

Malathion Registration Standard Submission of  
Toxicology Data on Behalf of the Malathion  
Reregistration Task Force  
#57875

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STUDY TITLE

Disposition and Metabolism of  $^{14}\text{C}$ -Labeled Malathion  
in Rats (Preliminary and Definitive Study)

DATA REQUIREMENT

85-1

AUTHOR

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STUDY DATE

December 20, 1989

PERFORMING LABORATORY

Midwest Research Institute  
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Kansas City, MO 64110

LABORATORY REPORT NUMBER

MRI Project No. 9354-B

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Cyanamid Company and A/S  
Cheminova

STATEMENT OF NO DATA CONFIDENTIALLY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

Company: American Cyanamid Company

Company Agent: Robert L. Linkfield Date: \_\_\_\_\_

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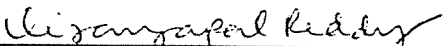
  
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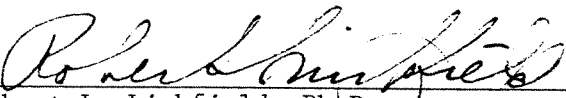
We submitted this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended and thereby consent to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the marking, "CONFIDENTIAL", this marking by itself conveys no supplemental claims of confidentiality under FIFRA sections 10(a) or 10(b). In submitting this material to the EPA according to the method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material (anywhere else in the world) that would have been claimed by the Company if this material had not been submitted to the EPA.

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## COMPLIANCE STATEMENT

These studies were conducted according to the Pesticide Assessment Guidelines (Federal Insecticide, Fungicide, and Rodenticide Act), Subdivision F, Section 85-1, and in accordance with EPA Good Laboratory Practice Standards of November 29, 1983 (FR 48 53946-53969).

  
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9345-B

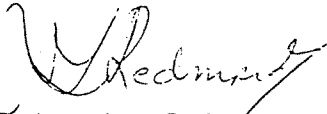
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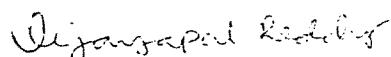
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The Final Report for "Disposition and Metabolism of  $^{14}\text{C}$ -Labeled Malathion in Rats" was prepared at Midwest Research Institute (MRI), 425 Volker Boulevard, Kansas City, Missouri 64110, under contract with the Malathion Task Force, MRI Project No. 9354-B. Meena B. Sonawane of Jellinek, Schwartz, Connolly and Freshman Inc. was the project monitor for the studies.

These studies were initiated on March 27, 1989, in the Life Sciences Department and completed by August 29, 1989. To the best of our knowledge, the studies were performed under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Good Laboratory Practice Standards.

  
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Date: Dec 20, '89

  
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## QUALITY ASSURANCE STATEMENT

Disposition and Metabolism of  $^{14}\text{C}$ -Labeled Malathion in Rats


MRI Project No. 9354-B

This study was inspected by the Quality Assurance Unit of Midwest Research Institute and reports were submitted to management and study director as follows:

<u>Phase</u>	<u>Date</u>
Protocol review	March 26, 1989
Body weights	May 2, 1989*
Dosing	May 11, 1989
General facility inspections	February 13, 1989*
	May 11, 1989*
	September 26, 1989*
Feces, urine collection	May 24, 1989*
Necropsy, blood sampling	May 26, 1989*
General facility and procedure inspection with Animal Care Committee	June 7, 1989*
Data audit	October 16-19, 1989*
Draft Report review	October 18-19, 1989*

\*Report to management.

The work reported herein was conducted in compliance with the Good Laboratory Practice Standards of the EPA as set forth in FR 48, 53946-53969, November 29, 1983. The report accurately presents the methods followed and the data generated during the study. Raw data and reports are stored in the MRI archives.

  
Eugene G. Podrebarac, Ph.D.  
Manager, Quality Assurance Unit

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## STUDY IDENTIFICATION

Test Material: Malathion

Sponsor: Malathion Reregistration Task Force  
P.O. Box 400  
Princeton, New Jersey 08540

Study Monitor: Ms. Meena Sonawane; Jellinek, Schwartz, Connolly and  
Freshman Inc.

Study Director: Vijayapal Reddy; Midwest Research Institute

Inlife Timetable: Initiation Date, Preliminary Study: 3/30/89  
Letter Report: 4/21/89  
Initiation Date, Definitive Study: 5/8/89  
Draft Report: 10/7/89  
Final Report: 12/20/89

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## SECTION 1

## SUMMARY

The disposition and metabolism of  $^{14}\text{C}$ -malathion was assessed in male and female Sprague-Dawley rats following single low (40 mg/kg body weight), high (800 mg/kg body weight), and multiple (40 mg/kg body weight x 15 days) oral doses. Malathion was rapidly absorbed, biotransformed, and excreted predominantly in urine and to some extent in the feces. Very low concentrations of radioactivity were present in tissues at 72 h. The metabolites excreted in urine and feces were primarily the mono and dicarboxylic acids of malathion.

The preliminary studies indicated that following treatment with low and high doses  $^{14}\text{C}$ -malathion, almost all of the administered radioactivity was eliminated in urine and feces within 72 h. Less than 1% of the initial dose was excreted in expired air. The definitive studies with male and female rats treated at low, high, and multiple dose levels of  $^{14}\text{C}$ -malathion demonstrated that urinary elimination of  $^{14}\text{C}$  was rapid and extensive in rats of both sexes. Approximately 80% to 86% of the doses were eliminated in urine within 24 h, with the exception of the high dose males, which excreted only ~ 62% of the total dose at this time. Elimination in feces was limited, ~ 4% to 10%, following low, high, and multiple doses. Cumulative excretion through both urine and feces (0 to 72 h) accounted for greater than 90% of the total initial dose in both sexes. Less than 1% of the administered dose was recovered in tissues and blood.

Four significant metabolites were observed in urine and feces during the high performance liquid chromatography (HPLC) analyses, and a total of 10 metabolites were identified by gas chromatography/mass spectroscopy (GC/MS). The major metabolites were identified as the  $\alpha$ - and  $\beta$ -monocarboxylic acids and the dicarboxylic acid of malathion. The other minor metabolites were identified as malaoxon, desmethyl malathion, O,O-dimethyl phosphorodithioic acid, O,O-dimethyl phosphorothioic acid, 2-mercaptosuccinic acid, fumaric acid, and monoethyl fumarate.

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## SECTION 2

## INTRODUCTION

The objective of this project was to conduct the metabolism studies required by the EPA's FIFRA Guidelines (85-1, 1982) for the reregistration of malathion. The project was designed using Sprague-Dawley rats exposed orally to  $^{14}\text{C}$ -malathion to (1) determine the amount and rate of absorption of malathion at different dose levels; (2) determine the pattern of distribution of malathion among tissue and fluid compartments at different dose levels; (3) identify and quantify the metabolites present at concentrations > 5% in samples of excreta; and (4) characterize the routes and rates of excretion.



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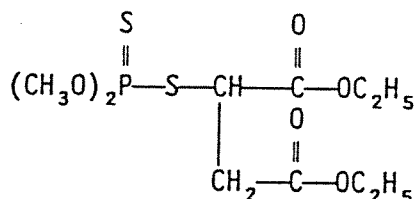
## SECTION 3

## MATERIALS AND METHODS

## 3.1 TEST SUBSTANCE

3.1.1 Identification of Test Substance (labeled and nonlabeled malathion)

- Malathion, CAS 121-75-5
- O,O-Dimethyl-S-(1,2-dicarbethoxyethyl)phosphorodithioate
- Molecular structure:



- Molecular weight:  
330.36
- Molecular formula:  
 $\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$
- Composition:  
C, 36.35%; H, 5.80%; O, 29.06%; P, 9.38%; S, 19.41%

3.1.2 Physical Characteristic

Deep brown to yellow liquid

- Nonlabeled malathion

Product No. W 60930-6038

Purity 94.6%

Stored at refrigerator temperature

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- $^{14}\text{C}$ -Labeled malathion

Lot No. C4

Purity 98%

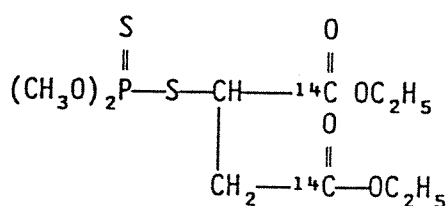
Specific activity 90.0  $\mu\text{Ci/mg}$ 

Stored at refrigerator temperature

3.1.3 Characterization and Purity

Nonlabeled malathion was supplied by American Cyanamid (Product No. W60930-6038). The chemical was characterized using the reverse phase HPLC system described below and gas chromatography/mass spectrometry.

$^{14}\text{C}$ -Malathion was prepared by Amersham Corporation (Arlington Heights, Illinois). The radiochemical purity was specified to be greater than 98% as determined by normal and reverse phase thin-layer chromatography (TLC). The radiochemical purity was reconfirmed by the reverse phase HPLC method at MRI (see below, Section 3.3.9.2).



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interessant med  
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## 3.2 TEST ANIMALS

Adult male and female Sprague-Dawley (CrI:CD BR) rats, 7 to 10 weeks old, weighing approximately 188 to 315 g (males) and 150 to 223 g (females) were used for the study. The species and strain of rats chosen is commonly used and preferred for this type of study. The animals were purchased from Charles River Breeding Laboratories Inc. Upon arrival, the animals were uniquely identified by numbered metal ear tags and acclimated for at least 7 days under test conditions. Prior to treatment, the attendant veterinarian examined and released the animals for the studies.

General procedures for animal care and housing were in accordance with DHEW Publication No. (NIH) 85-23, 1985, *Guide for the Care and Use of Laboratory Animals*, and the MRI *Manual for Animal Care*. Cages, racks, bedding, and feed containers were changed in accordance with MRI standard operating procedures.

The rats were housed in environmentally controlled rooms with 10 to 15 air changes per hour and a 12-h light/dark cycle per day. The rooms were maintained at a temperature of  $73^\circ \pm 2^\circ\text{F}$  and humidity of  $52\% \pm 7\%$ . The animals were housed in stainless steel suspension cages (two to three per cage) over Ab-Sorb-Dri® hardwood chip bedding (Ab-Sorb-Dri Company, Garfield, New Jersey) and were given Purina Certified Rodent Chow (Ralston Purina Company, Richmond, Indiana). Feed and tap water were provided *ad libitum*, except for ~ 16 h prior to and 4 h following a radioactive dose. There were

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no known contaminants present in the food or water that would have interfered with results of the study.

Prior to testing, the animals were randomized to each experimental group, using a computer-based weight stratification procedure. Within-group body weights were within two standard deviations of the mean; between-groups body weights were not significantly different ( $p < 0.05$ ). One day prior to testing, the animals were housed in individual metabolism cages.

### 3.3 METHODS

#### 3.3.1 Dose Preparation

The radioactive doses were prepared by addition of an appropriate quantity of  $^{14}\text{C}$ -malathion dissolved in toluene into a vial and evaporating the toluene to dryness under a gentle stream of nitrogen. Appropriate amounts of nonlabeled malathion and corn oil (vehicle) were added to the dried residue (labeled malathion) for final concentrations of 10 or 200 mg/mL for the 40 and 800 mg/kg dose group, respectively. The contents were thoroughly mixed for approximately 5 min using a Vortex mixer. For nonradioactive doses used for the multiple dose group, nonlabeled malathion was dissolved in corn oil and mixed to obtain a final concentration of 10 mg/mL. Doses were prepared fresh daily and administered at the 40 mg/kg level.

#### 3.3.2 Dosage and Treatment

$^{14}\text{C}$ -Labeled malathion was administered orally by gavage to rats at concentrations of 40 and 800 mg/kg body weight in a volume of 4 mL corn oil/kg body weight. The dosages were selected by the sponsor based on available toxicity information, including effects on blood cholinesterase. Actual doses administered to each rat were determined gravimetrically. Aliquots of the dosage solutions were counted before and after dosing to determine the amounts of radioactivity. The average final dose administered is given below.

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<u>Study</u>	<u>Sex</u>	<u>No. of Animals</u>	<u>Nominal Dose (mg/kg)</u>	<u>Actual Dose (mg/kg)</u> <sup>a,b</sup>	<u>Radioactivity Administered (<math>\mu</math>Ci/kg)<sup>a,d</sup></u>	<u>Specific Activity (dpm/<math>\mu</math>g)<sup>c</sup></u>
Preliminary	M	1	40	36.9	235.5	13,070
	F	1	40	37.4		
	M	1	800	796.4	259.5	720
	F	1	800	765.9	260.0	722
Definitive Single Dose	M	5	40	36.4	216.4	12,010
	F	5	40	37.1		
	M	5	800	755.3	235.6	654
	F	5	800	751.8		
Multiple Dose	M	5	40	35.1	207.3	11,504
	F	5	40	36.3		

- <sup>a</sup> Mean of five animals. See Appendix II, Tables II-1 to II-4, for individual animal values.
- <sup>b</sup>  $\frac{\mu\text{g administered}}{\text{body weight (g)}} = \text{dose (mg/kg)}$
- <sup>c</sup> Average of pre- and postdose samplings.
- <sup>d</sup>  $\mu\text{Ci/kg}$  numbers calculated by measuring three aliquots prior to and three aliquots postdosing.

### 3.3.3 Experimental Design

The study was conducted in two parts: a preliminary study followed by the definitive study. In the preliminary study, the rats were housed in glass metabolism cages for assessment of radioactivity in expired air, urine, and feces. The data from the preliminary study suggested that less than 1% of the dose was eliminated in expired air; therefore, the definitive studies were conducted in stainless steel metabolism cages for collection of urine and feces.

#### 3.3.3.1 Preliminary Study--

Two groups, each containing one male and one female rat, were treated orally with a single dose (40 or 800 mg/kg body weight) of  $^{14}\text{C}$ -malathion. Expired  $^{14}\text{C}$ -malathion was collected in 50% methanol, and  $^{14}\text{CO}_2$  was trapped with ethanalamine (5 M) in 2-methoxyethanol. Urine, feces, and expired air were collected and measured for radioactive content at 4, 8, 12, 24, 48, and 72 h after dosing. At 72 h, the animals were anesthetized with ether and exsanguinated by withdrawal of blood from the abdominal aorta.

### 3.3.3.2 Definitive Study--

Three groups, each containing five male and female rats, were used in these studies. One group was treated with a single oral dose of  $^{14}\text{C}$ -malathion at 40 mg/kg body weight; the second group was treated with a single oral dose of  $^{14}\text{C}$ -malathion at 800 mg/kg body weight. The third group was treated with a series of 15 single daily oral doses (40 mg/kg body weight) of the nonlabeled malathion, followed by a 16th dose with the radiolabeled malathion. The intravenous group was omitted since the test compound is insoluble in water or physiological saline at the doses selected. Following the administration of the radiolabeled dose (low, high, and multiple dose groups), urine and feces were collected and measured for radioactivity content at 4, 8, 12, 24, 48, and 72 h. At 72 h, all animals were killed as described previously for tissue sampling.

### 3.3.4 Sample Collection

During the preliminary study, urine, feces, and expired air containers were kept on dry ice. For the definitive studies, only the containers for the collection of urine were kept on dry ice. After each collection, the cages were rinsed with water, and these cage washings and urine were separately measured and analyzed. After exsanguination, the following tissues and organs were removed, rinsed with saline (0.9% NaCl), blotted with absorbent paper, weighed, and prepared for radiochemical analysis. The tissues examined were the following:

Liver	Spleen	Muscle (thigh)
Kidneys	Adrenals	Fat (retroperitoneal)
Lungs	Testes	Bone (femur)
Brain	Uterus	Skin
Heart	Ovaries	GI tract plus contents
		Residual carcass

Blood was kept on ice and tissues were kept in cold saline during the necropsy procedure. Portions of blood were centrifuged to separate plasma and red blood cells (RBCs). Prior to sampling, RBCs were washed three times with saline. Sample preparation and analyses were performed immediately after collection; or, after weighing, the samples were frozen (up to 4 weeks) until analyzed. All samples were measured gravimetrically; weights were recorded via an on-line data acquisition system.

### 3.3.5 Sample Preparation

Aliquots (250 to 500 mg) of whole blood, plasma, and red blood cells were analyzed for radioactivity. Total weights of urine, urine rinse, and expired air were obtained, and aliquots of samples were counted. Feces were weighed and homogenized in nine volumes of 10% v/v ethanol in water. Organs and the residual carcass, were weighed and homogenized in four volumes of 10% ethanol in water; the uterus was homogenized in nine volumes of 10% ethanol in water because of its small size. Aliquots (~ 500 mg) of all of the homogenates were measured and analyzed for radioactivity. Samples of fat, skin, bone, adrenals and ovaries were analyzed directly without homogenization. Duplicate samples were measured whenever possible, except for the adrenals and ovaries, which

were assayed as single samples, and the residual carcass homogenate, which was sampled in quadruplicate. Blood components, tissue, and fecal samples were combusted in a Packard Tricarb Sample Oxidizer (Model C306 or D306). Permafluor V in combination with Carbo-sorb (United Packard Technologies, Downers Grove, Illinois) was used as the scintillation fluid for combusted samples. Urine, cage washings, and expired air samples were counted directly in Phase Combining Scintillate (PCS, Amersham Corporation, Arlington Heights, Illinois).

### 3.3.6 Radioactivity Measurements

Vials were cooled for a minimum of 24 h before counting in a liquid scintillation counter (Packard Tricarb Model 2000CA). All samples were counted for a single 5-min period or until the counts per minute (cpm) reached 100,000. During both preliminary and definitive studies, some urine and cage washing samples contained high amounts of radioactivity resulting in a sample count time of < 0.5 min. Therefore, these samples were recounted with an upper threshold of 4 million cpm. Correction for background was carried out automatically by the scintillation counter. Background determinations were made from the average of natural counts of blank samples processed under the same conditions used throughout the experimental period. The counting efficiency was determined using the automatic external standard (AES) method. An AES versus efficiency curve was prepared by processing a commercial set of quenched standards through the counter under conditions used for these studies. The curve was stored in the counter and used for the automatic conversion of data from counts per minute (cpm) to disintegrations per minute (dpm). Sample values not within  $\pm 10\%$  of the mean were reassayed in duplicate except when the sample was not available or when radioactivity counts were low, i.e., less than two times the background. Background counts ranged from 37.7 to 57.2 cpm, depending on sample type.

### 3.3.7 Data Processing and Analyses

Weights and scintillation counting data acquired during the study were sequenced and merged into a data base. Individual calculations for each sample were performed as follows:

1. Counts per minute (cpm) for each sample were converted to dpm.

$$\frac{\text{cpm}}{\text{efficiency}} = \text{dpm/sample}$$

2. Disintegrations per minute (dpm) per gram was divided by the specific activity of the compound (dpm/ $\mu\text{g}$ ) to obtain the  $\mu\text{g}$  equivalents/g.

$$\frac{\text{dpm/g}}{\text{specific activity}} = \mu\text{g eq/g}$$

3. This ( $\mu\text{g eq/g}$ ) was multiplied by the total weight of the organ or excreta to obtain the total number of  $\mu\text{g}$  equivalents in the organ or excreta.

$$\mu\text{g eq/g} \times \text{total weight} = \mu\text{g eq/organ or excreta}$$

4. The  $\mu\text{g eq/organ or excreta}$  was divided by the total dose administered in order to obtain the percentage of the administered dose.

$$\frac{\mu\text{g eq/organ or excreta} \times 100}{\text{dose (in } \mu\text{g})} = \% \text{ of administered dose}$$

The percent of administered dose in blood, muscle, fat, skin and bone was calculated based on 7%, 40%, 11%, 16%, and 8%, respectively, of body weight. Percent recovery in plasma and red blood cells was calculated based on 60% and 40%, respectively, of total blood volume. These estimates were based on data from the published literature.<sup>1-3</sup>

Carbon-14 contents in blood and tissue are presented in terms of microgram equivalents per gram and percent of administered dose. The radioactivity recovered in urine and urine rinse was combined. Urinary and fecal elimination of radioactivity is presented in terms of microgram equivalents per total excreted and percent of dose eliminated during each sampling interval. The percent of dose recovered in whole blood, muscle, fat, skin, and bone was used to estimate total recovery of administered radioactivity. Radioactivity in the plasma and red blood cells was determined separately, but only values for the whole blood were used in recovery estimates.

In addition, radioactivity in carcasses was calculated but was not used in recovery estimates. This was done because the residual carcass contains portions of tissue (i.e., blood, bone, muscle, fat, skin) whose total weights can be closely estimated from percentage of body weights.

### 3.3.8 Biotransformation Studies

Standards of the monocarboxylic acid monoethyl ester (MCA) derivative (Lot No. CL28.966), the dicarboxylic acid (DCA) derivative of malathion (Lot No. CL28.397-2), and malaoxon (Lot No. CL28.966) were received from American Cyanamid. The structures of MCA and DCA were verified from gas chromatography/mass spectrometry analysis of the diazomethane and diazoethane derivatives. The structure of malaoxon was confirmed from the mass spectrum obtained from the GC/mass spectrometer.

### 3.3.9 Determination of Metabolic Profiles

#### 3.3.9.1 Sample Preparation--

3.3.9.1.1 Urine samples--Pooled (0 to 24 h postdose) urine samples were combined with an equal volume of acetonitrile, manually shaken for approximately 30 s, and then filtered through a 0.45- $\mu\text{m}$  Acrodisc-CR (Gelman,

Ann Arbor, Michigan) syringe-tip filtration system. Aliquots (100  $\mu$ L) of the filtrates were immediately assayed using the HPLC system.

3.3.9.1.2 Feces samples--Individual and pooled feces sample homogenates were weighed (300 to 700 mg) into glass vials containing 500  $\mu$ L of acetonitrile. The samples were manually shaken for approximately 30 s and then filtered through a 0.45- $\mu$ m Acrodisc-CR syringe-tip filtration system. Aliquots (100  $\mu$ L) of the filtrates were immediately assayed using the HPLC system.

### 3.3.9.2 HPLC System--

HPLC instrumentation for metabolic profile and purity analyses included a Perkin-Elmer Series 2 liquid chromatograph fitted with a Spherisorb 5- $\mu$ m ODS column (150 mm x 4.6 mm) and a RP-18 guard column (15 mm x 3.2 mm, Brownlee). Water and acetonitrile (ACN) containing trifluoroacetic acid (TFA, 0.1% for purity analyses or 1% for metabolic profile analyses) were used as the eluting solvents. The mobile phase gradient for metabolic profile analyses was programmed from water/ACN/TFA (90:10:1) to ACN/TFA (100:1) at a rate of 3%/min. Introduction of the analyte onto the column was performed manually by using a fixed-loop Rheodyne 7125 valve. Injection volumes were 20  $\mu$ L for purity analyses and 100  $\mu$ L for metabolic profile analyses. The chromatographic eluant was monitored for ultraviolet light absorbing species and carbon-14 content. The ultraviolet detection system was composed of a Perkin-Elmer Model LC-55B variable wavelength detector (detection wavelength of 230 nm) connected to the HPLC column and a Spectra-Physics Model 2470 integrator. The radiochemical detection system was composed of an on-line liquid scintillation flow detector (Radiomatic Flo-One Model HP) connected to the effluent port of the ultraviolet detector. The chromatographic eluate was combined with PCS scintillation cocktail and passed through a 500- $\mu$ L cell for detection of radioactivity. The detector's digital output in counts per minute (cpm) was transferred to a Radio Shack TRS-80 Model I computer (Tandy Corporation) using a LNW RS-232C universal asynchronous receiver/transmitter and processed using basic computer programs.

### 3.3.9.3 Recovery--

The recovery of radioactivity from the HPLC column was determined using urine (animal No. BL059, 12 h postdose) and feces (animal No. BL066, 4 h postdose) samples from the preliminary study. These samples were prepared and chromatographed using the methods described above and were found to be qualitatively identical to samples from the Definitive Study. The eluate-scintillation cocktail mixture obtained after injection of each sample (20  $\mu$ L for urine and 100  $\mu$ L of feces preparations) was collected for 60 min. This mixture was diluted to a known volume (250 mL) with scintillation cocktail and counted for the amount of radioactivity present. To determine the radioactivity actually injected onto the column, the injection device loop was filled with the same solution used in the analyses above and flushed directly into a volumetric flask (250 mL), diluted with scintillation cocktail, and counted for radioactivity. The recovery (%) of radioactivity through the entire system compared to that obtained directly from the injection loop was 99.7% for urine samples and 91.6% for feces samples. No loss of radioactivity arising from filtration was evident.



### 3.3.10 Metabolite Isolation and Identification

#### 3.3.10.1 Sample Preparation--

A pooled urine sample from Animal No. BL321 (high dose) was prepared by combining urine samples collected at 4, 8, 12, and 24 h postdose. An aliquot (2.0 mL) was acidified with 4 drops of concentrated hydrochloric acid and extracted with four 2.0-mL portions of diethyl ether. The ether extracts were combined, evaporated to dryness, and reconstituted in 1 mL of ether. An aliquot (200  $\mu$ L) of the ether was reacted with diazomethane dissolved in diethyl ether (200  $\mu$ L) for approximately 30 min; it was then evaporated to dryness and reconstituted in acetonitrile (250  $\mu$ L). A second aliquot of the ether-reconstituted extract (200  $\mu$ L) was reacted with diazoethane dissolved in diethyl ether (200  $\mu$ L), evaporated to dryness, and reconstituted in acetonitrile (250  $\mu$ L). Diazomethane and diazoethane derivatization of urine obtained from a control animal (BL290 male) was also performed using these same procedures.

The recovery of radioactivity from the urine of animal No. BL321 was 93% as determined from aliquots of the urine counted before and after the ether extraction. Similar studies for animal No. 6 (40 mg/kg, 0 to 24 h pooled) indicated a recovery of 83%.

A modified ether extraction procedure was utilized to isolate a radioactive peak eluting near the HPLC void volume (retention time ~ 3 min). Using this modification, the radioactive peak was retained in the aqueous (urine) phase and all other radioactive peaks were contained in the ether phase. The urine sample (2.0 mL, Animal No. 16, 0 to 24 h pooled) was acidified with 1% aqueous trifluoroacetic acid (1 mL) and extracted with diethyl ether (2 x 3.5 mL). The aqueous phase was lyophilized and the residue reconstituted in anhydrous ethanol (0.5 mL). Diazoethane (2.5 mL), prepared in diethylether, was added and the solution was allowed to react for approximately 30 min. The solution was evaporated to dryness and reconstituted in acetonitrile (300  $\mu$ L) for GC/MS analysis. The ether extracts of the urine sample were also combined, derivatized with diazoethane, evaporated, and reconstituted in acetonitrile (300  $\mu$ L) as described previously. Lyophilized urine and ether extracts from an undosed control animal (BL 290) were similarly prepared for analysis.

#### 3.3.10.2 Gas Chromatography/Mass Spectrometry--

GC/MS was performed on a Finnigan 4000 quadrupole mass spectrometer which was interfaced to a Hewlett-Packard Model 5890A gas chromatograph and an Incos 2400 Data System. Metabolite separations were obtained using a 30-m DB-5 capillary column (J&W Scientific, Folsom, California) and an on-column splitless (45 sec) injection system. The injector temperature was 270°C. The column temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 10°C/min. Reconstructed ion current chromatograms (RIC) mass spectra were obtained for the diazoethane and diazomethane ether extracts and the lyophilized urine sample preparations. MCA and DCA, prepared as methylated and ethylated derivatives, were used as reference standards. Additionally, malaoxon and malathion standards were also prepared and injected into the GC/MS.

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## SECTION 4

## RESULTS

## 4.1 IDENTIFICATION AND PURITY

Reverse phase HPLC results of nonlabeled malathion indicated a purity of 95.6%. The mass spectrum was consistent with structure of malathion (Figure 1). <sup>14</sup>C-Malathion as received from American Cyanamid had a stated radiochemical purity of 98%. Analysis of the solution by reverse phase HPLC method, developed in our laboratory, indicated a radiochemical purity of 97.9% (Figure 1).

## 4.2 PRELIMINARY STUDY

The data from disposition studies are shown in Tables II-5 through II-6. In the low and high dose groups, less than 1% (0.3% to 0.9%) of the total dose was eliminated in the expired air. In the low dose group, the male rat excreted 8.5% and the female rat excreted about 4% of the total dose in the feces. However, in the high dose group, both male and female rats excreted about 14% and 15%, respectively, of the total dose in the feces. Excretion by the urinary route appears to be predominant (~ 85% to 89% in the low dose group and 67% to 74% in the high dose group). The percentage of the dose excreted in the urine and feces together was 94% in the low dose (both the male and female) and 83% and 89% for the male and female, respectively, in the high dose group. In three out of the four animals (the exception was male No. 5), less than 1% of the total radioactivity remained in the carcass and other organs (Table II-13). Total radioactive recovery was greater than 90% in all animals except in the male rat from the high dose group, male No. 5, where it was 85%.

The preliminary data suggested that most of the radioactivity was eliminated by 72 h. Therefore, the definitive studies were conducted up to 72 h. Since less than 1% of the total dose was eliminated through the expired air, CO<sub>2</sub> was not collected in the definitive study and the definitive study was performed using the stainless steel metabolism cages.

## 4.3 DEFINITIVE STUDY

Urinary and fecal excretion of radioactivity for 72 h after a single oral dose of <sup>14</sup>C-malathion (40 mg or 800 mg/kg) or after multiple doses (40 mg/kg x 15) followed by single <sup>14</sup>C-malathion (40 mg/kg) administration are shown in Tables 1 and 2 for males and females, respectively. Individual animal data expressed as microgram equivalents and percent of dose administered are presented in Appendix II.

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With a single low dose (40 mg/kg) in males, urinary elimination of radioactivity averaged 83.8% of the administered dose. A similar percent (88.0%) of excretion was observed in female rats. The greatest proportion of the administered dose (77% and 81%; males and females) was excreted in urine within 12 h. The second largest amount of radioactivity was excreted between 12 and 24 h (3.9% and 4.4%; males and females). Only minimal amounts were excreted in urine between 24 and 72 h (less than 3%). Fecal elimination of  $^{14}\text{C}$ -malathion between 0 and 72 h was low in both males and females (10.9% and 5.9%). The cumulative excretion in urine and feces was 94.7% and 93.9% for male and females, respectively (Figures 2A and 2B).

In the high dose (800 mg/kg) males, urinary elimination of radioactivity averaged 76.1% of the dose of  $^{14}\text{C}$ -malathion. However, females excreted a slightly higher percent of radioactivity (85.2%) in urine than males did. Only 48.3% (males) and 67.1% (females) of the radioactivity were excreted within the first 12 h. By 24, 48, and 72 h, 62.5% and 81.1% (males and females); 73.6% and 84.0% (males and females); 76.1% and 85.2% (males and females), respectively, of the radioactivity were recovered. Fecal elimination of  $^{14}\text{C}$ -malathion between 0 and 72 h was 13.6% and 6.6% in males and females, respectively. The total dose excreted through urine and feces was 89.8% and 91.8% for males and females, respectively (Figures 2C and 2D).

Rats treated with a series of single daily oral doses (40 mg/kg) of the nonlabeled malathion followed by a 16th dose of radiolabeled malathion, excreted 84.5% (males) and 88.3% (females) of the dose in urine. The excretion profile was similar to the single low dose group. Excretion of  $^{14}\text{C}$ -malathion into urine was quite rapid and accounted for 75.7% and 79.3% (males and females) of radioactivity within the first 12 h, with only 6.5% and 7.2% (males and females) at 24 h. Only minimal amounts (~ 2%) were excreted in urine between 24 and 72 h. Fecal elimination between 0 and 72 h was 6.8% and 5.8% for males and females, respectively. Total dose eliminated through urine and feces was 91.3% and 94.1% for males and females, respectively (Figures 2E and 2F).

#### 4.4 BLOOD AND TISSUE RADIOACTIVITY LEVEL

The recovery of radioactivity in blood and tissue of rats treated with  $^{14}\text{C}$ -malathion expressed as microgram equivalents per gram and percent of the administered dose are presented in Table 3. Individual recoveries are shown in Appendix II, Tables II-14 through 19. In the low dose male and female rats, the majority of the dose was recovered in urine (~ 84% and 88%) and feces (~ 11% and 6%). Less than 1% of the administered dose was recovered in tissues. The carcass contained less than 0.5% of the administered dose. A similar disposition profile was observed in males and females of high and multiple dose groups. In both dosage groups, excretion of  $^{14}\text{C}$ -malathion by the urinary route appears predominant, and fecal excretion is the second major route. Very low amounts of radioactivity (less than 1%) was recovered in total tissues and carcass at 72 h (Table 4).

#### 4.5 BIOTRANSFORMATION STUDY

##### 4.5.1 Metabolic Profile Analysis

Four major peaks (greater than 5% of the total radioactivity) were detected in urine and feces samples using the HPLC system and sample preparation procedures developed (Figure 3). Two of these peaks were identified as MCA (both isomers; retention time ~ 25.5 min) and DCA (retention time ~ 19 min). This preliminary identification, followed by confirmatory identification by GC/MS described below, was based on the retention time coincidence to authentic standards. The peak designated as "A" in Figure 3 and Tables 5 and 6 (HPLC Metabolite Profiles, urine and feces, respectively) was subsequently found to contain multiple components; desmethyl malathion, 0,0-dimethyl phosphorothioic acid, and fumaric acid. The peak designated as "B" in the figures and tables was not directly correlated to other metabolites identified during GC/MS analyses. These metabolites included 2-mercaptosuccinic acid (as the disulfide), 0,0-dimethyl phosphorodithioic acid, and monoethyl fumarate. A minor metabolite associated with a radioactive peak eluting at approximately 22 min was identified as malaoxon. This peak was only observed in urine samples and because it was a minor metabolite (i.e., less than 2.1% of the total radioactivity), it was not reported in the Metabolite Profile Results.

The sample preparation for HPLC analyses involved mixing urine or fecal homogenates with a small volume of acetonitrile and then filtering the mixture. The filtrates were then used immediately for analysis. This method was found to recover 99.7% of the radioactivity from urine and 91.6% from feces samples.

