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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pyridate - Review of a Chronic Toxicity Study in Dogs

Tox Chem. No.: 716A
HED Project No.: 9-1485
MRID NO.: 410939-01

FROM: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

8-31-89

TO: Robert J. Taylor, PM 25
Fungicide - Herbicide Branch
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

9/5/89

Registrant: Agrolinz, Inc., Memphis, TN.

Action Requested:

Review a Chronic toxicity (one year) study in dogs.

Results and Recommendations:

Dynamac Corp. (W. McLellan) has completed the review of a chronic toxicity study in beagle dogs with Pyridate technical (DER attached). Briefly, the conduct of the study and major findings were as follows:

Groups of 5 male and 5 female dogs were administered (by capsule) Pyridate at the dose levels of 0, 5, 20 and 60 mg/kg/day. Since no toxicity was seen with these dose levels after 34 weeks on study, dose levels were escalated to 0, 30, 100 and 150 mg/kg/day between weeks 35 and 53 on study. Results indicate that systemic toxicity, in the form of excessive salivation, ataxia, mydriasis, dyspnea, tremors, increased respiration and prostration was observed at dose levels of 100 mg/kg/day or higher. Based on clinical signs of neurotoxicity the LEL was found to be 100 mg/kg/day, while the NOEL was approximately 20 mg/kg/day (lowest dose level given to animals for at least 34 weeks). (Note: a NOEL of 20 mg/kg/day was also established in the 90-day dog study which was used in setting dose levels for the chronic toxicity study).

The study was classified as Core - Minimum.

Tox Chem No. 176A Fyridate

File Last Updated _____

Study/Lab/Study #/Date	Material	EPA Accession/MRID No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Feeding - 1 year Species: dog Hazleton Labs. America Vienna, VA HLA No. 2495-100 5/2/89	Fyridate Technical (91.5% ai)	41093901	Systemic NOEL = 20 mg/kg/day based on excessive salivation, ataxia, mydriasis, dyspnea, tremors, increased respiration and prostration. Systemic LEL = 100 mg/kg/day Dose levels tested in male and female beagle dogs ~~20~~ 5, 20 and 60 mg/kg/day for the first 34 weeks on study. Dose levels were escalated after week 34 to 30, 100 and 150 mg/kg/day		Minimum (3)

EPA No.: 68D80056
DYNAMAC No.: 169-A
TASK No.: 1-69A
July 11, 1989

DATA EVALUATION RECORD

PYRIDATE

Chronic Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Prenta for
Date: July 11, 1989

4

EPA No.: 68D80056
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DATA EVALUATION RECORD

PYRIDATE

Chronic Toxicity Study in Dogs

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: July 11, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: July 11, 1989

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: July 11, 1989

Mike Ioannou, Ph.D.
EPA Reviewer and Acting
Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 8/24/89

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity study in dogs.

MRID NUMBER: 410939-01.

TEST MATERIAL: Technical pyridate.

SYNONYM(S): N/A.

STUDY NUMBER(S): HLA No. 2495-100.

SPONSOR: Agrolinz Inc., Memphis, TN.

TESTING FACILITY: Hazleton Laboratories America Inc., Vienna, VA.

TITLE OF REPORT: Chronic Toxicity Study in Dogs with Pyridate Technical.

AUTHOR(S): Bailey, D. E.

REPORT ISSUED: May 2, 1989.

CONCLUSIONS: The toxicity of pyridate was evaluated in a 12-month study with groups of five male and five female beagle dogs administered the test compound (by capsule) daily at initial levels of 0, 5, 20, and 60 mg/kg/day. Since there were no toxic signs or effects after 34 weeks of administration, the dose levels were escalated in steps so that the highest dose levels in the low-, mid-, and high-dose groups were 30, 100, or 150 mg/kg/day. In the mid- and high-dose groups there were neurologic signs including excessive salivation, ataxia, mydriasis, dyspnea, tremors, increased respiration, and prostration. No signs were seen in the 30-mg/kg group or in the mid-dose group until a level of 100 mg/kg/day was given (43 to 53 weeks). In the high-dose group, the threshold for clinical signs was 120 and 140 mg/kg/day in males and females, respectively. There were decreases in body weight gains in mid-dose dogs receiving 60 to 80 mg/kg/day and in high-dose dogs receiving greater than 120 mg/kg/day. There were no mortalities or effects on hematology, clinical chemistry, urinary parameters, or organ weights. One high-dose male had a histopathologic diagnosis of myeloid degeneration of the sciatic nerve, which was considered related to dosing. Based on clinical signs of neurotoxicity, the LOEL is 100 mg/kg/day and the NOEL is approximately 20 mg/kg/day.

Classification: CORE Minimum.

