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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: Review of a Draft Final Report on Rat Metabolism Studies with Rotenone and Request for Comments. (Reg. No. 6704-Q) Tox. Chem. No. 725.

TO: William Miller
Product Manager (16)
Registration Division (TS-767C)

THRU: Jane Harris, Ph. D., Section Head *JAH 9/6/85*
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Roger Gardner, Toxicologist
Review Section 6 *Roger Gardner 9-6-85*
Toxicology Branch *Sept 11/85*
Hazard Evaluation Division (TS-769) *9/10/85*

Actions Requested

In a letter dated May 8, 1984, the Fish and Wildlife Service (FWS) requested that a draft final report on rat metabolism studies be reviewed including suggestions for improvement of the report.

Recommendations and Conclusions

1. Practical limitations such as the low concentrations of radioactivity in collected samples and small sample sizes as well as an apparent sex difference with respect to acute oral toxicity suggest that statistical methods might not be appropriate (See Section III and the Appendix below).
2. Rotenone is primarily excreted in the feces after oral and intravenous doses (95 to 97% of the radioactivity recovered during the 144 hours following dosing and approximately 75% recovered within 48 to 72 hours). The route of administration, dose level, and number of doses had no apparent effect on the excretion pattern (see Section II and the Appendix). Enterohepatic circulation may also occur during excretion.

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I. Background

Rotenone is a plant root extract (derris or cube roots) which is used as an insecticide or piscicide. Its chemical name is [2R-(2a, 6a, 12a)]-1, 2, 12, 12a-tetrahydro-8, 9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano-(3, 4-yl-furo(2, 3-dibenzopyran-6, (6aH)-one. The principal active ingredient is associated with derris or cube resins (depending upon the source of the rotenone extract) which are also classified as active ingredients. Formulations contain rotenone and associated resins in a ratio of 1:2 (see Environmental Protection Agency unpublished report dated May, 1980. Rotenone: PreRPAR Review. Office of Pesticides and Toxic Substances.). Rotenone can be separated from the resins to a purity of 99.5%.

II. Summary of Metabolism Results

Results of the submitted report (see Appendix for detailed review) generally characterized the excretion pattern associated with single or repeated (14 consecutive days) low doses (0.01 mg/kg) and a single high dose (5 mg/kg) oral doses as well as a single low intravenous dose (0.01 mg/kg). The major route of excretion (95 to 97% of the administered dose) is in the feces, and female rats excrete administered radioactivity at a slower rate than males. The route of administration, dose level, and number of doses had no apparent effect on the excretion pattern.

There is a sex difference with respect to the acute oral toxicity of rotenone in that female rats are more sensitive than males. The calculated oral LD₅₀ for females is approximately 40 mg/kg, while that for males is approximately 100 mg/kg. Based on these results, the purified rotenone (95%) used in the metabolism study should be classified into Toxicity Category I.

III. Discussion

Radiolabeled residues in plasma samples from rats given the low oral doses could not be detected, and radioactivity was unextractable from feces of those rats. In addition, results from tissue analyses from rats given the 5 mg/kg dose contained relatively low concentrations (<0.5 ppm) within 96 to 144 hours after dosing. These results are consistent with the rapid distribution and excretion indicated by the pharmacokinetics experiments. Based on these considerations tissue analyses for animals sacrificed at 12 and 24 hours following dosing is more appropriate for characterization of absorption and distribution after dosing.

Variability in tissue and plasma results is expected at such low residue concentrations as those observed in the rotenone experiments (<1 ppm). That variability and the generally accepted practice of using 3 to 6 animals in each experiment are limitations considered in the selection of statistical analyses for results from metabolism studies like the one described in the Appendix. Techniques such as pooling of data from both sexes before weighted nonlinear regression analysis (a technique used in the pharmacokinetics experiment) may not be appropriate in view of the sex differences suggested by results from the acute toxicity studies. In the entero-hepatic circulation experiment, use of the exact permutations test (see Appendix page 4) is more appropriate for larger sample sizes, especially when the data vary as much as the results reported in the rotenone study and the samples are considered to be random.

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APPENDIX

Data Evaluation Record for the following study

Eiseman, J. L., and A. K. Thakur. April 13, 1984. General metabolism study for rotenone using rats. Unpublished report prepared by Hazleton Laboratories, Inc. (Project No. 419-137). Submitted by the U. S. Department of the Interior, U. S. Fish and Wildlife Service, National Fishery Research Laboratory. EPA Acc. No. 253333.