##### 4.5.1.1 Urine Sample Profiles--

The data from the HPLC analyses of urine samples are contained in Table 5. Data in this table include the animal number, dose group, animal sex, total cpm, and percentage of the total radioactivity of each of the four peaks observed by HPLC. Urine samples were collected for 48 h postdosing. Pooled urine samples were prepared by combining urine samples collected from 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h on a weight-per-weight basis. Selected individual time points were also assayed. Several samples were prepared and analyzed in replicate. The mean and standard deviation of these replicate analyses are also contained in the table.

A representative radiochromatogram from the metabolic profile analysis of animal 6 (40 mg/kg x 1, 0 to 24 h pooled) is contained in Figure 3. Additional radiochromatograms for animal 21 (800 mg/kg x 1, 0 to 24 h pooled), and animal 31 (40 mg/kg x 15, 0 to 24 h pooled) are contained in Appendix I, Figures AI-2 and AI-3.

The dicarboxylic acid (DCA) and monocarboxylic acid (MCA) derivatives of malathion were identified on the basis of their HPLC retention time coincidence to authentic standards before and after derivatization with diazomethane and diazoethane. Diazoethane treatment of the extract from Animal No. 21 (0-24 h, pooled) converted major metabolites to malathion (Figures 4 and 5).

Confirmatory gas chromatography/mass spectrometric analysis results are described in a subsequent section of this report.

A fifth radioactive peak, eluting between DCA and MCA at approximately 21 min, was also observed in the urine samples. This peak either represented less than 2.1% of the total chromatographed radioactivity or was not significantly different than the radioactive background observed during the analyses and therefore was not individually isolated. Retention-time data for a malaoxon standard (~ 22 min) and the identification of malaoxon as a minor metabolite during GC/MS analysis indicated that malaoxon may have contributed to the HPLC peak area. However, because the RIC peak and mass spectrum were weak relating to other identified metabolites, it was concluded that the malaoxon metabolite contributed to, but did not represent 100% of this peak.

#### 4.5.1.2 Fecal Sample Profiles--

The data from the HPLC analyses of fecal homogenates are contained in Table 6. Data in this table include the animal number, dose group, animal sex, and percentage of the total radioactivity of each of the four peaks observed by HPLC. Feces samples were collected for 48-h postdosing. Pooled feces homogenates for animals 6 and 11 were prepared by combining samples collected from 8 to 12 and 12 to 24 h on a weight-per-weight basis. No feces samples were available before 8-h postdose for the selected animals. Also, only limited sample mass was generally available during the 8- to 12-h collection; therefore, all individual feces were assayed. Several samples were reanalyzed after preparation. The mean and standard deviations of these analyses are also contained in the table.

The metabolic profiles observed for the fecal homogenates were comparable to those of urine samples. The only significant difference between the two sample types was that the total radioactivity injected into the HPLC for feces homogenates was much less than that of urine samples. Based on the background counts during analyses (HPLC background approximately 30 to 50 counts/min) and the counting error ( $\pm 10$  counts/min), individual peaks with integrated areas less than 60 counts/min were not considered quantitative and therefore are designated as "n.d., not detected".

#### 4.5.2 Metabolite Identification

Four major radioactive peaks were observed during metabolite profile analyses by HPLC. Two of these peaks were identified as MCA and DCA, based on the retention time coincidence to authentic standards. Confirmatory identification of these metabolites was performed by GC/MS. It was also observed that when diethyl ether extracts of acidified urine samples containing these metabolites were reacted with diazoethane, a major peak eluting with a retention time coincident to a  $^{14}\text{C}$ -malathion standard was observed. A representative radiochromatogram of the product obtained from reaction of a diethyl ether extract of pooled urine (0 to 24 h) from animal 21 (high dose) with diazoethane is contained in Figure 4. A radiochromatogram from analysis of  $^{14}\text{C}$ -malathion, obtained under identical conditions, is illustrated in Figure 5.

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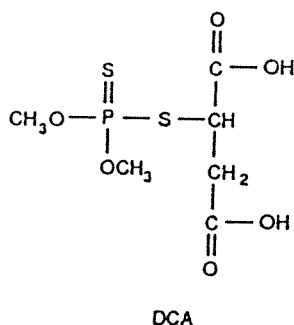
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Isolation of peak "A" in the HPLC metabolite profiles (Figure 3) and subsequent GC/MS analysis indicated this peak contained three metabolic products of malathion; O,O-dimethyl phosphorothioic acid, desmethyl malathion structures, and a fumaric acid moiety. The metabolites observed in the lyophilized urine preparation were also observed as weak spectra in the mass spectral RICs of the acidified ether extracts.

Metabolites associated with peak "B" (Figure 3) were not directly determined. This peak represented less than 4% of the total radioactivity in pooled (0 to 24 h) urine samples and was not present above background in feces samples. The contribution of this peak to the total radioactivity increased in the 24 to 48 h urine samples, however, this relative increase may be the result of an equilibrium phenomena from sample preparation and analysis (i.e., anhydride formation from DCA). Also, 2-mercaptosuccinic acid (as the disulfide), as discussed later, may elute in this region. Other metabolites identified during GC/MS analyses included O,O-dimethyl phosphorodithioic acid, and monoethyl fumarate.

The extraction and derivatization procedures (using 4 volumes ether/volume urine) were found to recovery 93% of the radioactivity from Animal No. 21 (0 to 24 h, pooled). The recovery of radioactivity from a similarly prepared sample from Animal No. 6 was 83%. The radioactivity remaining in these urine samples was observed to be proportional to the amount of peak "A" (Table 5). HPLC radiochromatograms indicated peak "A" was significantly diminished or absent in reconstituted ether extracts and retained in the aqueous portion remaining after extraction.

#### 4.5.2.1 Identification of DCA--



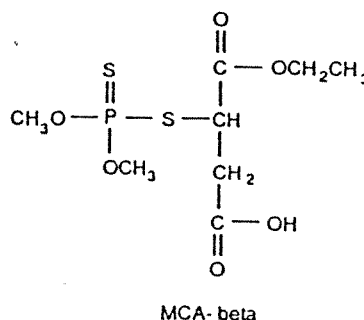
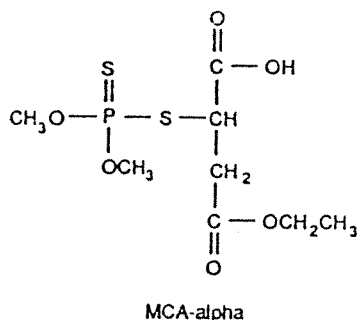
DCA, after derivatization with diazomethane, was identified as a major metabolite of malathion. This identity was confirmed by GC/MS comparison of retention time and mass spectral coincidence with a diazomethane-derivatized DCA standard. The retention time of the dimethyl derivative of the DCA standard was 23.6 min. The mass spectrum for a peak in the reconstructed ion current chromatogram (illustrated in Figure AI-4 for the ether extract at this retention time (23.7 min) was identical to that of the dimethyl DCA standard. Mass spectra for the standard and the metabolite are contained in Figure AI-5. The HPLC radiochromatogram of the diazomethane-treated ether extract is illustrated in Figure AI-6.

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Reaction of the ether extract with diazoethane resulted in a peak in the reconstructed ion current chromatogram with a retention time and mass spectral coincidence to a malathion standard. This result provided further confirmatory identification.

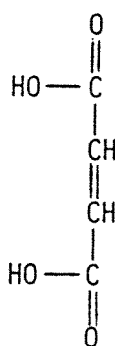
#### 4.5.2.2 Identification of MCA--



Two isomers of MCA were identified as major metabolites of malathion after derivatization to the ethyl methylcarboxy ester. This identification was confirmed by comparison of the GC/MS retention time and mass spectral coincidence with a diazomethane-derivatized MCA standard. The retention times of the monoethyl derivatives of the MCA standard (MCA- $\alpha$  and MCA- $\beta$ ) were 24.2 and 24.3 min. The isomeric configuration of the two isomers could not be determined from the mass spectral data. The information supplied by American Cyanamid indicated that the MCA standard contained 85% of the  $\alpha$  and 15% of the  $\beta$  isomer. Structural assignments were based on this information and on the relative RIC peak intensities observed during GC/MS analyses. The mass spectra of these peaks in the reconstructed ion current chromatogram (Figure AI-4, upper trace) for the ether extract at these same approximate retention times (24.3 and 24.4 min) were identical to those of the diazomethane-treated MCA standard. Mass spectra for the isomers in the derivatized MCA standard and the two metabolites are contained in Appendix I, Figures AI-7 (MCA- $\alpha$ ) and AI-8 (MCA- $\beta$ ).

The HPLC radiochromatogram of the diazomethane-treated ether extract is illustrated in Figure AI-6. HPLC analysis of the diazoethane-treated ether extract (Figure AI-2) resulted in a major peak with a retention time coincident to a <sup>14</sup>C-malathion standard.

## 4.5.2.3 Identification of Fumaric Acid Moieties--



Fumaric acid

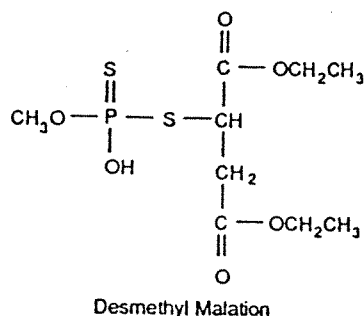
Fumaric acid was identified as a minor metabolite and a component of HPLC peak "A" after derivatization to the diethyl ester. The identification was based on GC/MS retention time and mass spectral coincidence with a diethyl fumarate standard. The retention time of the diethyl derivative of fumaric acid was 14.3 min. The mass spectrum of the peak in the reconstructed ion current chromatogram (Figure AI-9, upper trace) for the ether extract at this same retention time was identical to that of the authentic standard. Additionally, the spectra of the standard and metabolite were consistent with a reference spectrum. Mass spectra for the diethyl fumarate standard and the diethyl derivative of the metabolite are contained in Figure AI-10.

Peaks were also observed in the diazomethane-treated extracts (animal No. 21) with mass spectra consistent with the dimethyl and methyl ethyl ester of fumaric acid (retention times of 11.2 and 12.9 min, respectively, see Figure AI-15). This evidence indicates that the monoethyl ester of fumaric acid was also a minor metabolite of malathion. The spectrum of dimethyl fumarate was consistent with a literature reference.<sup>5</sup> The geometric configuration (*cis* and/or *trans*) of the fumaric acid metabolite could not be determined from the mass spectral data.

The HPLC retention time of the diethyl fumarate standard was approximately 24.1 min (using UV detection). Diethylfumarate and an additional peak thought to be monoethyl fumarate were observed with a retention time of 12.8 min after partial hydrolysis experiments with sodium hydroxide. No significant peaks (i.e., > 1% of the total radioactivity) were observed during the HPLC metabolite profile analysis at these retention times.



#### 4.5.2.4 Identification of Desmethyl Malathion--



Desmethyl malathion was tentatively identified as a minor metabolite and a component of HPLC peak "A" after derivatization to the ethylphospho ester. The mass spectrum of the peak eluting at 25.2 min in the reconstructed ion current chromatogram (Figure AI-11) was consistent with the structure of monoethyl desmethyl malathion; however, no authentic standard or reference spectrum was found for comparison. The mass spectrum for the metabolite is contained in Figure AI-12. The intensity and postulated identities of the major diagnostic ions in the spectrum are tabulated below:

<u>m/z</u>	<u>Intensity</u> (% of m/z 111)	<u>Postulated Identity</u>
344	Not observed	M <sup>+</sup> . (molecular ion)
298	2.0	M <sup>+</sup> . - CH <sub>3</sub> CH <sub>2</sub> OH (rearrangement ion)
270	3.9	M <sup>+</sup> . - COCH <sub>2</sub> CH <sub>3</sub> (alpha cleavage)
173	51.4	M <sup>+</sup> . - C <sub>3</sub> H <sub>8</sub> O <sub>2</sub> PS <sub>2</sub>
172	42.1	C <sub>3</sub> H <sub>9</sub> O <sub>2</sub> PS <sub>2</sub> <sup>+</sup> . (rearrangement ion)
143	25.7	m/z 173 - CH <sub>2</sub> O
139	30.1	CH <sub>3</sub> OP(S)OCH <sub>2</sub> CH <sub>3</sub> <sup>+</sup>
127	78.8	m/z 173 - CH <sub>3</sub> CH <sub>2</sub> OH
111	100	m/z 139 - C <sub>2</sub> H <sub>4</sub>
107	47.8	m/z 139 - CH <sub>3</sub> OH
99	40.5	m/z 127 - CO
79	79.3	m/z 139 - [S + C <sub>2</sub> H <sub>4</sub> ]

#### 4.5.2.5 Identification of Malaoxon--

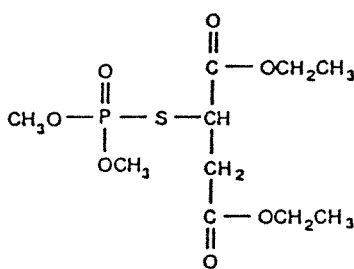
Malaoxon was identified as a minor metabolite by GC/MS analysis of the diazoethane-treated lyophilized urine sample and diethyl ether extracts. The mass spectrum of the peak eluting at 24.1 min in the RIC (Figure AI-13) was weak, but the major ions were consistent with the mass spectrum of a malaoxon

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standard, a reference spectrum,<sup>4</sup> and with the structure. The mass spectrum for the metabolite is contained in Figure AI-14.

The HPLC retention time of malaoxon was ~ 22 min (ex. Figure 3) using the system and parameters described in this report. A peak was observed during HPLC metabolite profile analysis eluting near this retention time (~ 21 min). However, because the intensity of the malaoxon spectra, obtained by GC/MS (Figure AI-4), was weak, it was concluded malaoxon was probably not the only metabolite contained under this HPLC peak.

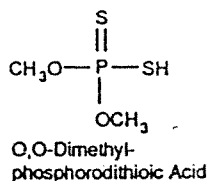


Malaoxon

Metabolite of  
Malathion - er  
IKKE market!

MS

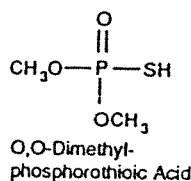
#### 4.5.2.6 Identification of O,O-Dimethyl Phosphorodithioic Acid--



O,O-Dimethyl phosphorodithioic acid was tentatively identified as a metabolite of malathion from GC/MS analysis of derivatized ether extracts. O,O-Dimethyl-S-ethyl phosphorodithioic acid had a retention time of 15.3 min and the O,O,S-trimethyl ester had a retention time of 14.2 min. The spectrum of the trimethyl ester was consistent with a literature reference.<sup>5</sup> The RIC obtained from analysis of the diazomethane-treated ether extract is illustrated in Figure AI-15. The mass spectrum of the metabolite (as the methylated derivative) is illustrated in Figure AI-16.

The HPLC retention time of O,O-dimethyl phosphorodithioic acid was not determined because a suitable, authentic standard could not be found.

#### 4.5.2.7 Identification of O,O-Dimethyl Phosphorothioic Acid--



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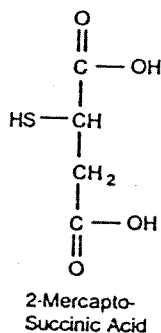
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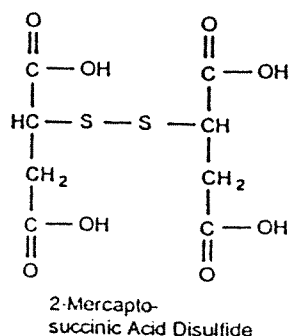
O,O-Dimethyl phosphorothioic acid was tentatively identified as a metabolite of malathion from GC/MS analysis of ether extracts. The O,O-dimethyl-S-ethyl ester was identified (after derivatization with diazoethane) and had a retention time of 14.0 min. The O,O,S-trimethyl ester was identified (after derivatization with diazomethane) and had a retention time of 12.8 min. The RIC obtained from analysis of the diazomethane-treated ether extract is illustrated in Figure AI-15. The mass spectrum of the metabolite (as the methylated derivative) is illustrated in Figure AI-17.

The HPLC retention time of O,O-dimethyl phosphorothioic acid was approximately 3 min using the system and parameters described in this report.

#### 4.5.2.8 2-Mercaptosuccinic Acid--



The retention time observed for a 2-mercaptosuccinic acid standard, which formed the diethyl ester S-ethyl sulfide on reaction with diazoethane, was 19.0 min. No corresponding peaks at this retention time were observed in the RIC for the diazoethane-treated ether extracts or lyophilized urine samples. However, a peak was observed in the diazoethane-treated ether extract with a retention time of 30.3 min and a mass spectrum consistent with that of the disulfide. The RIC from analysis of a diazoethane-treated ether extract is illustrated in Figure AI-18. The mass spectrum of the tentatively identified bis-(diethylsuccinate) disulfide is contained in Figure AI-19. The intensities and postulated identities of the major diagnostic ions in the spectrum are tabulated below.

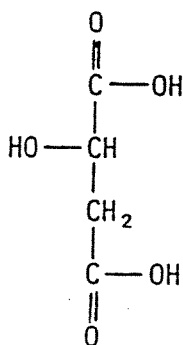


<u>m/z</u>	<u>Intensity</u> (% of m/z 55)	<u>Postulated Identity</u>
410	2.6	M <sup>+</sup> . (molecular ion)
364	1.4	M <sup>+</sup> . - CH <sub>3</sub> CH <sub>2</sub> OH (ethanol)
337	1.5	M <sup>+</sup> . - CH <sub>3</sub> CH <sub>2</sub> OCO
291	23.2	M <sup>+</sup> . - [CH <sub>3</sub> CH <sub>2</sub> OH + CH <sub>3</sub> CH <sub>2</sub> OCO.]
205	21.0	symmetrical disulfide cleavage
159	47.3	m/z 205 - ethanol*
131	64.5	m/z 205 - CH <sub>3</sub> CH <sub>2</sub> OCOH*
87	25.4	CH <sub>3</sub> CH <sub>2</sub> OCOCH <sub>2</sub> <sup>+</sup>
73	45.5	CH <sub>3</sub> CH <sub>2</sub> OCO <sup>+</sup> (carboxyethyl)
55	100	C <sub>3</sub> HO <sub>3</sub> <sup>+</sup>
45	38.5	CH <sub>3</sub> CH <sub>2</sub> O <sup>+</sup>

\* Rearrangement ion.

The HPLC retention times of 2-mercaptosuccinic acid disulfide and the ester derivatives could not be determined because no standards were readily available. However, these metabolites would retain the radioactive label and are predicted to have polarities compatible with peak "B" in the Metabolite Profile and therefore may contribute to the radioactivity detected at this retention time.

#### 4.5.3.9 Malic Acid (2-Hydroxybutanedioic Acid)--



Malic acid

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Malic acid, a potential metabolite of malathion, was not observed during GC/MS or HPLC analyses. The retention time of a diethyl malate standard (after derivatization with diazoethane) was 15.6 min. No corresponding peaks were observed in the ether extracts or the lyophilized urine preparations.

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## SECTION 5

## DISCUSSION AND CONCLUSION

The present study indicated that most of the radioactivity was eliminated by 72 h and that the main excretory route in both male and female rats was the urine. Fecal excretion was the second major excretory route of the compound. The expiration of  $^{14}\text{CO}_2$ , was a minor pathway of excretion; throughout the 72-h period, i.e., less than 1% of the initial dose was recovered in the expired air. Overall, in males and females at the end of 72-h period, urinary excretion of malathion (or metabolites) was similar in low, high, and multiple dose groups. However, in the low- and multiple-dose groups, most of the malathion was excreted in the urine between 4 to 8 h; and in the high-dose group, an equal percentage of the initial dose was eliminated at 4-h intervals up to 12 h. This may be because of saturation of the metabolism pathways and/or kidney excretory processes. Since most of the radioactivity was eliminated between 12 to 24 h, it appears that malathion is rapidly absorbed from the gastrointestinal tract, metabolized, and eliminated through urine and feces. The presence of radioactivity in the feces suggests possible excretion of malathion metabolites into the intestine via bile and/or unabsorbed malathion that was metabolized in the intestines. There is no evidence for accumulation of malathion (or metabolites) in the tissues. Very low concentrations of radioactivity are detected in tissues at 72 h after dosing. Concentration in the liver, though quite low, is higher than in other tissues; fat, skin, bone, and carcass have a somewhat higher amount of radioactivity at 72 h compared to the rest of the tissues.

Four major radioactive peaks were detected in urine and feces samples by HPLC analysis. A total of 10 metabolites were identified by GC/MS. It appears that malathion is rapidly biotransformed and excreted. The major metabolites found in urine were the  $\alpha$  and  $\beta$  monoacids, and the diacid of malathion. More than 80% of the recovered radioactivity consisted of these three metabolites. The remaining percent of radioactivity was distributed among seven other metabolites. The diacid was found to be the major metabolite in urine of both males and females in all dose groups. The  $\alpha$  and  $\beta$  monoacids together appear to be the second major metabolite(s). The metabolic profiles observed for the fecal homogenates were comparable to those of urine samples. The only significant difference of the two sample types was that the quantity (total radioactivity) of metabolites in fecal samples were lower than that of urine samples. The isolation of peak "A" in the HPLC metabolite profiles and subsequent GC/MS analysis indicated this peak contained three metabolic products of malathion, O,O-dimethyl phosphorothioic acid, desmethyl malathion, and a fumaric acid moiety. Malaaxon was identified as a minor metabolite which results from desulphuration of malathion. The other minor metabolites identified using GC/MS were 2-mercaptosuccinic acid, O,O-dimethyl

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phosphorodithioic acid, and monoethyl fumarate. The HPLC retention time of these metabolites could not be determined by using the present system.

The possible metabolic pathway of malathion in rats is shown in Figure 6. In liver, oxidative desulphuration of malathion by microsomal enzymes results in malaoxon which may be excreted in urine or can be further metabolized by phosphatases. Another pathway which involves the hydrolysis of carboxyester by tissue (possibly liver) or plasma carboxylesterases may result in MCA ( $\alpha$  and  $\beta$ ) and DCA. The latter appears to be the major pathway of malathion detoxification. Hydrolysis by phosphatases will result in O,O-dimethyl phosphorodithioic acid from malathion and O,O-dimethyl phosphorothioic acid from malaoxon. Metabolism of malathion may also occur through dealkylation, probably by glutathion *s*-transferases. Glutathione-dependent demethylation yields *s*-methylglutathione and the corresponding desmethyl phosphate compound.

In conclusion, malathion is rapidly absorbed and readily excreted in urine and feces. There is no evidence for accumulation of malathion or metabolites in the body. Malathion metabolites are mostly excreted in urine and, to some extent, in feces.

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## SECTION 6

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TABLE 1

RADIOACTIVITY IN EXCRETA OF MALE SPRAGUE-DAWLEY RATS FOLLOWING  
ORAL DOSES OF 14C-MALATHION (40 or 800 mg/kg) -a

Excretum	Time After Dosing (hr)	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
ug Equivalents Excreted							
Urine	4	857.314	543.249	24080	4774	3416.936	594.407
	8	3828.721	520.169	29938	4932	3310.466	523.659
	12	650.920	95.560	25006	5126	900.358	127.422
	24	273.358	35.147	23188	1435	662.972	73.044
	48	124.661	22.920	18143	11658	163.100	30.216
	72	45.446	8.553	4202	2216	62.120	14.246
Feces	4	-c		19 -d		-c	
	8	-c		19 -d		-c	
	12	193.874	37.818	3643	2194	25.925	25.297 -e
	24	456.759	61.246	13458	3300	647.489	63.304
	48	89.586	24.226	4042	819	100.721	23.058
	72	15.396	2.421	1226	664	28.834	12.447
Total	4	857.314	543.249	24088	4775	3416.936	594.407
	8	3828.721	520.169	29938	4932	3310.466	523.659
	12	844.795	92.493	28649	5429	915.913	137.416
	24	730.117	61.204	36646	2140	1310.461	89.307
	48	214.247	43.020	22185	12249	263.820	29.274
	72	60.841	10.628	5428	2870	90.954	25.089
Cumulative ug Equivalents Excreted							
Urine	4	857.314	543.249	24080	4774	3416.936	594.407
	8	4686.035	116.932	54018	8754	6727.402	297.104
	12	5336.955	72.692	79024	13335	7627.760	297.156
	24	5610.313	68.864	102212	12208	8290.732	356.758
	48	5734.974	58.320	120355	2871	8453.832	367.601
	72	5780.419	62.264	124557	3402	8515.952	368.497
Feces	4	-c		19 -d		-c	
	8	-c		19 -d		-c	
	12	193.874	37.818	3651	2201	25.925	25.297 -e
	24	650.633	43.770	17109	3572	663.043	76.977
	48	740.219	39.193	21152	2959	763.764	73.503
	72	755.615	39.276	22377	2307	792.598	70.099
Total	4	857.314	543.249	24088	4775	3416.936	594.407
	8	4686.035	116.932	25891	7483	6434.516	456.991
	12	5530.830	75.247	82675	14075	7350.429	466.043
	24	6260.946	66.259	119321	15708	8953.776	364.410
	48	6475.193	39.175	141507	4373	9217.596	365.844
	72	6536.034	47.130	146935	3012	9308.550	362.481

(continued)

(continued)

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TABLE 1 (Concluded)

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Excretum	Time After Dosing (hr)	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
		Percent of Dose					
Urine	4	12.409	7.921	14.720	2.924	33.782	5.636
	8	55.580	7.696	18.290	2.972	33.029	5.486
	12	9.435	1.394	15.330	3.189	8.976	1.327
	24	3.949	0.476	14.168	0.827	6.551	0.570
	48	1.804	0.328	11.093	7.137	1.609	0.284
	72	0.655	0.117	2.570	1.356	0.617	0.144
Feces	4	-c		0.005 -d		-c	
	8	-c		-c		-c	
	12	2.797	0.524	2.200	1.307	0.265	0.259
	24	6.632	0.906	8.257	2.049	5.345	1.249
	48	1.302	0.358	2.470	0.502	1.017	0.251
	72	0.222	0.034	0.750	0.406	0.289	0.128
Total	4	12.409	7.921	14.725	2.924	33.782	5.636
	8	55.592	7.704	18.290	2.972	33.029	5.486
	12	12.231	1.307	17.530	3.343	9.135	1.434
	24	10.582	0.874	22.426	1.359	11.896	0.919
	48	3.106	0.629	13.563	7.500	2.627	0.296
	72	0.877	0.146	3.320	1.757	0.906	0.257
Cumulative Percent of Dose							
Urine	4	12.409	7.921	14.720	2.924	33.782	5.636
	8	67.988	1.963	33.010	5.307	66.811	0.999
	12	77.422	1.392	48.340	8.184	75.787	0.497
	24	81.372	1.115	62.508	7.462	82.338	0.931
	48	83.175	0.885	73.601	1.653	83.947	0.803
	72	83.830	0.845	76.171	1.991	84.564	0.710
Feces	4	-c		0.005 -d		-c	
	8	-c		0.005 -d		-c	
	12	2.797	0.524	2.205	1.311	0.265	0.259
	24	9.429	0.614	10.462	2.185	5.504	1.365
	48	10.731	0.556	12.932	1.801	6.521	1.160
	72	10.954	0.553	13.682	1.401	6.811	1.168
Total	4	12.409	7.921	14.725	2.924	33.782	5.636
	8	68.000	1.973	32.658	5.184	66.811	0.999
	12	80.219	1.199	50.545	8.587	75.946	0.543
	24	90.801	0.927	72.971	9.604	87.842	1.017
	48	93.907	0.449	86.533	2.560	90.469	0.728
	72	94.784	0.347	89.853	1.665	91.375	0.639

a/ Mean and S.E. of five rats per group, except where indicated.

b/ Rats received 15 nonradiolabeled doses of malathion followed by a sixteenth dose of 14C-malathion.

c/ No feces during this time period.

d/ Mean of two rats.

e/ Mean and S.E. of three rats.

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TABLE 2

RADIOACTIVITY IN EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS FOLLOWING  
ORAL DOSES OF 14C-MALATHION (40 or 800 mg/kg) -a

Excretum	Time After Dosing (hr)	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
		ug Equivalents Excreted					
Urine	4	2765.826	624.077	33340	4829	3789.463	312.408
	8	1391.407	286.377	31677	5080	1731.185	480.893
	12	689.126	94.694	23588	3650	790.538	90.319
	24	260.117	30.925	18408	2592	578.806	76.297
	48	117.202	21.848	3778	1282	99.478	15.050
	72	32.808	5.710	1546	463	37.285	5.130
Feces	4	-c		-c		-c	
	8	-c		-c		-c	
	12	96.683	62.704	2574	1204	0.026 -d	
	24	193.309	52.420	4417	874	337.100	53.648
	48	56.738	12.439	1475	227	113.414	14.919
	72	8.096	1.817	248	106	12.305	1.379
Total	4	2765.826	624.077	33340	4829	3789.463	312.408
	8	1391.407	286.377	31677	5080	1731.185	480.893
	12	785.809	127.369	26162	4597	790.544	90.321
	24	453.426	63.339	22824	3358	915.906	79.711
	48	173.940	32.634	5254	1497	212.891	26.187
	72	40.904	6.603	1794	566	49.590	5.898
Cumulative ug Equivalents Excreted							
Urine	4	2765.826	624.077	33340	4829	3789.463	312.408
	8	4157.233	350.174	65017	5814	5520.648	246.100
	12	4846.359	295.547	88604	7896	6311.186	169.337
	24	5106.475	293.009	107012	6254	6889.992	116.988
	48	5223.677	284.917	110791	4988	6989.470	124.593
	72	5256.486	281.284	112336	4563	7026.755	125.363
Feces	4	-c		-c		-c	
	8	-c		-c		-c	
	12	96.683	62.704	2574	1204	0.026 -d	
	24	289.992	47.352	6991	520	337.105	53.652
	48	346.731	40.375	8466	605	450.519	47.096
	72	354.826	39.062	8714	631	462.824	46.938
Total	4	2765.826	624.077	33340	4829	3789.463	312.408
	8	4157.233	350.174	65017	5814	5520.648	246.100
	12	4943.042	306.804	91178	8246	6311.191	169.338
	24	5396.468	312.329	114003	6270	7227.098	161.323
	48	5570.408	301.677	119257	4789	7439.989	165.437
	72	5611.312	297.260	121051	4245	7489.579	166.684

(continued)

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TABLE 2 (Concluded)

Excretum	Time After Dosing (hr)	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
				Percent of Dose			
Urine	4	44.914	9.399	25.300	3.644	47.744	4.151
	8	24.339	6.241	24.075	3.826	21.648	5.932
	12	11.793	2.065	17.769	2.539	9.951	1.154
	24	4.402	0.554	14.043	2.128	7.298	1.005
	48	2.010	0.416	2.876	0.977	1.253	0.194
	72	0.567	0.125	1.175	0.353	0.470	0.066
Feces	4	-c		-c		-c	
	8	-c		-c		-c	
	12	1.630	1.028	1.926	0.885	0.000	-d
	24	3.214	0.880	3.374	0.699	4.223	0.644
	48	0.958	0.206	1.122	0.176	1.432	0.198
	72	0.138	0.032	0.190	0.081	0.155	0.018
Total	4	44.914	9.399	25.300	3.644	47.744	4.151
	8	24.339	6.241	24.075	3.826	21.648	5.932
	12	13.423	2.546	19.695	3.239	9.951	1.154
	24	7.616	1.030	17.417	2.751	11.521	1.021
	48	2.968	0.592	3.998	1.143	2.685	0.347
	72	0.706	0.145	1.365	0.432	0.625	0.077
				Cumulative Percent of Dose			
Urine	4	44.914	9.399	25.300	3.644	47.744	4.151
	8	69.253	3.405	49.376	4.529	69.392	2.858
	12	81.046	1.676	67.144	5.716	79.343	1.795
	24	85.448	1.395	81.187	4.546	86.640	1.218
	48	87.458	1.210	84.063	3.708	87.894	1.362
	72	88.025	1.141	85.238	3.393	88.364	1.390
Feces	4	-c		-c		-c	
	8	-c		-c		-c	
	12	1.630	1.028	1.926	0.885	0.000	-d
	24	4.844	0.747	5.301	0.370	4.223	0.644
	48	5.802	0.625	6.422	0.443	5.655	0.563
	72	5.940	0.603	6.612	0.469	5.810	0.561
Total	4	44.914	9.399	25.300	3.644	47.744	4.151
	8	69.253	3.405	49.376	4.529	69.392	2.858
	12	82.676	2.094	69.070	5.908	79.343	1.796
	24	90.292	1.635	86.488	4.632	90.863	1.619
	48	93.260	1.245	90.485	3.535	93.548	1.774
	72	93.966	1.130	91.851	3.138	94.173	1.809

a/ Mean and S.E. of five rats per group, except where indicated.

b/ Rats received 15 nonradiolabeled doses of malathion followed by a sixteenth dose of <sup>14</sup>C-malathion.

c/ No feces during this time period.

d/ One rat.