A. MATERIALS:

1. Test Compound: Pyridate technical; description: a brown oily liquid; batch No.: 2759523; purity: 91.5%.

2. Test Animals: Species: dog (Canis familiaris); strain: beagle; age: young adults; weight: males--6.6 to 9.2 kg, females--5.7 - 7.7 kg at initiation; source: Hazleton Research Products, Cumberland, VA.

B. STUDY DESIGN:

1. Animal Assignment: Dogs were quarantined for 2 weeks after receipt and were assigned to the following test groups by computerized weight randomization to ensure homogeneity of variance between groups:

Test group	Number of animals Males	Females	Dosage level (mg/kg/day)	Dosing duration (weeks)
1 Control	5	5	0	1-53
2 Low (LDT)	5	5	5	1-34
			10	35
			30	36-53
3 Mid (MDT)	5	5	20	1-34
			60	35-38
			80	39-42
			100	43-53[a]
4 High (HDT)	5	5	60	1-34
			100 (males only)	35
			120	36-38
			140	39-42
			150	43-53

[a]Dosed at 80 mg/kg for 2 days during week 49.

Dogs had been vaccinated against distemper, hepatitis, leptospirosis, parainfluenza, parvovirus, and rabies. They were individually housed in stainless steel cages in an environmentally controlled room with a 12-hour light/dark cycle.

2. Dosage Preparation: Capsules containing the appropriate amount of test material based on the most recent body weight were prepared weekly and stored refrigerated until dosing. Correction for purity of test compound was not considered in calculating the dose. Reserve samples (labeled as to dose) were sent to the sponsor for analysis. control dogs received empty capsules.

   Results: Material in the capsules was not analyzed. Purity of the test compound was analyzed prior to initiation and after 9 and 12 months. At the three intervals, the purity was 91.5, 91.3, and 94.6%, respectively.

3. Food and Water Consumption: Animals received food (Canine Diet Meal No. 5007) and water ad libitum.

4. Statistics: Changes in body weight (weeks 1-34, 35-42, and 43-52), hematology, clinical chemistry, and organ weight data were analyzed statistically. Levine's test of homogeneity of variance was used and homogeneous data were analyzed by ANOVA. Heterogeneous data were transformed using $\log_{10}$, square, square roots, reciprocal, arcsine, or rank transformation in that order. Comparisons between treated and control groups used Dunnett's test for equal variances for homogeneous data or Dunnett's test for unequal variances for data that remained heterogeneous after transformation.

5. Quality Assurance: A quality assurance statement was signed and dated May 2, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected once daily (3 to 4 hours after dosing) for clinical signs of toxicity. They were observed twice daily for signs of moribundity and mortality.

   Results: No dogs died during the study. There were no signs of toxicity in control or low-dose dogs. At doses of 100 mg/kg/day and greater (mid- and high-dose groups), clinical signs were characterized by excessive salivation, ataxia, mydriasis, dyspnea, tremors, increased respiration, and inability to stand. Frequency was greatest in high-dose animals and occurrence was more frequent in males than females. Table 1 summarizes incidence and frequency data. Other observations that were frequent were soft feces or diarrhea, sanguinous stools, emesis, alopecia, and skin sores. These latter observations were noted for most dogs in all groups and their incidence and frequency did not appear to increase with dosing.

2. Body Weight: Body weights were recorded weekly and mean body weight gains were calculated from individual weight data at the intervals between week 0-34, 34-42, and 42-52.

   Results: Table 2 summarizes body weight data. Body weight gains were similar in all groups during the first 34 weeks of the study and in low-dose males and females throughout the 52 weeks of the study. Between weeks 35 and 42 when the mid dose was 60-80 mg/kg/day and the high dose was 100-140 mg/kg/day, there was loss of weight that was significant ($p < 0.05$) in males at both doses and in females at the mid dose. Mean weight gains tended to be decreased

TABLE 1. Dose-Related Clinical Signs in Dogs Administered Pyridate[a]

Test group	Dose levels (mg/kg/day)	Males		Females	
		No. of dogs	No. of events	No. of dogs	No. of events
1	0	0	0	0	0
2	5-30	0	0	0	0
3	20-80	0	0	0	0
	100	4	7	3	4
4	60-100	0	0	0	0
	120	1	1	0	0
	140	2	2	1	1
	150	4	10	4	10

[a]Include ataxia, mydriasis, dyspnea, tremors, or prostration.

