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 (Unpublished report No. 4636 performed by Inveresk Research International, Musselburgh, Scotland, and submitted by Agrolinz, Memphis, TN; dated November, 1988.)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
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Signature: Roman J Pineda Jr
Date: June 7, 1989

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 (Unpublished report No. 4636 performed by Inveresk Research International, Musselburgh, Scotland, and submitted by Agrolinz, Memphis, TN; dated November, 1988.)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.

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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.

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.

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 ug equiv.ml^{-1}

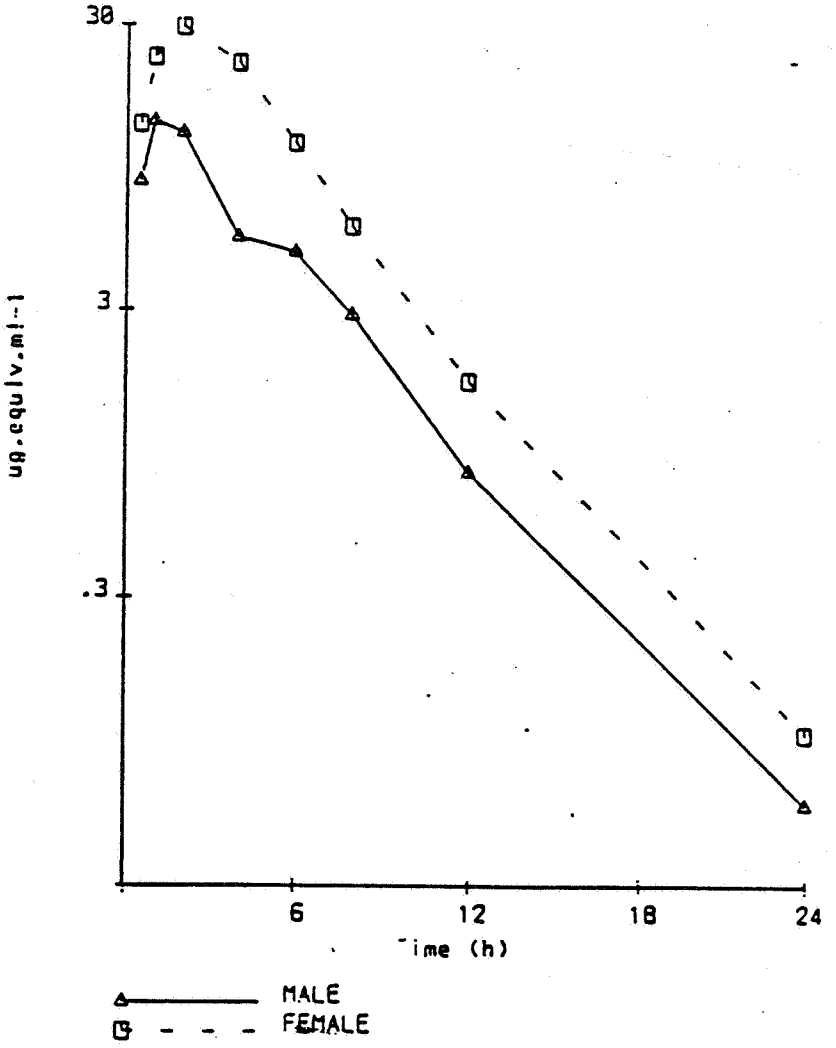

TABLE 4. Radioactive Residue Levels in Tissues of Rats at Various Intervals Following Oral Administration of [^{14}C]Pyridate at 20 mg/kg.

	[^{14}C] Residues (µg/g or mL) Postdosing (hours)[a]									
	1		6		24		48		96	
Tissue	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Brain	1.06	1.15	0.27	0.47	0.01	0.01	0.01	0.00	0.01	0.00
Fat	2.25	2.52	0.78	1.48	0.03	0.03	0.01	0.00	0.01	0.02
Bone	1.31	1.31	0.69	0.72	0.02	0.06	0.02	0.05	0.03	0.01
Heart	5.56	6.20	1.70	3.20	0.03	0.06	0.01	0.01	0.01	0.01
Liver	10.14	10.20	4.14	4.61	0.14	0.18	0.08	0.08	0.04	0.03
Lung	4.10	5.00	1.25	2.30	0.04	0.05	0.02	0.03	0.02	0.02
Kidney	31.25	20.03	9.52	12.37	0.39	0.73	0.17	0.15	0.07	0.09
Spleen	2.02	2.46	0.61	1.14	0.02	0.04	0.01	0.01	0.01	0.01
Gonads	1.88	4.64	0.79	2.27	0.05	0.07	0.01	0.01	0.01	0.16
GI tract	94.02	89.57	49.06	55.81	1.07	2.24	0.55	0.32	0.05	0.05
Muscle	2.48	2.50	1.00	1.51	0.15	0.08	0.02	0.04	0.01	0.02
Plasma	13.12	17.29	3.99	7.72	0.09	0.21	0.02	0.03	0.00	0.01
Blood	7.93	9.98	2.70	4.57	0.06	0.14	0.03	0.02	0.01	0.01

[a]Mean values from three animals/sex.

