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DATA EVALUATION RECORD

PYRIDATE

Subchronic Dermal Toxicity Study in Rats

REVIEWED BY:

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

Signature: William L. Richards
Date: June 7, 1989

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

Signature: Margaret E. Brower
Date: June 7, 1989

APPROVED BY:

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

Signature: Roman J. Pienta
Date: June 7, 1989

Mike Ioannou, Ph.D.
EPA Reviewer and Section
Head, Section I
Toxicology Branch II (TS-769C)

Signature: M Ioannou
Date: 6-12-89

DATA EVALUATION RECORD

STUDY TYPE: Subchronic dermal toxicity study in rats. GUIDELINE §82-2

MRID NUMBER: 409804-01.

TEST MATERIAL: Pyridate.

SYNONYM(S): O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate; Tough.

STUDY NUMBER(S): 437242.

SPONSOR: Agrolinz, Inc., Memphis, TN.

TESTING FACILITY: Inveresk Research International, Musselburgh, Scotland.

TITLE OF REPORT: 3 Week Dermal Toxicity Study in Rats.

AUTHOR(S): C. J. Perry and J. Duffen.

REPORT ISSUED: October 3, 1988.

CONCLUSIONS:

Groups of five male and five female Sprague-Dawley rats were dosed daily for 3 weeks by the dermal route with pyridate at dose levels of 0 or 1000 mg/kg/day. In animals that received 1000 mg/kg/day, the neat test compound was applied and (after 6 hours) removed (method not indicated) daily from clipped test sites. In control animals, the clipped test sites were untreated. A nonocclusive dressing was applied to the test sites of both treated and control rats. There were no treatment-related deaths or notable compound-related effects on body weight, food and water consumption, hematology and clinical chemistry parameters, or organ weights. The only compound-related effect was irritation of the treatment site, as evidenced at the gross level by skin encrustation in males and at the microscopic level by minimal to moderate epidermal hyperplasia and inflammation in both sexes, scab formation in males, and ulceration with moderate epidermal and dermal inflammation in one of the females. Because of the lack of sham treatment of test sites in control animals, the possible contribution of the daily application/removal process to the observed irritation at compound-treated test sites is unclear.

Classification: Core Guideline.

A. MATERIALS:

1. Test Compound: Pyridate Technical; description: oily liquid; batch No.: 2759523 CL11.344/AR22; purity: 91.5%.

2. Test Animals: Species: Rat; strain: Sprague-Dawley; age: 9 weeks; weight: males--265 g; females--200 g; source: Charles River UK Ltd., Manston, Kent.

B. STUDY DESIGN:

1. Animal Assignment: Animals were individually caged, identified by cage card and ear mark, and assigned randomly to the following test groups, after which they were acclimated to laboratory conditions for 12 days before treatment began:

Test group	Dermal Dose (mg/kg)	Males	Females
1 Control	0	5	5
2 High	1000	5	5

2. Dose Preparation: Dosing material was applied neat.

3. Preparation of Animal Skin: The animals' backs were clipped weekly, and the test material was applied daily at a dose volume of 1 mL/kg body weight over an area of approximately 10% of the total body surface area. The treated area was protected by a nonocclusive dressing and held in place by means of nonirritating tape. Dermal exposure was for approximately 6 hours/day for a minimum of 21 consecutive days; the site of exposure was cleaned of test material between daily applications. The control animals were clipped once per week and received a non-occlusive dressing daily but were undosed.

4. Food and Water Consumption: Animals received SDS Rat and Mouse (Modified) No. 1 Diet SQC Expanded and water ad libitum.

5. Statistics: The following procedures were utilized in analyzing the numerical data:

Hematology, clinical chemistry, and organ and body weight data were analyzed for homogeneity of variance by the F-max test. When the group variances appeared homogenous, a parametric ANOVA was used, and pairwise comparisons were made by Student's t-test using Fischer's F-protected Least Significant Difference (LSD). The data were transformed if appropriate. Organ weights were also analyzed conditional on body weight, i.e., by analysis of covariance. Histopathology data were analyzed by Fischer's Exact Probability Test.

6. Quality Assurance: A quality assurance statement was signed and dated December 20, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of morbidity and mortality. Due to results seen in previous toxicity studies, particular attention was paid to animals during the first 5 hours after dosing.

   Results: There were no compound-related deaths. The authors stated that scab formation and reddened skin were noted in some treated animals. The following incidences of clinically-observed skin lesions are summarized by the reviewers from data in individual animal data records: 1/5 control males had red skin; 4/5 treated males had red skin, and 5/5 had scabbing; no clinical findings were observed in control females; 2/5 treated females had red skin and scabbing. Animals were not scored numerically for dermal irritation. No other compound-related clinical signs of toxicity were seen.

