^{14}C-DIMETHOATE

THE BIOKINETICS AND METABOLISM IN THE RAT

Volume 1

Data requirement	US EPA Subdivision F, Guideline 85-1
Project identity	DTF 16
Study completed on	18 December 1995

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited with effect from 21 November 1995

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

18 December 1995

David Kirkpatrick, B.Sc. (Hons.), Ph.D., C.Chem., F.R.S.C.,
Study Director,
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Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to Management, Huntingdon Life Sciences are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	12 January 1993
Pre-experimental Period	-	-
Experimental Period	13 - 15 + 18 October 1993	18 October 1993
	1 December 1993	1 December 1993
	16 December 1993	20 December 1993
	24 January - 5 February 1994	7 February 1994
	2 June 1994	2 June 1994
	16 June 1994	17 June 1994
	1 September 1994	3 September 1994
	9 December 1994	12 December 1994
Date of reporting audit findings to the Study Director and Management		15 December 1995

D. J. Dams

16- December-1995

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SUMMARY

The biokinetics and metabolism of the insecticide dimethoate have been studied in rats following oral and dermal administration at nominal dose levels of 10 and 100 mg/kg bodyweight, and following intravenous administration at 10 mg/kg bodyweight.

The patterns of excretion of radioactivity in intact rats were similar after oral administration at 10 and 100 mg/kg bodyweight and intravenous administration at 10 mg/kg bodyweight. Radioactivity was mainly excreted in the urine in each case (85 - 91% dose excreted after 5 days) with small amounts lost in expired air (1.6 - 2.5% dose) and *via* the faeces (1.2 - 1.6% dose). Excretion was rapid, especially after intravenous administration (80% dose excreted after 6 hours). Pre-treatment of rats with non-radiolabelled dimethoate for 14 days did not alter the pattern of excretion of a subsequent 10 mg/kg bodyweight oral dose. Absorption of radioactivity by dermally-dosed rats (skin area: 10 cm^2) amounted to approximately 1 mg dimethoate/kg bodyweight at dose levels of both 10 and 100 mg/kg bodyweight. Absorbed radioactivity was excreted mainly in the urine. In rats with cannulated bile ducts biliary excretion represented 4 - 5% dose at dose levels of 10 and 100 mg/kg bodyweight.

Plasma kinetics of radioactivity after oral administration were similar between the sexes at 10 mg/kg bodyweight but displayed some differences at 100 mg/kg bodyweight. The mean times to reach peak plasma concentrations (C_{max}) of radioactivity were 0.5 hours in both sexes at 10 mg/kg bodyweight. At this dose level, plasma radioactivity concentrations declined biphasically thereafter with mean terminal half-lives in the range 42 - 59 hours. C_{max} values and mean areas under plasma radioactivity concentration against time curves (AUCs) were similar. At a dose level of 100 mg/kg bodyweight, the mean time to reach C_{max} was 0.5 hours in females and 0.25 hours in males. C_{max} values themselves and AUCs were greater in females than in males at 100 mg/kg bodyweight, such that AUCs increased by about 8 times in males but about 14 times in females from the low to the high dose level. Plasma radioactivity concentrations also declined biphasically at 100 mg/kg bodyweight (with similar terminal half-lives (36 - 46 hours) to those at 10 mg/kg bodyweight) apart from an increase in concentration seen after 6 hours. At a level of 100 mg/kg bodyweight, dimethoate was detectable in plasma up to 6 hours after administration.

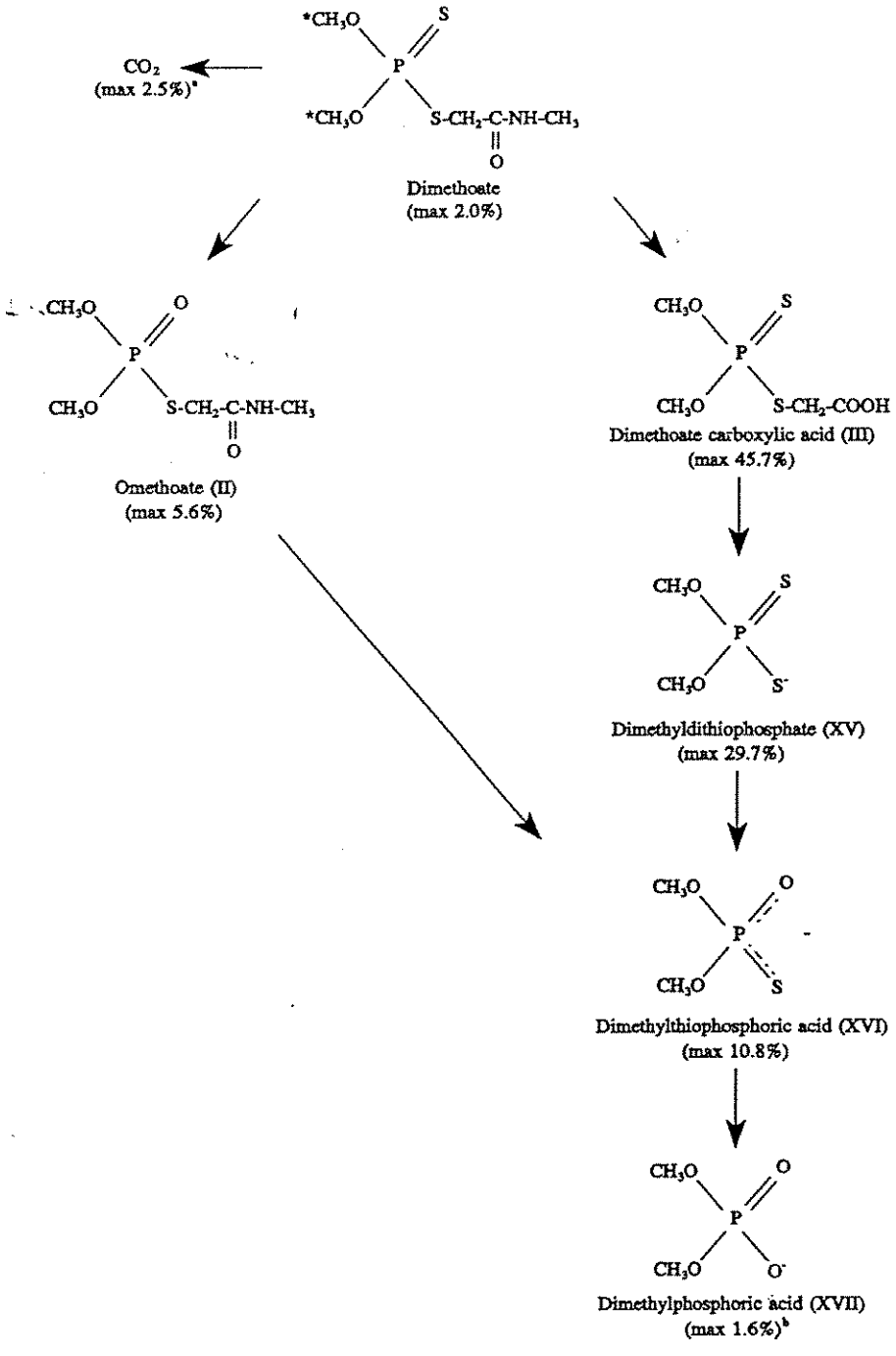
The distribution of radioactivity in tissues was similar in male and female animals. Highest concentrations were found in the liver and kidneys. After oral dosing at 10 mg/kg bodyweight, mean maximum concentrations of radioactivity in these tissues were in the range 9 - 25 mg dimethoate equivalents/kg (mg/kg). Concentrations in other tissues after 0.5 hours were generally much lower (1 - 6 mg/kg) with the lowest concentrations (*ca* 1 mg/kg) in the brain and fat. Tissue concentrations of radioactivity after multiple dosing at 10 mg/kg bodyweight were slightly elevated over those seen after a single dose. Concentrations in liver and kidney were in the range 13 - 28 mg/kg, with concentrations in other tissues in the range 2 - 7 mg/kg. After dosing at 100 mg/kg bodyweight, peak concentrations were generally 5 - 18 times higher than those measured after a single 10 mg/kg bodyweight dose, although higher ratios were observed for adrenals and fat in male rats. At each dose level rates of elimination of radioactivity were broadly similar in all tissues.

Qualitative investigations of the tissue distribution of radioactivity were made using whole-body autoradiography. This additionally indicated some accumulation of radioactivity in the Harderian gland, the intra- and exorbital lachrymal glands and the preputial gland. The central nervous system contained the lowest concentrations of radioactivity.

A number of metabolites of dimethoate were identified in urine by chromatographic and spectroscopic techniques. These showed that dimethoate was extensively metabolised, mainly (i) by initial cleavage of the C-N bond to yield dimethoate carboxylic acid and subsequently a number of thiophosphate and phosphate esters, but also (ii) by oxidation to its oxon analogue, omethoate. These metabolites, together with the maximum proportions of an administered dose that they represented, are shown in the proposed metabolic pathway, overleaf. Identified metabolites accounted for between 70 and 80% or an orally or intravenously administered dose.

DIAGRAM

Proposed biotransformation pathway for dimethoate in the rat



Values in parentheses are, unless stated otherwise, the maximum proportions of an administered dose the components represented in urine

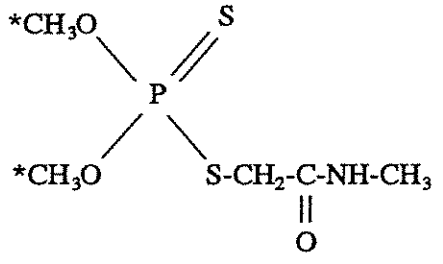
[a] present in expired air

[b] present in tissues only (tentative identification)

INTRODUCTION

Dimethoate, O,O-dimethyl-S-methylcarbamoylmethylphosphorodithioate, is a broad spectrum insecticide and acaricide used on a wide range of crops. This report described studies on the biokinetics and metabolism of dimethoate in rats. The objectives of the study were (i) to assess the extent of absorption, and the rates and routes of excretion, of radioactivity after oral, intravenous and dermal administration; (ii) to investigate the pharmacokinetics of the radiolabel and of dimethoate in plasma after oral administration; (iii) to investigate the distribution of the radiolabel in tissues and the rate of elimination of tissue radioactivity after single or multiple oral doses; and (iv) to obtain information on the biotransformation of dimethoate.

The study was conducted using dimethoate labelled with carbon-14 in the O-methyl groups, as shown below.



* denotes positions of the radiolabel

RELEVANT STUDY DATES

Protocol approval by:

Study Director (study initiation date)	6 January 1993
HRC Management	6 January 1993
Study Sponsor	16 January 1993

'In-life':

Date of first pre-test dose administration (experimental start date)	19 January 1993
Date of first main study dose administration	21 September 1993

Analytical work and other study procedures:

Start	22 September 1993
Finish (experimental termination date)	16 October 1995

Study completion date: 18 December 1995

See Appendix 1 for dates of dose administration (main study).

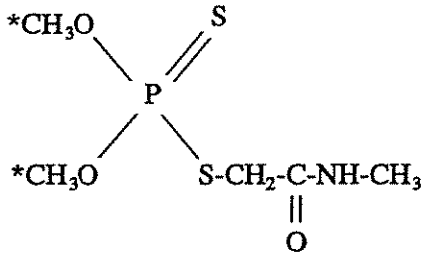
MATERIALS AND METHODS

TEST AND REFERENCE SUBSTANCES

Test substance general information

Common name:	Dimethoate.
Chemical name:	O,O-Dimethyl-S-methylcarbamoylmethyl-phosphorodithioate.
Molecular formula:	$C_5H_{12}NO_3PS_2$.
Molecular mass:	229.3.
Physical state (20°C):	Crystalline solid.
Solubility (water, 21°C):	23.8 g/litre.

Radiolabelled test substance



* denotes positions of the radiolabel

Position of radiolabelling:	In the two O-methyl carbon atoms.
Specific activity:	14.55 μCi/mg (32300 dpm/μg) (measured at the Huntingdon Research Centre).

Two batches of the radiolabelled test substance were supplied by Cheminova Agro A/S, Lemvig, Denmark. These had the batch identification: AC-DMT-SLUT. Both batches were purified at the Huntingdon Research Centre. See Appendix 2 for HRC Data Sheets.

Batch 1

Date of receipt:	13 July 1993.
Quantity received:	6.6 mCi.
HRC batch number:	MR-DTF17-3.

Batch 2

Date of receipt:	15 September 1993.
Quantity received:	6.5 mCi.
HRC batch number:	NPE-DTF17-5.

The radiochemical purity of ^{14}C-dimethoate was measured in each formulation prior to dose administration and was in all cases greater than 98%. Example TLC radiochromatograms are shown in Figure 1.

Mass spectra of purified ^{14}C-dimethoate (Batch 1) and of non-radiolabelled dimethoate are shown in Figure 2. The fragmentation pattern was consistent with that expected for dimethoate.

Following purification, each batch of ^{14}C-dimethoate was stored, in solution in ethyl acetate, at -20°C.

Non-radiolabelled test substance

Supplied by Cheminova Agro A/S:

Analytical grade

Identification code:	ST001-01.
Batch number:	00315-00.
Date of receipt:	7 December 1992.
Quantity:	2 g.
Storage:	-20°C.
Purity:	99.5%.
Expiry date:	18 May 1995.

Technical grade

Identification code:	PR020.
Batch number:	20522-00.
Date of receipt:	7 December 1992.
Quantity:	25 g.
Storage:	-20°C.

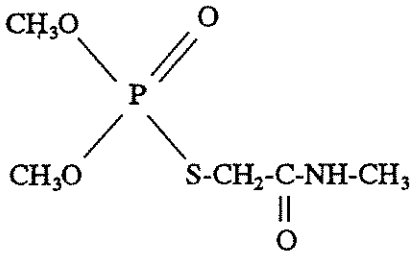
Purity: 99.1%.

Expiry date: 18 June 1995.

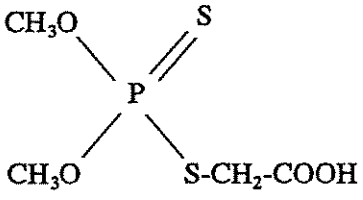
Both grades of non-radiolabelled dimethoate were used in dose formulations.

Reference substances

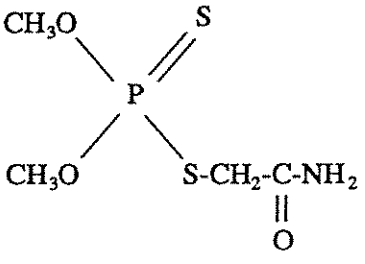
For use in the characterisation of metabolites, the following reference substances were supplied by Cheminova Agro A/S. Details of the receipt, quantity, purity, storage and expiry date of each substance are shown in Appendix 3.



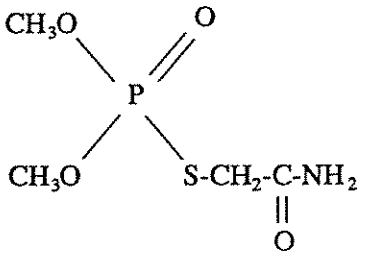
Reference substance II: omethoate
(Identification code: REF042-01; batch number: 234 108 038)



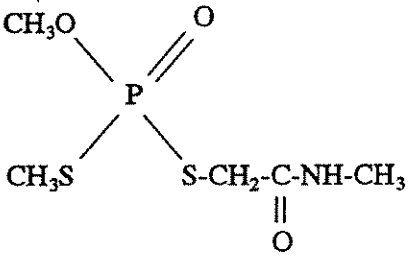
Reference substance III: dimethoate carboxylic acid
(Identification code: REF048-01; batch number: 279-ABB-40-1)



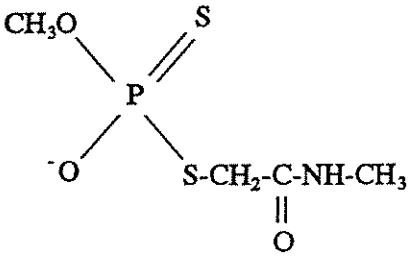
Reference substance VII: N-desmethyl-dimethoate
(Identification code: REF082-01; batch number: 319-BSe-26A)



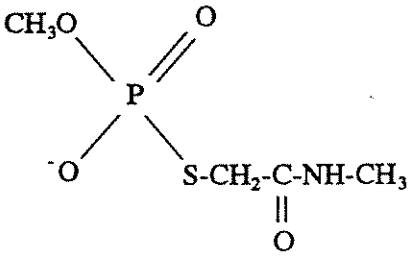
Reference substance VIII: N-desmethyl-omethoate
(Identification code: REF110-01; batch number: 352-OSJ-9F1)



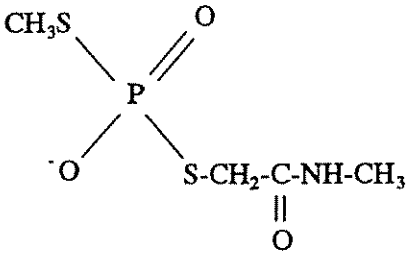
Reference substance IX: isodimethoate
(Identification code: REF043-02; batch number: 279-ABB-30)



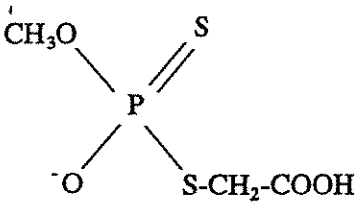
Reference substance X: O-desmethyl-dimethoate (dicyclohexylammonium salt)
(Identification code: REF053-01; batch number: 91-FVL-096)



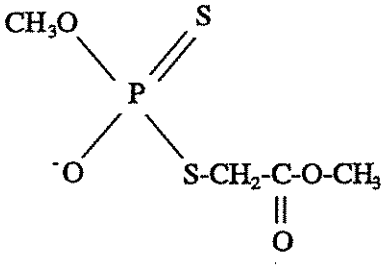
Reference substance XI: O-desmethyl-omethoate (potassium salt)
(Identification code: REF081-01; batch number: 324-OSJ-6A)



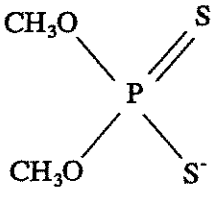
Reference substance XII: O-desmethyl-isodimethoate (dicyclohexylammonium salt)
(Identification code: REF051-01; batch number: 302-OSJ-10B)



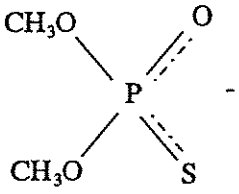
Reference substance XIII: O-desmethyl-dimethoate carboxylic acid (potassium salt)
(Identification code: REF079-01; batch number: 324-OSJ-16B)



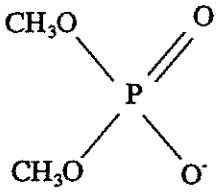
Reference substance XIV: desmethyl-MPEM (dicyclohexylammonium salt)
(Batch number: 91-FVL-12301)



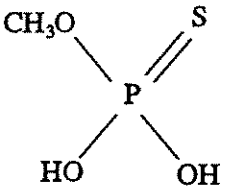
Reference substance XV: dimethyldithiophosphate (potassium salt)
(Identification code: REF057-01; batch number: 291-BSe-62A)



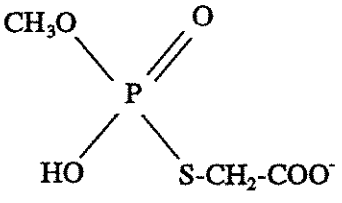
Reference substance XVI: dimethylthiophosphoric acid (dicyclohexylammonium salt)
(Identification code: REF083-01; batch number: 267-OSJ-54B)



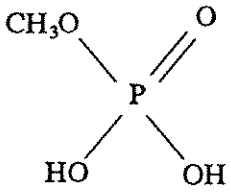
Reference substance XVII: dimethylphosphoric acid (sodium salt)
(Identification code: REF078-01; batch number: 302-OSJ-50B)



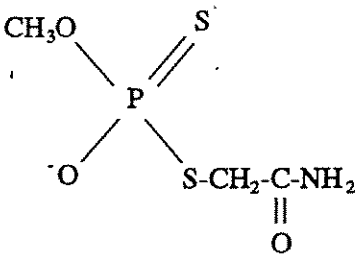
Reference substance XIX: monomethylthiophosphoric acid
(Identification code: REF109-01; batch number: 319-BSe-76A)



Reference substance XX: O-desmethyl-omethoate carboxylic acid (potassium salt)
(Identification code: REF105-01; batch number: 352-OSJ-56B)



Reference substance XXI: methylphosphoric acid (cyclohexylammonium salt)
(Identification code: REF084-01; batch number: 302-OSJ-92A)



Reference substance XXII: O-desmethyl-N-desmethyl-dimethoate (potassium salt)
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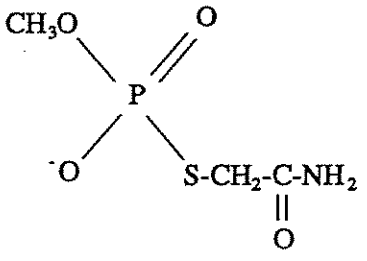


Reference substance XXIII: O-desmethyl-N-desmethyl-omethoate (potassium salt)
(Identification code: REF111-01; batch number: 352-OSJ-17A)

TEST ANIMALS

Species:	Rat.
Strain:	Wistar.
Sex:	Male and female.
Age (at administration of (first) radioactive dose):	7 - 10 weeks.
Bodyweight:	195 - 224 g (Appendix 4).

Supplier: Charles River UK Ltd, Margate, Kent, UK.

STUDY DESIGN

The various aspects of the biokinetics and metabolism of dimethoate in the rats are outlined in Table 1. ^{14}C-Dimethoate was administered either (a) orally, in solution in water, (b) intravenously, in solution in isotonic saline, or (c) dermally, as a suspension in 1% aqueous sodium carboxymethylcellulose solution. Two dose levels were employed, *viz* 10 mg/kg bodyweight and 100 mg/kg bodyweight.

Prior to the main study, a number of pre-test observation experiments were carried out using non-radiolabelled dimethoate, to establish dose levels for the main experiments. The results of these experiments are discussed in the section **PRE-TEST RESULTS AND DOSE SELECTION**.

ANIMAL EXPERIMENTATION

Animal husbandry

Animals were individually identified by indelible tail markings. The animals were provided with food (LAD 1 pellets, SDS, Witham, Essex, UK) and tap water (Anglian Water Services Ltd) *ad libitum*. (Example analytical data for the food and water are shown in Appendices 5 and 6 respectively.) In excretion-balance experiments using intact rats, for the period from 24 hours before dosing to sacrifice, animals were individually housed in glass metabolism cages, which facilitated the separation of urine and faeces. Air was passed through each cage and then through media designed to trap expired volatile radioactivity. (Dermally-dosed rats were housed in restraining cages from dosing until 6 hours after dosing, when they were transferred to metabolism cages.) Following dosing, bile duct-cannulated rats were housed in restraining cages, which facilitated the separate collection of urine, faeces and bile. In other experiments, animals were housed in groups of the same sex in stainless steel battery cages.

Dose preparation and administration

Dose formulations were freshly prepared for administration to each group of rats. Where, in a few instances, formulations were prepared for dosing on a number of days (maximum 3 days from the day of preparation), they were stored at -20°C and the radiochemical purity remeasured following the final administration.

Radiolabelled and non-radiolabelled dimethoate were mixed together in ethyl acetate to achieve the desired specific activity for the dosed material. The solvent was removed under a stream of nitrogen and the solid material redissolved in either high purity water (see Appendix 7 for specification) for oral doses, or isotonic saline (0.9% w/v aqueous sodium chloride solution) for intravenous doses. For dermal treatments, the solid material was suspended in 1% (w/v) aqueous sodium carboxymethylcellulose solution, using a glass homogenising tube and an electrically-driven Teflon pestle.

For oral dosing, animals were dosed by gastric intubation at a rate of *ca* 2.5 (experiment 5d), 3.3 (experiment 2d, radioactive dose) or, otherwise, 5 ml solution/kg bodyweight. For intravenous dosing, animals were dosed *via* a tail vein at a rate of *ca* 5 ml solution/kg bodyweight. For dermal dosing, animals were dosed at a rate of *ca* 1 ml suspension/kg bodyweight (0.2 ml/rat) over an area of about 10 cm^2. To accurately determine the quantity of radioactivity administered to each rat, further aliquots of dose solution or suspension were similarly syringed into volumetric flasks (250 ml). These were made up to volume with methanol or acetonitrile and triplicate aliquots of each (1 ml) taken for radioassay. In the experiment involving intravenous dosing, the administered dose was calculated from the weight of injected solution; the radioactivity concentration (dpm/g) of the dose solution was obtained from the radioassay of solutions (in volumetric flasks) of accurately weighed aliquots of the dose solution.

The specific activity of each radiodilution was calculated from the radioactivity concentration of the dose formulation (taking into account the specific activity of the undiluted ^{14}C-dimethoate) and the weight of non-radiolabelled dimethoate in the formulation.

The quantities of radioactivity administered to rats in each experiment, the specific activities of each dose formulation and the dose levels, in terms of mg/kg bodyweight, are shown in Appendix 8.

Sample collection: excretion-balance experiments

Urine was collected separately from each animal into receivers cooled in solid CO_2 at 0 - 6, 6 - 12, 12 - 24 hours and at 24-hour intervals up to 5 days after dosing. Faeces were collected separately from each animal at 24-hour intervals up to 5 days after dosing. Expired air was passed through two sequential traps containing 2-ethoxyethanol/ethanolamine (3 : 1, v/v). These solvents were exchanged after 6 and 24 hours, and then at 24-hour intervals up to 3 days after dosing. The interior of each cage was washed with water at the termination of the experiment (and additionally earlier, at 24 hours, in the pre-treated oral 10 mg/kg bodyweight experiment, in an attempt to improve the recovery of radioactivity).

Immediately prior to sacrifice, 5 days after dosing, the animals were lightly anaesthetised with halothane and a blood sample removed from the heart by cardiac puncture. The animals were then killed by cervical dislocation. Following sacrifice the stomach and remaining gastrointestinal tract (separately, including contents in each case), adrenal glands, brain, heart, kidneys, liver, lungs, ovaries (♀), pancreas, spleen, testes (♂), thyroid gland, uterus (♀), and samples of muscle, fat, bone, bone marrow and skin and the remaining carcass were taken for analysis.

Experiments with bile duct-cannulated rats

The operation was carried out with the animals under halothane-oxygen anaesthesia. The bile duct was cannulated with polythene tubing and the incision in the animal closed with sutures. Animals were dosed following recovery from the anaesthetic.

Urine and faeces were collected at 0 - 24 and 24 - 48 hours after dosing. Bile was collected at 0 - 3, 3 - 6, 6 - 12, 12 - 24 and 24 - 48 hours after dosing. The cages were washed at the termination of the experiment. At 48 hours the animals were killed by cervical dislocation and the gastrointestinal tract (including contents), liver and residual carcass were taken for analysis.

Excretion-balance experiments - dermal dosing

About 24 hours prior to application, an area on the back of each rat was shaved using electric clippers, avoiding any laceration or abrasion of the skin. Immediately prior to application, each rat was lightly anaesthetised with halothane-oxygen. The dose formulation was applied to an area of about 10 cm^2 and the treated area was protected with a plastic net cover attached by adhesive dressing to prevent loss and disturbance. The cover was not in contact with the treated area, which was open to the air. After dose application the animals were transferred to restraining cages, which allowed the separate collection of urine and faeces. At 6 hours the cover was removed and the treated area washed with cotton wool swabs soaked in soapy water (see Appendix 9 for specification). This treated area 'wash' was taken for analysis. The animals were then transferred to glass metabolism cages until sacrifice at 120 hours. Urine and faeces were collected separately at 6 - 24 and then at 24-hour intervals. At sacrifice the area of treated skin was removed; this, and the tissues/organs listed above for intact excretion-balance rats were taken for analysis. Dressings and covers removed from the rats after 6 and 120 hours were retained and taken for analysis. Cages were washed with water after 6 and 120 hours.

Prior to the main experiments, a pilot experiment was performed to assess procedures for removing unabsorbed dose from the skin. Four male animals were administered a 100 mg/kg bodyweight dose and after 4 minutes the treated areas of two animals were washed with cotton wool swabs soaked in soapy water and the treated areas of the other two animals were washed with swabs soaked in ethanol.

Sample collection - plasma radioactivity kinetics

Blood samples (*ca* 0.4 ml) were withdrawn from a tail vein into heparinised tubes at pre-dose, and at 0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours following dosing. In each experiment (10 and 100 mg/kg bodyweight), animals were divided into 3 groups of six (3 of each sex), such that samples were taken as follows:

Group 1: Pre-dose, 1, 6, 48, 120 hours.

Group 2: 0.25, 2, 12, 72, 144 hours.

Group 3: 0.5, 4, 24, 96, 168 hours.

In each sample the plasma was separated from the cells by centrifugation.

Sample collection - plasma parent compound kinetics

Groups of six rats (containing 3 of each sex) were sacrificed at 0.5, 2, 6 and 24 hours following dose administration. Immediately prior to sacrifice a blood sample was removed from each animal by cardiac puncture, and the plasma separated from the cells by centrifugation.

Sample collection - tissue distribution experiments

For quantitative experiments groups of animals (3 of each sex) were killed at 0.5, 2 and 48 hours after dosing. The method of sacrifice and the tissues taken for analysis were as described (above) for excretion-balance experiments.

For whole-body autoradiography, pairs of animals (1 of each sex), were sacrificed, by carbon dioxide asphyxiation, at 0.5, 2, 6, 48 and 120 hours after dosing.

Storage of test samples and reference substances

Blood and plasma samples were normally dispensed for radioassay on the day of sampling. Short-term storage (1 - 2 days) where necessary, was at 4°C. Volatile radioactivity trapping solvents were stored at ambient temperature prior to analysis. All other samples were stored at -20°C until taken for analysis.

The maximum duration of sample storage at -20°C from the time of collection to the measurement of radioactivity and initial chromatographic analysis is shown below. Many samples were analysed much sooner after collection than these periods indicate: for example, most urine samples were analysed chromatographically within 6 weeks of collection.

Experiments	Sample	Radioactivity measurement	Chromatography
Excretion-balance (2a - 2h)	Urine	15 days	28 weeks
	Faeces	7 weeks	-
	Tissues	8 weeks	-
	Carcass	21 weeks	-
Plasma parent compound kinetics (3c)	Plasma	1 day	8 weeks
Biliary excretion (4a, 4b)	Urine	14 days	-
	Bile	10 days	9 weeks
	Faeces	8 weeks	-
	Tissues	7 weeks	-
	Carcass	25 weeks	-
Tissue distribution (5b - 5d)	Tissues	6 weeks	31 weeks
	Carcass	26 weeks	-

The unlabelled reference substances were also stored, at -20°C, as neat compounds and in solution, from the date of their receipt until their expiry date (Appendix 3).

ANALYTICAL METHODS

Processing of samples for radioactivity measurement

Urine samples were allowed to thaw and were made up to volume with distilled water in volumetric flasks (typically 50 ml, although in some cases 10, 20 and 25 ml flasks were used). Duplicate aliquots (0.1, 0.5 or 1 ml) were radioassayed.

Bile samples were made up to volume with distilled water in volumetric flasks (10, 25 or 50 ml) and duplicate aliquots (1 ml) radioassayed.

The volumes of volatile radioactivity trapping solutions were measured and duplicate aliquots (1 ml) radioassayed.

Cage wash volumes were measured and duplicate aliquots (1 ml) radioassayed.

In dermal application experiments, the dressings and covers removed after 6 and 120 hours were extracted with methanol. The volumes of the extracts were measured and duplicate aliquots (1 ml) radioassayed. The soapy water washes were made up to volume with water in 100 ml volumetric flasks and 2 × 1 ml aliquots radioassayed.

Faeces samples were homogenised with sufficient water to give a smooth paste. The total homogenate weight was measured and triplicate portions (*ca* 0.4 g) taken for radioassay.

The sacrifice blood samples from the excretion-balance experiments and the tissue distribution experiments were divided into two portions, one of which was centrifuged to separate the plasma from the cells. Duplicate aliquots (*ca* 0.5 g) of whole-blood and of plasma were radioassayed, the plasma directly and the whole-blood after combustion. The blood samples from the plasma radioactivity and parent compound kinetics experiments were centrifuged and the plasma radioassayed (2 × 0.1 ml aliquots).

The weights of organs were recorded. The adrenals, bone marrow, ovaries and thyroid were taken directly for solubilisation. Brain, heart, kidneys, lungs, pancreas, testes, uterus and samples of bone, muscle, fat and skin were homogenised by chopping finely with scissors and triplicate portions (*ca* 0.1 - 0.4 g) were taken for solubilisation. Liver, spleen and gastrointestinal tracts and contents were also homogenised and triplicate portions taken for combustion.

Rat carcasses were solubilised in a cocktail of water, methanol and Triton X-405 (6 : 3 : 1, by volume) containing sodium hydroxide (80 g/l) for 24 hours at 55°C. On cooling, the total volumes were measured. Aliquots (1 ml) were neutralised with nitric acid prior to radioassay.

Measurement of radioactivity

Radioactivity was measured by liquid scintillation counting (LSC). Samples were measured using either model 1219 RackBeta Spectral or Wallac 1409 or 1410 (Pharmacia Biotech Ltd, Milton Keynes, UK) liquid scintillation counters with automatic external standard quench correction. Radioactivity in amounts of less than twice background levels was considered to be below the limit of accurate determination.

Liquid samples such as urine, bile, cage washes, plasma, tissue extracts and solutions associated with dose measurement were mixed with MI-31 scintillator cocktail (Canberra Packard Instrument Co Ltd, Pangbourne, Berkshire, UK) for measurement of radioactivity.

Portions of faecal homogenates, gastrointestinal tracts and contents, liver, spleen and aliquots of blood were combusted in oxygen using an automatic sample oxidiser (model 306 Mk 2 Tri-Carb®, Canberra Packard Instrument Company Ltd). The combustion products were absorbed into Optisorb 1 (9 ml) and mixed with Optisorb S (13 ml) scintillator (Fisons Scientific Equipment, Loughborough, Leicestershire, UK) for measurement of radioactivity. Recoveries of radioactivity from CFR 101 carbon-14 standards for sample oxidisers (Amersham International plc, Amersham, Buckinghamshire, UK) combusted in the oxidiser, exceeded 95%. Measurements of radioactivity were not corrected for oxidiser efficiency.

Whole small organs, portions of bone marrow and homogenates of tissues other than liver, spleen and gastrointestinal tract were treated with NCS-II solubiliser (1.5 ml, Amersham International plc) at 50 - 55°C for up to eighteen hours. When solubilisation was complete, Optisorb S scintillator (10 ml) and methanol (2 ml) were added to each sample. The samples were then left in darkness for about eighteen hours prior to liquid scintillation counting.

Sample LSC data and calculations are shown in Appendix 10.

Whole-body autoradiography

After sacrifice each rat was pinned out on a board and rapidly frozen by total immersion in a bath of solid CO_2/petroleum ether (bp 60 - 80°C) at about -80°C for approximately 30 minutes. After trimming off the legs and tail with a power saw, the frozen carcass was set in a block of aqueous 2% (w/v) carboxymethylcellulose at about -80°C and mounted on to the stage of a model OT/SEB base-sledge microtome in a cryostat with electrolinear drive (Bright Instrument Company Ltd, Huntingdon, Cambridgeshire, UK). The cryostat was maintained at about -20°C and 30 micron sagittal sections were obtained at several levels through the carcass between the level of the kidney and the level of the spinal cord.

The sections, mounted on 'Cellux' tape (National Diagnostics (UK) Ltd, Buckinghamshire, UK), were freeze-dried at 0.04 torr in a Lyolab BII freeze-dryer (Life Science Laboratories, Luton, Bedfordshire, UK) before placing them in contact with Kodak DEF/5 film (Kodak Ltd, Hemel Hempstead, Hertfordshire, UK) in light-tight cassettes.

After 50 days of exposure at -20°C, the autoradiographs were developed in LX-24 developer and fixed in FX-40 X-ray liquid fixative (Kodak Ltd).

One freeze-dried section at each level was mounted and used for reference purposes when evaluating autoradiographs.

The relative concentrations of radioactivity in the various tissues were estimated by visual inspection.

High performance liquid chromatography (HPLC)

HPLC was carried out using the following equipment:

Pump/gradient control:	Model SP8800 ternary pump system (Spectra-Physics Ltd, Hemel Hempstead, Hertfordshire, UK).
Injector:	Model U6K (Waters (UK) Ltd, Watford, Hertfordshire, UK).
Radiodetector:	Ramona 5D fitted with a 400 μl CaF_2 (X-cell) detector cell (LabLogic Systems Ltd, Sheffield, UK).
UV detector:	Spectromonitor D (LDC Analytical, Stone, Staffordshire, UK).
Fraction collector:	Model Frac-100 (Pharmacia Biotech Ltd).

The UV detector operated at a wavelength of 230 nm, except for chromatographic runs involving the detection of reference substance XVII, when a wavelength of 215 nm was used. The UV and radioactivity detectors were connected to a personal computer, employing LabChrom software (version 2.10) for the storage and manipulation of chromatographic data. Radiochromatograms were quantified where necessary, by the collection and radioassay of one-minute fractions of column eluate following sample injection. These LSC data were processed by an IBM XT personal computer. This calculated the proportion of the total net eluted radioactivity for each fraction and the recovery of radioactivity from the column. The column recovery was based upon the radioassay of separate aliquots of test solution to that injected. Column recoveries (not corrected for) were in all cases quantitative.

Chromatographic correspondence of reference substances with the radioactive metabolites of dimethoate (and dimethoate itself) was made after co-injection of the sample and a solution of the reference substance. Correspondence was assessed by examination of the radioactivity and UV absorbance chromatograms (taking into account the time delay between the radioactivity and absorbance detectors) and a comparison of the UV chromatogram with that obtained from the sample alone.

Retention times of dimethoate and reference substances are shown in Table 2.

HPLC method 1 - For the measurement of the relative proportions of radioactive components in urine, bile, plasma and tissue extracts:

Column: Nova-Pak C_{18} 4 μm Radial-Pak cartridge (100 mm × 8 mm, Waters (UK) Ltd).

Mobile phase:
A = Low UV PIC A (tetrabutylammonium hydrogen sulphate) (10 mM) (Waters (UK) Ltd).
B = Methanol.

Gradient (linear changes):

Time (minutes)	% A	% B
0	95	5
50	40	60
51	0	100
60	0	100

Flow rate: 1.0 ml/minute.

Temperature: Ambient.

HPLC method 2 - For the isolation of radioactive components in urine:

Column: As method 1.

Mobile phase:
A = 0.05M potassium phosphate buffer, pH 7.0.
B = Acetonitrile.

Gradient (linear changes):	Time (minutes)	% A	% B
	0	95	5
	15	70	30
	25	70	30

Flow rate: 1.0 ml/minute.

Temperature: Ambient.

HPLC method 3 - For the isolation of radioactive components in urine:

Column: Hamilton PRP-1 (250 mm × 4.1 mm, Hichrom Ltd, Reading, Berkshire, UK).

Mobile phase: 0.05M ammonium acetate, pH 6.5 (isocratic).

Flow rate: 1.0 ml/minute.

Temperature: Ambient.

Thin-layer chromatography (TLC)

Normal phase TLC was carried out on pre-layered, glass-backed Kieselgel 60 F_{254} plates, of layer thickness 0.25 mm (E Merck AG, Darmstadt, Germany). The developing solvent systems, employing chamber saturation, were (ratios by volume):

A. Hexane : acetone (1 : 1).

B. Ethyl acetate : toluene (4 : 1).

C. Dichloromethane : methanol (93 : 7).

D. Butan-1-ol : butan-2-one : water : ammonia solution (0.88 specific gravity) (6 : 3 : 2 : 1).

F. Chloroform : methanol : water (6 : 4 : 1).

Systems A, B and C were used to determine the radiochemical purity of ^{14}C-dimethoate in each dose formulation. Systems D and F were used for the separation of dimethoate and its radioactive metabolites in urine, bile, plasma and tissue extracts.

Radiochromatograms of the developed plates were obtained and quantified using an automatic TLC-Linear Analyser (model 2832 or 2842, Berthold Instruments (UK) Ltd, St Albans, Hertfordshire, UK) linked to an IBM-AT computer. Each radiochromatogram was divided into segments, defined as 'regions of interest' (ROIs). These ROIs, including background areas, were defined by the operator and their positions entered into the linear analyser computer. The computer calculated the average counts per channel for the background area, subtracted it from all other channels and calculated the percentage of total net radioactivity in each ROI.

Apposition autoradiographs of the TLC plates were obtained using Hyperfilm β-max X-ray film (Amersham International plc).

Alternatively, images of the developed plates were obtained using a Fujix BAS 2000 Bio Image Analyser (Fuji Photo Film Co Ltd, Japan) linked to a Solair computer. Imaging plates, coated with the photostimulable phosphor $BaFBr:Eu^{2+}$, were used to accumulate and store irradiated radioactive energy (from contact with the TLC plate). The image reader of the analyser then scanned the imaging plate with a fine laser beam; luminescence was emitted in proportion to the recorded incident radiation intensity. The luminescence was detected by a photomultiplier tube and converted to electrical signals which were stored as high resolution digital data to be recalled for a variety of analyses to be conduced by the operator. An associated 486 personal computer employing PC-BAS 1000 software (Version 1.0, Raytest Isotopenmessgerate GmbH) was used for the generation of linear scaled radiochromatograms; evaluation of these chromatograms, by the selection of 'regions of interest' was essentially as described for the linear analyser (above).

Reference substances were detected by spraying the developed TLC plate with one of the following reagents:

(a) 0.5% (w/v) palladium chloride in 0.1N hydrochloric acid, or,

(b) a mixture of (i) a 10% (w/v) aqueous solution of ammonium heptamolybdate (100 ml), (ii) a 5% (w/v) solution of ammonium vanadate in nitric acid (SG 1.42) : water (2 : 3) (100 ml) and (iii) 4N hydrochloric acid (170 ml).

For establishing chromatographic correspondence of reference substances with the radioactive metabolites of dimethoate the reference substances were co-chromatographed with the sample as follows. A solution (*ca* 10 mg/ml) of the reference substance was applied to the TLC plate as a 2 cm wide band. The sample was also applied as a 2 cm wide band, half of which overlapped with the reference substance band. Co-chromatographic correspondence was assessed by visual inspection of the TLC plate and its autoradiograph or Fujix image. Typical R_f values of the test and reference substances are shown in Table 2.

Limits of detection

Limits of detection and their calculation are shown in Appendix 11 for measurements of radioactivity and chromatographic analyses. Typical limits of detection (defined as a gross sample level of radioactivity of twice the background level) for radioactivity measurements were as follows:

Experiments	Sample	10 mg/kg bodyweight		100 mg/kg bodyweight	
		% dose	mg equiv/kg or l	% dose	mg equiv/kg or l
Excretion-balance, biliary excretion	Urine	0.003	0.001	0.002	0.007
	Faeces	0.008	0.007	0.004	0.045
	Trapping solutions	0.018	0.001	0.011	0.007
	Bile	0.0006	0.002	0.0004	0.014
Plasma kinetics	Plasma	-	0.018	-	0.11
Tissue distribution	*eg* Liver	0.005	0.011	0.003	0.072
	eg Thyroid	0.00008	0.15	0.00005	0.88

Mass spectrometry

Mass spectra of biotransformation products of dimethoate and of reference substances were obtained using a variety of techniques.

For direct insertion probe mass spectrometry, a VG 7070E mass spectrometer (VG Analytical, Manchester, UK) was employed. The sample was reconstituted in acetonitrile (80 μl) and a portion (1 μl) placed in a capillary tube in the probe of the spectrometer. The probe was introduced into the ion source of the mass spectrometer and was heated from 20°C - 350°C at a rate of 20°C/s to vapourise the sample. The ion source was operated in electron-ionisation (EI) mode with an electron energy of 70 eV and a trap current of 100 μA. The resolving power of the instrument was set at 1500 (10% valley definition). A mass range of m/z 500-50 was cyclically scanned at a rate of 2.5 s/decade during evaporation of the sample.

Gas chromatography-mass spectrometry employed a model 5890 gas chromatograph (Hewlett Packard, Bracknell, Berkshire, UK) interfaced with a VG TS250 mass spectrometer (VG Analytical).

A portion (*ca* 20 μg) of the sample was placed in a tapered glass vial and acetonitrile (10 μl) and N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA, 10 μl) were added. The vial was sealed and heated at 100°C for 10 minutes. An aliquot (1 μl) was injected into the split/splitless injector (operating in splitless mode) of the gas chromatograph which housed a DB5 capillary column (15 m × 0.25 mm id, J & W, Folsom, CA, USA) which was temperature programmed from 100°C to 300°C at 20°C/min after an initial hold time of 0.5 minutes. The final temperature was held for 2 minutes. The column was routed directly into the ion source of the mass spectrometer *via* the heated transfer line and re-entrant port, both of which were maintained at 250°C. The ion source was held at 200°C and was operated in EI mode with an electron energy of 70 eV and a trap current of 350 μA. The mass spectrometer was cyclically scanned between m/z 500-50 at a rate of 1 s/scan.

The above techniques were used to obtain spectra from urinary metabolites isolated using HPLC method 2. Ionspray mass spectrometry was used to identify a component isolated using HPLC method 3. A syringe pump (model 5555-22, Harvard Apparatus, South Natick, MA, USA) was used to introduce the sample (as HPLC column eluate), at a rate of 10 μl/minute, into the ionspray interface of a Sciex API III MS/MS instrument (Sciex, Ontario, Canada). Nitrogen (zero grade, BOC Ltd, Croydon, UK) was used as the nebuliser gas at a flow rate of 0.6 L/minute and nitrogen (Research grade, BOC Ltd) also at 0.6 L/minute, was used as the curtain gas. A negative ion ionspray mass spectrum over the mass range m/z 100-300 was recorded by summing several Q1 scans during infusion of the sample.

The ionspray mass spectra were examined to identify the likely $[M-H]^-$ ions for metabolites of dimethoate present in the sample. The $[M-H]^-$ ions of interest were then selected for MS/MS analysis in product ion scan mode. The Q1 mass analyser was set to pass only ions corresponding to the pre-selected precursor $[M-H]^-$. Ion dissociations were induced by colliding the precursor ions with argon (Research grade, BOC Ltd) The collision gas thickness was *ca* 3×10^{15} atoms/cm^2. A product ion spectrum was recorded by scanning the Q3 mass analyser across a mass range from m/z 10 to a point several mass units above the selected precursor mass. Several scans were summed to produce the daughter ion spectrum of sufficient signal intensity.

Pharmacokinetic analysis

Maximum plasma concentrations (C_{max}) and the times required to reach C_{max} (T_{max}) were the measured values. Areas under the plasma radioactivity concentration *versus* time curves (AUC) were calculated, by computer, up to the last concentration (at 168 hours) by the logarithmic-linear trapezoidal method. Terminal rate constants λ_z of radioactivity concentration decline were calculated by linear regression of data points selected by the operator (these were the 12-hour to 168-hour points in all cases). Terminal half-lives ($t_{½\lambda_z}$) were derived from $t_{½\lambda_z} = \ln 2/\lambda_z$. The computer software employed was KIN (v 5.1), written and validated at the Huntingdon Research Centre.

CHROMATOGRAPHIC ANALYSIS AND IDENTIFICATION OF METABOLITES

Chromatographic analysis of urine

Urine collected from animals during 48 hours after oral, intravenous and dermal doses of ^{14}C-dimethoate at nominal levels of 10 and 100 mg/kg bodyweight was combined (males and females separately). These samples were analysed using HPLC method 1 (from which quantitative data were obtained) and by TLC using systems D and F. Selected urine samples were co-chromatographed with dimethoate and the reference substances in these chromatographic systems.

Further portions of urine samples (buffered with sodium acetate buffer, pH 5.0) were incubated with β-glucuronidase/sulphatase enzyme (from *Helix pomatia*, type H-1, Sigma Chemical Co Ltd, Poole, Dorset, UK). The solutions incubated for ca 17 hours at 37°C. Control incubations were set up that omitted the enzyme. The activity of the enzyme was demonstrated by the production of a purple colouration on the addition of aqueous sodium hydroxide to an incubated solution containing buffered urine (as above), enzyme and also phenolphthalein glucuronide (indicating that the enzyme had hydrolysed the glucuronide). Aliquots of the incubated solutions, including controls, were analysed by TLC.

Chromatographic analysis of bile

Bile collected deep-frozen from animals during 12 hours after oral doses of ^{14}C-dimethoate at nominal levels of 10 and 100 mg/kg bodyweight was combined (males and females separately). These samples were analysed using HPLC method 1 (from which quantitative data were obtained) and by TLC using systems D and F. Further portions of selected bile samples were incubated with β-glucuronidase/sulphatase enzyme as described (above) for urine. Selected samples were also co-chromatographed (TLC) with samples of urine.

Chromatographic analysis of plasma

Plasma from rats sacrificed 0.5, 2, 6 and 24 hours following a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight (experiment 3c) was analysed. Plasma from the animals within each group was combined (males and females separately). Duplicate aliquots (100 µl) of each pooled sample were radioassayed. Prior to chromatographic analysis (HPLC method 1), a small quantity of ammonium sulphate was added to the sample to precipitate the plasma proteins. After sonication for 5 minutes the mixture was centrifuged and the supernatant removed. Duplicate aliquots (100 µl) of the supernatant were radioassayed to assess the recovery of radioactivity. Selected plasma samples were also co-chromatographed (TLC) with samples of urine, and (HPLC and TLC) with dimethoate.

Extraction and chromatographic analysis of kidney and liver

Kidneys and liver from rats sacrificed 0.5 hours after single or multiple oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight, or a single oral dose at a nominal level of 100 mg/kg bodyweight, were analysed (except male kidney at 100 mg/kg bodyweight where the 2-hour sample was analysed). Samples from the male and female animals were analysed separately.

For each group of animals, remaining kidney and liver (after removal of portions for radioassay) were combined and further homogenised. After removal of aliquots for radioassay, portions of pooled tissue were extracted, two or three times with methanol, by shaking on an orbital shaker, followed by centrifugation. The solid debris were weighed and radioassayed. The extracts were radioassayed, combined, then concentrated under nitrogen, to 5 ml (accurately measured in volumetric flasks). Duplicate aliquots (100 or 200 μl) of the concentrated solutions were radioassayed to check the recovery of radioactivity.

Aliquots of the kidney and liver extracts were analysed by TLC using solvent system D (from which quantitative data were obtained) and in some cases by HPLC (method 1). Selected kidney and liver extracts were co-chromatographed (TLC) together, and with samples of urine and reference substances.

Isolation of urinary metabolites

While HPLC method 1 provided a good separation of the urinary metabolites, the presence of the ion pair reagent meant that it could not easily be used for an initial separation prior to spectroscopic identification. Consequently, alternative liquid chromatographic systems were developed, which utilised conventional buffer gradients (methods 2 and 3). These did not provide as good a separation of the radioactive components as method 1 and only the major metabolites were adequately resolved.

Portions of urine from rats dosed at a level of 100 mg/kg bodyweight (experiments 2a or 2b) were injected into the chromatographic system (either HPLC method 2 or 3). The column eluate corresponding to the radioactive components was collected (each component separately). A number of chromatographic runs were carried out in this manner and the eluate corresponding to each separated component was combined.

In one experiment (using method 2), the eluate solutions were partitioned with ethyl acetate (following removal of the organic solvent from the solution, acidification of the remaining aqueous solution and addition of sodium chloride). The ethyl acetate fractions were dried ($MgSO_4$) and evaporated to dryness (N_2), prior to analysis by mass spectrometry.

In a separate experiment (using method 3) collected eluate solutions were analysed directly by mass spectrometry.

LOCATION OF STUDY RECORDS

All raw data and study-related documents generated during the course of the study at HRC, together with a copy of the final report, are lodged in the Huntingdon Life Sciences Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five-year retention period the Client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Client's knowledge.

Notebook reference numbers are MPK 93/3453, MPK 93/3454, MEC 935, MEC 9310, MEC 9322, MEC 9332, MEC 9338, MEC 9407, MEC 9416, MEC 9453, MEC 9485, MEC 94102, MEC 94125 and MEC 94176.

RESULTS

PRE-TEST RESULTS AND DOSE SELECTION

The oral and dermal routes were chosen as they are the potential routes for human exposure to the test substance. The intravenous route is also requested by EPA guidelines as the water solubility of the test substance permits dosing by this route. Relevant guidelines (OECD, EPA) also specify that two dose levels should be studied, a 'low' level which causes no toxic effects (No Observable Effect Level, NOEL) and a 'high' level which causes slight toxic effects (Low Observable Effect Level, LOEL).

The results of the pre-test (dose range-finding) experiments using non-radiolabelled dimethoate are summarised in Appendix 12. When dimethoate was administered orally at 100 mg/kg bodyweight, slight toxic effects (body tremors) were observed in male rats. As there were no observable effects at the lower dose level of 10 mg/kg bodyweight, these two levels were selected for oral administration. A dose level of 10 mg/kg bodyweight was also selected for intravenous administration to facilitate the comparison of results between these routes.

The highest dose level for dermal application that could be achieved with a workable suspension of the test substance was 250 mg/kg bodyweight. No toxic effects were observed at this level. It was felt, however, that practical constraints regarding the containment and accurate quantification of radioactive doses meant that increasing the dermal dose level beyond this would not be feasible. In view of this, dose levels of 10 and 100 mg/kg bodyweight were selected, to facilitate the comparison of results obtained after oral and dermal administration.

EXCRETION AND RETENTION OF RADIOACTIVITY

After oral administration of ^{14}C-dimethoate the patterns of excretion of radioactivity by rats were similar at each dose level used in the study, *viz* 10 and 100 mg/kg bodyweight, with no significant sex differences (Tables 3a and 3b). At both dose levels, from 89% to 95% of the dose was excreted within five days of administration. Most of this was excreted in the urine (most within 12 hours after administration at 10 mg/kg bodyweight, or 24 hours at 100 mg/kg bodyweight) with small amounts each lost in expired air (2.1 - 2.5%) and eliminated *via* the faeces (1.2 - 1.6%). Pre-treatment of rats with dimethoate at a level of 10 mg/kg bodyweight for 14 consecutive days did not affect the pattern of excretion of a subsequent radioactive dose.

A similar situation was observed in rats following intravenous administration at 10 mg/kg bodyweight (Tables 4a and 4b). Urinary excretion was even more rapid, with 80% dose excreted in the first 6 hours after administration.

Total retention of radioactivity by animals five days after oral or intravenous administration did not amount to more than 2% dose.

Absorption of radioactivity by dermally-dosed rats decreased as the rate of application was increased. Absorption amounted to 9 - 11% dose at an application rate of 10 mg/kg bodyweight (0.2 mg/cm^2), but only 1 - 2% dose at 100 mg/kg bodyweight (2 mg/cm^2) (Table 5a). In terms of weight equivalent of dimethoate, absorption was therefore similar at each dose level, *ie* approximately 1 mg dimethoate/kg bodyweight in each case (Table 5b). The unabsorbed radioactivity was mainly recovered in the washings of the treated skin done 6 hours after dose application. (A pilot experiment was first performed to determine the best procedure for recovering unabsorbed material. The results (Table 6) clearly show that soapy water was most effective at doing this and this was used in the main experiments.) Absorbed radioactivity was excreted mainly in the urine, an observation consistent with the pattern of excretion of radioactivity following oral or intravenous administration.