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TABLE 3

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF MALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING ORAL DOSES OF 14C-MALATHION -a

Tissue	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
	ug Equivalents/g					
Blood	0.219	0.016	3.602	0.526	0.212	0.006
Plasma	0.215	0.017	3.404	0.823	0.219	0.009
RBCs	0.144	0.005	3.012	0.193	0.138	0.005
Liver	0.822	0.070	16.968	2.009	1.282	0.087
Kidneys	0.315	0.005	6.355	0.940	0.453	0.015
Lungs	0.085	0.008	2.188	0.532	0.117	0.012
Brain	0.154	0.005	1.799	0.261	0.218	0.009
Heart	0.073	0.006	1.165	0.217	0.080	0.009
Spleen	0.156	0.009	3.650	0.700	0.236	0.009
Testes	0.193	0.009	2.383	0.455	0.215	0.011
Adrenals	0.350	0.044	5.904	0.966	0.220	0.056
Fat	0.363	0.072	5.754	0.892	0.238	0.013
Skin	0.231	0.011	6.296	1.750	0.273	0.045
Muscle	0.036	0.004	0.707	0.126	0.043	0.005
Bone	0.249	0.011	4.369	0.506	0.258	0.011
GI Tract	0.080	0.009	6.261	3.233	0.097	0.019
Carcass	0.148	0.010	6.041	1.589	0.193	0.020

(continued)

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TABLE 3 (Concluded)

Tissue/ Excretum	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
	Percent of Dose					
Blood -c	0.042	0.003	0.033	0.005	0.042	0.001
Plasma -c,d	0.025	0.002	0.019	0.005	0.026	0.001
RBCs -c,d	0.011	0.000	0.011	0.001	0.011	0.001
Liver	0.204	0.025	0.193	0.022	0.252	0.014
Kidneys	0.016	0.001	0.015	0.002	0.020	0.001
Lungs	0.002	0.000	0.002	0.000	0.002	0.000
Brain	0.007	0.001	0.003	0.001	0.006	0.000
Heart	0.001	0.000	0.001	0.000	0.001	0.000
Spleen	0.002	0.000	0.002	0.001	0.002	0.000
Testes	0.008	0.001	0.004	0.001	0.006	0.000
Adrenals	0.000	0.000	0.000	0.000	0.000	0.000
Fat -c	0.110	0.022	0.084	0.013	0.074	0.004
Skin -c	0.102	0.005	0.134	0.037	0.123	0.018
Muscle -c	0.039	0.004	0.037	0.006	0.048	0.005
Bone -c	0.055	0.002	0.046	0.005	0.059	0.002
GI Tract	0.053	0.006	0.158	0.066	0.044	0.009
Carcass -d	0.357	0.029	0.666	0.182	0.462	0.043
Urine	83.830	0.845	76.171	1.991	84.564	0.710
Feces	10.954	0.553	13.682	1.401	6.811	1.168
Recovery	95.425	0.331	90.565	1.661	92.054	0.637

a/ Mean and S.E. of five rats per group.

b/ Pretreated with 15 daily doses of nonlabeled malathion.

c/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

d/ Values not included in recovery estimates (see text).

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TABLE 4

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING ORAL DOSES OF 14C-MALATHION -a

Tissue	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16 -b	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
	ug Equivalents/g					
Blood	0.169	0.016	2.181	0.163	0.175	0.008
Plasma	0.154	0.018	1.956	0.205	0.174	0.016
RBCs	0.157	0.021	2.132	0.237	0.132	0.002
Liver	0.566	0.050	9.349	1.154	0.586	0.064
Kidneys	0.259	0.020	3.826	0.234	0.333	0.005
Lungs	0.052	0.002	0.694	0.211	0.076	0.010
Brain	0.098	0.008	1.001	0.101	0.116	0.010
Heart	0.052	0.016	0.754	0.107	0.057	0.006
Spleen	0.112	0.008	1.869	0.082	0.155	0.012
Ovaries	0.142	0.042	1.984	0.392	0.117	0.011
Uterus	0.108	0.014	1.416	0.271	0.046	0.007
Adrenals	0.211	0.055	3.017	0.315 -c	0.178	0.013
Fat	0.275	0.067	2.649	0.352	0.144	0.023
Skin	0.209	0.022	2.946	0.641	0.148	0.010
Muscle	0.028	0.004	0.382	0.061	0.021	0.005
Bone	0.176	0.015	2.541	0.120	0.165	0.009
GI Tract	0.094	0.014	1.885	0.593	0.051	0.005
Carcass	0.195	0.037	4.494	2.242	0.108	0.009

(continued)

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TABLE 4 (Concluded)

Tissue/ Excretum	40 mg/kg Dose X1		800 mg/kg Dose X1		40 mg/kg Dose X16-b	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
	Percent of Dose					
Blood -d	0.032	0.002	0.020	0.002	0.034	0.002
Plasma -d,e	0.015	0.001	0.011	0.001	0.020	0.002
RBCs -d,e	0.012	0.001	0.008	0.001	0.010	0.000
Liver	0.127	0.012	0.106	0.010	0.110	0.010
Kidneys	0.012	0.001	0.009	0.001	0.015	0.000
Lungs	0.002	0.000	0.002	0.000	0.002	0.000
Brain	0.005	0.000	0.002	0.000	0.005	0.000
Heart	0.001	0.000	0.000	0.000	0.001	0.000
Spleen	0.001	0.000	0.001	0.000	0.002	0.000
Ovaries	0.000	0.000	0.000	0.000	0.000	0.000
Uterus	0.001	0.000	0.000	0.000	0.001	0.000
Adrenals	0.000	0.000	0.000	0.000	0.000	0.000
Fat -d	0.080	0.018	0.039	0.005	0.044	0.007
Skin -d	0.091	0.011	0.062	0.014	0.065	0.004
Muscle -d	0.031	0.005	0.020	0.003	0.023	0.005
Bone -d	0.038	0.003	0.028	0.001	0.036	0.002
GI Tract	0.062	0.009	0.058	0.018	0.021	0.002
Carcass -e	0.457	0.094	0.501	0.257	0.244	0.023
Urine	88.025	1.141	85.238	3.393	88.364	1.390
Feces	5.905	0.625	6.612	0.469	5.810	0.561
Recovery	94.413	1.151	92.199	3.139	94.533	1.827

a/ Mean and S.E. of five rats per group.

b/ Pretreated with 15 daily doses of nonlabeled malathion.

c/ Mean and S.E. of four rats.

d/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively. 40% of blood volume, respectively.

e/ Values not included in recovery estimates (see text).



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TABLE 5 - MALATHION METABOLITE PROFILES FOR POOLED RAT URINE SAMPLES

Animal Number	Dose (mg/kg)	Sex	Sample	Total cpm	Concentration of Malathion Metabolites In Pooled Rat Urine (Percent of Total Radioactivity)			
					Peak A (~3 min)	Peak B (~18 min)	DCA (~19 min)	MCA (~25 min)
6	40 x 1	M	0-24 hr	63513	11.8	3.8	72.8	6.0
				42166	12.8	2.0	77.9	5.5
				Mean:	12.3	2.9	75.4	5.8
				Std. Dev.:	0.7	1.3	3.6	0.4
			24-48 hr	2209	18.2	81.8	nd	nd
				1552	18.0	20.9	59.0	nd
11	40 x 1	F	0-24 hr	53136	8.4	3.2	61.5	21.4
				52959	10.2	3.0	59.8	21.2
				Mean:	9.3	3.1	60.7	21.3
				Std. Dev.:	1.3	0.1	1.2	0.1
			24-48 hr	301	63.1	36.9	nd	nd
				280	75.7	2.1	22.1	nd
16	800 x 1	M	0-24 hr	158401	6.0	1.5	51.5	38.7
				3088	7.3	50.2	20.3	21.7
				424	22.6	52.8	24.5	0.0
				Mean:	15.0	51.5	22.4	10.9
				Std. Dev.:	7.6	0.8	1.8	10.8
			24-48 hr	1682	4.4	40.0	33.1	22.5
21	800 x 1	F	0-24 hr	99480	3.1	0.7	50.3	43.0
			24-48 hr	1682	4.4	40.0	33.1	22.5
26	40 x 15	M	0-24 hr	93552	10.3	3.3	68.8	10.8
			24-48 hr	2104	18.4	61.3	17.3	nd
31	40 x 15	F	0-24 hr	34016	8.1	3.1	67.5	19.5
			24-48 hr	716	14.5	54.7	30.7	nd

nd) not detected; estimated detection limit was 60 cpm

(a) Total cpm (background corrected) for radiochemical detection during HPLC analyses

(b) Retention Time

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TABLE 6 - MALATHION METABOLIC PROFILES FOR RAT FECES SAMPLES

Animal Number	Dose (mg/kg)	Sex	Time Interval	Total cpm (b)	Concentration of Malathion Metabolites in Rodent Feces Homogenates (Percent of Total Radioactivity) <sup>a</sup>			
					Peak A 3.0 min(c)	Peak B 18.0 min(c)	DCA 19.0 min(c)	MCA 25.5 min(c,d)
6	40 x 1	M	0-24 hr (Pooled)	392	22	nd	61	nd
			12-24 hr	78	86	nd	nd	nd
			24-48 hr	24	nd	nd	nd	nd
11	40 x 1	F	0-24 hr (Pooled)	15	nd	nd	nd	nd
			12-24 hr	52	nd	nd	nd	nd
			24-48 hr	0	nd	nd	nd	nd
16	800 x 1	M	8-12 hr	784	21	nd	nd	79
			12-24 hr	1032	17	nd	nd	82
			24-48 hr	0	nd	nd	nd	nd
21	800 x 1	F	8-12 hr	620	25	nd	26	49
			12-24 hr	0	nd	nd	nd	nd
			24-48 hr	0	nd	nd	nd	nd
			24-48 hr	21	nd	nd	nd	nd
26	40 x 15	M	8-12 hr	80	nd	nd	10	60
			8-12 hr	51	nd	nd	33	3
			12-24 hr	816	31	nd	62	6
			12-24 hr	344	17	nd	83	nd
			24-48 hr	0	nd	nd	nd	nd
			24-48 hr	0	nd	nd	nd	nd
31	40 x 15	F	12-24 hr	246	nd	nd	73	27
			12-24 hr	199	nd	nd	73	25
			24-48 hr	2	nd	nd	nd	nd

- a) Tabulated values should be considered semi-quantitative. Radiochromatograms from analysis contained significantly less radioactivity than could be precisely integrated.  
b) Total cpm (background corrected) for radiochemical detection during HPLC analyses  
c) Retention time  
d) Monocarboxylic Acid Isomers (alpha and beta) were not separated using this HPLC system.  
nd not detected, integrated radioactivity less than estimated detection limit (60 counts per minute)

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**<sup>14</sup>C-Malathion**  
Radiochemical Purity

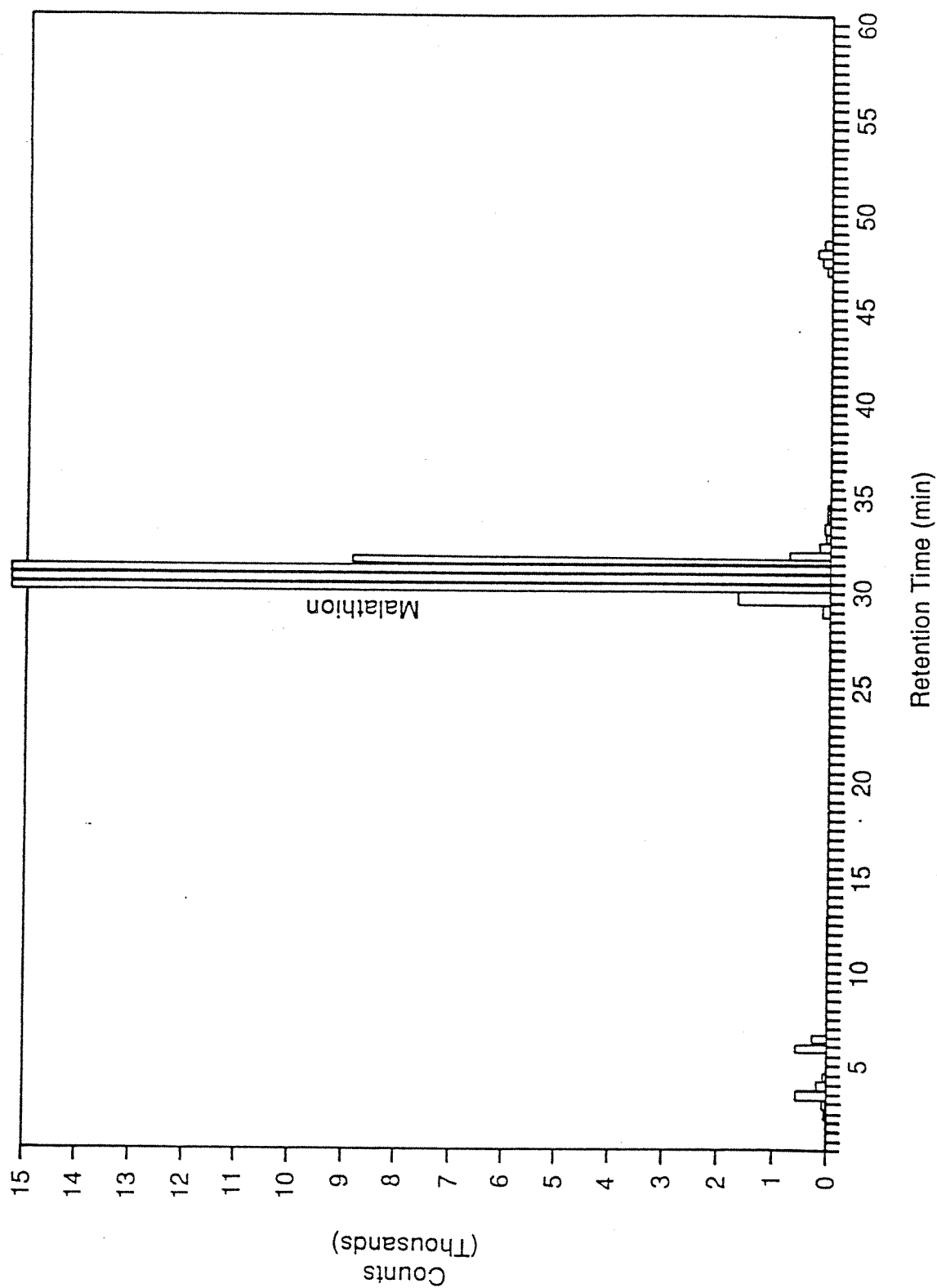


Figure 1. HPLC chromatogram for malathion radiochemical purity.

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Cumulative Percent of Dose

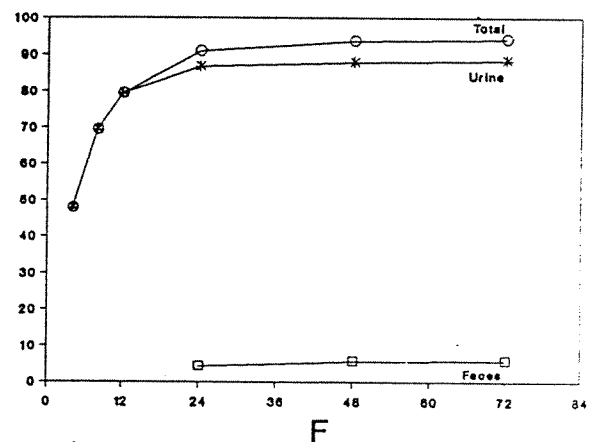
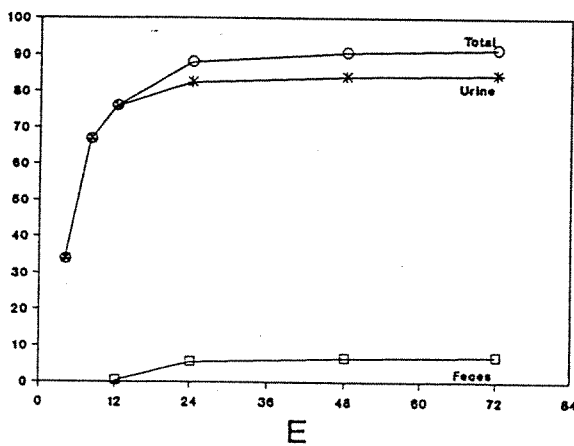
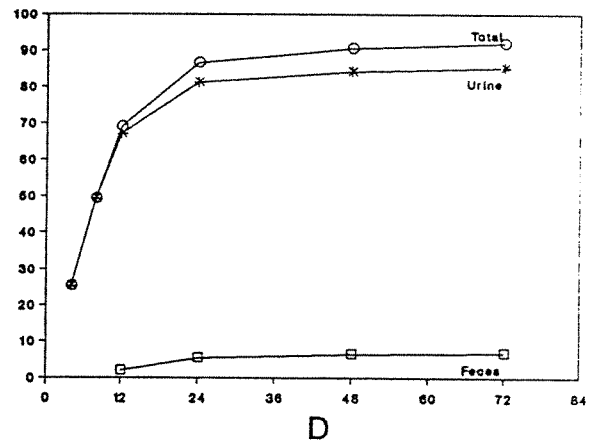
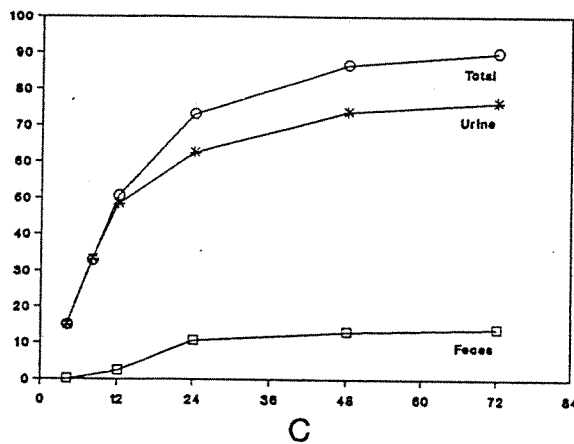
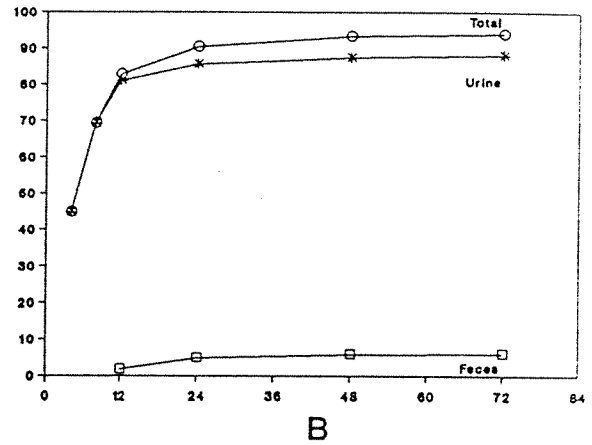
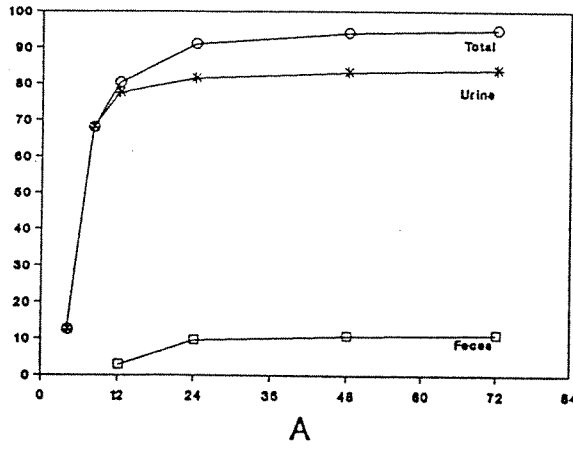


Figure 2. Recovery of  $^{14}\text{C}$ -malathion in excreta--A and B (males and females), 40 mg/kg; C and D (males and females), 800 mg/kg; E and F (males and females), 40 mg/kg x 16.

HPLC Radiochromatogram  
Urinary Metabolic Profile

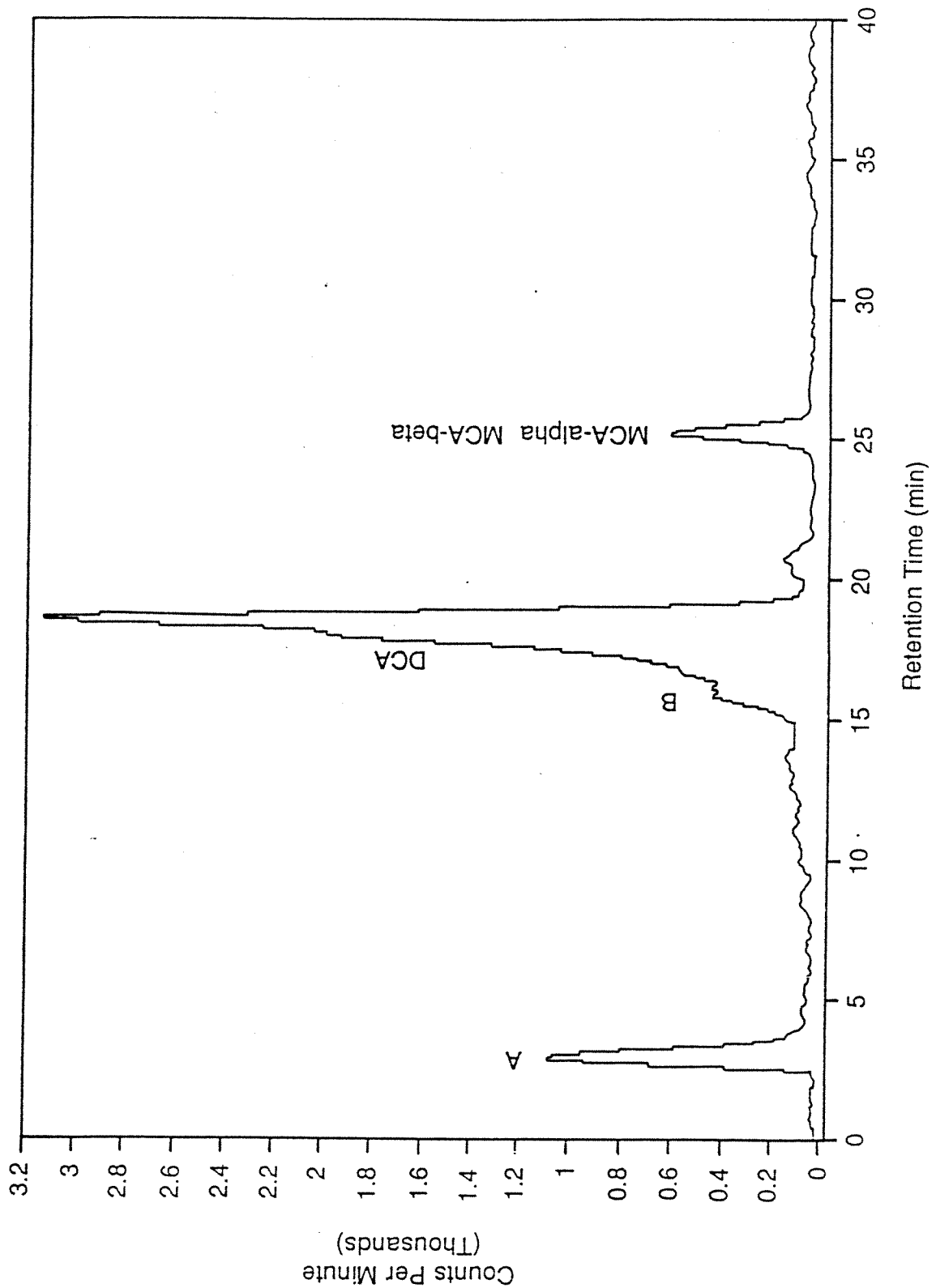


Figure 3. HPLC metabolite profile radiochromatogram for animal 6  
(40 mg/kg x 1).

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HPLC Radiochromatogram  
Diaoethane Product with 21U-24HP

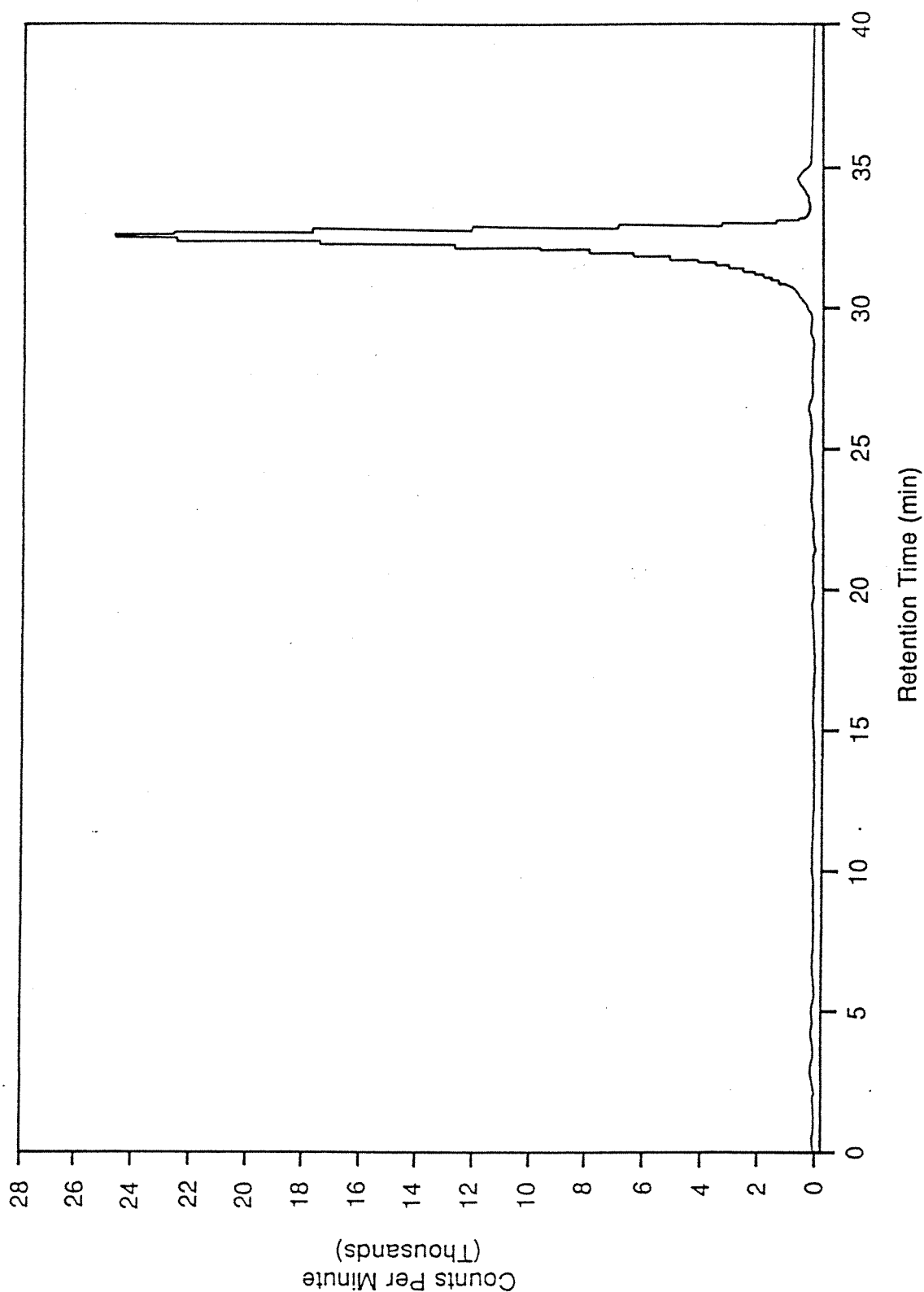


Figure 4. HPLC radiochromatogram of diazoethane-treated ether extract of urine from malathion-dosed rat (animal 21, 800 mg/kg).

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# HPLC Radiochromatogram Malathion Standard

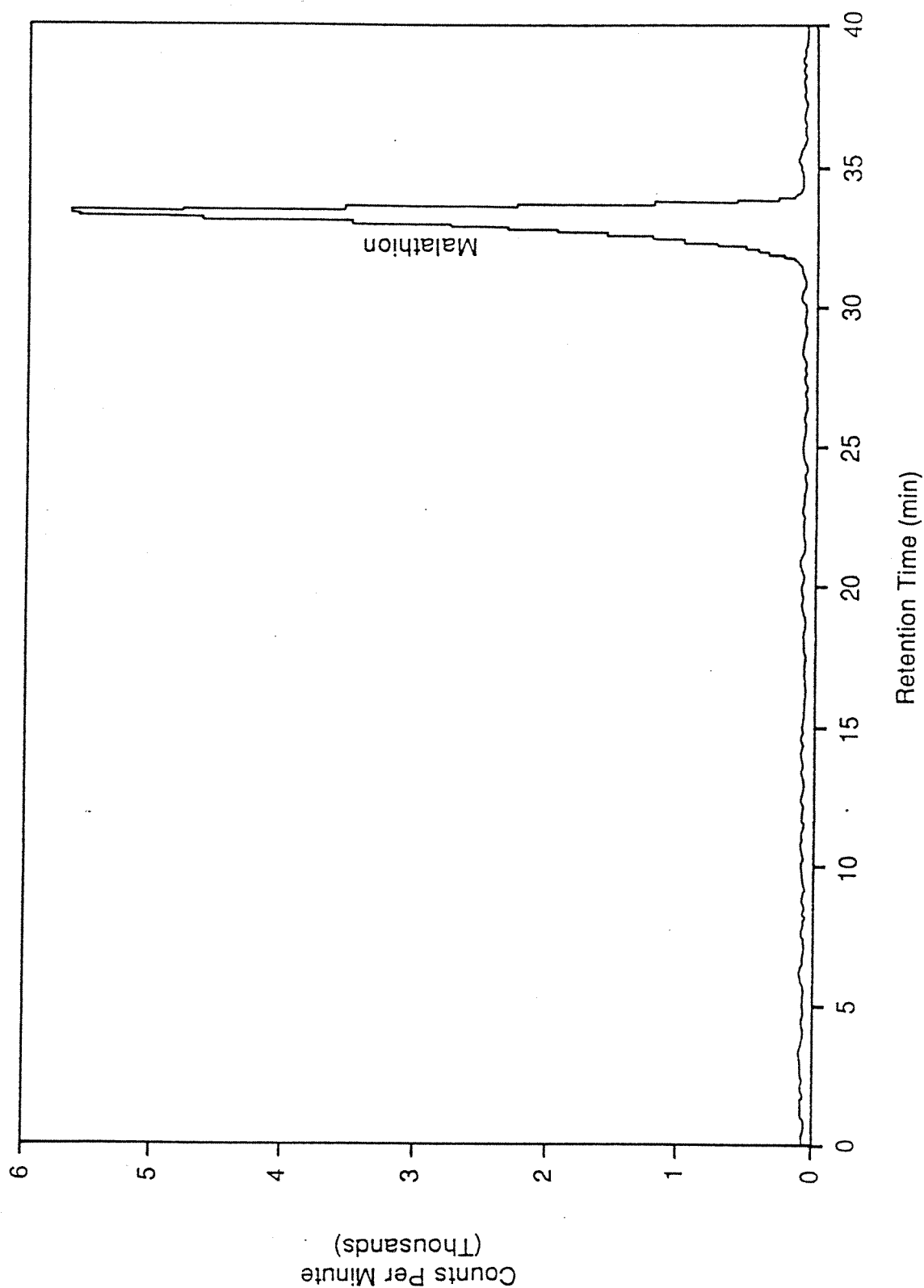


Figure 5. HPLC radiochromatogram of malathion standard.

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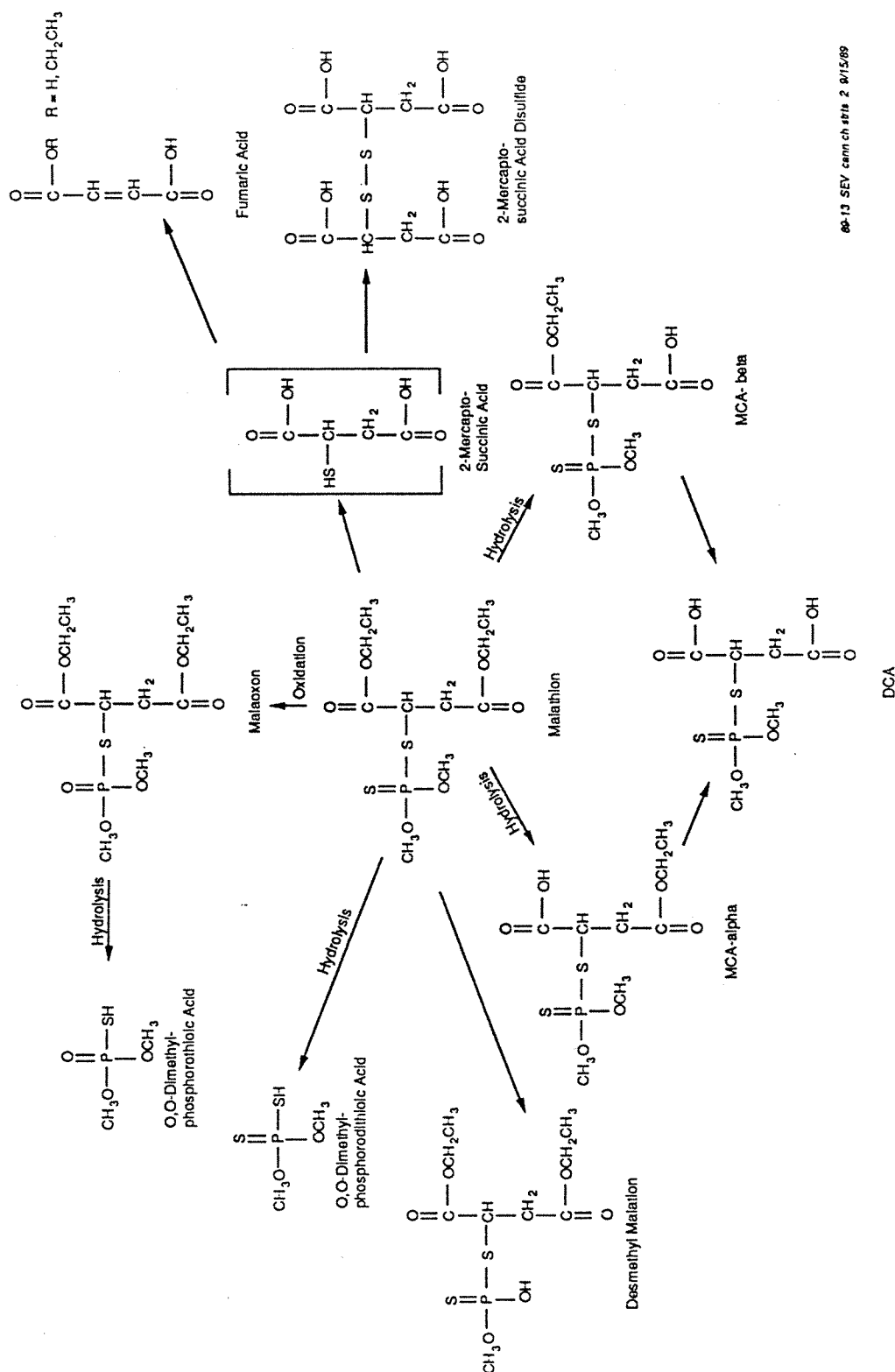


Figure 6. Possible metabolic pathway of malathion in Sprague-Dawley rats.