2. Body Weight: Animals were weighed prior to the first treatment and weekly thereafter.

   Results: Although there were no statistically significant differences in body weight or body weight gain in treated compared with control rats, body weights at week 3 were slightly less in treated (370 g, males; 254 g, females) than in control (397 g, males; 257 g, females) animals. Body weight gains for weeks 0 through 3 were substantially less in treated (29 g, males; 16 g, females) than in control (50 g, males; 24 g, females) animals.

3. Food and Water Consumption: Food consumption was recorded prior to the first treatment and weekly thereafter. Water consumption was monitored by visual inspection during the treatment period.

   Results: No compound-related effects on food or water consumption were noted.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus at week 3 for hematology and clinical analysis. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
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)
X Coagulation: thromboplastin time (PT)

Results: Table 1 shows selected mean hematology parameters. Although the Hepato Quick time was significantly ($p < 0.05$) extended in males and the lymphocyte count was significantly ($p < 0.05$) reduced in females dosed at 1000 mg/kg/day as compared with controls, these differences were small, confined to a single sex, and not considered biologically relevant by the study authors. The reviewers note that the Hepato Quick test and the parameter(s) it examines were not described by the study authors.

b. Clinical Chemistry

Electrolytes
X Calcium*
X Chloride*
Magnesium
X Phosphorus*
X Potassium*
X Sodium*

Enzymes
X Alkaline phosphatase (ALP)
Cholinesterase
Creatinine phosphokinase
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other
X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
Cholesterol
Globulins
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
Triglycerides

*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 1. Selected Mean Hematology Parameters (± S.D.) of Rats Treated Dermally with Pyridate for 3 Weeks[a]

Dose Group (mg/kg/day)	Total White Blood Cell Count (10^9/L)	Neutrophil (%)	Lymphocyte (10^9/L)	Hepato Quick (Sec)
		Males		
0	12.9 ± 4.3	1.6 ± 1.1	11.0 ± 3.5	25 ± 1
1000	15.4 ± 6.9	3.4 ± 1.8	11.4 ± 5.0	27 ± 1*
		Females		
0	10.8 ± 0.9	1.8 ± 0.8	8.5 ± 1.1	25 ± 1
1000	9.0 ± 2.3	2.1 ± 1.1	5.7 ± 2.2*	25 ± 1

*Significantly different from controls at $p < 0.05$.

[a]Units for hematology parameters were not reported.

Results: Table 2 shows selected mean clinical chemistry parameters. Significant ($p < 0.05$) decreases in blood urea nitrogen and serum chloride and significant ($p < 0.05$) increases in serum alanine aminotransferase and serum albumin were found in males dosed at 1000 mg/kg/day compared to controls. No significant changes in clinical chemistry parameters were noted in females. The significant differences in these parameters were small, confined to a single sex, and not considered biologically relevant. The authors stated that individual data were generally well within expected limits.

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals were killed and subjected 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		Aorta	X	Brain
	Salivary glands	X	Heart		Peripheral nerve (sciatic nerve)
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		Pituitary
	Duodenum	X	Spleen		Eyes (optic nerve)
	Jejunum		Thymus		
	Ileum				
	Cecum				
	Colon				
	Rectum		Urogenital		Glandular
XX	Liver+	XX	Kidneys+	XX	Adrenals
	Gallbladder		Urinary bladder		Lacrimal gland
	Pancreas	XX	Testes		Mammary gland
		X	Epididymides		Thyroids
			Prostate		Parathyroids
			Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
	Trachea		Uterus		
	Lung				
					Other
					Bone (sternum)
					Skeletal muscle
				X	Skin, normal+
				X	All gross lesions and masses+
				X	Skin, treated+

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. Selected Mean Clinical Chemistry Parameters (± S.D.) of Rats Treated Dermally with Pyridate for 3 Weeks[a]

Dose Group (mg/kg/day)	Blood Urea Nitrogen (mmoL/L)	Aspartate Amino-transferase (IU/L)	Alanine Amino-transferase (IU/L)	Chloride (mmoL/L)	Albumin (g/L)
			Males		
0	6.2 ± 0.4	107 ± 17	79 ± 8	106 ± 1	32 ± 1
1000	5.6 ± 0.2*	134 ± 22	91 ± 8*	105 ± 1*	34 ± 1*
			Females		
0	6.2 ± 0.8	93 ± 15	65 ± 7	106 ± 2	37 ± 2
1000	6.0 ± 0.7	97 ± 8	75 ± 11	106 ± 1	36 ± 2

*Significantly different from controls at $p < 0.05$.

[a]Units for clinical chemistry parameters were not reported.