Mean total recoveries of radioactivity from rats in the excretion-balance experiments described above were generally satisfactory (Tables 3 - 5) although some recoveries from individual animals were slightly low (see Appendix 13). Losses of radioactivity were probably incurred in the measurement of the initial (0 - 6 hours) urine sample which contained a high proportion of the total dose in a relatively small volume. Therefore any small physical loss of this sample, however small, would be significant. Recoveries from some rats in the oral 10 and 100 mg/kg bodyweight experiments were considered unacceptable and additional rats were treated. The results of the radioactivity measurements - not complete - made from the original animals are shown in Appendix 14.

BILIARY EXCRETION OF RADIOACTIVITY

Bile was a minor route of excretion of radioactivity in rats with cannulated bile ducts after oral administration of ^{14}C-dimethoate (Figure 3, Tables 7a, 7b, 8a and 8b).

During 48 hours after dosing at 10 and 100 mg/kg bodyweight, means of 4 - 5% dose were excreted in the bile, with excretion slightly more rapid in female rats than in male rats at the higher dose level. Biliary excretion was largely complete within 24 hours. Most of the remaining dose (82 - 87%) was excreted in the urine.

Most of the radioactivity excreted in the bile was subsequently re-absorbed in the gut (entero-hepatic recirculation); only 1 - 2% of an administered dose in intact rats was eliminated *via* the faeces (Table 3a).

PLASMA KINETICS OF RADIOACTIVITY

Plasma concentration versus time profiles of radioactivity following oral administration of ^{14}C-dimethoate are shown in Table 9 and illustrated in Figures 4 - 7. Pharmacokinetic parameters are shown in Table 10.

The time to reach peak plasma radioactivity concentration (T_{max}) was 0.5 hours in both sexes at a dose level of 10 mg/kg bodyweight and in female rats at 100 mg/kg bodyweight. This time was reached earlier (0.25 hours) in male rats at 100 mg/kg bodyweight. Mean peak plasma concentrations of radioactivity (C_{max}) were also similar between the sexes at 10 mg/kg bodyweight; 8.62 and 7.68 mg dimethoate equivalents/l in males and females respectively. Corresponding mean C_{max} values after dosing at 100 mg/kg bodyweight were 50.7 and 93.2 mg equiv/l, representing approximately six and twelve-fold increases respectively over the lower level.

At both dose levels, after reaching the peak concentration, radioactivity levels declined in an apparently biphasic manner, with the terminal phase extending from about 12 hours after dosing. The half-lives of the terminal phase were broadly similar for both sexes and at each dose level (36 - 59 hours) with slightly higher values seen in females. Terminal half-lives were calculated, in all cases, using the 12-hour to 168-hour concentrations. In rats dosed at 100 mg/kg bodyweight only, the initial rapid decline was followed by an increase in plasma concentration after 6 hours, to be followed by the terminal phase. This phenomenon looks genuine in that it appeared in both sexes and is presumably a consequence of the rate of absorption from the gut temporarily exceeding the rate of excretion in the urine during this period.

Mean areas under plasma radioactivity concentration against time curves (AUCs) were similar between males and females at 10 mg/kg bodyweight (49 mg equiv hr/l). For a ten-fold increase in dose level, the mean AUC in male rats increased by a factor of about 8 from the low to the high dose, but by about 14 in female rats.

PLASMA PARENT COMPOUND KINETICS

Concentrations of dimethoate in plasma following oral administration at 100 mg/kg bodyweight are shown in Figure 8 and Table 11. Concentrations fell rapidly from 6 - 7 mg/l 0.5 hours after administration, to 1 - 2 mg/l after 2 and 6 hours. At 24 hours after administration, plasma concentrations of dimethoate were below detectable levels (0.051 mg/l, see Appendix 11).

While total plasma radioactivity concentrations were similar between the sexes, there was a higher proportion of dimethoate in plasma in female rats (up to 2-fold after 2 and 6 hours). Also, the total plasma radioactivity concentration in female rats increased from 2 to 6 hours, corroborating the results of the plasma radioactivity kinetics experiment.

TISSUE DISTRIBUTION OF RADIOACTIVITY

Single oral dose, 10 mg/kg bodyweight

Mean tissue concentrations of radioactivity occurring at various times after a single oral dose at 10 mg/kg bodyweight are shown in Table 12 and are illustrated in Figures 9 and 10. The mean quantities of radioactivity (in terms of % dose) remaining in the tissues and carcasses of the rats are shown in Table 13. Mean tissue : plasma ratios are shown in Table 14. The tissue distribution of radioactivity was similar in male and female rats. Highest concentrations in almost all tissues occurred in animals sacrificed 0.5 hours after dosing. The highest radioactivity concentrations occurred in the kidneys and liver: in male rats the mean maximum radioactivity concentrations in kidney and liver were 20.0 and 8.57 mg dimethoate equivalents/kg (mg equiv/kg) respectively, while in females the corresponding mean concentrations were 24.6 and 11.7 mg equiv/kg. Mean maximum radioactivity concentrations in other tissues were between 1 and 5.5 mg equiv/kg, with the lowest mean maximum concentrations (*ca* 1 mg equiv/kg) occurring in brain and fat. Concentrations in tissues declined with time at similar rates in all cases (Figures 11 and 12). At 48 hours the concentration of radioactivity in all tissues was less than 1 mg equiv/kg.

Multiple oral dose, 10 mg/kg bodyweight

Mean tissue concentrations of radioactivity occurring at various times after the last of seven daily oral doses at 10 mg/kg bodyweight are shown in Table 15 and are illustrated in Figures 13 and 14. Mean tissue : plasma ratios are shown in Table 16. In female rats the highest concentrations in all tissues occurred in animals sacrificed 0.5 hours after the final dose, whereas in males, mean tissue concentrations were broadly similar at 0.5 and 2 hours, and declined thereafter. The highest radioactivity concentrations occurred in the kidneys and liver: in female rats the mean maximum concentrations in these tissues were 28.1 and 13.2 mg equiv/kg respectively, while in males corresponding values were *ca* 19 - 21 and *ca* 13 mg equiv/kg. Overall, mean maximum radioactivity concentrations were slightly higher (approximately 1.5 - 2 fold) than after a single 10 mg/kg bodyweight dose, ranging from 2 to 7 mg equiv/kg in other tissues. Again, lowest mean maximum concentrations (*ca* 2 mg equiv/kg) occurred in brain and fat. Concentrations in tissues declined with time at broadly similar rates (Figures 15 and 16). At 48 hours, the concentration of radioactivity in most tissues was *ca* 1 mg equiv/kg or below, although levels of 2 - 3 mg equiv/kg were present in liver and pancreas.

Single oral dose, 100 mg/kg bodyweight

Mean tissue concentrations of radioactivity occurring at various times after a single 100 mg/kg bodyweight dose are shown in Table 17 and are illustrated in Figures 17 and 18. Mean quantities of radioactivity remaining are shown in Table 18 and mean tissue : plasma ratios are shown in Table 19. Highest concentrations of radioactivity generally occurred in animals sacrificed 0.5 hours after dosing, although there were exceptions in male rats (adrenals, kidneys, pancreas and thyroid) where the highest concentrations were seen after 2 hours. In most tissues mean maximum levels of radioactivity were about 5 - 18 times higher than those measured after a single 10 mg/kg bodyweight dose, with slightly lower ratios occurring in female rats in all cases. In male rats peak concentrations in adrenals and fat were 30 and 51 times higher respectively than corresponding peak concentrations at 10 mg/kg bodyweight. The kidneys and liver were again the tissues in which the highest radioactivity concentrations occurred. In male rats mean maximum concentrations in kidney and liver were 205 and 73.0 mg equiv/kg respectively, while in females corresponding values were 127 and 59.4 mg equiv/kg. Mean maximum radioactivity concentrations in other tissues generally ranged up to about 36 mg equiv/kg, although levels in male adrenals and fat were somewhat higher (67.0 and 58.6 mg equiv/kg respectively). As at the lower dose level, concentrations in all tissues declined at broadly similar rates (Figures 19 and 20), such that at 48 hours the concentration of radioactivity in most tissues was between 1 and 3 mg equiv/kg, although levels in liver and pancreas remained at *ca* 4 - 5 and *ca* 8 mg equiv/kg respectively.

Excretion-balance experiments

The mean tissue concentrations of radioactivity occurring at 120 hours after an oral, intravenous or dermal dose are shown in Tables 20, 21 and 22 respectively. Following single oral or intravenous administration at 10 mg/kg bodyweight mean tissue concentrations at 120 hours were generally about or below 0.1 mg equiv/kg, although slightly higher levels (up to about 0.3 mg equiv/kg) were observed in some tissues (kidneys, liver and pancreas). Concentrations were broadly similar after single oral and intravenous administration, while they were generally slightly higher (up to about two-fold) after repeated dosing with non-radiolabelled dimethoate. Mean tissue concentrations 120 hours after a single oral dose at 100 mg/kg bodyweight were generally below about 3 mg equiv/kg, although higher levels (5 - 7 mg equiv/kg) were observed in the pancreas of both sexes and the thyroid of male rats. Following dermal administration, mean tissue concentrations 120 hours after sacrifice were in many cases less than the limit of accurate determination (especially at 100 mg/kg bodyweight), and in any event were no greater than 0.2 mg equiv/kg (10 mg/kg) or 0.9 mg equiv/kg (100 mg/kg).

Single oral dose, 10 mg/kg bodyweight (whole-body autoradiography)

The distribution of radioactivity in the tissues of rats was investigated qualitatively using whole-body autoradiography (Plates 1 - 10). Single male and female rats were sacrificed at various times after a single oral dose at 10 mg/kg bodyweight.

In general, the results corroborated those of the quantitative experiments. The highest levels of radioactivity occurred in tissues in those animals sacrificed the shortest times after dosing. At these times, the highest concentrations were present in the contents of the gastrointestinal tract and the liver and kidneys. The central nervous system contained the lowest tissue concentrations. There did appear to be some accumulation of radioactivity in the Harderian and the intra- and exorbital lachrymal glands. Even at 48 and 120 hours after dosing, concentrations in these glands were noticeably higher than those in the surrounding tissues, and appeared similar to those of liver and kidney. Higher than average levels of radioactivity were also observed in the preputial gland, up to 48 hours after dosing.

PROPORTIONS OF RADIOACTIVE COMPONENTS IN BIOLOGICAL SAMPLES

Urine

Urine collected up to 48 hours after oral, intravenous and dermal administration of ^{14}C-dimethoate was analysed. This represented most (83 - 91%) of the administered dose in orally and intravenously dosed rats, and most of the absorbed dose in dermally dosed rats.

Reverse phase HPLC employing a gradient of an ion pair reagent and methanol (HPLC method 1) provided a good separation of the radioactive urinary metabolites of dimethoate, and representative radiochromatograms are shown in Figures 21 - 23. TLC was used to corroborate chromatographic identifications of metabolites (made initially by HPLC) and the Fujix images and associated radiochromatograms of typical separations are shown in Figures 24 and 25.

At least eight metabolites of ^{14}C-dimethoate in urine were resolved by the HPLC method, as well as dimethoate itself. No qualitative differences in the metabolite profiles were observed after dosing *via* different routes or at different dose levels. Additionally, for each dose route/dose level, no significant sex differences in the proportions of the various metabolites were apparent (Tables 23 and 24).

In all cases, two metabolites represented most of the excreted radioactive dose, and these were initially identified, by co-chromatography with the authentic reference standards, as dimethyldithiophosphate (U7, reference substance XV) and dimethoate carboxylic acid (U9, reference substance III). Dimethyldithiophosphate represented between 20 and 30% of the total dose in orally and intravenously dosed rats, while dimethoate carboxylic acid similarly represented between 29 and 46% dose. The level of dimethoate carboxylic acid in the urine of these rats was about twice that of dimethyldithiophosphate after single oral administration at 10 and 100 mg/kg bodyweight and after intravenous administration (at 10 mg/kg bodyweight), whereas the levels of these two components were similar following pre-treatment of the rats with non-radiolabelled dimethoate at 10 mg/kg bodyweight for 14 days. A third metabolite (U4), representing between 4 and 11% of the total dose after oral or intravenous administration, was similarly identified as dimethylthiophosphoric acid (reference substance XVI), while a fourth (U2), representing between 1 and 6% dose in these rats was

identified as omethoate (reference substance II). Other components in urine included U1 (*ca* 4 - 7% dose in orally and intravenously dosed rats) and U3 and U6 (each *ca* 2 - 4% dose). None of these unidentified urinary metabolites corresponded to any of the available reference substances. The identities of dimethyldithiophosphate, dimethoate carboxylic acid and dimethylthiophosphoric acid were further confirmed by mass spectrometry (see below). (Isolation of U1 and U3 was attempted but was unsuccessful.)

In dermally dosed rats, the proportions of the radioactive components, in terms of % absorbed dose, were broadly similar to those following oral or intravenous administration (Table 24).

HPLC chromatograms demonstrating the correspondences described above, as well as with unchanged dimethoate, are shown in Figures 26 - 30. It should be borne in mind that HPLC co-chromatography was hindered by the necessity of using a relatively low detection wavelength (230 nm) due to the low UV absorbance of many of the reference substances. This increased the intensity of the UV profile of the urine sample itself, and indeed, one interfering UV peak was so intense that co-chromatography of urine with reference substance XV was not possible (Figure 29). Also, the very low absorbance of reference substance XVI is revealed in Figure 30, and so in this figure the UV chromatogram of the standard alone is included for clarity. A retention time could not be obtained for some of the reference substances (Table 2). In these cases their correspondence to any of the radioactive components in urine was ruled out by the use of thin-layer chromatography.

Incubation of urine samples with the enzyme β-glucuronidase/sulphatase had no effect on the chromatographic profiles of urine samples, indicating that conjugation of the radioactive metabolites with glucuronic acid or sulphate did not occur.

Unchanged dimethoate in urine accounted for between 1 and 2% dose in orally and intravenously dosed rats and *ca* 1% absorbed dose in dermally dosed rats.

Bile

Bile collected up to 12 hours after single oral doses at 10 and 100 mg/kg bodyweight was analysed. This represented between 3 and 5% of the total dose. In experiment 4a (100 mg/kg bodyweight), one female rat (no. 125) was sacrificed 29 hours after dose administration following poor recovery from the surgery. However, bile production from this rat was consistent with the others in its group and its bile was included for analysis.

Use of HPLC method 1 revealed a complex mixture of at least 13 radioactive components (Figure 31, Table 25). There were no qualitative and no significant quantitative differences between the two dose levels and no sex differences were apparent also. The major metabolite present in each case was dimethoate carboxylic acid (reference substance III) (1 - 2% dose) with lower amounts of dimethyldithiophosphate (reference substance XV) (0.2 - 0.4% dose). Dimethoate was present at levels of 0.1 - 0.2% dose. A large number of polar components (at least nine) were also found in bile (none of which accounted for more than 0.8% dose). None of these were affected by β-glucuronidase/sulphate enzyme. TLC analysis indicated that some may have been present in the urine as minor components.

Plasma

Plasma samples from the plasma parent compound kinetics experiment (3c) were analysed to determine the proportions of dimethoate and major metabolites. The recovery of radioactivity in the plasma following precipitation of the plasma proteins with ammonium sulphate was quantitative in all cases (94.4 - 101.9%) and thus no corrections were made. As with urine and bile, HPLC method 1 was the chromatographic method used (Figure 32, Table 26). This revealed that dimethoate carboxylic acid, dimethyldithiophosphate and dimethoate itself were major radioactive components in plasma. While dimethoate carboxylic acid (reference substance III) was the predominant metabolite present at 0.5 hours after dosing, the levels of this and of dimethyldithiophosphate (reference substance XV) were similar in subsequent samples (at least up to 6 hours). At 6 hours after dosing, with total plasma radioactivity concentrations of 11 - 13 mg equiv/l (in experiment 3c, Table 11), the concentrations of dimethoate carboxylic acid and dimethyldithiophosphate were both between 2 and 4 mg equiv/l, while the concentration of dimethoate was 1 - 2 mg/l.

In addition to these components, at least 6 other metabolites were resolved. The polar component, designated Pl/Pla, which appeared in some cases to comprise at least two components, increased in relative proportion with time, such that its concentration (2 - 4 mg equiv/l) did not significantly change between 0.5 and 6 hours after dosing. Co-chromatographic analysis with urine indicated that components P3 and P4 were probably omethoate (reference substance II) and dimethylthiophosphoric acid (reference substance XVI) respectively, and that components P5 and P6 were probably equivalent to the urinary components U5 and U6 respectively.

Tissues

Samples of kidney and liver which contained the highest levels of radioactivity after single and multiple oral doses at 10 mg/kg bodyweight and a single oral dose at 100 mg/kg bodyweight were selected for analysis. At 10 (single dose) and 100 mg/kg bodyweight, these were tissues taken from rats 0.5 hours after dosing, with the exception of male kidney at 100 mg/kg bodyweight where the highest mean concentration was seen after 2 hours. In the multiple radioactive dose experiment the tissues that were taken for analysis were those taken 0.5 hours after the final dose in all cases, although the mean concentration in male liver was slightly greater after 2 hours.

Extractability of tissue radioactivity with methanol was generally good (73 - 96%), particularly after single doses of ^{14}C-dimethoate, and less so after multiple dosing (Table 27). The values in Table 27 are proportions of total recovered radioactivity (% in extracts + % in residue = 100%); actual recoveries of radioactivity lay in the range 102.1 - 107.7%. Recoveries of radioactivity in concentrated extracts, prepared for chromatographic analysis were considered quantitative in all cases and no corrections were made. TLC solvent system D was used to separate and quantify the metabolites in kidney and liver, as the low levels of radioactivity in the samples meant that it was difficult to obtain HPLC radiochromatograms which were good enough for accurate quantification. Also, polar components, especially in extracts of liver, were inadequately resolved using HPLC. TLC solvent system F was used to corroborate component identifications made using system D, while chromatographic identification was further corroborated by co-chromatography of tissue extracts with urine.

In kidney, TLC resolved eleven radioactive components, including dimethoate carboxylic acid, dimethyldithiophosphate, dimethylthiophosphoric acid and dimethoate (Table 28). There were significant quantitative differences between dose levels. At 10 mg/kg bodyweight, dimethyldithiophosphate (XV) was the predominant species (40 - 41% kidney radioactivity,

0.7 - 0.8% dose), with lower levels of dimethoate carboxylic acid (III) (*ca* 15% kidney radioactivity, 0.3% dose). At the higher dose level this situation was reversed, with dimethoate carboxylic acid and dimethyldithiophosphate representing 35 - 48 and 10 - 22% kidney radioactivity (0.3 - 0.8% and 0.2% dose) respectively. A broadly similar situation to that seen after a single 10 mg/kg bodyweight dose was seen following multiple dosing with higher levels of dimethyldithiophosphate than of dimethoate carboxylic acid. Dimethoate represented between 2 and 7% kidney radioactivity (0.1% dose) at both dose levels, apart from in males following multiple dosing at 10 mg/kg bodyweight where an apparent value of 20% kidney radioactivity (0.3% dose) was observed. The levels of other extractable kidney metabolites were broadly similar between dose levels, including those of dimethylthiophosphoric acid (XVI) (*ca* 8 - 14% kidney radioactivity, 0.1 - 0.3% dose) and component K3 (6 - 9% kidney radioactivity, 0.1 - 0.2% dose). No other extractable metabolite represented more than 5% tissue radioactivity or 0.1% dose. A TLC Fujix image of typical separations of kidney metabolites is shown in Figure 33, with representative radiochromatograms shown in Figure 34.

Around 10 radioactive components in extracts of liver were resolved by the TLC system (Table 29). Dimethyldithiophosphate, dimethylthiophosphoric acid and component L4 were major components at both dose levels, representing 9 - 22, 12 - 19 and 15 - 29% liver radioactivity (0.4 - 1.0, 0.4 - 0.8 and 0.4 - 1.6% dose) respectively, while in contrast to kidney, dimethoate carboxylic acid was apparently not present at all in liver. A number of polar components (L1 - L3), which were only adequately resolved in some cases, together accounted for 11 - 15% liver radioactivity (0.4 - 0.7% dose); where they were resolved, no individual component represented more than 7% liver radioactivity or 0.3% dose. The only other quantitatively significant component was the unidentified L5, which represented between 6 and 15% liver radioactivity (0.3 - 0.6% dose). Dimethoate was present in all cases at levels of 1 - 4% liver radioactivity or 0.1 - 0.2% dose. A TLC Fujix image of typical separations of liver metabolites is shown in Figure 35, with representative radiochromatograms shown in Figure 36.

Components K3 in kidney and L4 in liver were possibly dimethylphosphoric acid. This reference substance (XVII) corresponded to these components in TLC system D (and to a quantitatively similar component in system F), but corroboration of this identification by co-chromatography with urine could not be made as this substance was not present in urine.

CHARACTERISATION OF METABOLITES

In addition to co-chromatographic analysis (HPLC and TLC) with reference substances, metabolites in urine were isolated using reverse-phase HPLC (methods 2 or 3) and characterised by mass spectrometry or gas chromatography-mass spectrometry. Confirmation of structures was obtained by comparison with reference substances. Isolated metabolites were re-chromatographed using HPLC method 1 to establish to which component in this system they corresponded.

In addition to those components described below, isolation of urinary metabolites U1 and U3 was also attempted. It was found, however, that the resolution of the urinary profile using either HPLC methods 2 or 3 was sufficient to enable only the isolation of the major components. No unidentified component in urine represented more than 7% dose.

No attempts were made to isolate tissue metabolites as their levels were so low. The maximum proportion of any single component in kidney was 0.8% dose, and similarly in liver 1.6% dose.

Component U4

This urinary metabolite was isolated using HPLC method 3 and characterised using ionspray mass spectrometry. Daughter ion spectra of the precursor ion of m/z 141 of both the major radioactive component in the isolate and of reference substance XVI are shown in Figure 37. Despite the presence of a daughter ion of m/z 59 in the isolate (presumably from some other component in the isolate) it can be concluded that this urinary metabolite is dimethylthiophosphoric acid.

Component U7

The mass spectra of the trimethylsilylated derivatives of both this component and of reference substance XV are shown in Figure 38. The close correspondence of the spectra to each other (the GC retention times were identical also - 2'31" - see text in Figure) indicate that this metabolite is dimethyldithiophosphate.

Component U9

The direct insertion probe mass spectra of both this component and of reference substance III are shown in Figure 39. The close correspondence of the spectra to each other indicate that the metabolite is dimethoate carboxylic acid.

BIOTRANSFORMATION PATHWAY OF DIMETHOATE

The proposed biotransformation pathway is shown in Figure 40. Dimethoate was metabolised in the rat *via* a number of oxidative and hydrolytic pathways. Quantitatively, the most important followed cleavage of the C-N bond to yield dimethoate carboxylic acid, which was then subsequently metabolised to dimethyldithiophosphate, dimethylthiophosphoric acid and dimethylphosphoric acid. Dimethoate was also converted to its oxon analogue, omethoate, which would then presumably be metabolised in an analogous manner. Metabolism *via* loss of the methoxy groups (yielding CO_2) was a minor process.

STORAGE STABILITY OF TEST SAMPLES AND REFERENCE SUBSTANCES

Various samples were analysed chromatographically at intervals during the study. For example, urine from experiment 2b (excretion-balance, oral, 100 mg/kg bodyweight) was analysed by HPLC in October 1993 (*ie* soon after its collection), in January 1994 and in November 1994 (Figure 41). Apart from some variation in the retention times of the radioactive components, there were clearly no qualitative or significant quantitative differences in the metabolite profile.

Some extracts of kidney and liver were also chromatographed on more than one occasion. For example, liver from male rats in experiment 5b (tissue distribution, 100 mg/kg bodyweight) was extracted in April 1994. These extracts were first chromatographed in July 1994 and then at later dates, up to March 1995 (Figure 42). Again, there was no significant change in the metabolite profile during this period.

On the basis of these results, it may be concluded that there was no evidence for the degradation of any metabolites in urine or in extracts of tissues during -20°C storage.

Bile and plasma samples were generally analysed chromatographically once only soon after collection.

The stability of the unlabelled reference substances was assessed by their chromatographic behaviour throughout the study. In most cases, essentially only one HPLC UV peak or TLC spot was observed, which for each substance did not vary with respect to retention time or R_f (Table 2).

CONCLUSIONS

Oral doses of ^{14}C-dimethoate at nominal levels of 10 and 100 mg/kg bodyweight and an intravenous dose at 10 mg/kg bodyweight were excreted largely in the urine (85 - 91% dose after 5 days), with lower amounts each in expired air (1.6 - 2.5% dose) and in the faeces (1.2 - 1.6% dose). Pre-treatment of rats with non-radiolabelled dimethoate did not affect the pattern of excretion of a subsequent 10 mg/kg bodyweight oral dose. Excretion was rapid, with most of the dose lost within 6 hours (intravenous dose), within 12 hours (oral 10 mg/kg bodyweight dose) or within 24 hours (oral 100 mg/kg bodyweight dose). No more than 2% dose was retained by animals five days after dosing. Biliary excretion of radioactivity by cannulated rats amounted to 4 - 5% of both 10 and 100 mg/kg bodyweight oral doses. Adsorption of radioactivity in dermally-treated rats decreased as the rate of application was increased, but in terms of weight equivalent of dimethoate absorbed, was similar at each dose level, *viz ca* 1 mg/kg bodyweight, or 0.02 mg/cm^2. Absorbed radioactivity was excreted mainly in the urine.

Plasma kinetics of radioactivity were similar between the sexes at 10 mg/kg bodyweight but showed some differences at 100 mg/kg bodyweight. At both dose levels, peak plasma concentrations were reached quickly, within 0.25 - 0.5 hours, and generally declined biphasically thereafter, with mean terminal half-lives in the range 36 - 59 hours. At the higher dose level, following the initial decline, plasma concentrations rose slightly after 6 hours, to be followed by the terminal phase. At 100 mg/kg bodyweight mean peak plasma radioactivity concentrations and areas under plasma radioactivity against time curves (AUCs) were higher in female rats, such that AUCs were about 8 times greater in males but 14 times greater in females than those at 10 mg/kg bodyweight. At a dose level of 100 mg/kg bodyweight, dimethoate was detectable in plasma up to 6 hours after administration; after 24 hours dimethoate was below the detectable limit of 0.051 mg/l.

Following single oral doses of ^{14}C-dimethoate, radioactivity concentrations were, with some exceptions, highest at the time of the first sacrifice (0.5 hours) and declined with time at broadly similar rates. The highest concentrations of radioactivity were found in the liver and kidneys. Following a 100 mg/kg bodyweight dose, peak radioactivity concentrations were about 5 - 18 times higher in most tissues than after a single 10 mg/kg bodyweight dose. Following multiple dosing with ^{14}C-dimethoate at 10 mg/kg bodyweight, peak radioactivity concentrations in most tissues were approximately 1.5 - 2 times higher than those following a single 10 mg/kg bodyweight dose. Qualitative analysis of the tissue distribution of radioactivity by whole-body autoradiography also revealed high concentrations of radioactivity in the Harderian gland, the intra- and exorbital lachrymal glands and the preputial gland. The central nervous system contained the lowest concentrations of radioactivity.

Metabolites of dimethoate excreted in urine were identified by mass spectrometry. Between 70 and 80% of an orally or intravenously administered dose was characterised in this way. Biliary, plasma and tissue metabolites were characterised by comparison with reference standards and urinary profiles. Biotransformation of dimethoate proceeded *via* various hydrolytic and oxidative pathways, mainly (i) by cleavage of the C-N bond to yield, initially, dimethoate carboxylic acid, and subsequently, a number of thiophosphate and phosphate esters, but also (ii) by oxidation to its oxon analogue, omethoate and (iii) (a minor pathway) loss of the methoxy groups, yielding CO_2. Radioactive urinary metabolites of dimethoate were not conjugated with glucuronic acid or sulphate.

FIGURE 1

Example thin-layer radiochromatograms of ^{14}C-dimethoate
(radiochemical purity determination)

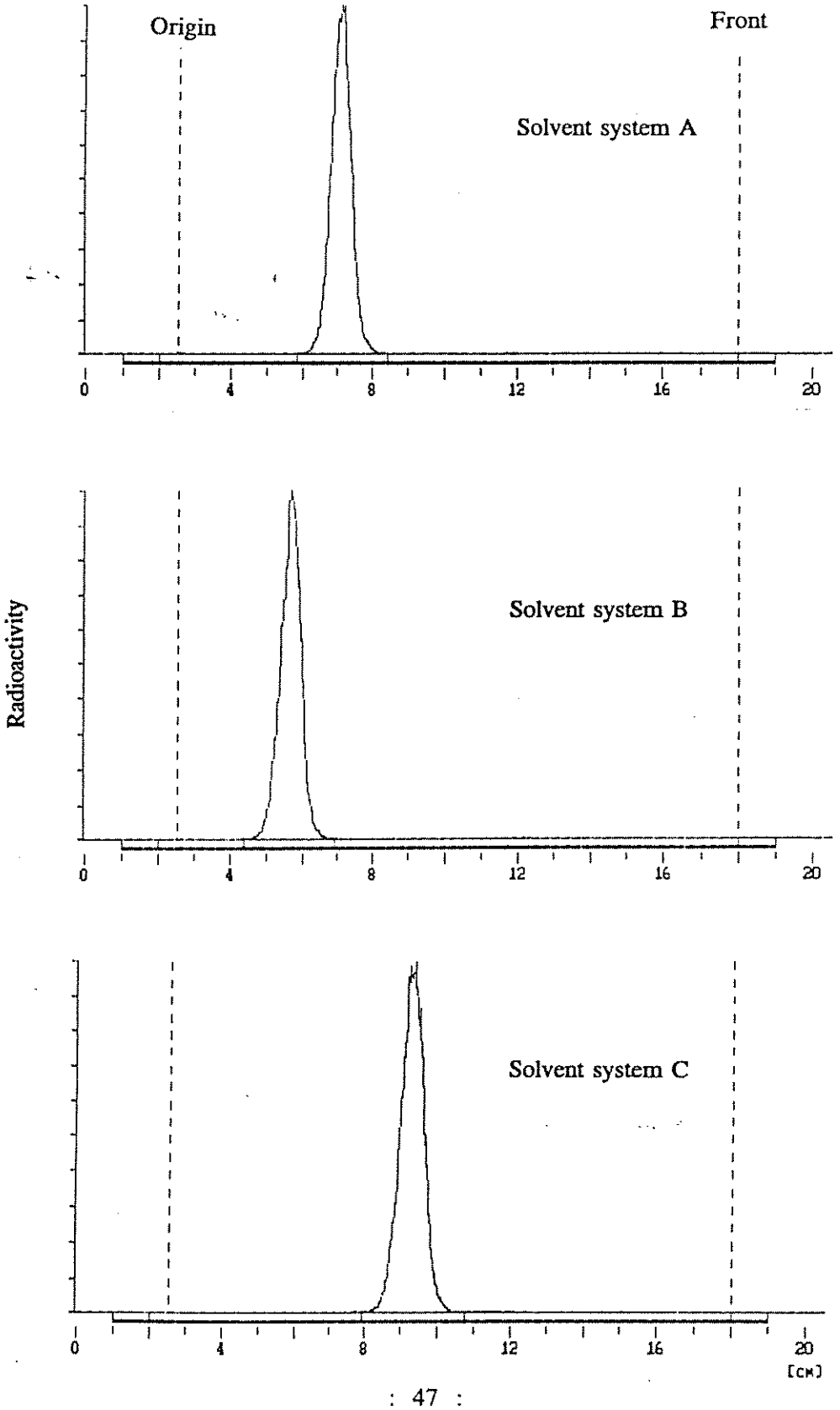


FIGURE 2

Electron impact mass spectra of ^{14}C-dimethoate and non-radiolabelled dimethoate

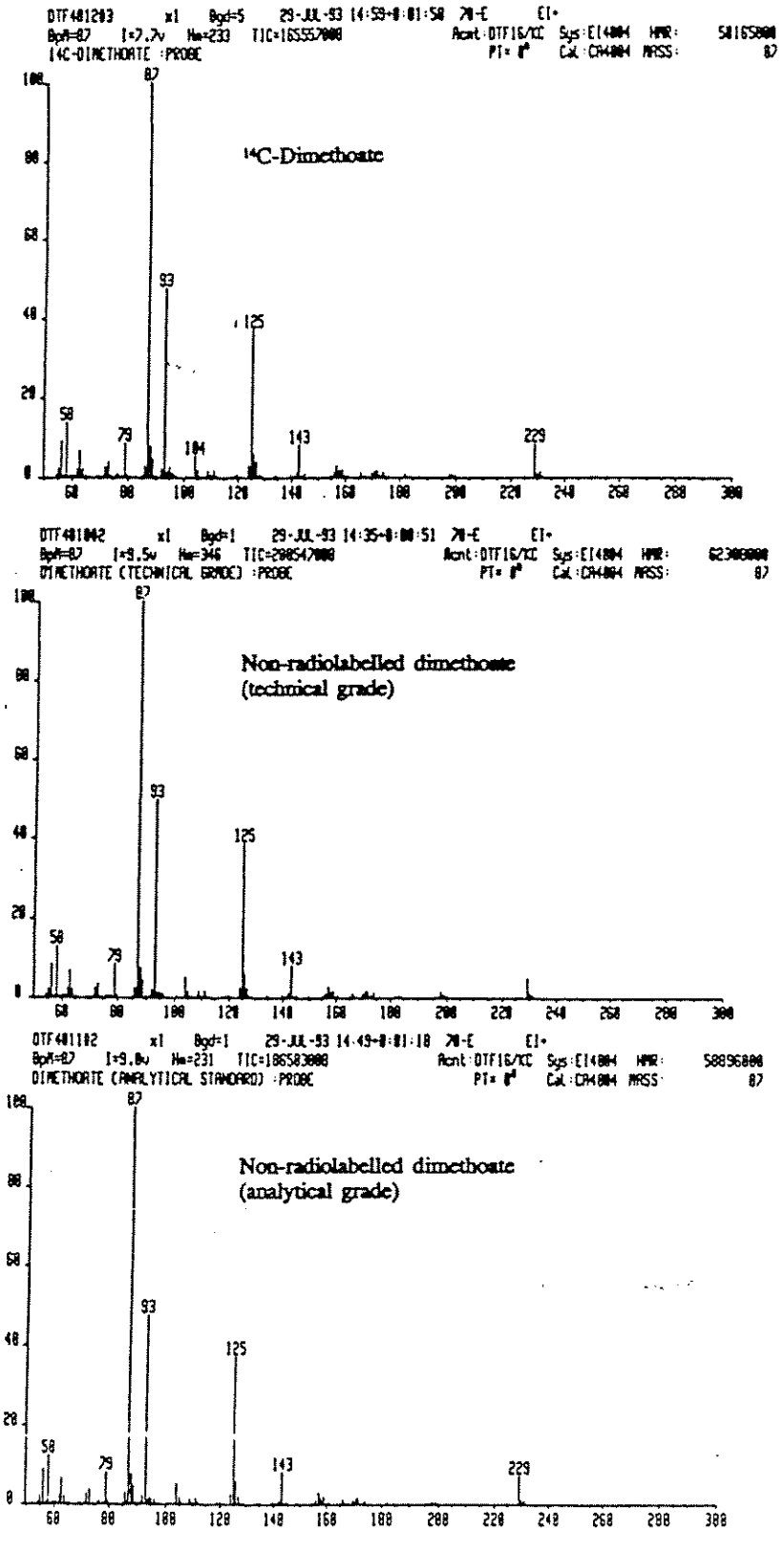
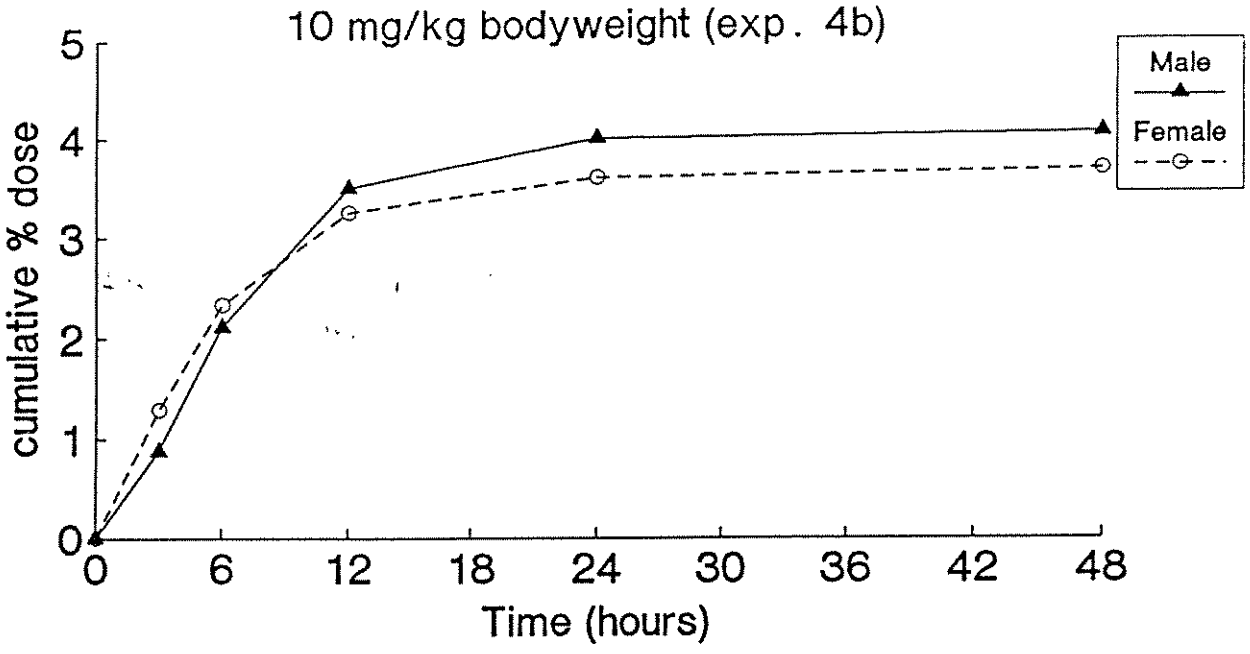


FIGURE 3

Mean cumulative excretion of radioactivity in bile following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight



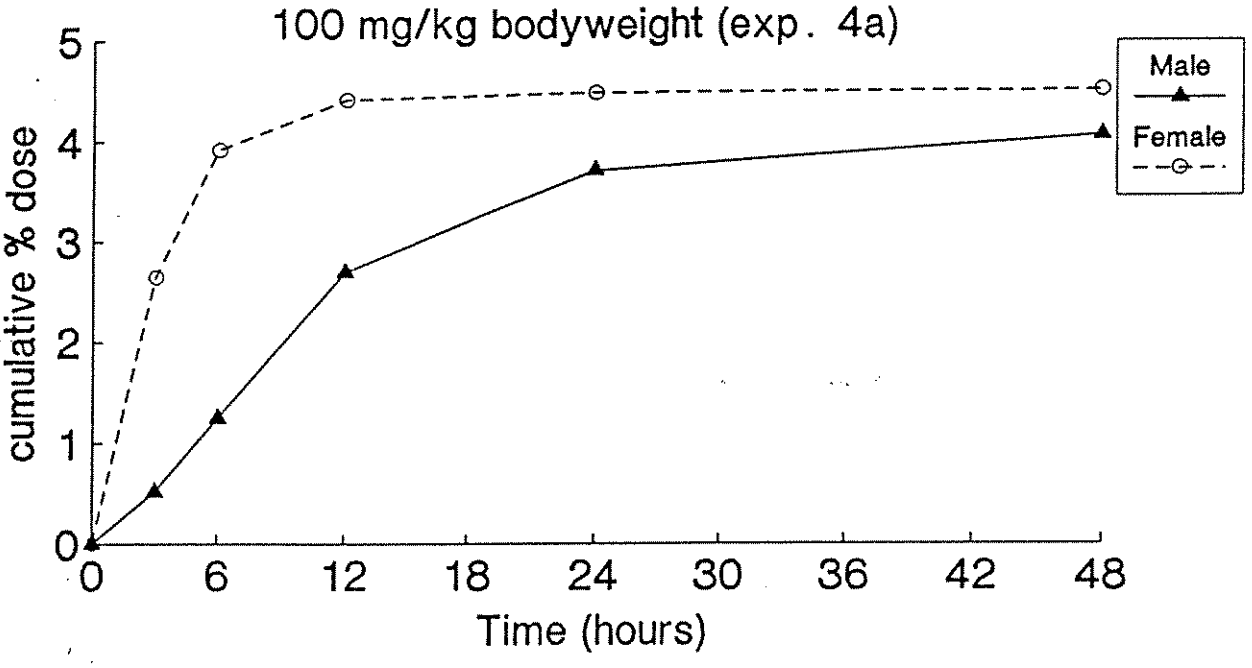


FIGURE 4

Mean plasma concentrations of radioactivity following a single oral dose at a nominal level of 10 mg/kg bodyweight

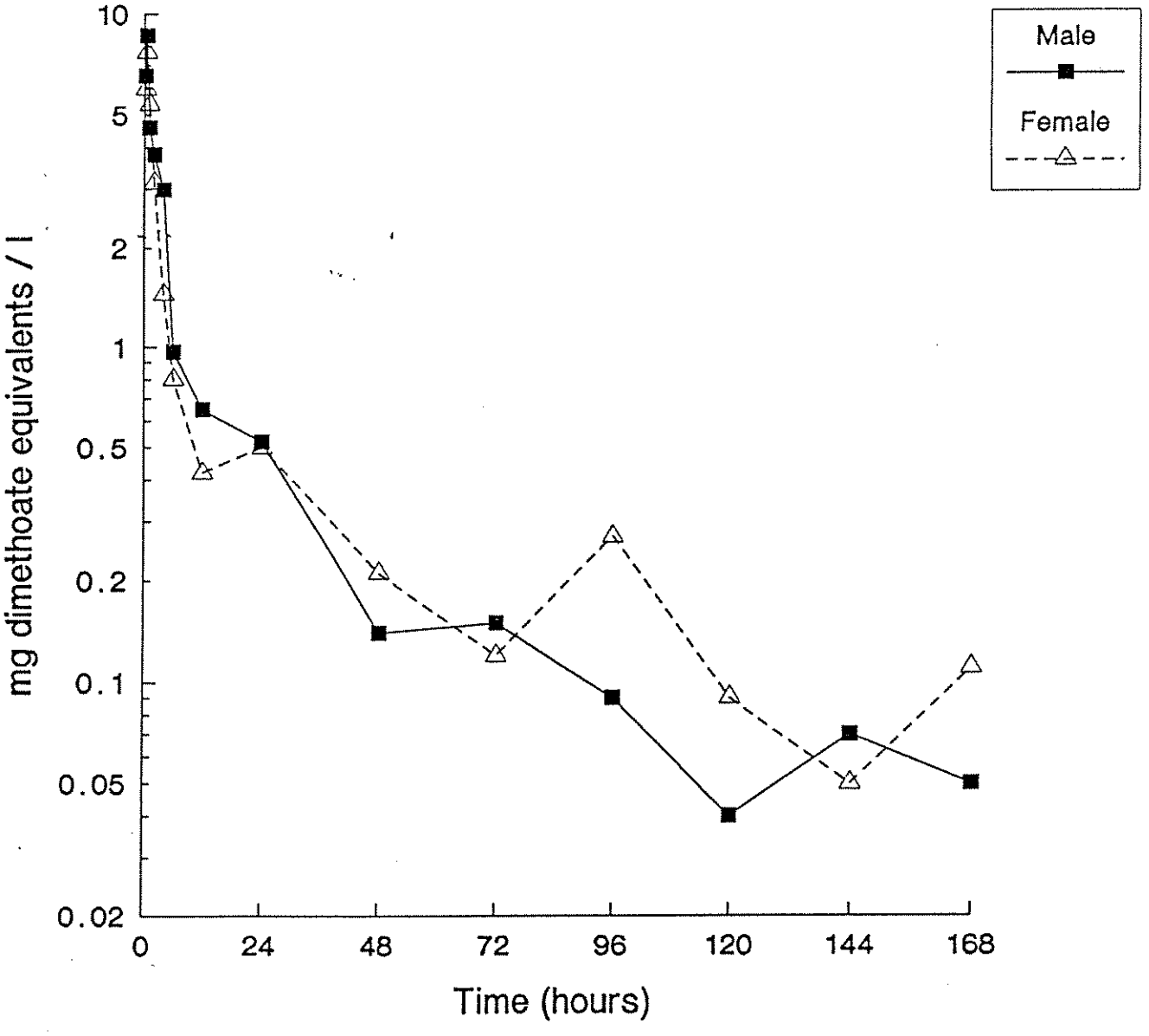


FIGURE 5

Mean plasma concentrations of radioactivity following a single oral dose at a nominal level of 100 mg/kg bodyweight

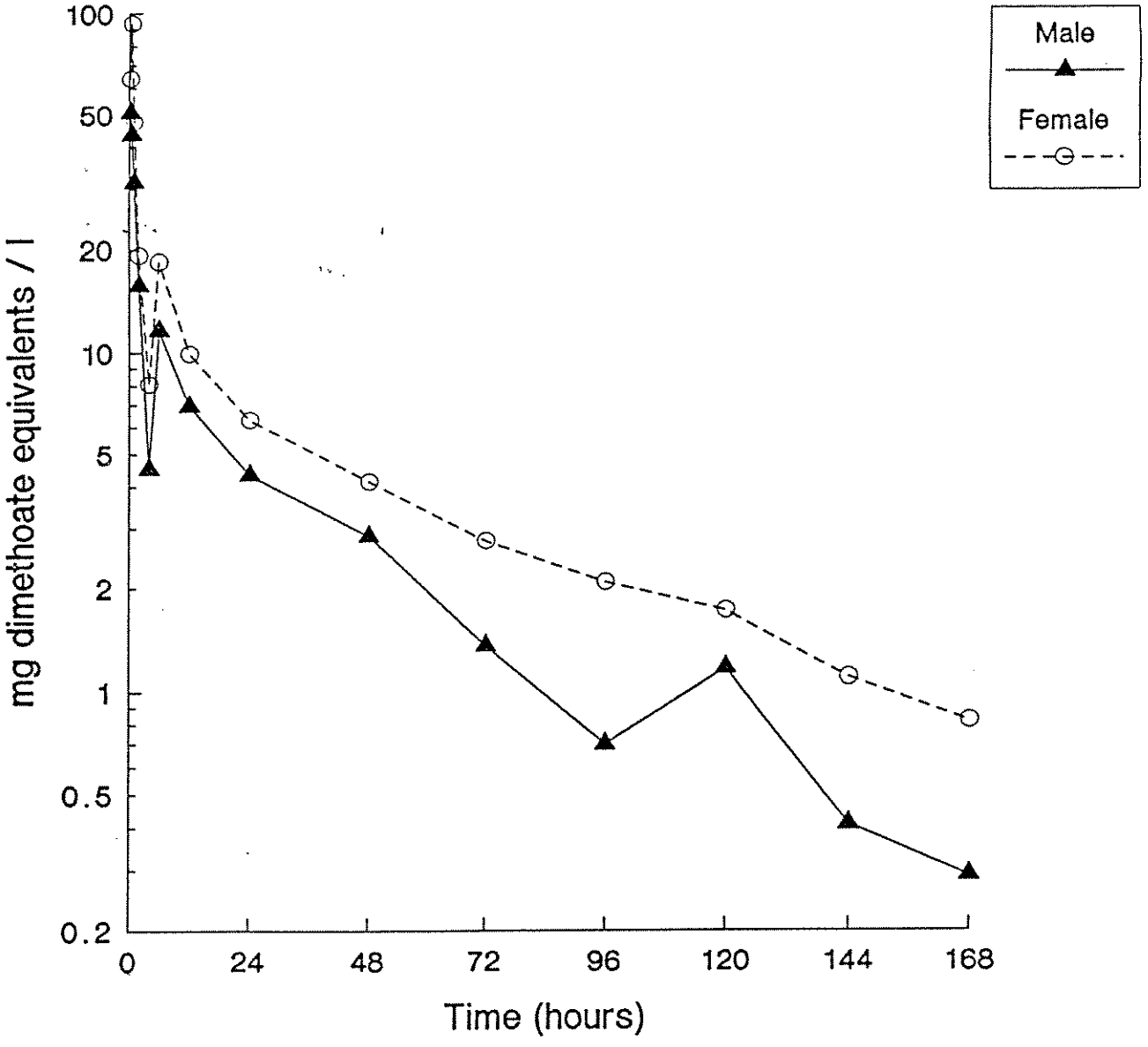


FIGURE 6

Mean plasma concentrations of radioactivity following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

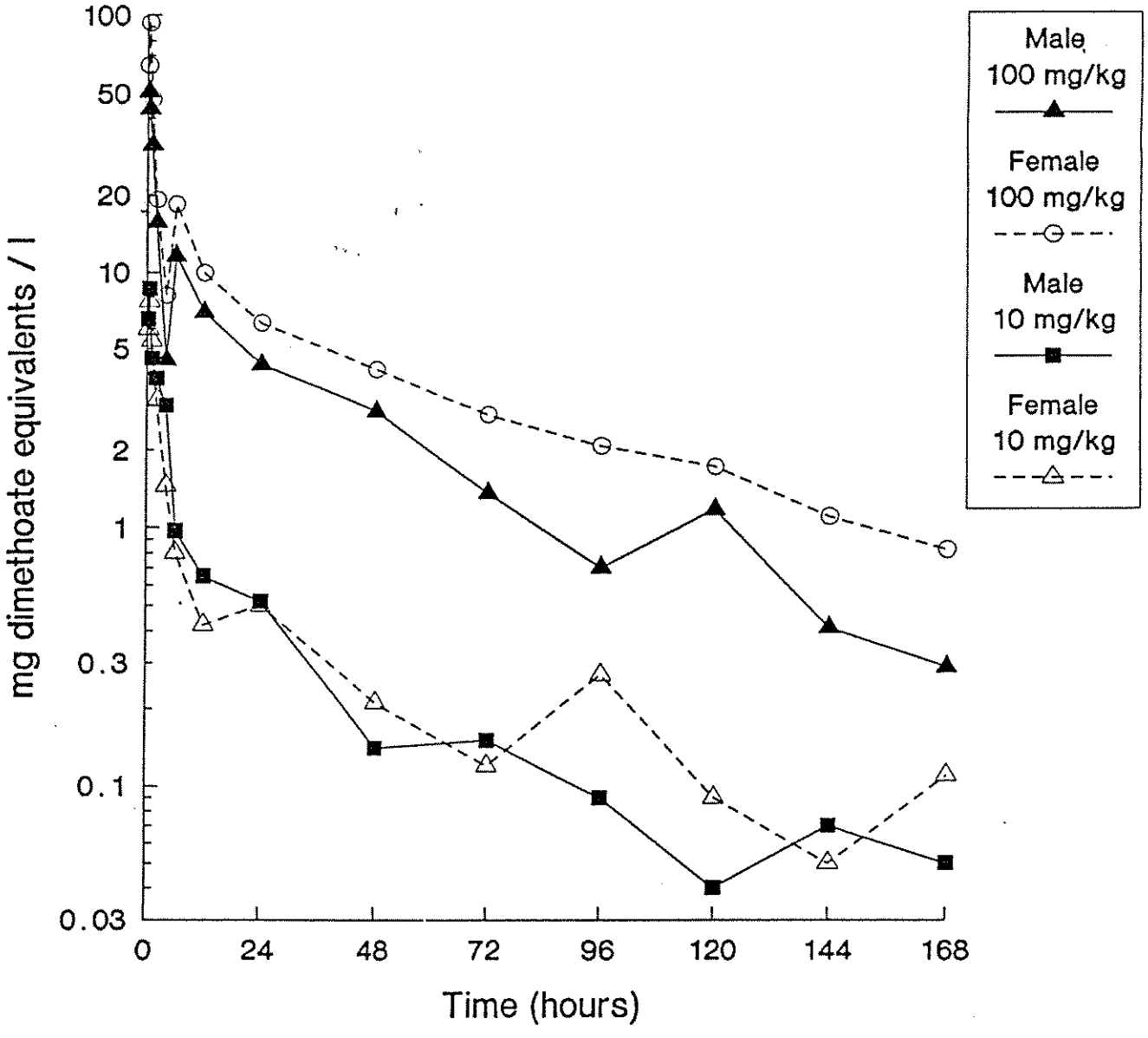


FIGURE 7

Mean plasma concentrations of radioactivity following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight (0 - 24 hours)