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APPENDIX I

METABOLISM DATA

DATA: 9354H30WQ2 #1485

SAMPLE: 2UL MWS 0.1MG/ML

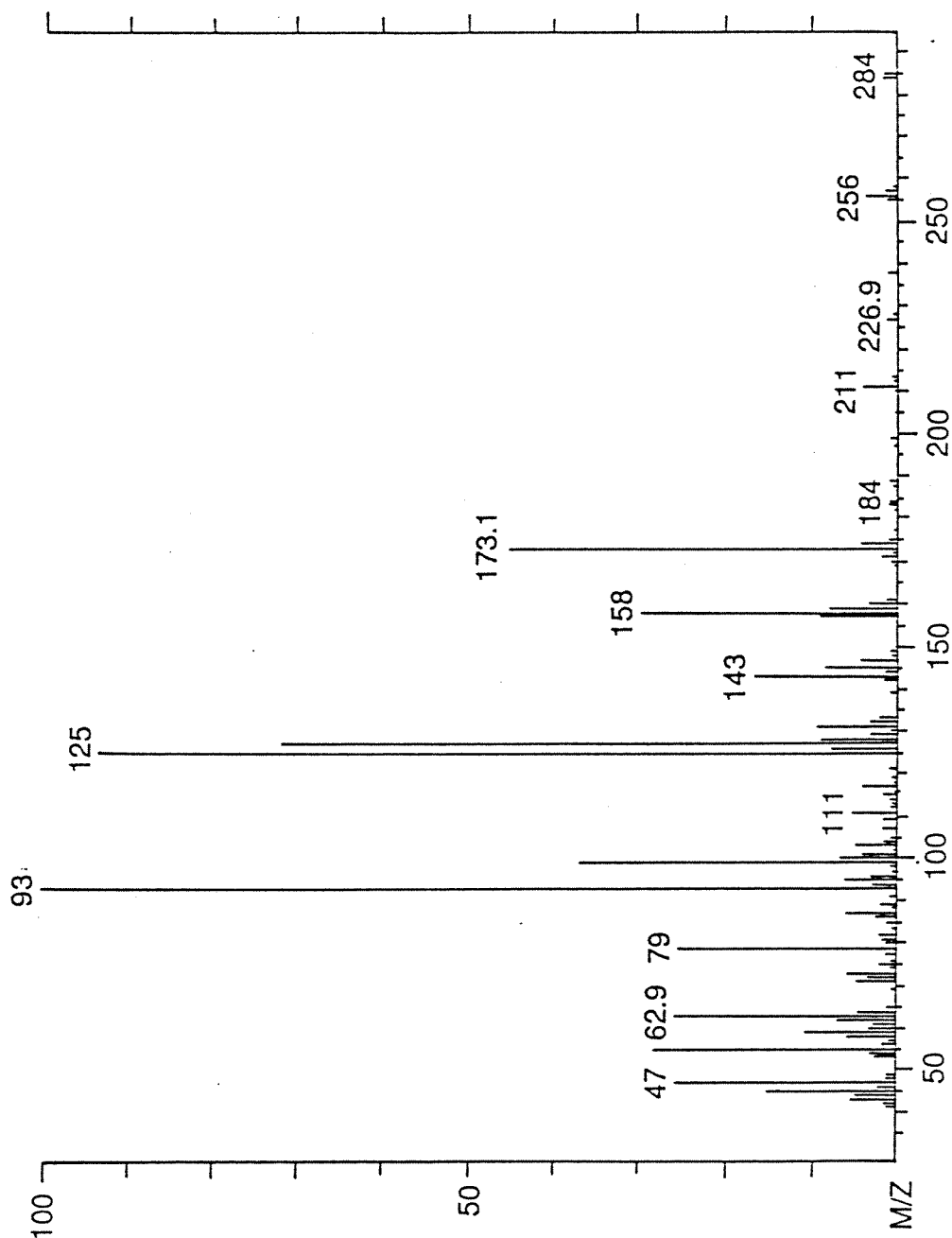


Figure AI-1. Mass spectrum of malathion standard.

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HPLC Radiochromatogram  
Urine for Animal No. 21 (0-24 hr)

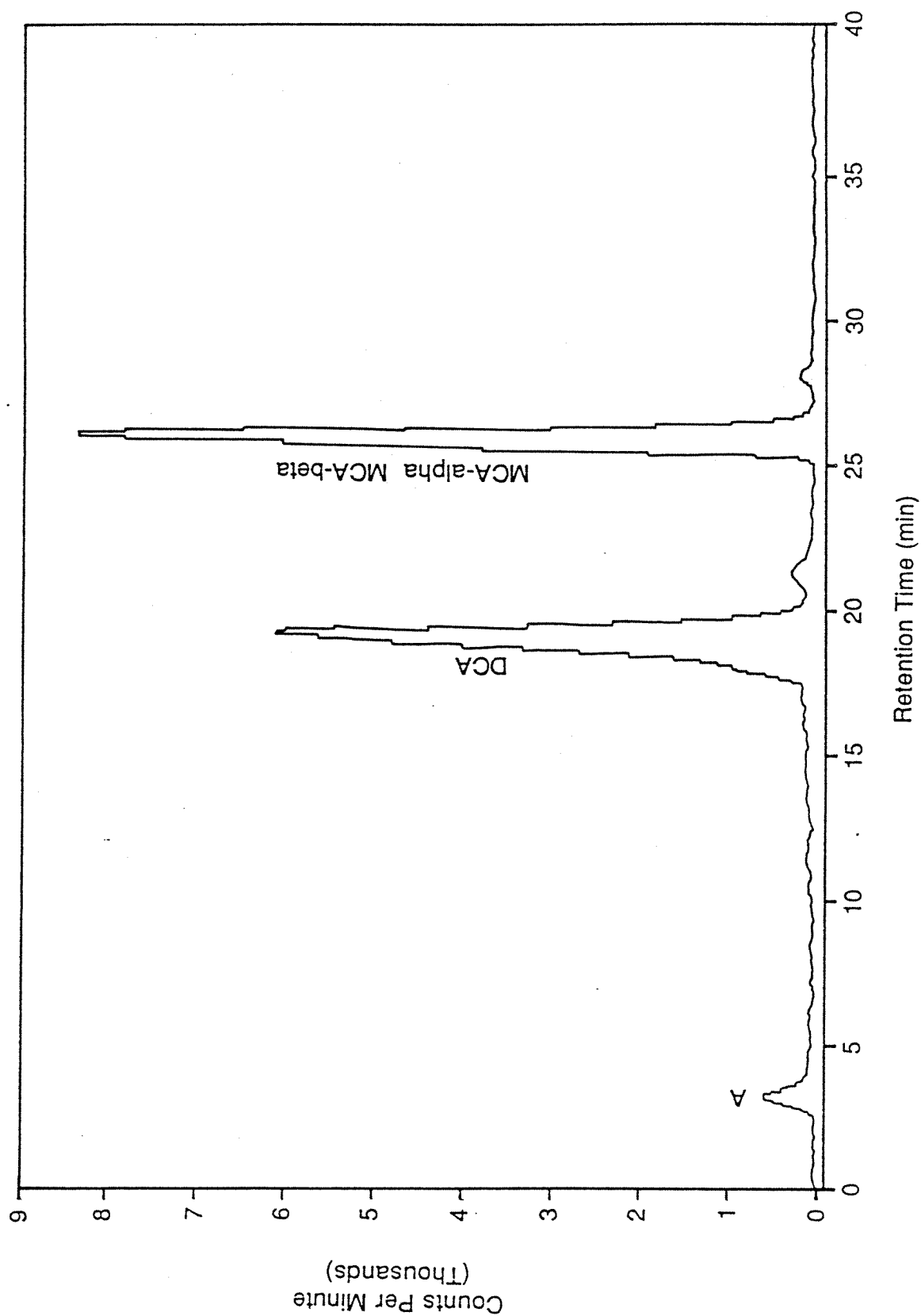


Figure AI-2. HPLC metabolite profile radiochromatogram for animal 21 (800 mg/kg x 1).

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HPLC Radiochromatogram  
Urine for Animal No. 31 (0-24 hr)

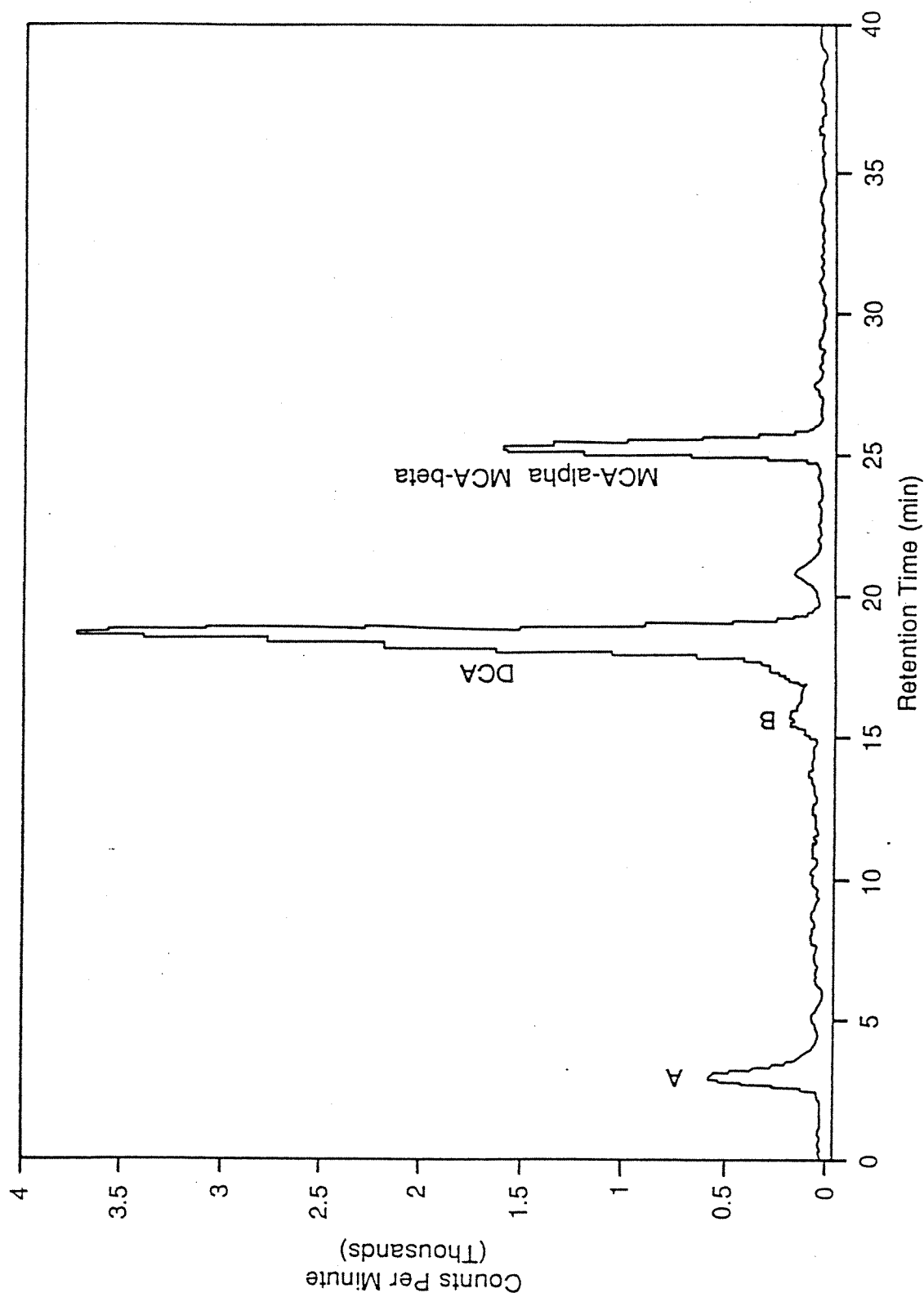


Figure AI-3. HPLC metabolite profile radiochromatogram for animal 31 (40 mg/kg x 15).

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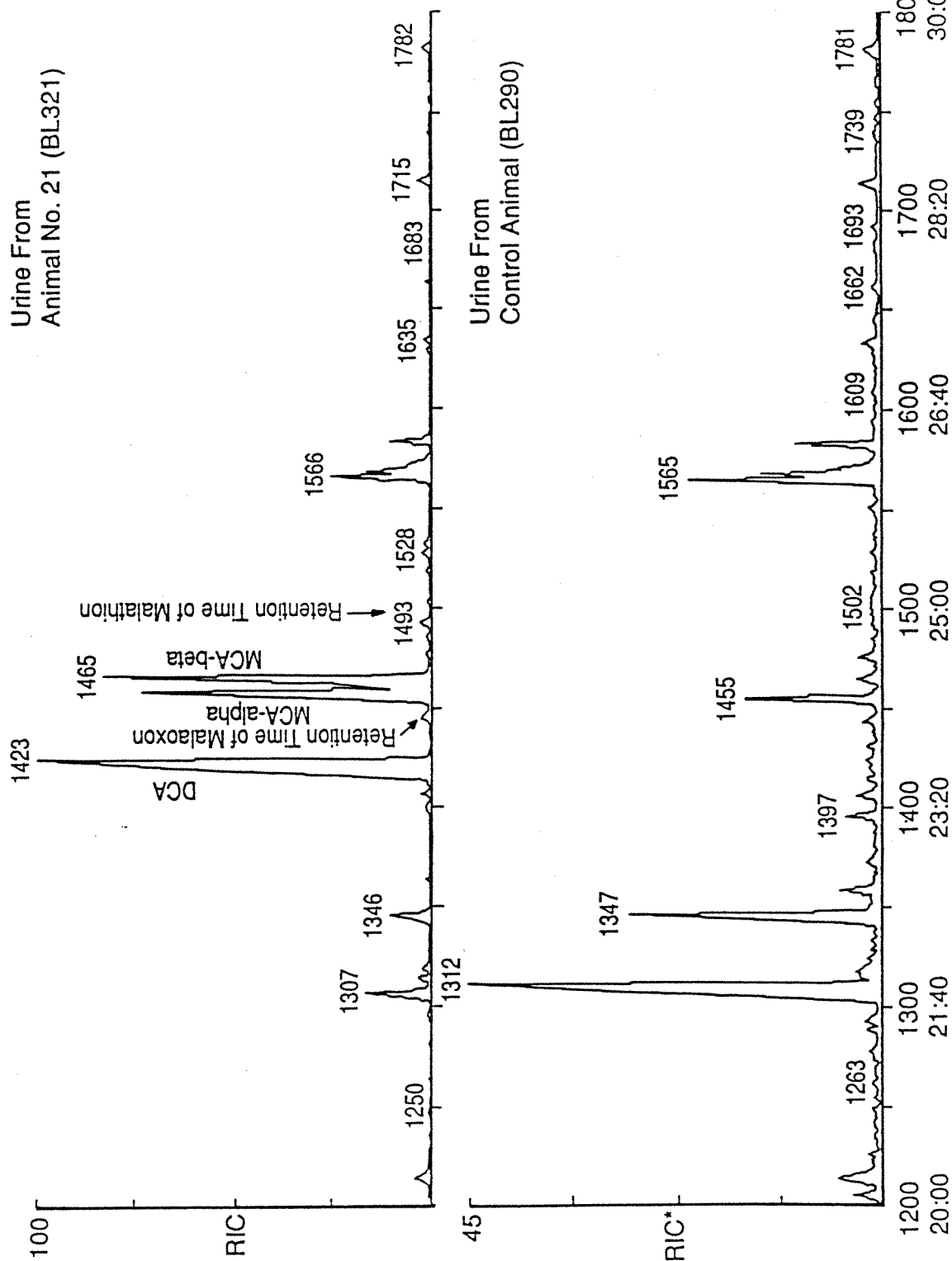


Figure AI-4. GC/MS RIC for diazomethane-treated ether extract of urine from malathion-dosed rat (animal 21, 800 mg/kg) (top) and similarly prepared control urine (bottom).

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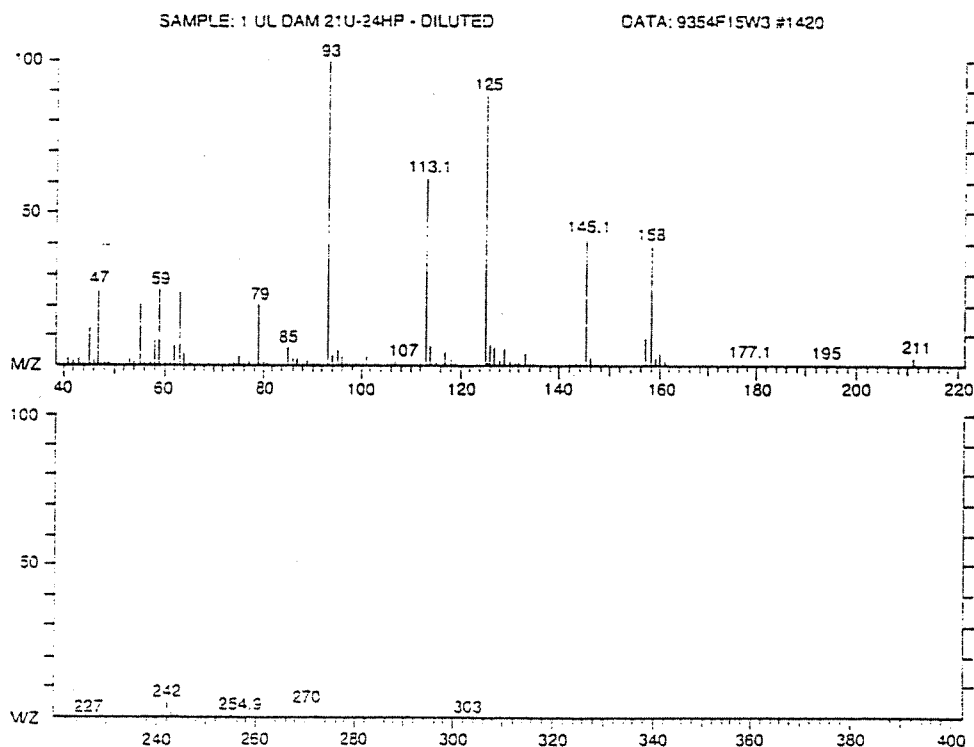
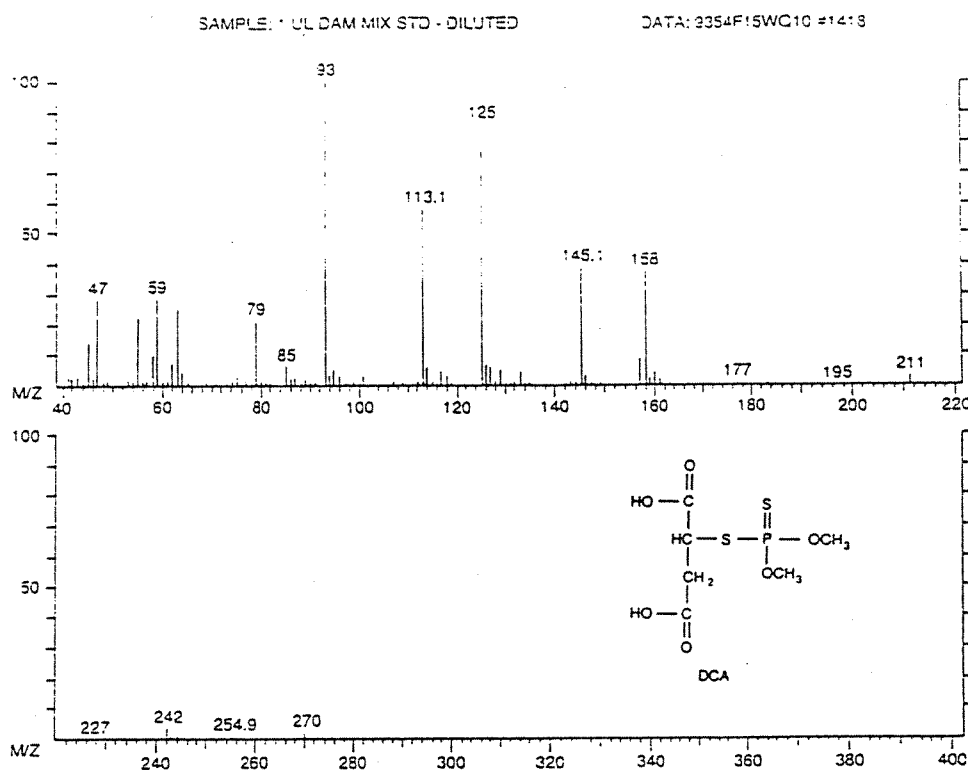


Figure AI-5. Mass spectra for DCA standard (top) and metabolite (bottom) identified (as the dimethylcarboxyester after reaction with diazomethane) in ether extracts of rat urine (animal 21, 800 mg/kg)

HPLC Radiochromatogram  
Diazomethane Product of 21U-24HP

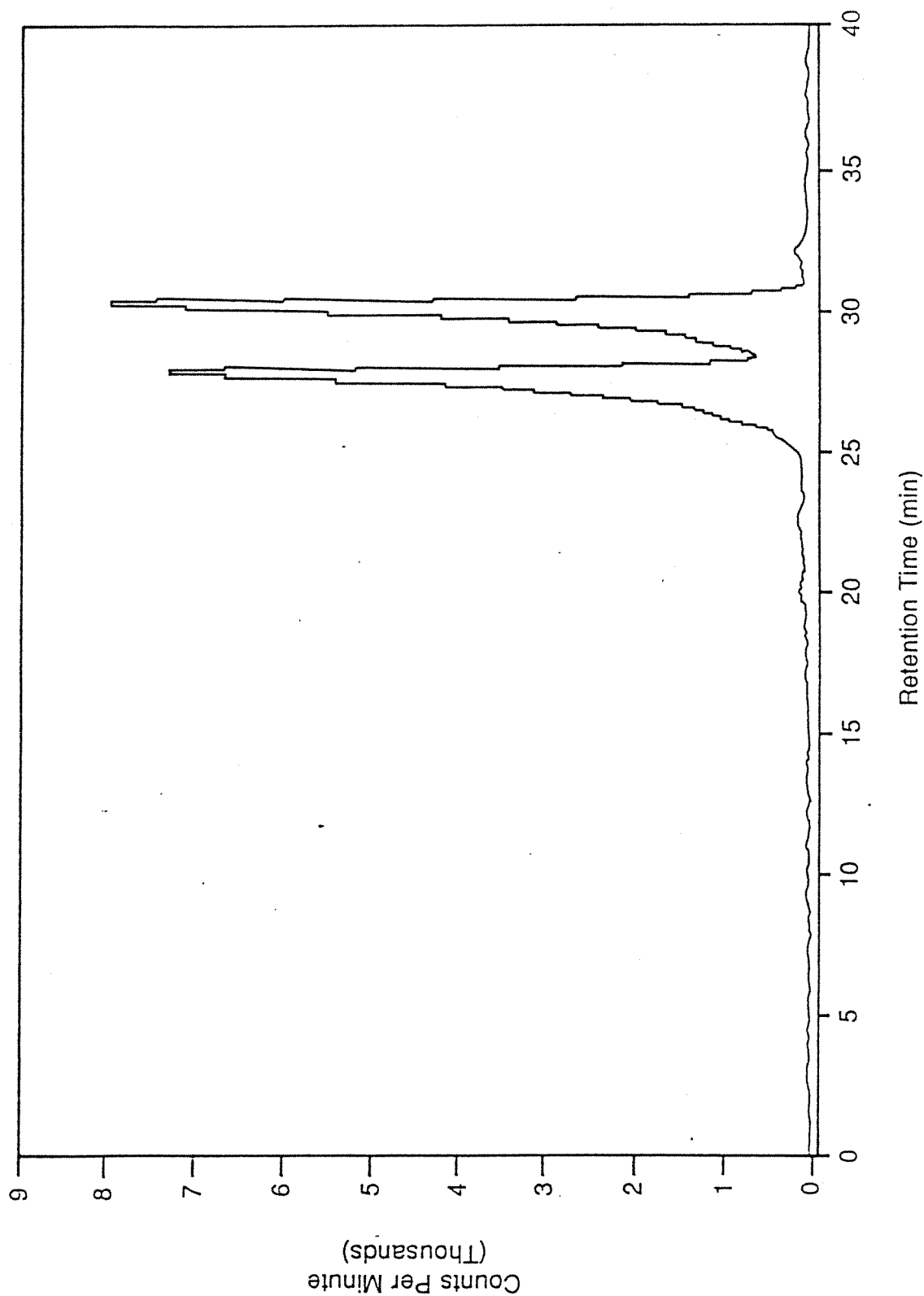


Figure AI-6. HPLC radiochromatogram for diazomethane-treated ether extract of rat urine (animal 21, 800 mg/kg).

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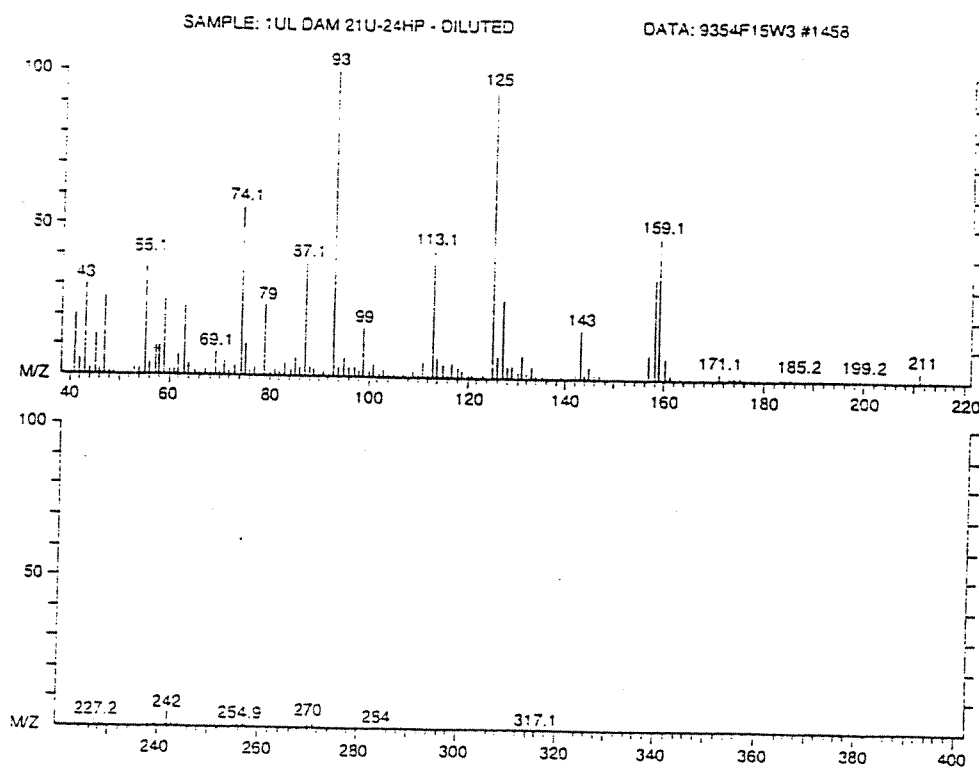
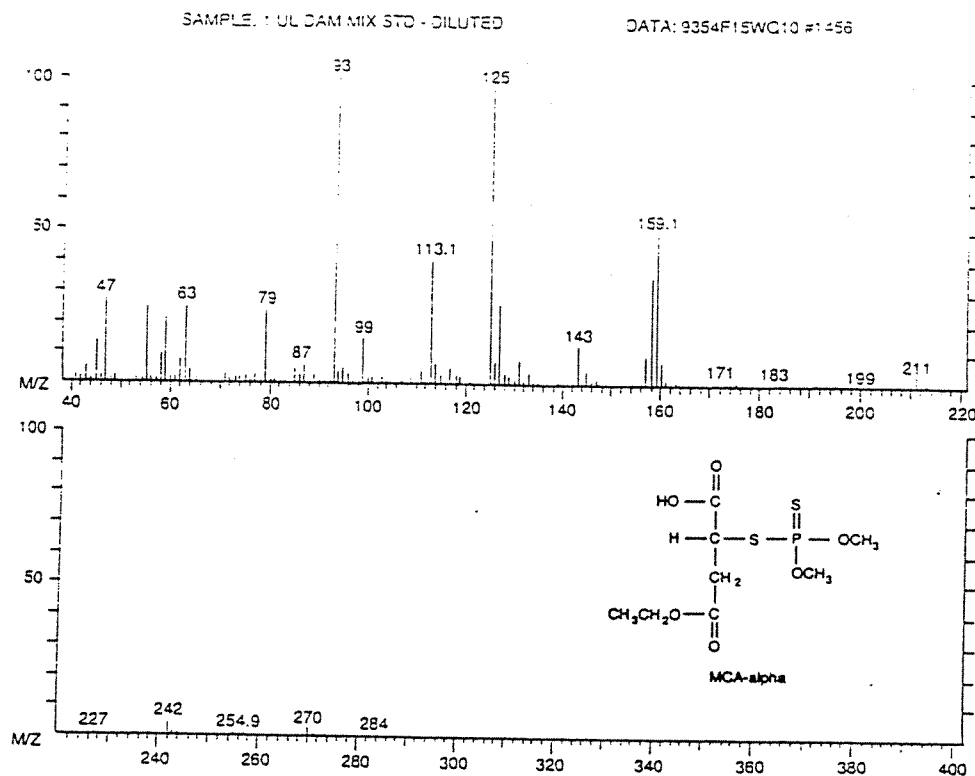


Figure AI-7. Mass spectra for MCA-alpha standard (top) and metabolite (bottom) identified (as the methyl ethyl carboxydiester after derivitization with diazomethane) in ether extracts of rat urine (animal 21, 800 mg/kg).



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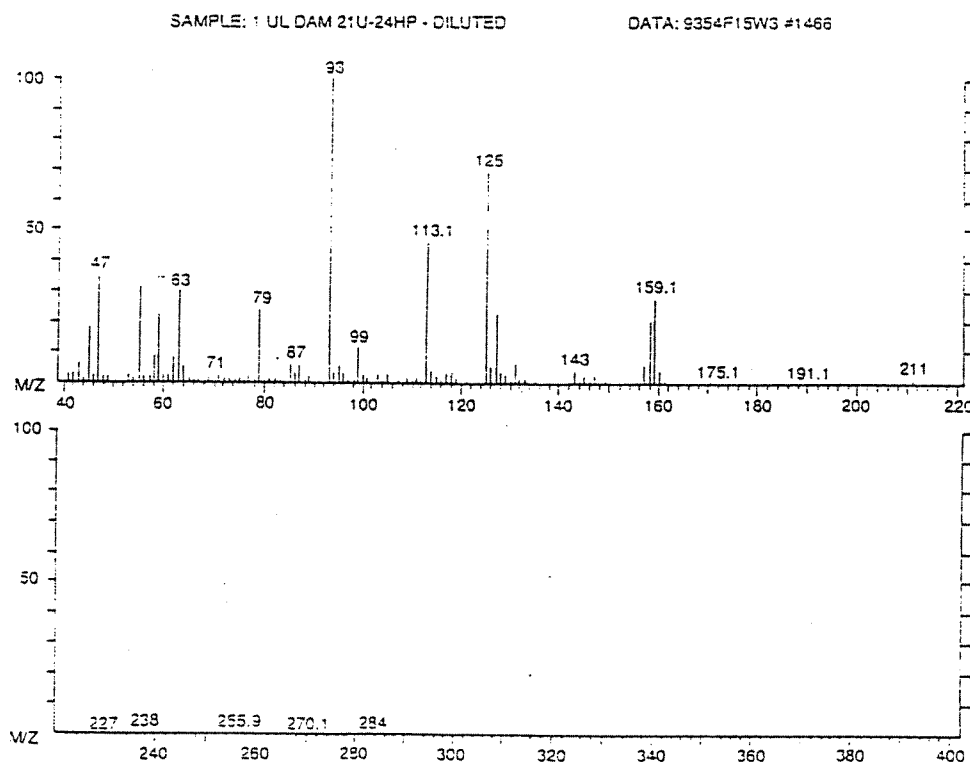
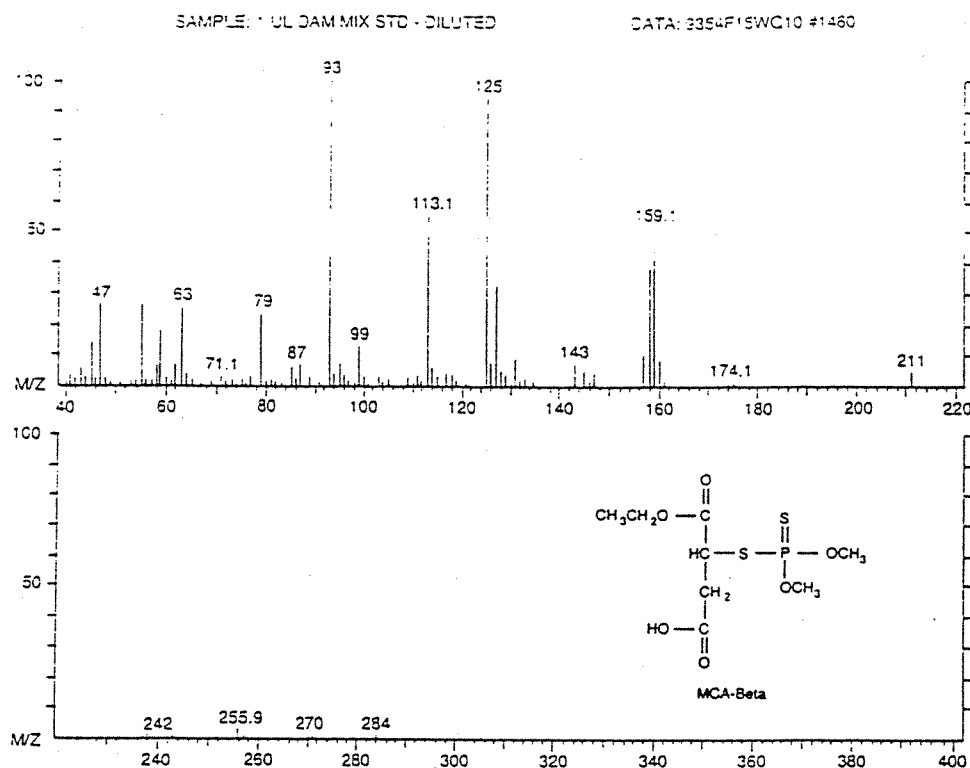


Figure AI-8. Mass spectra for MCA-beta standard (top) and metabolite (bottom) identified (as the methyl ethyl carboxydiester after derivitization with diazomethane) in ether extracts of rat urine (animal 21, 800 mg/kg).