TABLE 5. Percent Cumulative Excretion of Total Radioactivity in Male Rats Following Oral Administration [^{14}C]Pyridate

Study No.	Cumulative Excretion as Percent of Dose[a]			
	Urine	Feces	Cage Wash	Total
7) 200 mg/kg, Single Dose				
24 hours postdosing	68.22	25.74	3.88	97.84
96 hours postdosing	66.81	24.76	5.49	97.07
8) 600 mg/kg, Single Dose				
24 hours postdosing	48.87	18.94	5.04	72.85
96 hours postdosing	52.22	34.40	3.64	90.24

[a]Mean values from three males.

TABLE 6. Radioactive Residue Levels in Selected Tissues and Plasma of Male Rats at Various Intervals Following Oral Administration of [^{14}C]Pyridate

Tissue	[^{14}C] Residues (μg/g or mL) Postdosing (hours)[a]							
	200 mg/kg				600 mg/kg			
	1	6	24	96	1	6	24	96
Liver	53	43	2.5	0.3	63	111	65.8	1.3
Kidney	63	123	6.4	0.4	105	120	132.2	1.5
Fat	15	13	0.5	0.1	14	21	15.6	0.3
Brain	8	5	0.1	0.0	13	12	7.9	0.1
G.I. Tract	709	670	38.9	0.6	2515	3213	923.6	1.0
Plasma	130	71	2.6	0.0	114	143	119.9	0.1

[a]Mean values from three males.

glucuronide and accounted for about 23 to 50 percent of the applied radioactivity. CL9673 accounted for about 12 to 19 percent and an unknown accounted for 26 to 37 percent of the applied radioactivity (Tables 7 and 8). Unchanged parent compound was not detected. The unknown metabolite was further analyzed by mass spectroscopy and it was suggested to be a hydroxyphenyl derivative of CL9673.

Feces: For the same studies (Nos. 1, 2, 3, 7, and 8) described above, no CL9673-O- or N-glucuronides were detected. Unchanged parent compound was detected at about 14 to 35 percent of the applied radioactivity (Table 9). No parent compound was detected at the low dose. However, it is not clear whether it is the single or repeated-dose study (No. 1 or 2) since the text does not agree with the data in the table. CL9673 accounted for about 19 to 59 percent of the radioactivity and hydroxylated CL9673 accounted for 18 to 57 percent (Table 9).

Bile: Samples of pooled bile indicated that radioactivity was associated with highly polar metabolites. These were not identified.

Plasma: Samples from study No. 4 indicated the absence of unchanged parent compound. Almost all the radioactivity was associated with CL9673. TLC profiles for plasma samples from study No. 6 were similar to those from study No. 4. However, unchanged parent compound was detected in the 1-hour sample.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Following separate single oral administration of [^{14}C]-pyridate to rats at target dose levels of 20 and 200 mg/kg, radiolabeled components were apparently well absorbed. Urinary excretion of total radioactivity accounted for 70 to 80 percent of the administered dose. After single oral administration at a target dose level of 600 mg/kg, urinary excretion of total radioactivity accounted for a smaller proportion (52 percent) of the administered dose and this may suggest lower absorption at this higher dose level. The relative proportions of metabolites quantitated by TLC, however, did not differ significantly in 0- to 24-hour urine collected from rats dosed at 20, 200 and 600 mg/kg.

Data from bile duct cannulated rats (target dose level of 20 mg/kg) were consistent with high oral absorption. Excretion in bile represented less than 10 percent of the administered radioactivity; this was significantly lower than the proportion excreted via the feces at a similar dose level in nonsurgically treated rats. This may indicate

Table 7

Source: CBI Table 18, CBI p. 74.

TABLE 18

Radio-t.l.c. of 0-24 h Pooled Rat Urine

Solvent System: Acetone:chloroform:methanol:water:acetic acid, 75:10:10:5:2 (v/v/v/v/v)

Results expressed as % total radioactivity on plate

Rf	ca 0.37	ca 0.80	ca 0.88
Co-chromatograph	CL 9673-O-Glucuronide	CL 9673	Pyridate/Unknown
Sample			
20 mg.kg^{-1} Single Dose (Phase 1B)	31	68	
200 mg.kg^{-1} Single Dose (Phase 1D)	44	55	
200 mg.kg^{-1} Single Dose (Amendment 2, Low)	43	32	21
600 mg.kg^{-1} Single Dose (Amendment 2, High)	50	30	18
20 mg.kg^{-1} Multiple Dose (Phase 1C)	23	74	

TABLE 19

Radio-t.l.c. of 0-24 h Pooled Rat Urine

Solvent System: Chloroform:methanol, 9:1 (v/v)

Results expressed as % total radioactivity on plate

Rf	ca 0.38	ca 0.75	ca 0.21
Co-chromatograph	CL 9673	Pyridate	Unknown
Sample			
20 mg.kg^{-1} Single Dose (Phase 1B)	12	ND	35
200 mg.kg^{-1} Single Dose (Phase 1D)	15	ND	31
200 mg.kg^{-1} Single Dose (Amendment 2, Low)	19	ND	26
600 mg.kg^{-1} Single Dose (Amendment 2, High)	17	ND	28
20 mg.kg^{-1} Multiple Dose (Phase 1C)	16	ND	37

ND = Not detected

table 8

Source: CBI Table 19, CBI p. 75.

