CASWELL FILE

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

10

006708

MAY 11 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pyridate Herbicide - Review of a Teratogenicity Study in Rabbits - EPA Pesticide Petition Nos. 4G3047 and 5G3217

TOX Chem No.: 716A
MRID No.: 404632-01

FROM: Yiannakis M. Ioannou, Ph.D.
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Gilmore, Inc.
Memphis, TN

Toxicology Branch has completed the review of a study entitled "Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Pyridate Technical Administered as the Neat Test Substance Orally via Stomach Tube to New Zealand White Rabbits," submitted by the registrant in support of tolerances of Pyridate for corn, wheat, and rice.

Briefly, the conduct of this study, the results and conclusions were as follows.

New Zealand white virgin female rabbits (20/treatment) were artificially inseminated and on days 7 through 19 of presumed gestation were administered orally (by gavage) Pyridate Technical (89.5% ai) at dose levels of 0, 150, 300, or 600 mg/kg/day. Clinical signs of maternal toxicity, mortality, body weights, and food consumption were recorded throughout the study. All surviving animals were sacrificed on day 29 of gestation and a number of maternal and fetal parameters were measured. Results reported here indicate that the HDT (600 mg/kg/day) resulted in maternal toxicity in the form of body weight depression and increased number of abortions as compared to the control group. Fetal data indicate that Pyridate does not cause any developmental toxicity and does not appear to be teratogenic in rabbits.

The NOEL for maternal toxicity was considered to be the MDT (300 mg/kg/day) while the LEL was the HDT (600 mg/kg/day) for depression of body weights and increased abortions. The NOEL for developmental toxicity and teratogenicity was considered to be greater than 600 mg/kg/day (HDT).

The study was classified as Core-Guideline.

Reviewed By: Y.M. Ioannou
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: A.B. Kocialski
Section VII, Toxicology Branch (TS-769C)

006708

DATA EVALUATION REPORT

Study Type: Teratology (Rabbit)

TOX Chem No.: 716A

MRID No.: 404632-01

Test Material: Pyridate Technical

Synonyms: CL-11344; Lentagran

Study Number: Argus 512-001

Sponsor: Gilmore, Incorporated, Memphis, TN

Testing Facility: Argus Research Laboratories, Inc., Horsham, PA

Title of Report: Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Pyridate Technical Administered as the Neat Test Substance Orally via Stomach Tube to New Zealand White Rabbits

Author(s): Alan M. Hoberman

Report Issued: December 10, 1987

Conclusions:

Based on the available data, Pyridate Technical is not considered to be teratogenic in New Zealand White rabbits when administered at dose levels of 150, 300, or 600 mg/kg/day (days 7 through 19 of gestation). The NOEL for maternal toxicity (body weight depression) was 300 mg/kg/day. The NOEL for developmental toxicity was greater than 600 mg/kg/day, the HDT.

Classification: Core-Guideline

Materials and Methods:

New Zealand White [Hra:(NZW)SPF] virgin female rabbits (obtained from Hazelton Research Products, Denver, PA) approximately 5 months old and weighing approximately 2.8 to 4.5 kg each were used in this study. The animals were housed in individual cages and acclimated to laboratory conditions for 4 weeks. Eighty healthy female rabbits were randomly divided into four groups (20 rabbits per group) and each animal was identified by an ear tag bearing the animal's unique permanent number. The animals were kept in a room with a temperature of 64 to 70 °F, relative humidity of 48 to 58 percent, 12 air changes per hour, and a 12-hour light/dark cycle.

