6/28/89.

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

007303

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pyridate - Review of Registrant's Safety Evaluation Assessment of Pyridate; Evaluation of the Registrant's Reply to EPA Comments Concerning a Mutagenicity Study (UDS Assay); and Review of a Metabolism Study

Tox Chem No.: 716A
HED Project Nos.: 9-0982
9-0983

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THRU: Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II (HFAS)
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MRID Nos.: 409862-01 and 409718-01

Registrant: Agrolinz, Inc., Memphis, Tennessee

Action Requested

Review and comment on the Registrant's account of "Pyridate safety evaluation", evaluate the Registrant's reply to EPA comments on a mutagenicity study (unscheduled DNA synthesis assay); and review a rat metabolism Study.

Conclusions and Recommendations

A. Pyridate Safety Evaluation - The registrant presented a brief account on pyridate safety evaluation. This account is based on all available data on pyridate submitted to the Agency and reviewed by Toxicology Branch. Based on the available data (acceptable acute, subchronic, chronic toxicity and oncogenicity, terotogenicity, reproduction, mutagenicity and metabolism studies) we agree with the Registrant's contention that pyridate appears to have a high margin of safety. However, since the data base for pyridate

is not complete (mouse oncogenicity not submitted; 1-year chronic toxicity dog, under review) a final assessment for pyridate safety cannot be made at present.

B. Mutagenicity Study - Registrant's Response to EPA's Comments. A Mutagenicity Study, titled " in vivo - in vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay" was recently reviewed (12-05-88) by Toxicology Branch and classified as unacceptable due to several deficiencies concerning mainly the following points.

1. Only male rats were used for the assay.

2. No information was provided as to the absorption of pyridate from the GI tract at high enough concentrations to cause cytotoxicity of the target cells.

3. No documentation was provided to show that storage of slides coated with Kodak NTB2 emulsion for 3 days was sufficient time to develop enough nuclear grains from a weakly positive chemical.

In the present reply, the Registrant addressed all 3 points raised by the Agency and satisfactory explanations and/or additional information were provided so that all issues raised by the Agency are now resolved. The study is thus upgraded to Acceptable.

Rat Metabolism Study. The Registrant submitted a comprehensive metabolism study with pyridate in rats. The study was reviewed by Dynamac and the DER is attached. Briefly the conduct of the study and the major findings were as follows:

Male and female Sprague-Dawley rats were administered single oral (gavage) doses of ^{14}C-Pyridate (radiopurity greater than 97%) at levels of 20, 200, or 600 mg/kg body weight. A multiple exposure study was also conducted at a dose level of 20 mg/kg/day of nonradioactive Pyridate for 14 days followed by a single oral administration of ^{14}C-Pyridate. The absorption, distribution, metabolism, and excretion of Pyridate were investigated at selected time points. Metabolites were isolated (from urine) and identified using a variety of acceptable analytical techniques.

Results

At single oral dose levels of 20 and 200 mg/kg, Pyridate was rapidly absorbed through the gastrointestinal tract distributed to all major tissues examined and greater than 93 percent of the administered dose was excreted in urine and feces within 96 hours, in both sexes. The major portion of Pyridate-derived

radioactivity (approximately 80%) was excreted in the urine. No significant difference in excretion was seen between the single dose levels of 20 and 200 mg/kg and the multiple dose of 20 mg/kg/day. At the high dose of 600 mg/kg, the percent of Pyridate excreted in the urine and feces was approximately 68 percent within 24 hours and 87 percent within 96 hours.

Pyridate distribution in the major tissues was studied at selected time points after oral administration of 20, 200, or 600 mg/kg of Pyridate to male and female rats. At all dose levels tested, the highest concentration of Pyridate (as percent of administered dose) was found in the kidney, liver, and plasma. Clearance of radioactivity from these tissues was almost complete within 24 hours with the 20 and 200 mg/kg dose levels (less than 0.1% of the dose remaining in these tissues); however, with the dose of 600 mg/kg, clearance from all tissues was slow with significant concentrations still remaining in these tissues at the 24-hour time point although, clearance was almost complete by 96 hours. These results suggest that the high dose (600 mg/kg) was either more slowly absorbed from the gastrointestinal tract and/or more slowly metabolized (due possibly to saturation of metabolizing enzymes) as compared to the dose levels of 20 or 200 mg/kg.