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DATA EVALUATION RECORD

1. CHEMICAL: Rotenone
2. TEST MATERIAL: Two lots of radiolabeled Rotenone were used. The label was in the -6-alpha position, and the specific activities were 16.0 mCi/mM or 24.65 mg/mCi (lot no. 800507) and 13.1 mCi/mM or 30.12 mg/mCi (lot no. 801110). Lot no. 800507 was recrystallized in carbon tetrachloride to 94.64% and a specific activity of 32.8479 uCi/mg. The purity of unlabeled rotenone was reported to be 99.23% as determined by liquid chromatography.
3. STUDY/ACTION TYPE: Metabolism - rats
4. STUDY IDENTIFICATION: Eiseman, J. L., and A. K. Thakur. April 13, 1984. General metabolism study for rotenone using rats. Unpublished report prepared by Hazleton Laboratories, Inc. (Project No. 419-137). Submitted by the U. S. Department of the Interior, U. S. Fish and Wildlife Service, National Fishery Research Laboratory. EPA Acc. No. 253333.
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7. CONCLUSIONS: The acute oral LD₅₀ for male rats was reported to be 102 + 12.6 mg/kg, and that for females was reported to be 39.5 + 2.21 mg/kg. These results suggest that the test substance should be classified into Toxicity Category I.

The major route of excretion after single low oral or intravenous doses (0.01 mg/kg), repeated low doses (0.01 mg/kg), or a single high oral dose (5 mg/kg) is the feces (95 to 97% of the excreted radioactivity). The majority of the metabolites are unidentified polar compounds which were unaffected by glucuronidase or aryl sulfatase. Most of the administered radioactivity (approximately 80 to 90%) is recovered within 48 hours after dosing.

Core classification: Acute study---minimum
Metabolism study---acceptable

8. MATERIALS AND METHODS

Test species: Nine to seven week old male and female Sprague-Dawley rats were used for the experiments.

Analytical procedures: Urine, fecal, and cage debris samples were collected 8, 24, 48, 72, 96, 120, and 144 hours after dosage administration unless otherwise described below. Expired air samples were taken at 8, 24, and 48 hours after dosing. Blood samples were drawn from the tail vein of each animal 2, 5, 15, 30, and 45 minutes as well as 1, 2, 3, 6, 8, and 24 hours after intravenous (i. v.) administration and 5, 15, and 30 minutes as well as 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours after oral dosing with the labeled test substance in the excretion balance and pharmacokinetics experiments.

Urine samples were mixed directly with scintillation cocktail for counting, while fecal, tissue, and organ samples were homogenized and oxidized. Diethanolamine in ethanol was used to trap $^{14}\text{CO}_2$ in expired air from test rats. The $^{14}\text{CO}_2$ from combusted samples was trapped and counted in Oxiflour[®] CO_2 . All samples were counted by liquid scintillation. Tissues were also autoradiographed.

Samples of urine and feces were analyzed by thin layer chromatography (TLC) to determine the presence of metabolites. Urine was concentrated under a stream of nitrogen, and the concentrate was spotted onto TLC plates. The plates were developed in benzene:methanol (90:10, v/v) and autoradiographed. Radiolabeled areas on the chromatogram were quantified by liquid scintillation.

Fecal homogenates were adjusted to pH 12 with sodium hydroxide and extracted with methanol. The extracts were analyzed using TLC and autoradiography in a manner similar to that used for urine concentrates. Rotenone and oxidation products were used as standards in the TLC analyses.

Fecal homogenates were also incubated with aryl sulfatase (2 units) or beta-glucuronidase (10,000 units) for 16 hours at 37° C. These samples and an untreated homogenate were then analyzed as described above.

Experimental procedures---Acute oral toxicity: Groups of 10 male and 10 female rats were given a single oral dose of 10, 25, 50, 75, or 150 mg rotenone (unlabeled) per kg body weight. The test substance was administered by gavage in corn oil.

8. MATERIALS AND METHODS (continued)

All animals that died during the study or that were sacrificed at termination of the study were subjected to a gross necropsy.

The LD₅₀'s and their 95% confidence limits were calculated by a maximum likelihood method using the probit transformation according to the report.