All female animals selected for this study were artificially inseminated with spermatozoa from four untreated proven male breeders (male animals were of the same source and strain as the female animals). Three hours prior to insemination, each female rabbit was intravenously administered with 20 USP units/kg of human chorionic gonadotropin (HCG). A volume of 0.25 mL of semen with a concentration of 6.0 x 10^6 spermatozoa was used to inseminate each rabbit. The day of artificial insemination was designated as day 0 of presumed gestation. The insemination of all animals lasted 4 days, with five different animals from each group inseminated each day. Pyridate Technical, a dark-brown viscous liquid with a purity of 89.5 percent and a specific gravity of 1.16, was administered orally (via a stomach tube) as the neat (undiluted) test substance to the artificially inseminated female rabbits on days 7 through 19 of presumed gestation, at dose levels of 0, 150, 300, or 600 mg/kg/day at dosage volumes of 0.517, 0.129, 0.259, and 0.517 mL/kg for the control (deionized water), low, mid-, and high-dose levels tested.

All animals were offered 180 g of Certified Rabbit Chow #5322 (Ralston Purina) daily throughout the gestation period. Water was available to all animals ad libitum. During gestation days 0 through 6 all animals were observed for general appearance and viability. During the treatment period (days 7 through 19 of gestation), all animals were examined twice daily for clinical signs of toxicity, abortion, and/or deliveries. Daily observations continued on days 20 through 29 of gestation. Body weights were recorded on day 0 and on days 7 through 29 of gestation. Feed consumption was recorded daily throughout the study.

Necropsy was performed on all rabbits that died, aborted, or prematurely delivered on the day the event occurred. On day 29 of gestation, all surviving female rabbits on study were killed by intravenous administration of T-61® Euthanasia solution.

The abdomen was dissected, and specific organs were removed and examined as follows:

1. Ovaries - Corpora lutea identified and counted in each ovary.

2. Uterus - Weighed and examined for pregnancy, number and placement of implantations, early and late resorptions, and live and dead fetuses.

Fetuses from each doe were weighed and examined for gross external changes. Live fetuses were killed with intraperitoneal injection of Uthol® (pentobarbital sodium) and then examined for sex identification and visceral alterations. Tissues of interest were preserved in neutral buffered 10% formalin. All fetuses were stained with alizarin red-5 and examined for skeletal malformations.

Statistical Analysis (abstracted from the original report):

"Adult data were evaluated with the individual rabbit as the unit measured. Litter values were used in evaluation of Caesarean-sectioning and fetal ossification site data. The incidence of fetal alterations was examined in terms of the litter and fetal percentages.

"Maternal clinical sign and necropsy observation data and the incidences of pregnancy, death and total resorption were analyzed using the Variance Test for Homogeneity of the Binomial Distribution.

"Maternal body weight, body weight change, feed consumption, uterine weight, fetal body weight, anomaly average and ossification data were based on surviving pregnant does and their litters and analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance. If the Analysis of Variance was significant and appropriate, i.e., it passed Bartlett's Test ($P > 0.05$), then Dunnett's Test was used to identify the statistical significance of individual groups. If Bartlett's Test was significant ($P \leq 0.05$), and there were greater than 75% ties in a group, then Fisher's Exact Test was used.

"Data obtained at Caesarean-sectioning of the does were evaluated using the Kruskal-Wallis Test; in cases where statistical significance occurred ($P \leq 0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of individual groups.

"Proportion data for Caesarean-sectioning and fetal observations were analyzed using the Variance Test for Homogeneity of the Binomial Distribution."

Results:

Mortality and Clinical Observations - Several mated female rabbits died during the course of the study as follows: control group - one animal found dead on day 11 of gestation; LDT - two animals found dead on days 9 and 14 of gestation, respectively; HDT - one animal found dead on day 11 of gestation. Based on the author's evaluation, none of the deaths were due to the test article, and they appeared to be accidental. The major clinical signs of toxicity reported were the occurrence of dried feces at the HDT or the absence of feces at the HDT (600 mg/kg/day). The number of animals with dried feces at the HDT was statistically significantly higher than controls (9/20 vs. 0/20 for the HDT and controls, respectively). The duration of occurrence of dried feces was also statistically significantly longer at the HDT (39 days) compared to controls (0 days). Similarly, the number of animals at the HDT with no feces was statistically significantly higher than controls (3/20 vs. 0/20 for the HDT and controls, respectively), and of longer duration (12 days for HDT and 0 days for controls).