TABLE 20

Radio-t.l.c. of 0-24 h Pooled Rat Faeces

Solvent System: Chloroform:methanol, 9:1 (v/v)

Results expressed as % total radioactivity on plate

Rf	ca 0.40	ca 0.75	ca 0.26
Co-chromatograph	CL 9673	Pyridate	Unknown
Sample			
20 mg.kg^{-1} Single Dose (Phase 1B)	31	27	32
200 mg.kg^{-1} Single Dose (Phase 1D)	33	35	18
200 mg.kg^{-1} Single Dose (Amendment 2, Low)	33	22	28
600 mg.kg^{-1} Single Dose (Amendment 2, High)	57	14	20
20 mg.kg^{-1} Multiple Dose (Phase 1C)	19	ND	57

ND = Not detected

table 9

Source: CBI Table 20, CBI p. 76.

indicate that some unabsorbed radiolabeled material is being excreted in feces of nonsurgically treated rats. The apparent presence of unchanged pyridate in feces of rats after administration at this dose level appears to support this view.

Concentration of total radioactivity in plasma indicate relatively rapid absorption following oral administration at a target dose level of 20 mg/kg. Maximum concentrations of radioactivity were observed at 1 to 2 hours postdose. By 24 hours postdose, levels were at or near background. Plasma radioactivity levels observed following single oral administration of 20 mg/kg appeared to indicate generally similar kinetics to those of the low-dose group; but following single oral administration at a target dose of 600 mg/kg, plasma levels of radioactivity at 24 hours were disproportionately high in comparison with those observed at the lower dose levels. This slow elimination of radioactivity is consistent with excretion data, which indicate that a significant proportion of the administered dose remains unexcreted at 24 hours postdosing.

Radioactivity appeared to be distributed rapidly; in animals treated at target dose levels of 20 and 200 mg/kg, maximum levels of radioactivity were observed in tissues at 1 and 6 hours postdose. Levels were generally highest in the organs of excretion. The levels of radioactivity detected in brain remained substantially lower than plasma concentration at all dose levels. By 96 hours postdose (dose levels of 20, 200 and 600 mg/kg), levels of radioactivity were at or near background in all tissues examined. Some radioactivity did, however, appear to persist in kidney. The levels of radioactivity at 168 hours did not appear to be influenced by multiple, daily oral administration. The elimination of radioactivity from tissues in the first 24 hours postdose was influenced dramatically by increasing the dose level to 600 mg/kg and levels of radioactivity were disproportionately higher in this high dose group. This finding is highly relevant to toxicology studies with pyridate at dose levels of 600 mg/kg/day. It appears that at such high dose levels an alteration in the kinetics of pyridate-related components occurs and this change appears to be responsible for the altered detoxication process.

Pyridate appears to be rapidly and extensively metabolized following oral administration to rats. In plasma following single oral administration at a target dose level of 20 mg/kg, typically no components with similar chromatographic character to pyridate were detected. The major radiolabeled component detected in plasma was chromatographically similar to CL9673, the hydrolysis product of pyridate.

Three radiolabeled components were present in all samples of urine analyzed; the principal component co-chromatographed with the O-glucuronide of CL9673. A significant proportion of urinary radioactivity appeared to correlate to CL9673. A third component did not co-chromatograph with any of the reference standards available, and analysis of this component by mass spectrometry indicated that it was a phenyl ring-hydroxylated derivative of CL9673.

Extracts of feces were also examined by TLC and significant quantities of CL9673, pyridate, and hydroxylated CL9673 were detected in all samples.

The metabolites detected in urine and feces are all highly polar in character and would be expected to be excreted rapidly. Rapid excretion has been clearly demonstrated; at dose levels of 20 mg/kg, >95 percent of administered radioactivity was recovered by 24 hour postdose and, in addition, no bioaccumulation of radioactivity has been observed.

B. A quality assurance statement was signed and dated December 30, 1988.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study was adequately conducted and the conclusions of the authors are supported by the data presented. Adequate number of rats were used in each of the studies. The selection of dose levels was also appropriate to detect saturation at the 600-mg/kg dose. Repeated dosing revealed little, if any, potential for bioaccumulation or induction of liver enzymes. Biliary excretion indicated that a certain proportion of the compound is excreted via the bile. However, the identity of the metabolites was not determined. The results of the time-course studies can also be used to determine the elimination kinetics of pyridate.

A deficiency was noted in the study and was associated with the TLC procedures used and the incomplete reporting of the TLC findings. In addition, enzyme hydrolysis could have been utilized, especially with the bile to determine the extent of conjugation.

These studies fulfill EPA guidelines, despite the above-mentioned deficiencies.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix 1, Protocol, CBI pp. 106 to 128.

APPENDIX A

Protocol