Results:

a. Organ Weights: There were no differences in the absolute weights of the adrenals, kidneys, liver, testes, or ovaries of animals dosed at 1000 mg/kg/day as compared to controls. However, following adjustment for differences in final body weight (covariance analysis), the liver of male and female rats dosed at 1000 mg/kg/day showed a slight but significant ($p < 0.05$, males; $p < 0.01$, females) increase compared with controls (Table 3). The reviewers attribute this significant covariance of liver weight to the nonsignificantly reduced body weights at week 3 and body weight gain during weeks 0 through 3; the reviewers also note the absence of corroborative liver gross findings and histopathology (see below) and therefore consider these changes not to be biologically relevant.

b. Gross Pathology: Encrustation of the skin was noted in 3/5 males, and darkened areas were noted in one rat of each sex dosed at 1000 mg/kg/day. No controls exhibited these lesions.

c. Microscopic Pathology:

1) Nonneoplastic: Table 4 shows representative nonneoplastic findings. Compound-related changes in the skin at the treatment site were observed in 8/10 rats dosed at 1000 mg/kg/day. The only significant ($p < 0.05$) effect, compared with controls, was minimal to moderate epidermal hyperplasia in 4/5 rats of each sex. Minimal to moderate inflammation was seen at the treatment site of two dosed rats of each sex. In addition, scab formation was seen in two dosed males and ulceration with moderate dermal and epidermal inflammation was seen in one dosed female. No marked treatment-related microscopic findings were noted in other tissues.

2) Neoplastic: No neoplastic changes were noted.

TABLE 3. Selected Mean Organ Weights (± S.D.)[a] and Covariance of Organ Weights (± S.E.) of Rats Treated Dermally with Pyridate for 3 Weeks

Dose Level (mg/kg/day)	Liver	
	Absolute (g)	Covariance Analysis
	Males	
0	16.39 ± 0.67	15.60 ± 0.38
1000	16.68 ± 2.02	17.47 ± 0.38*
	Females	
0	10.07 ± 0.70	9.98 ± 0.12
1000	10.53 ± 0.31	10.61 ± 0.12**

[a]Based on five rats/sex/group.

*Significantly different from controls at $p < 0.05$.

**Significantly different from controls at $p < 0.01$.

TABLE 4. Representative Nonneoplastic Findings in Rats Treated Dermally with Pyridate for 3 Weeks

	Dose Level (mg/kg/day)			
	Males		Females	
	0	1000	0	1000
Liver	(5)[a]	(5)	(5)	(5)
Periportal inflammation	1	1	0	0
Microfoci of inflammatory cells	3	1	1	1
Heart	(5)	(5)	(5)	(5)
Pericarditis	0	0	0	2
Kidneys	(5)	(5)	(5)	(5)
Basophilic tubules	0	0	1	0
Mineral deposits	0	0	3	2
Spleen	(5)	(5)	(5)	(5)
Increased extramedullary hemopoiesis	0	0	0	1
Skin/subcutis, untreated	(5)	(5)	(5)	(5)
Inflammation	1	0	0	0
Skin/treatment site	(5)	(5)	(5)	(5)
Ulceration	0	0	0	1
Inflammation	1	2	0	2
Epidermal hyperplasia	0	4*	0	4*
Scab(s)	0	2	0	0

[a]Figures in parentheses represent number of animals from which tissue was examined.

*Significantly different from controls at $p < 0.05$.

D. STUDY AUTHORS' CONCLUSIONS:

Groups of five male and five female rats were dosed daily for 3 weeks by the dermal route with pyridate at dose levels of 0 or 1000 mg/kg/day. The only changes that could be attributed to treatment with pyridate were observed in the skin at the dosing site. At the gross level, scab formation and reddened skin were observed. At the cellular level was evidence of minimal to moderate inflammatory changes and minimal to mild epidermal hyperplasia; these were consistent with gross pathology and clinical signs observations. All findings are considered to be consistent with the effects of a mild irritant. There were no compound-related deaths or notable intergroup differences in body weight, food and water consumption, hematology and clinical chemistry parameters, organ weights, or clinical signs other than the evidence of irritation at the application site.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were generally adequate. Animals were not scored for dermal irritation, but scoring is not a recommendation of the Pesticide Assessment Guidelines (1982) for the repeated dose dermal toxicity study. Clinically observed dermal lesions were not summarized by the study authors. The Hepato Quick test, included with hematology parameters in the study report, was not defined. The reviewers agree with the authors that the only evidence of compound-related toxicity in this single-dose study (1000 mg/kg/day) was irritation at the treatment site, as evidenced by minimal to moderate epidermal hyperplasia, scab formation, and inflammation in both sexes, and ulceration with moderate epidermal and dermal inflammation in 1/5 treated females. The reviewers note, however, that nothing was applied to test sites in control rats whereas compound was applied and (after 6 hours) removed daily from the test sites of treated animals. Thus, because of the absence of sham treatment of control rats, the possible contribution of the daily application/removal process to the observed irritation at compound-treated test sites cannot be determined.