Abortions and Premature Delivery - Four out of 18 pregnant rabbits (22.2%) of the HDT aborted on days 19, 24, or 26 of gestation. These abortions appeared to be treatment-related and were statistically significantly higher than the corresponding control groups. One female from the high-dose group prematurely delivered its litter on day 28 of gestation. This event appeared to be treatment-related.

Gross Necropsy - A variety of gross pathological lesions were seen in all groups of animals on study. The only gross change, however, that was treatment-related was the persistent odor of the test article in the gastric contents of animals treated with 600 mg/kg/day of Pyridate. The incidence of this change was statistically significantly higher in the HDT compared to controls.

Maternal Body Weights - Average body weight gains were statistically significantly lower in females administered 600 mg/kg/day of Pyridate between days 7 and 20 of the study. Average body weights of females of the HDT were statistically significantly lower than controls beginning on day 17 of study and lasting until sacrifice (day 29). Body weights for the low- and mid-dose groups were comparable to controls throughout the study (Table 1).

Maternal Feed Consumption - The average feed consumption was numerically lower in the HDT as soon as dosing started (days 7 through 9) and statistically significantly lower than controls from day 10 through day 24 of the study (Table 2).

Table 1

Effect of Pyridate on Maternal Body Weight and Body Weight Change

Body Weight (kg) Reported on Day:	Dose (mg/kg/day) 0	150	300	600
	1/			
0	3.75±0.25	3.75+0.25	3.72±0.30	3.69±0.31
7	3.92±0.27	3.90±0.29	3.90±0.34	3.86±0.35
14	4.07±0.29	4.01±0.33	4.03±0.34	3.84±0.39
17	4.12±0.29	4.04±0.35	4.07±0.37	3.76±0.40**
20	4.16±0.34	4.09±0.37	4.06±0.37	3.68±0.40**
21	4.18±0.32	4.10±0.38	4.08±0.37	3.68±0.40**
24	4.20±0.32	4.16±0.37	4.12±0.39	3.71±0.40**
29	4.18+0.28	4.18+0.36	4.17+0.31	3.78+0.41**
Body Weight Change (kg)				
Days 0-7	+0.17±0.17	+0.15±0.08	+0.18±0.09	+0.17±0.09
Days 10-14	+0.10±0.05	+0.08±0.06	+0.10±0.06	-0.07±0.32**
Days 17-20	+0.05±0.08	+0.05±0.06	-0.01±0.09	-0.11±0.12**
Days 7-20	+0.23+0.13	+0.21+0.12	+0.15+0.12	-0.17+0.30**

1/ Mean ± S.D.

** Significantly different from the control value; $p \leq 0.01$.

Table 2

Effect of Pyridate on Maternal Feed Consumption

Feed Consumption (g/day) on Day:	Dose (mg/kg/day) 0	150	300	600
0-7	175.6±12.7 [1/]	171.6±13.9	173.4±22.4	173.4±20.6
8-9	170.3±22.6	168.3±22.1	174.4±15.4	143.8±48.2
10-14	165.6±31.6	162.9±29.6	166.1±20.9	114.5±64.8**
14-17	163.2±37.9	156.6±31.5	152.4±36.4	69.8±67.5**
17-20	157.3±46.9	156.0±32.1	133.8±52.1	44.4±59.6**
21-24	141.8±41.9	147.2±35.8	127.4±58.4	62.2±67.0**
7-20	165.1±30.8	161.4±27.1	157.5±27.2	92.2±56.7**
20-29	110.0±37.1	125.0±33.7	120.1±52.7	80.0±55.8
7-29	141.1+28.3	145.4+26.0	143.4+32.4	107.0+45.3***

1/ Mean ± S.D.