The sponsor reported the isolation and identification of Pyridate metabolites from rat urine. Using thin layer chromatography (TLC) and different solvent systems, three metabolites were isolated from urine. Two of the metabolites were identified (using authentic standards) as CL-9673 (the hydrolysis product of Pyridate), and CL-9673-O-glucuronide. These two metabolites accounted for 35-69 percent of the radioactivity in urine. A third metabolite (representing 26-37 percent of the radioactivity in urine) was identified (using mass spectrometry) as the hydroxylated derivative of CL-9673. None of the radioactivity in the urine cochromatographed with the parent compound, Pyridate.

Conclusions

Based on results presented here, it appears that Pyridate, when administered orally to male or female rats at dose levels of 20 or 200 mg/kg, (single doses), or at 20 mg/kg, multiple exposure, is rapidly absorbed from the gastrointestinal tract, distributed to all major tissues examined metabolized and cleared from all tissues rapidly so that 24 hours postdosing only very low concentrations are present in these tissues. Most of the Pyridate-derived radioactivity is excreted in the urine (> 80%) and consists of three Pyridate metabolites identified as CL-9673, CL-9673-O-glucuronide and the hydroxylated derivative of CL-9673.

At the dose level 600 mg/kg (single oral dose), Pyridate appears to be absorbed at a slower rate than lower dose levels, distributed to the same tissues as lower dose levels, and eliminated from these tissues at a slower rate.

Classification: Core-Guideline

EPA: 68D80056
DYNAMAC No.: 169-C
TASK No.: 1-69C
June 7, 1989

DATA EVALUATION RECORD

PYRIDATE

Metabolism in Rats

STUDY IDENTIFICATION: Cameron, B. D., Fisher, J., Johnston, A. M., and Scott, G. The absorption, distribution, metabolism and excretion of [^{14}C]pyridate in the rat. (Unpublished report No. 4636 performed by Inveresk Research International, Musselburgh, Scotland, and submitted by Agrolinz, Memphis, TN; dated November, 1988.) MRID No. 409862-01.

APPROVED BY:

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

Signature: Ramon J. Prieto Jr.
Date: June 7, 1989

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1. CHEMICAL: Pyridate; O-[6-chloro-3-phenyl-4-(4,5-^{14}C)-pyridazinyl-)]-S-octyl-carbonothionate.

2. TEST MATERIAL: [^{14}C]Pyridate was from batch No. 14.C CL 11344-04-87 with a specific activity of 28.34 μCi/mg and a radiochemical purity of 97 percent. The test material was labeled on C_4 and C_5 of the pyridazinyl ring as follows:

3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Cameron, B. D., Fisher, J., Johnston, A. M., and Scott, G. The absorption, distribution, metabolism and excretion of [^{14}C]pyridate in the rat. (Unpublished report No. 4636 performed by Inveresk Research International, Musselburgh, Scotland, and submitted by Agrolinz, Memphis, TN; dated November, 1988.) MRID No. 409862-01.

5. REVIEWED BY:

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7. CONCLUSIONS:

The metabolism of [^{14}C]pyridate was studied in male and female Sprague-Dawley rats following oral administration. A total of eight experiments were conducted. Total recovery of radioactivity 96 hours following oral administration of [^{14}C]pyridate at 20, 200, or 600 (males only) mg/kg was >90 percent. At the low and mid doses, most of the radioactivity was eliminated within 24 hours postdosing. No apparent sex- or dose-related differences were noted. At all doses, most of the radioactivity was eliminated in the urine (52 to 84 percent of the dose) with lower amounts eliminated in the feces (11 to 34 percent). The percent radioactivity eliminated in urine decreased as the dose increased and this was accompanied by an increase in radioactivity eliminated in the feces. Saturation kinetics were noted at the 600-mg/kg dose. [^{14}C]CO_2 accounted for less than 0.07 percent of the administered dose. At 20 mg/kg, biliary excretion accounted for 6 to 8 percent of the dose in males and females 24 hours postdosing. Radioactive tissue residues were associated mainly with the carcass and accounted for less than 0.2 percent of the dose 96 to 168 hours postdosing. There were no apparent differences in elimination patterns and tissue residues in rats administered 20 mg/kg [^{14}C]pyridate following oral administration of unlabeled material at 20 mg/kg/day for 14 days when compared to results obtained from rats given a single dose of the [^{14}C] test material. Radioactive residue levels in plasma of male and female rats receiving 20 mg/kg were highest 1 to 2 hours postdosing, with higher residues found in females (29.6 µg/mL) than males (13.7 µg/mL). However, at 600 mg/kg the highest residue levels were noted 6 hours postdosing. At all doses, plasma [^{14}C] levels dropped to <0.1 µg/mL by 96 hours postdosing. As with plasma, radioactivity in tissues of dosed rats was highest 1 hour after receiving 20 or 200 mg/kg and 6 hours after receiving 600 mg/kg. Radioactivity in the kidneys of male rats receiving 200 and 600 mg/kg peaked at 6 and 24 hours after dosing, respectively. The highest residue levels were found in the kidney, liver, and plasma as well as the gastrointestinal tract and were dose-dependent. By 96 hours postdosing, residue levels decreased to less than 1.5 µg/g.