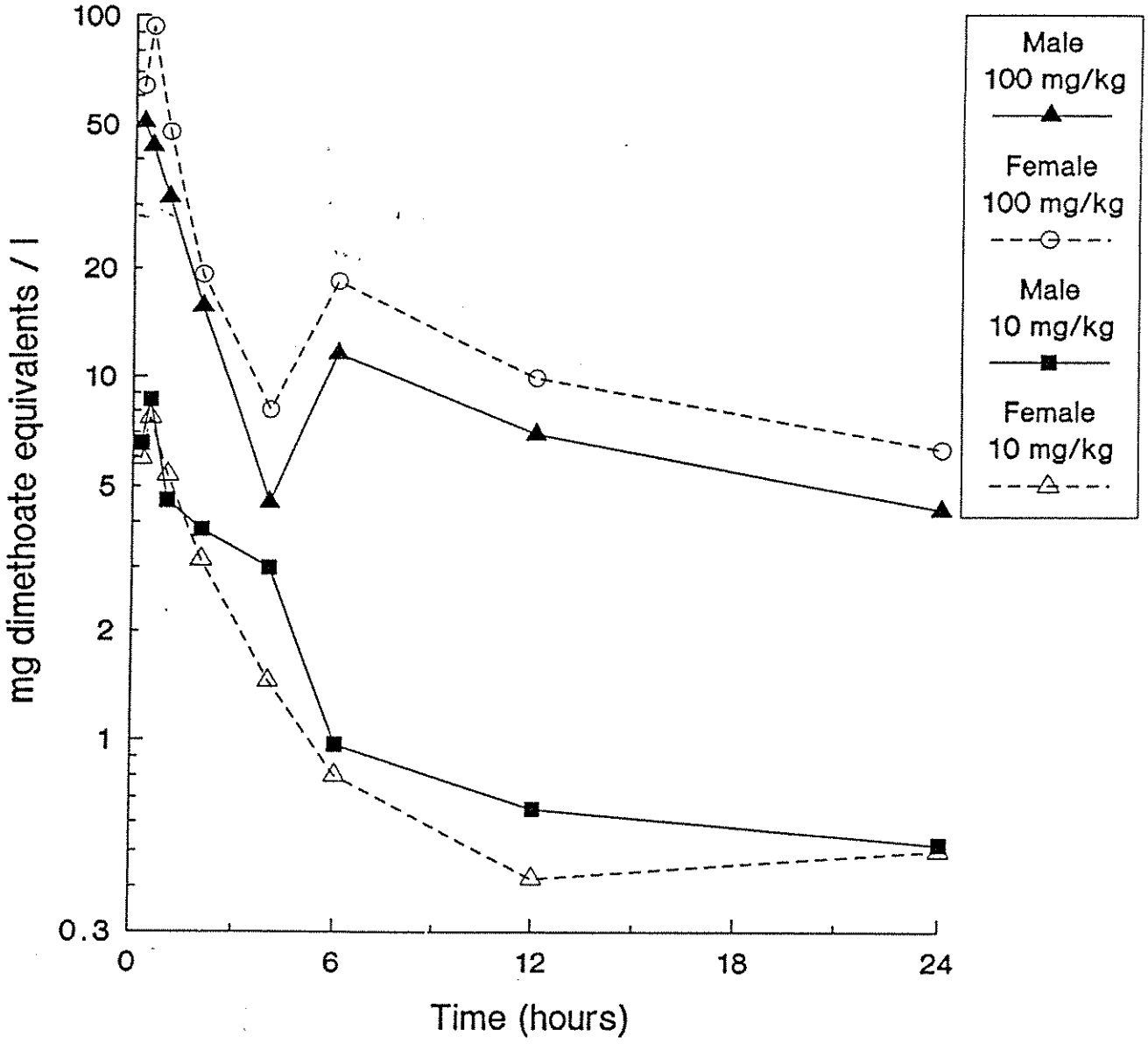
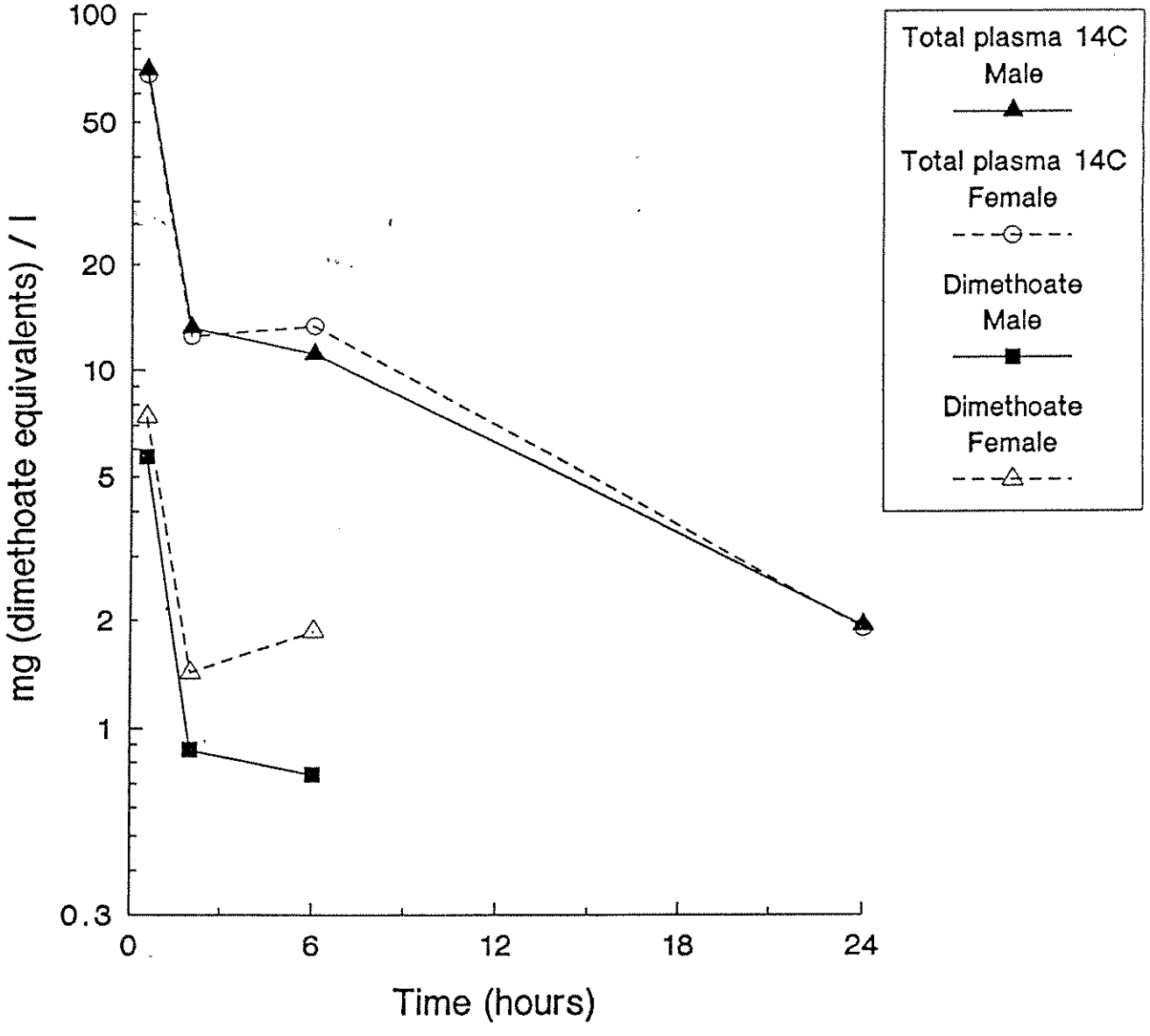


FIGURE 8

Mean plasma concentrations of dimethoate following a single oral dose at a nominal level of 100 mg/kg bodyweight



Plasma dimethoate at 24 hours was below the limit of detection (0.051 mg/l)

FIGURE 9

Mean tissue concentrations of radioactivity in male rats following a single oral dose at a nominal level of 10 mg/kg bodyweight

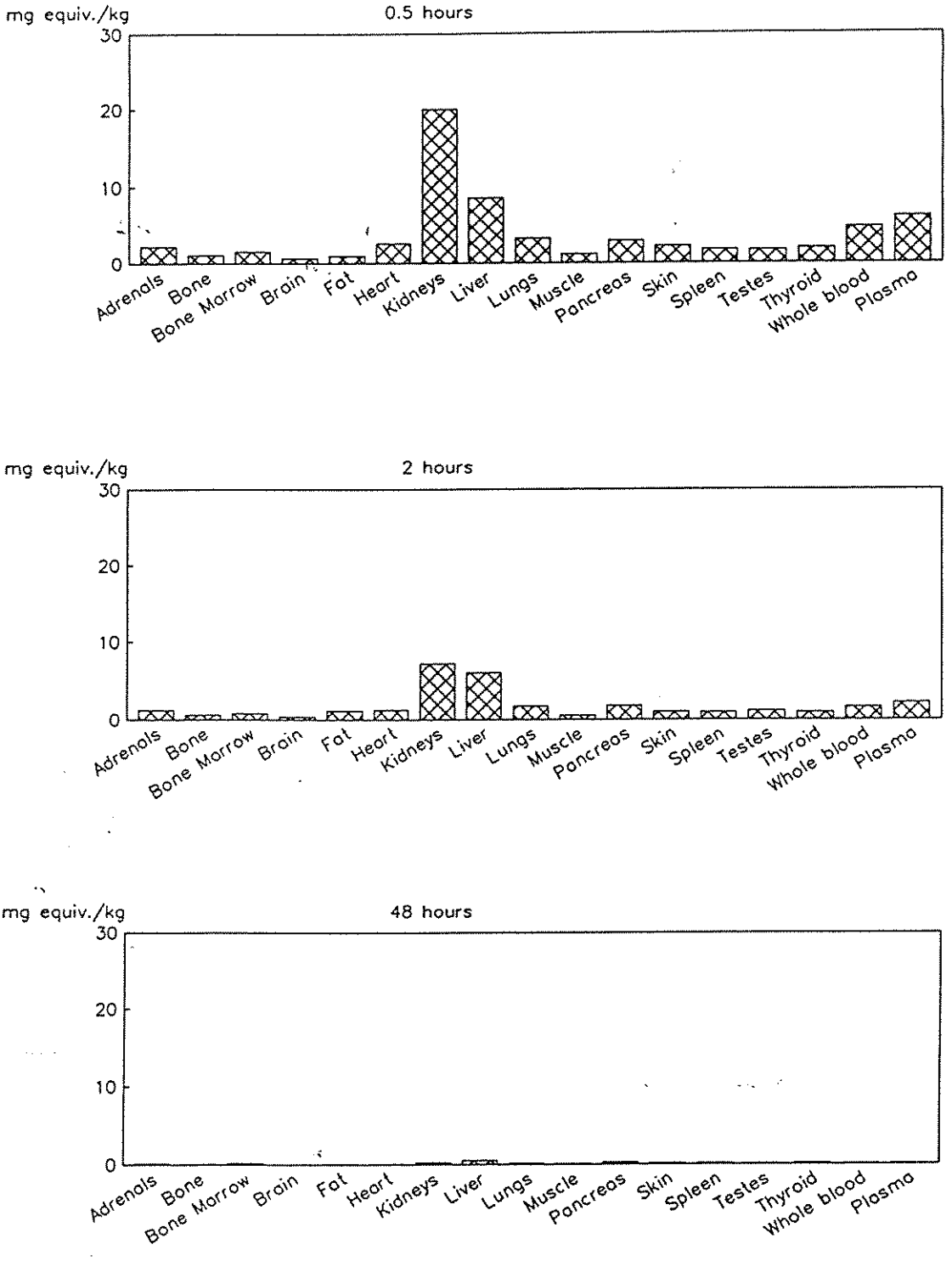


FIGURE 10

Mean tissue concentrations of radioactivity in female rats following a single oral dose at a nominal level of 10 mg/kg bodyweight

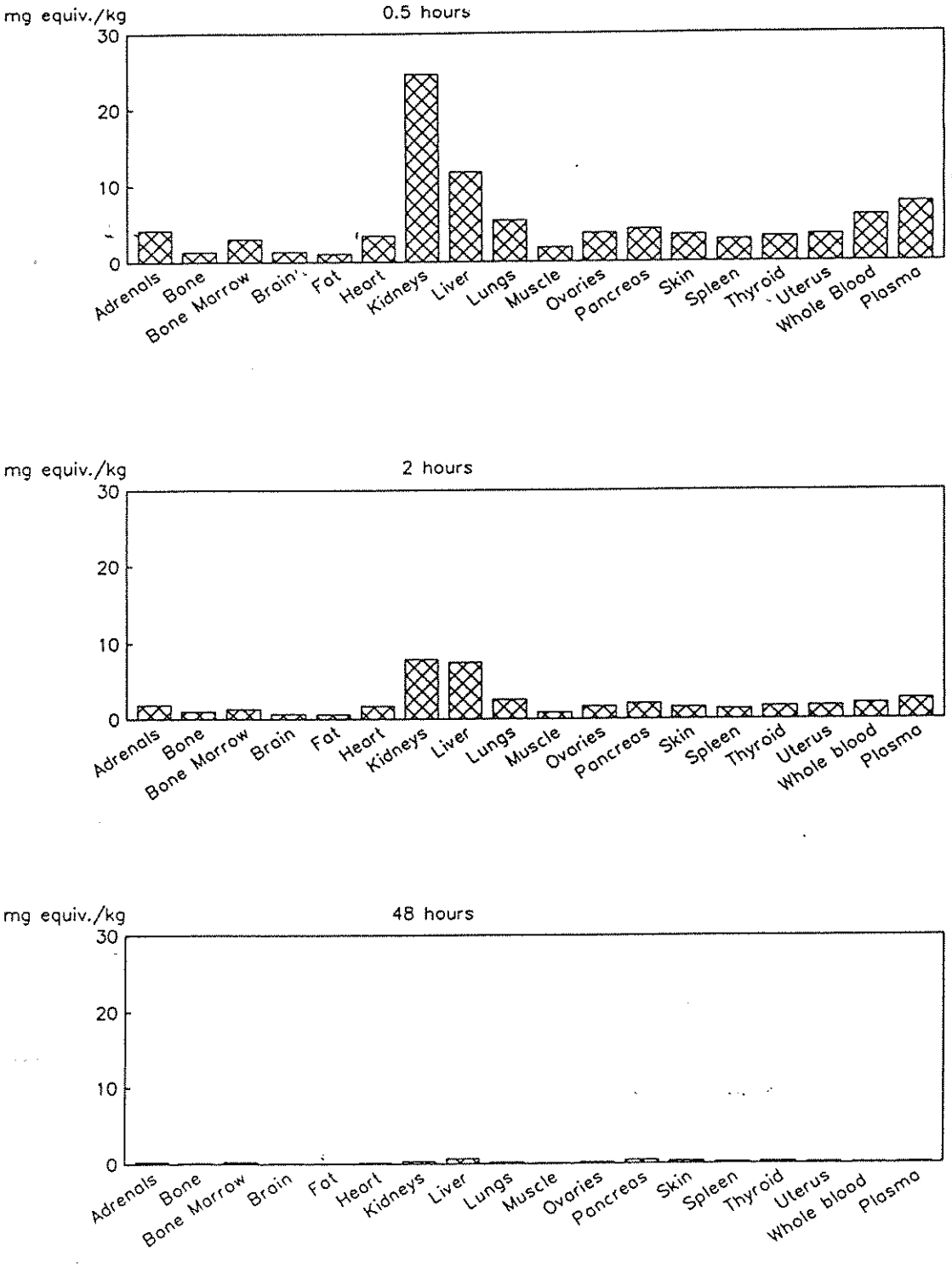


FIGURE 11

Changes in mean tissue concentrations of radioactivity with time in male rats following a single oral dose at a nominal level of 10 mg/kg bodyweight

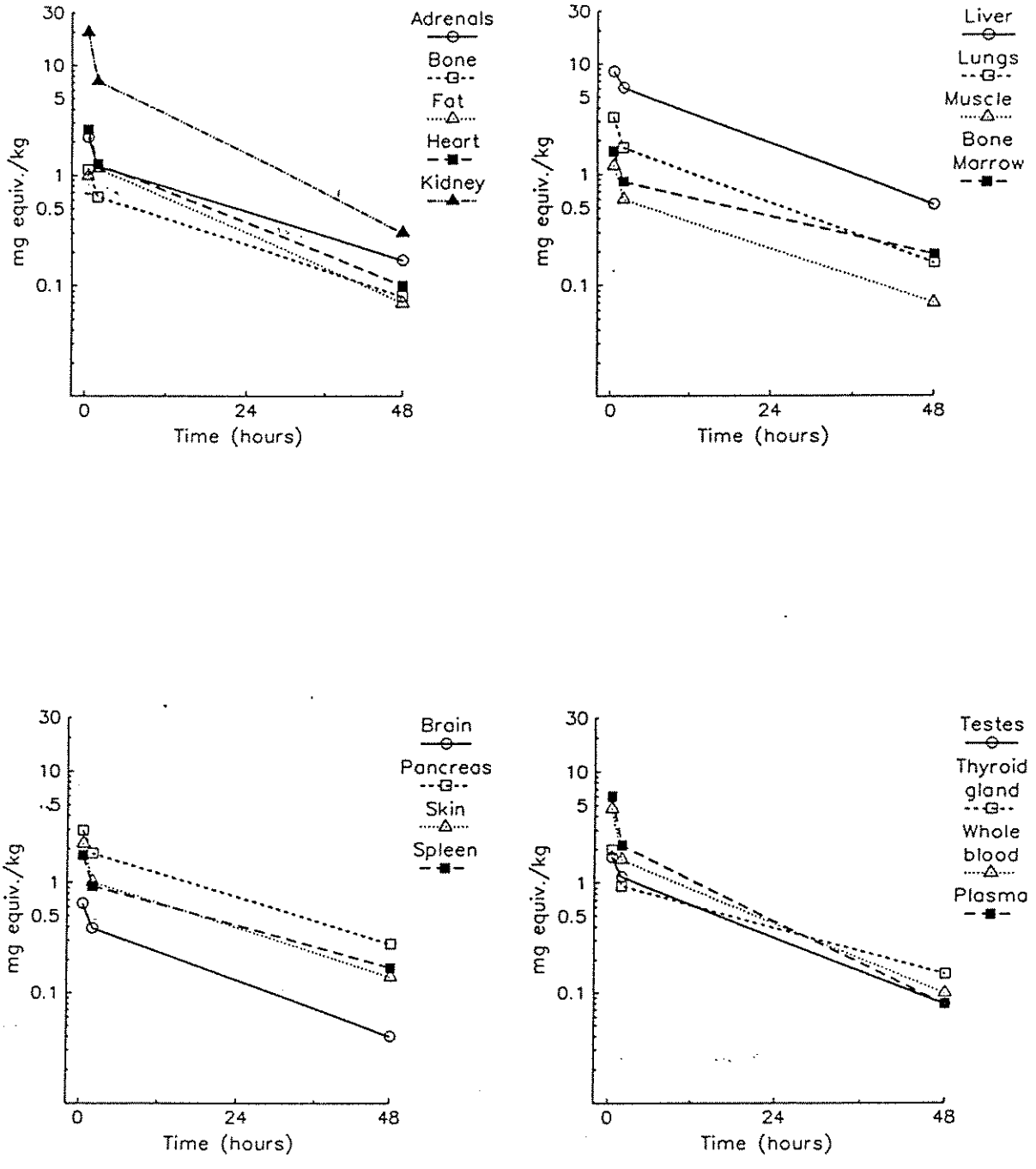


FIGURE 12

Changes in mean tissue concentrations of radioactivity with time in female rats following a single oral dose at a nominal level of 10 mg/kg bodyweight

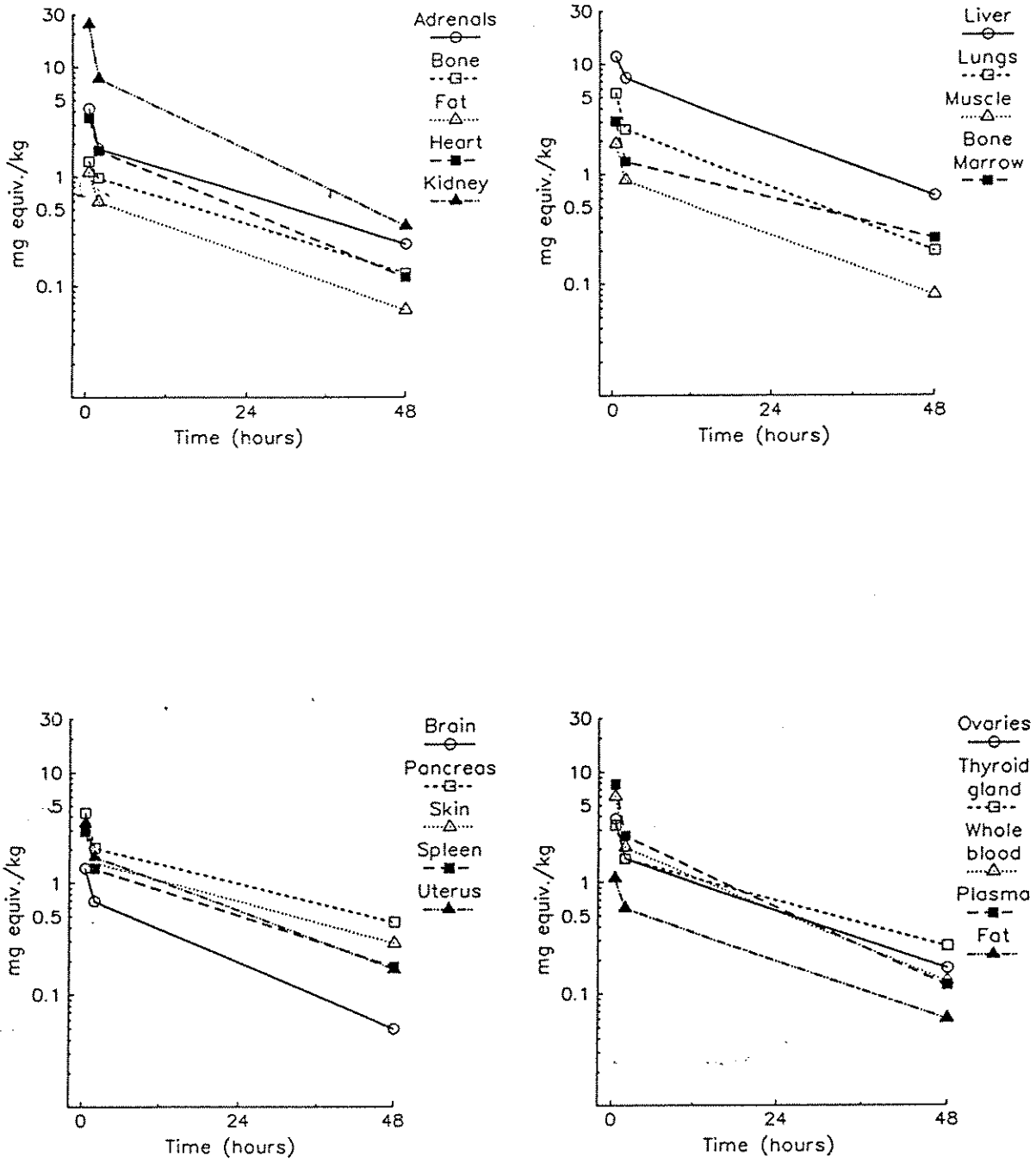
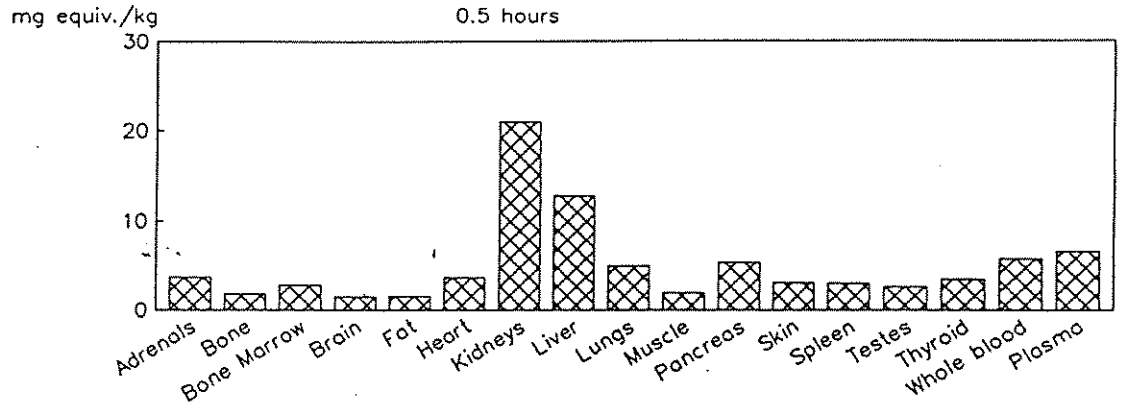
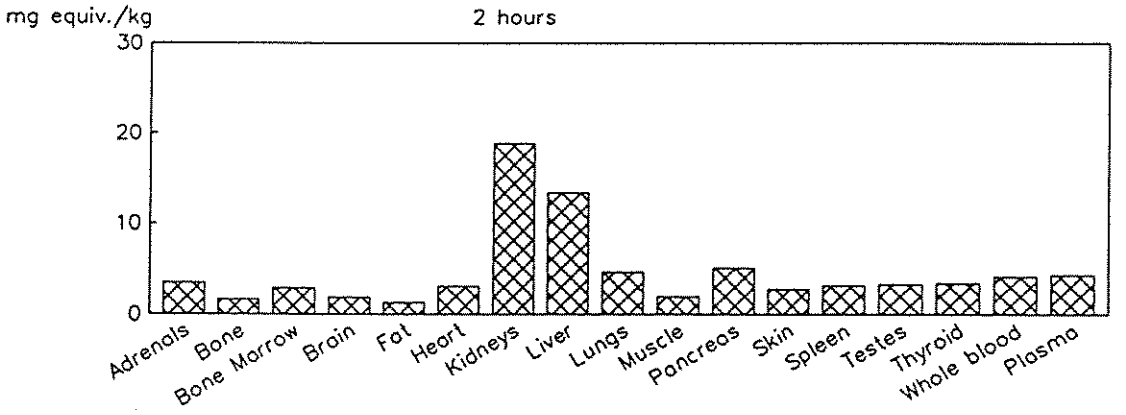


FIGURE 13

Mean tissue concentrations of radioactivity in male rats following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight





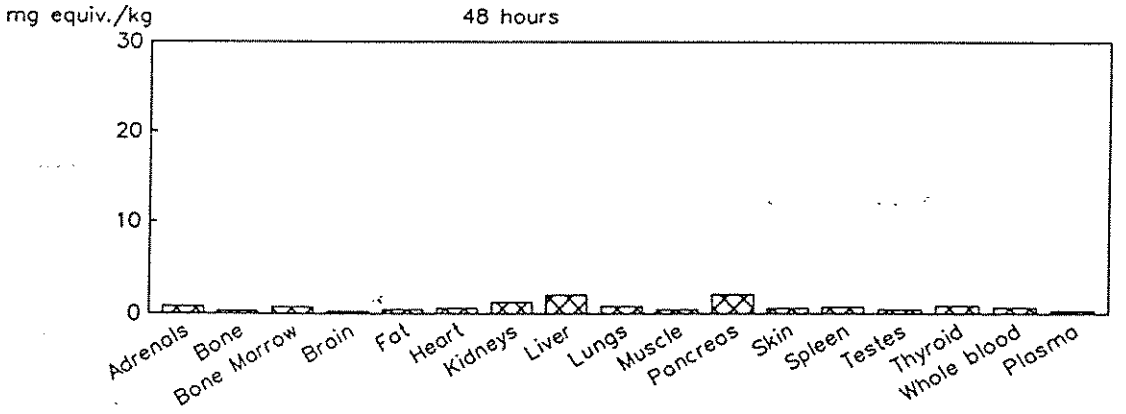


FIGURE 14

Mean tissue concentrations of radioactivity in female rats following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

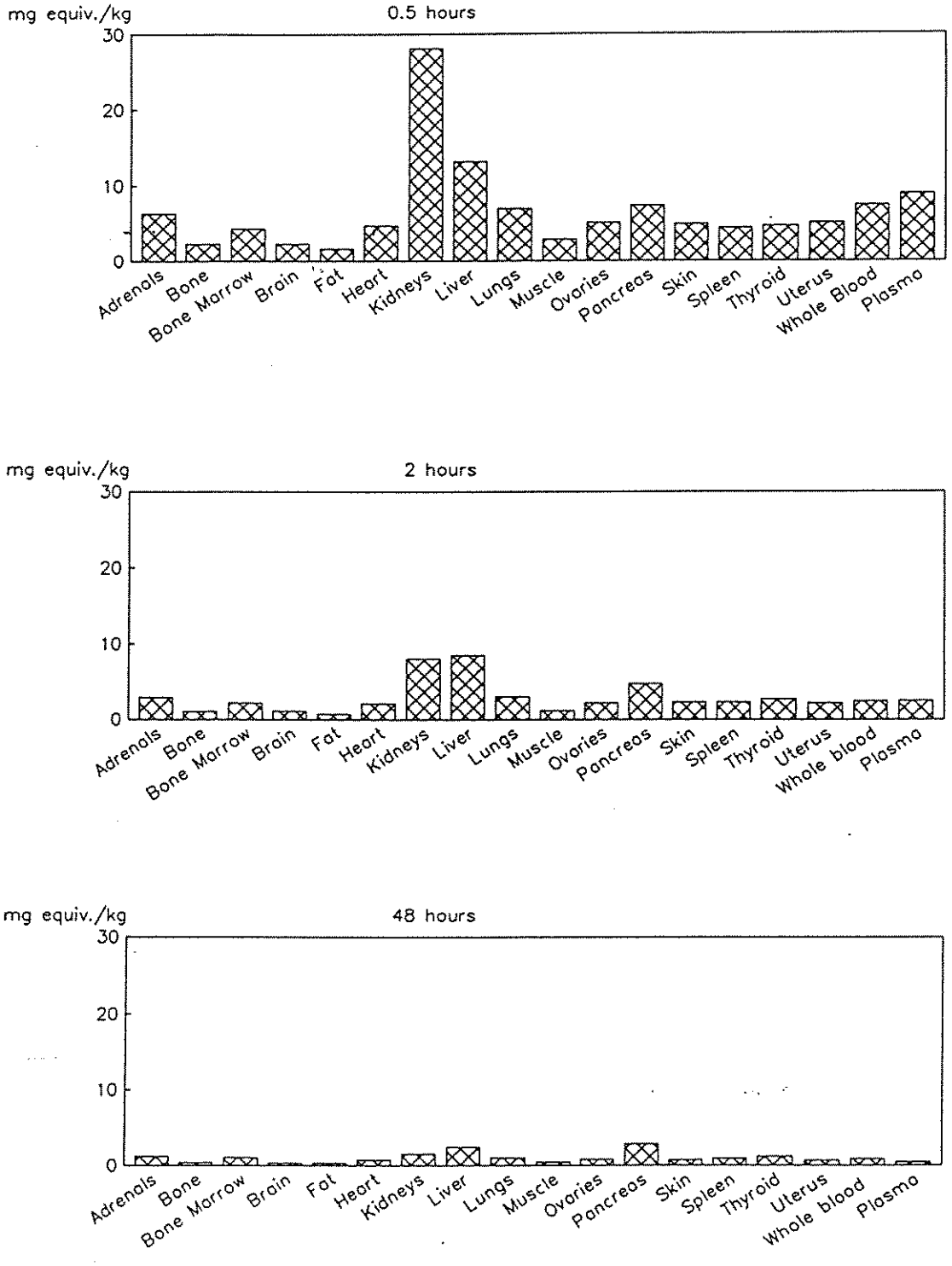


FIGURE 15

Changes in mean tissue concentrations of radioactivity with time in male rats following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

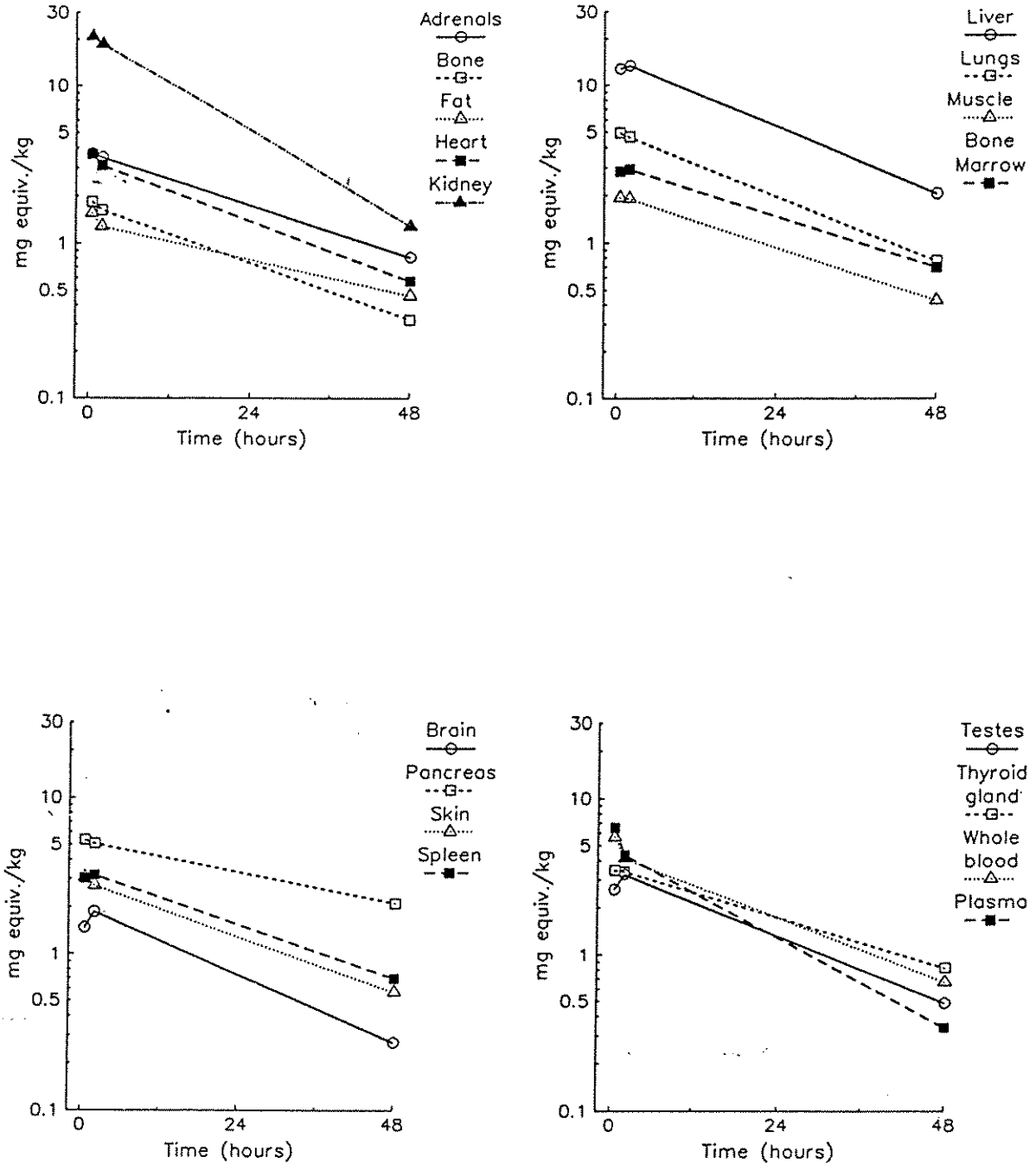


FIGURE 16

Changes in mean tissue concentrations of radioactivity with time in female rats following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

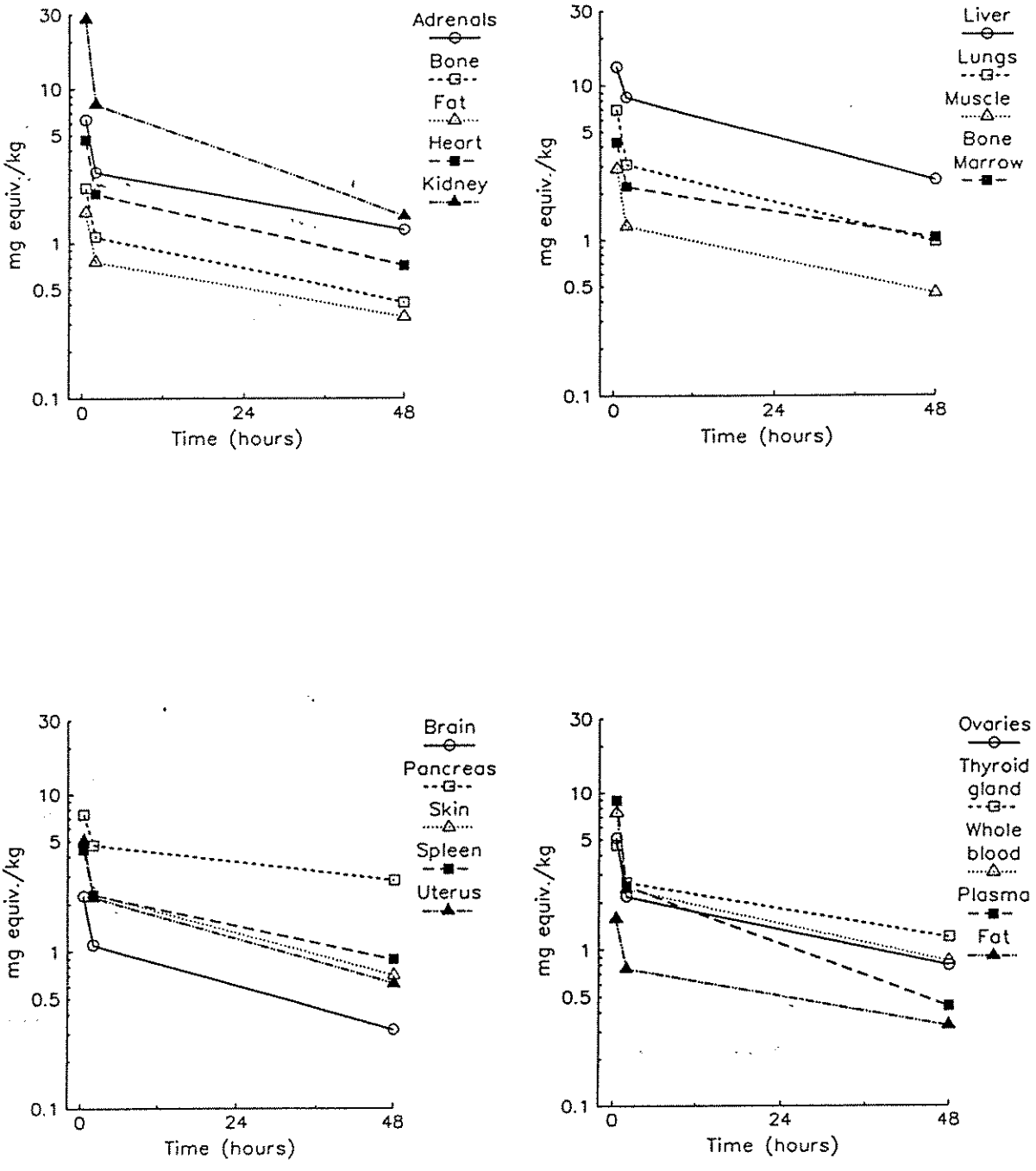


FIGURE 17

Mean tissue concentrations of radioactivity in male rats following a single oral dose at a nominal level of 100 mg/kg bodyweight

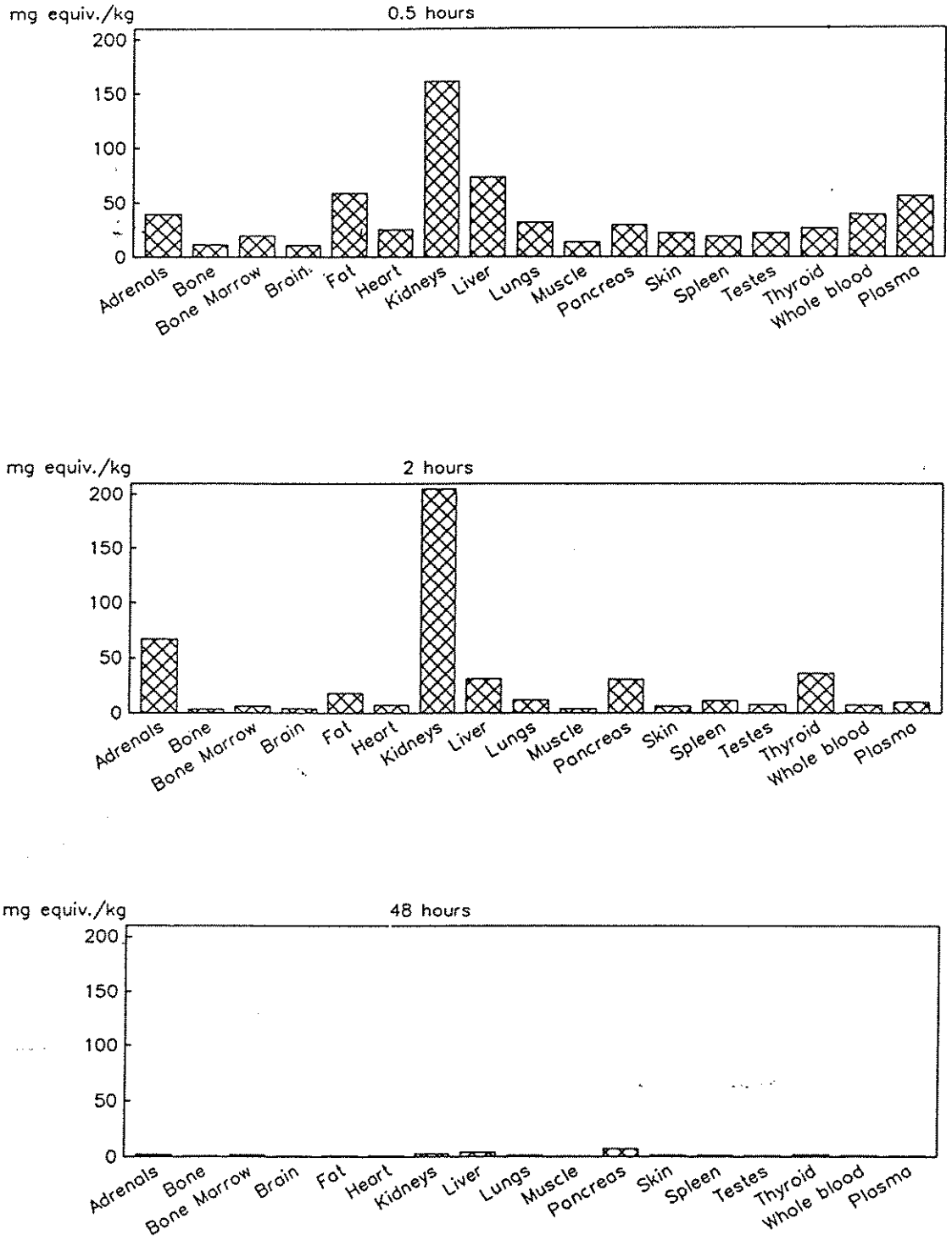
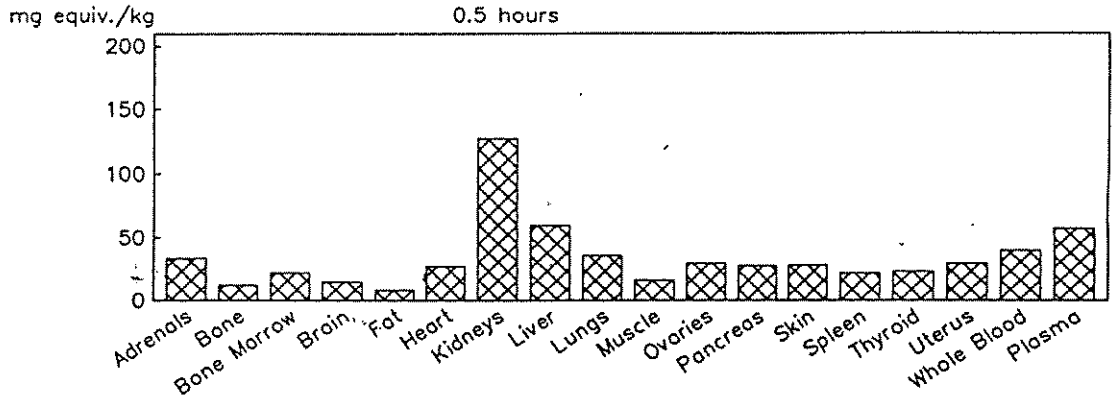
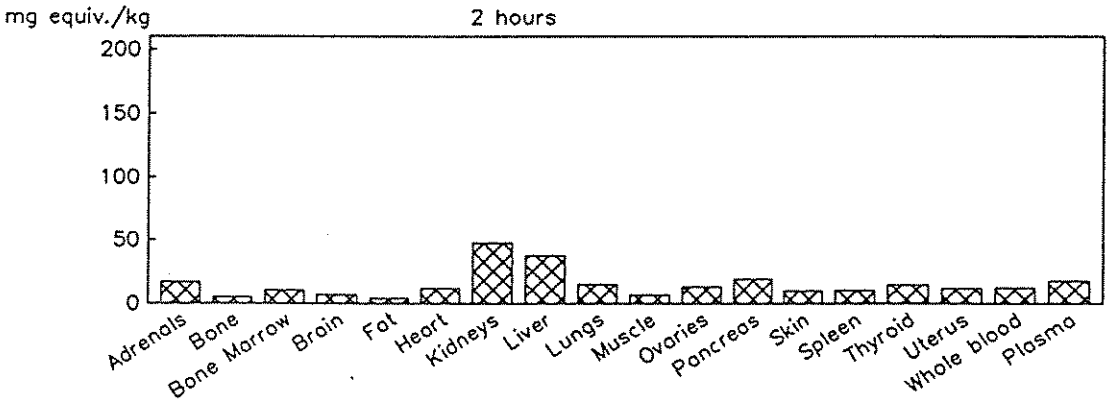


FIGURE 18

Mean tissue concentrations of radioactivity in female rats following a single oral dose at a nominal level of 100 mg/kg bodyweight





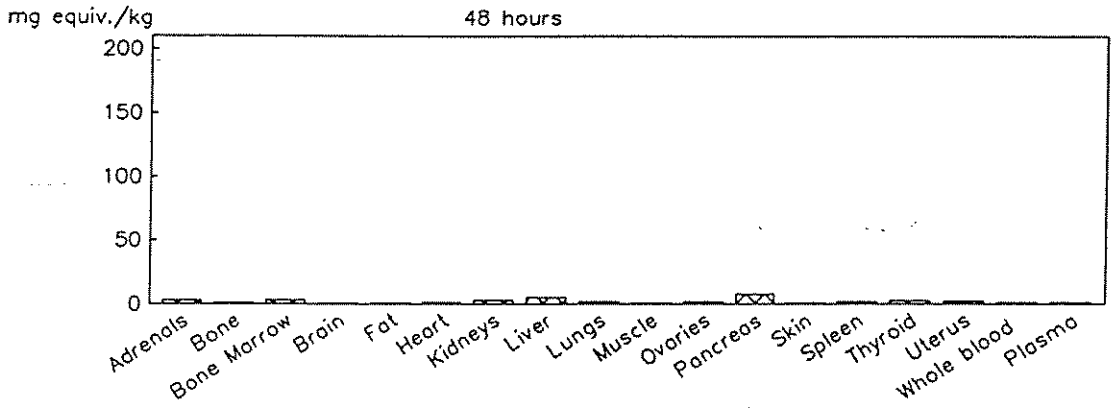


FIGURE 19

Changes in mean tissue concentrations of radioactivity with time in male rats following a single oral dose at a nominal level of 100 mg/kg bodyweight

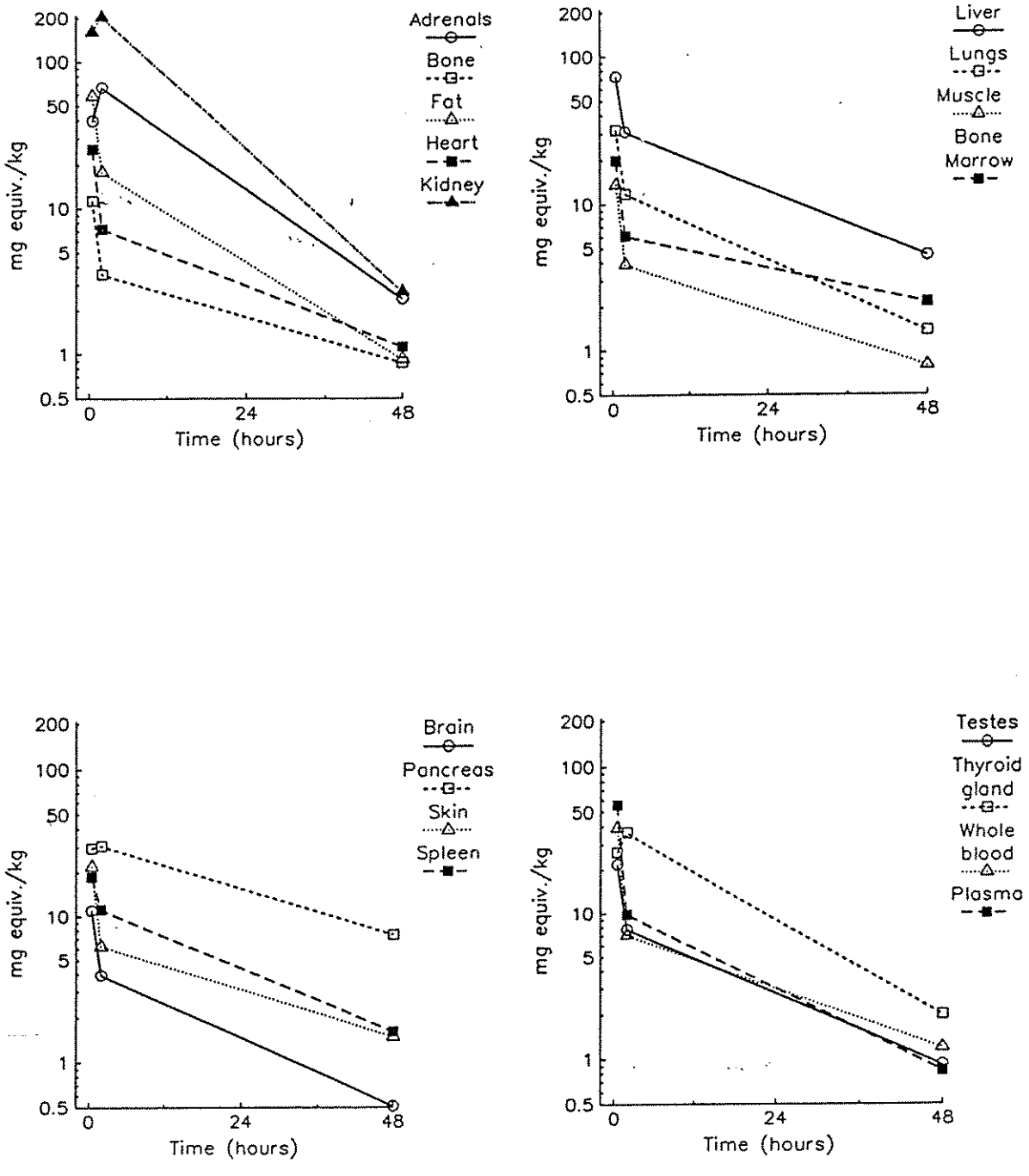


FIGURE 20

Changes in mean tissue concentrations of radioactivity with time in female rats following a single oral dose at a nominal level of 100 mg/kg bodyweight