Excretion balance and pharmacokinetics studies: According to the report there was a preliminary excretion balance study in which one rat of each sex per group was used, while in the main study 5 per sex per group were used. There were 6 rats of each sex used in each group for the pharmacokinetics studies.

Dosages administered were as follows:

A single intravenous (i. v.) dose of labeled rotenone at 0.01 mg/kg

A single oral dose of labeled rotenone at 0.01 mg/kg

Single daily doses of 0.01 mg unlabeled rotenone per kg for 14 consecutive days followed 24 hours after the last dose with a single 0.01 mg/kg dose of labeled test substance.

A single oral dose of 5 mg labeled test substance per kg body weight

All animals that died during the study or that were sacrificed at termination of the study were subjected to a gross necropsy. The tissues and organs which were collected after sacrifice of the animals included brain, gonads, liver, kidney, bone, heart, thymus, spleen, bone marrow, adrenals, lungs, fat, and skeletal muscle. These tissues and the residual carcass were kept at -70° C for analysis.

The results from the intravenous phase of the pharmacokinetics studies were analyzed by weighted nonlinear regression, and the data were fitted to an equation based on a two-compartment model for intravenous administration of the compound. A similar type of analysis was done on the data from the oral portion of the study, and the results were fitted to a two-compartment model with first order absorption of the test substance.

Enterohepatic circulation studies: Groups of 6 rats of each sex were given an oral dose of 5 mg/kg or an intravenous dose

8. MATERIALS AND METHODS (continued)

of 0.01 mg/kg radiolabeled test substance. Eighteen hours after the oral dose was administered the animals were anesthetized and a midline laparotomy was performed on each. Blood samples were taken from the hepatic portal vein where it enters the liver and from the hepatic vein distal to the liver. Blood samples were also taken by cardiac puncture from each animal. The livers were also removed for analysis. Similar procedures were applied to the second group of rats 6 hours after i. v. administration of the test substance.

The results from this study were analyzed by a two-sample exact permutations test for dependent measures because of heterogeneity according to the authors.

9. REPORTED RESULTS

Acute toxicity: The most frequently reported signs of toxicity noted by the investigators included tremors, prostration, labored breathing, and soft feces. Survivors appeared normal 4 to 7 days after dosing, and most deaths occurred within 2 days after dosing. The reported incidence of mortalities is summarized as follows:

Sex	Dose (mg/kg)				
	150	75	50	25	10
Males	7/10	3/10	2/10	2/10	0/10
Females	10/10	8/10	9/10	2/10	0/10
Total	17/20	11/20	11/20	4/10	0/20

The most frequent observations at gross necropsy were mottled appearance of the lungs and liver, dark red clotted material throughout the intestines, and distended atria in the heart. The authors noted that the observations in the lungs and intestines were treatment-related and were found in animals dying within 5 days after dosing.

The calculated LD₅₀ for males was reported to be 102 ± 12.6 mg/kg, and that for females was reported to be 39.5 ± 2.21 mg/kg.

Preliminary study (Excretion balance study): Table 1 summarizes results of the preliminary excretion balance study with respect to excretion, and Table 2 summarizes tissue levels. There were no detectable levels of radioactivity found in the expired air collected after dosing.

Table 1

Excretion of ¹⁴C-labeled rotenone in rats
(% of dose, preliminary study)

Time of Sample	Urine		Feces	
	Male	Female	Male	Female
<u>0.01 mg/kg intravenous dose</u>				
0-24	0.88	0.95	36.03	19.67
24-48	0.47	1.07	53.57	55.66
48-72	0.14	0.68	14.50	20.32
72-96	0.08	0.45	8.88	7.22
96-120	---**	---**	4.49	4.49
120-144	0.02	0.27	3.16	6.09
<u>0.01 mg/kg oral dose</u>				
0-8	0.94	0.79	----***	----***
8-24	0.88	0.28	31.58	0.89
24-48	0.31	0.54	44.52	22.16
48-72	0.11	0.33	8.29	51.14
72-96	0.05	0.16	4.17	6.89
<u>0.01 mg/kg oral dose****</u>				
0-8	0.36	0.99	----***	----***
8-24	0.97	0.93	63.76	59.20
24-48	0.20	0.34	17.80	41.74
48-72	0.12	0.11	4.06	9.51
72-96	0.10	0.11	3.28	4.89
96-120	0.09	0.12	7.73	5.50
<u>5 mg/kg oral dose</u>				
0-8	0.06	0.03	----***	----***
8-24	1.08	1.22	35.17	4.66
24-48	0.20	1.36	40.01	71.33
48-72	0.08	0.09	3.67	18.08
72-96	0.03	---**	0.99	1.11
96-120	0.02	---**	0.64	0.24
120-144	0.01		0.12	0.20

*Sample not collected.