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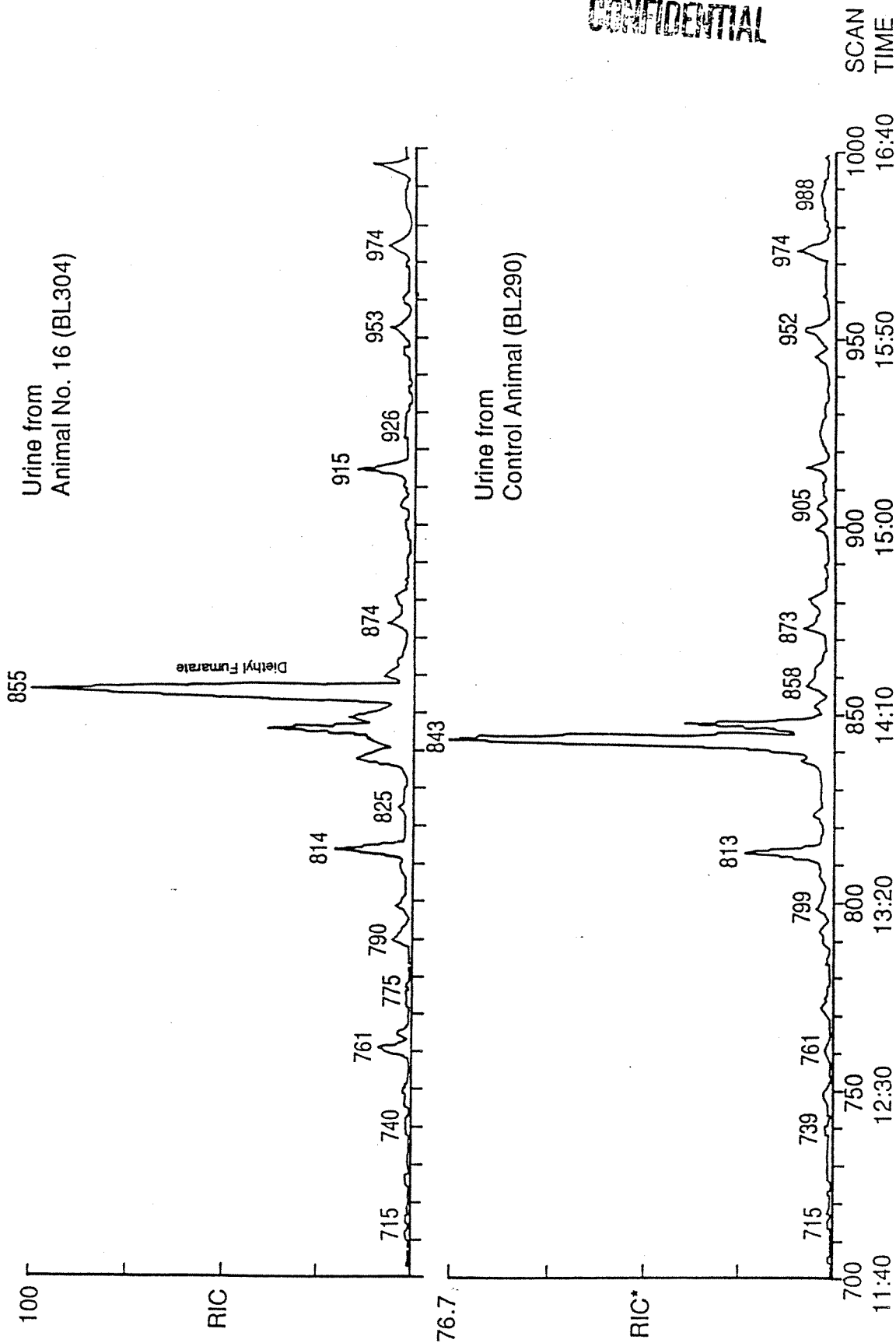


Figure AI-9. GC/MS RIC for diazoethane-treated ether extract of urine from malathion-dosed rat (animal 16, 800 mg/kg) (top) and similarly prepared control urine (bottom).

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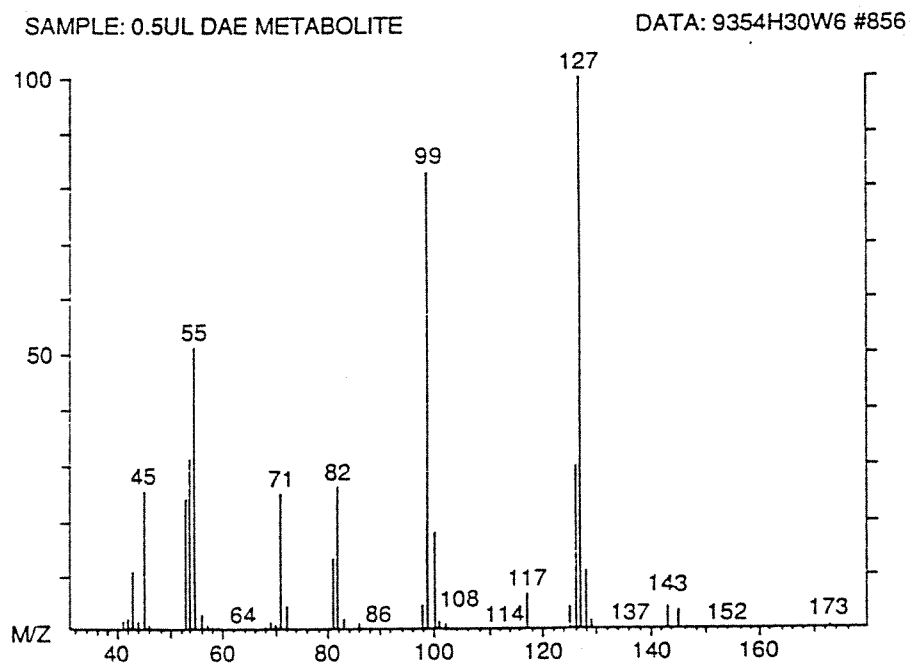
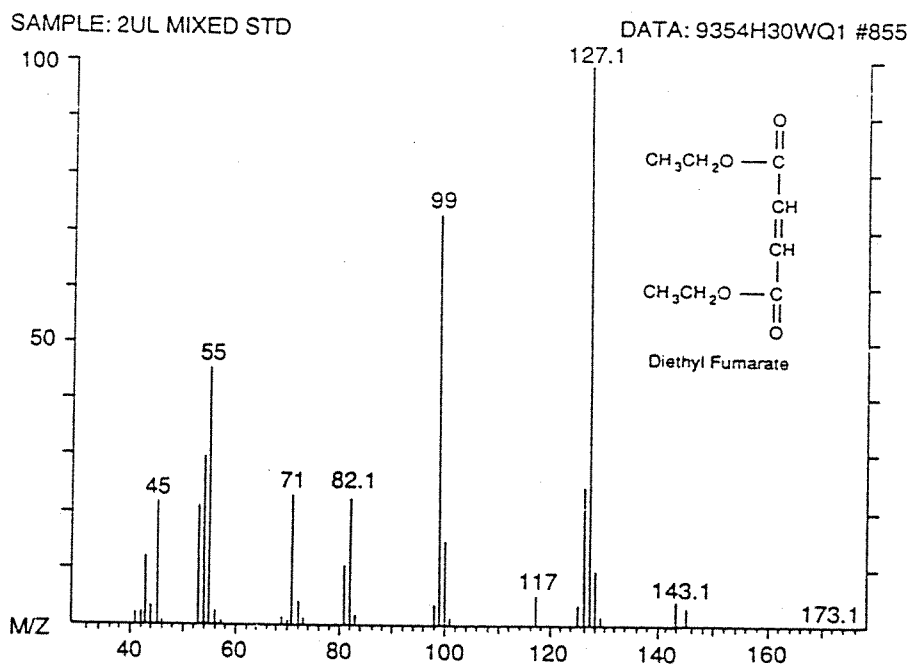


Figure AI-10. Mass spectra for diethyl fumarate standard and metabolite identified (as the diethyl ester after derivitization with diazoethane) in ether extracts of rat urine (animal 16, 800 mg/kg).

SAMPLE: 2UL DAE METABOLITE

DATA: 9354H30W4 #840,9354H30W3

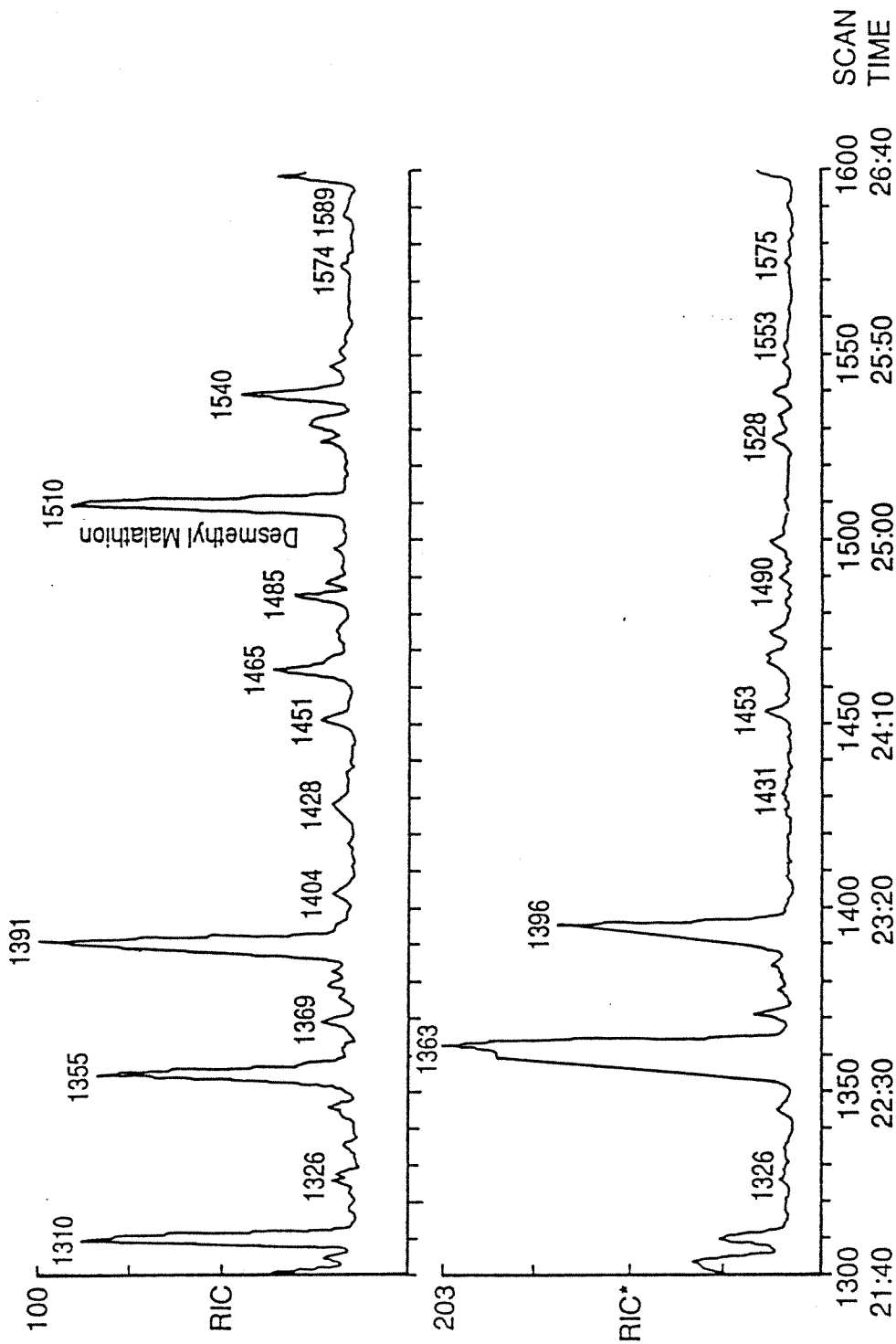


Figure AI-11. GC/MS RIC for diazoethane-treated lyophilized urine from malathion-dosed rat (animal 16, 800 mg/kg) (top) and similarly prepared control urine.

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SAMPLE: 2UL DAE METABOLITE

DATA: 9354H30W4 #1510

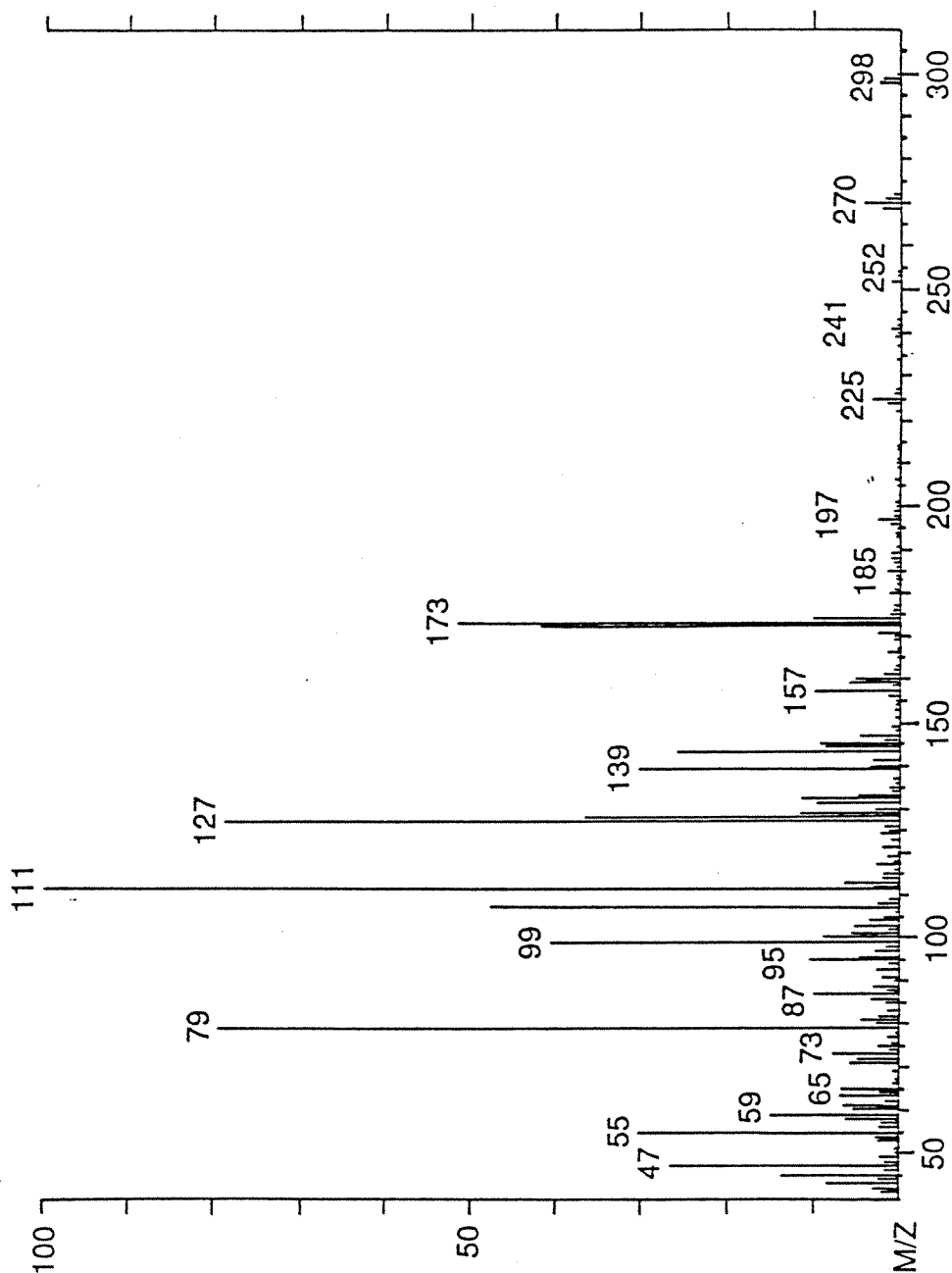


Figure AI-12. Mass spectrum for desmethyl malathion metabolite tentatively identified (as methylethyl phosphorodiester after derivitization with diazoethane) in lyophilized rat urine (animal 16, 800 mg/kg).

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SAMPLE: 2UL 16U24HP ET20 EXTR DATA: 9354H30W2 #1, 9354H30W1

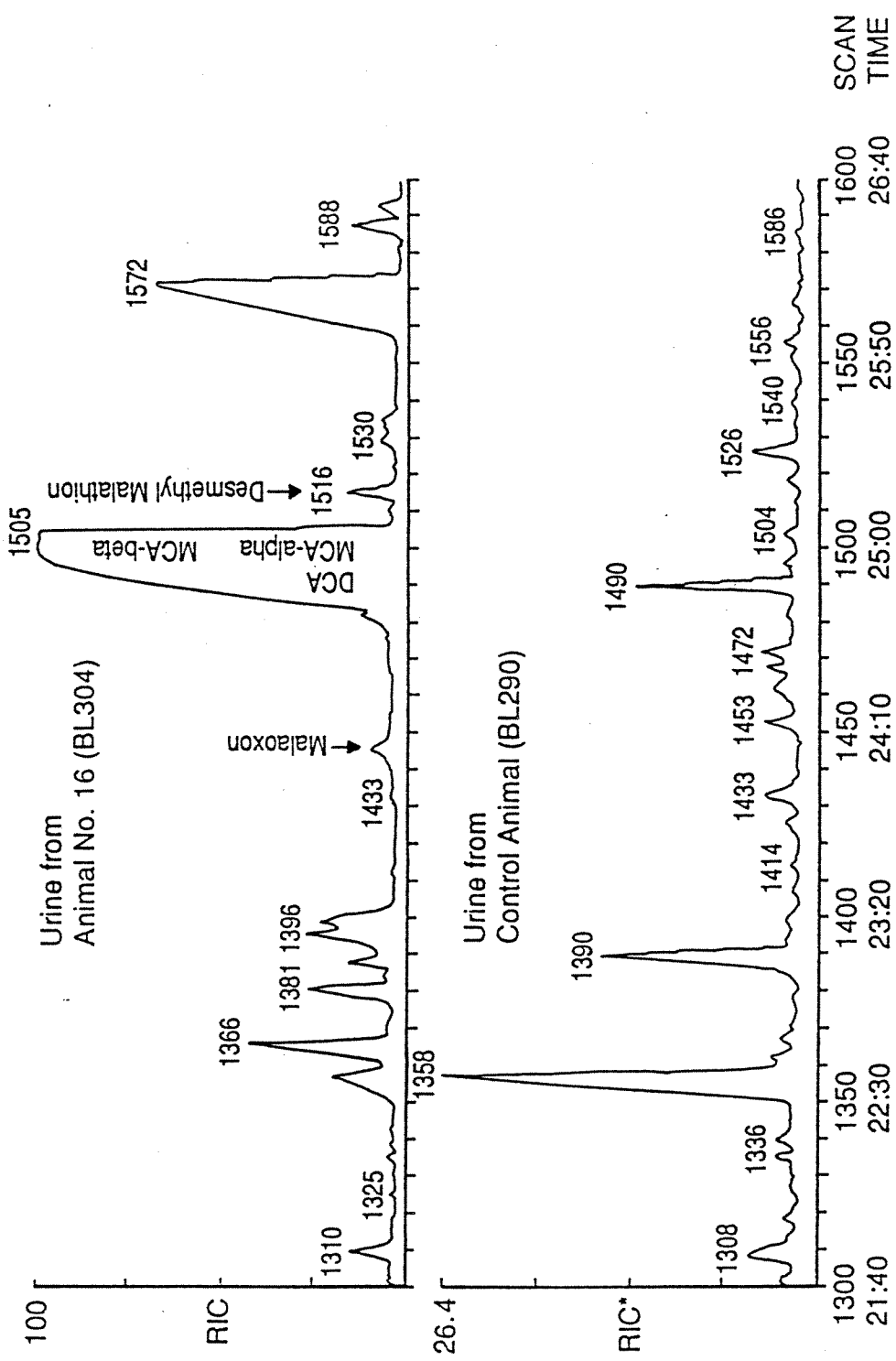


Figure AI-13. GC/MS RIC for diazoethane-treated ether extract of urine from malathion-dosed rat (animal 16, 800 mg/kg) (top) and similarly prepared control urine.

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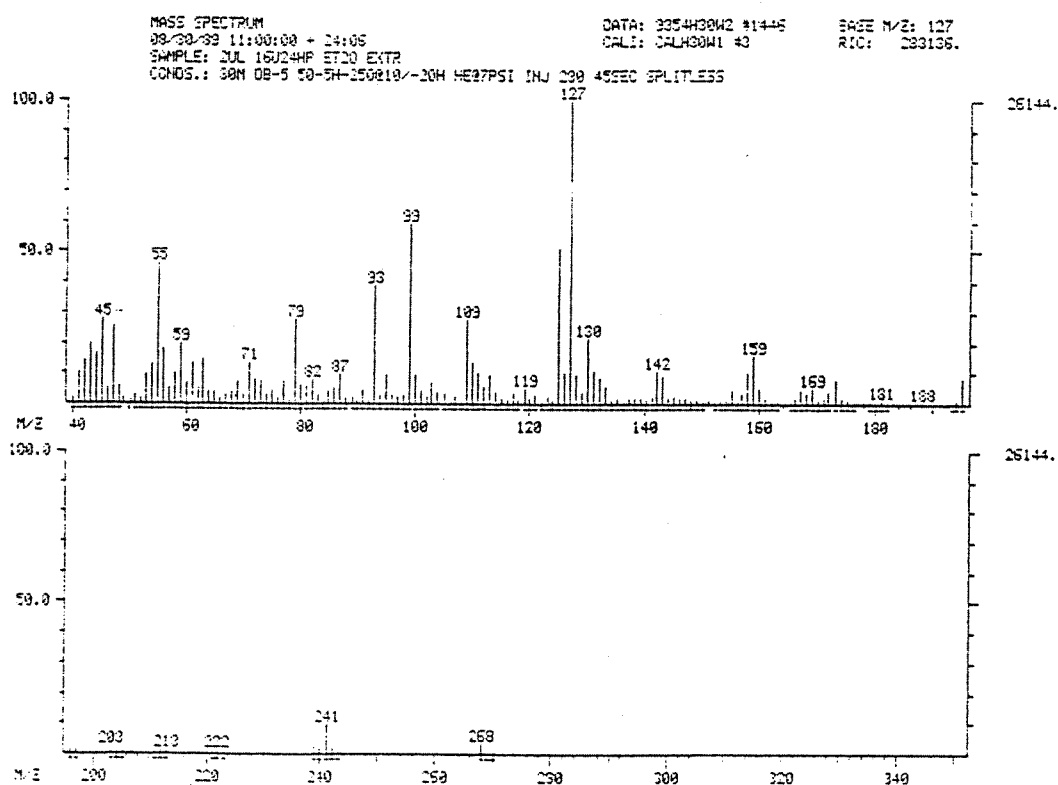
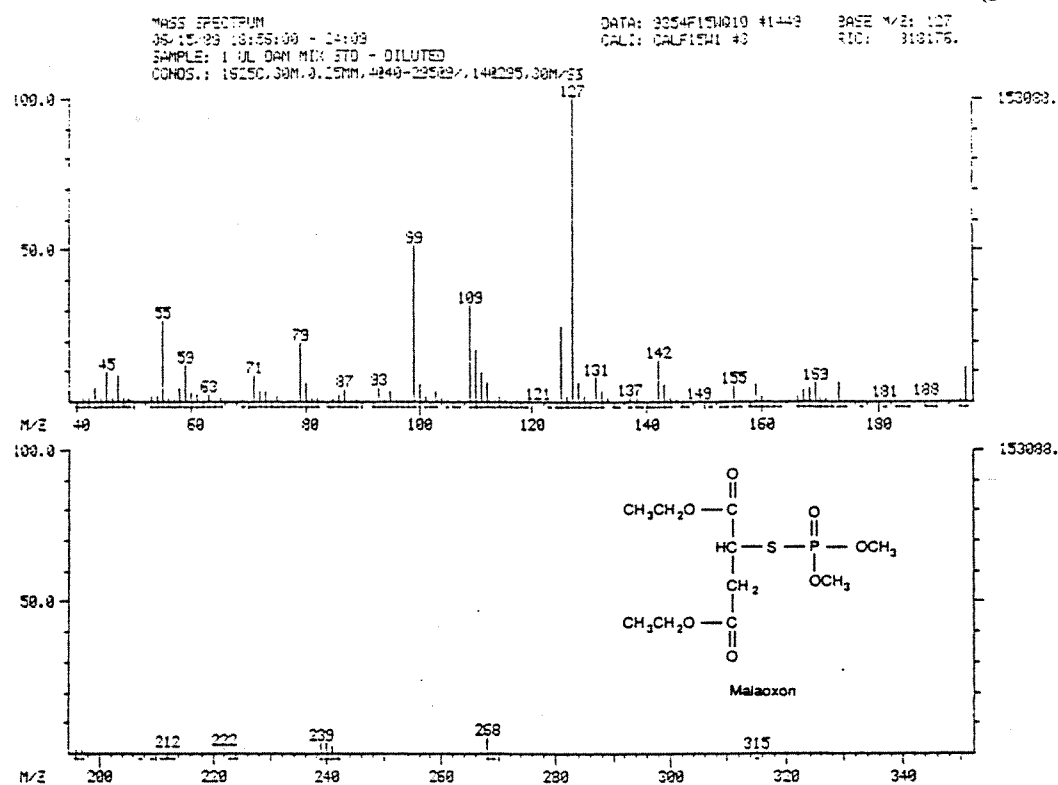
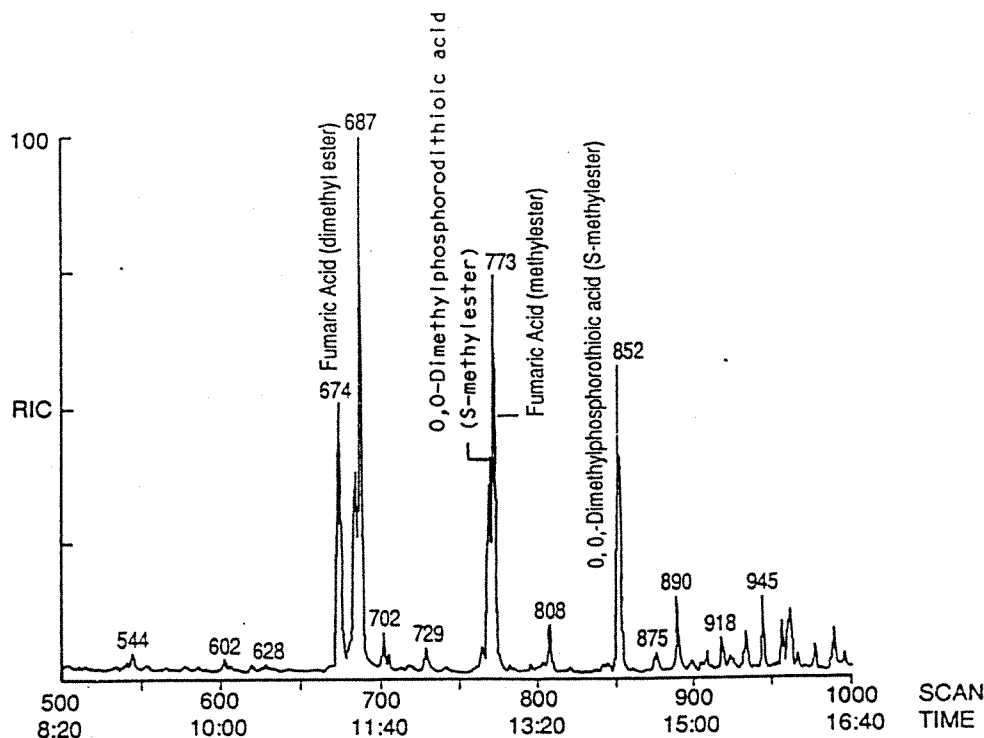


Figure AI-14. Mass spectrum for malaoxon standard (top) and metabolite (bottom) identified in ether extract of urine from malathion-dosed rat (animal 16, 800 mg/kg).

SAMPLE: 1 UL DAM 21U-24HP - DILUTED

DATA: 9354F15W3 #1

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SAMPLE: 1 UL DAM CONTROL URINE EXTRACT-DILUTED DATA: 9354F15W4 #1

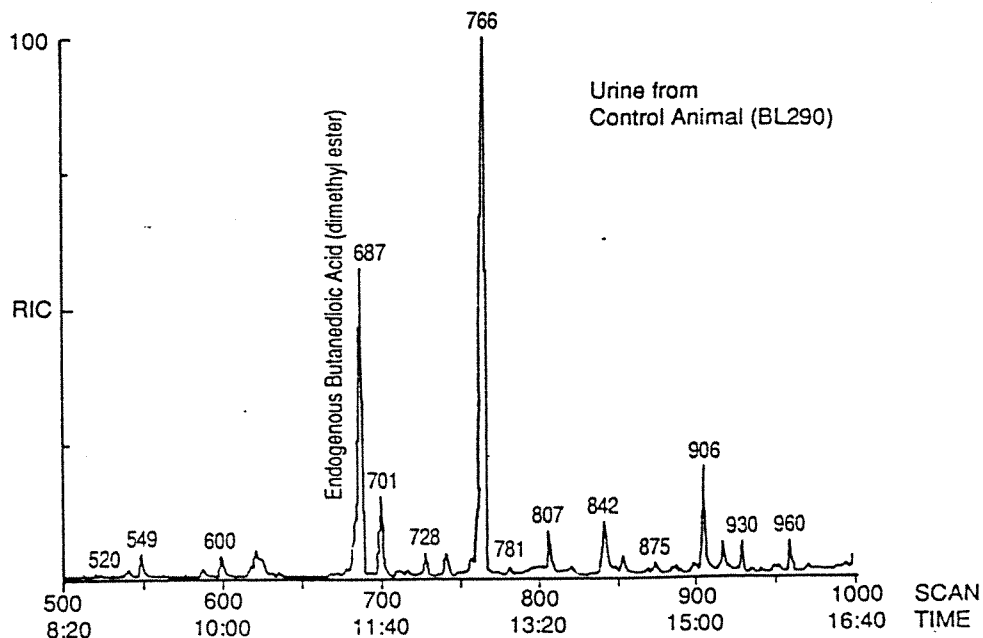


Figure AI-15. GC/MS RIC for diazomethane-treated ether extract of urine from malathion-dosed rat (animal 21, 800 mg/kg) (top) and similarly prepared control urine (bottom).



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SAMPLE: 1 UL DAM 21U-24HP - DILUTED

DATA: 9354F15W3 #852

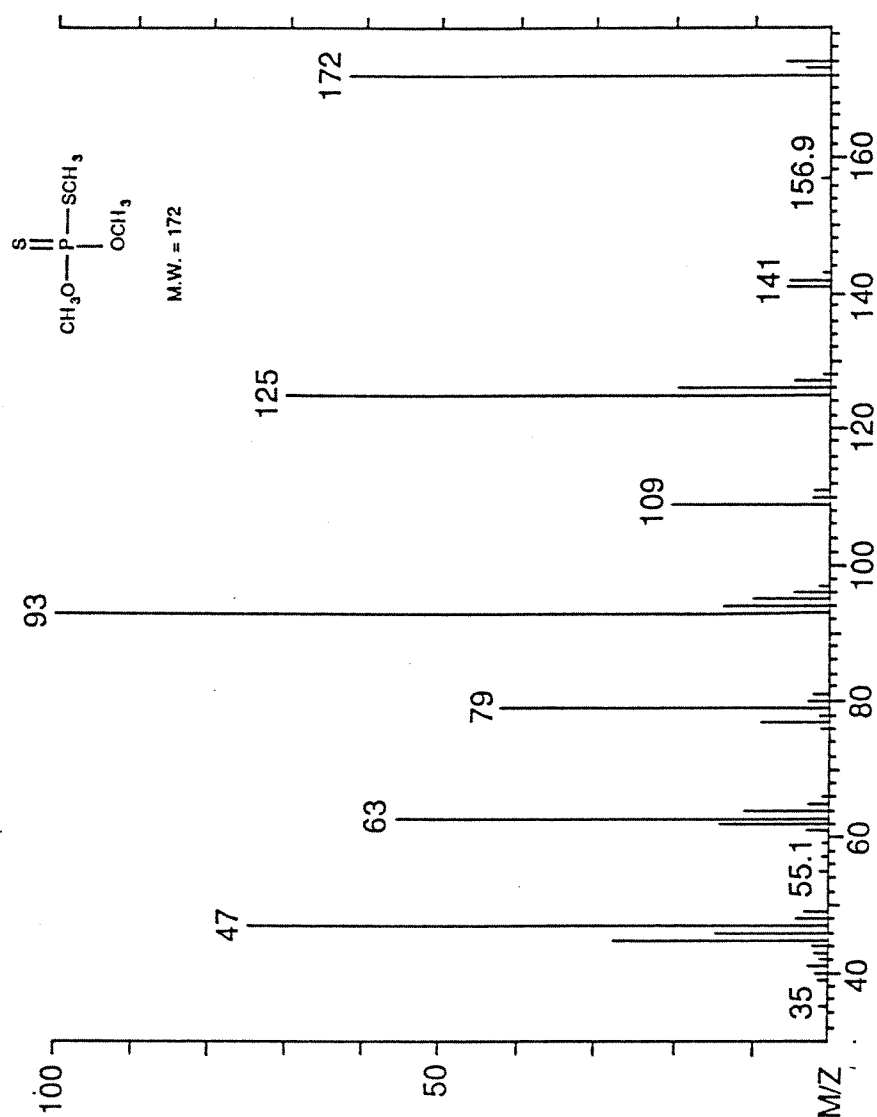


Figure AI-16. Mass spectrum of O,O-dimethyl phosphorodithioic acid identified (as O,O,S-trimethyl phosphoro ester after derivitization with diazomethane) in ether extract of urine from malathion-dosed rat (animal 21, 800 mg/kg).