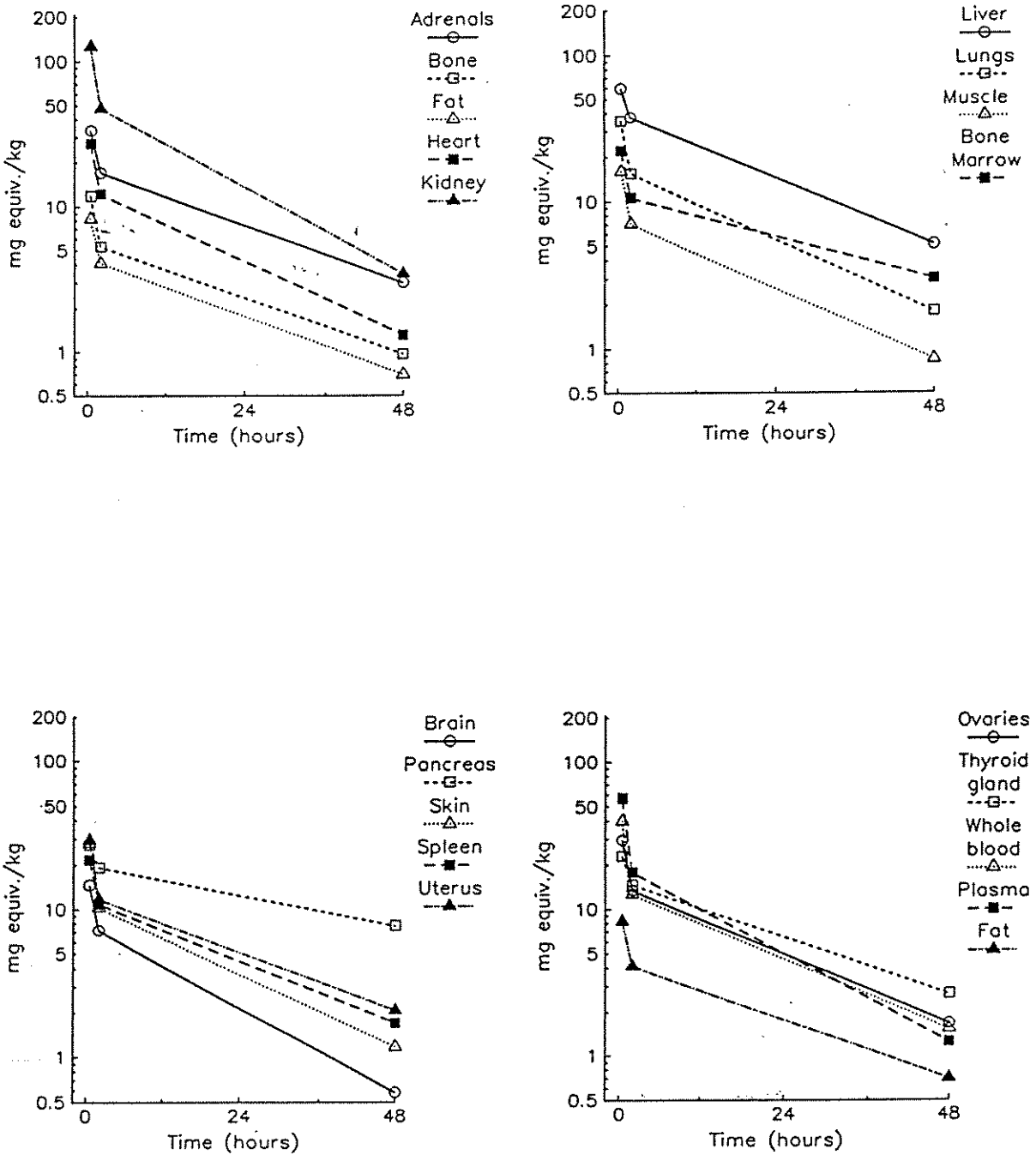


FIGURE 21

HPLC (method 1) radiochromatograms of urine

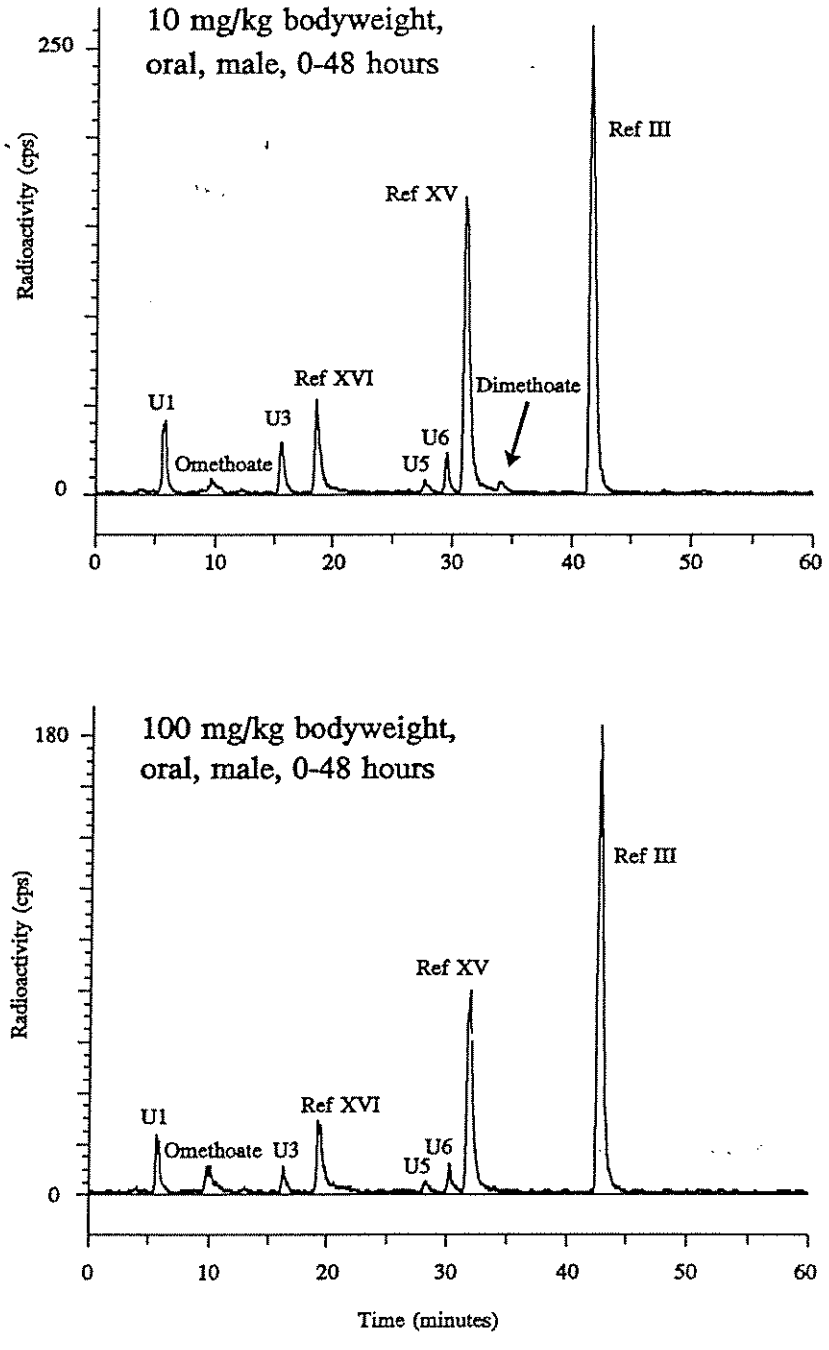


FIGURE 22

HPLC (method 1) radiochromatograms of urine

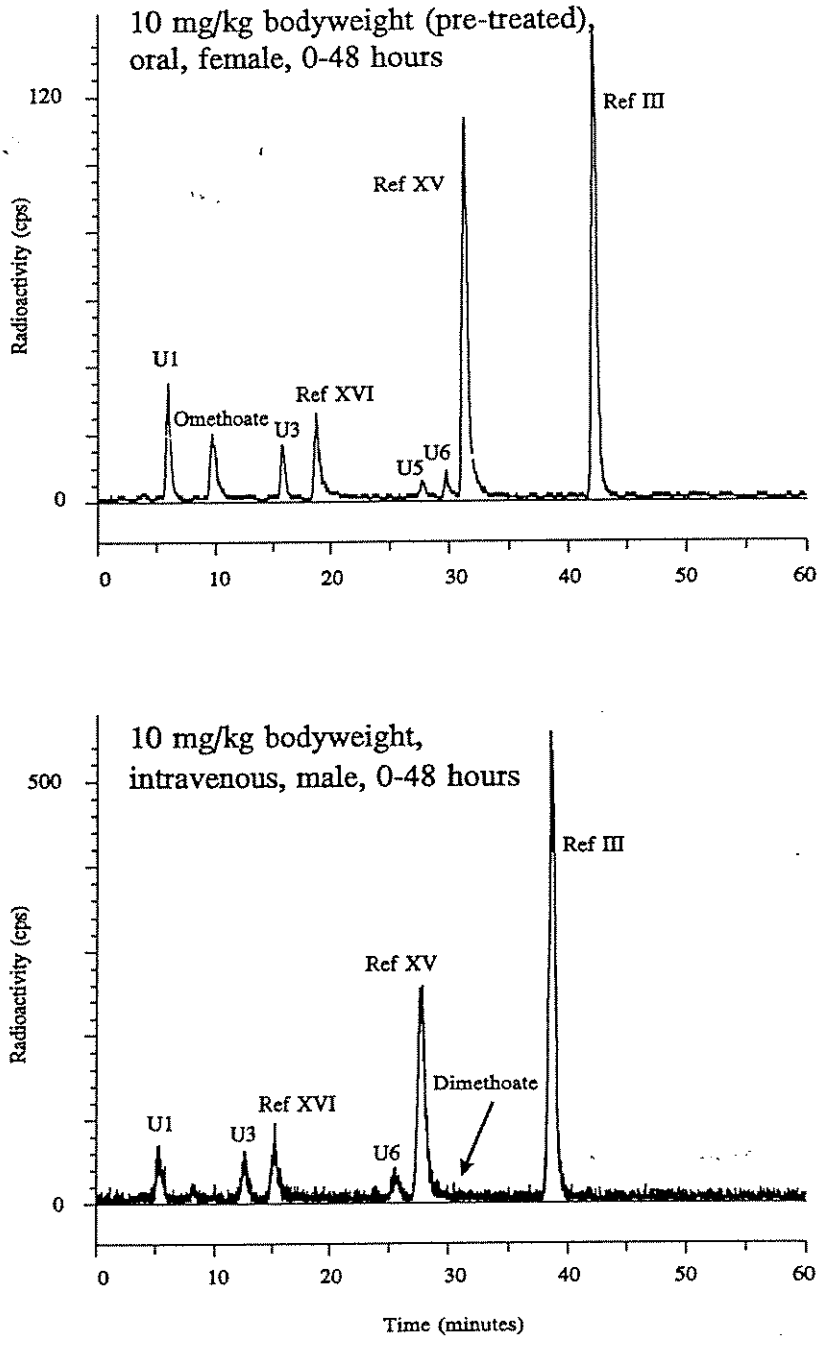


FIGURE 23

HPLC (method 1) radiochromatograms of urine from dermally-dosed rats

100 mg/kg bodyweight, female, 0-48 hours

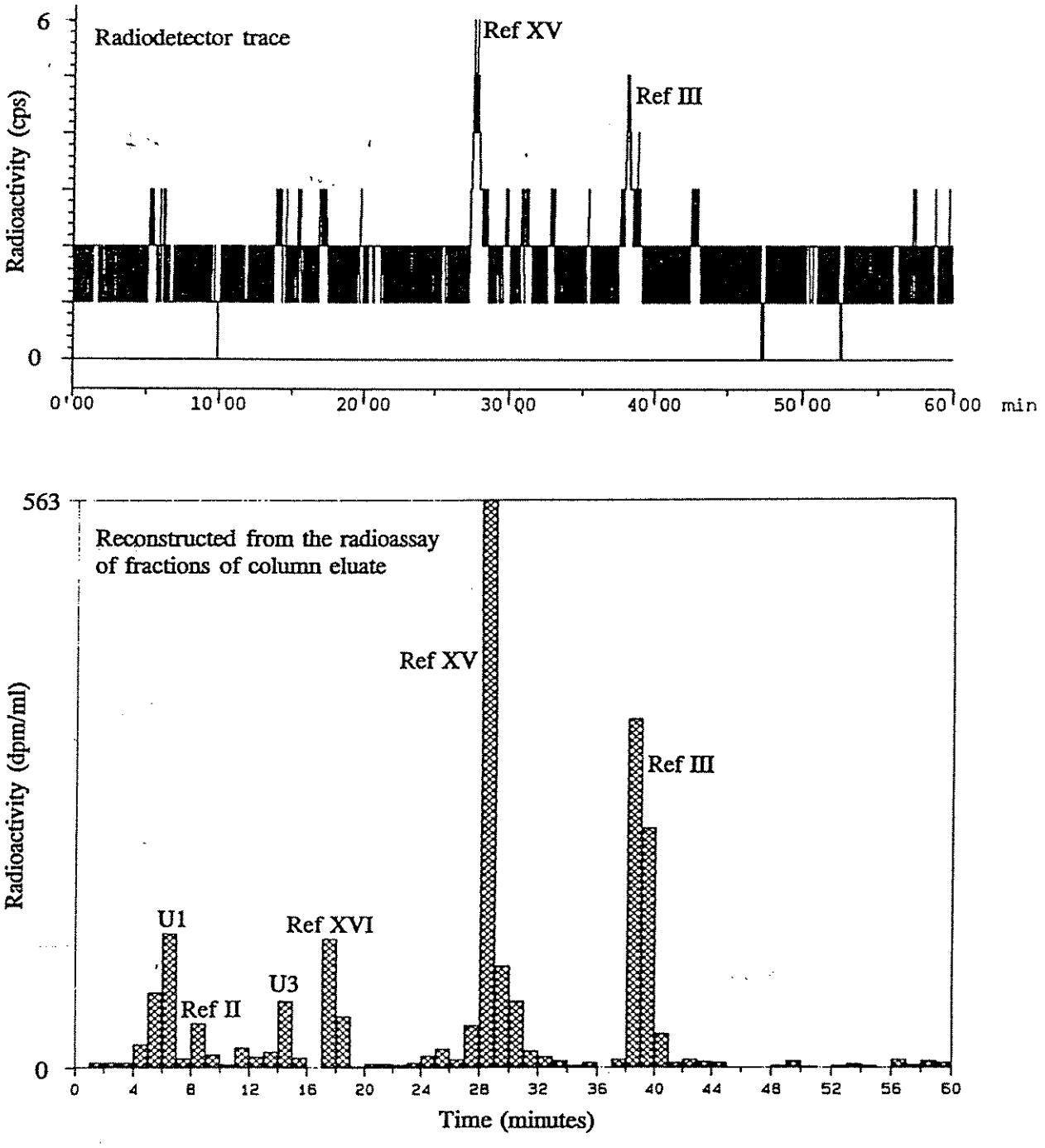
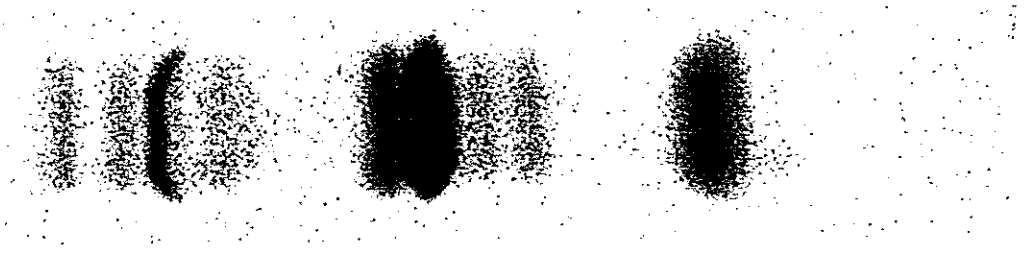


FIGURE 24

TLC (system D) Fujix image and radiochromatogram of urine

100 mg/kg bodyweight, oral,
male, 0-24 hours (exp. 2a)



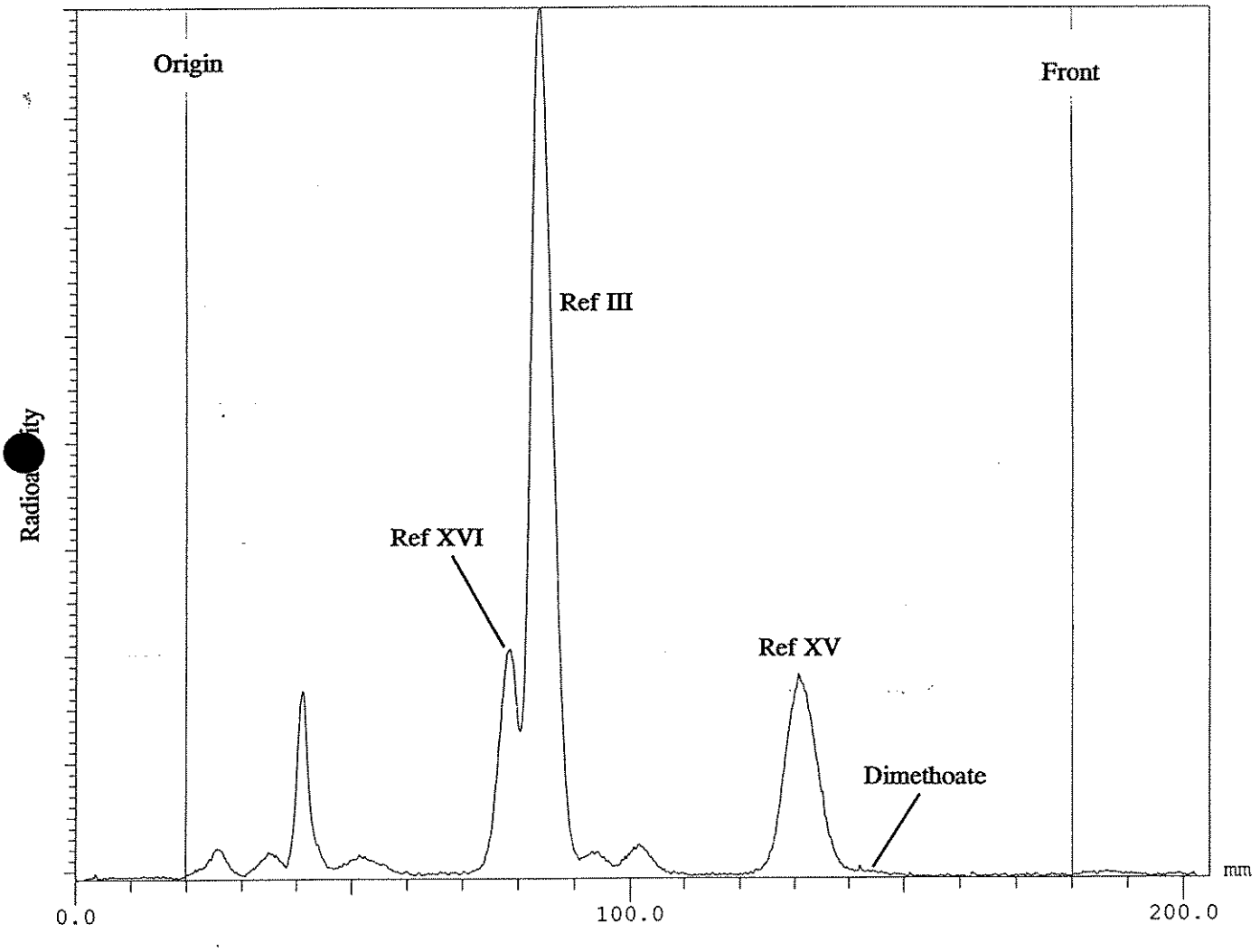
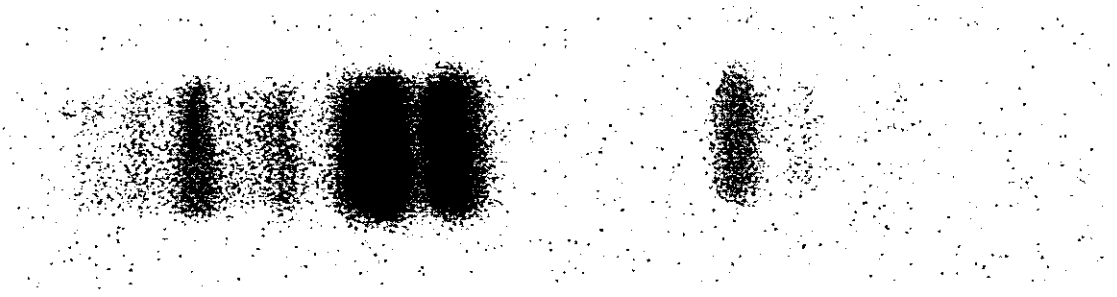


FIGURE 25

TLC (system F) Fujix image and radiochromatogram of urine

100 mg/kg bodyweight, oral,
female, 0-24 hours (exp. 2a)



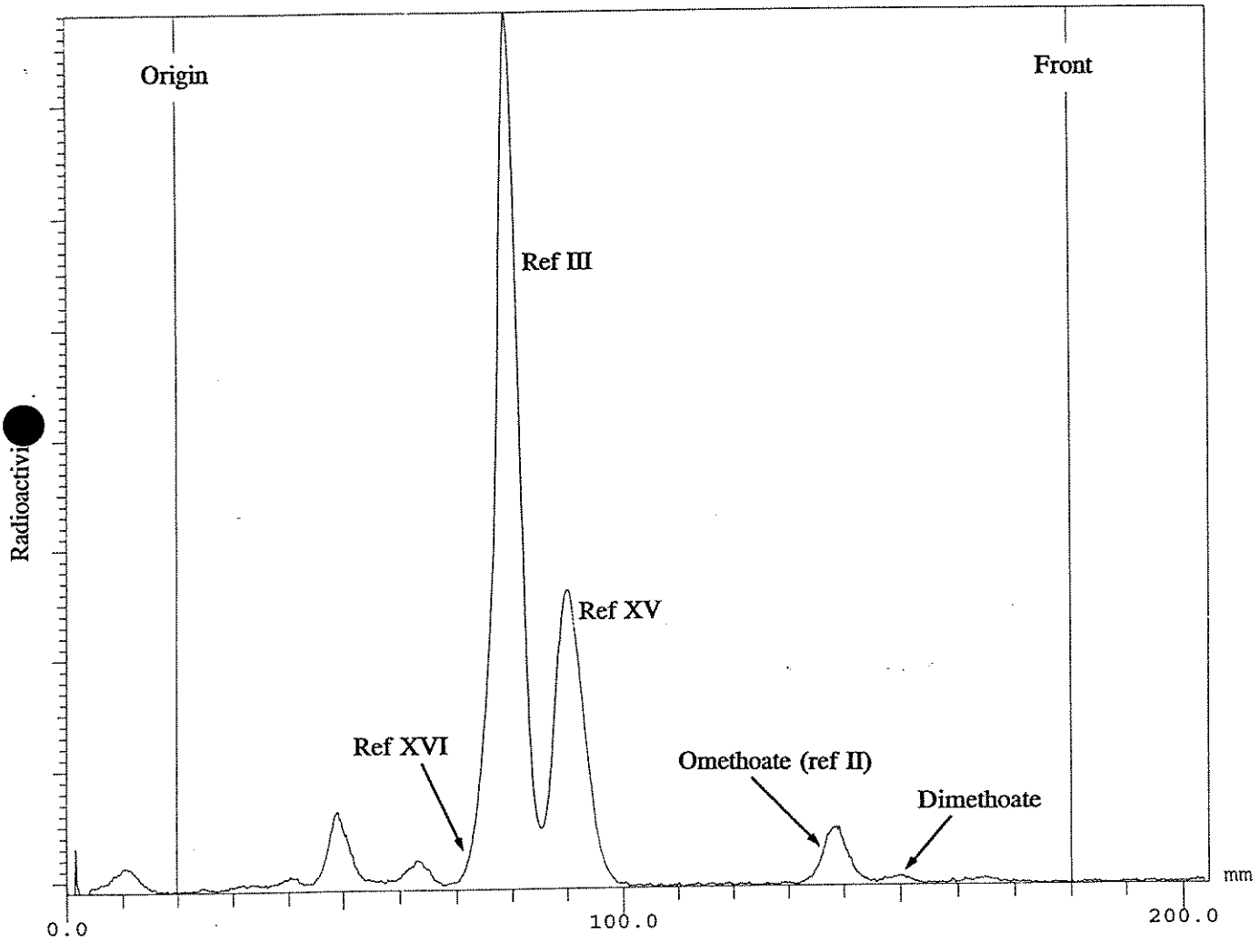


FIGURE 26

HPLC (method 1) chromatograms of urine and dimethoate

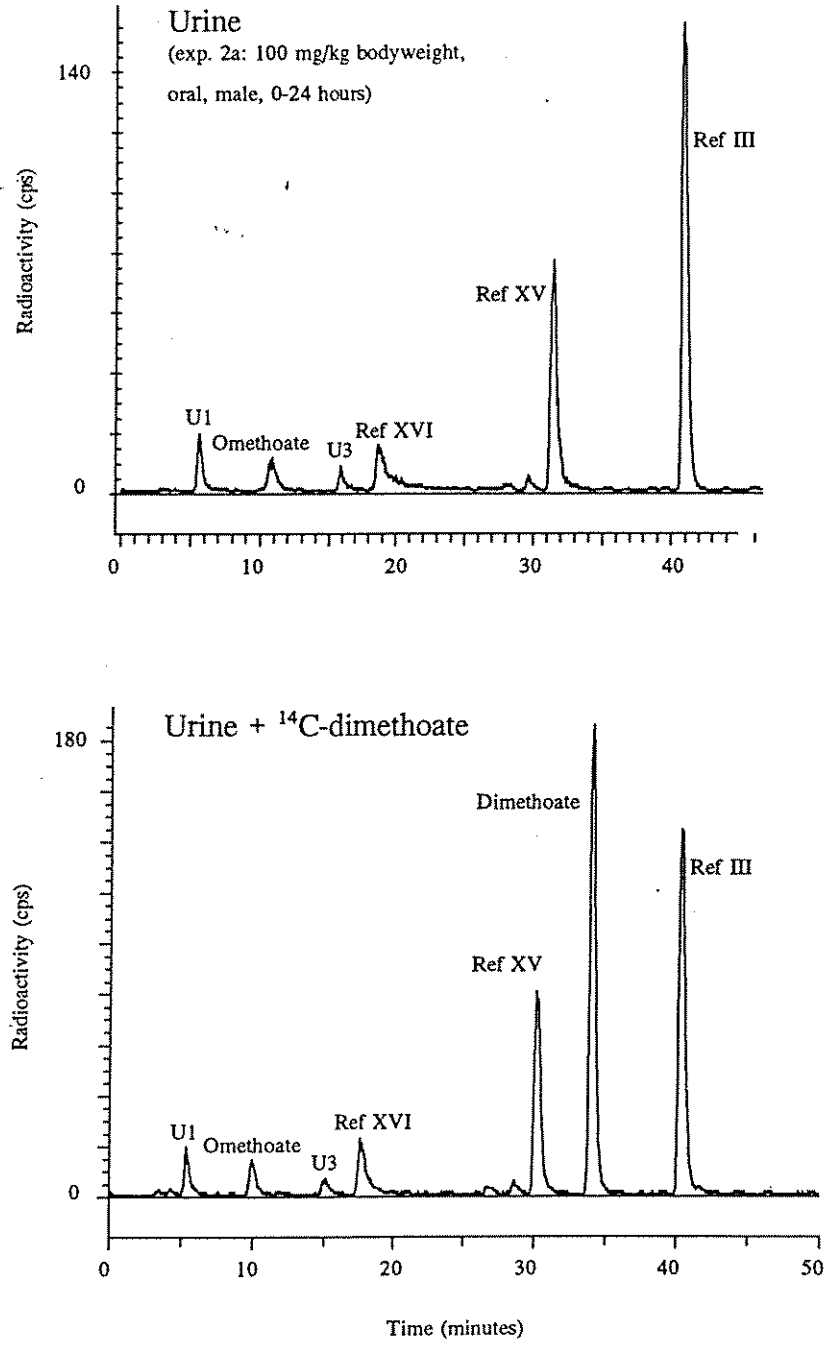


FIGURE 27

HPLC (method 1) chromatograms of urine and reference substance II

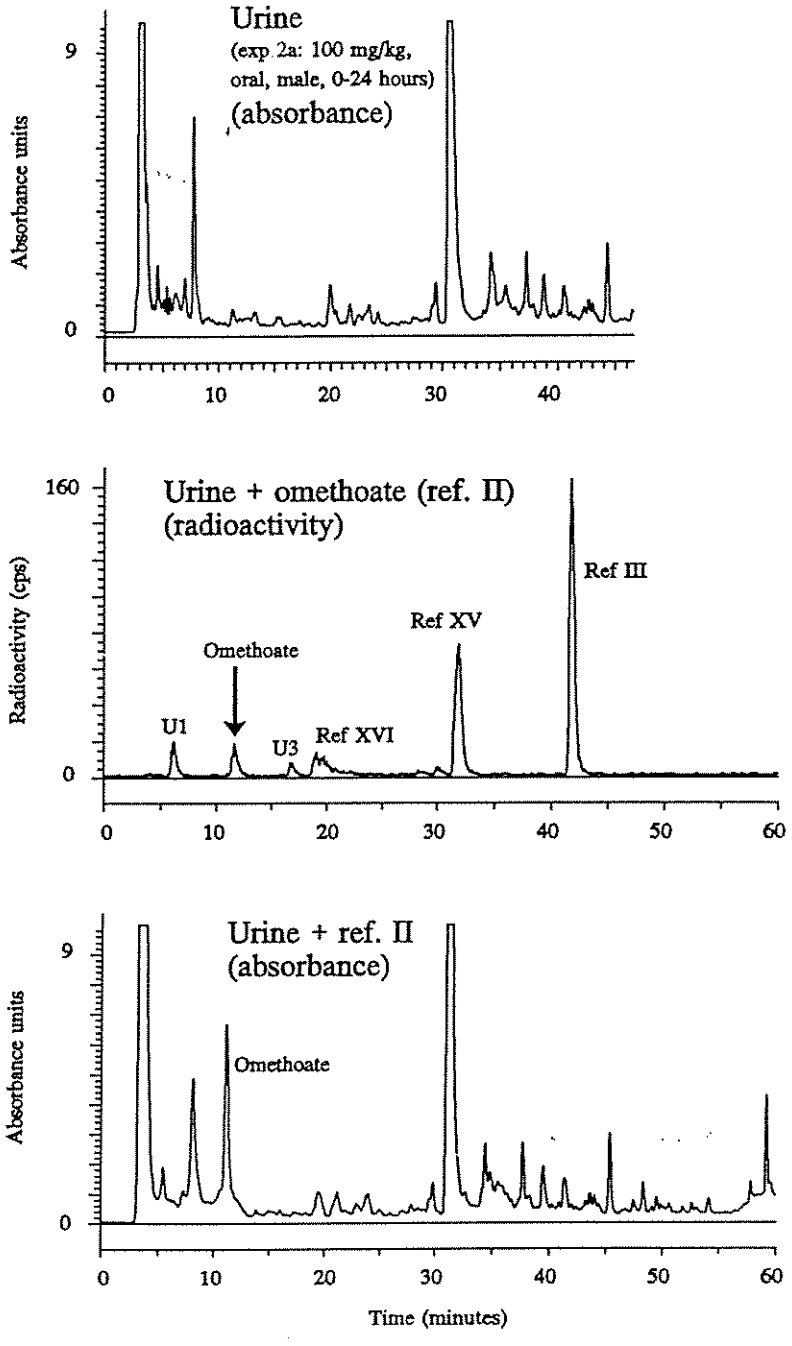


FIGURE 28

HPLC (method 1) chromatograms of urine and reference substance III

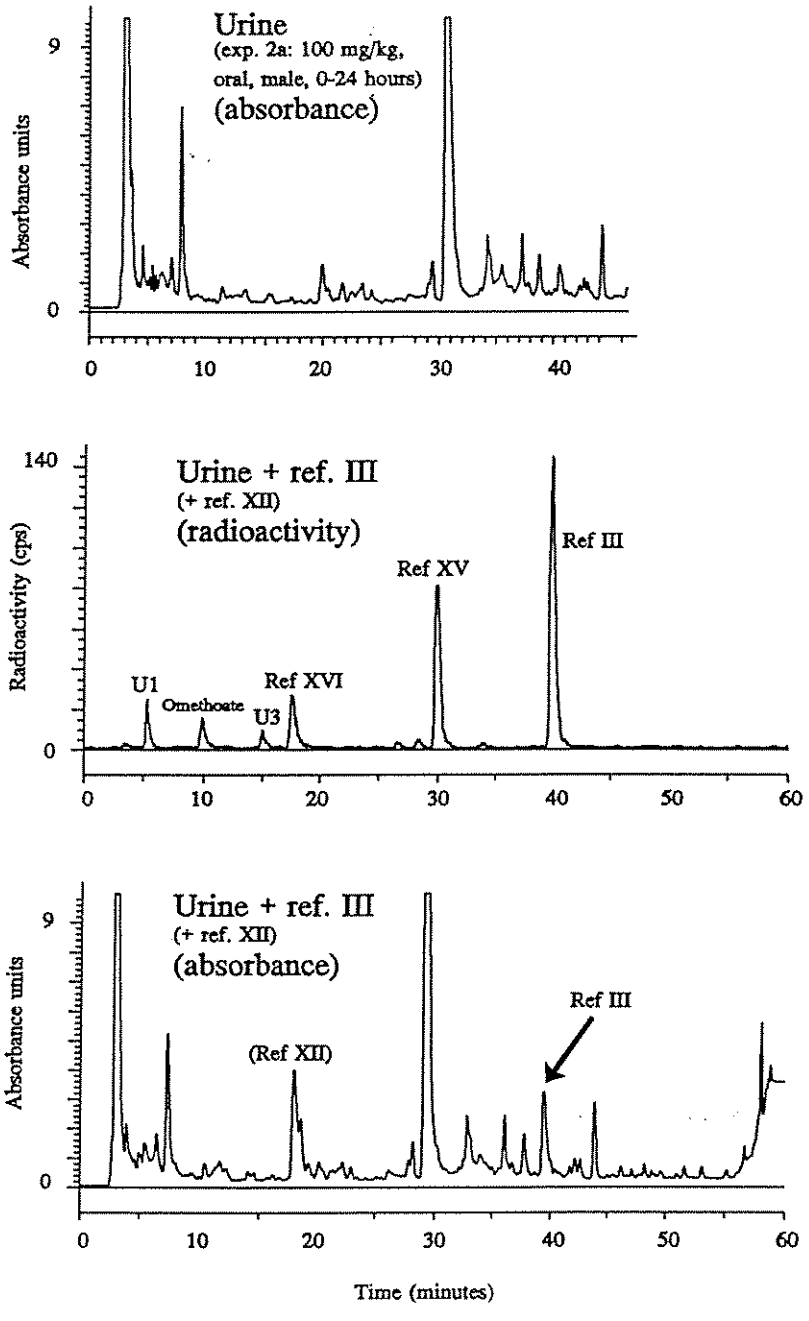


FIGURE 29

HPLC (method 1) chromatograms of urine and reference substance XV

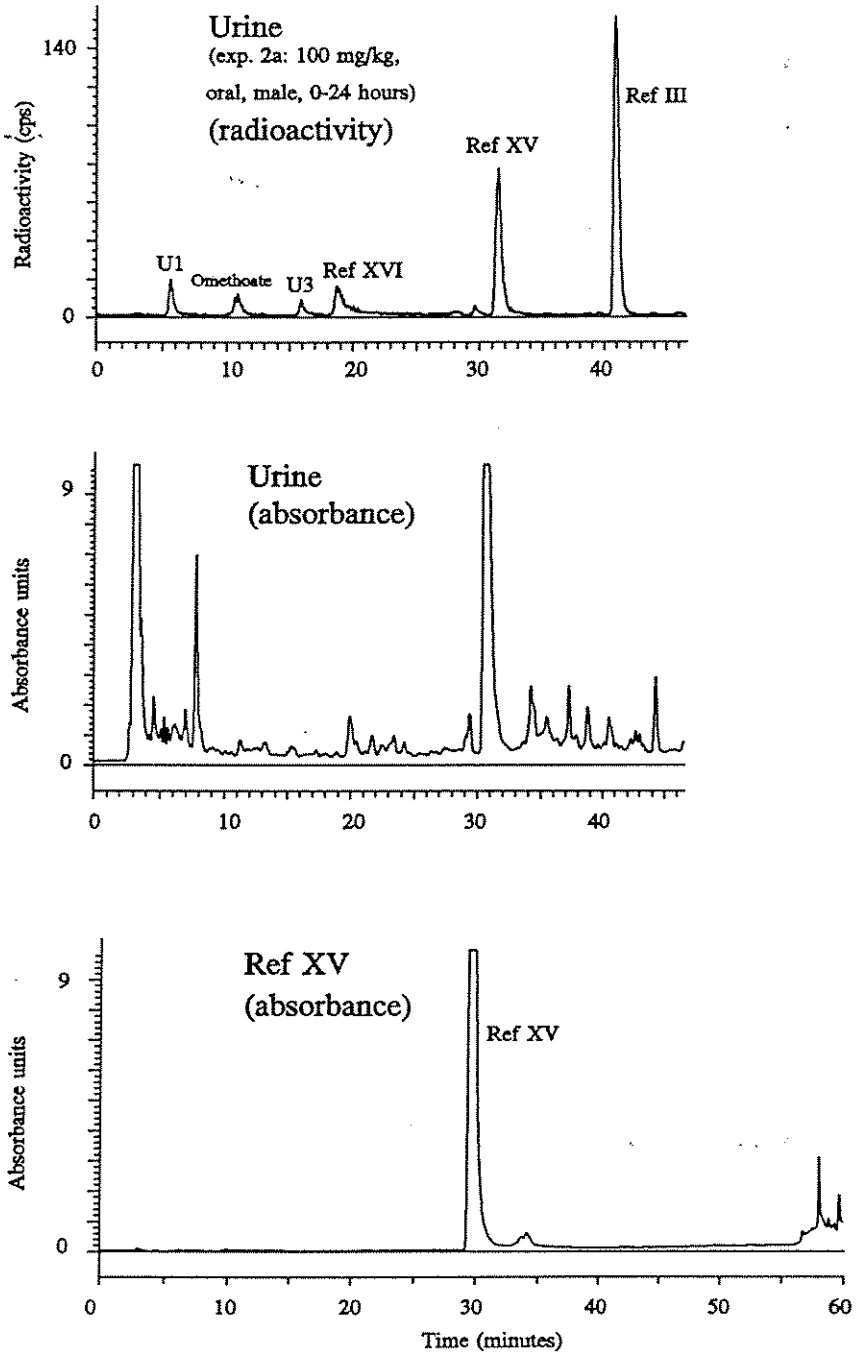


FIGURE 30

HPLC (method 1) chromatograms of urine and reference substance XVI

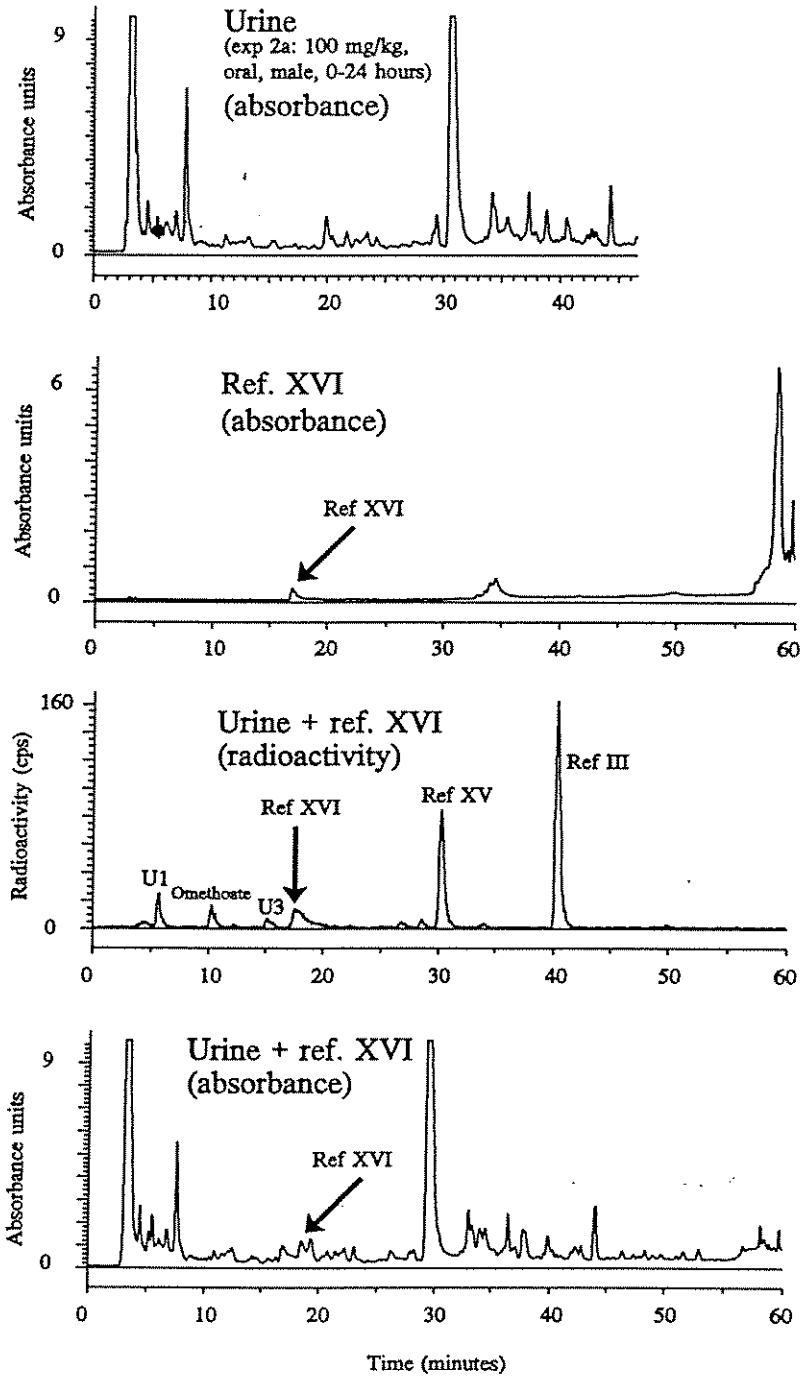


FIGURE 31

HPLC (method 1) radiochromatograms of bile

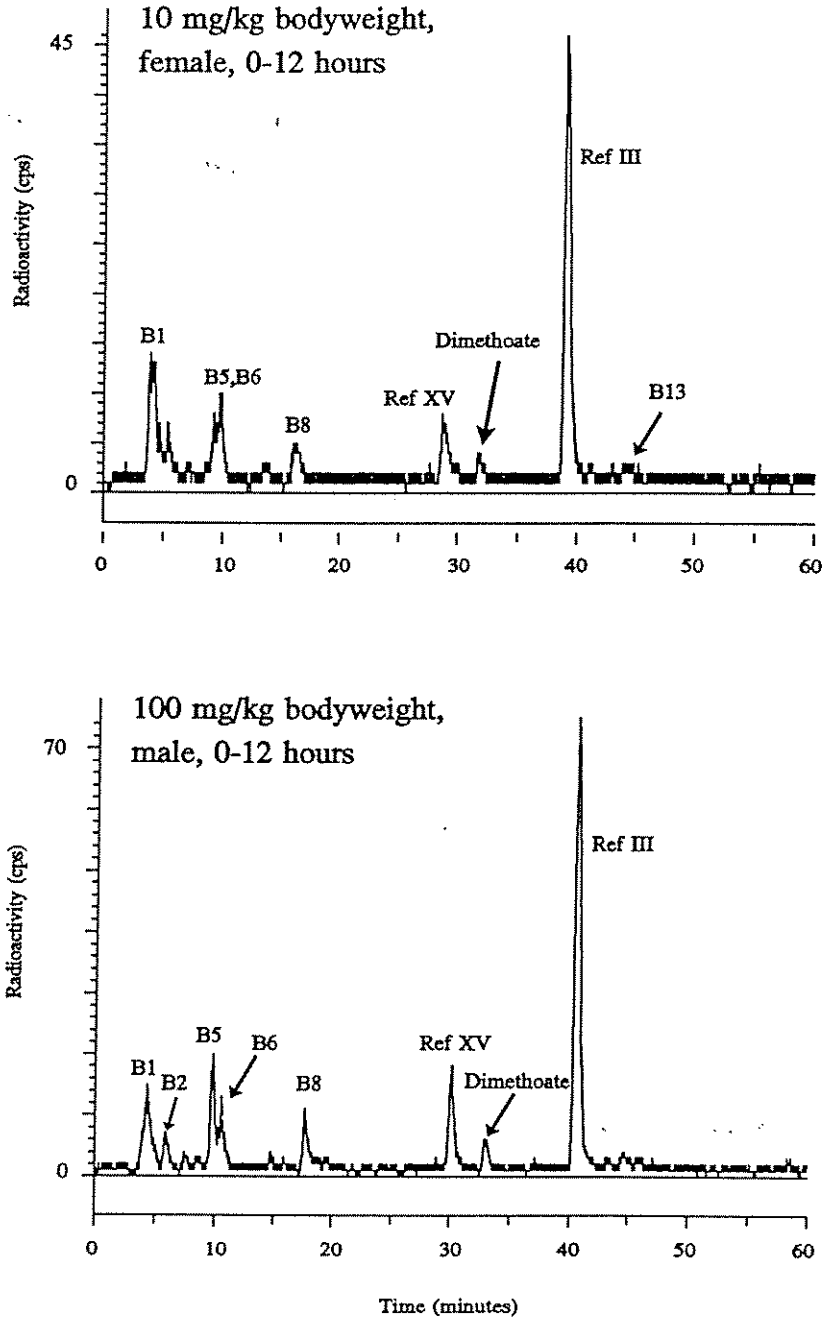


FIGURE 32

HPLC (method 1) radiochromatograms of plasma

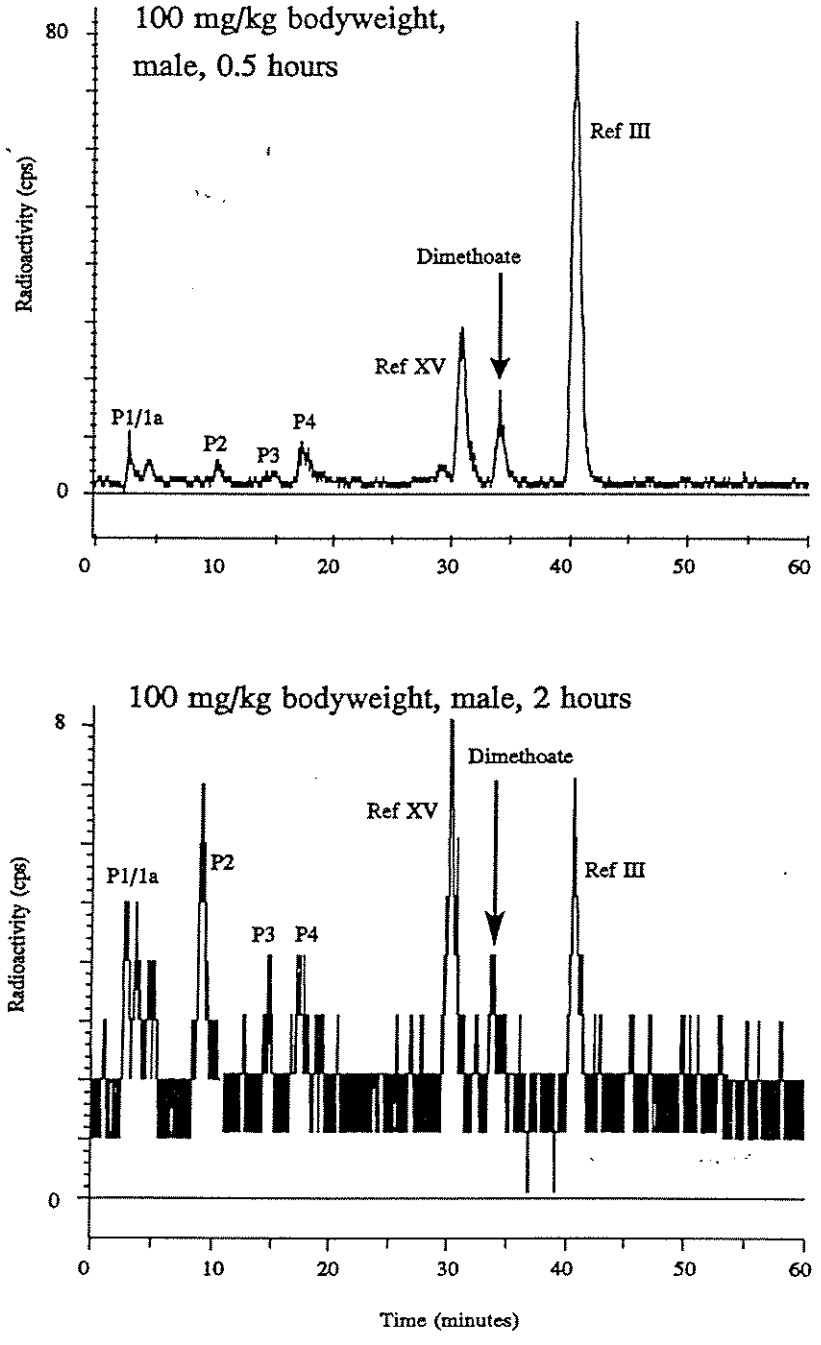


FIGURE 33

TLC (system D) Fujix image of extracts of kidney

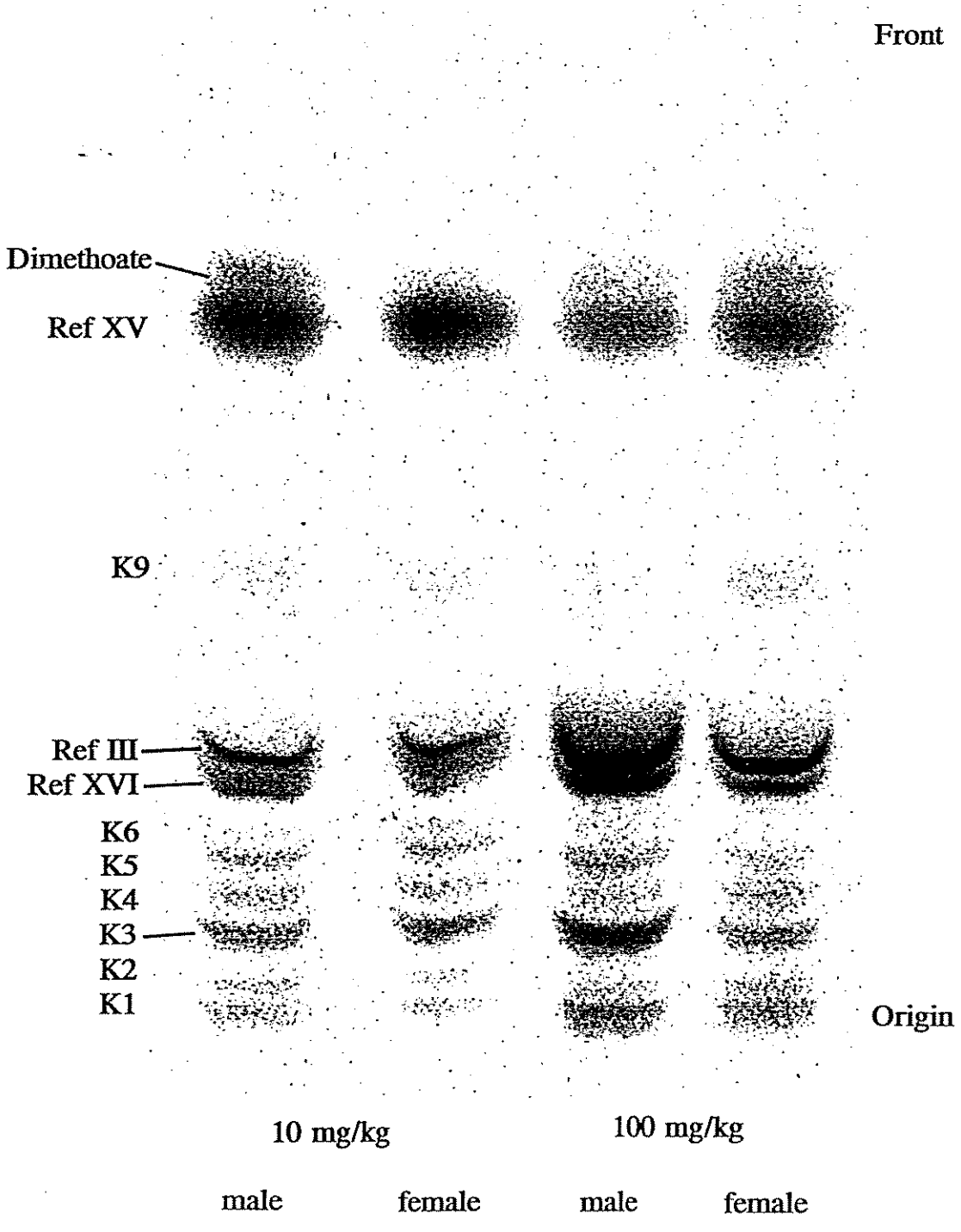
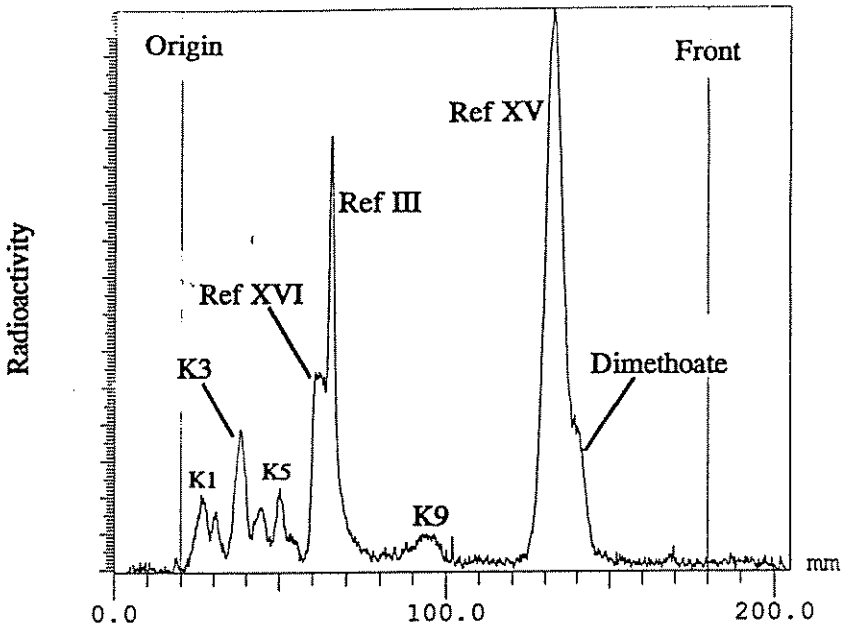


FIGURE 34

TLC (system D) radiochromatograms of extracts of kidney

10 mg/kg bodyweight, male, 0.5 hours sacrifice



100 mg/kg bodyweight, male, 2 hours sacrifice

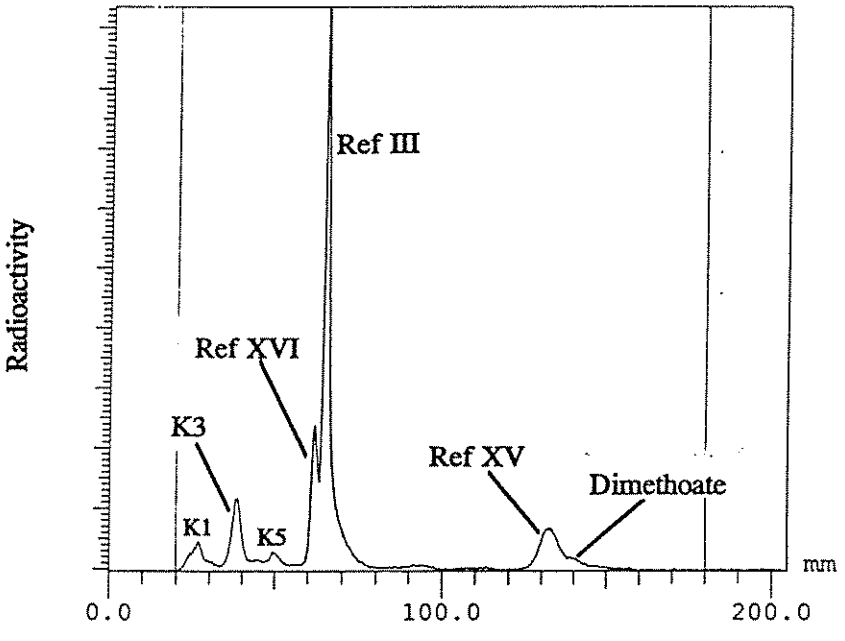


FIGURE 35

TLC (system D) Fujix image of extracts of liver

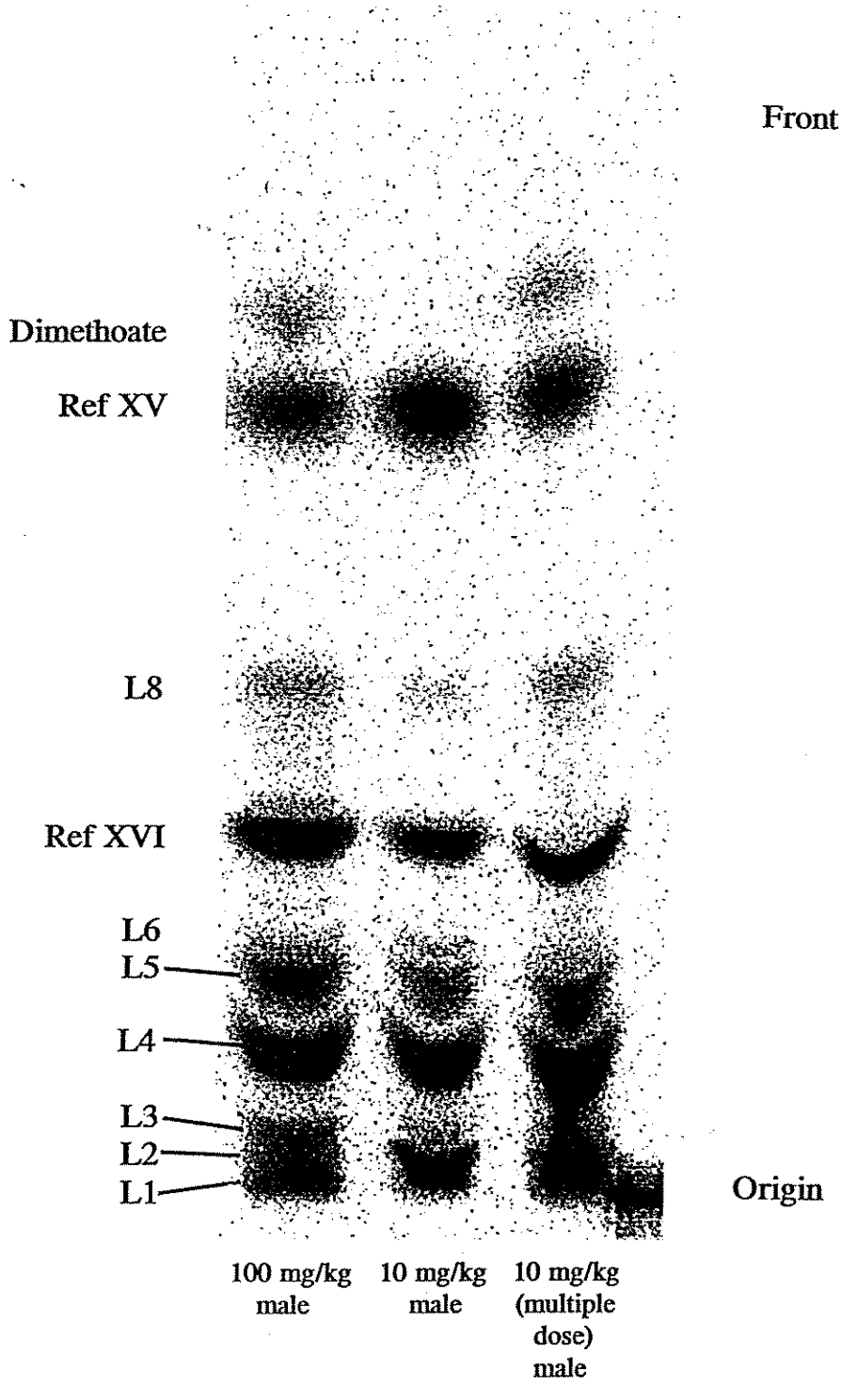
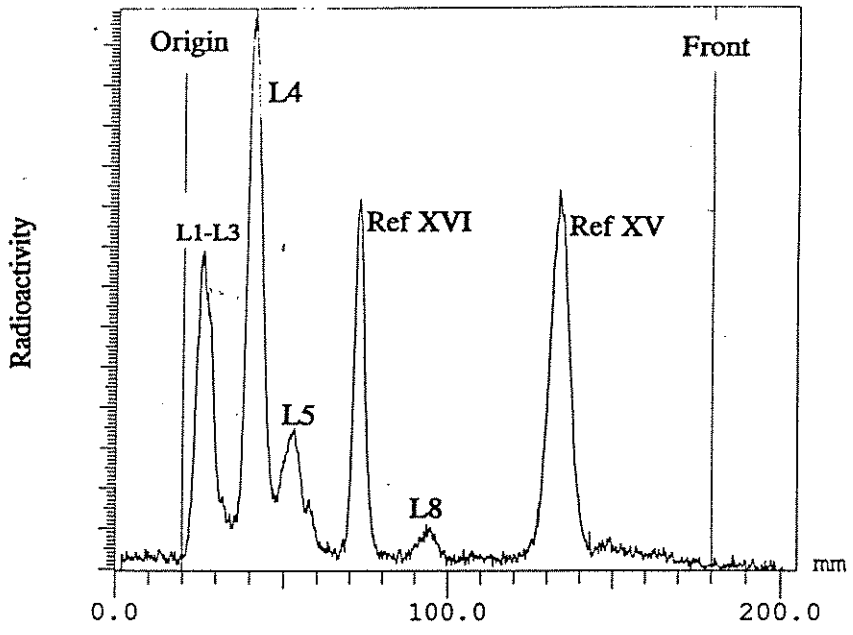


