites were recorded. Implantation sites (including resorptions) were consecutively numbered from the left distal uterine horn to the opposite distal horn. Maternal tissues obtained from Cesarean section were preserved for possible histopathological examination. The remainder of the carcass was discarded. In the absence of macroscopic evidence of implantation, uteri were subjected to the "ammonium sulfide" technique of Salewski in order further to examine for implantation sites.

4. Fetal Examination - Fetuses were weighed and tagged individually. External examination included eyes, palate, external orifices, and crown-rump length (of late resorptions only). Each fetus was sexed internally and examined viscerally (modification of Staples technique) to include the heart and major vessels. The brain was examined by a mid-coronal slice. Skeletal examination by the Dawson Alizarin Red S technique was conducted at low power magnification. External, visceral, and skeletal findings were recorded as developmental variations or malformations. The laboratory historical data (vehicle or untreated control) from 47 rabbit teratology studies are appended to the Report.

D. Results:

1. Maternal Data - Maternal clinical observations and weight changes are reproduced in Tables 2 and 4 (attached). In the high-dose group, seven dams died or were sacrificed in extremis. Before gestation day 29, four additional females aborted and were sacrificed: one control, one mid dose, and two dams at high dose. The Report notes that spontaneous abortions are not uncommon for this species and strain.

Prostration and ataxia were occasionally observed at h dose prior to death or sacrifice. Anogenital stain and matting were somewhat increased at top dose, relat to other dose groups. Hair loss occurred in all d groups, and was not dose related in that it occurred only one or two animals per dose group (although number of occurrences was centered at low and mid dos Decreased defecation and urination, however, occur mainly at top dose. One animal at top dose deposi wet, red material on the cage paper. Appearance mucoid feces was elevated at both low and high do relative to control.

On day 29 the mean maternal body weights at low a (particularly) high dose were reduced, relative control. Body weight gains during organogenesis (days to 18) were 25, 45, 32, and -179 g for the control, a ascending dose groups, respectively. Maternal weig data for other periods were too erratic for val interpretation.

Of the four females that aborted, the control fema aborted one placenta. The mid-dose female aborted c normally developed fetus and had seven placentas <u>utero</u>. Also, this female showed fluid in the thorac cavity, and the stomach mucosa were "reddened." At t dose one female aborted two placentas and one ear resorption, while the other abortion produced one la resorption, with nine late resorptions and two placent <u>in utero</u>.

For all seven dams at top dose that died or we sacrificed <u>in extremis</u> the prevalent necropsy findin were as follows: pale, soft, or mottled kidneys, pa and soft liver, dark red areas in the stomach, and da red lungs. These females showed a high degree postimplantation loss. Other necropsy findings in t dams were considered typical of the species and strai and were not dose related. These findings included sma gallbladder (one dam in vehicle control); extreme lobul tion of the liver, fluid in thoracic cavity and inte tines, and stomach ulceration (one dam at low dose fluid in thoracic cavity and intestines, red foci stomach, and cystic kidneys (one dam each at mid dose and fluid in the intestines (one dam at high dose).

2. <u>Fetal Data</u> - Cesarean section data (Table 5, attached) that "mean viable fetuses" was somewhat reduced at high relative to control value: 4.9 versus 6.7 (historical control 3.8 to 9.6). Mean postimplantation loss was

increased at high dose: 1.8 versus 0.5 in control, and 0. to 1.9 in historical control. Fetal weights showed no effect of treatment.

The patterns of fetal alterations show no evidence of treatment effect (See Tables 6 and 9). Thus, one fetus at high dose (only) showed absence of kidney and ureter. Con truncus arteriosus occurred (in separate litters) in one fetus at control and low dose, and in two fetuses at mid dose. Skeletal effects included fused sternebrae at mid d (3 fetuses in one litter), and vertebral anomaly (with or without associated rib anomaly), which occurred in 3 fetus of separate litters at low dose and one fetus at mid dose. None of these incidences was significantly above control value.