**Amount not detectable.

***Collected from 0-24 hours.

****Radiolabeled dose followed 14 days administration of unlabeled test substance.

Table 2

Tissue levels (ng rotenone/g tissue)
at various hours after dosing of 0.01 mg/kg
for 14 days (preliminary study)

<u>Tissue</u>	<u>96</u>	<u>120</u>	<u>144</u>	<u>144*</u>
<u>Males</u>				
Brain	2.7103	2.0716	ND**	ND**
Gonads	3.0689	2.0199	7.2922	7.4768
Heart	11.3707	ND**	176.12	ND**
Kidney	2.8521	ND**	32.122	21.960
Liver	8.3487	5.1964	50.294	3.2276
Thymus	8.8701	ND**	ND**	ND**
Spleen	7.9016	ND**	ND**	ND**
Bone	10.289	1.1733	11.510	6.0665
Muscle	8.7047	ND**	41.966	ND**
Fat	40.064	11.549	ND**	ND**
Lungs	3.8568	ND**	ND**	ND**
Adrenals	28.670	10.773	9.5622	ND**
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	ND**	ND**	ND**	ND**
<u>Females</u>				
Brain	ND**	7.5862	ND**	ND**
Gonads	ND**	ND**	8.1448	ND**
Heart	10.179	9.6381	28.364	ND**
Kidney	ND**	1.5942	14.962	4.7627
Liver	6.1068	4.8050	17.097	ND**
Thymus	14.235	6.8505	ND**	ND**
Spleen	10.544	ND**	ND**	ND**
Bone	2.7902	2.8578	3.9674	5.7885
Muscle	6.5207	ND**	ND**	ND**
Fat	72.354	ND**	ND**	ND**
Lungs	6.0806	3.6081	ND**	9.5130
Adrenals	8.4346	7.2594	ND**	4.5008
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	ND**	ND**	ND**	ND**

*Intravenous dose of 0.01 mg/kg.
**Not detectable.

9. REPORTED RESULTS (continued)

Excretion balance study: Tables 3, 4, and 5 summarize the results of the main study with respect to total recovery of radiolabeled rotenone in the excreta, cage washings, and cage debris.

Table 3

Summary of excreted radioactivity
(mean percentage of the dose)

<u>Dose and route</u>	<u>Feces</u>	<u>Urine</u>	<u>Cage washings</u>	<u>Cage debris</u>
<u>Males</u>				
0.01 mg/kg (i. v.)	77.14 (+2.330)	2.92 (+0.359)	0.04 (+0.049)	2.53 (+0.626)
0.01 mg/kg (oral)	95.88 (+3.259)	2.28 (+0.455)	0.13 (+0.150)	ND*
0.01 mg/kg (oral)**	89.86 (+8.657)	3.29 (+1.403)	0.14 (+0.078)	4.02 (+1.739)
5 mg/kg (oral)	75.07 (+4.669)	2.98 (+1.001)	0.11 (+0.038)	4.07 (+2.859)
<u>Females</u>				
0.01 mg/kg (i. v.)	78.17 (+3.665)	2.94 (+0.661)	0.08 (+0.106)	7.71 (+3.736)
0.01 mg/kg (oral)	79.14 (+11.483)	4.12 (+0.834)	0.10 (+0.056)	ND*
0.01 mg/kg (oral)**	94.15 (+9.512)	2.65 (+0.592)	0.09 (+0.026)	16.66 (+3.082)
5 mg/kg (oral)	67.87 (+14.331)	3.13 (+1.190)	0.09 (+0.032)	10.30 (+8.180)

*Not detectable

**Radiolabeled dose administered after 14 consecutive daily doses of unlabeled test substance.

Table 4

Summary of mean tissue levels (ng/g tissue)
144 hours after dosing of rats given ¹⁴C-labeled
rotenone