TABLE 2. **Mean Body Weights and Weight Gain Data at Selected Intervals in Dogs Administered Pyridate for 52 Weeks**

Final dose level (mg/kg/day)	Mean weight (kg ± SD) at week: 0	34	42	52	Mean weight gain (kg) between weeks: 0-34	35-42	43-52	0-52[a]
				Males				
0	7.5 ± 0.75	11.6 ± 0.76	11.8 ± 0.93	12.1 ± 1.10	4.1	0.2	0.3	4.6
30	8.5 ± 0.89	11.7 ± 1.43	11.9 ± 1.40	12.0 ± 1.34	3.2	0.1	0.1	3.5
100	8.0 ± 0.41	12.7 ± 1.62	12.1 ± 1.06	11.8 ± 0.79	4.7	-0.6*	-0.3	3.9
150	8.1 ± 0.61	12.5 ± 1.52	12.1 ± 1.25	12.1 ± 1.45	4.4	-0.4*	-0.1	4.0
				Females				
0	7.0 ± 0.70	9.9 ± 1.11	10.1 ± 1.13	10.5 ± 1.23	2.9	0.2	0.4	3.5
30	6.5 ± 0.44	9.3 ± 0.81	9.1 ± 0.58	9.3 ± 0.57	2.8	-0.1	0.2	2.8
100	6.7 ± 0.36	9.8 ± 1.75	9.2 ± 1.58	9.0 ± 1.97	3.1	-0.6*	-0.1	2.4
150	6.5 ± 0.71	8.9 ± 1.40	8.9 ± 1.49	8.8 ± 1.74	2.4	0	-0.1	2.3

*Significantly different from control weight gain ($p \leq 0.05$).

[a]These values were calculated by the reviewers from the individual animal data.

from week 43 through week 53 in all dosed groups (low dose, mid dose, and high dose were 30, 100, and 150 mg/kg/day, respectively) when compared to controls, but the changes did not reach a level of significance.

3. Food Consumption and Compound Intake: Consumption was determined weekly for each dog.

Results: Food consumption tended to be reduced in mid- and high-dose females from week 35 to study termination. Between weeks 35 and 42, mean weekly consumption was 1.6 and 1.8 kg in mid- and high-dose females compared to 2.2 kg/week in controls; between weeks 43 to 52, mean consumption was 1.8 and 1.9 kg/week in mid- and high-dose females compared to 2.6 kg/week in controls. Statistically significant differences were not found.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to initiation of the study and during week 52 of dosing.

Results: At termination, there did not appear to be any abnormality related to compound administration. One mid-dose male exhibited bilateral, tortuous pulsating vessels of the fundus. It was reported that the other abnormalities observed were also present prior to initiation. Neither summary nor individual animal data were presented.

5. Hematology and Clinical Chemistry: Blood was collected prior to study initiation and during weeks 12, 26, and 52 for hematology and clinical analysis from all dogs. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)

Results: There were no effects of biologic importance related to dosing. MCV was slightly ($p<0.05$) increased at weeks 12, 26, and 52 and MCH at weeks 26 and 52 ($p<0.05$) in high-dose females when compared to controls. However, the changes were not accompanied by effects on HCT, HGB, or RBC and there were no corresponding effects in males.

*Recommended by Subdivision F (October 1982) Guidelines.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium*	X	Albumin*
X	Chloride*		Albumin/globulin ratio
	Magnesium*	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Cholesterol*
X	Sodium*	X	Globulins
		X	Glucose*
	Enzymes	X	Total bilirubin*
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total carbon dioxide
X	Creatinine phosphokinase*		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)*		
X	Serum aspartate aminotransferase (SGOT)*		
	Gamma glutamyltransferase (GGT)		

Results: There were no effects of biological importance on clinical chemistry parameters. Total globulin was significantly lower ($p<0.05$) than controls in high-dose females at weeks 12 and 52; however, the globulin values were indirectly derived by subtracting the value for albumin from the total serum protein and there were no remarkable findings for either of these parameters nor was there an effect in males.

6. Urinalysis: Urine was collected from fasted animals at 12, 26, and 52 weeks. The CHECKED (X) parameters were examined:

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Reducing substances
X	Protein*	X	Urobilinogen

Results: A review of the individual animal data did not reveal any toxicologically important effects.

*Recommended by Subdivision F (October 1982) Guidelines.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	X Aorta[+]	XX Brain[+]
X Salivary glands[+]	XX Heart[+]	X Peripheral nerve (sciatic nerve)[+a]
X Esophagus[+]	X Bone marrow[+]	X Spinal cord (3 levels)
X Stomach[+]	X Lymph nodes[+]	X Pituitary[+]
X Duodenum[+]	X Spleen[+]	X Eyes (optic nerve)[+]
X Jejunum[+]	X Thymus[+]	
X Ileum[+]		
X Cecum[+]		
X Colon[+]		
X Rectum[+]		
XX Liver[+]	Urogenital	Glandular
X Gallbladder[+]	XX Kidneys[+]	XX Adrenals[+]
X Pancreas[+]	X Urinary bladder[+]	Lacrimal gland
	XX Testes[+]	X Mammary gland[+]
Respiratory	X Epididymides	XX Thyroids[+]
X Trachea[+]	X Prostate	XX Parathyroids[+]
X Lung[+]	X Seminal vesicle	Harderian glands
	X Ovaries	
	Uterus[+]	