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SAMPLE: 1 UL DAM 21U-24HP - DILUTED

DATA: 9354F15W3 #770

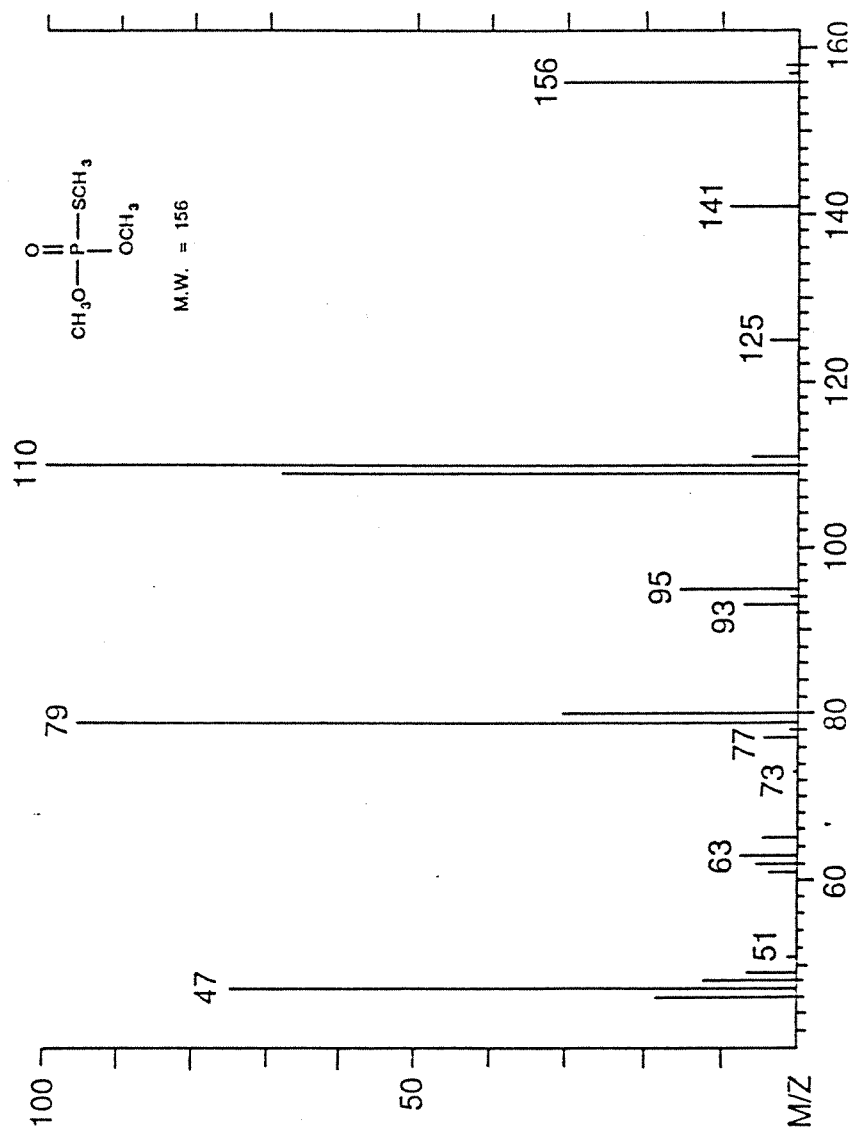


Figure AI-17. Mass spectrum of O,O-dimethyl phosphorothioic acid tentatively identified (as O,O,S-trimethyl phosphoro ester after derivitization with diazomethane) in ether extract of urine from malathion-dosed rat (animal 21, 800 mg/kg).

SAMPLE: 2UL 16U24HP ET20 EXTR

DATA: 9354H30W2 #1446,9354H30W1

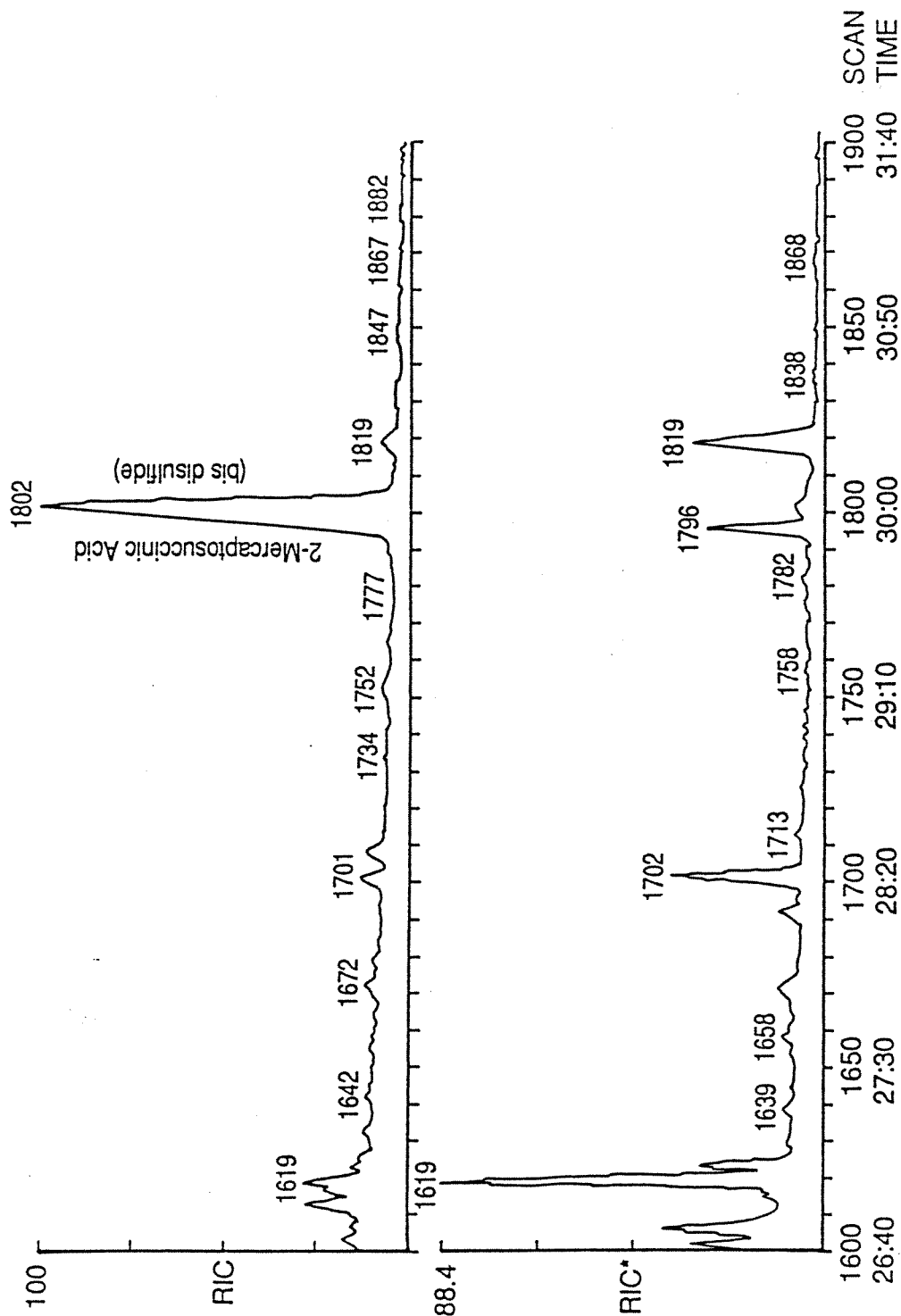


Figure AI-18. GC/MS RIC for diazoethane-treated lyophilized urine from malathion-dosed rat (animal 16, 800 mg/kg) (top) and similarly prepared control urine (bottom).

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SAMPLE: 2UL 16U24HP ET20 EXTR

DATA: 9354H30W2 #1902

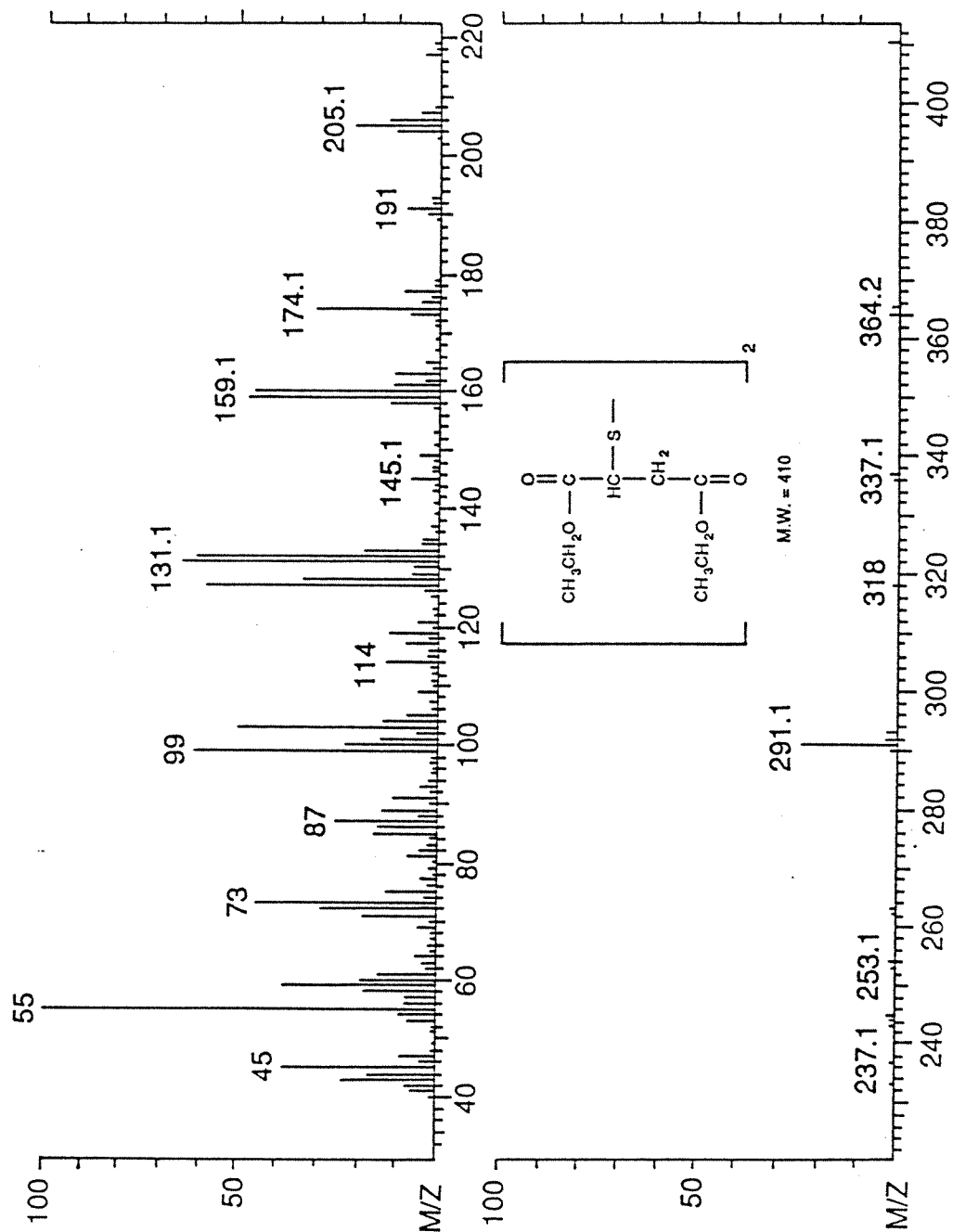


Figure AI-19. Mass spectrum of tentatively identified 2-mercapto-succinic acid metabolite (as bis-(diethylsuccinate)disulfide after derivitization with diazoethane) in ether extract of urine from malathion-dosed rat

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APPENDIX II

INDIVIDUAL ANIMAL DISPOSITION DATA

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TABLE II-1

DOSING INFORMATION FOR SPRAGUE-DAWLEY RATS TREATED WITH <sup>14</sup>C-MALATHION  
AT A NOMINAL DOSE LEVEL OF 40 OR 800 mg/kg : PRELIMINARY STUDY

Dose Group	Animal No.	Sex	Actual Dose (mg/kg)	Body Wt. (g)	ug Admin.	DPM Admin.
40 mg/kg	1	Male	36.91	238.4	8800	115016000
	2	Female	37.40	163.9	6130	80119100
800 mg/kg	5	Male	785.47	231.2	181600	121546000
	4	Female	765.99	156.4	119800	86495600

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TABLE II-2

DOSING INFORMATION FOR SPRAGUE-DAWLEY RATS TREATED WITH  
14C-MALATHION AT A NOMINAL DOSE LEVEL OF 40 mg/kg

Animal No.	Sex	Actual Dose (mg/kg)	Body Wt. (g)	ug Admin.	DPM Admin.
6	Male	35.869	189.3	6790	81547900
7		36.378	188.3	6850	82268500
8		36.410	195.0	7100	85271000
9		36.902	184.0	6790	81547900
10		35.917	193.5	6950	83469500
11	Female	39.459	166.5	6570	78905700
12		39.396	158.9	6260	75182600
13		36.358	168.6	6130	73621300
14		36.346	157.1	5710	68577100
15		34.109	150.4	5130	61611300

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TABLE II-3

DOSING INFORMATION FOR SPRAGUE-DAWLEY RATS TREATED WITH  
14C-MALATHION AT A NOMINAL DOSE LEVEL OF 800 mg/kg

Animal No.	Sex	Actual Dose (mg/kg)	Body Wt. (g)	ug Admin.	DPM Admin.
16	Male	762.945	210.5	160600	105032400
17		758.714	220.9	167600	109610400
18		757.885	215.6	163400	106863600
19		758.621	217.5	165000	107910000
20		738.532	218.0	161000	105294000
21	Female	765.599	177.9	136200	89074800
22		749.129	172.2	129000	84366000
23		761.962	165.1	125800	82273200
24		730.300	180.2	131600	86066400
25		751.789	181.7	136600	89336400



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TABLE II-4

DOSING INFORMATION FOR SPRAGUE-DAWLEY RATS TREATED WITH  
14C-MALATHION AT A NOMINAL DOSE LEVEL OF 40 mg/kg -a

Animal No.	Sex	Actual Dose (mg/kg)	Body Wt. (g)	ug Admin.	DPM Admin.
26	Male	33.661	284.9	9590	110323360
27		35.030	278.9	9770	112394080
28		36.952	315.0	11640	133906560
29		34.610	282.0	9760	112279040
30		35.497	269.6	9570	110093280
31	Female	36.444	214.3	7810	89846240
32		35.865	218.6	7840	90191360
33		35.925	219.9	7900	90881600
34		36.820	218.9	8060	92722240
35		36.400	223.9	8150	93757600

a/ Following 15 doses of nonlabeled malathion (40 mg/kg/day).

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TABLE II-5

RADIOACTIVITY IN EXPIRED AIR OF MALE AND FEMALE SPRAGUE-DAWLEY RATS  
FOLLOWING A SINGLE ORAL DOSE OF 14C-MALATHION : PRELIMINARY STUDY

Chemical Component	Time After Dosing (hr)	40 mg/kg Dose		800 mg/kg Dose	
		No. 1 (M)	No. 2 (F)	No. 5 (M)-a	No. 4 (F)
		Percent of Dose			
Parent Compound	4	0.001	0.000	0.039	0.030
	8	0.001	0.004	0.026	0.022
	12	0.000	0.002	0.018	0.016
	24	0.004	0.006	0.046	0.028
	48	0.004	0.018	0.064	0.027
	72	0.000	0.006	0.042	0.020
CO2	4	0.500	0.134	0.079	0.192
	8	0.154	0.092	0.099	0.042
	12	0.133	0.025	0.143	0.040
	24	0.091	0.028	0.047	0.021
	48	0.030	0.023	0.047	0.012
	72	0.015	0.029	0.024	0.017
Cumulative Percent of Dose					
Parent Compound	4	0.001	0.000	0.039	0.030
	8	0.002	0.004	0.064	0.052
	12	0.002	0.006	0.082	0.068
	24	0.006	0.012	0.128	0.096
	48	0.010	0.030	0.192	0.123
	72	0.010	0.036	0.234	0.143
CO2	4	0.500	0.134	0.079	0.192
	8	0.654	0.226	0.178	0.234
	12	0.787	0.251	0.321	0.274
	24	0.878	0.279	0.368	0.295
	48	0.908	0.302	0.414	0.307
	72	0.923	0.331	0.439	0.324

a/ Values for No. 5 (M) adjusted to reflect a partial loss of dose (see text).

TABLE II-6

RADIOACTIVITY IN EXCRETA OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FOLLOWING  
A SINGLE ORAL DOSE OF 14C-MALATHION : PRELIMINARY STUDY

Excrement	Time After Dosing (hr)	40 mg/kg Dose		800 mg/kg Dose		40 mg/kg Dose		800 mg/kg Dose	
		No. 1 (M)	No. 2 (F)	No. 5 (M)	-a No. 4 (F)	No. 1 (M)	No. 2 (F)	No. 5 (M)	-a No. 4 (F)
		ug Equivalents Excreted				Percent of Dose			
Urine	4	4883.106	0.012	3517.118	14925.959	55.490	0.000	8.271	29.153
	8	1786.632	4935.650	4900.802	10461.592	20.303	80.516	9.117	8.733
	12	292.498	314.403	7344.235	6020.661	3.324	5.129	22.849	5.026
	24	449.273	189.020	6528.870	12204.096	5.105	3.084	22.350	26.881
	48	87.526	53.233	7457.942	4404.440	0.994	0.868	4.563	3.677
	72	10.371	9.753	2751.964	701.030	0.118	0.159	1.684	0.585
Feces	4	-b	-b	-b	4923.196	-b	-b	-b	4.110
	8	-b	-b	-b	-b	-b	-b	-b	-b
	12	8.951	-b	-b	1291.188	0.102	-b	-b	1.078
	24	732.427	151.751	3030.970	6972.825	8.323	2.476	7.973	5.820
	48	10.320	57.281	9047.325	4732.409	0.117	0.934	5.536	3.950
	72	3.878	39.179	1700.235	354.019	0.044	0.639	1.040	0.296
Total	4	4883.106	0.012	3517.118	19849.155	55.490	0.000	8.271	33.263
	8	1786.632	4935.650	4900.802	10461.592	20.303	80.516	9.117	8.733
	12	301.449	314.403	7344.235	7311.849	3.426	5.129	22.849	6.104
	24	1181.700	340.771	9559.840	19176.921	13.428	5.560	30.323	32.701
	48	97.846	110.514	16505.267	9136.849	1.111	1.802	10.099	7.627
	72	14.249	48.932	4452.199	1055.049	0.162	0.798	2.724	0.881
(continued)									

(continued)

TABLE II-6 (Concluded)

Excretum	Time After Dosing (hr)	40 mg/kg Dose		800 mg/kg Dose		40 mg/kg Dose		800 mg/kg Dose	
		No. 1 (M)	No. 2 (F)	No. 5 (M)	-a No. 4 (F)	No. 1 (M)	No. 2 (F)	No. 5 (M)	-a No. 4 (F)
		Cumulative ug Equivalents Excreted				Cumulative Percent of Dose			
Urine	4	4883.106	0.012	3517.118	14925.959	55.490	0.000	8.271	29.153
	8	6669.738	4935.662	8417.920	25387.551	75.793	80.516	17.388	37.886
	12	6962.236	5250.065	15762.155	31408.212	79.117	85.645	40.237	42.912
	24	7411.509	5439.085	22291.025	43612.308	84.222	88.729	62.587	69.793
	48	7499.035	5492.318	29748.967	48016.748	85.216	89.597	67.150	73.470
	72	7509.406	5502.071	32500.931	48717.778	85.334	89.756	68.834	74.055
Feces	4	-b	-b	-b	4923.196	-b	-b	-b	4.110
	8	-b	-b	-b	4923.196	-b	-b	-b	4.110
	12	8.951	-b	-b	6214.384	0.102	-b	-b	5.188
	24	741.378	151.751	3030.970	13187.209	8.425	2.476	7.973	11.008
	48	751.698	209.032	12078.295	17919.618	8.542	3.410	13.509	14.958
	72	755.576	248.211	13778.530	18273.637	8.586	4.049	14.549	15.254
Total	4	4883.106	0.012	3517.118	19849.155	55.490	0.000	8.271	33.263
	8	6669.738	4935.662	8417.920	30310.747	75.793	80.516	17.388	41.996
	12	6971.187	5250.065	15762.155	37622.596	79.219	85.645	40.237	48.100
	24	8152.887	5590.836	25321.995	56799.517	92.647	91.205	70.560	80.801
	48	8250.733	5701.350	41827.262	65936.366	93.758	93.007	80.659	88.428
	72	8264.982	5750.282	46279.461	66991.415	93.920	93.805	83.383	89.309

a/ Values for No. 5 (M) adjusted to reflect a partial loss of dose (see text).

b/ No feces during this time period.

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TABLE II-7

RADIOACTIVITY IN EXCRETA OF MALE SPRAGUE-DAWLEY RATS FOLLOWING  
A SINGLE ORAL DOSE OF <sup>14</sup>C-MALATHION (40 mg/kg)

Excretum	Time							
	After Dosing (hr)	No. 6	No. 7	No. 8	No. 9	No. 10	Mean	S.E.
-----								
ug Equivalents Excreted								
-----								
Urine	4	4.199	0.656	1682.596	2597.339	1.778	857.314	543.249
	8	5033.808	4401.703	3047.480	2206.965	4453.650	3828.721	520.169
	12	300.337	867.588	656.614	762.618	667.445	650.920	95.560
	24	189.759	228.494	365.160	234.219	349.158	273.358	35.147
	48	154.037	90.254	133.837	57.908	187.267	124.661	22.920
	72	33.626	31.560	72.928	30.887	58.227	45.446	8.553
Feces	4	-a	-a	-a	-a	-a		-a
	8	-a	-a	-a	-a	-a		-a
	12	177.085	91.794	305.814	145.632	249.047	193.874	37.818
	24	370.468	697.400	371.504	419.849	424.572	456.759	61.246
	48	177.985	59.452	64.268	42.846	103.379	89.586	24.226
	72	14.818	8.795	19.132	11.948	22.286	15.396	2.421
Total	4	4.199	0.656	1682.596	2597.339	1.778	857.314	543.249
	8	5033.808	4401.703	3047.480	2206.965	4453.650	3828.721	520.169
	12	477.422	959.382	962.428	908.250	916.492	844.795	92.493
	24	560.227	925.894	736.664	654.068	773.730	730.117	61.204
	48	332.022	149.706	198.105	100.754	290.646	214.247	43.020
	72	48.444	40.355	92.060	42.835	80.513	60.841	10.628
Cumulative ug Equivalents Excreted								
-----								
Urine	4	4.199	0.656	1682.596	2597.339	1.778	857.314	543.249
	8	5038.007	4402.359	4730.076	4804.304	4455.428	4686.035	116.932
	12	5338.344	5269.947	5386.690	5566.922	5122.873	5336.955	72.692
	24	5528.103	5498.441	5751.850	5801.141	5472.031	5610.313	68.864
	48	5682.140	5588.695	5885.687	5859.049	5659.298	5734.974	58.320
	72	5715.766	5620.255	5958.615	5889.936	5717.525	5780.419	62.264
Feces	4	-a	-a	-a	-a	-a		-a
	8	-a	-a	-a	-a	-a		-a
	12	177.085	91.794	305.814	145.632	249.047	193.874	37.818
	24	547.553	789.194	677.318	565.481	673.619	650.633	43.770
	48	725.538	848.646	741.586	608.327	776.998	740.219	39.193
	72	740.356	857.441	760.718	620.275	799.284	755.615	39.276
Total	4	4.199	0.656	1682.596	2597.339	1.778	857.314	543.249
	8	5038.007	4402.359	4730.076	4804.304	4455.428	4686.035	116.932
	12	5515.429	5361.741	5692.504	5712.554	5371.920	5530.830	75.247
	24	6075.656	6287.635	6429.168	6366.622	6145.650	6260.946	66.259
	48	6407.678	6437.341	6627.273	6467.376	6436.296	6475.193	39.175
	72	6456.122	6477.696	6719.333	6510.211	6516.809	6536.034	47.130

(continued)

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TABLE II-7 (Concluded)

Excretum	Time After Dosing (hr)	Percent of Dose					Mean	S.E.
		No. 6	No. 7	No. 8	No. 9	No. 10		
Urine	4	0.061	0.010	23.699	38.252	0.025	12.409	7.921
	8	74.136	64.258	42.922	32.504	64.081	55.580	7.696
	12	4.424	12.666	9.249	11.232	9.603	9.435	1.394
	24	2.795	3.335	5.143	3.450	5.024	3.949	0.476
	48	2.269	1.317	1.885	0.853	2.694	1.804	0.328
	72	0.495	0.461	1.027	0.454	0.837	0.655	0.117
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	2.608	1.340	4.307	2.145	3.583	2.797	0.524
	24	5.456	10.181	5.232	6.183	6.109	6.632	0.906
	48	2.621	0.868	0.905	0.631	1.487	1.302	0.358
	72	0.218	0.128	0.269	0.176	0.321	0.222	0.034
Total	4	0.061	0.010	23.699	38.252	0.025	12.409	7.921
	8	74.197	64.258	42.922	32.504	64.081	55.592	7.704
	12	7.032	14.006	13.556	13.377	13.186	12.231	1.307
	24	8.251	13.516	10.375	9.633	11.133	10.582	0.874
	48	4.890	2.185	2.790	1.484	4.181	3.106	0.629
	72	0.713	0.589	1.296	0.630	1.158	0.877	0.146
Cumulative Percent of Dose								
Urine	4	0.061	0.010	23.699	38.252	0.025	12.409	7.921
	8	74.197	64.258	66.621	70.756	64.106	67.988	1.963
	12	78.621	76.924	75.870	81.988	73.709	77.422	1.392
	24	81.416	80.259	81.013	85.438	78.733	81.372	1.115
	48	83.685	81.576	82.898	86.291	81.427	83.175	0.885
	72	84.180	82.037	83.925	86.745	82.264	83.830	0.845
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	2.608	1.340	4.307	2.145	3.583	2.797	0.524
	24	8.064	11.521	9.539	8.328	9.692	9.429	0.614
	48	10.685	12.389	10.444	8.959	11.179	10.731	0.556
	72	10.903	12.517	10.713	9.135	11.500	10.954	0.553
Total	4	0.061	0.010	23.699	38.252	0.025	12.409	7.921
	8	74.258	64.258	66.621	70.756	64.106	68.000	1.973
	12	81.229	78.264	80.177	84.133	77.292	80.219	1.199
	24	89.480	91.780	90.552	93.766	88.425	90.801	0.927
	48	94.370	93.965	93.342	95.250	92.606	93.907	0.449
	72	95.083	94.554	94.638	95.880	93.764	94.784	0.347

a/ No feces during this time period.

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TABLE II-8

RADIOACTIVITY IN EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS FOLLOWING  
A SINGLE ORAL DOSE OF <sup>14</sup>C-MALATHION (40 mg/kg)

Excretum	Time							Mean	S.E.
	After Dosing (hr)	No. 11	No. 12	No. 13	No. 14	No. 15			
ug Equivalents Excreted									
Urine	4	4070.236	3530.589	3125.639	2652.160	450.507	2765.826	624.077	
	8	962.285	821.217	1264.233	1464.587	2444.711	1391.407	286.377	
	12	591.007	735.259	712.807	414.643	991.913	689.126	94.694	
	24	247.266	359.885	169.740	241.426	282.266	260.117	30.925	
	48	93.613	167.666	49.114	115.921	159.697	117.202	21.848	
	72	34.241	25.253	20.763	29.998	53.786	32.808	5.710	
Feces	4	-a	-a	-a	-a	-a		-a	
	8	-a	-a	-a	-a	-a		-a	
	12	47.792	0.039	336.595	0.024	98.966	96.683	62.704	
	24	264.024	191.956	86.313	349.600	74.653	193.309	52.420	
	48	61.023	85.514	11.516	70.854	54.784	56.738	12.439	
	72	6.852	12.767	1.920	8.858	10.082	8.096	1.817	
Total	4	4070.236	3530.589	3125.639	2652.160	450.507	2765.826	624.077	
	8	962.285	821.217	1264.233	1464.587	2444.711	1391.407	286.377	
	12	638.799	735.298	1049.402	414.667	1090.879	785.809	127.369	
	24	511.290	551.841	256.053	591.026	356.919	453.426	63.339	
	48	154.636	253.180	60.630	186.775	214.481	173.940	32.634	
	72	41.093	38.020	22.683	38.856	63.868	40.904	6.603	
Cumulative ug Equivalents Excreted									
Urine	4	4070.236	3530.589	3125.639	2652.160	450.507	2765.826	624.077	
	8	5032.521	4351.806	4389.872	4116.747	2895.218	4157.233	350.174	
	12	5623.528	5087.065	5102.679	4531.390	3887.131	4846.359	295.547	
	24	5870.794	5446.950	5272.419	4772.816	4169.397	5106.475	293.009	
	48	5964.407	5614.616	5321.533	4888.737	4329.094	5223.677	284.917	
	72	5998.648	5639.869	5342.296	4918.735	4382.880	5256.486	281.284	
Feces	4	-a	-a	-a	-a	-a		-a	
	8	-a	-a	-a	-a	-a		-a	
	12	47.792	0.039	336.595	0.024	98.966	96.683	62.704	
	24	311.816	191.995	422.908	349.624	173.619	289.992	47.352	
	48	372.839	277.509	434.424	420.478	228.403	346.731	40.375	
	72	379.691	290.276	436.344	429.336	238.485	354.826	39.062	
Total	4	4070.236	3530.589	3125.639	2652.160	450.507	2765.826	624.077	
	8	5032.521	4351.806	4389.872	4116.747	2895.218	4157.233	350.174	
	12	5671.320	5087.104	5439.274	4531.414	3986.097	4943.042	306.804	
	24	6182.610	5638.945	5695.327	5122.440	4343.016	5396.468	312.329	
	48	6337.246	5892.125	5755.957	5309.215	4557.497	5570.408	301.677	
	72	6378.339	5930.145	5778.640	5348.071	4621.365	5611.312	297.260	

(continued)

TABLE II-8 (Concluded)

Excretum	Time After Dosing (hr)	Percent of Dose						
		No. 11	No. 12	No. 13	No. 14	No. 15	Mean	S.E.
Urine	4	61.952	56.400	50.990	46.447	8.782	44.914	9.399
	8	14.647	13.119	20.624	25.649	47.655	24.339	6.241
	12	8.995	11.745	11.628	7.262	19.336	11.793	2.065
	24	3.763	5.749	2.768	4.228	5.502	4.402	0.554
	48	1.425	2.679	0.801	2.030	3.113	2.010	0.416
	72	0.521	0.403	0.339	0.526	1.048	0.567	0.125
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	0.727	0.001	5.491	0.000	1.929	1.630	1.028
	24	4.019	3.066	1.408	6.123	1.455	3.214	0.880
	48	0.929	1.366	0.188	1.241	1.068	0.958	0.206
	72	0.104	0.204	0.031	0.155	0.197	0.138	0.032
Total	4	61.952	56.400	50.990	46.447	8.782	44.914	9.399
	8	14.647	13.119	20.624	25.649	47.655	24.339	6.241
	12	9.722	11.746	17.119	7.262	21.265	13.423	2.546
	24	7.782	8.815	4.176	10.351	6.957	7.616	1.030
	48	2.354	4.045	0.989	3.271	4.181	2.968	0.592
	72	0.625	0.607	0.370	0.681	1.245	0.706	0.145
Cumulative Percent of Dose								
Urine	4	61.952	56.400	50.990	46.447	8.782	44.914	9.399
	8	76.599	69.519	71.614	72.096	56.437	69.253	3.405
	12	85.594	81.264	83.242	79.358	75.773	81.046	1.676
	24	89.357	87.013	86.010	83.586	81.275	85.448	1.395
	48	90.782	89.692	86.811	85.616	84.388	87.458	1.210
	72	91.303	90.095	87.150	86.142	85.436	88.025	1.141
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	0.727	0.001	5.491	0.000	1.929	1.630	1.028
	24	4.746	3.067	6.899	6.123	3.384	4.844	0.747
	48	5.675	4.433	7.087	7.364	4.452	5.802	0.625
	72	5.779	4.637	7.118	7.519	4.649	5.940	0.603
Total	4	61.952	56.400	50.990	46.447	8.782	44.914	9.399
	8	76.599	69.519	71.614	72.096	56.437	69.253	3.405
	12	86.321	81.265	88.733	79.358	77.702	82.676	2.094
	24	94.103	90.080	92.909	89.709	84.659	90.292	1.635
	48	96.457	94.125	93.898	92.980	88.840	93.260	1.245
	72	97.082	94.732	94.268	93.661	90.085	93.966	1.130

a/ No feces during this time period.