Tissue	0.01 mg/kg			5 mg/kg
	i. v.	oral	oral*	oral
<u>Males</u>				
Brain	5.1082	1.3486	ND**	17.946
Gonads	1.4341	0.5713	ND**	11.482
Heart	2.0992	ND**	0.9520	75.972
Kidney	0.3358	1.3924	ND**	19.192
Liver	2.3677	ND**	ND**	31.209
Thymus	ND**	ND**	ND**	23.355
Spleen	ND**	ND**	ND**	26.046
Bone	0.7455	3.3354	ND**	16.935
Muscle	ND**	1.5085	ND**	22.314
Fat	ND**	0.824	ND**	115.01
Lungs	ND**	7.5284	ND**	18.980
Adrenals	3.306	9.2172	2.5702	41.783
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	5.038	6.2818	ND**	ND**
<u>Females</u>				
Brain	0.8017	2.0941	0.9528	14.595
Gonads	0.2656	2.6070	ND**	90.057
Heart	4.8264	0.3984	ND**	102.87
Kidney	6.0169	3.9599	ND**	43.812
Liver	ND**	0.7510	ND**	42.061
Thymus	ND**	3.3798	ND**	19.839
Spleen	ND**	2.8590	ND**	22.593
Bone	7.8243	ND**	3.6457	14.622
Muscle	1.1860	ND**	ND**	47.616
Fat	3.0358	ND**	ND**	42.862
Lungs	5.7169	ND**	ND**	35.624
Adrenals	7.1615	6.3667	ND**	61.078
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	ND**	1.6330	2.8958	11.203

*14-day administration of 0.01 mg/kg.
**Not detectable.

Table 5

Group mean per cent of dose represented by tissue levels

Tissue	0.01 mg/kg			5 mg/kg
	i. v.	oral	oral*	oral
<u>Males</u>				
Brain	0.36	0.11	ND**	0.00
Gonads	0.16	0.06	ND**	0.00
Heart	0.08	ND**	0.03	0.00
Kidney	0.04	0.14	ND**	0.00
Liver	0.35	ND**	ND**	0.03
Thymus	ND**	ND**	ND**	0.00
Spleen	ND**	ND**	ND**	0.00
Bone	0.00	0.02	ND**	0.00
Muscle	ND**	0.03	ND**	0.00
Fat	ND**	0.16	ND**	0.01
Lungs	ND**	0.55	ND**	0.00
Adrenals	0.01	0.01	0.00	0.00
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	0.19	0.22	ND**	ND**
<u>Females</u>				
Brain	0.08	0.23	0.06	.595
Gonads	0.00	0.01	ND**	.057
Heart	0.20	0.02	ND**	.87
Kidney	0.68	0.45	ND**	.812
Liver	ND**	0.41	ND**	.061
Thymus	ND**	0.13	ND**	.839
Spleen	ND**	0.07	ND**	.593
Bone	0.08	ND**	0.02	.622
Muscle	0.05	ND**	ND**	.616
Fat	0.07	ND**	ND**	.862
Lungs	0.39	ND**	ND**	.624
Adrenals	0.00	0.01	ND**	.078
Residual carcass	ND**	ND**	ND**	ND**
Bone marrow	ND**	0.09	0.11	.203

*14-day administration of 0.01 mg/kg.
 **Not detectable.

9. REPORTED RESULTS (continued)

Pharmacokinetics study: The authors noted that no detectable levels of radiolabeled residues were found in the plasma of rats given a single or multiple oral doses (0.01 mg/kg). They attributed this problem to the small volume (100 ul) of blood sampled.

Data for individual animals given a 0.01 mg/kg intravenous dose or a 5 mg/kg oral dose are reproduced in Appendix A below. The investigators used a two-compartment model to interpret the results. In the case of the oral dose, the model was based on the assumption of first order absorption. Data from males and females were pooled for the analysis.

The distribution/elimination half life (rapid phase) after the intravenous dose was estimated to be 1.1 hours, and the biological half life (slow phase) was calculated to be approximately 14 hours. According to the report, these values in animals given the oral dose were similar. The absorption half life (rapid phase) was calculated to be 2.4 hours, while the biological half life was found to be 18 hours.

Enterohepatic circulation study: The group mean ratios for hepatic/cardiac plasma concentrations (ng equivalents/g plasma) were calculated from the reported individual animal data. Table 6 summarizes the results of those calculations.