FIGURE 36

TLC (system D) radiochromatograms of extracts of liver

10 mg/kg bodyweight, male, 0.5 hours sacrifice



100 mg/kg bodyweight, female, 0.5 hours sacrifice

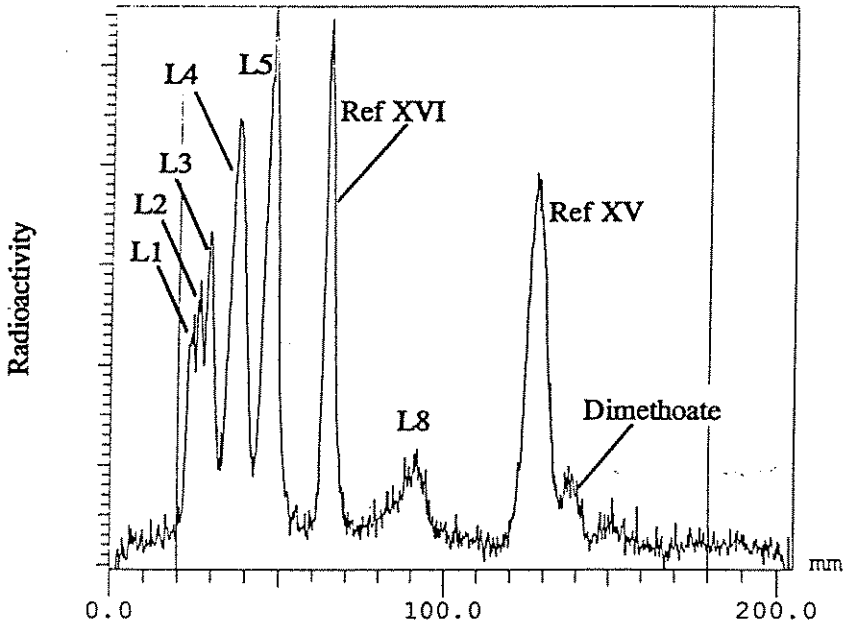
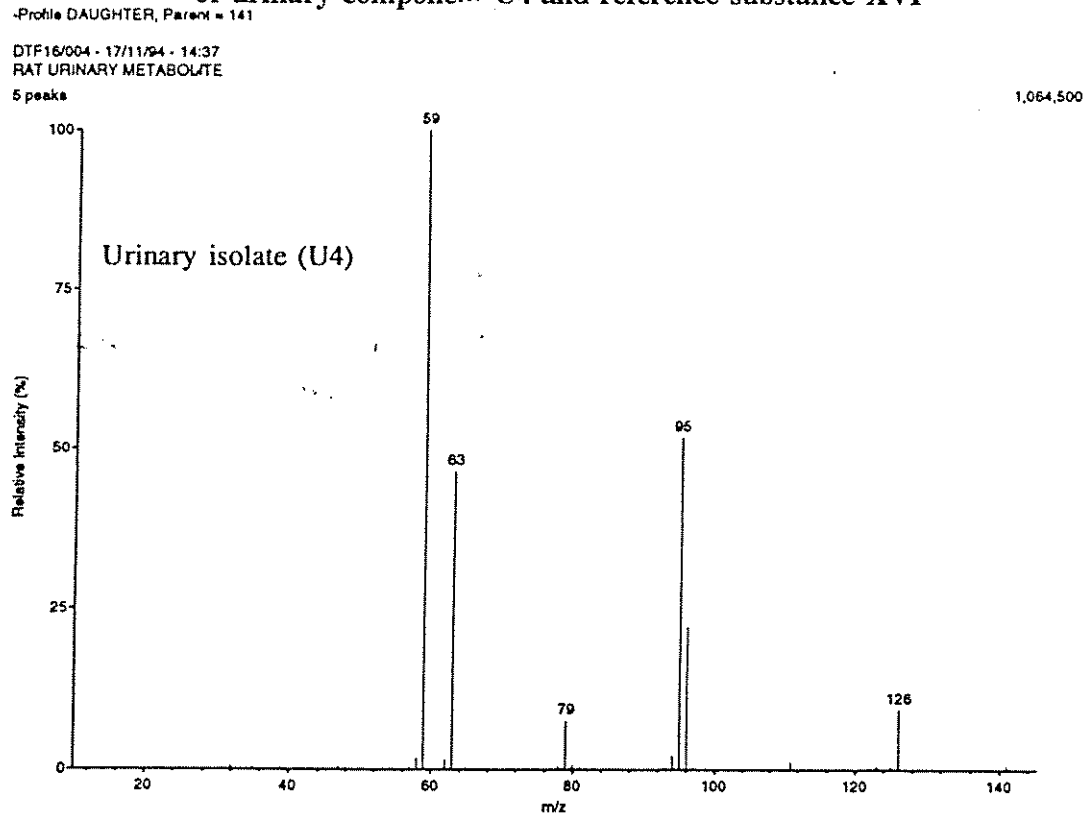


FIGURE 37

Daughter ion mass spectra of the precursor ion m/z 141 of urinary component U4 and reference substance XVI



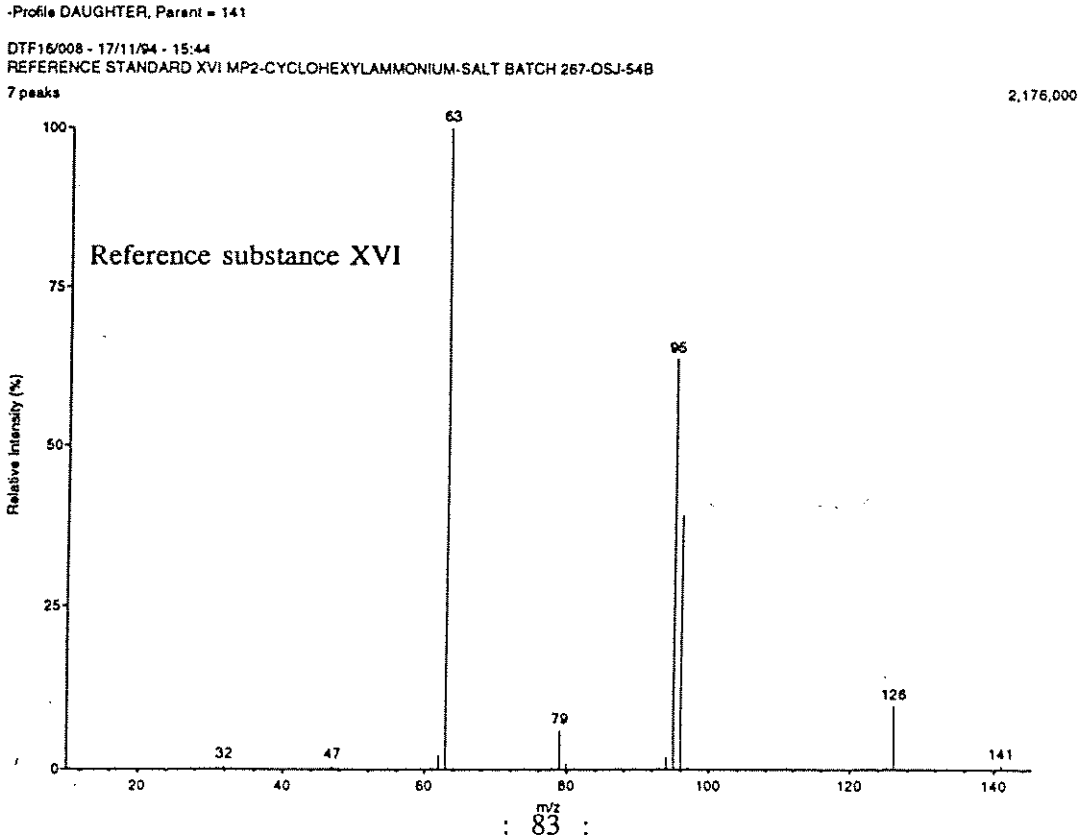


FIGURE 38

Mass spectra of the trimethylsilylated derivatives of urinary component U7 and reference substance XV

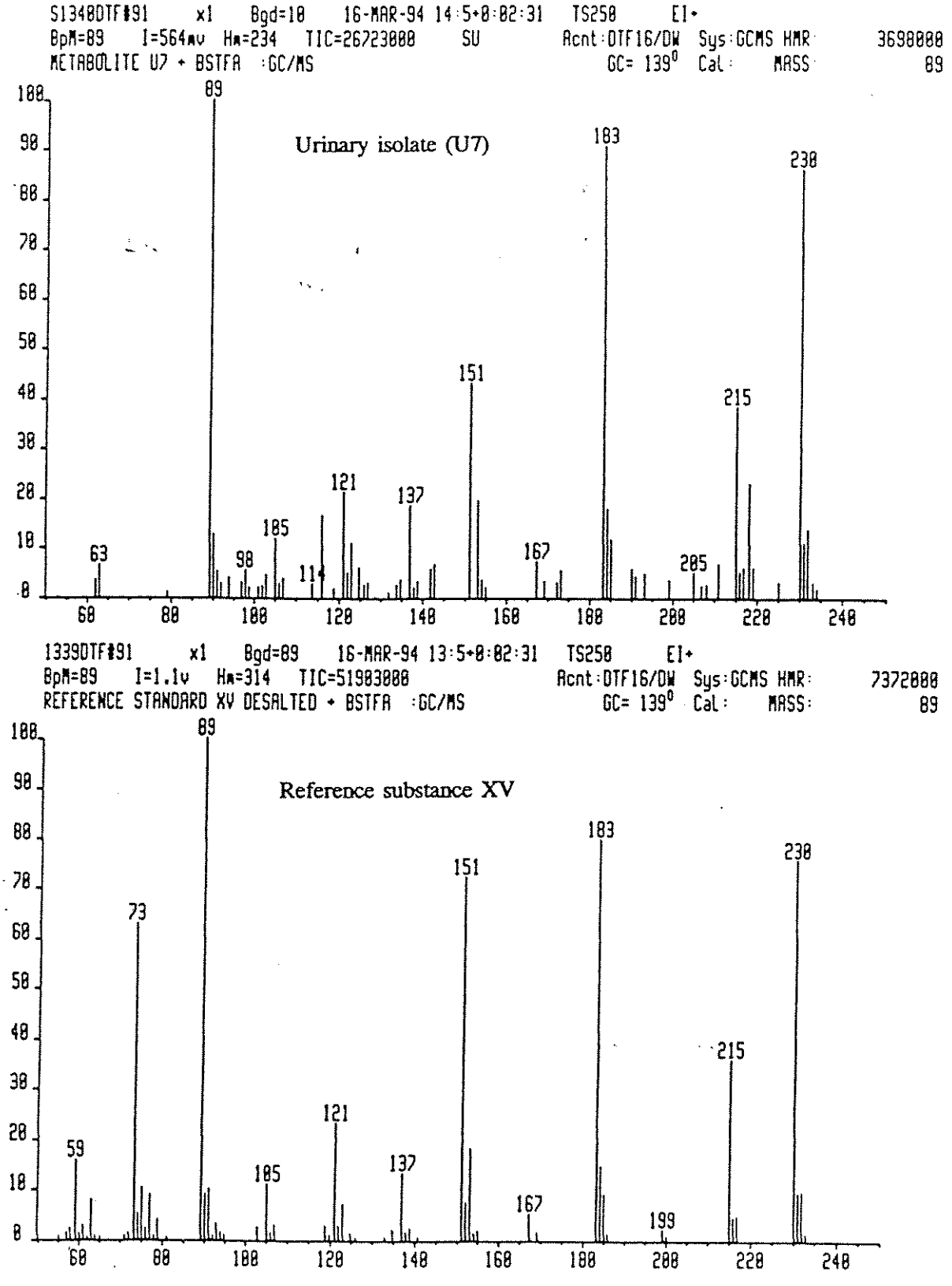


FIGURE 39

Mass spectra of urinary component U9 and reference substance III

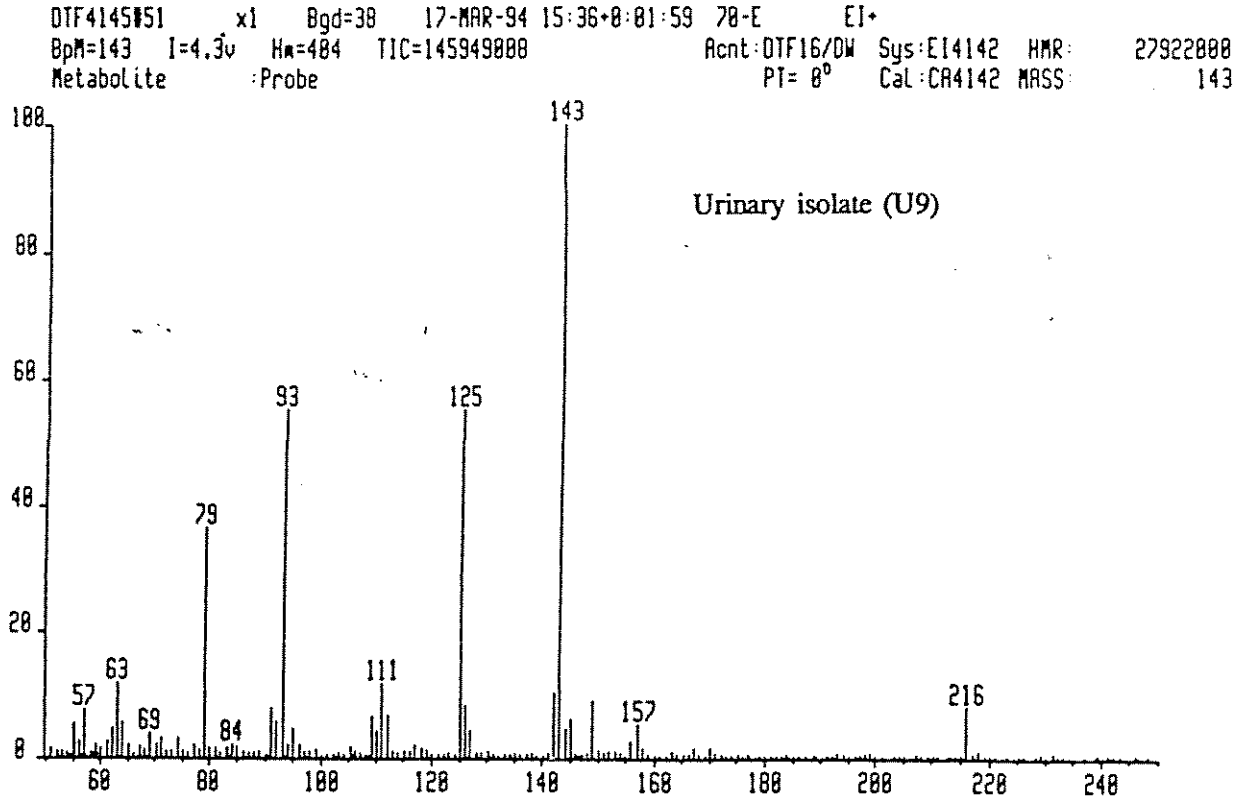
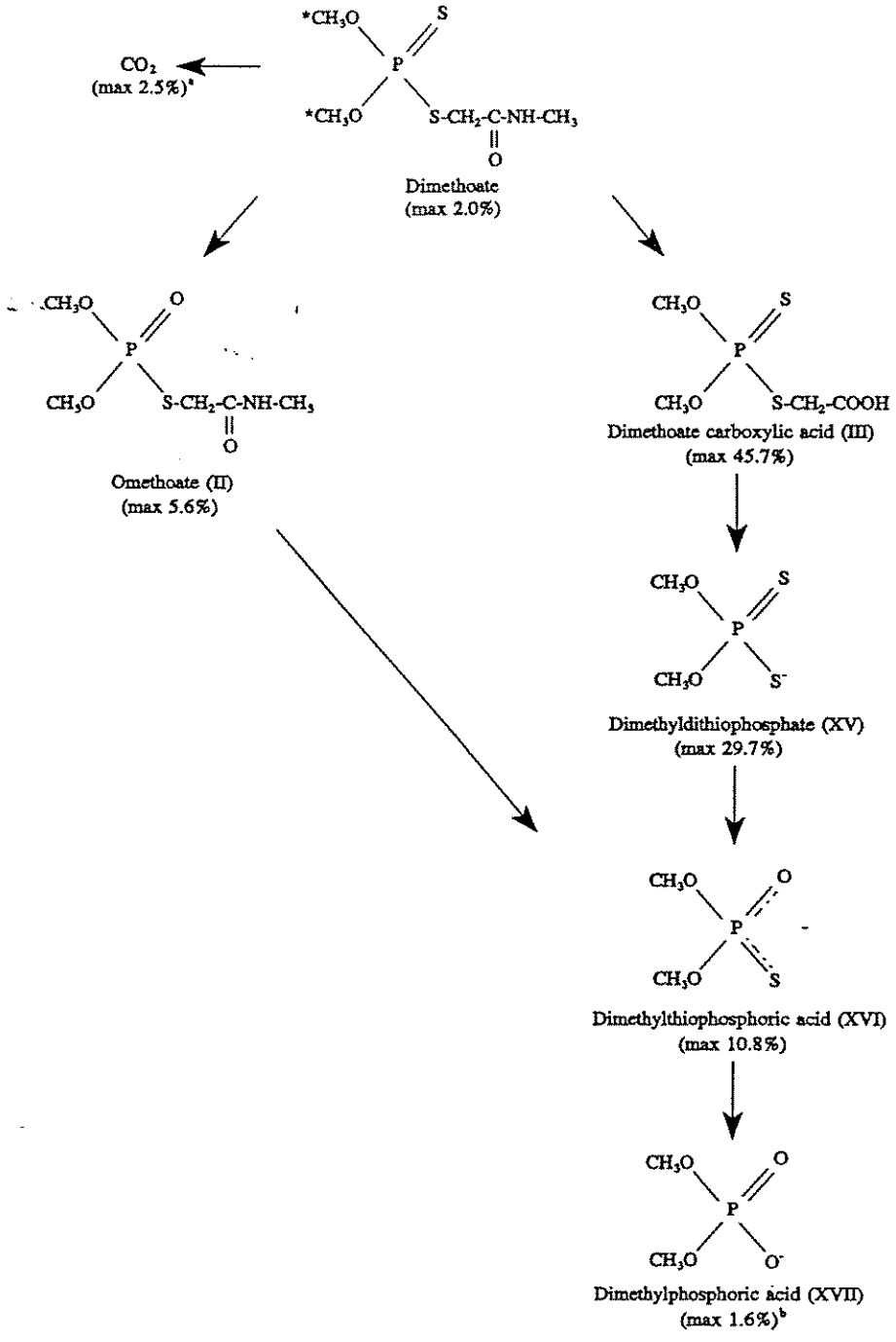


FIGURE 40

Proposed biotransformation pathway for dimethoate in the rat



Values in parentheses are, unless stated otherwise, the maximum proportions of an administered dose the components represented in urine

[a] present in expired air

[b] present in tissues only (tentative identification)

FIGURE 41

HPLC (method 1) radiochromatograms of urine (storage stability)

100 mg/kg bodyweight, oral, male, 0-48 hours (exp. 2b)

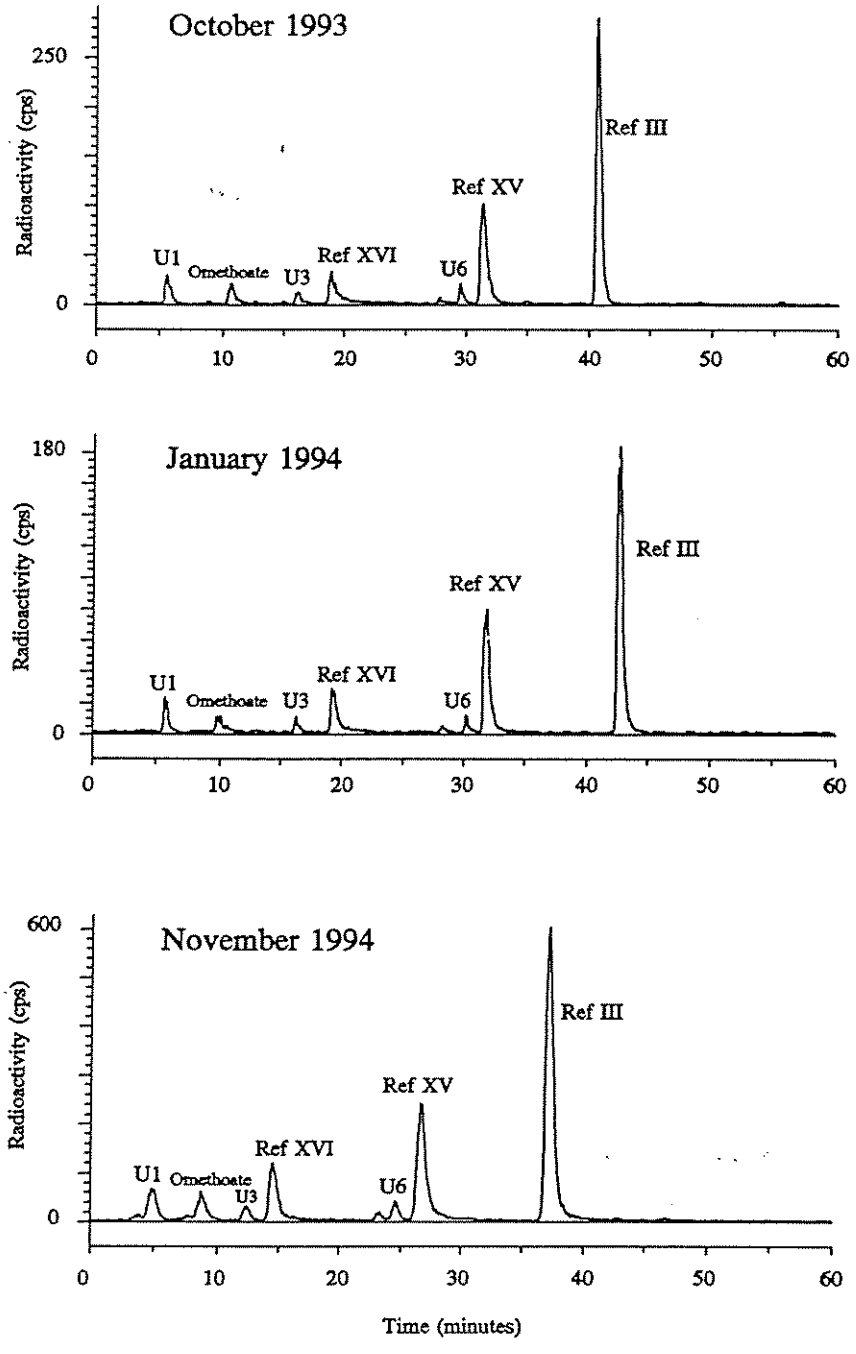
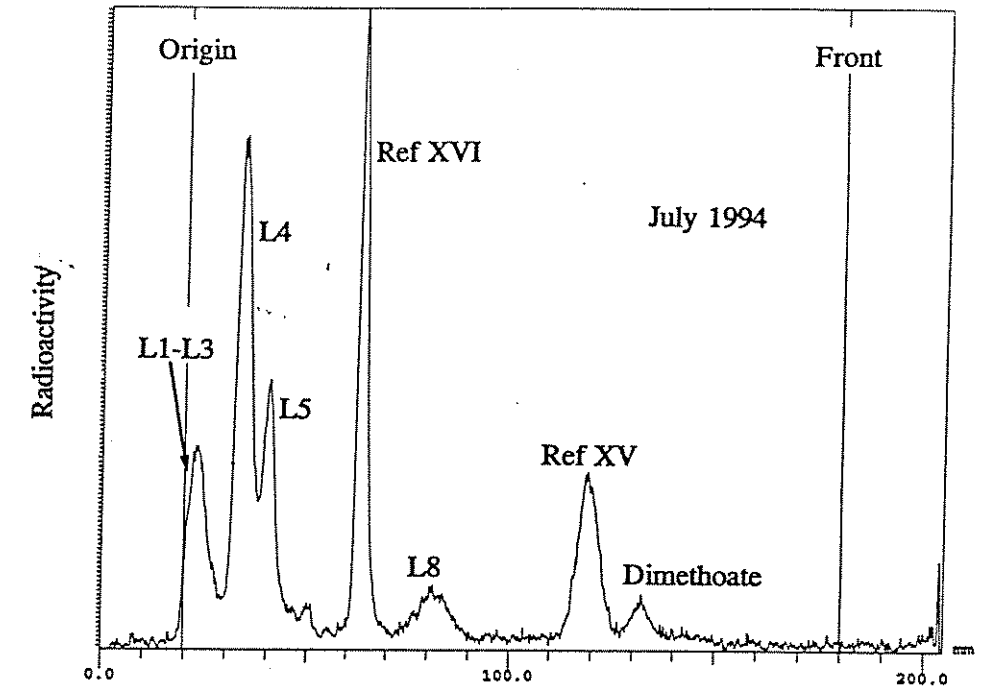


FIGURE 42

TLC (system D) radiochromatograms of extracts of liver (storage stability)

100 mg/kg bodyweight, male, 2 hours sacrifice



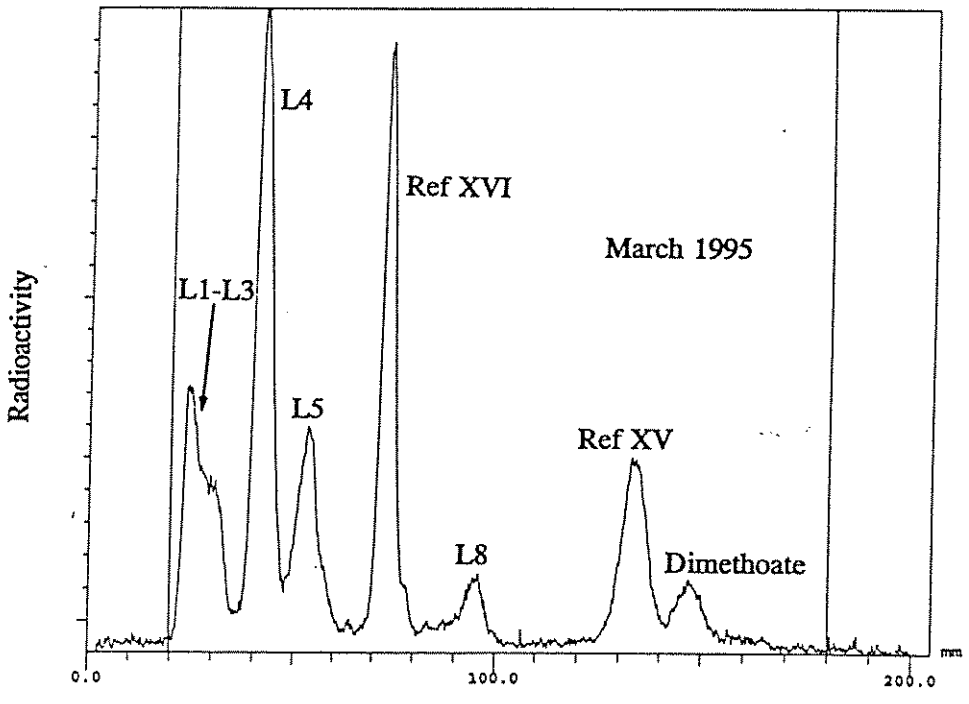


TABLE 1

Summary of animal experimentation and dosing

Experiment number	Experiment type	Nominal dose level (mg/kg bodyweight)	Dose route	Number of animals	Animal identification numbers
1a	Pre-test observation (non-radioactive)	100	Oral	2♂, 2♀	A, B, C, D
1b	Pre-test observation (non-radioactive)	10	Intravenous	2♂, 2♀	E, F, G, H
-	Pre-test observation (non-radioactive)	10	Oral	2♂, 2♀	Q, R, S, T
-	Pre-test observation (non-radioactive)	100	Dermal	2♂, 2♀	U, V, W, X
-	Pre-test observation (non-radioactive)	250	Dermal	2♂, 2♀	A1 - A4
2a	Excretion-balance (pilot)	100	Oral	2♂, 2♀	I, J, K, L
2b	Excretion-balance	100	Oral	5♂, 5♀	1, 3 - 6, 8, 9, 198 - 200
2c	Excretion-balance	10	Oral	5♂, 5♀	201 - 210
2d	Excretion-balance[a]	10	Oral	5♂, 5♀	21 - 30
2e	Excretion-balance	10	Intravenous	5♂, 5♀	31 - 40
2f	Pre-test (washing procedure)	100	Dermal	4♂	M, N, O, P
2g	Excretion-balance	10	Dermal	5♂, 5♀	41 - 50
2h	Excretion-balance	100	Dermal	5♂, 5♀	R51 - R60
3a	Plasma radioactivity kinetics	100	Oral	9♂, 9♀	61 - 78
3b	Plasma radioactivity kinetics	10	Oral	9♂, 9♀	79 - 96
3c	Plasma parent compound kinetics	100	Oral	12♂, 12♀	97 - 120
4a	Biliary excretion	100	Oral	3♂, 3♀	121 - 126
4b	Biliary excretion	10	Oral	3♂, 3♀	127 - 132
5a	Whole-body autoradiography	10	Oral	5♂, 5♀	133 - 142
5b	Tissue distribution (single dose)	100	Oral	9♂, 9♀	143 - 160
5c	Tissue distribution (single dose)	10	Oral	9♂, 9♀	161 - 178
5d	Tissue distribution (multiple dose[b])	10	Oral	9♂, 9♀	179 - 196

a rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

b rats received doses of ^{14}C-dimethoate once daily for 7 consecutive days

TABLE 2

Typical TLC R_f values and HPLC retention times of the test and reference substances

Substance	TLC system D	TLC system F	HPLC method 1
	R_f	R_f	Retention time (minutes)
Dimethoate (I)	0.76	0.85	34
II	a	0.77	10
III	0.32	0.37	42
VII	0.70	0.80	31
VIII	0.09	d	b
IX	0.17	0.82	10
X	0.33	a	27
XI	0.16	0.27	9
XII	0.30	0.70	18
XIII	0.07	0.16	28
XIV	0.36	0.71	36, 51[c]
XV	0.69	0.45	31
XVI	0.25	a	19
XVII	0.11	0.17	21
XIX	0.02	d	6
XX	0.03	d	b
XXI	0.01	0.17	b
XXII	0.27	0.40	23
XXIII	0.14	d	b

a extensive smearing meant that an accurate R_f value could not be determined
b insufficient UV absorbance meant that a retention time could not be determined
c two peaks were observed
d not determined

TABLE 3a

Mean excretion and retention of radioactivity following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		10 mg/kg bodyweight[a]		100 mg/kg bodyweight	
Animal numbers	201 - 205♂	206 - 210♀	21 - 25♂	26 - 30♀	1, 3, 4, 5, 198♂	6, 8, 9, 199, 200♀
Urine (hours)						
0 - 6	69.3	71.9	71.6	71.1	59.0	52.0
6 - 12	17.1	6.67	15.1	10.5	19.6	23.7
12 - 24	2.64	2.97	2.65	3.92	9.12	10.7
24 - 48	1.72	1.60	0.63	1.20	1.89	2.37
48 - 72	0.26	1.02	0.19	0.81	0.58	0.97
72 - 96	0.17	0.56	0.09	0.37	0.20	0.35
96 - 120	0.07	0.36	0.11	0.28	0.15	0.26
Cage wash (hours)						
24	NM	NM	0.23	0.46	NM	NM
120	0.10	0.29	0.05	0.25	0.27	0.45
Total urine and cage wash	91.3	85.4	90.6	88.9	90.8	90.8
Expired air (hours)						
0 - 6	0.91	0.98	0.82	0.82	} 2.06	} 2.13
6 - 24	1.00	0.90	0.99	1.10		
24 - 48	0.14	0.19	0.19	0.23	0.27	0.27
48 - 72	0.06	0.11	0.08	0.12	0.11	0.13
Total expired air	2.10	2.17	2.07	2.28	2.44	2.53
Faeces (hours)						
0 - 24	0.86	1.02	1.09	0.77	1.00	0.88
24 - 48	0.19	0.26	0.13	0.23	0.28	0.35
48 - 72	0.05	0.14	0.05	0.08	0.10	0.13
72 - 96	0.03	0.08	0.02	0.09	0.04	0.07
96 - 120	0.02	0.05	0.01	0.05	0.03	0.04
Total faeces	1.15	1.56	1.30	1.22	1.45	1.45
Total excreted	94.6	89.1	94.0	92.4	94.7	94.8
Carcass						
GIT	0.04	0.06	0.04	0.06	0.05	0.06
Kidneys	0.01	0.01	0.02	0.02	0.01	0.01
Liver	0.13	0.11	0.13	0.11	0.12	0.09
Residual carcass	0.50	1.26	0.85	1.53	0.96	1.71
Total carcass	0.67	1.45	1.04	1.72	1.14	1.88
Total recovery	95.3	90.6	95.0	94.1	95.9	96.6

Results are expressed as % dose

a rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

GIT gastrointestinal tract and contents

NM not measured

See Appendix 13, Tables 1 - 6 for data from individual animals

TABLE 3b

Mean excretion and retention of radioactivity following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		10 mg/kg bodyweight[a]		100 mg/kg bodyweight	
Animal numbers	201 - 205♂	206 - 210♀	21 - 25♂	26 - 30♀	1, 3, 4, 5, 198♂	6, 8, 9, 199, 200♀
Urine (hours)						
0 - 6	1.43	1.57	2.37	1.73	12.3	10.9
6 - 12	0.354	0.145	0.500	0.255	4.08	4.98
12 - 24	0.055	0.065	0.088	0.095	1.90	2.25
24 - 48	0.036	0.035	0.021	0.029	0.39	0.50
48 - 72	0.005	0.022	0.006	0.020	0.12	0.20
72 - 96	0.004	0.012	0.003	0.009	0.04	0.07
96 - 120	0.001	0.008	0.004	0.007	0.03	0.05
Cage wash (hours)						
24	NM	NM	0.008	0.011	NM	NM
120	0.002	0.006	0.002	0.006	0.06	0.09
Total urine and cage wash	1.89	1.86	3.00	2.16	18.9	19.1
Expired air (hours)						
0 - 6	0.019	0.021	0.027	0.020	} 0.43	} 0.45
6 - 24	0.021	0.020	0.033	0.027		
24 - 48	0.003	0.004	0.006	0.006	0.06	0.06
48 - 72	0.001	0.002	0.003	0.003	0.02	0.03
Total expired air	0.043	0.047	0.069	0.055	0.51	0.53
Faeces (hours)						
0 - 24	0.018	0.022	0.036	0.019	0.21	0.18
24 - 48	0.004	0.006	0.004	0.006	0.06	0.07
48 - 72	0.001	0.003	0.002	0.002	0.02	0.03
72 - 96	0.001	0.002	0.001	0.002	0.01	0.01
96 - 120	<0.001	0.001	<0.001	0.001	0.01	0.01
Total faeces	0.024	0.034	0.043	0.030	0.30	0.30
Total excreted	1.96	1.94	3.11	2.25	19.7	19.9
Carcass						
GIT	0.001	0.001	0.001	0.001	0.01	0.01
Kidneys	<0.001	<0.001	0.001	<0.001	<0.01	<0.01
Liver	0.003	0.002	0.004	0.003	0.02	0.02
Residual carcass	0.010	0.027	0.028	0.037	0.20	0.36
Total carcass	0.014	0.032	0.034	0.042	0.24	0.39
Total recovery	1.97	1.98	3.14	2.29	19.9	20.3

Results are expressed as mg dimethoate equivalents

[a] rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

GIT gastrointestinal tract and contents

NM not measured

Results were obtained by multiplying the mean proportion of the administered dose in the sample (from Table 3a) by the (mean) mg dimethoate administered to the rats (from Appendix 8, Table 1). (In the 100 mg/kg bodyweight experiment, weighted mean doses of 20.8 mg (♂) and 21.0 mg (♀) were used)

TABLE 4a

Mean excretion and retention of radioactivity following a single intravenous dose at a nominal level of 10 mg/kg bodyweight

Animal numbers	31 - 35♂	36 - 40♀
Urine (hours)		
0 - 6	80.6	80.4
6 - 12	4.43	4.32
12 - 24	1.97	2.05
24 - 48	0.73	1.18
48 - 72	0.26	0.41
72 - 96	0.19	0.31
96 - 120	0.34	0.26
Cage wash	0.41	0.44
Total urine and cage wash	88.9	89.4
Expired air (hours)		
0 - 6	0.93	0.89
6 - 24	0.71	0.58
24 - 48	0.12	0.15
48 - 72	<0.04	<0.04
Total expired air	1.76	1.62
Faeces (hours)		
0 - 24	0.84	0.88
24 - 48	0.17	0.26
48 - 72	0.08	0.09
72 - 96	0.03	0.07
96 - 120	0.03	0.05
Total faeces	1.15	1.35
Total excreted	91.8	92.4
Carcass		
GIT	0.04	0.06
Kidneys	0.01	0.01
Liver	0.11	0.09
Residual carcass	0.65	0.81
Total carcass	0.81	0.96
Total recovery	92.6	93.3

Results are expressed as % dose
GIT gastrointestinal tract and contents
See Appendix 13, Tables 7 - 8 for data from individual animals

TABLE 4b

Mean excretion and retention of radioactivity following a single intravenous dose at a nominal level of 10 mg/kg bodyweight

Animal numbers	31 - 35♂	36 - 40♀
Urine (hours)		
0 - 6	1.65	1.65
6 - 12	0.091	0.089
12 - 24	0.040	0.042
24 - 48	0.015	0.024
48 - 72	0.005	0.008
72 - 96	0.004	0.006
96 - 120	0.007	0.005
Cage wash	0.008	0.009
Total urine and cage wash	1.82	1.83
Expired air (hours)		
0 - 6	0.019	0.018
6 - 24	0.015	0.012
24 - 48	0.002	0.003
48 - 72	<0.001	<0.001
Total expired air	0.036	0.033
Faeces (hours)		
0 - 24	0.017	0.018
24 - 48	0.003	0.005
48 - 72	0.002	0.002
72 - 96	0.001	0.001
96 - 120	0.001	0.001
Total faeces	0.024	0.028
Total excreted	1.88	1.89
Carcass		
GIT	0.001	0.001
Kidneys	<0.001	<0.001
Liver	0.002	0.002
Residual carcass	0.013	0.017
Total carcass	0.017	0.020
Total recovery	1.90	1.91

Results are expressed as mg dimethoate equivalents
GIT gastrointestinal tract and contents
Results were obtained by multiplying the mean proportion of the administered dose in the sample (from Table 4a) by the mg dimethoate administered to the rats (from Appendix 8, Table 1)

TABLE 5a

Mean excretion and retention of radioactivity following a single dermal dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	41 - 45♂	46 - 50♀	R51 - R55♂	R56 - R60♀
Urine (hours)				
0 - 6	4.55	5.51	0.65	0.67
6 - 24	2.86	2.19	0.31	0.33
24 - 48	0.27	0.28	0.05	0.09
48 - 72	0.12	0.11	0.02	0.05
72 - 96	0.07	0.07	0.01	0.04
96 - 120	0.05	0.08	0.01	0.03
Cage wash (hours)				
6	0.25	0.91	0.07	0.06
120	0.10	0.12	0.02	0.04
Total urine and cage wash	8.27	9.26	1.13	1.32
Faeces (hours)				
0 - 6	0.11	0.45	0.03	0.16
6 - 24	0.05	0.04	0.01	0.01
24 - 48	0.03	0.05	<0.01	0.01
48 - 72	0.01	0.02	<0.01	0.01
72 - 96	0.01	0.01	<0.01	0.01
96 - 120	0.01	0.03	<0.01	0.01
Total faeces	0.21	0.58	0.05	0.13
Carcass				
GIT	0.02	0.03	<0.01	0.01
Kidneys	<0.01	<0.01	<0.01	<0.01
Liver	0.01	0.01	<0.01	0.01
Residual carcass	0.79	0.72	0.08	0.20
Total carcass	0.76	0.76	0.11	0.22
Total absorbed	9.19	10.6	1.18	1.64
Skin wash (6 hours)	62.5	62.1	84.1	83.7
Dressing extracts				
6 hours	2.50	2.33	1.10	3.36
120 hours	0.92	0.83	0.25	0.28
Treated skin	17.3	13.3	3.65	2.18
Total recovery	92.5	89.1	90.2	91.1

Results are expressed as % dose
GIT gastrointestinal tract and contents
See Appendix 13, Tables 9 - 12 for data from individual animals

TABLE 5b

Mean excretion and retention of radioactivity following a single dermal dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	41 - 45♂	46 - 50♀	R51 - R55♂	R56 - R60♀
Urine (hours)				
0 - 6	0.086	0.104	0.13	0.13
6 - 24	0.054	0.041	0.06	0.07
24 - 48	0.005	0.005	0.01	0.02
48 - 72	0.002	0.002	<0.01	0.01
72 - 96	0.001	0.001	<0.01	0.01
96 - 120	0.001	0.002	<0.01	0.01
Cage wash (hours)				
6	0.005	0.017	0.01	0.01
120	0.002	0.002	<0.01	0.01
Total urine and cage wash	0.155	0.174	0.22	0.26
Faeces (hours)				
0 - 6	0.002	0.008	0.01	0.03
6 - 24	0.001	0.001	<0.01	<0.01
24 - 48	0.001	0.001	<0.01	<0.01
48 - 72	<0.001	<0.001	<0.01	<0.01
72 - 96	<0.001	<0.001	<0.01	<0.01
96 - 120	<0.001	0.001	<0.01	<0.01
Total faeces	0.004	0.011	0.01	0.03
Carcass				
GIT	<0.001	0.001	<0.01	<0.01
Kidneys	<0.001	<0.001	<0.01	<0.01
Liver	<0.001	<0.001	<0.01	<0.01
Residual carcass	0.015	0.014	0.02	0.04
Total carcass	0.014	0.014	0.02	0.04
Total absorbed	0.173	0.199	0.23	0.33
Skin wash (6 hours)	1.18	1.17	16.7	16.7
Dressing extracts				
6 hours	0.047	0.044	0.22	0.67
120 hours	0.017	0.016	0.05	0.06
Treated skin	0.325	0.250	0.73	0.43
Total recovery	1.74	1.68	17.9	18.1

Results are expressed as mg dimethoate equivalents
GIT gastrointestinal tract and contents
Results were obtained by multiplying the mean proportion of the administered dose in the sample (from Table 5a) by the mg dimethoate administered to the rats (from Appendix 8, Table 1)

TABLE 6

Recoveries of radioactivity in the skin washes of rats following a single dermal dose at a nominal level of 100 mg/kg bodyweight (pilot experiment)

Animal identification	Skin wash solution	Recovery of radioactivity
M♂	Soapy water	91.4
N♂	Soapy water	91.2
O♂	Ethanol	66.4
P♂	Ethanol	59.3

Results are expressed as % dose
Washes were made 4 minutes after dose administration to a 10 cm^2 skin area (*ie* 2 mg/cm^2)

TABLE 7a

Mean excretion and retention of radioactivity in bile duct cannulated rats following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	127 - 129♂	130 - 132♀	121 - 123♂	124, 126♀
Bile (hours)				
0 - 3	0.88	1.29	0.52	2.65
3 - 6	1.24	1.04	0.73	1.28
6 - 12	1.39	0.93	1.45	0.50
12 - 24	0.50	0.35	1.01	0.08
24 - 48	0.05	0.07	0.35	0.03
Total bile	4.05	3.68	4.07	4.52
Urine (hours)				
0 - 24	77.9	74.0	71.6	83.5
24 - 48	3.45	5.95	9.11	1.91
Cage wash	1.43	1.62	1.84	1.59
Total urine and cage wash	82.8	81.6	82.6	87.0
Faeces (hours)				
0 - 24	0.43	1.48	0.51	1.75
24 - 48	0.97	1.82	1.84	0.94
Total faeces	1.40	3.31	2.39	2.69
Carcass				
GIT	0.11	0.09	0.24	0.06
Liver	0.29	0.20	0.24	0.13
Residual carcass	2.61	1.99	2.29	1.88
Total carcass	3.00	2.28	2.77	2.07
Total recovery	91.3	90.8	91.8	96.3

Results are expressed as % dose
GIT gastrointestinal tract and contents
See Appendix 13, Tables 13 - 16 for data from individual animals

TABLE 7b

Mean excretion and retention of radioactivity in bile duct cannulated rats following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	127 - 129♂	130 - 132♀	121 - 123♂	124, 126♀
Bile (hours)				
0 - 3	0.018	0.026	0.11	0.55
3 - 6	0.026	0.021	0.15	0.27
6 - 12	0.029	0.019	0.30	0.10
12 - 24	0.010	0.007	0.21	0.02
24 - 48	0.001	0.001	0.07	0.01
Total bile	0.083	0.075	0.85	0.94
Urine (hours)				
0 - 24	1.60	1.52	14.9	17.4
24 - 48	0.071	0.122	1.89	0.40
Cage wash	0.029	0.033	0.38	0.33
Total urine and cage wash	1.71	1.67	17.2	18.1
Faeces (hours)				
0 - 24	0.009	0.030	0.11	0.36
24 - 48	0.020	0.037	0.38	0.20
Total faeces	0.029	0.068	0.50	0.56
Carcass				
GIT	0.002	0.002	0.05	0.01
Liver	0.006	0.004	0.05	0.03
Residual carcass	0.054	0.041	0.48	0.39
Total carcass	0.062	0.047	0.58	0.43
Total recovery	1.88	1.86	19.1	20.0

Results are expressed as mg dimethoate equivalents
GIT gastrointestinal tract and contents
Results were obtained by multiplying the mean proportion of the administered dose in the sample (from Table 7a) by the mg dimethoate administered to the rats (from Appendix 8, Table 1)

TABLE 8a

Mean cumulative excretion of radioactivity in bile following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	127 - 129♂	130 - 132♀	121 - 123♂	124 - 126♀
Time (hours)				
0 - 3	0.88	1.29	0.52	2.54
0 - 6	2.11	2.33	1.25	4.00
0 - 12	3.50	3.25	2.70	4.59
0 - 24	4.00	3.60	3.71	4.77
0 - 48	4.05	3.68	4.07	4.81

Results are expressed as cumulative % dose
See Appendix 13, Tables 17 - 20 for data from individual animals

TABLE 8b

Mean cumulative excretion of radioactivity in bile following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	127 - 129♂	130 - 132♀	121 - 123♂	124 - 126♀
Time (hours)				
0 - 3	0.018	0.026	0.11	0.53
0 - 6	0.043	0.048	0.26	0.83
0 - 12	0.072	0.067	0.56	0.95
0 - 24	0.082	0.074	0.77	0.99
0 - 48	0.083	0.075	0.85	1.00

Results are expressed as cumulative mg dimethoate equivalents
Results were obtained by multiplying the mean cumulative proportion of the administered dose in the sample (from Table 8a) by the mg dimethoate administered to the rats (from Appendix 8, Table 1)

TABLE 9

Mean concentrations of radioactivity in plasma following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Sex	Male	Female	Male	Female
Time (hours)				
0.25	6.54	5.96	50.7	64.0
0.5	8.62	7.68	43.6	93.2
1	4.58	5.37	31.5	47.6
2	3.81	3.14	15.7	19.2
4	2.99	1.45	4.52	8.08
6	0.97	0.80	11.6	18.4
12	0.65	0.42	6.95	9.92
24	0.52	0.50	4.32	6.30
48	0.14	0.21	2.84	4.13
72	0.15	0.12	1.36	2.75
96	0.09	0.27	0.70	2.08
120	0.04	0.09	1.17	1.72
144	0.07	0.05	0.41	1.10
168	0.05	0.11	0.29	0.82

Results are expressed as mg dimethoate equivalents/l
See Appendix 13, Tables 21 - 24 for data from individual animals

TABLE 10

Pharmacokinetic parameters of plasma radioactivity

Dose level (mg/kg bodyweight)	Sex	C_{max} (mg equiv/l)	T_{max} (hours)	AUC (mg equiv.hr/l)	Terminal half-life[a] (hours)
10	Male	8.62	0.5	49.4	42.0
10	Female	7.68	0.5	48.9	59.3
100	Male	50.7	0.25	417.0	36.1
100	Female	93.2	0.5	686.6	46.4

AUC area under the curve
Parameters were derived from meaned data shown in Table 9
[a] calculated using the 12 - 168 hours data

TABLE 11

Concentrations of dimethoate in plasma following a single oral dose at a nominal level of 100 mg/kg bodyweight

Males

Time (hours)	Animal numbers	Mean total radioactivity concentration in plasma (mg equiv/l)	Proportion of plasma radioactivity associated with dimethoate (%)	Plasma dimethoate (mg/l)
0.5	97 - 99♂	70.0	8.2	5.7
2	103 - 105♂	13.2	6.6	0.87
6	109 - 111♂	11.1	6.7	0.74
24	115 - 117♂	1.93	a	<0.051

Females

Time (hours)	Animal numbers	Mean total radioactivity concentration in plasma (mg equiv/l)	Proportion of plasma radioactivity associated with dimethoate (%)	Plasma dimethoate (mg/l)
0.5	100 - 102♀	67.7	11.0	7.4
2	106 - 108♀	12.5	11.4	1.43
6	112 - 114♀	13.3	14.0	1.86
24	118 - 120♀	1.90	a	<0.051

a less than the limit of detection (equivalent to a gross dpm of twice the background dpm) in a single fraction of column eluate (Appendix 11)

TABLE 12

Mean tissue concentrations of radioactivity following a single oral dose at a nominal level of 10 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	161 - 163♂	164 - 166♀	167 - 169♂	170 - 172♀	173 - 175♂	176 - 178♀
Adrenal glands	2.22	4.23	1.20	1.83	0.17	0.24
Bone	1.12	1.39	0.64	0.98	0.08	0.13
Bone marrow	1.61	3.04	0.86	1.31	0.19	0.26
Brain	0.65	1.36	0.39	0.69	0.04	0.05
Fat	0.99	1.10	1.16	0.59	0.07	0.06
Heart	2.60	3.47	1.26	1.75	0.10	0.12
Intestines and contents	6.20	6.71	4.73	6.70	0.18	0.81
Kidneys	20.0	24.6	7.22	7.91	0.30	0.35
Liver	8.57	11.7	6.11	7.53	0.53	0.63
Lungs	3.28	5.47	1.73	2.58	0.16	0.20
Muscle	1.19	1.89	0.60	0.88	0.07	0.08
Ovaries	-	3.77	-	1.64	-	0.17
Pancreas	2.94	4.33	1.83	2.08	0.28	0.45
Skin	2.24	3.56	1.02	1.53	0.14	0.29
Spleen	1.76	2.93	0.93	1.35	0.17	0.18
Stomach and contents	146	178	83.0	82.0	0.08	0.25
Testes	1.72	-	1.14	-	0.08	-
Thyroid gland	1.99	3.31	0.94	1.64	0.15	0.27
Uterus	-	3.52	-	1.73	-	0.17
Whole-blood	4.70	6.08	1.63	2.08	0.10	0.13
Plasma	6.09	7.81	2.20	2.65	0.08	0.12

Results are expressed as mg dimethoate equivalents/kg
See Appendix 13, Tables 25 - 27 for data from individual animals

TABLE 13

Mean quantities of radioactivity in tissues and remaining carcasses following a single oral dose at a nominal level of 10 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	161 - 163♂	164 - 166♀	167 - 169♂	170 - 172♀	173 - 175♂	176 - 178♀
GIT	54.2	46.8	28.1	14.6	0.18	0.67
Kidneys	1.70	1.87	0.58	0.61	0.03	0.03
Liver	4.25	5.46	2.96	3.41	0.31	0.29
Whole-blood[a]	3.29	4.29	1.14	1.46	0.08	0.09
Plasma[a]	2.44	3.15	0.88	1.06	0.04	0.05
Other tissues	0.68	0.83	0.41	0.40	0.04	0.03
Residual carcass[b]	11.9	17.9	8.18	10.7	0.90	3.32
Total[c]	74.7	75.2	40.8	30.3	1.50	4.40

Results are expressed as % dose

GIT gastrointestinal tract and contents

a calculated on the basis that whole-blood and plasma accounted for 7% and 4% respectively of rat bodyweight (International Commission on Radiological Protection, Report of Committee 2 (1959))

b the residual carcass contained some whole-blood

c total of GIT, kidneys, liver, whole-blood, other tissues and residual carcass. This value takes into account the whole-blood remaining within the residual carcass