TABLE II-9

RADIOACTIVITY IN EXCRETA OF MALE SPRAGUE-DAWLEY RATS FOLLOWING  
 A SINGLE ORAL DOSE OF 14C-MALATHION (800 mg/kg)

Excretum	Time After Dosing (hr)	-----						
		No. 16	No. 17	No. 18	No. 19	No. 20	Mean	S.E.
-----								
ug Equivalents Excreted								
-----								
Urine	4	34229	26077	9749	33571	16773	24080	4774
	8	31722	40512	11256	31985	34217	29938	4932
	12	36331	21106	7122	32164	28307	25006	5126
	24	18637	24633	27367	23082	22222	23188	1435
	48	2112	7427	64532	7502	9143	18143	11658
	72	1250	2468	13000	1602	2692	4202	2216
Feces	4	-a	37	-a	2	-a	19	
	8	-a	-a	-a	-a	-a	-a	
	12	3796	12087	1090	883	360	3643	2194
	24	19364	9676	2218	18615	17417	13458	3300
	48	1324	3239	6124	4739	4786	4042	819
	72	548	435	3869	785	491	1226	664
Total	4	34229	26113	9749	33573	16773	24088	4775
	8	31722	40512	11256	31985	34217	29938	4932
	12	40127	33193	8213	33047	28667	28649	5429
	24	38002	34309	29585	41697	39639	36646	2140
	48	3436	10665	70655	12241	13929	22185	12249
	72	1798	2903	16869	2387	3182	5428	2870
Cumulative ug Equivalents Excreted								
-----								
Urine	4	34229	26077	9749	33571	16773	24080	4774
	8	65951	66589	21005	65556	50990	54018	8754
	12	102282	87695	28127	97720	79297	79024	13335
	24	120919	112328	55494	120802	101518	102212	12208
	48	123031	119755	120025	128304	110661	120355	2871
	72	124281	122222	133026	129906	113352	124557	3402
Feces	4	-a	37	-a	2	-a	19	
	8	-a	37	-a	2	-a	19	
	12	3796	12124	1090	885	360	3651	2201
	24	23161	21800	3308	19500	17777	17109	3572
	48	24484	25039	9432	24239	22563	21152	2959
	72	25033	25474	13301	25024	23054	22377	2307
Total	4	34229	26113	9749	33573	16773	24088	4775
	8	15951	16589	21005	55556	20357	25891	7483
	12	106078	99819	29217	98605	79656	82675	14075
	24	144080	134128	58802	140302	119295	119321	15708
	48	147516	144793	129457	152544	133224	141507	4373
	72	149313	147697	146327	154930	136406	146935	3012

(continued)

TABLE II-9 (Concluded)

Excretum	Time						Mean	S.E.
	After Dosing (hr)	No. 16	No. 17	No. 18	No. 19	No. 20		
Percent of Dose								
Urine	4	21.313	15.559	5.966	20.346	10.418	14.720	2.924
	8	19.752	24.172	6.888	19.385	21.252	18.290	2.972
	12	22.622	12.593	4.358	19.493	17.582	15.330	3.189
	24	11.604	14.698	16.749	13.989	13.802	14.168	0.827
	48	1.315	4.431	39.493	4.547	5.679	11.093	7.137
	72	0.779	1.472	7.956	0.971	1.672	2.570	1.356
Feces	4	-a	0.022	-a	0.001	-a	0.005	
	8	-a	-a	-a	-a	-a	-a	
	12	2.364	7.212	0.667	0.535	0.224	2.200	1.307
	24	12.057	5.773	1.357	11.282	10.818	8.257	2.049
	48	0.824	1.932	3.748	2.872	2.973	2.470	0.502
	72	0.341	0.260	2.368	0.476	0.305	0.750	0.406
Total	4	21.313	15.581	5.966	20.347	10.418	14.725	2.924
	8	19.752	24.172	6.888	19.385	21.252	18.290	2.972
	12	24.986	19.805	5.025	20.028	17.806	17.530	3.343
	24	23.661	20.471	18.106	25.271	24.620	22.426	1.359
	48	2.139	6.363	43.241	7.419	8.652	13.563	7.500
	72	1.120	1.732	10.324	1.447	1.977	3.320	1.757
Cumulative Percent of Dose								
Urine	4	21.313	15.559	5.966	20.346	10.418	14.720	2.924
	8	41.065	39.731	12.854	39.731	31.670	33.010	5.307
	12	63.687	52.324	17.212	59.224	49.252	48.340	8.184
	24	75.291	67.022	33.961	73.213	63.054	62.508	7.462
	48	76.606	71.453	73.454	77.760	68.733	73.601	1.653
	72	77.385	72.925	81.410	78.731	70.405	76.171	1.991
Feces	4	-a	0.022	-a	0.001	-a	0.005	
	8	-a	0.022	-a	0.001	-a	0.005	
	12	2.364	7.234	0.667	0.536	0.224	2.205	1.311
	24	14.421	13.007	2.024	11.818	11.042	10.462	2.185
	48	15.245	14.939	5.772	14.690	14.015	12.932	1.801
	72	15.586	15.199	8.140	15.166	14.320	13.682	1.401
Total	4	21.313	15.581	5.966	20.347	10.418	14.725	2.924
	8	39.303	39.731	12.854	39.731	31.670	32.658	5.184
	12	66.051	59.558	17.879	59.760	49.476	50.545	8.587
	24	89.712	80.029	35.985	85.031	74.096	72.971	9.604
	48	91.851	86.392	79.226	92.450	82.748	86.533	2.560
	72	92.971	88.124	89.550	93.897	84.725	89.853	1.665

a/ No feces during this time period.

TABLE II-10

RADIOACTIVITY IN EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS FOLLOWING  
A SINGLE ORAL DOSE OF 14C-MALATHION (800 mg/kg)

Excretum	Time After Dosing (hr)						Mean	S.E.
		No. 21	No. 22	No. 23	No. 24	No. 25		
		ug Equivalents Excreted						
		-----						
Urine	4	45015	44259	29911	20980	26534	33340	4829
	8	18597	32833	37192	22784	46979	31677	5080
	12	34766	23993	14808	16693	27678	23588	3650
	24	12872	12878	25812	22653	17824	18408	2592
	48	2654	2234	3219	8834	1951	3778	1282
	72	939	1356	1029	3378	1026	1546	463
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	6874	2900	5	2459	632	2574	1204
	24	2067	3219	7084	4219	5495	4417	874
	48	1435	988	1590	2271	1092	1475	227
	72	89	173	245	656	78	248	106
Total	4	45015	44259	29911	20980	26534	33340	4829
	8	18597	32833	37192	22784	46979	31677	5080
	12	41640	26893	14813	19152	28311	26162	4597
	24	14939	16096	32896	26872	23319	22824	3358
	48	4089	3222	4809	11105	3043	5254	1497
	72	1029	1530	1274	4033	1104	1794	566
		Cumulative ug Equivalents Excreted						
		-----						
Urine	4	45015	44259	29911	20980	26534	33340	4829
	8	63611	77092	67104	43763	73513	65017	5814
	12	98377	101085	81912	60457	101191	88604	7896
	24	111249	113963	107724	83110	119015	107012	6254
	48	113903	116197	110943	91944	120967	110791	4988
	72	114842	117554	111972	95321	121993	112336	4563
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	6874	2900	5	2459	632	2574	1204
	24	8941	6119	7089	6678	6127	6991	520
	48	10376	7107	8679	8949	7219	8466	605
	72	10465	7280	8924	9605	7297	8714	631
Total	4	45015	44259	29911	20980	26534	33340	4829
	8	63611	77092	67104	43763	73513	65017	5814
	12	105251	103985	81916	62916	101824	91178	8246
	24	120190	120082	114813	89787	125143	114003	6270
	48	124279	123304	119622	100893	128186	119257	4789
	72	125307	124834	120896	104926	129290	121051	4245

(continued)

TABLE II-10 (Concluded)

Excretum	Time After Dosing (hr)	Percent of Dose					Mean	S.E.
		No. 21	No. 22	No. 23	No. 24	No. 25		
Urine	4	33.050	34.309	23.777	15.942	19.424	25.300	3.644
	8	13.654	25.452	29.565	17.313	34.392	24.075	3.826
	12	25.526	18.599	11.771	12.685	20.262	17.769	2.539
	24	9.451	9.982	20.519	17.214	13.048	14.043	2.128
	48	1.948	1.732	2.558	6.713	1.429	2.876	0.977
	72	0.690	1.051	0.818	2.566	0.751	1.175	0.353
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	5.047	2.248	0.004	1.869	0.463	1.926	0.885
	24	1.517	2.495	5.631	3.206	4.023	3.374	0.699
	48	1.054	0.766	1.264	1.726	0.799	1.122	0.176
	72	0.065	0.134	0.195	0.499	0.057	0.190	0.081
Total	4	33.050	34.309	23.777	15.942	19.424	25.300	3.644
	8	13.654	25.452	29.565	17.313	34.392	24.075	3.826
	12	30.573	20.847	11.775	14.554	20.725	19.695	3.239
	24	10.968	12.477	26.150	20.420	17.071	17.417	2.751
	48	3.002	2.498	3.822	8.439	2.228	3.998	1.143
	72	0.755	1.185	1.013	3.065	0.808	1.365	0.432
Cumulative Percent of Dose								
Urine	4	33.050	34.309	23.777	15.942	19.424	25.300	3.644
	8	46.704	59.761	53.342	33.255	53.816	49.376	4.529
	12	72.230	78.360	65.113	45.940	74.078	67.144	5.716
	24	81.681	88.342	85.632	63.154	87.126	81.187	4.646
	48	83.629	90.074	88.190	69.867	88.555	84.063	3.708
	72	84.319	91.125	89.008	72.433	89.306	85.238	3.393
Feces	4	-a	-a	-a	-a	-a	-a	-a
	8	-a	-a	-a	-a	-a	-a	-a
	12	5.047	2.248	0.004	1.869	0.463	1.926	0.885
	24	6.564	4.743	5.635	5.075	4.486	5.301	0.370
	48	7.618	5.509	6.899	6.801	5.285	6.422	0.443
	72	7.683	5.643	7.094	7.300	5.342	6.612	0.469
Total	4	33.050	34.309	23.777	15.942	19.424	25.300	3.644
	8	46.704	59.761	53.342	33.255	53.816	49.376	4.529
	12	77.277	80.608	65.117	47.809	74.541	69.070	5.908
	24	88.245	93.085	91.267	68.229	91.612	86.488	4.632
	48	91.247	95.583	95.089	76.668	93.840	90.485	3.535
	72	92.002	96.768	96.102	79.733	94.648	91.851	3.138

a/ No feces during this time period.

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TABLE II-11

RADIOACTIVITY IN EXCRETA OF MALE SPRAGUE-DAWLEY RATS TREATED  
WITH MULTIPLE ORAL DOSES OF MALATHION (40 mg/kg/day) -a

Excretum	Time							
	After Dosing (hr)	No. 26	No. 27	No. 28	No. 29	No. 30	Mean	S.E.
ug Equivalents Excreted								
Urine	4	2387.922	1640.527	4522.092	3962.878	4571.259	3416.936	594.407
	8	4303.195	4572.891	3351.933	2526.973	1797.338	3310.466	523.659
	12	457.139	1222.314	924.878	850.370	1047.091	900.358	127.422
	24	620.844	554.009	889.950	485.994	764.064	662.972	73.044
	48	229.304	94.960	222.385	179.559	89.290	163.100	30.216
	72	77.731	38.597	71.139	101.442	21.693	62.120	14.246
Feces	4	-b	-b	-b	-b	-b		-b
	8	-b	-b	-b	-b	-b		-b
	12	1.113	76.515	0.146	-b	-b	25.925	25.297
	24	477.667	870.305	633.205	609.331	646.935	647.489	63.304
	48	80.563	53.963	75.080	107.631	186.367	100.721	23.058
	72	20.192	5.822	23.441	77.183	17.532	28.834	12.447
Total	4	2387.922	1640.527	4522.092	3962.878	4571.259	3416.936	594.407
	8	4303.195	4572.891	3351.933	2526.973	1797.338	3310.466	523.659
	12	458.252	1298.829	925.024	850.370	1047.091	915.913	137.416
	24	1098.511	1424.314	1523.155	1095.325	1410.999	1310.461	89.307
	48	309.867	148.923	297.465	287.190	275.657	263.820	29.274
	72	97.923	44.419	94.580	178.625	39.225	90.954	25.089
Cumulative ug Equivalents Excreted								
Urine	4	2387.922	1640.527	4522.092	3962.878	4571.259	3416.936	594.407
	8	6691.117	6213.418	7874.025	6489.851	6368.597	6727.402	297.104
	12	7148.256	7435.732	8798.903	7340.221	7415.688	7627.760	297.156
	24	7769.100	7989.741	9688.853	7826.215	8179.752	8290.732	356.758
	48	7998.404	8084.701	9911.238	8005.774	8269.042	8453.832	367.601
	72	8076.135	8123.298	9982.377	8107.216	8290.735	8515.952	368.497
Feces	4	-b	-b	-b	-b	-b		-b
	8	-b	-b	-b	-b	-b		-b
	12	1.113	76.515	0.146	-b	-b	25.925	25.297
	24	478.780	946.820	633.351	609.331	646.935	663.043	76.977
	48	559.343	1000.783	708.431	716.962	833.302	763.764	73.503
	72	579.535	1006.605	731.872	794.145	850.834	792.598	70.099
Total	4	2387.922	1640.527	4522.092	3962.878	4571.259	3416.936	594.407
	8	6691.117	6213.418	7874.025	5025.421	6368.597	6434.516	456.991
	12	7149.369	7512.247	8799.049	5875.791	7415.688	7350.429	466.043
	24	8247.880	8936.561	10322.204	8435.546	8826.687	8953.776	364.410
	48	8557.747	9085.484	10619.669	8722.736	9102.344	9217.596	365.844
	72	8655.670	9129.903	10714.249	8901.361	9141.569	9308.550	362.481

(continued)

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TABLE II-11 (Concluded)

Excretum	Time							Mean	S.E.
	After Dosing (hr)	No. 26	No. 27	No. 28	No. 29	No. 30			
Percent of Dose									
Urine	4	24.901	16.791	38.849	40.603	47.767	33.782	5.636	
	8	44.871	46.806	28.796	25.892	18.781	33.029	5.486	
	12	4.767	12.511	7.946	8.713	10.942	8.976	1.327	
	24	6.474	5.671	7.646	4.980	7.984	6.551	0.570	
	48	2.391	0.972	1.911	1.839	0.933	1.609	0.284	
	72	0.810	0.395	0.612	1.039	0.227	0.617	0.144	
Feces	4	-b	-b	-b	-b	-b	-b	-b	
	8	-b	-b	-b	-b	-b	-b	-b	
	12	0.012	0.783	0.001	-b	-b	0.265	0.259	
	24	4.981	8.908	5.440	6.243	1.152	5.345	1.249	
	48	0.840	0.552	0.645	1.103	1.947	1.017	0.251	
	72	0.211	0.060	0.201	0.791	0.183	0.289	0.128	
Total	4	24.901	16.791	38.849	40.603	47.767	33.782	5.636	
	8	44.871	46.806	28.796	25.892	18.781	33.029	5.486	
	12	4.779	13.294	7.947	8.713	10.942	9.135	1.434	
	24	11.455	14.579	13.086	11.223	9.136	11.896	0.919	
	48	3.231	1.524	2.556	2.942	2.880	2.627	0.296	
	72	1.021	0.455	0.813	1.830	0.410	0.906	0.257	
Cumulative Percent of Dose									
Urine	4	24.901	16.791	38.849	40.603	47.767	33.782	5.636	
	8	69.772	63.597	67.645	66.495	66.548	66.811	0.999	
	12	74.539	76.108	75.591	75.208	77.490	75.787	0.497	
	24	81.013	81.779	83.237	80.188	85.474	82.338	0.931	
	48	83.404	82.751	85.148	82.027	86.407	83.947	0.803	
	72	84.214	83.146	85.760	83.066	86.634	84.564	0.710	
Feces	4	-b	-b	-b	-b	-b	-b	-b	
	8	-b	-b	-b	-b	-b	-b	-b	
	12	0.012	0.783	0.001	-b	-b	0.265	0.259	
	24	4.993	9.691	5.441	6.243	1.152	5.504	1.365	
	48	5.833	10.243	6.086	7.346	3.099	6.521	1.160	
	72	6.044	10.303	6.287	8.137	3.282	6.811	1.168	
Total	4	24.901	16.791	38.849	40.603	47.767	33.782	5.636	
	8	69.772	63.597	67.645	66.495	66.548	66.811	0.999	
	12	74.551	76.891	75.592	75.208	77.490	75.946	0.543	
	24	86.006	91.470	88.678	86.431	86.626	87.842	1.017	
	48	89.237	92.994	91.234	89.373	89.506	90.469	0.728	
	72	90.258	93.449	92.047	91.203	89.916	91.375	0.639	

a/ Fifteen consecutive daily doses of nonlabeled malathion followed by a sixteenth dose of 14C-malathion.

b/ No feces during this time period.

TABLE II-12

RADIOACTIVITY IN EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS TREATED  
WITH MULTIPLE ORAL DOSES OF MALATHION (40 mg/kg/day) -a

Excretum	Time After Dosing (hr)	-----						
		No. 31	No. 32	No. 33	No. 34	No. 35	Mean	S.E.
-----								
ug Equivalents Excreted								
-----								
Urine	4	4163.453	3903.342	4515.020	2664.820	3700.679	3789.463	312.408
	8	883.534	2105.891	352.510	2931.998	2381.991	1731.185	480.893
	12	857.194	565.496	1033.572	898.907	597.523	790.538	90.319
	24	867.975	445.195	593.482	504.861	482.518	578.806	76.297
	48	134.507	116.836	48.056	112.357	85.632	99.478	15.050
	72	49.620	39.521	26.849	46.439	23.998	37.285	5.130
Feces	4	-b	-b	-b	-b	-b	-b	-b
	8	-b	-b	-b	-b	-b	-b	-b
	12	-b	-b	-b	0.026	-b	0.026	
	24	302.090	268.983	194.658	484.934	434.836	337.100	53.648
	48	157.151	138.227	100.961	75.506	95.223	113.414	14.919
	72	11.495	17.227	10.770	12.947	9.086	12.305	1.379
Total	4	4163.453	3903.342	4515.020	2664.820	3700.679	3789.463	312.408
	8	883.534	2105.891	352.510	2931.998	2381.991	1731.185	480.893
	12	857.194	565.496	1033.572	898.933	597.523	790.544	90.321
	24	1170.065	714.178	788.140	989.795	917.354	915.906	79.711
	48	291.658	255.063	149.017	187.863	180.855	212.891	26.187
	72	61.115	56.748	37.619	59.386	33.084	49.590	5.898
Cumulative ug Equivalents Excreted								
-----								
Urine	4	4163.453	3903.342	4515.020	2664.820	3700.679	3789.463	312.408
	8	5046.987	6009.233	4867.530	5596.818	6082.670	5520.648	246.100
	12	5904.181	6574.729	5901.102	6495.725	6680.193	6311.186	169.337
	24	6772.156	7019.924	6494.584	7000.586	7162.711	6889.992	116.988
	48	6906.663	7136.760	6542.640	7112.943	7248.343	6989.470	124.593
	72	6956.283	7176.281	6569.489	7159.382	7272.341	7026.755	125.363
Feces	4	-b	-b	-b	-b	-b	-b	-b
	8	-b	-b	-b	-b	-b	-b	-b
	12	-b	-b	-b	0.026	-b	0.026	
	24	302.090	268.983	194.658	484.960	434.836	337.105	53.652
	48	459.241	407.210	295.619	560.466	530.059	450.519	47.096
	72	470.736	424.437	306.389	573.413	539.145	462.824	46.938
Total	4	4163.453	3903.342	4515.020	2664.820	3700.679	3789.463	312.408
	8	5046.987	6009.233	4867.530	5596.818	6082.670	5520.648	246.100
	12	5904.181	6574.729	5901.102	6495.751	6680.193	6311.191	169.338
	24	7074.246	7288.907	6689.242	7485.546	7597.547	7227.098	161.323
	48	7365.904	7543.970	6838.259	7673.409	7778.402	7439.989	165.437
	72	7427.019	7600.718	6875.878	7732.795	7811.486	7489.579	166.684

(continued)

TABLE II-12 (Concluded)

Excretum	Time							Mean	S.E.
	After Dosing (hr)	No. 31	No. 32	No. 33	No. 34	No. 35			
Percent of Dose									
Urine	4	53.309	49.788	57.152	33.062	45.407	47.744	4.151	
	8	11.313	26.861	4.462	36.377	29.227	21.648	5.932	
	12	10.975	7.213	13.083	11.152	7.332	9.951	1.154	
	24	11.114	5.678	7.512	6.264	5.921	7.298	1.005	
	48	1.723	1.490	0.608	1.394	1.051	1.253	0.194	
	72	0.635	0.504	0.340	0.576	0.295	0.470	0.066	
Feces	4	-b	-b	-b	-b	-b	-b		
	8	-b	-b	-b	-b	-b	-b		
	12	-b	-b	-b	0.000	-b	0.000		
	24	3.868	3.431	2.464	6.017	5.335	4.223	0.644	
	48	2.012	1.763	1.278	0.937	1.168	1.432	0.198	
	72	0.147	0.220	0.136	0.161	0.111	0.155	0.018	
Total	4	53.309	49.788	57.152	33.062	45.407	47.744	4.151	
	8	11.313	26.861	4.462	36.377	29.227	21.648	5.932	
	12	10.975	7.213	13.083	11.152	7.332	9.951	1.154	
	24	14.982	9.109	9.976	12.281	11.256	11.521	1.021	
	48	3.735	3.253	1.886	2.331	2.219	2.685	0.347	
	72	0.782	0.724	0.476	0.737	0.406	0.625	0.077	
Cumulative Percent of Dose									
Urine	4	53.309	49.788	57.152	33.062	45.407	47.744	4.151	
	8	64.622	76.649	61.614	69.439	74.634	69.392	2.858	
	12	75.597	83.862	74.697	80.591	81.966	79.343	1.795	
	24	86.711	89.540	82.209	86.855	87.887	86.640	1.218	
	48	88.434	91.030	82.817	88.249	88.938	87.894	1.362	
	72	89.069	91.534	83.157	88.825	89.233	88.364	1.390	
Feces	4	-b	-b	-b	-b	-b	-b		
	8	-b	-b	-b	-b	-b	-b		
	12	-b	-b	-b	0.000	-b	0.000		
	24	3.868	3.431	2.464	6.017	5.335	4.223	0.644	
	48	5.880	5.194	3.742	6.954	6.503	5.655	0.563	
	72	6.027	5.414	3.878	7.115	6.614	5.810	0.561	
Total	4	53.309	49.788	57.152	33.062	45.407	47.744	4.151	
	8	64.622	76.649	61.614	69.439	74.634	69.392	2.858	
	12	75.597	83.862	74.697	80.591	81.966	79.343	1.796	
	24	90.579	92.971	84.673	92.872	93.222	90.863	1.619	
	48	94.314	96.224	86.559	95.203	95.441	93.548	1.774	
	72	95.096	96.948	87.035	95.940	95.847	94.173	1.809	

a/ Fifteen consecutive daily doses of nonlabeled malathion followed by a sixteenth dose of 14C-malathion.

b/ No feces during this time period.



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TABLE II-13

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF MALE AND  
FEMALE SPRAGUE-DAWLEY RATS 72 H FOLLOWING A SINGLE ORAL  
DOSE OF 14C-MALATHION : PRELIMINARY STUDY

Tissue	40 mg/kg Dose		800 mg/kg Dose	
	No. 1 (M)	No. 2 (F)	No. 5 (M)-a	No. 4 (F)
	ug Equivalents/g			
Blood	0.212	0.182	3.887	3.481
Plasma	0.176	0.218	4.220	3.682
RBCs	0.179	0.119	0.432 *	2.857
Liver	1.310	0.669	27.106	16.580
Kidneys	0.613	0.600	6.950	10.975 *
Lungs	0.213	0.132	2.938	2.343 *
Brain	0.188	0.231	1.834	2.381
Heart	0.107	0.050 *	1.592	0.189 *
Spleen	0.281	0.212	2.608 *	2.161
Gonads	0.221	0.161	2.334	3.680
Uterus	na	0.116 *	na	1.434
Adrenals	0.380	0.230	5.498	8.310 *
Fat	0.305	0.066 *	6.660	4.700
Skin	0.344	0.196	14.953	3.547
Muscle	0.085 *	0.035 *	0.907 *	0.193
Bone	0.356	0.204	4.276	2.722
GI Tract	0.110	0.454	5.903	2.341
Carcass	0.174	0.110	17.243	7.508

(continued)

TABLE II-13 (Concluded)

Tissue/ Excretum	40 mg/kg Dose		800 mg/kg Dose	
	No. 1 (M)	No. 2 (F)	No. 5 (M)-a	No. 4 (F)
	Percent of Dose			
Blood -b	0.040	0.034	0.034	0.032
Plasma -b,c	0.020	0.025	0.022	0.020
RBCs -b,c	0.014	0.009	0.001 *	0.010
Liver	0.213	0.064	0.244	0.110
Kidneys	0.019	0.020	0.013	0.015 *
Lungs	0.003	0.002	0.002	0.002 *
Brain	0.004	0.009	0.002	0.004
Heart	0.001	0.000 *	0.001	0.000 *
Spleen	0.002	0.001	0.001 *	0.001
Gonads	0.007	0.000	0.003	0.000
Uterus	na	0.001 *	na	0.000
Adrenals	0.000	0.000	0.000	0.000 *
Fat -b	0.091	0.019 *	0.093	0.067
Skin -b	0.149	0.084	0.304	0.074
Muscle -b	0.092 *	0.038 *	0.047 *	0.010
Bone -b	0.077	0.044	0.043	0.028
GI Tract	0.038	0.091	0.129	0.035
Carcass -c	0.385	0.207	1.840	0.804
Expired Air	0.933	0.367	0.673	0.467
Urine	85.334	89.756	68.834	74.055
Feces	8.586	4.049	14.549	15.254
Recovery	95.589	94.579	84.972	90.154

a/ Values for No. 5 (M) adjusted to reflect a partial loss of dose (see text).

b/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

c/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.

na Not applicable.

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TABLE II-14

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF MALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING A SINGLE ORAL DOSE OF <sup>14</sup>C-MALATHION (40 mg/kg)

Tissue	No. 6	No. 7	No. 8	No. 9	No. 10	Mean	S.E.
	ug Equivalents/g						
Blood	0.197	0.184	0.276	0.211	0.226	0.219	0.016
Plasma	0.190	0.182	0.278	0.203	0.220	0.215	0.017
RBCs	0.158	0.127	0.142	0.150	0.142	0.144	0.005
Liver	0.693	0.886	0.623	0.918	0.991	0.822	0.070
Kidneys	0.318	0.308	0.304	0.313	0.333	0.315	0.005
Lungs	0.087 *	0.075 *	0.076 *	0.072 *	0.113	0.085	0.008
Brain	0.147	0.148	0.147	0.158	0.170	0.154	0.005
Heart	0.085 *	0.054 *	0.064 *	0.075 *	0.086 *	0.073	0.006
Spleen	0.156 *	0.128 *	0.145 *	0.173 *	0.180 *	0.156	0.009
Testes	0.186	0.172	0.182	0.206	0.220	0.193	0.009
Adrenals	0.364	0.272	0.256	0.353	0.504	0.350	0.044
Fat	0.359	0.302	0.176	0.361	0.617	0.363	0.072
Skin	0.221	0.226	0.201	0.240	0.265	0.231	0.011
Muscle	0.033 *	0.039 *	0.027 *	0.051 *	0.031 *	0.036	0.004
Bone	0.223	0.236	0.244	0.252	0.288	0.249	0.011
GI Tract	0.084 *	0.055 *	0.111	0.067 *	0.085 *	0.080	0.009
Carcass	0.136	0.156	0.115	0.164	0.168	0.148	0.010

(continued)

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TABLE II-14 (Concluded)

Tissue/ Excretum	No. 6	No. 7	No. 8	No. 9	No. 10	Mean	S.E.
Percent of Dose							
Blood -a	0.038	0.035	0.053	0.040	0.044	0.042	0.003
Plasma -a,b	0.022	0.021	0.032	0.023	0.026	0.025	0.002
RBCs -a,b	0.012	0.010	0.011	0.011	0.011	0.011	0.000
Liver	0.185	0.242	0.118	0.219	0.258	0.204	0.025
Kidneys	0.016	0.017	0.014	0.015	0.018	0.016	0.001
Lungs	0.002 *	0.002 *	0.002 *	0.002 *	0.003	0.002	0.000
Brain	0.007	0.008	0.006	0.007	0.009	0.007	0.001
Heart	0.001 *	0.001 *	0.001 *	0.001 *	0.001 *	0.001	0.000
Spleen	0.002 *	0.001 *	0.001 *	0.002 *	0.002 *	0.002	0.000
Testes	0.007	0.006	0.007	0.009	0.009	0.008	0.001
Adrenals	0.000	0.000	0.000	0.000	0.001	0.000	0.000
Fat -a	0.110	0.091	0.053	0.108	0.189	0.110	0.022
Skin -a	0.099	0.099	0.088	0.104	0.118	0.102	0.005
Muscle -a	0.036 *	0.042 *	0.029 *	0.055 *	0.034 *	0.039	0.004
Bone -a	0.050	0.052	0.054	0.055	0.064	0.055	0.002
GI Tract	0.066 *	0.041 *	0.040	0.046 *	0.070 *	0.053	0.006
Carcass -b	0.344	0.384	0.251	0.395	0.409	0.357	0.029
Urine	84.180	82.037	83.925	86.745	82.264	83.830	0.845
Feces	10.903	12.517	10.713	9.135	11.500	10.954	0.553
Recovery	95.702	95.191	95.104	96.543	94.584	95.425	0.331

a/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

b/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.

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TABLE II-15

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING A SINGLE ORAL DOSE OF 14C-MALATHION (40 mg/kg)

Tissue	No. 11	No. 12	No. 13	No. 14	No. 15	Mean	S.E.
ug Equivalents/g							
Blood	0.219	0.182	0.168	0.155	0.120	0.169	0.016
Plasma	0.213	0.166	0.151	0.130	0.109	0.154	0.018
RBCs	0.225	0.160	0.156	0.151	0.091	0.157	0.021
Liver	0.440	0.702	0.557	0.656	0.475	0.566	0.050
Kidneys	0.308	0.283	0.257	0.257	0.191	0.259	0.020
Lungs	0.055 *	0.060	0.048 *	0.052 *	0.046 *	0.052	0.002
Brain	0.093 *	0.085 *	0.117 *	0.118	0.077 *	0.098	0.008
Heart	0.107 *	0.057 *	0.051 *	0.033 *	0.012 *	0.052	0.016
Spleen	0.122 *	0.130	0.104	0.118 *	0.085 *	0.112	0.008
Ovaries	0.128	0.305	0.106	0.096	0.074	0.142	0.042
Uterus	0.129 *	0.131 *	0.130 *	0.090 *	0.059	0.108	0.014
Adrenals	0.266	0.344	0.215	0.221	0.010 *	0.211	0.055
Fat	0.296	0.527	0.189	0.154	0.210	0.275	0.067
Skin	0.177	0.234	0.165	0.187	0.283	0.209	0.022
Muscle	0.016 *	0.037 *	0.036 *	0.033 *	0.020 *	0.028	0.004
Bone	0.183	0.206	0.192	0.180	0.118	0.176	0.015
GI Tract	0.101	0.133	0.052 *	0.080	0.106	0.094	0.014
Carcass	0.157	0.108	0.208	0.173	0.329	0.195	0.037

(continued)

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TABLE II-15 (Concluded)

Tissue/ Excretum	No. 11	No. 12	No. 13	No. 14	No. 15	Mean	S.E.
Percent of Dose							
Blood -a	0.039	0.032	0.032	0.030	0.025	0.032	0.002
Plasma -a,b	0.013	0.018	0.017	0.015	0.013	0.015	0.001
RBCs -a,b	0.016	0.011	0.012	0.012	0.007	0.012	0.001
Liver	0.083	0.146	0.131	0.145	0.131	0.127	0.012
Kidneys	0.013	0.014	0.012	0.011	0.011	0.012	0.001
Lungs	0.002 *	0.002 *	0.002 *	0.002 *	0.002 *	0.002	0.000
Brain	0.004	0.005 *	0.006	0.006	0.004 *	0.005	0.000
Heart	0.001 *	0.001 *	0.001 *	0.000 *	0.000 *	0.001	0.000
Spleen	0.001 *	0.002	0.001	0.001 *	0.001 *	0.001	0.000
Ovaries	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Uterus	0.001 *	0.001 *	0.001 *	0.001 *	0.001	0.001	0.000
Adrenals	0.000	0.000	0.000	0.000	0.000 *	0.000	0.000
Fat -a	0.083	0.147	0.057	0.047	0.068	0.080	0.018
Skin -a	0.072	0.095	0.073	0.082	0.133	0.091	0.011
Muscle -a	0.016 *	0.038 *	0.039 *	0.036 *	0.024 *	0.031	0.005
Bone -a	0.037	0.042	0.042	0.040	0.028	0.038	0.003
GI Tract	0.059	0.079	0.034 *	0.050	0.086	0.062	0.009
Carcass -b	0.337	0.259	0.479	0.407	0.801	0.457	0.094
Urine	91.303	90.095	87.150	86.142	85.436	88.025	1.141
Feces	5.779	4.637	7.118	7.527	4.464	5.905	0.625
Recovery	97.493	95.337	94.699	94.120	90.414	94.413	1.151

a/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

b/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.