The major metabolite found in urine was the CL9673-O-glucuronide which accounted for about 23 to 50 percent of the radioactivity. CL9673 [6-chloro-4-hydroxy-3-phenylpyridazinyl] accounted for about 12 to 19 percent and a hydroxylated CL9673 accounted for 26 to 37 percent. Pyridate was not detected in the urine but was found in the feces and accounted for 0 to 35 percent of the radioactivity. CL9673 and hydroxylated CL9673 were also detected in the feces. Almost all of the radioactivity in plasma was identified as CL9673.

These studies are acceptable and fulfill EPA guidelines.

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Items 8 through 10--see footnote [1].

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. [^{14}C]Pyridate was apparently purified by preparative thin-layer chromatography (TLC) to a radiopurity of ≥97 percent.

2. Male and female Charles River CD remote Sprague-Dawley rats (age and source not specified) were used in the studies. It was not reported whether the animals were acclimated to laboratory conditions prior to dosing.

3. Table 1 lists eight metabolic studies performed with groups of three to six female and/or male rats. For these studies, appropriate amounts of [^{14}C]pyridate and unlabeled pyridate were mixed in corn oil and administered by gavage. The doses received were determined by radioassay of "mock doses" taken at the time of dosing. Following dosing, rats were housed in all-glass metabolism cages designed for the separate collection of urine and feces. During studies with bile duct cannulated rats, the animals were held in restraining cages to enable collection of bile. Cages were rinsed at the time of feces collection. Urine was collected into containers cooled by solid CO_2 for the first 48 hours. Elimination of CO_2 was determined in one male and one female rat.

Samples of urine, cage wash, plasma, and dosing solutions were radioassayed directly by liquid scintillation counting (LSC). The LSC was equipped with an automatic quench correction using the external standard channels ratio. A limit of 30 dpm above background was used for reliable determination of radioactivity. Feces and tissues were homogenized and aliquots were combusted prior to radioassay. Whole blood was also combusted prior to radioassay.

Urine, feces, plasma and bile were further analyzed by TLC for metabolites as follows: pooled plasma was collected at various intervals from experiments 4 and 6, deprotenized with acetone, and centrifuged; the supernatant was then analyzed by TLC.

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[1]Only items appropriate to this DER have been included.

TABLE 1. Metabolic Studies Performed with Male and Female Rats Dosed Orally With [^{14}C]Pyridate

Study No.	Target Dose (mg/kg)	No. of doses	No. of Animals/ Sex	[^{14}C] determined in
1	20	Single	6	Urine, feces, CO_2, and tissues after 168 hours
2	20	Repeat (14)[a]	6	Urine, feces, CO_2, and tissues after 168 hours
3	200	Single	6	Urine, feces, CO_2, and tissues after 168 hours
4	20	Single	6	Plasma after 0.5 to 24 hours
5	20	Single	3	Bile after 1 to 24 hours, urine, feces, and carcass after 24 hours
6	20	Single	3x5	Tissues and blood after 1 to 96 hours
7	200	Single	3x4 M[b]	Urine, feces, and tissues after 1 to 96 hours
8	600	Single	3x4 M[b]	Urine, feces, and tissues after 1 to 96 hours

[a]Rats received single oral doses of unlabeled pyridate at 20 mg/kg/day for 14 days followed with a single dose of [^{14}C]pyridate.