The precent fetuses and litters with variations are reproduced in the attached Table 9 from the Report. The patterns of neither fetal nor litter incidences show treat ment effect. Thus, mid dose (only) shows somewhat levated fetal and litter mean incidences (compared to control values) for major blood vessel variation: 13.3% fetuses i 27.8% litters vs. 5.3% and 23.5% for control. Retrocaval ureter is somewhat elevated (on a litter basis only) at lo and mid dose, with mean values of 15.0% and 16.7%, respec- tively, and 11.8% in control. All litters at top dose sho 13th full rib(s), but litter control was 94.1% and the fet incidence was also similar to control. The incidence of 2 presacral vertebrae was somewhat elevated above control on litter basis (only) at all doses, but there was no dose response. Likewise, the percentages of 13th rudimentary rib(s) were somewhat elevated from litter control value at all doses, but again there was no dose response. Incidence of bent hyoid arch(es) were somewhat elevated over control, but only at low and high dose on a litter basis, and only at top dose on a fetal basis. The variation of "sternebrae with thread-like attachment" showed an elevated litter and fetal incidence at both top dose and mid dose. And, finally, incidence of sternebrae malaligned (slight or moderate) was somewhat elevated at top dose on a litter basis.

None of the incidences of fetal malformations or variations was significantly greater than control values, according to the Report.

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Arsenic Acid Review

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Appendix

Range-Finding Study, MRID No. 404639-03, for Rabbit Teratology Study with Arsenic Acid (75%) (MRID No. 405588-02).

Five dose groups, each containing five bred New Zealand White rabbits, received gavage administration once daily during gestation days 6 through 18. The experimental protocol was similar to that of the succeeding main study with exception of the dose range and size of dose groups, and the fact that fetal data (malformations and variations) were not reported. The dosage levels were 0, 1, 3, 6, 9, and 12 mg/kg/day.

At the doses above 3 mg/kg/day all animals succumbed before term, with exception of one female in the 6 mg/kg/day group. All of these unscheduled deaths were considered compound-related, based on the similarity of necropsy findings. At the other doses all dams survived with exception of a single mortality at 1 mg/kg/day. This death was not considered compound-related, primarily due to the absence of clinical signs and to the lack of deaths at the next higher dose. Clinical signs at the higher doses included decreased defecation and urination, brown ano-genital matting, mucoid feces, and soft stool. Hair loss was reported in two dams at the 3 mg/kg/day group, but in none of the other groups, including controls. Body weight loss was severe in the top three dose groups prior to death. In the other two dose groups and controls, the weight changes (overall and at various periods) were highly erratic and not useful for interpretation.

Cesarean data show an increased postimplantation loss at low dose (1 mg/kg/day), where one litter gave five late resportions, and two litters each showed one early resorption. These resorptions may be compared to the one late resorption in control and the one early resportion at the 3 mg/kg/day level.

Prevalent necropsy findings in animals succumbing to compound effects included dark red lungs, mucosal exudate and red areas in the stomach, pale and soft liver and kidneys, and pale and/or soft spleen. Other findings in the top two dose groups included dark red thymus, dark red cortico-medullary junction in the kidneys, and green fluid in the intestines. For the low dose female found dead, only a fluid-filled thoracic cavity and dark red lungs were reported, suggesting to the author of the Report that the death was not compound-related. Of the dams in the 3 mg/kg/day group, one showed accentuated lobulation of the liver, and one had dark green fluid in the cecum. (The fluid in the cecum is stated by the Report as a possible treatment-related effect.) Two surviving females in the 1 mg/kg/day group showed reddened stomach mucosa. One control female had reddened stomach mucosa and three controls showed accentuated lobulation of the liver.

Reviewer's Conclusion: (range-finding study)

NOEL for maternal toxicity not observed.

LOEL (developmental and maternal toxicity) = 1 mg/kg/day (LDT) (increased postimplantation loss and reddened stomach mucosa; at 3 mg/kg/day there was alopecia and dark green fluid in the cecum).

The dosage intervals in this study are too small.