TABLE 14

Mean ratios of tissue to plasma concentrations of radioactivity following a single oral dose at a nominal level of 10 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	161 - 163♂	164 - 166♀	167 - 169♂	170 - 172♀	173 - 175♂	176 - 178♀
Adrenal glands	0.37	0.54	0.57	0.68	2.08	2.07
Bone	0.18	0.18	0.29	0.36	1.01	1.13
Bone marrow	0.26	0.39	0.39	0.50	2.35	2.26
Brain	0.11	0.17	0.18	0.26	0.47	0.46
Fat	0.16	0.14	0.59	0.22	0.79	0.55
Heart	0.43	0.44	0.58	0.67	1.28	1.07
Intestines and contents	1.02	0.86	2.19	2.56	2.17	6.94
Kidneys	3.26	3.14	3.37	2.96	3.67	3.03
Liver	1.40	1.50	2.79	2.89	6.54	5.49
Lungs	0.53	0.70	0.79	0.97	1.94	1.71
Muscle	0.19	0.24	0.27	0.34	0.84	0.70
Ovaries	-	0.48	-	0.62	-	1.49
Pancreas	0.48	0.56	0.87	0.79	3.42	3.81
Skin	0.37	0.45	0.47	0.59	1.67	2.65
Spleen	0.29	0.37	0.43	0.52	2.04	1.58
Stomach and contents	24.1	23.0	38.3	32.7	0.97	2.21
Testes	0.28	-	0.54	-	1.04	-
Thyroid gland	0.33	0.42	0.43	0.60	1.78	2.33
Uterus	-	0.45	-	0.66	-	1.42
Whole-blood	0.77	0.78	0.74	0.79	1.22	1.13
Plasma	1.00	1.00	1.00	1.00	1.00	1.00

See Appendix 13, Tables 28 - 30 for data from individual animals

TABLE 15

Mean tissue concentrations of radioactivity following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	179 - 181♂	182 - 184♀	185 - 187♂	188 - 190♀	191 - 193♂	194 - 196♀
Adrenal glands	3.70	6.34	3.50	2.89	0.81	1.21
Bone	1.84	2.27	1.63	1.10	0.32	0.41
Bone marrow	2.83	4.29	2.92	2.22	0.70	1.03
Brain	1.48	2.25	1.86	1.11	0.27	0.32
Fat	1.56	1.59	1.29	0.76	0.46	0.33
Heart	3.66	4.70	3.11	2.09	0.57	0.71
Intestines and contents	9.39	10.8	7.54	8.45	0.84	1.71
Kidneys	21.0	28.1	18.8	8.03	1.28	1.48
Liver	12.8	13.2	13.4	8.45	2.05	2.43
Lungs	4.94	7.00	4.66	3.07	0.77	0.97
Muscle	1.94	2.89	1.91	1.23	0.43	0.45
Ovaries	-	5.13	-	2.19	-	0.80
Pancreas	5.36	7.43	5.09	4.74	2.11	2.85
Skin	3.09	4.89	2.74	2.34	0.57	0.71
Spleen	3.04	4.41	3.19	2.30	0.70	0.90
Stomach and contents	250	240	84.4	67.2	0.83	1.39
Testes	2.62	-	3.27	-	0.49	-
Thyroid gland	3.48	4.63	3.41	2.68	0.83	1.21
Uterus	-	5.10	-	2.21	-	0.63
Whole-blood	5.70	7.47	4.16	2.44	0.67	0.85
Plasma	6.54	8.96	4.36	2.55	0.34	0.44

Results are expressed as mg dimethoate equivalents/kg
See Appendix 13, Tables 31 - 33 for data from individual animals

TABLE 16

Mean ratios of tissue to plasma concentrations of radioactivity following the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	179 - 181♂	182 - 184♀	185 - 187♂	188 - 190♀	191 - 193♂	194 - 196♀
Adrenal glands	0.61	0.72	0.82	1.13	2.36	2.74
Bone	0.29	0.25	0.39	0.43	0.93	0.94
Bone marrow	0.45	0.48	0.69	0.87	2.03	2.36
Brain	0.25	0.25	0.42	0.44	0.79	0.73
Fat	0.25	0.18	0.32	0.30	1.32	0.75
Heart	0.58	0.53	0.73	0.82	1.67	1.62
Intestines and contents	1.59	1.19	1.92	3.32	2.42	3.89
Kidneys	3.21	3.12	4.10	3.12	3.74	3.35
Liver	2.00	1.49	3.23	3.33	5.99	5.50
Lungs	0.78	0.79	1.14	1.20	2.25	2.20
Muscle	0.31	0.33	0.45	0.48	1.26	1.02
Ovaries	-	0.58	-	0.86	-	1.81
Pancreas	0.86	0.84	1.27	1.88	6.18	6.49
Skin	0.49	0.55	0.65	0.92	1.67	1.62
Spleen	0.49	0.50	0.75	0.90	2.03	2.04
Stomach and contents	40.8	26.5	26.3	26.1	2.44	3.17
Testes	0.43	-	0.77	-	1.44	-
Thyroid gland	0.55	0.52	0.78	1.04	2.43	2.74
Uterus	-	0.57	-	0.86	-	1.43
Whole-blood	0.88	0.84	0.95	0.95	1.95	1.93
Plasma	1.00	1.00	1.00	1.00	1.00	1.00

See Appendix 13, Tables 34 - 36 for data from individual animals

TABLE 17

Mean tissue concentrations of radioactivity following a single oral dose at a nominal level of 100 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	143 - 145♂	146 - 148♀	149 - 151♂	152 - 154♀	155 - 157♂	158 - 160♀
Adrenal glands	39.8	33.6	67.0	17.1	2.39	2.99
Bone	11.3	11.9	3.56	5.35	0.87	0.97
Bone marrow	19.8	22.2	6.10	10.7	2.15	3.01
Brain	11.0	14.6	3.94	7.25	0.51	0.58
Fat	58.6	8.32	17.9	4.12	0.92	0.70
Heart	25.5	27.2	7.28	12.3	1.12	1.31
Intestines and contents	43.8	74.5	36.2	51.7	1.89	3.97
Kidneys	161	127	205	47.9	2.68	3.44
Liver	73.0	59.4	31.0	37.6	4.46	5.12
Lungs	31.9	35.6	11.8	15.5	1.37	1.80
Muscle	13.7	16.0	3.91	7.10	0.79	0.85
Ovaries	-	29.5	-	13.5	-	1.67
Pancreas	29.5	27.3	30.7	19.3	7.51	7.79
Skin	22.1	28.0	6.25	10.3	1.49	1.18
Spleen	18.7	21.6	11.2	10.9	1.61	1.72
Stomach and contents	1130	2170	879	873	1.32	2.85
Testes	21.9	-	7.79	-	0.92	-
Thyroid gland	26.6	23.0	36.4	14.7	2.00	2.66
Uterus	-	29.4	-	11.8	-	2.09
Whole-blood	39.1	39.9	7.11	12.7	1.19	1.52
Plasma	55.7	57.2	9.90	17.9	0.84	1.25

Results are expressed as mg dimethoate equivalents/kg
See Appendix 13, Tables 37 - 39 for data from individual animals

TABLE 18

Mean quantities of radioactivity in tissues and remaining carcasses following a single oral dose at a nominal level of 100 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	143 - 145♂	146 - 148♀	149 - 151♂	152 - 154♀	155 - 157♂	158 - 160♀
GIT	58.2	53.1	43.0	27.1	0.20	0.28
Kidneys	1.29	0.91	1.59	0.35	0.02	0.02
Liver	3.65	2.64	1.39	1.60	0.24	0.20
Whole-blood[a]	2.77	2.77	0.50	0.88	0.09	0.10
Plasma[a]	2.26	2.27	0.40	0.71	0.04	0.05
Other tissues	0.74	0.63	0.35	0.32	0.06	0.05
Residual carcass[b]	12.2	13.0	5.22	8.83	1.16	1.87
Total[c]	77.7	71.7	51.8	38.6	1.73	2.48

Results are expressed as % dose

GIT gastrointestinal tract and contents

a calculated on the basis that whole-blood and plasma accounted for 7% and 4% respectively of rat bodyweight (International Commission on Radiological Protection, Report of Committee 2 (1959))

b the residual carcass contained some whole-blood

c total of GIT, kidneys, liver, whole-blood, other tissues and residual carcass. This value takes into account the whole-blood remaining within the residual carcass

TABLE 19

Mean ratios of tissue to plasma concentrations of radioactivity following a single oral dose at a nominal level of 100 mg/kg bodyweight

Sacrifice time	0.5 hours		2 hours		48 hours	
Animal numbers	143 - 145♂	146 - 148♀	149 - 151♂	152 - 154♀	155 - 157♂	158 - 160♀
Adrenal glands	0.74	0.59	6.31	0.96	2.84	2.37
Bone	0.21	0.21	0.36	0.30	1.03	0.77
Bone marrow	0.37	0.39	0.62	0.61	2.55	2.39
Brain	0.21	0.25	0.40	0.41	0.60	0.46
Fat	1.07	0.15	1.67	0.23	1.08	0.56
Heart	0.46	0.47	0.74	0.69	1.32	1.04
Intestines and contents	0.89	1.30	3.95	2.87	2.24	3.11
Kidneys	2.89	2.22	19.2	2.69	3.17	2.74
Liver	1.37	1.03	3.19	2.12	5.30	4.09
Lungs	0.59	0.62	1.18	0.86	1.63	1.43
Muscle	0.26	0.28	0.40	0.40	0.93	0.67
Ovaries	-	0.51	-	0.75	-	1.33
Pancreas	0.55	0.48	3.07	1.08	8.70	6.14
Skin	0.41	0.49	0.64	0.57	1.76	0.94
Spleen	0.35	0.38	1.10	0.61	1.90	1.37
Stomach and contents	22.8	38.1	94.4	54.1	1.58	2.31
Testes	0.40	-	0.79	-	1.09	-
Thyroid gland	0.49	0.40	3.16	0.77	2.36	2.17
Uterus	-	0.51	-	0.65	-	1.68
Whole-blood	0.71	0.70	0.72	0.71	1.40	1.21
Plasma	1.00	1.00	1.00	1.00	1.00	1.00

See Appendix 13, Tables 40 - 42 for data from individual animals

TABLE 20

Mean tissue concentrations of radioactivity in rats sacrificed 120 hours following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		10 mg/kg bodyweight[a]		100 mg/kg bodyweight	
Animal numbers	201 - 205♂	206 - 210♀	21 - 25♂	26 - 30♀	1, 3, 4, 5, 198♂	6, 8, 9, 199, 200♀
Adrenal glands	0.11	0.12	0.20	0.24	2.03	2.01
Bone	0.07	0.07	0.05	0.05	0.41	0.37
Bone marrow	0.07	0.10	0.20	0.16	2.97	1.64
Brain	0.04	0.05	0.06	0.07	0.41	0.50
Fat	0.06	0.08	0.03	0.04	0.72	0.48
Heart	0.08	0.10	0.11	0.13	0.83	1.07
Intestines and contents	0.04	0.07	0.07	0.10	0.47	0.70
Kidneys	0.15	0.19	0.26	0.26	1.60	1.75
Liver	0.24	0.28	0.34	0.34	2.16	2.11
Lungs	0.10	0.14	0.15	0.17	0.91	1.19
Muscle	0.06	0.07	0.08	0.08	0.65	0.68
Ovaries	-	0.08	-	0.14	-	1.03
Pancreas	0.19	0.26	0.19	0.37	5.15	6.63
Skin	0.09	0.11	0.11	0.44	0.77	0.82
Spleen	0.08	0.10	0.14	0.16	0.87	1.17
Stomach and contents	0.03	0.09	0.14	0.14	0.30	0.49
Testes	0.06	-	0.10	-	0.63	-
Thyroid gland	<0.14	0.13	0.18	0.23	5.18	1.99
Uterus	-	0.11	-	0.12	-	0.76
Whole-blood	0.06	0.09	0.11	0.14	0.79	1.19
Plasma	0.03	0.04	0.06	0.07	0.36	0.52

Results are expressed as mg dimethoate equivalents/kg

[a] rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

See Appendix 13, Tables 43 - 48 for data from individual animals

TABLE 21

Mean tissue concentrations of radioactivity in rats sacrificed 120 hours following a single intravenous dose at a nominal level of 10 mg/kg bodyweight

Animal numbers	31 - 35♂	36 - 40♀
Adrenal glands	0.11	0.11
Bone	0.03	0.02
Bone marrow	0.06	0.07
Brain	0.03	0.03
Fat	0.04	0.03
Heart	0.06	0.07
Intestines and contents	0.03	0.07
Kidneys	0.13	0.13
Liver	0.20	0.20
Lungs	0.08	0.09
Muscle	0.05	0.05
Ovaries	-	0.07
Pancreas	0.17	0.29
Skin	0.07	0.06
Spleen	0.06	0.07
Stomach and contents	0.03	0.07
Testes	0.05	-
Thyroid gland	<0.12	<0.11
Uterus	-	0.06
Whole-blood	0.06	0.07
Plasma	0.03	0.04

Results are expressed as mg dimethoate equivalents/kg
See Appendix 13, Tables 49 - 50 for data from individual animals

TABLE 22

Mean tissue concentrations of radioactivity in rats sacrificed 120 hours following a single dermal dose at nominal levels of 10 and 100 mg/kg bodyweight

Dose level	10 mg/kg bodyweight		100 mg/kg bodyweight	
Animal numbers	41 - 45♂	46 - 50♀	R51 - R55♂	R56 - R60♀
Adrenal glands	<0.09	<0.03	<0.31	<0.16
Bone	<0.02	0.02	<0.10	<0.10
Bone marrow	<0.05	<0.07	<0.59	<0.63
Brain	<0.01	0.01	<0.06	0.06
Fat	<0.02	<0.02	<0.09	<0.10
Heart	<0.01	<0.01	<0.07	<0.08
Intestines and contents	0.01	0.03	<0.07	0.13
Kidneys	0.01	0.02	<0.07	<0.07
Liver	0.02	0.03	<0.07	0.08
Lungs	0.01	0.02	<0.07	<0.07
Muscle	0.01	0.02	<0.05	<0.05
Ovaries	-	<0.02	-	<0.10
Pancreas	0.02	0.03	<0.07	0.08
Skin	0.02	0.12	0.20	0.30
Spleen	<0.02	<0.02	<0.08	<0.09
Stomach and contents	<0.02	0.04	<0.08	<0.08
Testes	<0.01	-	<0.05	-
Thyroid gland	<0.15	<0.14	<0.88	<0.74
Uterus	-	<0.02	-	<0.12
Whole-blood	0.01	0.01	<0.03	<0.03
Plasma	0.01	0.01	<0.02	<0.02

Results are expressed as mg dimethoate equivalents/kg
See Appendix 13, Tables 51 - 54 for data from individual animals

TABLE 23

Proportions of radioactive components in urine following single oral, intravenous and dermal doses

Component[b]	Oral		Oral		Oral		Intravenous		Dermal		Dermal	
	10 mg/kg bodyweight		10 mg/kg bodyweight[a]		100 mg/kg bodyweight		10 mg/kg bodyweight		10 mg/kg bodyweight		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
U1 (6)	5.2	5.0	6.5	6.0	4.8	3.8	4.3	3.9	0.6	0.5		0.1
Omethoate (ref II) (10)	1.5	2.5	3.2	5.6	3.7	3.7	1.3	1.8	0.2	0.2	0.1	<0.1
U2a (13)	0.3	0.2	0.3	0.3	0.4	0.3	d	d	d	d		<0.1
U3 (16)	4.1	4.0	4.2	3.6	2.2	2.0	3.7	3.7	0.3	0.3	<0.1	<0.1
Ref XVI (19)	8.3	5.7	10.8	7.3	8.7	4.7	6.5	4.0	0.8	0.6	0.1	0.1
U5 (28)	0.9	0.7	1.2	1.2	1.0	1.3	0.9	0.7	0.2	<0.1	<0.1	<0.1
U6 (30)	2.5	2.1	2.1	1.5	2.3	1.9	3.7	1.8		0.1	<0.1	
Ref XV (31)	26.6	25.2	29.7	27.4	20.3	22.1	22.5	24.1	2.9	3.2	0.3	0.4
Dimethoate (34)	1.4	0.7	0.9	0.6	0.7	2.0	0.4	0.5	0.1	0.1	<0.1	<0.1
Ref III (42)	37.8	35.1	29.1	31.0	43.2	44.4	42.7	45.7	2.5	2.8	0.3	0.3
Others[c]	2.3	1.9	2.2	2.1	2.3	2.7	1.7	1.7	0.2	0.2	0.2	0.1

Results are expressed as % dose

0 - 48 hours pooled samples were analysed

Components were resolved and quantified using HPLC method 1

For proportions in the urine of dermally-dosed rats, expressed as % absorbed dose, see Table 24

a rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

b shown in parentheses are typical retention times (in minutes)

c regions of the chromatogram containing no discrete radioactive components

d included in 'others'

TABLE 24

Proportions of radioactive components in urine following a single dermal dose at nominal levels of 10 and 100 mg/kg bodyweight

Component[a]	10 mg/kg bodyweight		100 mg/kg bodyweight	
	Male	Female	Male	Female
U1 (6)	6.4	5.1	6.4	7.2
Omethoate (ref II) (10)	1.9	1.9		1.8
U2a (13)	c	c		0.6
U3 (16)	3.3	2.6	2.8	2.9
Ref XVI (19)	8.3	5.6	6.0	5.6
U5 (28)	1.7	0.2	1.8	1.1
U6 (30)		0.5	2.4	
Ref XV (31)	31.5	30.3	23.7	24.0
Dimethoate (34)	1.3	0.8	0.6	0.9
Ref III (42)	27.2	26.7	25.1	19.2
Others[b]	2.0	1.7	16.8	3.3

Results are expressed as % absorbed dose (from Table 5)
0 - 48 hours pooled samples were analysed
Components were resolved and quantified using HPLC method 1

a shown in parentheses are typical retention times (in minutes)
b regions of the chromatogram containing no discrete radioactive components
c included in 'others'

TABLE 25

Proportions of radioactive components in bile following a single oral dose at nominal levels of 10 and 100 mg/kg bodyweight

Component[a]	10 mg/kg bodyweight		100 mg/kg bodyweight	
	Male	Female	Male	Female
B1 (4)	0.8	0.6	0.3	0.7
B2 (6)				
B3 (7)	0.1	<0.1	0.1	0.2
B4 (8)				
B5 (10)	0.2	0.4	0.4	0.7
B6 (11)				
B7 (15)	0.1	0.1	<0.1	0.1
B8 (17)	0.3	0.2	0.2	0.2
B9 (19)	<0.1		<0.1	0.1
Ref XV (31)	0.3	0.3	0.2	0.4
Dimethoate (34)	0.1	0.1	0.1	0.2
Ref III (42)	1.4	1.4	1.1	1.9
B13 (45)	0.1	0.1	0.1	0.1
Others[b]	0.2	0.1	0.2	0.2

Results are expressed as % dose
0 - 12 hours pooled samples were analysed
Components were resolved and quantified using HPLC method 1

a shown in parentheses are typical retention times (in minutes)

b regions of the chromatogram containing no discrete radioactive components

TABLE 26

Proportions of radioactive components in plasma following a single oral dose at a nominal level of 100 mg/kg bodyweight

Component[a]	Male			Female		
	0.5 hours	2 hours	6 hours	0.5 hours	2 hours	6 hours
P1/Pla (3-5)	4.3	3.1	2.7	2.6	2.0	3.0
P2 (5)	1.7	2.0	0.7	1.6	1.5	0.8
P3 (10)	1.2	0.4	0.2	1.4	0.4	0.3
P4 (19)	4.3	1.0	0.6	2.2	0.6	0.6
P5 (28)	0.6	0.1	0.1	0.9	0.1	0.2
P6 (30)	2.0	0.1	0.1	1.8	0.2	0.3
Ref XV (31)	12.0	2.7	1.9	13.9	3.3	2.7
Dimethoate (34)	5.7	0.9	0.7	7.4	1.4	1.9
Ref III (42)	35.8	2.1	3.1	32.7	2.4	3.8
Others[b]	2.3	0.8	0.9	3.2	0.7	<0.1

Results are expressed as mg dimethoate equivalents/l

Pooled samples from experiment 3c (plasma parent compound kinetics) were analysed: 24-hour samples contained radioactivity in insufficient quantities for accurate quantification of metabolites

Components were resolved and quantified using HPLC method 1

a shown in parentheses are typical retention times (in minutes)

b regions of the chromatogram containing no discrete radioactive components

TABLE 27

Proportions of tissue radioactivity in extracts analysed by TLC

Tissue	10 mg/kg bodyweight (single dose)		10 mg/kg bodyweight (multiple dose)		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female
Kidneys	94.6	93.1	77.0	82.8	95.0	96.4
Liver	87.4	85.9	73.8	72.8	89.8	86.9

Results are expressed as % tissue radioactivity
Tissues from the 0.5 hours sacrifice were analysed in all cases with the exception of male kidneys at 100 mg/kg bodyweight which were taken from the 2 hours sacrifice

TABLE 28

Proportions of radioactive components in kidneys following single and multiple oral doses

Expressed as % kidney radioactivity

Component[a]	10 mg/kg bodyweight (single dose)		10 mg/kg bodyweight (multiple dose)		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female
K1 (0.03)	3.0	2.3	2.7	2.3	4.0	4.7
K2 (0.07)	2.0	1.5			1.1	2.0
K3 (0.11)	6.0	7.5	7.1	6.6	9.3	5.5
K4 (0.15)	2.9	3.0	2.7	0.6	1.6	3.2
K5 (0.19)	2.7	3.3			2.7	2.3
K6 (0.22)	1.3	1.6	0.4		0.9	0.7
Ref XVI (0.25)	7.9	24.1	14.4	13.1	10.2	7.7
Ref III (0.32)	15.1		8.7	18.4	48.0	35.0
K9 (0.47)	3.3	3.5	d	d	1.7	3.5
Ref XV (0.69)	40.1	40.8	16.0	34.4	9.8	21.5
Dimethoate (0.76)	7.3	2.2	20.2	4.1	2.3	6.4
Others[b]	2.9	3.3	4.8	3.3	3.3	4.0
Not extracted	5.4	6.9	23.0	17.2	5.0	3.6

Expressed as % dose

Component[a]	10 mg/kg bodyweight (single dose)		10 mg/kg bodyweight (multiple dose)[c]		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female
K1 (0.03)	0.05	0.04	0.05	0.05	0.06	0.04
K2 (0.07)	0.03	0.03			0.02	0.02
K3 (0.11)	0.10	0.14	0.12	0.13	0.15	0.05
K4 (0.15)	0.05	0.06	0.05	0.01	0.03	0.03
K5 (0.19)	0.05	0.06			0.04	0.02
K6 (0.22)	0.02	0.03	0.01		0.01	0.01
Ref XVI (0.25)	0.14	0.45	0.24	0.26	0.16	0.07
Ref III (0.32)	0.26		0.14	0.37	0.76	0.32
K9 (0.47)	0.06	0.06	d	d	0.03	0.03
Ref XV (0.69)	0.68	0.76	0.27	0.69	0.16	0.20
Dimethoate (0.76)	0.12	0.04	0.34	0.08	0.04	0.06
Others[b]	0.05	0.06	0.08	0.07	0.05	0.04
Not extracted	0.09	0.12	0.29	0.29	0.08	0.03

Components were resolved and quantified using TLC system D

a shown in parentheses are typical R_f values

b regions of the chromatogram containing no discrete radioactive components

c results expressed as % Day 7 dose

d not apparent; included in 'others'

TABLE 29

Proportions of radioactive components in liver following single and multiple oral doses

Expressed as % liver radioactivity

Component[a]	10 mg/kg bodyweight (single dose)		10 mg/kg bodyweight (multiple dose)		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female
L1 (0.01)	14.7	10.6	13.3	13.2	7.1	3.6
L2 (0.04)					2.5	2.9
L3 (0.06)					5.3	7.0
L4 (0.11)	23.9	29.3	21.8	17.9	23.8	15.4
L5 (0.16)	6.9	10.7	7.4	5.9	9.5	15.4
L6 (0.21)	1.7	1.0	1.9	2.1	1.8	1.9
Ref XVI (0.28)	12.1	13.9	12.1	14.6	19.2	13.2
L8 (0.43)	1.8	3.2	2.8	1.5	3.3	5.3
Ref XV (0.66)	22.3	12.9	9.5	9.3	10.8	17.0
Dimethoate (0.75)	1.5	1.5	2.8	1.2	3.8	2.4
Others[b]	2.5	2.9	2.3	7.0	2.8	2.7
Not extracted	12.6	14.1	26.2	27.2	10.2	13.1

Expressed as % dose

Component[a]	10 mg/kg bodyweight (single dose)		10 mg/kg bodyweight (multiple dose)[c]		100 mg/kg bodyweight	
	Male	Female	Male	Female	Male	Female
L1 (0.01)	0.63	0.58	0.73	0.74	0.26	0.10
L2 (0.04)					0.09	0.08
L3 (0.06)					0.19	0.19
L4 (0.11)	1.0	1.6	1.2	0.99	0.87	0.41
L5 (0.16)	0.29	0.58	0.41	0.33	0.35	0.41
L6 (0.21)	0.07	0.05	0.10	0.12	0.06	0.05
Ref XVI (0.28)	0.51	0.76	0.67	0.81	0.70	0.35
L8 (0.43)	0.08	0.18	0.15	0.08	0.12	0.14
Ref XV (0.66)	0.95	0.70	0.53	0.52	0.39	0.45
Dimethoate (0.75)	0.06	0.08	0.15	0.07	0.14	0.06
Others[b]	0.11	0.16	0.13	0.39	0.10	0.07
Not extracted	0.47	0.66	1.1	1.1	0.33	0.30

Components were resolved and quantified using TLC system D

a shown in parentheses are typical R_f values

b regions of the chromatogram containing no discrete radioactive components

c results expressed as % Day 7 dose

WHOLE-BODY AUTORADIOGRAPHS

Key to abbreviations

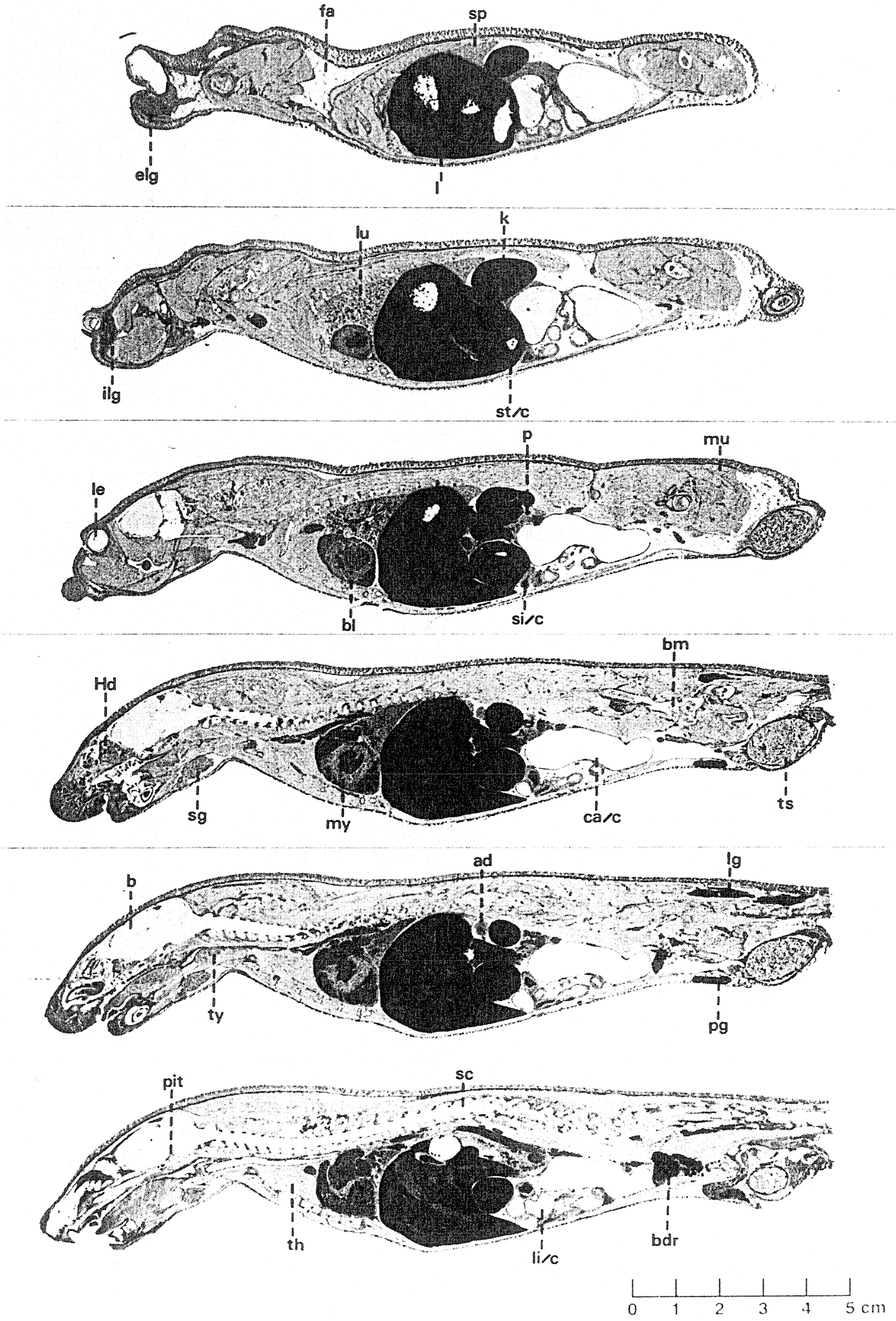
ad	adrenal gland
art	artefact
b	brain
bdr	bladder
bl	blood
bm	bone marrow
ca/c	caecum contents
elg	exorbital lachrymal gland
fa	fat
Hd	Harderian gland
ilg	intraorbital lachrymal gland
k	kidney
l	liver
le	lens
lg	ligaments
li/c	large intestine contents
lu	lung
mu	muscle
my	myocardium
p	pancreas
pg	preputial gland
pit	pituitary
sc	spinal cord
sg	salivary gland
si/c	small intestine contents
si/m	small intestine mucosa
sp	spleen
st/c	stomach contents
st/m	stomach mucosa
th	thymus
ty	thyroid
ts	testes
ut	uterus