[b]Only males were used.

Pooled urine samples collected 24 hours posttreatment from experiments 1, 2, 3, 7, and 8 were analyzed directly by TLC. Pooled bile samples collected 24 hours posttreatment from experiment 5 were taken to near dryness and the residue was redissolved in methanol; the methanol solution was then analyzed. Samples of pooled feces collected 24 hours posttreatment from experiments 1, 2, 3, 7, and 8 were sonicated in acetone. The mixture was centrifuged and the supernatant was analyzed by TLC. All samples were compared chromatographically with reference standards of pyridate, CL9673 (6-chloro-3-phenyl-4-hydroxypyridazinyl), and CL9673-O- and N-glucuronides using two solvent systems.

A sample of urine from male rats in experiment 8 was purified and analyzed by mass spectrometry.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Disposition of Radioactivity in Rats After Receiving a Single Dose at 20 mg/kg (Study No. 1): Total recovery of [^{14}C]pyridate accounted for >99 percent of the administered dose (Table 2). Most of the radioactivity was eliminated within 24 hours postdosing. About 82 percent was eliminated in the urine and about 14 percent in the feces 96 hours postdosing, with no apparent sex-related differences. Only ≤0.06 percent of the dose was eliminated as [^{14}C]CO_2. Radioactive residues in carcasses and tissues accounted for about 0.3 percent of the dose 7 days postdosing. The highest tissue residues were found in the kidney, liver, fat, and whole blood (≤0.023 µg Eq/g, Table 3).

B. Disposition of Radioactivity in Rats after Receiving Repeated Doses at 20 mg/kg (Study No. 2): Total [^{14}C]recovery, [^{14}C]elimination patterns, and [^{14}C]residue levels in tissues following the administration of [^{14}C]pyridate to rats receiving pyridate for 14 days at 20 mg/kg/day were similar to those obtained from rats receiving a single dose (Tables 2 and 3).

C. Disposition of Radioactivity in Rats after Receiving a Single Dose at 200 mg/kg (Study No. 3): The results of this study were similar to those obtained in rats receiving the low dose (Tables 2 and 3). However, rats receiving the high dose eliminated slightly more radioactivity in the feces and slightly less radioactivity in the urine. In addition, total recovery in females was only 91 percent.

TABLE 2. Percent Recovery of Radiocarbon in Urine, Feces, and Tissues of Rats Following Oral Administration of [^{14}C]Pyridate

Study No.	Dose Administered (mg/kg)	Percent Recovery of Administered Dose[a]					
		Urine	Feces	Cage Wash	Tissues	CO_2	Total
(1) 20 mg/kg Single dose							
Male	18.5	81.80	14.54	2.97	0.33	0.02	99.64
Female	20.1	81.44	13.62	3.88	0.25	0.06	99.21
(2) 20 mg/kg Repeated dose[b]							
Male	23.5	83.81	12.43	2.27	0.11	0.03	98.65
Female	18.8	79.86	11.01	5.94	0.42	0.07	97.30
(3) 200 mg/kg Single dose							
Male	199	76.88	17.05	2.87	0.19	0.14	97.13
Female	223	69.02	19.05	2.61	0.17	0.03	90.85

[a]Mean values from six animals/sex, except CO_2 where values are from one animal/sex. Cumulative recoveries for urine, feces, and cage wash were 96 hours postdosing, whereas cumulative recoveries for CO_2 and tissues were 24 and 168 hours postdosing.

[b]Rats received single oral doses of unlabeled pyridate of 20 mg/kg/day for 14 days followed with a single dose of [^{14}C]pyridate.