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TABLE II-16

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF MALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING A SINGLE ORAL DOSE OF 14C-MALATHION (800 mg/kg)

Tissue	No. 16	No. 17	No. 18	No. 19	No. 20	Mean	S.E.
	ug Equivalents/g						
Blood	3.069	3.062	5.679	3.366	2.835	3.602	0.526
Plasma	2.020	2.919	6.639	2.663	2.781	3.404	0.823
RBCs	3.569	2.856	2.915	3.281	2.438	3.012	0.193
Liver	17.356	13.288	23.870	17.704	12.624	16.968	2.009
Kidneys	5.028	5.361	10.016	6.250	5.122	6.355	0.940
Lungs	1.692 *	1.901	4.273	1.792	1.282 *	2.188	0.532
Brain	1.807	1.374	2.761	1.758	1.296 *	1.799	0.261
Heart	0.626 *	0.971 *	1.939	1.230 *	1.061 *	1.165	0.217
Spleen	3.293	2.772	6.346	3.453	2.384	3.650	0.700
Testes	2.385	1.678 *	3.893	2.699	1.260 *	2.383	0.455
Adrenals	4.560	5.529	9.607	5.628	4.195	5.904	0.966
Fat	5.764	8.220	3.476	7.178	4.133	5.754	0.892
Skin	3.214	12.374	4.021	3.774	8.099	6.296	1.750
Muscle	0.848 *	0.774 *	0.465 *	1.066 *	0.383 *	0.707	0.126
Bone	4.347	3.459	6.046	4.139	3.615	4.321	0.461
GI Tract	1.017 *	2.709	18.944	4.791	3.843	6.261	3.233
Carcass	2.629	6.731	6.717	2.833	11.297	6.041	1.589

(continued)

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TABLE II-16 (Concluded)

Tissue/ Excretum	No. 16	No. 17	No. 18	No. 19	No. 20	Mean	S.E.
	Percent of Dose						
Blood -a	0.028	0.028	0.052	0.031	0.027	0.033	0.005
Plasma -a,b	0.011	0.016	0.037	0.015	0.016	0.019	0.005
RBCs -a,b	0.013	0.011	0.011	0.012	0.009	0.011	0.001
Liver	0.201	0.155	0.265	0.202	0.140	0.193	0.022
Kidneys	0.013	0.013	0.024	0.015	0.012	0.015	0.002
Lungs	0.002 *	0.002	0.004	0.002	0.001 *	0.002	0.000
Brain	0.004	0.003	0.005	0.003	0.002 *	0.003	0.001
Heart	0.000 *	0.001 *	0.001	0.001 *	0.001 *	0.001	0.000
Spleen	0.001	0.001	0.005	0.001	0.001 *	0.002	0.001
Testes	0.004	0.003 *	0.006	0.004	0.002	0.004	0.001
Adrenals	0.000	0.000	0.000	0.000	0.000 *	0.000	0.000
Fat -a	0.083	0.119	0.050	0.104	0.062	0.084	0.013
Skin -a	0.067	0.261	0.085	0.080	0.175	0.134	0.037
Muscle -a	0.044 *	0.041 *	0.025 *	0.056 *	0.021 *	0.037	0.006
Bone -a	0.046	0.036	0.064	0.044	0.039	0.046	0.005
GI Tract	0.035 *	0.081	0.409	0.150	0.115	0.158	0.066
Carcass -b	0.285	0.744	0.699	0.314	1.288	0.666	0.182
Urine	77.385	72.925	81.410	78.731	70.405	76.171	1.991
Feces	15.586	15.199	8.140	15.166	14.320	13.682	1.401
Recovery	93.499	88.868	90.545	94.590	85.323	90.565	1.661

a/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

b/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.



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TABLE II-17

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING A SINGLE ORAL DOSE OF <sup>14</sup>C-MALATHION (800 mg/kg)

Tissue	No. 21	No. 22	No. 23	No. 24	No. 25	Mean	S.E.
	ug Equivalents/g						
Blood	2.015	2.791	1.867	2.222	2.009	2.181	0.163
Plasma	1.701	2.387	1.557	2.518	1.619	1.956	0.205
RBCs	2.160	2.938	1.993	1.456	2.111	2.132	0.237
Liver	9.591	10.971	7.326	6.281	12.577	9.349	1.154
Kidneys	3.320	4.355	3.211	4.078	4.166	3.826	0.234
Lungs	0.652 *	0.088 *	1.415 *	0.633 *	0.682 *	0.694	0.211
Brain	0.971 *	1.094 *	0.744 *	0.864 *	1.331 *	1.001	0.101
Heart	1.039 *	0.980 *	0.569 *	0.523 *	0.658 *	0.754	0.107
Spleen	1.864	2.026	1.677 *	1.699 *	2.078	1.869	0.082
Ovaries	1.611	3.526	1.373	1.807	1.603	1.984	0.392
Uterus	2.245 *	1.307 *	0.827 *	0.895 *	1.806 *	1.416	0.271
Adrenals	3.537	2.831	2.205	-c	3.495	3.017	0.315
Fat	3.687	2.977	2.802	2.133	1.647 *	2.649	0.352
Skin	3.218	3.143	2.139	1.192 *	5.036	2.946	0.641
Muscle	0.355 *	0.602 *	0.265 *	0.404 *	0.282 *	0.382	0.061
Bone	2.495	3.030	2.456	2.413	2.811	2.641	0.120
GI Tract	1.038 *	1.456 *	0.903 *	4.158	1.871	1.885	0.593
Carcass	1.782	2.745	1.367	13.363	3.215	4.494	2.242

(continued)

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TABLE II-17 (Concluded)

Tissue/ Excretum	No. 21	No. 22	No. 23	No. 24	No. 25	Mean	S.E.
	Percent of Dose						
Blood -a	0.018	0.026	0.017	0.021	0.019	0.020	0.002
Plasma -a,b	0.009	0.013	0.009	0.014	0.009	0.011	0.001
RBCs -a,b	0.008	0.011	0.007	0.006	0.008	0.008	0.001
Liver	0.109	0.123	0.090	0.075	0.132	0.106	0.010
Kidneys	0.008	0.010	0.008	0.011	0.010	0.009	0.001
Lungs	0.001 *	0.002 *	0.003 *	0.001 *	0.001 *	0.002	0.000
Brain	0.002 *	0.002 *	0.002 *	0.002 *	0.003 *	0.002	0.000
Heart	0.001 *	0.001 *	0.000 *	0.000 *	0.000 *	0.000	0.000
Spleen	0.001	0.001	0.001 *	0.001 *	0.001	0.001	0.000
Ovaries	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uterus	0.001 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000	0.000
Adrenals	0.000	0.000	0.000	-c	0.000	0.000	0.000
Fat -a	0.053	0.044	0.040	0.032	0.024 *	0.039	0.005
Skin -a	0.067	0.067	0.045	0.026 *	0.107	0.062	0.014
Muscle -a	0.019 *	0.032 *	0.014 *	0.022 *	0.015 *	0.020	0.003
Bone -a	0.026	0.032	0.026	0.026	0.030	0.028	0.001
GI Tract	0.037 *	0.044 *	0.031 *	0.129	0.051	0.058	0.018
Carcass -b	0.186	0.305	0.150 *	1.517	0.349	0.501	0.257
Urine	84.319	91.125	89.008	72.433	89.306	85.238	3.393
Feces	7.683	5.643	7.094	7.299	5.342	6.612	0.469
Recovery	92.345	97.152	96.379	80.078	95.041	92.199	3.139

a/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

b/ Values not included in recovery estimates (see text).

c/ Sample lost during oxidizing.

\* Based on insignificant counts, i.e., less than two times the background.

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TABLE II-18

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF MALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING MULTIPLE DOSES OF MALATHION (40 mg/kg/day) -a

Tissue	No. 26	No. 27	No. 28	No. 29	No. 30	Mean	S.E.
	ug Equivalents/g						
Blood	0.223	0.204	0.228	0.205	0.198	0.212	0.006
Plasma	0.219	0.228	0.247	0.204	0.198	0.219	0.009
RBCs	0.147	0.131	0.122	0.145	0.145	0.138	0.005
Liver	1.185	1.019	1.402	1.283	1.521	1.282	0.087
Kidneys	0.488	0.446	0.482	0.446	0.405	0.453	0.015
Lungs	0.130	0.132	0.145	0.092 *	0.085 *	0.117	0.012
Brain	0.219	0.203	0.229	0.214	0.204	0.214	0.005
Heart	0.083 *	0.069 *	0.109	0.053 *	0.084 *	0.080	0.009
Spleen	0.243	0.212	0.266	0.230	0.227	0.236	0.009
Testes	0.216	0.223	0.247	0.194	0.210	0.218	0.009
Adrenals	0.292	0.296	0.000 *	0.264	0.246	0.220	0.056
Fat	0.191	0.266	0.254	0.225	0.252	0.238	0.013
Skin	0.223	0.228	0.452	0.247	0.213	0.273	0.045
Muscle	0.047 *	0.028 *	0.056 *	0.039 *	0.043 *	0.043	0.005
Bone	0.265	0.255	0.297	0.236	0.239	0.258	0.011
GI Tract	0.122	0.055 *	0.112	0.147	0.048 *	0.097	0.019
Carcass	0.236	0.168	0.244	0.148	0.168	0.193	0.020

(continued)

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TABLE II-18 (Concluded)

Tissue/ Excretum	No. 26	No. 27	No. 28	No. 29	No. 30	Mean	S.E.
Percent of Dose							
Blood -b	0.046	0.041	0.043	0.041	0.039	0.042	0.001
Plasma -b,c	0.027	0.027	0.028	0.025	0.023	0.026	0.001
RBCs -b,c	0.012	0.010	0.009	0.012	0.011	0.011	0.001
Liver	0.251	0.202	0.271	0.250	0.287	0.252	0.014
Kidneys	0.023	0.019	0.018	0.021	0.017	0.020	0.001
Lungs	0.002	0.002	0.002	0.002 *	0.002 *	0.002	0.000
Brain	0.007	0.007	0.006	0.006	0.006	0.006	0.000
Heart	0.001 *	0.001 *	0.001	0.001 *	0.001 *	0.001	0.000
Spleen	0.002	0.002	0.002	0.002	0.002	0.002	0.000
Testes	0.006	0.006	0.007	0.006	0.006	0.006	0.000
Adrenals	0.000	0.000	0.000 *	0.000	0.000	0.000	0.000
Fat -b	0.062	0.084	0.076	0.071	0.078	0.074	0.004
Skin -b	0.106	0.104	0.196	0.114	0.096	0.123	0.018
Muscle -b	0.056 *	0.032 *	0.060 *	0.045 *	0.048 *	0.048	0.005
Bone -b	0.063	0.058	0.064	0.055	0.054	0.059	0.002
GI Tract	0.063	0.028 *	0.048	0.061	0.019 *	0.044	0.009
Carcass -c	0.580	0.409	0.551	0.364	0.408	0.462	0.043
Urine	84.214	83.146	85.760	83.066	86.634	84.564	0.710
Feces	6.044	10.303	6.287	8.137	3.282	6.811	1.168
Recovery	90.946	94.035	92.841	91.878	90.571	92.054	0.633

a/ Fifteen consecutive daily doses of nonlabeled malathion followed by a sixteenth dose of 14C-malathion.

b/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

c/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.

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TABLE II-19

RADIOACTIVITY IN BLOOD, TISSUES, AND EXCRETA OF FEMALE SPRAGUE-DAWLEY RATS  
72H FOLLOWING MULTIPLE DOSES OF MALATHION (40 mg/kg/day) -a

Tissue	No. 31	No. 32	No. 33	No. 34	No. 35	Mean	S.E.
ug Equivalents/g							
Blood	0.174	0.181	0.156	0.164	0.201	0.175	0.008
Plasma	0.154	0.213	0.152	0.141	0.211	0.174	0.016
RBCs	0.138	0.131	0.126	0.131	0.133	0.132	0.002
Liver	0.803	0.643	0.424	0.545	0.516	0.586	0.064
Kidneys	0.350	0.328	0.337	0.320	0.331	0.333	0.005
Lungs	0.081 *	0.111	0.053 *	0.070 *	0.067 *	0.076	0.010
Brain	0.132	0.139	0.095	0.091 *	0.125	0.116	0.010
Heart	0.063 *	0.067 *	0.036 *	0.053 *	0.064 *	0.057	0.006
Spleen	0.182	0.176	0.116	0.141	0.162	0.155	0.012
Ovaries	0.113	0.151	0.082	0.109	0.129	0.117	0.011
Uterus	0.072 *	0.035 *	0.047 *	0.040 *	0.038 *	0.046	0.007
Adrenals	0.198	0.176	0.137	0.167	0.213	0.178	0.013
Fat	0.212	0.172	0.088 *	0.102	0.146	0.144	0.023
Skin	0.137	0.172	0.119	0.146	0.165	0.148	0.010
Muscle	0.038 *	0.024	0.018 *	0.006 *	0.019 *	0.021	0.005
Bone	0.169	0.182	0.135	0.182	0.155	0.165	0.009
GI Tract	0.072 *	0.043 *	0.043 *	0.047 *	0.050 *	0.051	0.005
Carcass	0.100	0.136	0.084 *	0.099	0.119	0.108	0.009

(continued)

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TABLE II-19 (Concluded)

Tissue/ Excretum	No. 31	No. 32	No. 33	No. 34	No. 35	Mean	S.E.
Percent of Dose							
Blood -b	0.033	0.035	0.030	0.031	0.039	0.034	0.002
Plasma -b,c	0.018	0.025	0.018	0.016	0.024	0.020	0.002
RBCs -b,c	0.011	0.010	0.010	0.010	0.010	0.010	0.000
Liver	0.128	0.134	0.081	0.103	0.102	0.110	0.010
Kidneys	0.016	0.014	0.016	0.014	0.015	0.015	0.000
Lungs	0.002 *	0.003	0.001 *	0.002 *	0.002 *	0.002	0.000
Brain	0.005	0.005	0.004	0.004 *	0.005	0.005	0.000
Heart	0.001 *	0.001 *	0.000 *	0.001 *	0.001 *	0.001	0.000
Spleen	0.002	0.002	0.001	0.001	0.002	0.002	0.000
Ovaries	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uterus	0.001 *	0.000 *	0.001 *	0.000 *	0.001 *	0.001	0.000
Adrenals	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fat -b	0.064	0.053	0.027 *	0.030	0.044	0.044	0.007
Skin -b	0.060	0.077	0.053	0.063	0.072	0.065	0.004
Muscle -b	0.041 *	0.027	0.021 *	0.007 *	0.021 *	0.023	0.005
Bone -b	0.037	0.041	0.030	0.040	0.034	0.036	0.002
GI Tract	0.030 *	0.020 *	0.021 *	0.021 *	0.026 *	0.024	0.002
Carcass -c	0.224	0.318	0.191 *	0.211	0.278	0.244	0.023
Urine	89.069	91.534	83.157	88.825	89.233	88.364	1.390
Feces	6.027	5.414	3.878	7.115	6.614	5.810	0.561
Recovery	95.516	97.360	87.321	96.257	96.211	94.533	1.827

a/ Fifteen consecutive daily doses of nonlabeled malathion followed by a sixteenth dose of <sup>14</sup>C-malathion.

b/ Percent of dose calculations based on 7, 11, 16, 40, and 8% of body weight for blood, fat, skin, muscle, and bone, respectively. Plasma and RBC calculations based on 60 and 40% of blood volume, respectively.

c/ Values not included in recovery estimates (see text).

\* Based on insignificant counts, i.e., less than two times the background.

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APPENDIX III

STUDY PROTOCOL, AMENDMENTS, AND DEVIATIONS



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### Study Protocol

## Disposition and Metabolism of $^{14}\text{C}$ -Labeled Malathion in Rats

### Sponsor

Malathion Registration Task Force  
Robert L. Linkfield, Ph.D., Chair  
American Cyanamid Company  
Agricultural Research Division

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### Sponsor's Representative

Jellinek, Schwartz, Connolly & Freshman, Inc.  
1350 New York Avenue, N.W., Suite 400  
Washington, D.C. 20005  
(202) 783-3388

Data Requirements  
Pesticide Assessment Guidelines  
Subdivision F, Section 85-1

### Testing Laboratory

Midwest Research Institute  
425 Volker Boulevard  
Kansas City, Missouri 64110  
(816) 753-7600

Study Director  
V. J. Reddy, Ph.D.

MRI Project No. 9354-B  
JSCF Study No. 56

March 27, 1989

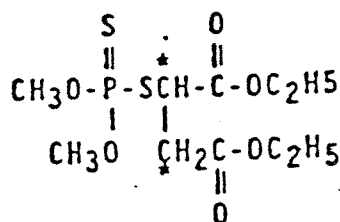


## STUDY PROTOCOL

### Disposition and Metabolism of $^{14}\text{C}$ -Labeled Malathion in Rats

#### 1.0 Objectives

The objectives of these studies are to determine the absorption, tissue distribution, elimination, and biotransformation of  $^{14}\text{C}$ -labeled malathion in male and female Sprague-Dawley rats. These studies will be conducted according to FIFRA guidelines (85-1).



Malathion  
\* Position of the  $^{14}\text{C}$  label

#### 2.0 Materials and Methods

##### 2.1 Animals

Adult male and female Sprague-Dawley (Cr1:CD BR) rats, weighing 175 to 275 g, will be used for the study. The species and strain of rats chosen is commonly used and is the preferred species for this type of study. The rats will be purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. Upon arrival, the animals will be uniquely identified by metal eartag and will be acclimated for a minimum of 7 days under test conditions. An attendant veterinarian will examine and release the animals for the studies. Prior to testing, the animals will be randomized to each experimental group using a computer-based body weight stratification procedure. Within-group body weights will not vary by more than  $\pm 2$  standard deviations of the mean; between group body weights of each sex will not be statistically different ( $p < 0.5$ ).

The animals will be housed in environmentally controlled rooms with 10 to 15 air changes per hour. The rooms will be maintained at a temperature of  $72 \pm 3^\circ\text{F}$  and humidity of  $50 \pm 20\%$ , on a 12-hr light/dark cycle per day. During the acclimation period, the rats will be housed individually in stainless steel suspension cages over Ab-Sorb-Dri hardwood chip bedding (Ab-Sorb-Dri Company, Garfield, New Jersey). Throughout the study the animals will be provided with Purina certified rodent chow (No. 5002) and tap water ad libitum, except for -16 hr prior to and 4 hr following a radioactive dose. No contaminants are known or assumed to be present in the food or water that would interfere with results of the study.

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Animal care and housing will be in accordance with DHHS Publication No. (NIH) 85-23, 1985, "Guidelines for the Care and Use of Laboratory Animals," and the MRI "Manual for Animal Care."

## 2.2 Test Compounds

The  $^{14}\text{C}$ -labeled and nonlabeled malathion will be supplied by the sponsor, who will also supply information on the physical properties, chemical composition, purity, stability, etc. Upon arrival, the test materials will be stored frozen or under specified conditions which minimize decomposition. The radiochemical purity of the  $^{14}\text{C}$  malathion will be confirmed at MRI prior to dosing. All unused test material will be returned to the sponsor or designee upon completion of the studies.

## 2.3 Dosage and Treatment

Mixtures of  $^{14}\text{C}$ -labeled and nonlabeled malathion will be administered orally to 3 groups of rats. Oral dosing will be used since it is the potential exposure route. The intravenous group will be omitted since the test compound is insoluble, at the doses selected, in water or physiological saline. The rats will receive single gavage doses of the radiolabeled material at a low (40 mg/kg body weight) or high (800 mg/kg body weight) dose level. An additional group of rats will receive, at the low level, oral doses of the nonlabeled material once-daily for 14 consecutive days, followed by a fifteenth dose of the radiolabeled material. The dose mixtures will contain appropriate quantities of labeled material, e.g., 200 uCi/kg (~50 uCi/rat). The test compound will be dissolved in corn oil. The rats will be treated at a volume of 4 ml/kg (~1 ml/rat). Duplicate predose and postdose samples of the dosing solutions for each group will be taken at the time of dose administration to determine the amount of radioactivity administered.

## 2.4 Experimental Design

A total of 34 rats (17 males and 17 females) will be used in the studies. Four rats (2 males and 2 females) will be used in a preliminary study to define the rates of  $^{14}\text{C}$  elimination. These rats will receive single doses of  $^{14}\text{C}$ -malathion orally at the low and high dose level. The animals will be housed in glass metabolism cages for the separate collection of expired air, urine, and feces (at 4, 8, 12, 24, 48 and 72 hr after dosing). The animals will be observed frequently throughout the study; cageside observations will be recorded once-daily (a.m.). All animals will be sacrificed for tissue sampling at 72 hr following treatment.

Following assessment of data generated from the preliminary studies, a definitive study will be conducted in 15 male and 15 female rats. If results from the preliminary study indicate that >5% of the dose is eliminated in the expired air, then the definitive studies will be performed using glass metabolism cages; otherwise, the animals will be housed in stainless steel metabolism cages for the separate collection of urine and feces. The animals will be acclimated to the metabolism cages for 24 hr prior to dosing. All rats will be weighed before dosing and prior to sacrifice. Mortality and morbidity will be checked twice daily, and health and appearance will be checked once daily.

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Three groups of five male and five female rats each will receive the test compound using the following dosing regimens: single oral dose at the low dose level; single oral dose at the high dose level; and 14 daily oral doses with the nonlabeled test compound followed by a fifteenth dose of the radiolabeled test material at the low dose level. Following dosing with the radiolabeled test compound, urine, feces, (and expired air, if necessary) will be collected at 4, 8, 12, and 24 hr, and at 24 hr intervals up to the time determined from the preliminary study when >90% of the administered dose has been eliminated. At this time, animals will be sacrificed for tissue analysis (see below).

#### Study Design<sup>a</sup>

Study	No. of Rats	Sex	Treatment	Sacrifice Time	Samples Collected
Preliminary <sup>b</sup>	1	M	p.o. 40 mg/kg	72 hr	Air, urine, feces, tissue
	1	F	p.o. 40 mg/kg	72 hr	Air, urine, feces, tissue
	1	M	p.o. 800 mg/kg	72 hr	Air, urine, feces, tissue
	1	F	p.o. 800 mg/kg	72 hr	Air, urine, feces, tissue
Definitive	5	M	p.o. 40 mg/kg	TBD <sup>d</sup>	(Air) <sup>d</sup> , Urine, feces, tissue
	5	F	p.o. 40 mg/kg	TBD	(Air) <sup>d</sup> , Urine, feces, tissue
	5	M	p.o. 800 mg/kg	TBD	(Air) <sup>d</sup> , Urine, feces, tissue
	5	F	p.o. 800 mg/kg	TBD	(Air) <sup>d</sup> , Urine, feces, tissue
	5	M	X15 p.o. 40 mg/kg <sup>c</sup>	TBD	(Air) <sup>d</sup> , Urine, feces, tissue
	5	F	X15 p.o. 40 mg/kg <sup>c</sup>	TBD	(Air) <sup>d</sup> , Urine, feces, tissue

<sup>a</sup> Since, at the doses selected, the test compound is insoluble in water or in physiological saline, no intravenous studies will be performed.

<sup>b</sup> All animals will be sacrificed for tissue sampling at 72 hr following treatment.

<sup>c</sup> Animals will be treated with once-daily 40 mg/kg oral doses of the nonlabeled test compound for 14 consecutive days, followed by a fifteenth dose of the radiolabeled test compound.

<sup>d</sup> To be determined.

#### 2.5 Sample Collection

Any expired <sup>14</sup>C-malathion will be collected in 50% methanol and <sup>14</sup>CO<sub>2</sub> and will be trapped with 5 M ethanolamine in 2-methoxyethanol. Urine and feces will be collected in containers and kept on dry ice. Feces will be weighed and an appropriate amount of homogenizing solvent (10% ethanol) will be added. These samples will be refrigerated until processed. After each collection, the cages will be rinsed with water; the cage washings will be separately measured and analyzed. At sacrifice, animals will be anesthetized with ether and exsanguinated by withdrawal of blood from the abdominal aorta. Organs and tissues will be grossly examined. The following tissues will be removed from all animals, washed with saline, blotted with absorbent paper, weighed, and prepared for radiochemical analyses. In addition, any tissues showing gross pathological changes will be processed:

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Liver	Spleen	Muscle (thigh)
Kidneys	Adrenals	Fat (retroperitoneal)
Lungs	Testes	Bone (femur)
Brain	Uterus	Skin
Heart	Ovaries	GI Tract plus contents
		Residual carcass

Portions of blood samples will be centrifuged to separate plasma and red blood cells. Blood and tissues will be kept on ice during the necropsy procedure. Sample preparation and analyses will be performed immediately after collection, or after weighing, the samples will be frozen until analyzed. Tissue and excreta will be stored under appropriate conditions for metabolic studies (see below).

## 2.6 Sample Preparation and Analyses

Aliquots (250 to 500 mg) of whole blood, plasma, and red blood cells will be analyzed for total radioactivity. Total weights of expired air trappings, urine, and urine washings will be measured and samples (~500 mg) will be counted. Tissues weighing >0.25 g will be homogenized in four volumes of an appropriate solvent (e.g., water or ethanol:water, 10:90). Feces will be homogenized in nine volumes of the solvent. Aliquots (~500 mg) of the homogenates will be measured and analyzed. Samples of fat and skin (40 to 120 mg), and the adrenals, ovaries, and bone will be analyzed directly without homogenization.

Tissues, blood components, and fecal samples will be combusted using a Packard Tricarb Sample Oxidizer (Model C306 or D306). Permafluor V in combination with Carbosorb (Packard Instrument Company, Downers Grove, Illinois) will be used as the scintillation cocktail for combusted samples. Expired air trappings, urine, and urine washings will be counted directly in Phase Combining Scintillate (PCS, Amersham, Arlington Heights, Illinois). Counting will be performed in a Packard Tri-carb liquid scintillation counter (Model 4530 or 2000CA). All samples will be counted for a minimum of 5 min or to 100,000 counts per min (cpm). Correction for background and efficiency will be performed.

Samples will be analyzed in duplicate whenever possible. Assays not within +/- 10% of the mean of the duplicates will be reassayed in duplicate except when the sample is no longer available or when radioactivity counts are low and nonsignificant, i.e., less than 100 disintegrations per min (dpm).

## 2.7 Data Processing and Analyses

All weighings of excreta and tissue will be performed utilizing an on-line data acquisition system. Weighing data will be sequenced and merged with scintillation counting data using DataEase™ software (DataEase International, Inc., Trumbull, Connecticut) and an IBM-XT computer.

## 2.8 Biotransformation Studies

A high performance liquid chromatographic (HPLC) method will be developed and validated to separate and quantitate the metabolites of malathion

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in selected urine and feces samples. Pooled 0-24 hr or 0-48 hr excreta samples from one rat per sex per dose group will be utilized for the analyses. Samples will be mixed with either methanol or acetonitrile and filtered. Total radioactivity determinations will be performed on aliquots of the diluted samples and on any residue collected in the filter to assure that all metabolites are present in the filtrate of the diluted samples. HPLC analyses will then be performed on the diluted samples. Detected peaks will be quantitated using on-line radiochemical detection. The retention time of the peaks will be compared to those of nonradioactive standards of all available metabolites. Selected samples will be hydrolyzed enzymatically and chromatographed to tentatively identify (retention time comparison with known standards) conjugates present in the samples. Peaks containing major metabolites will be collected, derivatized with diazomethane, and analyzed by gas chromatography/mass spectrometry (GC/MS).

### 3.0 Reporting

Appropriate methods will be used to tabulate and graphically illustrate the  $^{14}\text{C}$  contents of blood and tissues in terms of microgram equivalents per gram and/or percent of administered dose. Cumulative amounts of dose eliminated in expired air, urine, and feces will be calculated and tabulated or presented graphically. Metabolic profiles and quantitative analyses of metabolites will be presented. Individual animal data will be presented as appendices. Data from each treatment group will be expressed as mean +/- standard error.

The final report will contain detailed descriptions of methodology used throughout the study (e.g., dose preparations, methods used for counting radioactivity, counting efficiency), as well as results and discussion of findings.

### 4.0 Quality Assurance

Quality assurance of the critical phases and the data generated in the study will be monitored both within the program and externally by the Institute's Quality Assurance Unit. The research conducted in this program will be in accordance with EPA Good Laboratory Practice standards of November 29, 1983, FIFRA (Federal Register, 48, 53946-53969). After completion of the study, all remaining samples will be shipped to the sponsor. All raw data will be stored in the MRI archives.

### 5.0 Personnel Safety

The general safety policies of the Institute are established and enforced by the Institute Safety Committee, the Carcinogen Safety Committee, and the Radiation Safety Committee. All activities will be conducted in appropriately equipped limited access laboratories. Since this compound is a toxin with known cholinesterase inhibitory properties, special precautions (two pairs of gloves, disposable laboratory suits, etc.) will be employed when handling the chemical, dosed animals, and the biological samples generated in this study.

**COMMERCIAL  
CONFIDENTIAL****6.0 Study Personnel**

This program will be conducted in the Life Sciences Department of Midwest Research Institute, Dr. Monaem El-hawari, Director. Dr. V. J. Reddy, Toxicologist, will serve as Study Director. The animal studies will be conducted by Mr. Timothy Freeman, Assistant Biologist, Ms Terri Douglas, Associate Biologist, Mr. Reynaldo Lopez, Senior Technician, and Mr. Brant Mowry, Technician. Mr. Edward Williams, Supervisor, Animal Care, will be responsible for animal care activities under the direction of Dr. Elizabeth Evans, Veterinarian. The biotransformation studies will be performed by Mr. Frank Pallas, Staff Chemist and Mr. Larry Litle, Associate Chemist, under the direction of Dr. Evelyn Murrill, Principal Advisor. Ms. Maxine Stoltz, Senior Toxicologist and Program Manager, will provide technical and administrative support. Dr. Eugene Podrebarac, Manager, Quality Assurance, will monitor conformity with GLP regulations.

**7.0 Study Schedule**

Preliminary Study Initiation: March 28, 1989  
 Analytical Studies Initiation: April 4, 1989  
 Preliminary Study Completion: May 31, 1989  
 Progress Report: June 15, 1989  
 Definitive Study Initiation: July 1, 1989  
 Definitive Study Completion: November 15, 1989  
 Draft report: January 15, 1990  
 Final report: Four weeks after return of draft report

**8.0 Protocol Approvals**

Vijayapal Reddy March 27, 1989  
 Study Director (MRI) Date

Monaem El-hawari 3/27/89  
 Department Director (MRI) Date

Robert L. Smith 4/7/89  
 Sponsor Date  
 (Malathion Registration Task Force)



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9354-B

MIDWEST RESEARCH INSTITUTE

425 Volker Boulevard

Kansas City, Missouri 64110

Telephone (816) 753-7600

PROTOCOL AMENDMENT  
NO. 1

MRI PROJECT NO.: 9354-B

DATE EFFECTIVE: May 1, 1989

STUDY TITLE: Disposition and Metabolism of  $^{14}\text{C}$ -Labeled Malathion in Rats

SPONSOR: Malathion Reregistration Task Force

PROTOCOL REFERENCE: Part 2.6 Sample Preparation and Analyses. All samples will be counted for a minimum of 5 minutes or to 100,000 counts per minute (cpm).

CHANGE: Any sample that reaches the cpm threshold of 100,000 in less than 0.5 minutes will be recounted with a cpm threshold of 4,000,000.

REASON FOR CHANGE: The counting time for samples with high radioactivity was less than 10 seconds based on the threshold of 100,000 cpm or counting time of 5 minutes. This time was too short to obtain reliable counts for samples with high radioactivity.

APPROVED:

Vijaya Pal Reddy  
(Signature)  
Study Director  
Midwest Research Institute

May 24, 1989  
(Date)

ACKNOWLEDGED:

Robert H. Hinkle  
(Signature)  
Study Sponsor  
Malathion Reregistration Task Force

8/3/89  
(Date)



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MIDWEST RESEARCH INSTITUTE  
425 Volker Boulevard  
Kansas City, Missouri 64110  
Telephone (816) 753-7600

PROTOCOL AMENDMENT  
NO. 2

MRI PROJECT NO.: 9354-B  
DATE EFFECTIVE: May 1, 1989  
STUDY TITLE: Disposition and Metabolism of <sup>14</sup>C-Labeled Malathion in Rats  
SPONSOR: Malathion Reregistration Task Force  
PROTOCOL REFERENCE: Title Page

Sponsor's Representative:

Jellinek, Schwartz, Connolly & Freshman, Inc.  
1350 New York Avenue, N.W.  
Suite 400  
Washington, D.C. 20005

CHANGE: Title Page

Sponsor's Representative:

Jellinek, Schwartz, Connolly & Freshman, Inc.  
1015 15th Street, N.W.  
Suite 500  
Washington, D.C. 20005

REASON FOR CHANGE: Sponsor's representative moved offices to another location.

APPROVED:

Vijayapal Reddy  
(Signature)  
Study Director  
Midwest Research Institute

May 24, 1989  
(Date)

ACKNOWLEDGED:

Robert Smith  
(Signature)  
Study Sponsor  
Malathion Reregistration Task Force

8/3/89  
(Date)





COMMERCIAL  
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9354-B

MIDWEST RESEARCH INSTITUTE

425 Volker Boulevard

Kansas City, Missouri 64110

Telephone (816) 753-7600

Date: May 24, 1989

DEVIATION TO THE PROTOCOL

MRI PROJECT NO.: 9354-B

STUDY TITLE: Disposition and Metabolism of  $^{14}\text{C}$ -Labeled Malathion in Rats

SPONSOR: Malathion Reregistration Task Force

PROTOCOL REFERENCE: Experimental Design, Definitive Study. . . . and 14 daily oral doses with the non-labeled test compound followed by a fifteenth dose of the radio-labeled test material at the low dose level.

DATE OF DOSING: May 22, 1989

DATE OF DEVIATION: May 22, 1989

DEVIATION: Fifteen rather than 14 daily oral doses with the non-labeled test compound were given, followed by a dose of the radio-labeled test material.

REASON FOR DEVIATION: The rats were not fasted prior to dosing with the radio-labeled test material on day 15. Therefore, an additional dose of non-labeled test compound was given upon consulting with the JSCF study monitor.

IMPACT ON STUDY: None. According to EPA guideline (#85-1) for the metabolism study, the repeated dosing of non-labeled test material is done over a period of at least 14 days. One additional day of dosing with non-labeled test material will not make any impact on the outcome of the study.

APPROVED:

*Ujayanpal Reddy*

(Signature)  
Study Director  
Midwest Research Institute

*May 24, 1989*

(Date)

ACKNOWLEDGED:

*Robert H. Smith*

(Signature)  
Study Sponsor  
Malathion Reregistration Task Force

*8/3/89*

(Date)