AUTORADIOGRAPHS

Plate 1

Male rat – single oral dose

0.5 hour sacrifice

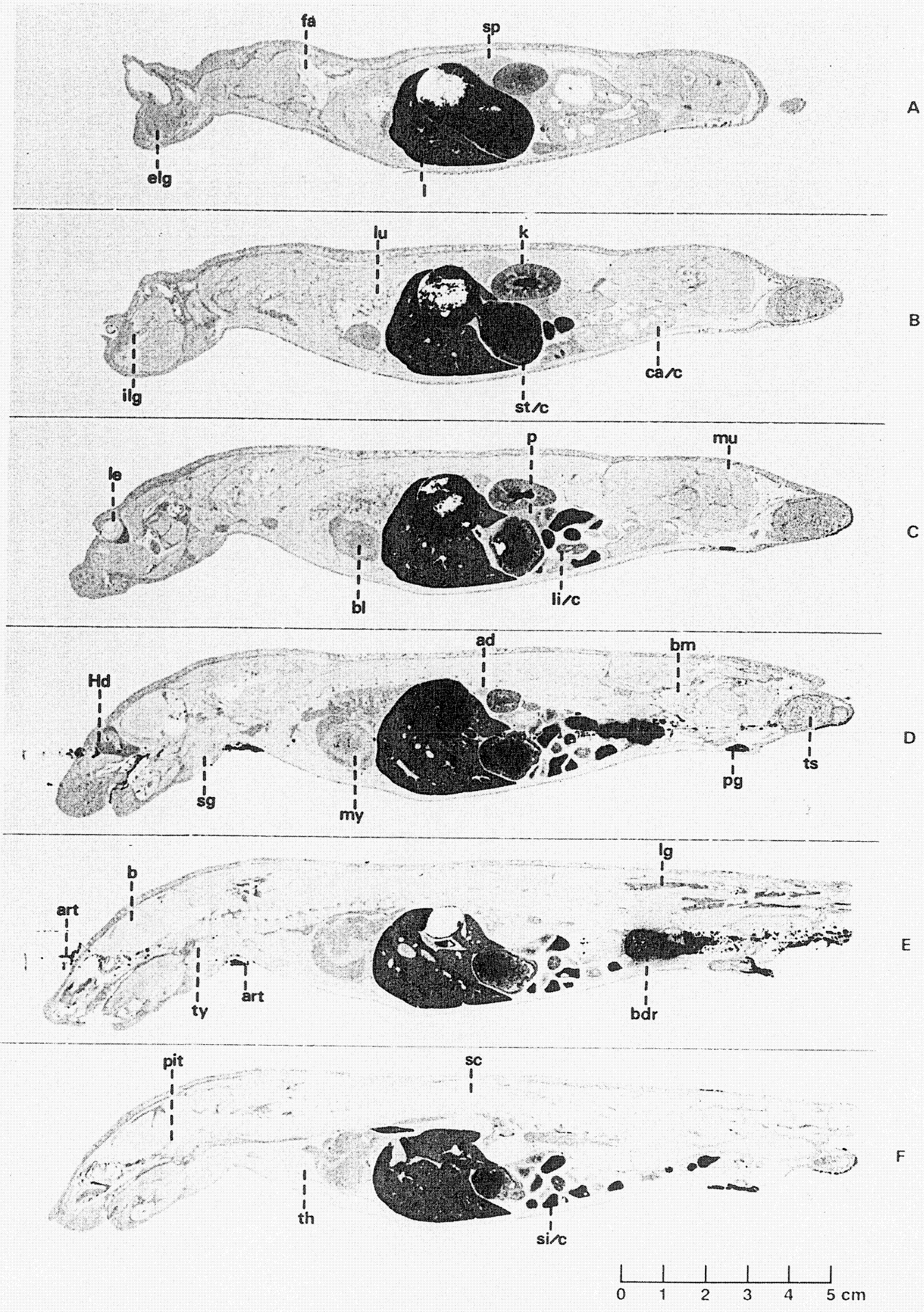


AUTORADIOGRAPHS

Plate 2

Male rat – single oral dose

2 hours sacrifice

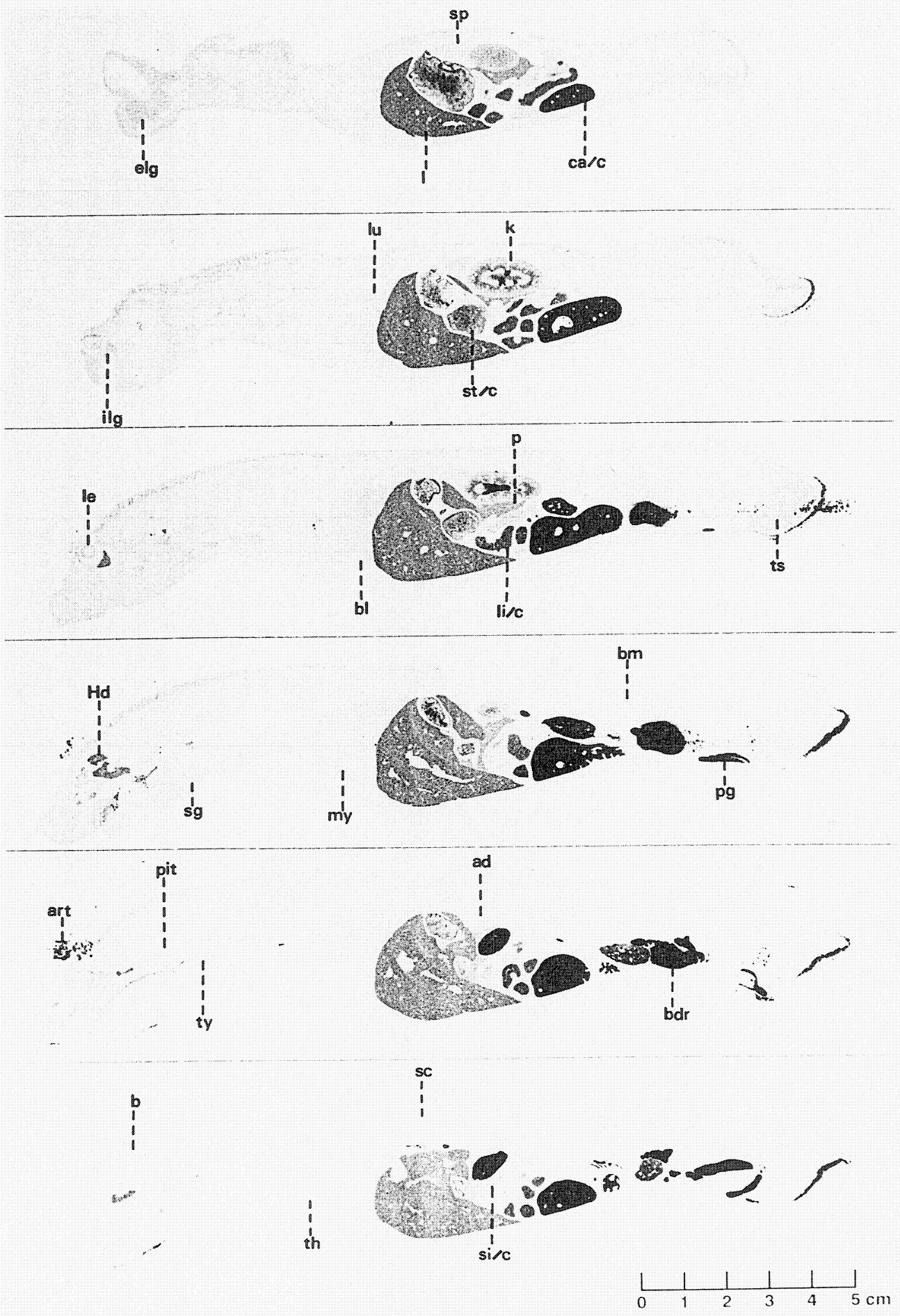


AUTORADIOGRAPHS

Plate 3

Male rat – single oral dose

6 hours sacrifice

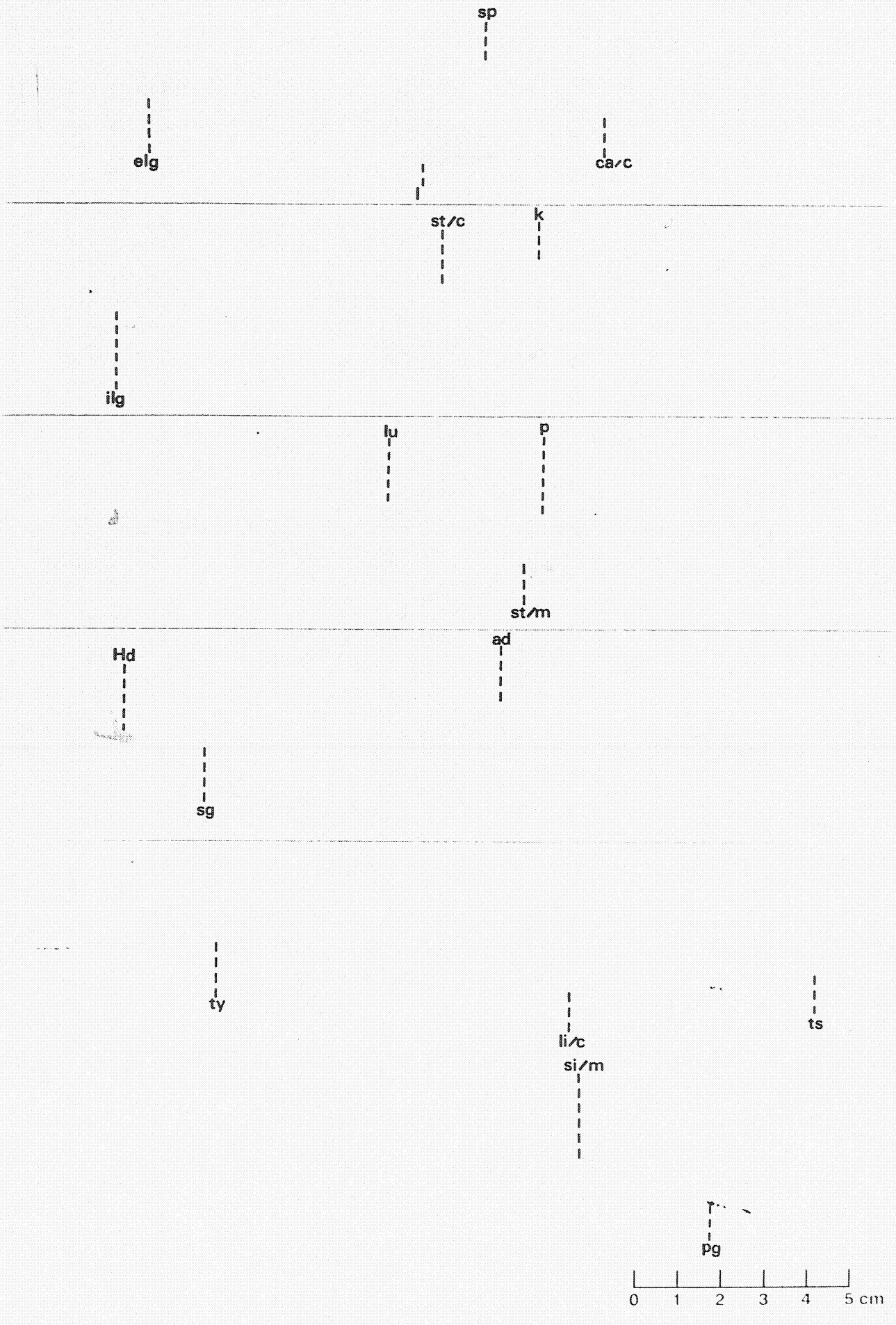


AUTORADIOGRAPHS

Plate 4

Male rat – single oral dose

48 hours sacrifice

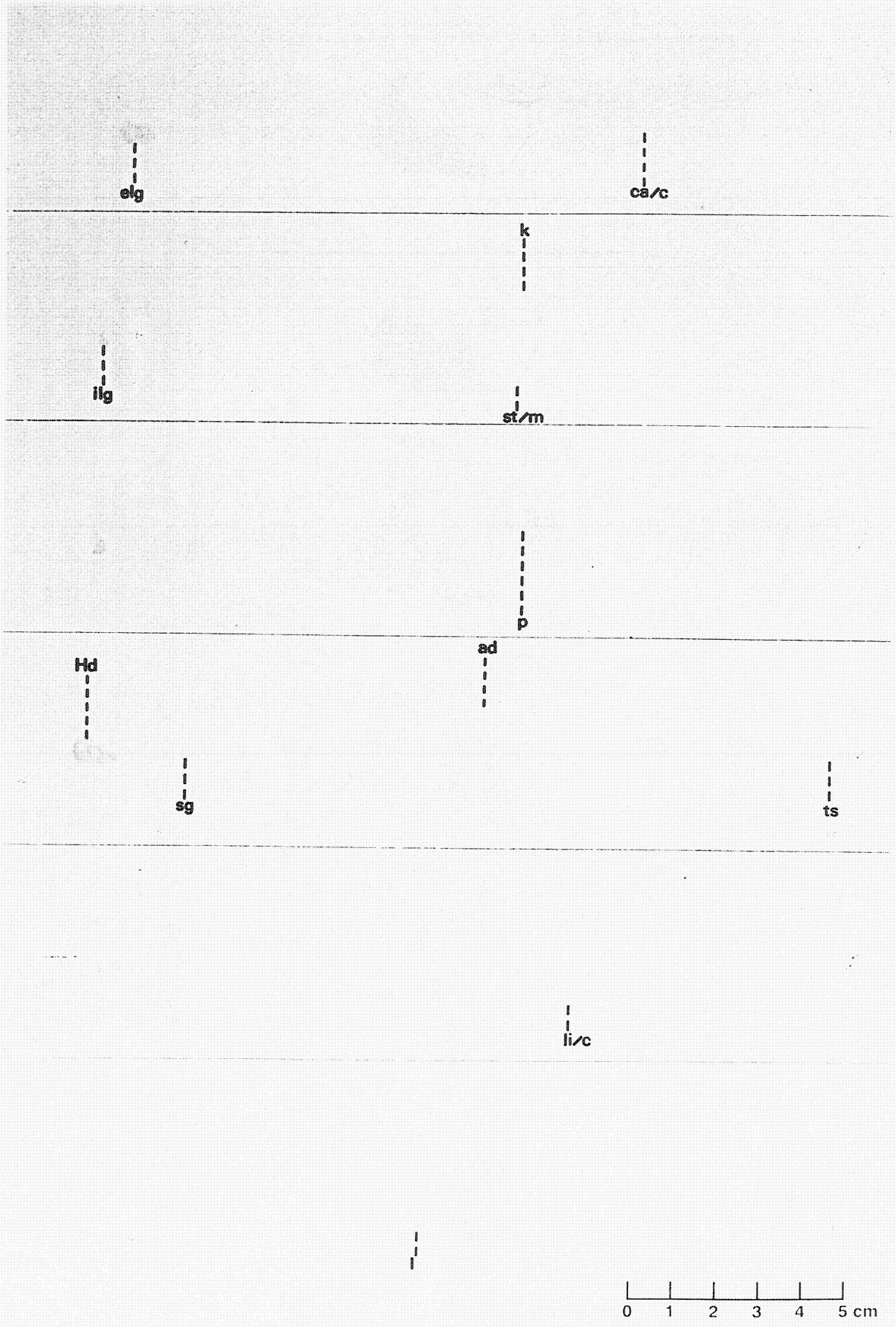


AUTORADIOGRAPHS

Plate 5

Male rat – single oral dose

120 hours sacrifice

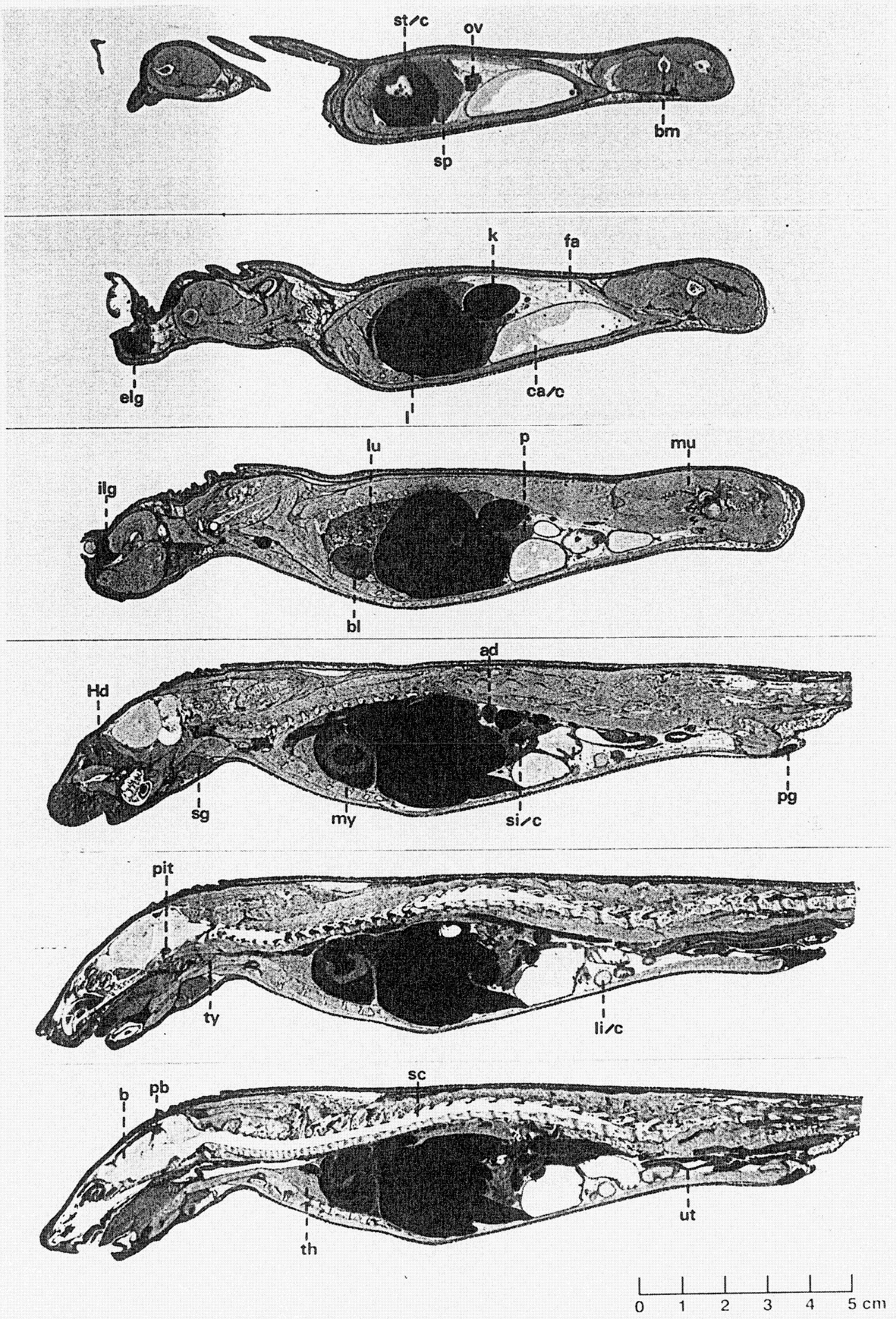


AUTORADIOGRAPHS

Plate 6

Female rat – single oral dose

0.5 hour sacrifice

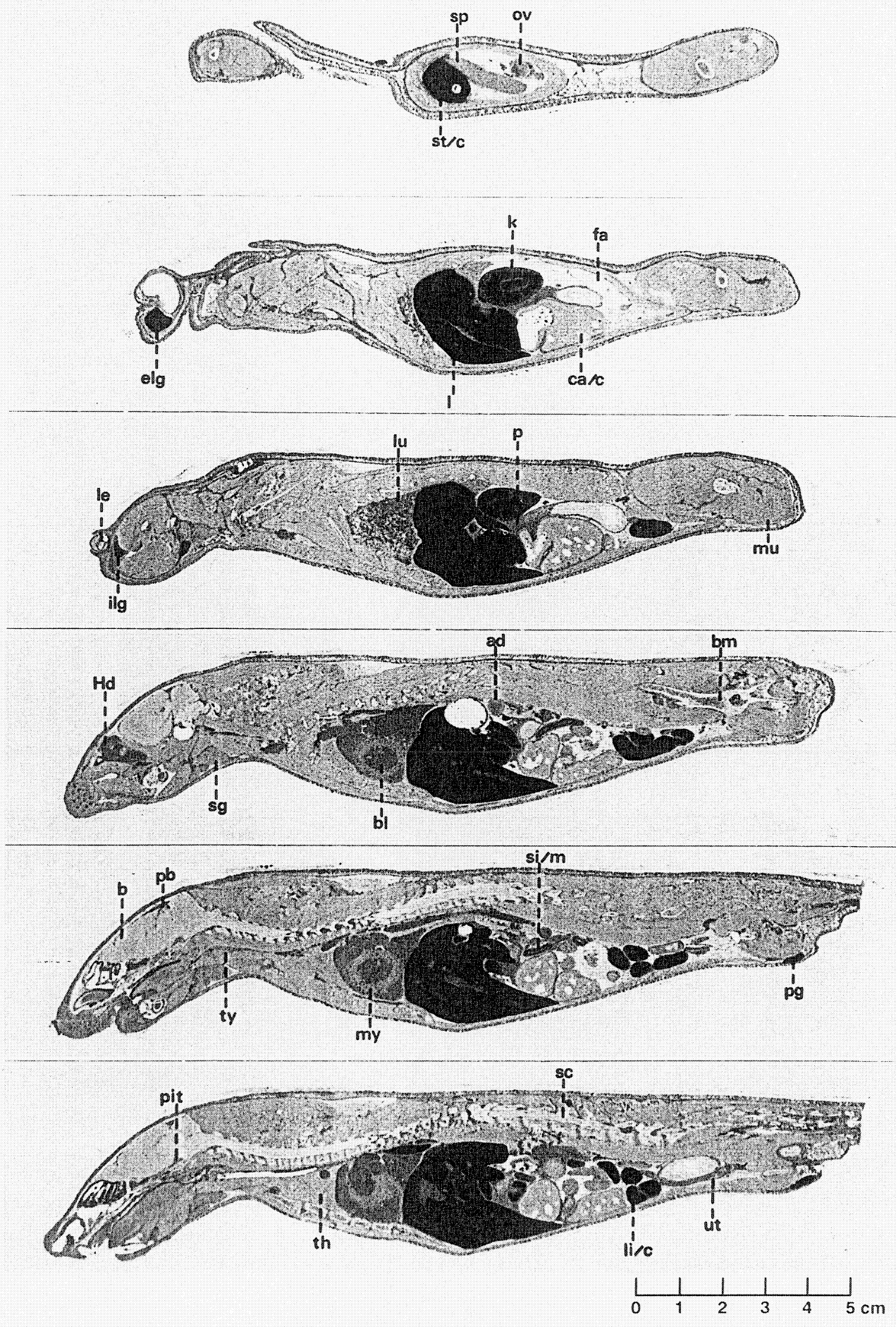


AUTORADIOGRAPHS

Plate 7

Female rat – single oral dose

2 hours sacrifice

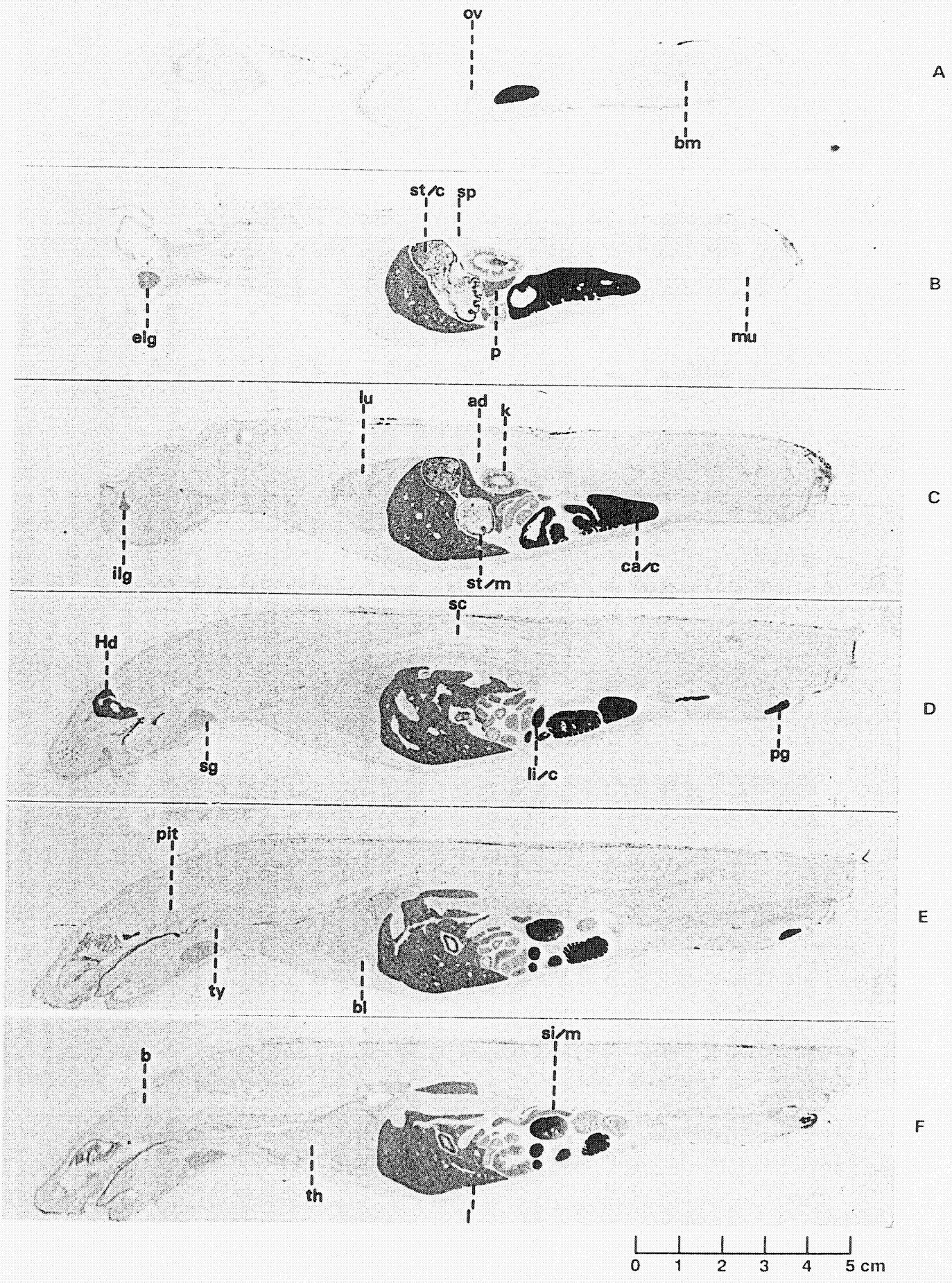


AUTORADIOGRAPHS

Plate 8

Female rat – single oral dose

6 hours sacrifice

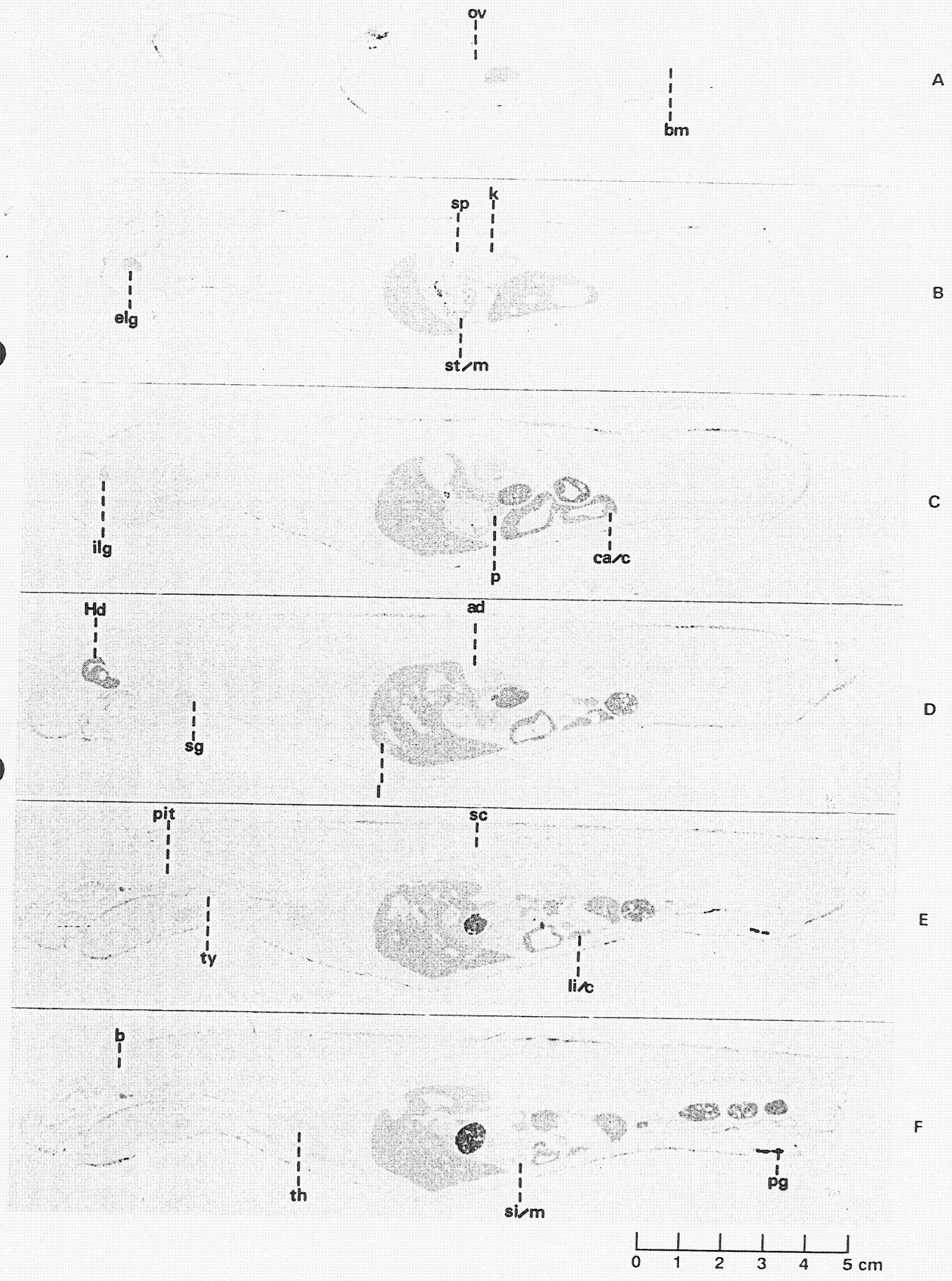


AUTORADIOGRAPHS

Plate 9

Female rat – single oral dose

48 hours sacrifice

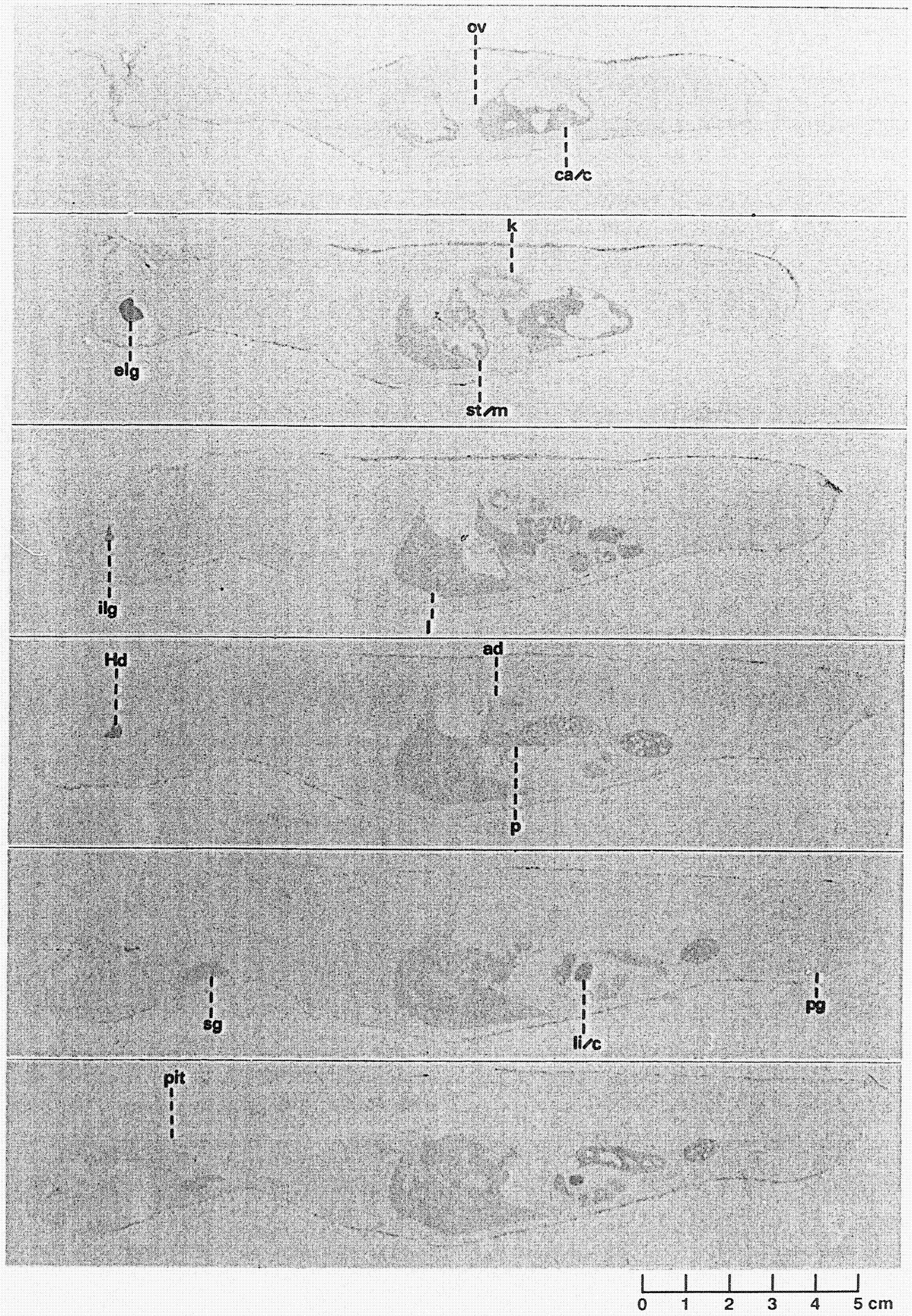


AUTORADIOGRAPHS

Plate 10

Female rat – single oral dose

120 hours sacrifice



^{14}C-DIMETHOATE

THE BIOKINETICS AND METABOLISM IN THE RAT

Volume 2

Data requirement	US EPA Subdivision F, Guideline 85-1
Project identity	DTF 16
Study completed on	18 December 1995

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited with effect from 21 November 1995

Sponsor

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Testing facility

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Study Director

Dr. D. Kirkpatrick

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TABLES (continued)

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TABLES (continued)

APPENDIX 1

Dates of dose administration (main study)

Experiment number	Date(s)
2a	21 September 1993
2b	13 October 1993, 30 November 1993
2c	30 November 1993
2d	15 December 1993[a]
2e	23 March 1994
2f	11 April 1994
2g	7 November 1994
2h	2 June 1994
3a	13 October 1993
3b	3 November 1993
3c	10 January 1994
4a	24 January 1994, 27 January 1994
4b	21 February 1994, 24 February 1994
5a	21 February 1994
5b	24 January 1994
5c	12 July 1994
5d	16 August 1994[b]

a radioactive dose
b first dose

APPENDIX 2

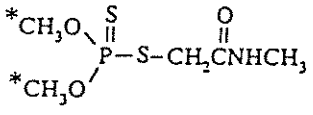
Data sheets for ^{14}C-dimethoate

Batch 1

HRC

METABOLISM AND ENVIRONMENTAL CHEMISTRY GROUP

Custom Synthesis Data Sheet

Compound:	[0-Methyl-^{14}C]Dimethoate
Structure:	
	(* Denotes position of radiolabel)
Dimethoate Task Force Batch No:	AC-DMT-SLUT
Repurified Batch No:	MR-DTF17-3
Total Radioactivity:	6.05 mCi
Specific Activity:	14.55 μCi/mg
Radiochemical purity:	99%
Method of Analysis:	Radiochemical purity determined by TLC radioscanning on pre-coated silica gel plates (Merck 5714) using the following solvent systems: Dichloromethane : methanol (93:7, v/v) Purity : 99.72%

APPENDIX 2

(continued)

Batch 1

HRC

Ethyl acetate : toluene (4:1, v/v)
Purity : 99.65%

Hexane : acetone (1:1, v/v)
Purity : 99.65%

Chemical Identity: The mass spectrum of [0-methyl-^{14}C]dimethoate was essentially identical to authentic dimethoate.

Chemical purity: No chemical impurities were detected by TLC (detection by UV at 254nm and iodine vapour) using the above solent systems.

Date of analysis: 17 September 1993

Analysed by ..... Date 21/10/93
Dr M Roberts
Radiochemist
Metabolism & Environmental Chemistry Group

Checked/Verified by M.R. Huckstep Date 19/10/93
Dr M R Huckstep
Senior Radiochemist
Metabolism & Environmental Chemistry Group

Approved by ..... Date 22/10/93
Dr D R Hawkins
Head of Group
Metabolism & Environmental
Chemistry Group

APPENDIX 2

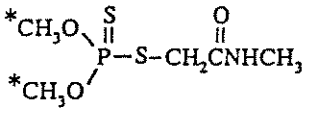
(continued)

HRC

Batch 2

METABOLISM AND ENVIRONMENTAL CHEMISTRY GROUP

Custom Synthesis Data Sheet

Compound:	[0-Methyl-^{14}C]Dimethoate
Structure:	
	(* Denotes position of radiolabel)
Dimethoate Task Force Batch No:	AC-DMT-SLUT
Repurified Batch No:	NPE-DTF17-5
Total Radioactivity:	5.83 mCi
Specific Activity:	14.55 μCi/mg (see data sheet for Batch No MR-DTF17-3)
Radiochemical purity:	99%
Method of Analysis:	Radiochemical purity determined by TLC radioscanning on pre-coated silica gel plates (Merck 5714) using the following solvent systems: Dichloromethane : methanol (93:7, v/v) Purity : 99.52%

APPENDIX 2

(continued)

HRC

Batch 2

Ethyl acetate : toluene (4:1, v/v)
Purity : 99.35%

Hexane : acetone (1:1, v/v)
Purity : 99.24%

Chemical Identity:	The mass spectrum of [0-methyl-^{14}C]dimethoate was essentially identical to authentic dimethoate.
Chemical purity:	No chemical impurities were detected by TLC (detection by UV at 254nm and iodine vapour) using the above solent systems.
Date of analysis:	24 September 1993

Analysed by ....N. Eggleton..... Date 14-10-93.....
Dr N P Eggleton
Radiochemist
Metabolism & Environmental Chemistry Group

Checked/Verified by M. R. Huckstep..... Date 14-10-93.....
Dr M R Huckstep
Senior Radiochemist
Metabolism & Environmental Chemistry Group

Approved by .....Date 15-10-93.....
Dr D R Hawkins
Head of Group
Metabolism & Environmental
Chemistry Group

APPENDIX 3

Details of receipt, storage and purity of the reference substances

Reference substance	Date of receipt	Quantity	Purity	Storage	Expiry date
II	7 December 1992	0.5 g	95.4%	-20°C	16 December 1997
III	7 December 1992	0.5 g	93.3%	-20°C	17 January 1998
VII	17 June 1993	0.5 g	97.7%	-20°C	9 June 1996
VIII	8 December 1994	0.5 g	96.2%	-20°C	19 December 1997
IX	7 December 1992	0.5 g	90.4%	-20°C	17 January 2000
X	7 December 1992	0.5 g	91.2%	-20°C	9 May 2000
XI	17 June 1993	0.5 g	98.6%	-20°C	9 June 1996
XII	7 December 1992	0.5 g	98.2%	-20°C	8 May 2000
XIII	17 June 1993	0.5 g	92.0%	-20°C	9 June 1996
XIV	7 December 1992	0.5 g	99.9%	-20°C	28 July 1995
XV	7 December 1992	0.5 g	98.2%	-20°C	6 October 1995
XVI	5 January 1993 17 June 1993	0.5 g 0.5 g	97.9%	-20°C	9 June 1996
XVII	5 January 1993 17 June 1993	0.5 g 0.5 g	98.4%	-20°C	31 March 1996
XIX	8 December 1994	0.5 g	98.8%	-20°C	19 December 1997
XX	8 December 1994	0.5 g	89.5%	-20°C	15 August 1997
XXI	17 June 1993	0.5 g	40.6%[a]	-20°C	9 June 1996
XXII	17 June 1993	0.5 g	94.9%	-20°C	9 June 1996
XXIII	8 December 1994	0.5 g	96.4%	-20°C	20 December 1997

a free acid

APPENDIX 4

Bodyweights of rats

TABLE 1

Bodyweights of rats in single dose experiments

Animal number/ sex	Weight at dosing (g)	Weight at sacrifice (g)
A♂	205	204
B♂	203	205
C♀	203	204
D♀	205	205
E♂	195	202
F♂	197	206
G♀	197	200
H♀	199	201
I♂	199	219
J♂	197	232
K♀	203	214
L♀	204	215
M♂	215	a
N♂	219	a
O♂	214	a
P♂	212	a
Q♂	200	210
R♂	204	213
S♀	201	205
T♀	204	208
U♂	207	216
V♂	210	215
W♀	207	209
X♀	209	212
A1♂	220	226
A2♂	222	230
A3♀	215	218
A4♀	216	219
1♂	207	242
2♂	211	245
3♂	196	232
4♂	205	233
5♂	200	250
6♀	203	217
7♀	203	207
8♀	216	240
9♀	203	212
10♀	205	217
11♂	210	234
12♂	216	243
13♂	218	241
14♂	213	238
15♂	213	246
16♀	207	201
17♀	203	203
18♀	203	223
19♀	202	206
20♀	205	224
31♂	204	233
32♂	202	237
33♂	205	239
34♂	201	226
35♂	202	233
36♀	205	215
37♀	201	214
38♀	206	216
39♀	206	214
40♀	203	208
41♂	208	235
42♂	214	246
43♂	210	248
44♂	209	234
45♂	209	244
46♀	216	213
47♀	214	229
48♀	212	229
49♀	216	220
50♀	212	225
R51♂	206	237
R52♂	206	244
R53♂	202	236
R54♂	203	206
R55♂	204	219
R56♀	205	219
R57♀	207	231
R58♀	202	218
R59♀	208	203
R60♀	205	227
61♂	219	250[b]
62♂	215	239[b]
63♂	218	241[b]
64♀	212	212[b]
65♀	220	225[b]
66♀	212	219[b]
67♂	216	264[b]
68♂	218	256[b]
69♂	217	237[b]
70♀	218	225[b]
71♀	214	217[b]
72♀	218	234[b]
73♂	217	248[b]
74♂	214	243[b]
75♂	216	251[b]
76♀	210	212[b]
77♀	211	223[b]
78♀	215	220[b]
79♂	217	276[b]
80♂	222	270[b]
81♂	214	264[b]
82♀	211	227[b]
83♀	218	218[b]
84♀	209	215[b]
85♂	211	260[b]
86♂	215	259[b]
87♂	219	264[b]

a the weight was the same as that at dosing

b bodyweight at 5 days after dosing; rats were subsequently sacrificed 7 days after dosing

APPENDIX 4

(continued)

TABLE 1

(continued)

Animal number/ sex	Weight at dosing (g)	Weight at sacrifice (g)
88♀	214	227[b]
89♀	213	227[b]
90♀	213	220[b]
91♂	217	259[b]
92♂	219	262[b]
93♂	219	261[b]
94♀	214	224[b]
95♀	209	217[b]
96♀	216	218[b]
97♂	209	a
98♂	206	a
99♂	206	a
100♀	212	a
101♀	216	a
102♀	216	a
103♂	208	a
104♂	204	a
105♂	209	a
106♀	213	a
107♀	213	a
108♀	214	a
109♂	208	a
110♂	205	a
111♂	208	a
112♀	216	a
113♀	216	a
114♀	209	a
115♂	210	208
116♂	204	203
117♂	205	203
118♀	213	214
119♀	214	212
120♀	219	208
121♂	208	180
122♂	211	177
123♂	207	169
124♀	206	190
125♀	203	198
126♀	204	194
127♂	202	195
128♂	204	199
129♂	206	183
130♀	212	196
131♀	206	195
132♀	206	192
133♂	204	a
134♀	214	a
135♂	207	a
136♀	212	a
137♂	209	a
138♀	206	a
139♂	209	221
140♀	210	207
141♂	200	246
142♀	211	230
143♂	209	a
144♂	207	a
145♂	215	a
146♀	204	a
147♀	207	a
148♀	209	a
149♂	206	a
150♂	208	a
151♂	210	a
152♀	208	a
153♀	203	a
154♀	208	a
155♂	204	218
156♂	213	223
157♂	204	208
158♀	211	203
159♀	206	202
160♀	208	206
161♂	213	a
162♂	213	a
163♂	213	a
164♀	211	a
165♀	215	a
166♀	217	a
167♂	212	a
168♂	215	a
169♂	212	a
170♀	217	a
171♀	212	a
172♀	209	a
173♂	215	238
174♂	217	236
175♂	219	237
176♀	218	221
177♀	208	214
178♀	219	213
198♂	214	236
199♀	218	220
200♀	217	218
201♂	203	214
202♂	200	222
203♂	202	227
204♂	201	229
205♂	205	221
206♀	209	216
207♀	212	220
208♀	212	229
209♀	206	214
210♀	211	214

a the weight was the same as that at dosing
b bodyweight at 5 days after dosing; rats were subsequently sacrificed 7 days after dosing

APPENDIX 4

(continued)

TABLE 2

Bodyweights of rats in the tissue accumulation experiments

Experiment 2d

Animal number/ sex	Day 1	Day 3	Day 6	Day 8	Day 10	Day 12	Day 15*	Sacrifice
21♂	209	221	248	270	287	307	324	301
22♂	200	216	241	259	276	293	313	312
23♂	205	220	244	266	283	296	316	317
24♂	205	225	254	274	295	312	343	320
25♂	207	224	248	267	279	300	318	312
26♀	208	216	215	227	227	233	237	223
27♀	211	221	223	229	237	244	249	242
28♀	214	217	222	223	235	228	244	237
29♀	210	218	218	216	232	226	237	231
30♀	206	211	212	208	225	216	235	230

* day of radioactive dose

Experiment 5d

Animal number/ sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Sacrifice
179♂	218	224	231	239	250	257	266	a
180♂	208	209	214	220	228	233	242	a
181♂	224	232	245	248	259	264	274	a
182♀	215	223	225	218	225	228	226	a
183♀	211	212	206	212	214	216	213	a
184♀	213	214	225	222	218	226	232	a
185♂	215	223	232	238	245	253	260	a
186♂	212	217	227	233	240	249	257	a
187♂	214	221	229	233	244	253	258	a
188♀	212	208	219	221	219	218	225	a
189♀	207	212	207	212	215	218	220	a
190♀	213	222	224	224	219	226	229	a
191♂	214	226	232	240	252	259	269	292
192♂	221	231	239	241	254	261	265	289
193♂	212	226	230	234	244	250	259	278
194♀	211	206	213	210	210	207	210	216
195♀	203	212	207	207	213	216	212	221
196♀	208	213	216	208	209	215	213	219

a the weight was the same as that at dosing on Day 7

APPENDIX 5

Example certificate of analysis for animal diet

SDS
SPECIAL DIETS SERVICES

SPECIAL QUALITY CONTROL OF SMALL ANIMAL DIETS

CERTIFICATE OF ANALYSIS

PRODUCT: LAD 1 SQC

BATCH NO: 8488 PREMIX BATCH NO: 125/87

DATE OF MANUFACTURE: 27-OCT-92

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	11.0	%	Fluoride	39	mg/kg	1.0 mg/kg
Crude Fat	4.1	%	Nitrate as NaNO3	27	mg/kg	1.0 mg/kg
Crude Protein	21.6	%	Nitrite as NaNO2	2.7	mg/kg	1.0 mg/kg
Crude Fibre	1.6	%	Lead	0.57	mg/kg	0.25 mg/kg
Ash	5.1	%	Arsenic	0.59	mg/kg	0.2 mg/kg
Calcium	0.88	%	Cadmium	0.13	mg/kg	0.05 mg/kg
Phosphorus	0.69	%	Mercury	0.02	mg/kg	0.01 mg/kg
Sodium	0.45	%	Selenium	0.26	mg/kg	0.05 mg/kg
Chloride	0.45	%				
Potassium	0.86	%				
Magnesium	0.17	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	172	mg/kg				
Copper	14	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	72	mg/kg	Total D.D.T	Non Detected	mcg/kg	1.0 mcg/kg
Zinc	81	mg/kg	Dieldrin	Non Detected	mcg/kg	1.0 mcg/kg
			Lindane	4	mcg/kg	1.0 mcg/kg
			Heptachlor	Non Detected	mcg/kg	1.0 mcg/kg
			Malathion	Non Detected	mcg/kg	20.0 mcg/kg
Vitamin A	17.3	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	88	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Presumptive E.coli	Non Detected	per grm	Absent in 20 grm
			E.coli Type 1	Non Detected	per grm	Absent in 20 grm
			Fungal Units	Non Detected	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed R S F [illegible]

Dated 12/11/92

APPENDIX 6

Example analytical data for drinking water

The following analytical data cover the period 1 July 1994 to 31 September 1994 and relate to the Huntingdon North supply zone, in which Huntingdon Research Centre is situated. The data were supplied by Anglian Water Services Ltd.

Parameter	Units	Number of samples	PCV	Concentration or value		
				Minimum	Mean	Maximum
Colour	pt/co	16	20.0	<1.0	<1.0	<1.0
Turbidity	ftu	19	4.00	0.070	0.24	1.6
Odour (25°C)	Dil no	3	3.00	<1.0	<1.0	<1.0
Taste (25°C)	Dil no	3	3.00	<1.0	<1.0	<1.0
Temperature	°C	29	25.0	14	18	25
Hydrogen ion	pH	19	5.5 - 9.5	7.5	7.6	7.9
Nitrate as NO_3	mg/l	16	50.0	17	19	22
Nitrite as NO_2	mg/l	15	0.100	<0.0099	<0.094	0.29
Ammonium	mg/l	16	0.500	<0.026	<0.089	0.21
Aluminium	µg/l	16	200	<10	<11	18
Iron	µg/l	16	200	<10	<40	250
Manganese	µg/l	16	50.0	<2.0	<2.5	7.0
Phosphorus	µg/l	16	2200	390	480	630
Lead	µg/l	7	50.0	1.0	1.4	1.6
Azinphos-mthyl	µg/l	5	0.100	<0.020	<0.020	<0.020
Carboph'othion	µg/l	5	0.100	<0.010	<0.010	<0.010
Chlorfenv'phos	µg/l	5	0.100	<0.010	<0.010	<0.010
Chlorpyrifos	µg/l	5	0.100	<0.010	<0.010	<0.010
Diazinon	µg/l	5	0.100	<0.010	<0.010	<0.010
Dichlorvos	µg/l	5	0.100	<0.010	<0.010	<0.010
Dimethoate	µg/l	5	0.100	<0.030	<0.030	<0.030
Fenitrothion	µg/l	5	0.100	<0.010	<0.010	<0.010
Malathion	µg/l	5	0.100	<0.010	<0.010	<0.010
Mevinphos	µg/l	5	0.100	<0.010	<0.010	<0.010
Parathion	µg/l	5	0.100	<0.010	<0.010	<0.010
Pyri'os methyl	µg/l	5	0.100	<0.010	<0.010	<0.010
Chlorotoluron	µg/l	8	0.100	<0.020	<0.020	<0.020
Diuron	µg/l	8	0.100	<0.020	<0.020	<0.020
Isoproturon	µg/l	8	0.100	<0.020	<0.020	<0.020
Linuron	µg/l	8	0.100	<0.020	<0.020	<0.020
Monuron	µg/l	8	0.100	<0.020	<0.020	<0.020
Propyzamide	µg/l	10	0.100	<0.030	<0.030	<0.030
Aldrin	µg/l	2	0.100	<0.010	<0.010	<0.010
Alpha - HCH	µg/l	2	0.100	<0.010	<0.010	<0.010
Dieldrin	µg/l	2	0.100	<0.010	<0.010	<0.010
Endosulph. tot	µg/l	2	0.100	0	0	0
Endrin	µg/l	2	0.100	<0.010	<0.010	<0.010
Gamma - HCH	µg/l	2	0.100	<0.010	<0.010	<0.010

PCV prescribed concentration or value (maximum unless otherwise stated)

APPENDIX 6

(continued)

Parameter	Units	Number of samples	PCV	Concentration or value		
				Minimum	Mean	Maximum
Heptachlor	µg/l	2	0.100	<0.010	<0.010	<0.010
Heptac. epoxide	µg/l	2	0.100	<0.010	<0.010	<0.010
Hexach'benzene	µg/l	2	0.100	<0.010	<0.010	<0.010
Isodrin	µg/l	2	0.100	<0.020	<0.020	<0.020
PP'-DDE	µg/l	2	0.100	<0.010	<0.010	<0.010
PP'-DDT	µg/l	2	0.100	<0.010	<0.010	<0.010
PP'-DDD (TDE)	µg/l	2	0.100	<0.010	<0.010	<0.010
2,3,6-TBA	µg/l	10	0.100	<0.020	<0.023	<0.050
2,4-D	µg/l	10	0.100	<0.050	<0.050	<0.050
2,4,5-T	µg/l	10	0.100	<0.050	<0.050	<0.050
Bentazone	µg/l	10	0.100	<0.020	<0.020	<0.020
Dicamba	µg/l	10	0.100	<0.050	<0.050	<0.050
Dichloroprop	µg/l	10	0.100	<0.020	<0.020	<0.020
MCPA	µg/l	10	0.100	<0.020	<0.020	<0.020
MCPB	µg/l	10	0.100	<0.050	<0.050	<0.050
MCPP (mecoprop)	µg/l	10	0.100	<0.020	<0.020	<0.020
Bromoxynil	µg/l	4	0.100	<0.050	<0.050	<0.050
Ioxynil	µg/l	4	0.100	<0.050	<0.050	<0.050
Atrazine	µg/l	10	0.100	<0.030	<0.030	<0.030
Prometryne	µg/l	10	0.100	<0.030	<0.030	<0.030
Propazine	µg/l	10	0.100	<0.030	<0.030	<0.030
Simazine	µg/l	10	0.100	<0.030	<0.030	<0.030
Terbutryne	µg/l	10	0.100	<0.030	<0.030	<0.030
Trietazine	µg/l	10	0.100	<0.030	<0.030	<0.030
Pesticides tot	µg/l	12	0.500	0	0	0
Total coliforms	/dl	29	0.0000	0	0	0
Faecal coliforms	/dl	29	0.0000	0	0	0
Colony -37°C 1D	/ml	29	-	0	0.14	1.0
Colony -22°C 7D	/ml	29	-	2.0	86	420
Chlorine total	mg/l	29	-	0.090	0.26	0.45
Conductivity	µS/cm	19	MEA 1500	620	630	640
Chloride	mg/l	16	MEA 400	60	62	65
Tetrac'methane	µg/l	3	MEA 3.00	<0.10	<0.10	<0.10
Trichl'oethene	µg/l	3	MEA 30.0	<0.40	<0.40	<0.40
Tetrach'ethene	µg/l	3	MEA 10.0	<0.30	<0.30	<0.30
Trihalo'thanes	µg/l	1	100	46	46	46
111-Tric'thane	µg/l	3	-	<0.30	<0.30	<0.30
T-BME	µg/l	3	-	<0.10	<0.10	<0.10

PCV prescribed concentration or value (maximum unless otherwise stated: MEA annual mean)

APPENDIX 7

Specification* of the water used to prepare oral dose solutions

Water 'Super Purity Solvent' (code H-950), from Romil Chemicals Ltd, 63 Ashby Road, Shepshed, Loughborough, Leicestershire, LE12 9BS, UK:

Non-volatile matter:	0.0005% max.

Maximum absorbance of largest eluted peak:

220 nm:	0.050 AU.
236 nm:	0.002 AU.
254 nm:	0.002 AU.

(determined by passing 40 ml water through a C18 reverse phase column and eluting with a linear water 'Romil H-950'/acetonitrile 'Romil H-049' gradient at 5% per minute at a flow rate of 2 ml per minute).

* supplied by Romil Chemicals Ltd

APPENDIX 8

Quantities of radioactivity administered to rats

TABLE 1

Single radioactive dose experiments

Experiment number	Animal numbers	Administered dose			Mean dose level (mg/kg bodyweight)
		Specific activity (dpm/μg)	dpm/rat ($\times 10^7$)	mg/rat	
2a	I, J, K, L	1592	3.1465	19.8	99
2b	1, 3 - 6, 8 - 9,	2833	5.8430	20.6	101
	198 - 200	2847	6.1900	21.7	100
2c	201 - 210	17156	3.5220(♂)	2.07(♂)	10.2
		17156	3.7322(♀)	2.18(♀)	10.4
2d	21 - 30	11339	3.7525(♂)[a]	3.31(♂)[a]	10.3
		11339	2.7577(♀)[a]	2.43(♀)[a]	10.1
2e	31 - 40	16554	3.3982[a]	2.05[a]	10.0
2f	M, N, O, P	1881	3.7539	20.0	93
2g	41 - 50	15645	2.9334	1.88	8.9
2h	R51 - R60	2700	5.3830	19.9	97
3a	61 - 78	2833	6.1383	21.7	100
3b	79 - 96	17124	3.5465	2.07	9.6
3c	97 - 120	2737	5.7607	21.0	100
4a	121 - 126	2838	5.9095(♂)	20.8(♂)	100
		2838	5.9086(♀)	20.8(♀)	102
4b	127 - 132	16418	3.3856(♂)	2.06(♂)	10.1
		16418	3.3579(♀)	2.05(♀)	9.9
5a	133 - 142	16418	3.3856	2.06	9.9
5b	143 - 160	2838	5.9095	20.8	100
5c	161 - 178	16685	3.5490	2.13	10.0

[a] mean values. Rats were administered individual doses

APPENDIX 8

(continued)

TABLE 2

Multiple radioactive dose tissue distribution experiment (5d)

Animal number/ sex	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg	dpm/rat ($\times 10^7$)	mg/kg
179♂	2.9798	10.2	3.0340	10.1	3.1423	10.1	3.2507	10.1	3.4913	10.2	3.5467	10.1	3.7129	10.3
180♂	2.8173	10.1	2.8173	10.0	2.9256	10.2	2.9798	10.1	3.1588	10.2	3.2142	10.1	3.3804	10.2
181♂	3.0340	10.1	3.1423	10.1	3.3049	10.0	3.3590	10.1	3.6021	10.2	3.6575	10.2	3.8238	10.3
182♀	2.9256	10.1	3.0340	10.1	3.0340	10.0	2.9798	10.2	3.1034	10.1	3.1588	10.2	3.1588	10.3
183♀	2.8714	10.1	2.8714	10.1	2.8173	10.2	2.8714	10.1	2.9925	10.3	2.9925	10.2	2.9371	10.1
184♀	2.8714	10.0	2.9256	10.2	3.0340	10.0	3.0340	10.2	3.0479	10.3	3.1588	10.3	3.2142	10.2
185♂	2.9256	10.1	3.0340	10.1	3.1423	10.1	3.2507	10.2	3.3804	10.1	3.4913	10.1	3.6021	10.2
186♂	2.8714	10.1	2.9256	10.0	3.0881	10.1	3.1423	10.0	3.3250	10.2	3.4359	10.1	3.5467	10.2
187♂	2.9256	10.2	2.9780	10.0	3.0881	10.0	3.1423	10.0	3.3804	10.2	3.4913	10.1	3.6021	10.2
188♀	2.8714	10.1	2.8173	10.3	2.9798	10.1	2.9798	10.0	3.0479	10.2	3.0479	10.3	3.1034	10.1
189♀	2.8173	10.1	2.8714	10.1	2.8173	10.1	2.8714	10.1	2.9925	10.2	3.0479	10.3	3.0479	10.2
190♀	2.8714	10.0	3.0340	10.2	3.0340	10.1	3.0340	10.1	3.0479	10.2	3.1588	10.3	3.1588	10.1
191♂	2.9256	10.2	3.0811	10.2	3.1423	10.1	3.2507	10.1	3.4913	10.2	3.6021	10.2	3.7129	10.1
192♂	2.9798	10.0	3.1423	10.1	3.2507	10.1	3.2507	10.0	3.5467	10.2	3.6021	10.1	3.6575	10.1
193♂	2.8714	10.1	3.0881	10.2	3.1423	10.2	3.1965	10.2	3.3804	10.2	3.4913	10.2	3.6021	10.2
194♀	2.8714	10.1	2.8173	10.2	2.8714	10.0	2.8714	10.2	2.9371	10.3	2.8817	10.2	2.9371	10.3
195♀	2.7631	10.1	2.8714	10.1	2.8173	10.1	2.8173	10.1	2.9371	10.1	2.9925	10.2	2.9371	10.2
196♀	2.8173	10.1	2.8714	10.0	2.9256	10.1	2.8173	10.1	2.8817	10.1	2.9925	10.2	2.9371	10.1

APPENDIX 9

Composition of the soap used in dermal application experiments

An approx 10% solution of liquid soap (pg 'Deluxe 55', A&I Janatorial Supplies Ltd, Great Paxton, St Neots, Huntingdon, Cambridgeshire) in tap water was used to wash the treated areas of skin in dermal application experiments.

The stated composition of the soap was as follows:

Potassium salts of mixed fatty acids
Sodium sulphate
Sodium chloride
Sodium EDTA
Ethylene glycol monosterate
Bromo chlorophen
Coconut diethanolamide
Sodium lauryl ether sulphate
Perfume
Dye

APPENDIX 10

Sample raw data and calculations

QUANTIFICATION OF ADMINISTERED DOSES

Example: experiment 2b (excretion-balance, single oral dose at a nominal level of 100 mg/kg bodyweight, rats 1, 3 - 6, 8 - 9)

Three portions (0.95 ml) of dose solution, identical to those administered to the rats, were dispensed into 250 ml volumetric flasks containing methanol. These were made up to volume and triplicate aliquots (1 ml) radioassayed. The LSC data are shown below.

Dose portion	Aliquot (1 ml of 250 ml)	Counting time (seconds)	Counts per minute (cpm)	Disintegrations per minute (dpm)
1	1	240	204385	233617
	2	240	204564	233742
	3	240	203582	231985
2	1	240	210984	241051
	2	240	212393	242237
	3	240	210344	239853
3	1	240	199007	226678
	2	240	199434	227622
	3	240	199007	226931
Background		240	26	29 (mean 28)
		240	23	26

The mean net radioactivity measurement (from all nine aliquots) was 233718 dpm/ml.

The total radioactivity in 250 ml of solution, *ie* the quantity of radioactivity administered to each rat, was therefore:

$$233718 \text{ dpm/ml} \times 250 \text{ ml} = 5.8430 \times 10^7 \text{ dpm}$$

The specific activity of the dose material was 2833 dpm/μg (Appendix 5, Table 1).

Therefore, each rat received:

$$\frac{5.8430 \times 10^7 \text{ dpm}}{2833 \text{ dpm}/\mu\text{g}} = 20.6 \text{ mg dimethoate}$$

The mean bodyweight of this group of rats at the time of dosing was 204 g (Appendix 1, Table 1).

Therefore, the mean dose, in terms of bodyweight, received by this group of rats was:

$$\frac{20.6 \text{ mg}}{204 \text{ g}} = 101 \text{ mg/kg}$$

APPENDIX 10

(continued)

TOTAL RADIOACTIVITY IN URINE

Example: rat 3♂ (experiment 2b)

Time period (hours)	Total volume (ml)	Aliquot volume (ml)	Gross dpm per aliquot	Background dpm	Mean net dpm/ml	% dose
0 - 6	10	0.1	285455	28	2811740	48.1
		0.1	276949	28		
6 - 12	10	0.1	118514	28	1209375	20.7
		0.1	123417	28		
12 - 24	20	0.5	235115	15	470846	16.1
		0.5	235761	15		
24 - 48	25	0.5	39216	15	78090	3.34
		0.5	38904	15		
48 - 72	25	1.0	16724	15	16570	0.71
		1.0	16446	15		
72 - 96	25	1.0	3313	15	3228	0.14
		1.0	3172	15		
96 - 120	25	1.0	3404	15	3387	0.14
		1.0	3399	15		

For example, % dose in the 0 - 6 hours sample:

$$\frac{\left[\frac{285455 \text{ dpm/0.1 ml} + 276949 \text{ dpm/0.1 ml}}{2} - 28 \text{ dpm}\right] \times 10 \times 10}{5.8430 \times 10^7 \text{ dpm}} \times 100$$

$$= 48.1\%$$

APPENDIX 10

(continued)

TOTAL RADIOACTIVITY IN FAECES

Example: rat 3♂ (experiment 2b)

Time period (hours)	Total homogenate weight (g)	Aliquot weight (g)	Gross dpm per aliquot	Background dpm	Mean net dpm/g	% dose
0 - 24	19.63	0.3988	13391	51		
		0.3981	13184	51	33236	1.12
		0.4163	13901	51		
24 - 48	20.86	0.3997	4014	51		
		0.4228	4105	51	10036	0.36
		0.4105	4404	51		
48 - 72	20.41	0.4184	1281	51		
		0.4234	1238	51	2832	0.10
		0.4143	1191	51		
72 - 96	13.12	0.4012	682	51		
		0.3911	686	51	1626	0.04
		0.4111	742	51		
96 - 120	20.01	0.4053	332	51		
		0.4143	333	51	706	0.02
		0.4235	366	51		

For example, % dose in the 0 - 24 hours sample:

$$\frac{\left[\frac{\left[\frac{13391 \text{ dpm} - 51 \text{ dpm}}{0.3988 \text{ g}}\right] + \left[\frac{13184 \text{ dpm} - 51 \text{ dpm}}{0.3981 \text{ g}}\right] + \left[\frac{13901 \text{ dpm} - 51 \text{ dpm}}{0.4163 \text{ g}}\right]}{3}\right] \times 19.63 \text{ g}}{5.8430 \times 10^{7} \text{ dpm}} \times 100$$

$$= 1.12\%$$

APPENDIX 10

(continued)

CONCENTRATIONS OF RADIOACTIVITY IN TISSUES

Example: rat 3♂ (experiment 2b)

Tissue	Total weight (g)	Aliquot weight (g)	Gross dpm per aliquot	Background dpm	Mean net dpm/g	% dose	Concentration (μg dimethoate equiv/g)
Kidneys	1.8346	0.1950	1109	30			
		0.1847	1019	30	5448	0.02	1.92
		0.1758	989	30			
Liver	12.1070	0.2538	1913	49			
		0.2126	1615	49	7246	0.15	2.56
		0.2281	1652	49			

For example, concentration of radioactivity in kidneys:

$$\frac{5448 \text{ dpm/g}}{2833 \text{ dpm/}\mu\text{g}} = 1.92 \text{ mg dimethoate equivalents/kg}$$

(The value of the specific activity (2833 dpm/μg) was taken from Appendix 8, Table 1.)

APPENDIX 10

(continued)

HPLC ANALYSIS

Following sample injection, one-minute fractions of column eluate were collected and radioassayed. For example, the computer-processed LSC data (retyped) for the 0 - 48 hours male pooled urine sample from experiment 2c (excretion-balance, single oral 10 mg/kg bodyweight dose) are shown below. No correction was made for column recovery. The chromatogram is shown in Figure 21 of the main report.

RUN 126 Male urine 0 - 48 hour (Experiment 2c(R))

Study no.:	DTF 16	Flow rate:	1 ml/min
Background:	30	Dpm injected:	93857
Dpm eluted:	94264	Recovery:	100.4%

Fraction	Dpm/ fraction	Dpm - blank	
1	32	<30 (2)	
2	27	<30 (-3)	
3	25	<30 (-5)	
4	80	50	
5	4793	4763	
6	291	261	U1 (5.7%)
7	336	306	
8	269	239	
9	132	102	
10	283	253	
11	1187	1157	Omethoate (ref II) (1.7%)
12	257	227	
13	308	278	U2a (0.3%)
14	159	129	
15	90	60	
16	385	355	
17	3639	3609	U3 (4.5%)
18	267	237	
19	254	224	
20	6789	6759	
21	1376	1346	Ref XVI (9.2%)
22	389	359	
23	229	199	
24	143	113	
25	183	153	
26	117	87	
27	128	98	
28	120	90	
29	985	955	U5 (1.0%)
30	283	253	U6 (2.7%)
31	2346	2316	U6
32	13198	13168	
33	13068	13038	Ref XV (29.3%)
34	1419	1389	
35	934	904	
36	584	554	Dimethoate (1.5%)
37	139	109	
38	134	104	
39	107	77	
40	90	60	
41	79	49	
42	92	62	
43	32406	32376	
44	6306	6276	
45	451	421	Ref III (41.6%)
46	135	105	
47	81	51	
48	65	35	
49	148	118	
50	68	38	
51	75	45	
52	190	160	
53	106	76	
54	36	<30 (6)	
55	44	<30 (14)	
56	40	<30 (10)	
57	46	<30 (16)	
58	34	<30 (4)	
59	47	<30 (17)	
60	40	<30 (10)	

APPENDIX 10

(continued)

The background was calculated as the mean of the dpm values of the first two fractions.

For example, proportion of ref III in the sample:

$$= \frac{32376 + 6276 + 421 + 105}{94264} \times 100$$

$$= 41.6\%$$

Proportion as % dose:

$$= \frac{41.6}{100} \times \frac{90.76}{100} \times 100$$

$$= 37.8\%$$

The value of 90.76% (% dose in the urine up to 48 hours after administration) was taken from Table 3 of the main report.

APPENDIX 10

(continued)

TLC ANALYSIS

The Fujix evaluation report (retyped) for the TLC radiochromatogram of the extracts of male kidney taken 2 hours after dosing at 100 mg/kg bodyweight (experiment 5b) is shown below. The chromatogram is shown in Figure 34 of the main report.

Image: COH92 – OB.IMG File: 925BKIDM
Date: 19-10.94 Time: 10:38:26
Origin: 19.8 mm Front: 180.0 mm

No.	Name	From (mm)	To (mm)	Max (PSL)	Pos (mm)	R_f	Sum (PSL)	ROI (%)	Sum - BGK (PSL)	ROI - BGK (%)
1	BGK1	10.2	18.4	1.73	13.4		705.97			
2	K1	19.8	28.7	48.88	26.6	0.03	1753.00	4.70	977.38	4.21
3	K2	28.7	33.0	18.13	29.0	0.07	644.21	1.73	280.52	1.21
4	K3	33.0	42.0	119.03	38.4	0.11	3040.60	8.15	2259.57	9.74
5	K4	42.0	46.8	19.38	44.0	0.15	814.96	2.18	397.47	1.71
6	K5	46.8	52.9	31.79	49.4	0.19	1187.73	3.18	647.50	2.79
7	K6	52.9	57.3	12.94	53.1	0.22	602.48	1.62	218.43	0.94
8	Ref XVI	57.3	62.9	240.53	61.9	0.25	2988.47	8.01	2498.88	10.77
9	Ref III	62.9	78.1	937.03	64.9	0.32	13049.55	34.98	11716.16	50.51
10		78.1	87.3	6.92	84.3	0.39	1035.30	2.78	225.06	0.97
11	K9	87.3	101.5	9.86	91.5	0.47	1665.37	4.46	410.05	1.77
12		101.5	123.5	7.74	113.3	0.58	2226.32	5.97	288.32	1.24
13	Ref XV	123.5	137.5	69.74	132.3	0.69	3653.89	9.80	2401.95	10.35
14	Dimethoate	137.5	144.7	24.07	137.5	0.76	1217.65	3.26	571.62	2.46
15		144.7	179.6	7.81	147.3	0.89	3423.81	9.18	304.21	1.31
16	BGK2	180.8	192.0	2.65	184.8		1020.05			
	Remainder						3708.96		73.46	

Total contents: 41012.30 PSL
Contents regions: 37303.34 PSL
Remainder: 9.04%
Total contents - BKG: 23270.57 PSL
Contents regions - BKG: 23197.11 PSL
Remainder - BKG: 0.32%
BG1 (PSL/mm): 86.037
BG2 (PSL/mm): 90.931

APPENDIX 10

(continued)

The percentage tissue radioactivity associated with each component in the extracts was calculated as the product of the net % chromatographed radioactivity in the ROI (taken from the right-most column of the table) and the % analysed tissue radioactivity in the extracts.

For example, reference substance III:

$$= \frac{50.51}{100} \times \frac{95.0}{100} \times 100$$

$$= 48.0\%$$

The value of 95.0 (% analysed tissue radioactivity in the extracts) was taken from Table 27 of the main report.

APPENDIX 11

Limits of detection

Shown in this Appendix are calculations of limits of detection of radioactivity measurements of excreta and tissues, and of those associated with chromatographic analyses. Unless stated otherwise, the calculations employ representative total sample weights and volumes, and aliquot sizes, and a background radioactivity value of 20 dpm is assumed (50 dpm for combustion/LSC). Two dose values are also used: 3.3×10^7 dpm and 5.6×10^7 dpm, and two specific activity values: 17000 dpm/μg and 2800 dpm/μg which were approximately those used in the 10 and 100 mg/kg bodyweight experiments, respectively (Appendix 8). Actual limits varied slightly, due to differences in one or more of the above values.

The limit of detection was assumed to be equal to a gross sample level of radioactivity of twice the background level.

RADIOACTIVITY MEASUREMENTS

Urine

Total volume: 50 ml (typical).

Aliquot size: 1 ml (typical).

$$\text{Limit of detection} = \frac{\left[\frac{50 \text{ ml}}{1 \text{ ml}}\right] \times [(2 \times 20 \text{ dpm}) - 20 \text{ dpm}]}{3.3 \text{ or } 5.6 \times 10^7 \text{ dpm}} \times 100$$

$$= 0.003\% \text{ dose (10 mg/kg bodyweight) or}$$
$$0.002\% \text{ dose (100 mg/kg bodyweight)}$$

$$\text{Limit of detection} = \frac{\left[\frac{1}{1 \text{ ml}}\right] \times [(2 \times 20 \text{ dpm}) - 20 \text{ dpm}]}{2800 \text{ or } 17000 \text{ dpm}/\mu\text{g}}$$

$$= 0.001 \text{ mg dimethoate equivalents/l (10 mg/kg bodyweight) or}$$
$$0.007 \text{ mg dimethoate equivalents/l (100 mg/kg bodyweight)}$$

APPENDIX 11

(continued)

Faeces

Total homogenate weight: 20 g (typical).

Aliquot size: 0.4 g (typical).

$$\text{Limit of detection} = \frac{\left[\frac{20\text{ g}}{0.4\text{ g}}\right] \times [(2 \times 50\text{ dpm}) - 50\text{ dpm}]}{3.3 \text{ or } 5.6 \times 10^7\text{ dpm}} \times 100$$

= 0.008% dose (10 mg/kg bodyweight) or
0.004% dose (100 mg/kg bodyweight)

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.4\text{ g}}\right] \times [(2 \times 50\text{ dpm}) - 50\text{ dpm}]}{2800 \text{ or } 17000\text{ dpm}/\mu\text{g}}$$

= 0.007 mg dimethoate equivalents/kg (10 mg/kg bodyweight) or
0.045 mg dimethoate equivalents/kg (100 mg/kg bodyweight)

Trapping solutions

Total volume: 300 ml (trap 1); 150 ml (trap 2) (typical).

Aliquot size: 1 ml.

$$\text{Limit of detection} = \frac{\left[\frac{300 \text{ or } 150\text{ ml}}{1\text{ ml}}\right] \times [(2 \times 20\text{ dpm}) - 20\text{ dpm}]}{3.3 \text{ or } 5.6 \times 10^7\text{ dpm}} \times 100$$

= 0.018 (trap 1) or 0.009 (trap 2) % dose (10 mg/kg bodyweight) or
0.011 (trap 1) or 0.005 (trap 2) % dose (100 mg/kg bodyweight)

$$\text{Limit of detection} = \frac{\left[\frac{1}{1\text{ ml}}\right] \times [(2 \times 20\text{ dpm}) - 20\text{ dpm}]}{2800 \text{ or } 17000\text{ dpm}/\mu\text{g}}$$

= 0.001 mg dimethoate equivalents/l (10 mg/kg bodyweight) or
0.007 mg dimethoate equivalents/l (100 mg/kg bodyweight)

APPENDIX 11

(continued)

Bile

Total volume: 5 ml.

Aliquot size: 0.5 ml.

$$\text{Limit of detection} = \frac{\left[\frac{5\text{ ml}}{0.5\text{ ml}}\right] \times [(2 \times 20\text{ dpm}) - 20\text{ dpm}]}{3.3 \text{ or } 5.6 \times 10^7\text{ dpm}} \times 100$$

= 0.0006% dose (10 mg/kg bodyweight) or
0.0004% dose (100 mg/kg bodyweight)

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.5\text{ ml}}\right] \times [(2 \times 20\text{ dpm}) - 20\text{ dpm}]}{2800 \text{ or } 17000\text{ dpm}/\mu\text{g}}$$

= 0.002 mg dimethoate equivalents/l (10 mg/kg bodyweight) or
0.014 mg dimethoate equivalents/l (100 mg/kg bodyweight)

Plasma (experiments 3a, 3b)

Specific activity: 17124 dpm/μg (10 mg/kg bodyweight) or
2833 dpm/μg (100 mg/kg bodyweight).

Aliquot size: 0.1 ml.

Background: 31 dpm (actual).

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.1\text{ ml}}\right] \times [(2 \times 31\text{ dpm}) - 31\text{ dpm}]}{\text{specific activity (dpm}/\mu\text{g)}}$$

= 0.018 mg dimethoate equivalents/l (10 mg/kg bodyweight) or
0.11 mg dimethoate equivalents/l (100 mg/kg bodyweight)

APPENDIX 11

(continued)

Tissues

eg Liver (experiment 5c, 10 mg/kg bodyweight, tissue distribution)

Total weight: 10 g (typical).

Aliquot size: 0.23 g (typical).

Background: 43 dpm (actual).

Dose: 3.5490×10^7 dpm.

Specific activity: 16685 dpm/μg.

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.23\ \text{g}}\right] \times [(2 \times 43\ \text{dpm}) - 43\ \text{dpm}]}{16685\ \text{dpm}/\mu\text{g}}$$

$$= 0.011 \text{ mg dimethoate equivalents/kg}$$

$$\text{Limit of detection} = \frac{\left[\frac{10\ \text{g}}{0.23\ \text{g}}\right] \times [(2 \times 43\ \text{dpm}) - 43\ \text{dpm}]}{3.5490 \times 10^7\ \text{dpm}} \times 100$$

$$= 0.005\% \text{ dose}$$

eg Liver (experiment 5b, 100 mg/kg bodyweight, tissue distribution)

Total weight: 10 g (typical).

Aliquot size: 0.23 g (typical).

Background: 47 dpm (actual).

Dose: 5.9095×10^7 dpm.

Specific activity: 2838 dpm/μg.

APPENDIX 11

(continued)

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.23\ \text{g}}\right] \times [(2 \times 47\ \text{dpm}) - 47\ \text{dpm}]}{2838\ \text{dpm}/\mu\text{g}}$$

$$= 0.072\ \text{mg dimethoate equivalents/kg}$$

$$\text{Limit of detection} = \frac{\left[\frac{10\ \text{g}}{0.23\ \text{g}}\right] \times [(2 \times 47\ \text{dpm}) - 47\ \text{dpm}]}{5.9095 \times 10^7\ \text{dpm}} \times 100$$

$$= 0.003\%\ \text{dose}$$

***eg* Thyroid gland (experiment 5c, 10 mg/kg bodyweight, tissue distribution)**

Total weight: 0.012 g (typical).

Aliquot size: Entire amount taken for radioassay.

Background: 30 dpm (actual).

Dose: 3.5490×10^7 dpm.

Specific activity: 16685 dpm/μg.