Other
X Bone (sternum and femur)[+]
X Skeletal muscle[+]
X Skin.
X All gross lesions and masses

Results:

a. Organ Weights: Absolute and relative organ weights were similar in dosed and control groups in both sexes. No statistically significant changes were indicated in the summary tables.

b. Gross Pathology: There were no gross findings that were considered related to dosing. The only findings recorded were an ovarian cyst in one control and one low-dose female, thickened uterine wall in two low-dose females, and hair loss in one or two dogs of each group and sex, including controls.

[+]Recommended by Subdivision F (October 1982) Guidelines.
[a]Longitudinal and cross section.

c. Microscopic Pathology:

1) Nonneoplastic: The only histologic finding considered related to dosing was a slight degenerative myelopathy of the sciatic nerve found in one high-dose male; this dog also had increased hematopoiesis in the spleen. Slight subacute inflammation of the brain was seen in one control and one high-dose male, one control and one low-dose female, and two high-dose females. Slight or minimum inflammatory changes in other organs were sporadically found (one or less dogs/group); the tissues included thyroids, prostate, lung, liver, and kidney.

2) Neoplastic: There were no neoplasms or preneoplastic lesions.

D. STUDY AUTHOR'S CONCLUSIONS:

Significant findings in this study were clinical observations characterized by excessive salivation, dyspnea, mydriasis, rapid respiration, ataxia, and inability to stand, or prostration. The signs were intermittent and occurred in the mid- and high-dose groups after the doses had been raised to 100 mg/kg/day (mid dose) or 120 to 150 mg/kg/day (high dose). There were decreased body weight gains in mid- and high-dose males at doses above 100 mg/kg/day. In the mid-dose group, dose concentrations were raised from 20 mg/kg/day to 60, 80, or 100 mg/kg/day at weeks 35, 39, and 43 weeks, respectively; in the high-dose group, the doses were raised from 60 mg/kg/day to 100 in males only and 120, 140, or 150 mg/kg/day at weeks 35, 36, 39, and 43, respectively.

There were no effects of dosing on hematology, clinical chemistry, urinary parameters, or organ weights. Gross pathology findings were not related to dosing. The only compound-related histopathologic change was a degenerative myelopathy of the sciatic nerve in one high-dose male. This same lesion was seen in four of eight dogs that received 200 mg/kg/day in a 3-month study. The threshold level for dramatic nonlethal clinical signs (neurologic) was 80 mg/kg/day. The signs were more related to dose level rather than length of dosing, suggesting that the test compound did not accumulate.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

This study is a repeat of a 12-month dog study in which no effects were seen after administering 62 mg/kg/day pyridate. In the present study, no toxicity was observed after dosing for levels of up to 60 mg/kg/day for 35 weeks. In order to achieve an effect level, the doses were escalated in the low-, mid-, and high-dose groups. Although, this is not a recommended procedure, it is our assessment that the dose escalation should not affect the study classification.

The conduct and reporting of the study were, in general, adequate. Ophthalmologic findings were not tabulated. Clinical findings tabulated for each day and week were noted for each occurrence; this was critical since the effect level depended on adequate data on clinical findings. Individual animal data and summary data were provided for body weights, food consumption, clinical laboratory findings, organ weights, and gross and histopathology findings.

We agree with the study author's conclusions that nonlethal clinical effects of neurotoxicity were the most important and clear effect in the study. This was a threshold effect and there was a lack of a clear dose-response effect. The weight gain data clearly show that there were weight losses in mid- and high-dose males and females from week 35 to study termination. Weight gain was also depressed in low-dose females but the effect was slight, was not statistically significant, and cannot be unequivocally related to dosing. The effects on food consumption were slight and not statistically significant but they indicate a trend toward decreased consumption in the mid- and high-dose groups. There was clearly no effect of dosing on clinical laboratory parameters or on organ weights. The only histologic effect that can be related to dosing was the degenerative myelopathy of the sciatic nerve in one high-dose male. The incidence of this finding may have increased if a higher dose level were used, as evidenced from data on a subchronic study at 200 mg/kg/day reported by the author. However, it is possible that using a dose as high as 200 mg/kg/day may have resulted in mortality.

The LOEL for this study is 100 mg/kg/day and the NOEL is 20 mg/kg/day based on clinical signs of neurotoxicity.