Table 6

Group mean hepatic/cardiac blood concentrations and liver concentrations (ng equivalents/g liver) of ¹⁴C-labeled rotenone in rats sacrificed 6 or 18 hours after dosing

	Intravenous*		Oral**	
	Males	Females	Males	Females
Hepatic/cardiac concentration	1.7497 (+0.9717)	1.7058 (+1.0025)	2.2218 (-0.9717)	1.5868 (+0.3753)
Liver concentration	131.77 (+80.831)	80.121 (+22.173)	926.65 (+170.29)	1014.2 (+508.92)

*Six hours after a 0.01 mg/kg dose

**Eighteen hours after a 5 mg/kg dose

The higher concentrations of radioactivity in hepatic portal plasma than that in cardiac plasma and the higher concentrations found in the liver than in other tissues indicated enterohepatic circulation.

9. REPORTED RESULTS (continued)

Identification of metabolites: Chromatography of the ^{14}C -rotenone standard indicated that the parent had an Rf value of approximately 0.93, and one band accounted for >90% of the radioactivity. Four other minor bands were observed also, and they accounted for <8% of the radioactivity according to the report. The band representing rotenone was designated "S9" by the authors.

HPLC of fecal extracts from rats treated with the low dose intravenously or orally did not have radioactivity, and a metabolite profile could not be determined.

Fecal extracts from rats given the 5 mg/kg dose orally contained up to 7 radioactive bands, but the authors noted that the same bands were not always present in all animals. For this reason, individual animal data are presented in Appendix B below.

The majority of the metabolites were characterized as polar and did not migrate. The band associated with those metabolites was designated "S0" and represented 40.82 to 72.99% and from 33.48 to 65.76% of the applied radioactivity for males and females, respectively. There was no radioactivity which could be associated with the parent compound (see Appendix B).

Urine samples from the animals treated with the 0.01 mg/kg doses did not contain enough radioactivity for chromatographic analysis. Results of urine sampled from male rats given the 5 mg/kg dose indicated that 69.67 to 93.37% of the radioactivity on the chromatographic plates was associated with the polar metabolites designated "S0" by the investigators. That fraction of the radioactivity in samples from female rats accounted for 43.51 to 94.88%.

Pretreatment of fecal extracts with glucuronidase and aryl sulfatase did not change the metabolite profile.

The report stated that cochromatography with known standards indicated that the band designated "S8" was associated with rotenolone, and the band designated "S1" was associated with dehydrorotenolone. The authors stated that efforts to separate or move the "S0" band with a more polar solvent system resulted in smearing of the band on the TLC plates. Therefore, most of the metabolites remained unidentified.

10. DISCUSSION

As noted on page 10 above, radiolabeled residues in plasma

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10. DISCUSSION (continued)

samples from rats given the low oral doses could not be detected. Radioactivity was also unextractable from feces of those rats (see page 11 above). In addition, results from tissue analyses (see Tables 2, 4, and 5) from rats given the 5 mg/kg dose contained relatively low residue concentrations (<0.5 ppm) within 96 to 144 hours after dosing. These results are consistent with the rapid distribution and excretion indicated by the results from pharmacokinetics experiments. Based on these considerations tissue analyses for animals sacrificed at 12 and 24 hours following dosing is more appropriate for characterization of absorption and distribution after dosing.

Variability in tissue and plasma results is expected at such low residue concentrations as those observed in the rotenone experiments (<1 ppm). That variability and the generally accepted practice of using 3 to 6 animals in each experiment are limitations considered in the selection of statistical analyses for results from metabolism studies like the one described here. Techniques such as pooling of data from both sexes before weighted nonlinear regression analysis (see page 3 above) may not be appropriate in view of the sex differences suggested by results from the acute toxicity studies. In the enterohepatic circulation experiment, use of the exact-permutations test (see page 4 above) is more appropriate for larger sample sizes, especially when the data vary as much as the results reported in the rotenone study.

Although limitations in experimental design exist, the results show that the feces is the main route of excretion of oral and intravenous doses (95 to 97% of the radioactivity recovered in excreta). The route of administration, dose level, and number of doses had no apparent effect on the excretion pattern.

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APPENDIX A

Reported individual animal plasma concentrations
for radiolabeled residues of rats given a single
i. v. dose (0.01 mg/kg) or a single oral dose (5 mg/kg)
of rotenone