** Significantly different from the control value; $p \leq 0.01$.

Reproductive Parameters and Litter Data - As can be seen in Table 3, none of the reproductive parameters measured in this study was adversely affected by Pyridate administration. The lower number of females with viable fetuses observed in the high-dose group was mainly due to the fact that four females in this group aborted their fetuses on days 19 to 26 on study. The abortion was attributable to Pyridate administration (600 mg/kg/day). Similarly, litter data (Table 3) appeared to be comparable between the Pyridate-treated groups and the control group.

Fetal Alteration Data - There was no statistically significant difference between Pyridate-treated and control groups as far as fetal external anomalies, soft tissue anomalies, or skeletal anomalies (skeletal variations and/or malformations) were concerned.

Table 3

Summary of Maternal Reproductive Parameters and Litter Data

Parameter	Dose (mg/kg/day) 0	150	300	600
No. of female rabbits tested	20	20	20	20
No. of pregnant rabbits	19	19	18	18
No. of females with viable fetuses	18	17	15	12
No. of corpora lutea/litter	9.9±2.0	10.3±2.1	9.6±2.3	9.0±1.9
No. of implantations/litter	8.4±1.8	7.9±2.6	6.4±3.1	7.8±2.0
No. of live fetuses/litter	7.7±1.7	7.2±2.4	5.9±3.2	7.2±1.5
No. of early resorptions	0.6±0.9	0.4±0.7	0.4±0.5	0.2±0.4
No. of late resorptions	0.2±0.4	0.4±0.8	0.0±0.0	0.2±0.6
Total No. of iive fetuses	138	123	101	87
No. of male fetuses	67	67	46	43
Fetal body weights(g)-males	42.80±6.50	44.96±7.83	44.86±7.19	40.49±9.49
- females	42.82±6.41	42.96±6.77	45.11±6.68	38.55±10.72
% of male fetuses/litter	48.3±16.1	54.8±12.3	47.1±24.6	49.7±17.8

Discussion:

The present study has investigated the possible teratogenic potential of Pyridate Technical in rabbits. Results presented here have indicated that no maternal mortality could be attributed to the test article. A number of toxic effects were, however, observed in the test animals receiving the high dose (600 mg/kg/day) of the test article. These effects included the statistically significant:

1. Increase in abortions;
2. Increase in the incidence of dried feces;
3. Decrease in body weights and body weight gains; and
4. Decrease in feed consumption.

These data indicate that the high dose tested was an acceptable dose for the MTD. The HDT is also considered to be the LEL for maternal toxicity while the MDT (300 mg/kg/day) is considered to be the NOEL for maternal toxicity. Administration of the test article did not have any effect on any of the measured reproductive parameters.

Although abortions can be considered in some cases to be an expression of developmental toxicity, in the present study the abortions observed in the high-dose group (600 mg/kg/day) were the result of maternal toxicity (decreased feed consumption and increased weight loss) since the aborted fetuses were in all cases alive and normal (according to the authors). Similarly, litter data were comparable between the treated and control groups.

Fetal data indicate that Pyridate Technical is not teratogenic in rabbits. Thus, the incidence of external anomalies, soft tissue anomalies, and skeleton anomalies (malformations and/or variations) in fetuses obtained from Pyridate-treated females, was comparable to the concurrent as well as the historical controls.

Conclusions:

Based on the available data reported here, Pyridate Technical is not teratogenic when administered to New Zealand white pregnant rabbits on days 7 through 19 of gestation at dose levels of 150, 300, or 600 mg/kg/day. The LEL for maternal toxicity was established at 600 mg/kg/day, the HDT (depression of body weights and body weight gains), while the NOEL was the MDT (300 mg/kg/day). Administration of Pyridate does not appear to cause any developmental toxicity. Thus, the NOEL for developmental toxicity is higher than 600 mg/kg/day, the HDT.

Classification: Core-Guideline