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TABLE 3. Radioactive Residue Levels in Tissue of Rats 168 Hours Following Oral Administration of [^{14}C]Pyridate

Tissue	[^{14}C]Residues (µg/g or mL)[a] in rats receiving					
	20 mg/kg[b]		20 mg/kg[c]		200 mg/kg	
	Male	Female	Male	Female	Male	Female
Bone	0.020	0.010	0.052	0.023	0.06	0.21
Brain	0.009	0.002	0.009	0.005	0.01	0.03
Fat	0.012	0.001	0.009	0.010	0.07	0.06
Heart	0.006	0.033	0.006	0.005	0.05	0.04
Kidney	0.023	0.012	0.009	0.030	0.19	0.30
Liver	0.017	0.007	0.039	0.014	0.15	0.16
Lung	0.006	0.006	0.016	0.005	0.04	0.06
Muscle	0.009	0.005	0.009	0.007	0.06	0.11
Spleen	0.008	0.009	0.003	0.010	0.02	0.12
Gonads	0.003	0.001	0.004	0.018	0.01	0.15
Plasma	0.001	0.001	0.000	0.000	0.01	0.00
Whole Blood	0.016	0.013	0.031	0.025	0.23	0.29

[a]Mean values for six animals/sex.

[b]Single oral dose.

[c]Rats received single oral doses of unlabeled pyridate of 20 mg/kg/day for 14 days followed by a single dose of [^{14}C]pyridate.

D. Plasma [^{14}C] Residue Levels in Rats Receiving a Single Dose at 20 mg/kg (Study No. 4): The actual doses administered to male and female rats were 17.83 and 18.88 mg/kg. Following oral administration of [^{14}C], plasma [^{14}C] levels peaked at 1 to 2 hours postdosing accounting for 13.7 and 29.6 µg Eq/mL in males and females, respectively (Figure 1). Thereafter, [^{14}C] residue levels decreased gradually with time.

E. Biliary Excretion Study in Rats Receiving a Single Dose at 20 mg/kg (Study No. 5): The actual doses administered to groups of three male and three female rats were 18.6 and 18.2 mg/kg, respectively. Total recovery of radioactivity 24 hours postdosing accounted for 80 to 104 percent of the dose. The average biliary excretion accounted for 8 and 6 percent of the dose in males and females, respectively.

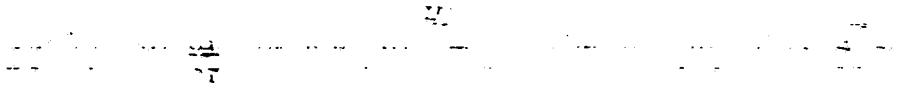
F. [^{14}C] Residue Levels in Rat Tissues at Various Intervals after Receiving a Single Dose at 20 mg/kg (Study No. 6): The actual doses administered to 15 male and 15 female rats were 16.81 and 17.49 mg/kg. The highest [^{14}C] residue levels were found 1 hour after dosing and decreased thereafter (Table 4). The gastrointestinal (GI) tract, liver, kidney, and plasma contained the highest levels of radioactivity (10 to 94 µg/g) 1 hour postdosing. By 24 hours the residue levels in tissues were ≤2.24 µg/g.

G. Disposition of Radioactivity in Male Rats at Various Intervals after Receiving a Single Dose at 200 µg/kg (Study No. 7): The actual dose administered to 12 male rats was 173.1 mg/kg. Total recovery of radioactivity in urine and feces after 24 and 96 hours postdosing was over 97 percent (Table 5). Radioactive residue levels were highest 1 hour postdosing and decreased thereafter, except for kidney levels (Table 6) where radioactivity increased to 123 µg/g at 6 hours postdosing and decreased thereafter. At 96 hours postdosing, residue levels in tissues were ≤0.6 µg/g.

H. Disposition of Radioactivity in Male Rats at Various Intervals after Receiving a Single Dose at 600 µg/kg (Study No. 8): The actual dose administered to 12 male rats was 542 mg/kg. Total recovery of radioactivity in urine and feces after 24 and 96 hours postdosing were 73 and 90 percent, respectively (Table 5). Radioactive residue levels were highest 6 hours postdosing and decreased thereafter, except for kidney. At 96 hours postdosing, residue levels in tissues were ≤1.5 µg/g (Table 6).

I. Metabolite Identification:

Urine: Three radiolabeled components were observed consistently in all urine samples examined (study Nos. 1, 2, 3, 7, and 8). The major component was CL9763-O-



Source: CBI Figure 7, CBI p. 84.

FIGURE 7

Plasma Levels of Total Radioactivity Following Single Oral Administration of [^{14}Cl-Pyridate to 6 Male and 6 Female Sprague-Dawley CD Rats at a Target Dose Level of 20 mg.kg^{-1}

Results expressed as µg equiv.ml^{-1}