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.012\ \text{g}}\right] \times [(2 \times 30\ \text{dpm}) - 30\ \text{dpm}]}{16685\ \text{dpm}/\mu\text{g}}$$

$$= 0.15\ \text{mg dimethoate equivalents/kg}$$

$$\text{Limit of detection} = \frac{[(2 \times 30\ \text{dpm}) - 30\ \text{dpm}]}{3.5490 \times 10^7\ \text{dpm}} \times 100$$

$$= 0.00008\%\ \text{dose}$$

***eg* Thyroid gland (experiment 5b, 100 mg/kg bodyweight, tissue distribution)**

Total weight: 0.012 g (typical).

Aliquot size: Entire amount taken for radioassay.

APPENDIX 11

(continued)

Background: 30 dpm (actual).

Dose: 5.9095×10^7 dpm.

Specific activity: 2838 dpm/μg.

$$\text{Limit of detection} = \frac{\left[\frac{1}{0.012\ \text{g}}\right] \times [(2 \times 30\ \text{dpm}) - 30\ \text{dpm}]}{2838\ \text{dpm}/\mu\text{g}}$$

$$= 0.88\ \text{mg dimethoate equivalents/kg}$$

$$\text{Limit of detection} = \frac{[(2 \times 30\ \text{dpm}) - 30\ \text{dpm}]}{5.9095 \times 10^7\ \text{dpm}} \times 100$$

$$= 0.00005\%\ \text{dose}$$

HPLC

It was assumed that low levels of radioactive components in urine, bile, plasma, etc, would be eluted in a single fraction of column eluate. The background level of radioactivity in a single fraction of eluate was typically 25 dpm. The quantity of radioactivity injected onto the column in each analysis varied throughout the study. For example, for urine samples from excretion-balance experiments this ranged from *ca* 2000 dpm (experiment 2h) to 42000 - 94000 dpm (experiments 2b - 2e). Limits of detection in these cases were therefore approximately:

$$\text{Exp 2h:} \quad \frac{[(2 \times 25\ \text{dpm}) - 25\ \text{dpm})}{2000\ \text{dpm}} \times 100$$

$$= 1.3\%\ \text{sample radioactivity}$$

As the urine sample from this experiment contained *ca* 1% dose, the limit was equivalent to *ca* 0.013% dose.

$$\text{Exp 2c:} \quad \frac{[(2 \times 25\ \text{dpm}) - 25\ \text{dpm}]}{94000\ \text{dpm}} \times 100$$

$$= 0.027\%\ \text{sample radioactivity}$$

As the urine sample from this experiment contained *ca* 90% dose, the limit was equivalent to *ca* 0.025% dose.

APPENDIX 11

(continued)

Limit of detection of dimethoate in plasma (experiment 3c)

It was assumed that low levels of dimethoate in plasma would be eluted in a single fraction of HPLC column eluate.

The net dpm in one fraction of eluate in which dimethoate could be expected to be found was <28 dpm (for both male and female 24-hour samples).

At a specific activity of the administered ^{14}C-dimethoate of 2737 dpm/μg (Appendix 8, Table 1), and an HPLC injection corresponding in each case to 200 μl plasma, the limit of detection was therefore:

$$\frac{[(2 \times 28 \text{ dpm}) - 28 \text{ dpm}]}{2737 \text{ dpm}/\mu\text{g}} \times \frac{1000}{200} = 0.051 \text{ mg/l plasma}$$

TLC

The limit of detection was assumed to be equal to 1% of the net chromatographed radioactivity, irrespective of the absolute quantity of radioactivity applied to the plate. In terms of % dose or % tissue radioactivity, the limit therefore varied depending upon the % dose or % tissue radioactivity in the sample. For example, in experiments 5b, 5c and 5d, tissue extracts contained *ca* 80 - 90% tissue radioactivity. In these cases the limit of detection was thus *ca* 0.8 - 0.9% tissue radioactivity.

APPENDIX 12

Results of pre-test (dose range-finding) experiments

Dose route	Dose level (mg/kg bodyweight)	Observable signs	
		Male	Female
Intravenous	10	No	No
Oral	10	No	No
Oral	100	Yes	No
Dermal	100	No	No
Dermal	250	No	No

The effects seen in the male rats after oral administration at 100 mg/kg bodyweight were described as slight body tremors at about 2 - 3 hours after dosing

APPENDIX 13

Data from individual animals

Shown in this appendix are data from individual animals from which Tables 3a, 4a, 5a, 7a, 8a, 9, 12, 14, 15, 16, 17, 19, 20, 21 and 22 of the main report are derived.

TABLES

Excretion and retention of radioactivity by rats

1. Oral dose, 10 mg/kg bodyweight, males
2. Oral dose, 10 mg/kg bodyweight, females
3. Oral dose, 10 mg/kg bodyweight (pre-treated), males
4. Oral dose, 10 mg/kg bodyweight (pre-treated), females
5. Oral dose, 100 mg/kg bodyweight, males
6. Oral dose, 100 mg/kg bodyweight, females
7. Intravenous dose, 10 mg/kg bodyweight, males
8. Intravenous dose, 10 mg/kg bodyweight, females
9. Dermal dose, 10 mg/kg bodyweight, males
10. Dermal dose, 10 mg/kg bodyweight, females
11. Dermal dose, 100 mg/kg bodyweight, males
12. Dermal dose, 100 mg/kg bodyweight, females

Excretion and retention of radioactivity and cumulative biliary excretion of radioactivity by rats with cannulated bile ducts

13. Excretion and retention, 10 mg/kg bodyweight, males
14. Excretion and retention, 10 mg/kg bodyweight, females
15. Excretion and retention, 100 mg/kg bodyweight, males
16. Excretion and retention, 100 mg/kg bodyweight, females
17. Cumulative biliary excretion, 10 mg/kg bodyweight, males
18. Cumulative biliary excretion, 10 mg/kg bodyweight, females
19. Cumulative biliary excretion, 100 mg/kg bodyweight, males
20. Cumulative biliary excretion, 100 mg/kg bodyweight, females

Concentrations of radioactivity in the plasma of rats

21. 10 mg/kg bodyweight, males
22. 10 mg/kg bodyweight, females
23. 100 mg/kg bodyweight, males
24. 100 mg/kg bodyweight, females

APPENDIX 13

(continued)

Concentrations of radioactivity in the tissues of rats sacrificed at various times after administration at 10 mg/kg bodyweight

25. 0.5 hours
26. 2 hours
27. 48 hours

Ratios of tissue to plasma concentrations of radioactivity in rats sacrificed at various times after administration at 10 mg/kg bodyweight

28. 0.5 hours
29. 2 hours
30. 48 hours

Concentrations of radioactivity in the tissues of rats sacrificed at various times after the last of seven daily radioactive doses at 10 mg/kg bodyweight

31. 0.5 hours
32. 2 hours
33. 48 hours

Ratios of tissue to plasma concentrations of radioactivity in rats sacrificed at various times after the last of seven daily radioactive doses at 10 mg/kg bodyweight

34. 0.5 hours
35. 2 hours
36. 48 hours

Concentrations of radioactivity in the tissues of rats sacrificed at various times after administration at 100 mg/kg bodyweight

37. 0.5 hours
38. 2 hours
39. 48 hours

Ratios of tissue to plasma concentrations of radioactivity in rats sacrificed at various times after administration at 100 mg/kg bodyweight

40. 0.5 hours
41. 2 hours
42. 48 hours

APPENDIX 13

(continued)

Concentrations of radioactivity in the tissues of rats sacrificed 120 hours after administration of ^{14}C-dimethoate

43. Oral dose, 10 mg/kg bodyweight, males
44. Oral dose, 10 mg/kg bodyweight, females
45. Oral dose, 10 mg/kg bodyweight (pre-treated), males
46. Oral dose, 10 mg/kg bodyweight (pre-treated), females
47. Oral dose, 100 mg/kg bodyweight, males
48. Oral dose, 100 mg/kg bodyweight, females
49. Intravenous dose, 10 mg/kg bodyweight, males
50. Intravenous dose, 10 mg/kg bodyweight, females
51. Dermal dose, 10 mg/kg bodyweight, males
52. Dermal dose, 10 mg/kg bodyweight, females
53. Dermal dose, 100 mg/kg bodyweight, males
54. Dermal dose, 100 mg/kg bodyweight, females

APPENDIX 13

(continued)

TABLE 1

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	201♂	202♂	203♂	204♂	205♂	Mean	sd
Urine (hours)							
0 - 6	59.5	71.0	62.0	78.2	75.9	69.3	8.3
6 - 12	23.2	16.4	22.2	14.0	9.49	17.1	5.7
12 - 24	2.87	2.35	3.55	2.25	2.17	2.64	0.58
24 - 48	0.75	0.50	1.93	0.56	4.85	1.72	1.84
48 - 72	0.22	0.22	0.37	0.17	0.30	0.26	0.08
72 - 96	0.15	0.16	0.35	0.09	0.09	0.17	0.11
96 - 120	0.08	0.07	0.10	0.05	0.05	0.07	0.02
Cage wash	0.10	0.10	0.16	0.05	0.09	0.10	0.04
Total urine and cage wash	86.9	90.8	90.7	95.4	92.9	91.3	3.1
Expired air (hours)							
0 - 6	1.12	0.81	0.78	0.93	0.89	0.91	0.13
6 - 24	1.67	0.71	0.70	0.99	0.95	1.00	0.40
24 - 48	0.22	0.11	0.10	0.13	0.12	0.14	0.05
48 - 72	0.08	0.04	0.06	0.05	0.06	0.06	0.01
Total expired air	3.09	1.67	1.64	2.10	2.02	2.10	0.59
Faeces (hours)							
0 - 24	0.78	0.81	0.72	0.90	1.08	0.86	0.14
24 - 48	0.24	0.14	0.13	0.14	0.30	0.19	0.08
48 - 72	0.08	0.03	0.07	0.06	0.03	0.05	0.02
72 - 96	0.04	0.02	0.04	0.02	0.02	0.03	0.01
96 - 120	0.02	0.01	0.03	0.02	0.01	0.02	0.01
Total faeces	1.16	1.01	0.99	1.14	1.44	1.15	0.18
Total excreted	91.1	93.5	93.3	98.6	96.4	94.6	2.9
Carcass							
GIT	0.05	0.03	0.03	0.04	0.03	0.04	0.01
Kidneys	0.02	0.01	0.01	0.01	0.01	0.01	<0.01
Liver	0.17	0.11	0.10	0.12	0.13	0.13	0.03
Residual carcass	0.74	0.30	0.53	0.51	0.41	0.50	0.16
Total carcass	0.98	0.45	0.67	0.68	0.58	0.67	0.20
Total recovery	92.1	93.9	94.0	99.3	97.0	95.3	2.9

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 2

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	206♀	207♀	208♀	209♀	210♀	Mean	sd
Urine (hours)							
0 - 6	74.5	71.0	75.5	72.2	66.5	71.9	3.5
6 - 12	6.82	5.11	5.19	7.68	8.55	6.67	1.52
12 - 24	2.12	2.00	2.34	3.67	4.73	2.97	1.19
24 - 48	1.46	0.80	1.89	1.47	2.37	1.60	0.58
48 - 72	1.09	0.48	1.01	0.47	2.03	1.02	0.64
72 - 96	0.29	0.19	0.47	1.37	0.48	0.56	0.47
96 - 120	0.19	0.12	0.79	0.45	0.23	0.36	0.27
Cage wash	0.19	0.18	0.47	0.20	0.42	0.29	0.14
Total urine and cage wash	86.7	79.9	87.7	87.5	85.3	85.4	3.2
Expired air (hours)							
0 - 6	0.92	1.08	0.89	0.81	1.19	0.98	0.15
6 - 24	0.71	0.98	0.92	0.94	0.93	0.90	0.10
24 - 48	0.12	0.13	0.22	0.18	0.28	0.19	0.06
48 - 72	0.06	0.09	0.13	0.09	0.17	0.11	0.04
Total expired air	1.81	2.28	2.16	2.02	2.57	2.17	0.28
Faeces (hours)							
0 - 24	3.51	0.24	0.13	0.29	0.95	1.02	1.43
24 - 48	0.15	0.17	0.30	0.27	0.42	0.26	0.11
48 - 72	0.07	0.09	0.18	0.13	0.23	0.14	0.07
72 - 96	0.04	0.04	0.13	0.08	0.13	0.08	0.05
96 - 120	0.03	0.05	0.08	0.03	0.08	0.05	0.03
Total faeces	3.80	0.59	0.82	0.80	1.81	1.56	1.34
Total excreted	92.3	82.8	90.6	90.3	89.7	89.1	3.7
Carcass							
GIT	0.05	0.06	0.07	0.06	0.08	0.06	0.01
Kidneys	0.01	0.01	0.01	0.01	0.02	0.01	<0.01
Liver	0.10	0.10	0.11	0.12	0.13	0.11	0.01
Residual carcass	0.84	0.83	1.26	1.16	2.20	1.26	0.56
Total carcass	1.00	1.00	1.45	1.35	2.43	1.45	0.59
Total recovery	93.3	83.8	92.1	91.7	92.1	90.6	3.8

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 3

Excretion and retention of radioactivity in pre-treated* male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	21♂	22♂	23♂	24♂	25♂	Mean	sd
Urine (hours)							
0 - 6	66.3	78.2	75.1	80.2	58.1	71.6	9.2
6 - 12	23.9	7.80	8.24	7.22	28.2	15.1	10.1
12 - 24	2.44	3.16	2.61	1.59	3.43	2.65	0.71
24 - 48	0.41	0.89	0.63	0.53	0.70	0.63	0.18
48 - 72	0.11	0.24	0.30	0.13	0.18	0.19	0.08
72 - 96	0.07	0.11	0.12	0.06	0.09	0.09	0.03
96 - 120	0.05	0.07	0.21	0.03	0.20	0.11	0.09
Cage wash (hours)							
24	0.17	0.47	0.28	0.10	0.15	0.23	0.15
120	0.03	0.05	0.04	0.08	0.05	0.05	0.02
Total urine and cage wash	93.5	91.0	87.5	89.9	91.1	90.6	2.2
Expired air (hours)							
0 - 6	0.73	0.53	1.06	0.95	0.85	0.82	0.20
6 - 24	0.82	0.77	1.16	1.10	1.08	0.99	0.18
24 - 48	0.15	0.16	0.21	0.21	0.20	0.19	0.03
48 - 72	0.07	0.07	0.08	0.09	0.08	0.08	0.01
Total expired air	1.77	1.53	2.51	2.35	2.21	2.07	0.41
Faeces (hours)							
0 - 24	0.61	0.80	1.17	1.56	1.30	1.09	0.38
24 - 48	0.11	0.18	0.06	0.19	0.11	0.13	0.05
48 - 72	0.03	0.07	0.04	0.02	0.08	0.05	0.03
72 - 96	0.01	0.03	0.01	0.03	0.03	0.02	0.01
96 - 120	0.01	0.02	0.01	0.01	0.02	0.01	0.01
Total faeces	0.77	1.10	1.29	1.81	1.54	1.30	0.40
Total excreted	96.0	93.6	91.3	94.1	94.9	94.0	1.8
Carcass							
GIT	0.04	0.05	0.04	0.04	0.04	0.04	<0.01
Kidneys	0.02	0.02	0.02	0.02	0.02	0.02	0.00
Liver	0.10	0.13	0.15	0.12	0.13	0.13	0.02
Residual carcass	0.74	1.02	0.80	0.81	0.87	0.85	0.11
Total carcass	0.90	1.22	1.01	0.99	1.06	1.04	0.12
Total recovery	96.9	94.8	92.3	95.1	95.9	95.0	1.7

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation
* rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

APPENDIX 13

(continued)

TABLE 4

Excretion and retention of radioactivity in pre-treated* female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	26♀	27♀	28♀	29♀	30♀	Mean	sd
Urine (hours)							
0 - 6	72.7	70.1	77.6	63.9	71.1	71.1	4.9
6 - 12	8.11	11.0	9.11	13.2	11.2	10.5	2.0
12 - 24	3.58	5.14	2.07	5.28	3.54	3.92	1.32
24 - 48	1.46	1.66	0.65	1.66	0.58	1.20	0.54
48 - 72	1.92	0.70	0.50	0.74	0.21	0.81	0.65
72 - 96	0.47	0.37	0.38	0.32	0.31	0.37	0.06
96 - 120	0.17	0.20	0.22	0.18	0.65	0.28	0.21
Cage wash (hours)							
24	0.50	0.67	0.18	0.55	0.40	0.46	0.18
120	0.17	0.29	0.29	0.38	0.12	0.25	0.10
Total urine and cage wash	89.1	90.1	91.0	86.2	88.1	88.9	1.9
Expired air (hours)							
0 - 6	0.82	0.76	0.61	0.79	1.13	0.82	0.19
6 - 24	1.14	1.08	0.85	1.02	1.42	1.10	0.21
24 - 48	0.26	0.27	0.15	0.26	0.23	0.23	0.05
48 - 72	0.14	0.12	0.09	0.14	0.11	0.12	0.02
Total expired air	2.36	2.23	1.70	2.21	2.89	2.28	0.42
Faeces (hours)							
0 - 24	0.69	0.55	0.68	1.04	0.88	0.77	0.19
24 - 48	0.31	0.29	0.13	0.36	0.04	0.23	0.14
48 - 72	0.08	0.06	0.04	0.14	0.09	0.08	0.04
72 - 96	0.13	0.10	0.06	0.13	0.05	0.09	0.04
96 - 120	0.06	0.04	0.04	0.06	0.05	0.05	0.01
Total faeces	1.27	1.04	0.95	1.73	1.11	1.22	0.31
Total excreted	92.7	93.4	93.7	90.2	92.1	92.4	1.4
Carcass							
GIT	0.06	0.07	0.04	0.06	0.07	0.06	0.01
Kidneys	0.02	0.02	0.01	0.02	0.02	0.02	<0.01
Liver	0.10	0.12	0.09	0.11	0.13	0.11	0.02
Residual carcass	1.65	1.48	0.90	2.55	1.09	1.53	0.64
Total carcass	1.83	1.69	1.04	2.74	1.31	1.72	0.65
Total recovery	94.5	95.1	94.7	92.9	93.4	94.1	0.9

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation
* rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

APPENDIX 13

(continued)

TABLE 5

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	1♂	3♂	4♂	5♂	198♂	Mean	sd
Urine (hours)							
0 - 6	57.7	48.1	65.1	64.4	59.5	59.0	6.8
6 - 12	16.3	20.7	18.6	21.8	20.8	19.6	2.2
12 - 24	11.7	16.1	4.22	3.76	9.80	9.12	5.21
24 - 48	2.34	3.34	1.47	0.84	1.47	1.89	0.97
48 - 72	0.78	0.71	0.71	0.40	0.32	0.58	0.21
72 - 96	0.31	0.14	0.27	0.14	0.16	0.20	0.08
96 - 120	0.25	0.14	0.16	0.11	0.11	0.15	0.06
Cage wash	0.52	0.27	0.26	0.15	0.13	0.27	0.16
Total urine and cage wash	89.9	89.5	90.8	91.6	92.3	90.8	1.2
Expired air (hours)							
0 - 24	2.21	2.30	1.58	2.37	1.86	2.06	0.33
24 - 48	0.32	0.34	0.29	0.22	0.16	0.27	0.07
48 - 72	0.14	0.14	0.10	0.10	0.08	0.11	0.03
Total expired air	2.67	2.78	1.97	2.69	2.10	2.44	0.38
Faeces (hours)							
0 - 24	1.09	1.12	0.86	1.09	0.82	1.00	0.14
24 - 48	0.40	0.36	0.14	0.16	0.35	0.28	0.12
48 - 72	0.17	0.10	0.10	0.07	0.06	0.10	0.04
72 - 96	0.06	0.04	0.04	0.03	0.04	0.04	0.01
96 - 120	0.05	0.02	0.02	0.02	0.02	0.03	0.01
Total faeces	1.77	1.64	1.16	1.37	1.29	1.45	0.25
Total excreted	94.3	93.9	93.9	95.7	95.7	94.7	0.9
Carcass							
GIT	0.06	0.05	0.04	0.04	0.05	0.05	0.01
Kidneys	0.02	0.02	0.01	0.01	0.01	0.01	0.01
Liver	0.13	0.15	0.10	0.12	0.11	0.12	0.02
Residual carcass	1.11	0.92	0.85	0.72	1.20	0.96	0.19
Total carcass	1.32	1.14	1.00	0.89	1.37	1.14	0.20
Total recovery	95.7	95.1	94.9	96.6	97.1	95.9	0.9

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 6

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	6♀	8♀	9♀	199♀	200♀	Mean	sd
Urine (hours)							
0 - 6	58.7	54.7	56.7	37.8	52.2	52.0	8.3
6 - 12	19.9	22.6	18.9	33.9	23.2	23.7	6.0
12 - 24	9.87	8.94	8.41	19.0	7.04	10.7	4.8
24 - 48	2.07	3.11	1.94	1.61	3.12	2.37	0.70
48 - 72	0.48	1.45	0.78	0.98	1.14	0.97	0.37
72 - 96	0.23	0.41	0.35	0.28	0.47	0.35	0.10
96 - 120	0.19	0.30	0.30	0.10	0.39	0.26	0.11
Cage wash	0.51	0.38	0.96	0.09	0.33	0.45	0.32
Total urine and cage wash	92.0	91.9	88.3	93.8	87.9	90.8	2.6
Expired air (hours)							
0 - 24	1.70	2.55	2.56	2.01	1.85	2.13	0.40
24 - 48	0.24	0.34	0.31	0.24	0.22	0.27	0.05
48 - 72	0.10	0.18	0.15	0.11	0.11	0.13	0.03
Total expired air	2.04	3.07	3.02	2.36	2.18	2.53	0.48
Faeces (hours)							
0 - 24	0.77	0.93	1.64	0.23	0.81	0.88	0.50
24 - 48	0.23	0.44	0.23	0.39	0.45	0.35	0.11
48 - 72	0.05	0.27	0.16	0.06	0.09	0.13	0.09
72 - 96	0.03	0.09	0.08	0.05	0.09	0.07	0.03
96 - 120	0.02	0.04	0.04	0.03	0.05	0.04	0.01
Total faeces	1.10	1.77	2.15	0.76	1.49	1.45	0.55
Total excreted	95.1	96.7	93.5	96.9	91.6	94.8	2.2
Carcass							
GIT	0.04	0.07	0.06	0.05	0.07	0.06	0.01
Kidneys	0.01	0.01	0.02	0.02	0.01	0.01	0.01
Liver	0.09	0.10	0.10	0.09	0.08	0.09	0.01
Residual carcass	0.80	2.28	2.13	1.08	2.27	1.71	0.71
Total carcass	0.94	2.46	2.31	1.24	2.43	1.88	0.73
Total recovery	96.0	99.2	95.8	98.1	94.0	96.6	2.0

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 7

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single intravenous dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	31♂	32♂	33♂	34♂	35♂	Mean	sd
Urine (hours)							
0 - 6	78.7	79.3	79.5	77.5	88.0	80.6	4.2
6 - 12	4.61	5.54	4.48	4.86	2.67	4.43	1.07
12 - 24	3.17	1.96	1.47	1.83	1.42	1.97	0.71
24 - 48	1.30	0.53	0.68	0.73	0.43	0.73	0.34
48 - 72	0.34	0.27	0.26	0.28	0.16	0.26	0.06
72 - 96	0.23	0.12	0.19	0.27	0.14	0.19	0.06
96 - 120	0.20	0.08	1.17	0.14	0.09	0.34	0.47
Cage wash	0.29	0.21	1.18	0.24	0.12	0.41	0.44
Total urine and cage wash	88.8	88.0	88.9	85.9	93.0	88.9	2.6
Expired air (hours)							
0 - 6	1.02	1.14	0.72	0.85	0.93	0.93	0.16
6 - 24	0.77	0.85	0.72	0.46	0.75	0.71	0.15
24 - 48	0.15	0.11	0.10	0.11	0.12	0.12	0.02
48 - 72	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	-
Total expired air	1.94	2.10	1.54	1.42	1.80	1.76	0.28
Faeces (hours)							
0 - 24	0.81	1.06	1.49	0.45	0.38	0.84	0.46
24 - 48	0.17	0.12	0.29	0.22	0.07	0.17	0.09
48 - 72	0.06	0.05	0.07	0.18	0.02	0.08	0.06
72 - 96	0.03	0.02	0.04	0.05	0.02	0.03	0.01
96 - 120	0.03	0.01	0.06	0.02	0.01	0.03	0.02
Total faeces	1.10	1.26	1.95	0.92	0.50	1.15	0.53
Total excreted	91.9	91.4	92.4	88.2	95.3	91.8	2.5
Carcass							
GIT	0.04	0.03	0.05	0.03	0.04	0.04	0.01
Kidneys	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Liver	0.11	0.13	0.10	0.10	0.12	0.11	0.01
Residual carcass	0.81	0.60	0.64	0.67	0.53	0.65	0.10
Total carcass	0.97	0.77	0.80	0.81	0.70	0.81	0.10
Total recovery	92.9	92.1	93.2	89.0	96.0	92.6	2.5

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 8

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single intravenous dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	36♀	37♀	38♀	39♀	40♀	Mean	sd
Urine (hours)							
0 - 6	76.6	84.8	80.2	80.8	79.7	80.4	2.9
6 - 12	3.53	3.38	5.83	3.92	4.93	4.32	1.04
12 - 24	2.89	1.66	1.74	2.11	1.86	2.05	0.50
24 - 48	1.42	1.14	0.70	1.40	1.22	1.18	0.29
48 - 72	0.39	0.40	0.32	0.64	0.31	0.41	0.13
72 - 96	0.29	0.24	0.11	0.64	0.26	0.31	0.20
96 - 120	0.19	0.18	0.08	0.54	0.32	0.26	0.18
Cage wash	0.42	0.32	0.21	0.88	0.37	0.44	0.26
Total urine and cage wash	85.7	92.1	89.2	90.9	89.0	89.4	2.4
Expired air (hours)							
0 - 6	0.69	1.09	0.89	1.06	0.72	0.89	0.19
6 - 24	0.69	0.54	0.77	0.40	0.51	0.58	0.15
24 - 48	0.15	0.16	0.14	0.14	0.17	0.15	0.01
48 - 72	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	-
Total expired air	1.53	1.79	1.80	1.60	1.40	1.62	0.17
Faeces (hours)							
0 - 24	1.08	1.11	0.66	0.84	0.73	0.88	0.20
24 - 48	0.37	0.22	0.24	0.34	0.13	0.26	0.10
48 - 72	0.13	0.07	0.08	0.09	0.10	0.09	0.02
72 - 96	0.05	0.03	0.03	0.19	0.04	0.07	0.07
96 - 120	0.05	0.03	0.03	0.07	0.05	0.05	0.02
Total faeces	1.68	1.46	1.04	1.53	1.05	1.35	0.29
Total excreted	88.9	95.4	92.0	94.1	91.4	92.4	2.5
Carcass							
GIT	0.04	0.04	0.05	0.06	0.09	0.06	0.02
Kidneys	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Liver	0.08	0.10	0.09	0.07	0.09	0.09	0.01
Residual carcass	0.80	0.67	0.72	1.24	0.63	0.81	0.25
Total carcass	0.93	0.82	0.87	1.38	0.82	0.96	0.24
Total recovery	89.9	96.2	92.9	95.4	92.2	93.3	2.5

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 9

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	41♂	42♂	43♂	44♂	45♂	Mean	sd
Urine (hours)							
0 - 6	8.08	3.14	3.19	3.47	4.87	4.55	2.10
6 - 24	3.07	2.95	1.72	3.77	2.78	2.86	0.74
24 - 48	0.25	0.28	0.19	0.21	0.44	0.27	0.10
48 - 72	0.12	0.13	0.09	0.11	0.15	0.12	0.02
72 - 96	0.07	0.08	0.06	0.05	0.08	0.07	0.01
96 - 120	0.05	0.04	0.03	0.04	0.09	0.05	0.02
Cage wash (hours)							
6	0.29	0.32	0.10	0.17	0.36	0.25	0.11
120	0.13	0.08	0.06	0.08	0.14	0.10	0.03
Total urine and cage wash	12.1	7.02	5.44	7.90	8.91	8.27	2.49
Faeces (hours)							
0 - 6	<0.01	0.33	0.02	0.02	0.15	0.11	-
6 - 24	0.07	0.04	0.04	0.03	0.09	0.05	0.03
24 - 48	0.04	0.03	0.03	0.02	0.05	0.03	0.01
48 - 72	0.02	0.01	<0.01	<0.01	0.02	0.01	-
72 - 96	<0.01	<0.01	<0.01	<0.01	0.02	0.01	-
96 - 120	0.02	<0.01	<0.01	<0.01	<0.02	0.01	-
Total faeces	0.15	0.41	0.09	0.07	0.33	0.21	0.15
Carcass							
GIT	0.01	<0.02	<0.02	<0.02	<0.01	0.02	-
Kidneys	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Liver	0.02	0.01	<0.01	0.01	0.02	0.01	-
Residual carcass	3.10	<0.14	<0.14	<0.14	0.45	0.79	-
Total carcass	3.13	0.01	<0.18	0.01	0.47	0.76	-
Total absorbed	15.3	7.44	5.53	7.98	9.71	9.19	3.73
Skin wash (6 hours)	45.9	73.2	74.4	60.2	58.9	62.5	11.7
Dressing extracts							
6 hours	3.25	0.04	0.03	9.14	0.06	2.50	3.96
120 hours	1.49	0.56	0.64	0.69	1.24	0.92	0.41
Treated skin	26.7	12.0	14.6	14.4	18.9	17.3	5.8
Total recovery	92.7	93.2	95.2	92.4	88.8	92.5	2.3

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 10

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	46♀	47♀	48♀	49♀	50♀	Mean	sd
Urine (hours)							
0 - 6	6.29	6.03	6.19	6.31	2.73	5.51	1.56
6 - 24	2.72	2.09	2.00	2.11	2.01	2.19	0.30
24 - 48	0.35	0.25	0.23	0.22	0.34	0.28	0.06
48 - 72	0.16	0.10	0.09	0.10	0.12	0.11	0.03
72 - 96	0.10	0.06	0.06	0.05	0.07	0.07	0.02
96 - 120	0.17	0.07	0.05	0.04	0.05	0.08	0.05
Cage wash (hours)							
6	0.84	0.77	1.22	0.53	1.17	0.91	0.29
120	0.24	0.07	0.08	0.10	0.11	0.12	0.07
Total urine and cage wash	10.9	9.44	9.92	9.46	6.60	9.26	1.60
Faeces (hours)							
0 - 6	0.66	<0.01	0.55	0.55	0.46	0.45	-
6 - 24	0.11	0.02	0.01	0.03	0.03	0.04	0.04
24 - 48	0.07	0.04	0.04	0.02	0.06	0.05	0.02
48 - 72	0.01	0.02	0.02	0.02	0.02	0.02	<0.01
72 - 96	0.01	0.01	0.01	<0.01	0.01	0.01	-
96 - 120	0.09	0.02	<0.01	<0.01	0.01	0.03	-
Total faeces	0.95	0.11	0.63	0.62	0.59	0.58	0.30
Carcass							
GIT	0.08	0.03	0.01	0.01	<0.01	0.03	-
Kidneys	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Liver	0.01	0.02	0.01	0.01	0.01	0.01	<0.01
Residual carcass	1.96	0.67	0.33	0.27	0.36	0.72	0.71
Total carcass	2.05	0.72	0.35	0.29	0.37	0.76	0.74
Total absorbed	13.9	10.3	10.9	10.4	7.56	10.6	2.3
Skin wash (6 hours)	52.9	66.2	59.6	65.5	66.2	62.1	5.8
Dressing extracts							
6 hours	4.64	0.03	2.89	0.11	3.96	2.33	2.15
120 hours	1.59	0.39	0.79	0.94	0.45	0.83	0.48
Treated skin	17.8	8.58	14.7	13.0	12.2	13.3	3.4
Total recovery	90.8	85.5	88.9	89.9	90.4	89.1	2.1

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 11

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	R51♂	R52♂	R53♂	R54♂	R55♂	Mean	sd
Urine (hours)							
0 - 6	0.45	0.82	0.58	0.80	0.60	0.65	0.16
6 - 24	0.43	0.26	0.25	0.36	0.24	0.31	0.08
24 - 48	0.06	0.04	0.02	0.04	0.07	0.05	0.02
48 - 72	0.02	0.01	0.01	0.02	0.03	0.02	0.01
72 - 96	0.01	0.01	0.01	0.01	0.01	0.01	0.00
96 - 120	0.01	0.01	<0.01	0.01	0.01	0.01	-
Cage wash (hours)							
6	0.12	0.07	0.04	0.03	0.07	0.07	0.04
120	0.02	0.02	0.01	0.02	0.03	0.02	0.01
Total urine and cage wash	1.12	1.24	0.92	1.29	1.06	1.13	0.15
Faeces (hours)							
0 - 6	<0.01	0.09	0.02	<0.01	0.01	0.03	-
6 - 24	0.01	0.01	0.01	<0.01	0.02	0.01	-
24 - 48	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
48 - 72	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
72 - 96	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
96 - 120	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Total faeces	0.01	0.10	0.03	<0.06	0.03	0.05	-
Carcass							
GIT	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Kidneys	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Liver	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Residual carcass	0.10	<0.08	<0.08	<0.08	<0.08	0.08	-
Total carcass	0.10	<0.11	<0.11	<0.11	<0.11	0.11	-
Total absorbed	1.23	1.34	0.95	1.29	1.09	1.18	0.16
Skin wash (6 hours)	82.2	81.5	86.9	84.0	85.7	84.1	2.3
Dressing extracts							
6 hours	0.07	1.97	2.87	0.25	0.36	1.10	1.25
120 hours	0.25	0.36	0.19	0.33	0.11	0.25	0.10
Treated skin	3.70	4.17	3.15	3.46	3.78	3.65	0.38
Total recovery	87.5	89.3	94.1	89.3	91.0	90.2	2.5

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 12

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	R56♀	R57♀	R58♀	R59♀	R60♀	Mean	sd
Urine (hours)							
0 - 6	0.49	1.03	0.41	0.54	0.89	0.67	0.27
6 - 24	0.53	0.37	0.21	0.17	0.38	0.33	0.14
24 - 48	0.06	0.23	0.06	0.04	0.08	0.09	0.08
48 - 72	0.02	0.16	0.02	0.03	0.03	0.05	0.06
72 - 96	0.02	0.12	0.02	0.02	0.02	0.04	0.04
96 - 120	0.02	0.09	0.02	0.02	0.02	0.03	0.03
Cage wash (hours)							
6	0.07	0.12	0.02	0.03	0.06	0.06	0.04
120	0.01	0.11	0.01	0.04	0.03	0.04	0.04
Total urine and cage wash	1.22	2.23	0.77	0.89	1.51	1.32	0.58
Faeces (hours)							
0 - 6	NS	0.09	NS	0.03	0.35	0.16	0.17
6 - 24	0.01	0.02	<0.01	0.01	0.01	0.01	-
24 - 48	0.01	0.01	<0.01	0.01	0.01	0.01	-
48 - 72	<0.01	0.01	<0.01	<0.01	<0.01	0.01	-
72 - 96	<0.01	0.01	<0.01	<0.01	<0.01	0.01	-
96 - 120	<0.01	0.01	<0.01	0.01	<0.01	0.01	-
Total faeces	0.02	0.15	<0.05	0.06	0.37	0.13	-
Carcass							
GIT	<0.01	0.03	<0.01	0.01	<0.01	0.01	-
Kidneys	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Liver	<0.01	0.01	<0.01	<0.01	<0.01	0.01	-
Residual carcass	0.08	0.59	0.11	0.15	<0.08	0.20	-
Total carcass	0.08	0.63	0.11	0.16	<0.11	0.22	-
Total absorbed	1.32	3.01	0.88	1.11	1.88	1.64	0.85
Skin wash (6 hours)	76.6	79.7	91.5	82.1	88.4	83.7	6.2
Dressing extracts							
6 hours	5.88	0.21	0.06	10.4	0.23	3.36	4.65
120 hours	0.13	0.34	0.19	0.19	0.55	0.28	0.17
Treated skin	1.89	2.23	2.04	2.09	2.67	2.18	0.30
Total recovery	85.8	85.5	94.7	95.9	93.7	91.1	5.1

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation
NS no sample

APPENDIX 13

(continued)

TABLE 13

Excretion and retention of radioactivity in male rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	127♂	128♂	129♂	Mean	sd
Bile (hours)					
0 - 3	0.39	0.60	1.64	0.88	0.67
3 - 6	1.69	1.07	0.95	1.24	0.40
6 - 12	1.60	1.39	1.17	1.39	0.22
12 - 24	0.43	0.89	0.17	0.50	0.36
24 - 48	0.04	0.07	0.05	0.05	0.02
Total bile	4.15	4.02	3.98	4.05	0.09
Urine (hours)					
0 - 24	77.1	84.2	72.5	77.9	5.9
24 - 48	4.65	2.39	3.31	3.45	1.14
Cage wash	1.25	0.49	2.55	1.43	1.04
Total urine and cage wash	83.0	87.1	78.4	82.8	4.4
Faeces (hours)					
0 - 24	0.66	0.48	0.16	0.43	0.25
24 - 48	1.07	0.52	1.32	0.97	0.41
Total faeces	1.73	1.00	1.48	1.40	0.37
Carcass					
GIT	0.12	0.09	0.11	0.11	0.02
Liver	0.34	0.27	0.26	0.29	0.04
Residual carcass	2.61	1.68	3.53	2.61	0.93
Total carcass	3.07	2.04	3.90	3.00	0.93
Total recovery	92.0	94.1	87.7	91.3	3.3

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 14

Excretion and retention of radioactivity in female rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	130♀	131♀	132♀	Mean	sd
Bile (hours)					
0 - 3	1.35	1.49	1.03	1.29	0.24
3 - 6	0.74	1.26	1.11	1.04	0.27
6 - 12	1.08	0.68	1.02	0.93	0.22
12 - 24	0.65	0.25	0.15	0.35	0.26
24 - 48	0.15	0.04	0.03	0.07	0.07
Total bile	3.97	3.72	3.34	3.68	0.32
Urine (hours)					
0 - 24	78.9	81.7	61.4	74.0	11.0
24 - 48	4.50	2.06	11.3	5.95	4.79
Cage wash	0.73	0.82	3.30	1.62	1.46
Total urine and cage wash	84.1	84.6	76.0	81.6	4.8
Faeces (hours)					
0 - 24	3.67	0.16	0.62	1.48	1.91
24 - 48	0.67	1.25	3.55	1.82	1.52
Total faeces	4.34	1.41	4.17	3.31	1.64
Carcass					
GIT	0.12	0.09	0.07	0.09	0.03
Liver	0.18	0.23	0.18	0.20	0.03
Residual carcass	1.52	1.60	2.85	1.99	0.75
Total carcass	1.82	1.92	3.10	2.28	0.71
Total recovery	94.3	91.6	86.6	90.8	3.9

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 15

Excretion and retention of radioactivity in male rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	121♂	122♂	123♂	Mean	sd
Bile (hours)					
0 - 3	0.07	0.45	1.05	0.52	0.49
3 - 6	0.47	0.82	0.89	0.73	0.23
6 - 12	1.91	1.23	1.22	1.45	0.40
12 - 24	1.08	0.99	0.96	1.01	0.06
24 - 48	0.09	0.39	0.58	0.35	0.25
Total bile	3.62	3.88	4.70	4.07	0.56
Urine (hours)					
0 - 24	84.1	68.1	62.7	71.6	11.1
24 - 48	2.53	14.1	10.7	9.11	5.95
Cage wash	1.11	1.18	3.22	1.84	1.20
Total urine and cage wash	87.7	83.4	76.6	82.6	5.6
Faeces (hours)					
0 - 24	0.52	1.11	0.02	0.51	0.55
24 - 48	0.72	1.50	3.29	1.84	1.32
Total faeces	1.24	2.61	3.31	2.39	1.05
Carcass					
GIT	0.08	0.17	0.47	0.24	0.20
Liver	0.19	0.27	0.26	0.24	0.04
Residual carcass	1.59	2.26	3.02	2.29	0.72
Total carcass	1.86	2.70	3.75	2.77	0.95
Total recovery	94.5	92.6	88.4	91.8	3.1

Results are expressed as % dose
GIT gastrointestinal tract and contents
sd standard deviation

APPENDIX 13

(continued)

TABLE 16

Excretion and retention of radioactivity in female rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	124♀	125♀	126♀	Mean*
Bile (hours)				
0 - 3	1.12	2.34	4.17	2.65
3 - 6	1.59	1.82	0.96	1.28
6 - 12	0.73	0.77	0.26	0.50
12 - 24	0.12	0.39	0.03	0.08
24 - 48	0.03	0.07	0.02	0.03
Total bile	3.59	5.39	5.44	4.52
Urine (hours)				
0 - 24	83.5	26.9	83.5	83.5
24 - 48	2.40	a	1.41	1.91
Cage wash	2.20	13.3	0.97	1.59
Total urine and cage wash	88.1	40.2	85.9	87.0
Faeces (hours)				
0 - 24	1.78	0.41	1.71	1.75
24 - 48	0.95	a	0.93	0.94
Total faeces	2.73	0.41	2.64	2.69
Carcass				
GIT	0.07	1.02	0.05	0.06
Liver	0.14	0.56	0.12	0.13
Residual carcass	2.03	31.0	1.72	1.88
Total carcass	2.24	32.6	1.89	2.07
Total recovery	96.7	78.6	95.9	96.3

Results are expressed as % dose
GIT gastrointestinal tract and contents
* rat 125♀ excluded
a rat 125♀ was sacrificed after 29 hours: no sample collected

APPENDIX 13

(continued)

TABLE 17

Cumulative excretion of radioactivity in bile by male rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	127♂	128♂	129♂	Mean	sd
Time (hours)					
0 - 3	0.39	0.60	1.64	0.88	0.67
0 - 6	2.08	1.67	2.59	2.11	0.46
0 - 12	3.68	3.06	3.76	3.50	0.38
0 - 24	4.11	3.95	3.93	4.00	0.10
0 - 48	4.15	4.02	3.98	4.05	0.09

Results are expressed as cumulative % dose
sd standard deviation

APPENDIX 13

(continued)

TABLE 18

Cumulative excretion of radioactivity in bile by female rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	130♀	131♀	132♀	Mean	sd
Time (hours)					
0 - 3	1.35	1.49	1.03	1.29	0.24
0 - 6	2.09	2.75	2.14	2.33	0.37
0 - 12	3.17	3.43	3.16	3.25	0.15
0 - 24	3.82	3.68	3.31	3.60	0.26
0 - 48	3.97	3.72	3.34	3.68	0.32

Results are expressed as cumulative % dose
sd standard deviation

APPENDIX 13

(continued)

TABLE 19

Cumulative excretion of radioactivity in bile by male rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	121♂	122♂	123♂	Mean	sd
Time (hours)					
0 - 3	0.07	0.45	1.05	0.52	0.49
0 - 6	0.54	1.27	1.94	1.25	0.70
0 - 12	2.45	2.50	3.16	2.70	0.40
0 - 24	3.53	3.49	4.12	3.71	0.35
0 - 48	3.62	3.88	4.70	4.07	0.56

Results are expressed as cumulative % dose
sd standard deviation

APPENDIX 13

(continued)

TABLE 20

Cumulative excretion of radioactivity in bile by female rats with cannulated bile ducts after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	124♀	125♀	126♀	Mean	sd
Time (hours)					
0 - 3	1.12	2.34	4.17	2.54	1.54
0 - 6	2.71	4.16	5.13	4.00	1.22
0 - 12	3.44	4.93	5.39	4.59	1.02
0 - 24	3.56	5.32	5.42	4.77	1.05
0 - 48	3.59	5.39[a]	5.44	4.81	1.05

Results are expressed as cumulative % dose
sd standard deviation
a rat 125♀ was sacrificed after 29 hours

APPENDIX 13

(continued)

TABLE 21

Concentrations of radioactivity in plasma of male rats
after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	79♂	80♂	81♂	85♂	86♂	87♂	91♂	92♂	93♂	Mean	sd
Group	1	1	1	2	2	2	3	3	3		
Time (hours)											
0.25	-	-	-	4.98	8.96	5.69	-	-	-	6.54	2.12
0.5	-	-	-	-	-	-	6.55	10.2	9.11	8.62	1.87
1	3.89	4.38	5.47	-	-	-	-	-	-	4.58	0.81
2	-	-	-	3.36	2.77	5.30	-	-	-	3.81	1.32
4	-	-	-	-	-	-	1.83	4.42	2.71	2.99	1.32
6	0.88	0.66	1.37	-	-	-	-	-	-	0.97	0.36
12	-	-	-	0.34	1.13	0.48	-	-	-	0.65	0.42
24	-	-	-	-	-	-	0.20	0.80	0.55	0.52	0.30
48	0.12	0.16	0.14	-	-	-	-	-	-	0.14	0.02
72	-	-	-	0.07	0.23	0.15	-	-	-	0.15	0.08
96	-	-	-	-	-	-	0.06	0.09	0.11	0.09	0.03
120	0.03	0.04	0.04	-	-	-	-	-	-	0.04	0.01
144	-	-	-	0.04	0.11	0.05	-	-	-	0.07	0.04
168	-	-	-	-	-	-	0.03	0.10	0.03	0.05	0.04

Results are expressed as mg dimethoate equivalents/l
sd standard deviation

APPENDIX 13

(continued)

TABLE 22

Concentrations of radioactivity in plasma of female rats
after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	82♀	83♀	84♀	88♀	89♀	90♀	94♀	95♀	96♀	Mean	sd
Group	1	1	1	2	2	2	3	3	3		
Time (hours)											
0.25	-	-	-	4.56	6.07	7.26	-	-	-	5.96	1.35
0.5	-	-	-	-	-	-	8.72	7.24	7.07	7.68	0.91
1	4.59	6.30	5.21	-	-	-	-	-	-	5.37	0.87
2	-	-	-	3.18	3.00	3.23	-	-	-	3.14	0.12
4	-	-	-	-	-	-	1.31	1.68	1.37	1.45	0.20
6	0.71	0.81	0.89	-	-	-	-	-	-	0.80	0.09
12	-	-	-	0.30	0.43	0.52	-	-	-	0.42	0.11
24	-	-	-	-	-	-	0.64	0.62	0.24	0.50	0.23
48	0.19	0.19	0.24	-	-	-	-	-	-	0.21	0.03
72	-	-	-	0.11	0.10	0.14	-	-	-	0.12	0.02
96	-	-	-	-	-	-	0.20	0.43	0.19	0.27	0.14
120	0.06	0.09	0.11	-	-	-	-	-	-	0.09	0.03
144	-	-	-	0.04	0.04	0.08	-	-	-	0.05	0.02
168	-	-	-	-	-	-	0.07	0.19	0.07	0.11	0.07

Results are expressed as mg dimethoate equivalents/l
sd standard deviation

APPENDIX 13

(continued)

TABLE 23

Concentrations of radioactivity in plasma of male rats
after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	61♂	62♂	63♂	67♂	68♂	69♂	73♂	74♂	75♂	Mean	sd
Group	1	1	1	2	2	2	3	3	3		
Time (hours)											
0.25	-	-	-	64.0	56.2	32.0	-	-	-	50.7	16.7
0.5	-	-	-	-	-	-	40.6	45.3	45.0	43.6	2.6
1	30.6	38.5	25.3	-	-	-	-	-	-	31.5	6.6
2	-	-	-	14.5	13.1	19.4	-	-	-	15.7	3.3
4	-	-	-	-	-	-	3.61	4.95	5.01	4.52	0.79
6	10.9	12.5	11.3	-	-	-	-	-	-	11.6	0.8
12	-	-	-	7.98	7.53	5.35	-	-	-	6.95	1.41
24	-	-	-	-	-	-	3.09	5.37	4.49	4.32	1.15
48	1.54	5.03	1.94	-	-	-	-	-	-	2.84	1.91
72	-	-	-	1.55	1.26	1.27	-	-	-	1.36	0.16
96	-	-	-	-	-	-	0.75	0.68	0.68	0.70	0.04
120	0.52	2.38	0.61	-	-	-	-	-	-	1.17	1.05
144	-	-	-	0.42	0.38	0.44	-	-	-	0.41	0.03
168	-	-	-	-	-	-	0.22	0.35	0.29	0.29	0.07

Results are expressed as mg dimethoate equivalents/l
sd standard deviation

APPENDIX 13

(continued)

TABLE 24

Concentrations of radioactivity in plasma of female rats after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	64♀	65♀	66♀	70♀	71♀	72♀	76♀	77♀	78♀	Mean	sd
Group	1	1	1	2	2	2	3	3	3		
Time (hours)											
0.25	-	-	-	75.0	59.7	57.2	-	-	-	64.0	9.6
0.5	-	-	-	-	-	-	76.2	109	94.3	93.2	16.4
1	67.9	44.4	30.4	-	-	-	-	-	-	47.6	18.9
2	-	-	-	14.9	16.5	26.1	-	-	-	19.2	6.1
4	-	-	-	-	-	-	6.11	10.6	7.53	8.08	2.29
6	11.7	27.8	15.7	-	-	-	-	-	-	18.4	8.4
12	-	-	-	12.6	6.46	10.7	-	-	-	9.92	3.14
24	-	-	-	-	-	-	5.83	8.31	4.77	6.30	1.82
48	3.71	3.38	5.30	-	-	-	-	-	-	4.13	1.03
72	-	-	-	3.21	2.12	2.91	-	-	-	2.75	0.56
96	-	-	-	-	-	-	2.42	2.77	1.05	2.08	0.91
120	2.47	0.67	2.03	-	-	-	-	-	-	1.72	0.94
144	-	-	-	0.66	0.63	2.01	-	-	-	1.10	0.79
168	-	-	-	-	-	-	0.74	0.99	0.72	0.82	0.15

Results are expressed as mg dimethoate equivalents/l
sd standard deviation

APPENDIX 13

(continued)

TABLE 25

Concentrations of radioactivity in the tissues of rats sacrificed 0.5 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	161♂	162♂	163♂	Mean (♂)	164♀	165♀	166♀	Mean (♀)
Adrenal glands	1.74	2.34	2.58	2.22	3.02	5.00	4.68	4.23
Bone	0.85	1.34	1.16	1.12	1.15	1.53	1.48	1.39
Bone marrow	1.34	1.72	1.77	1.61	2.30	3.07	3.76	3.04
Brain	0.49	0.72	0.74	0.65	0.88	1.66	1.53	1.36
Fat	0.76	0.90	1.31	0.99	1.01	1.17	1.12	1.10
Heart	2.24	2.91	2.64	2.60	3.11	3.56	3.73	3.47
Intestines and contents	5.55	5.31	7.73	6.20	6.34	5.70	8.08	6.71
Kidneys	15.5	22.9	21.5	20.0	26.1	20.8	26.9	24.6
Liver	7.79	9.17	8.74	8.57	9.83	12.7	12.7	11.7
Lungs	2.70	3.76	3.38	3.28	4.51	5.76	6.13	5.47
Muscle	0.95	1.29	1.32	1.19	1.48	2.06	2.14	1.89
Ovaries	-	-	-	-	3.16	3.90	4.26	3.77
Pancreas	2.42	3.79	2.62	2.94	3.29	5.29	4.41	4.33
Skin	1.86	2.31	2.56	2.24	3.26	3.49	3.92	3.56
Spleen	1.43	1.86	2.00	1.76	2.32	3.27	3.19	2.93
Stomach and contents	157	130	150	146	180	203	152	178
Testes	1.30	1.88	1.99	1.72	-	-	-	-
Thyroid gland	1.88	2.15	1.95	1.99	2.80	3.69	3.44	3.31
Uterus	-	-	-	-	2.91	3.45	4.21	3.52
Whole-blood	4.37	5.25	4.49	4.70	5.83	5.94	6.46	6.08
Plasma	5.71	6.68	5.89	6.09	7.68	7.43	8.31	7.81

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 26

Concentrations of radioactivity in the tissues of rats sacrificed 2 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	167♂	168♂	169♂	Mean (♂)	170♀	171♀	172♀	Mean (♀)
Adrenal glands	1.11	0.97	1.51	1.20	2.63	1.78	1.09	1.83
Bone	0.76	0.63	0.52	0.64	0.70	1.77	0.48	0.98
Bone marrow	0.98	0.85	0.75	0.86	1.41	1.59	0.94	1.31
Brain	0.43	0.39	0.34	0.39	0.86	0.70	0.51	0.69
Fat	0.52	0.49	2.47	1.16	0.83	0.55	0.40	0.59
Heart	1.52	1.22	1.04	1.26	2.03	1.90	1.33	1.75
Intestines and contents	4.78	4.70	4.70	4.73	5.61	9.39	5.10	6.70
Kidneys	7.72	5.79	8.16	7.22	8.94	9.70	5.09	7.91
Liver	7.23	5.44	5.66	6.11	8.45	8.19	5.94	7.53
Lungs	1.94	1.85	1.40	1.73	3.42	2.58	1.75	2.58
Muscle	0.69	0.59	0.51	0.60	1.01	0.97	0.67	0.88
Ovaries	-	-	-	-	1.95	1.77	1.19	1.64
Pancreas	1.61	1.42	2.45	1.83	2.27	2.47	1.51	2.08
Skin	1.15	1.00	0.92	1.02	1.68	1.67	1.23	1.53
Spleen	1.02	0.90	0.87	0.93	1.61	1.47	0.98	1.35
Stomach and contents	97.2	66.2	85.7	83.0	117	47.7	81.3	82.0
Testes	1.03	1.03	1.36	1.14	-	-	-	-
Thyroid gland	1.22	0.85	0.76	0.94	2.24	1.74	0.94	1.64
Uterus	-	-	-	-	1.97	2.00	1.23	1.73
Whole-blood	1.98	1.46	1.44	1.63	2.42	2.38	1.45	2.08
Plasma	2.55	2.20	1.84	2.20	3.06	3.05	1.83	2.65

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 27

Concentrations of radioactivity in the tissues of rats sacrificed 48 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	173♂	174♂	175♂	Mean (♂)	176♀	177♀	178♀	Mean (♀)
Adrenal glands	0.18	0.15	0.18	0.17	0.30	0.20	0.22	0.24
Bone	0.08	0.08	0.09	0.08	0.14	0.13	0.11	0.13
Bone marrow	0.21	0.16	0.21	0.19	0.30	0.23	0.26	0.26
Brain	0.05	0.03	0.04	0.04	0.06	0.04	0.06	0.05
Fat	0.07	0.07	0.06	0.07	0.07	0.06	0.06	0.06
Heart	0.11	0.09	0.11	0.10	0.14	0.10	0.13	0.12
Intestines and contents	0.17	0.17	0.19	0.18	0.97	0.62	0.84	0.81
Kidneys	0.33	0.29	0.29	0.30	0.40	0.29	0.37	0.35
Liver	0.56	0.54	0.50	0.53	0.70	0.53	0.67	0.63
Lungs	0.18	0.14	0.15	0.16	0.21	0.17	0.22	0.20
Muscle	0.07	0.06	0.07	0.07	0.10	0.06	0.08	0.08
Ovaries	-	-	-	-	0.21	0.13	0.18	0.17
Pancreas	0.32	0.25	0.27	0.28	0.55	0.34	0.45	0.45
Skin	0.14	0.14	0.13	0.14	0.25	0.33	0.30	0.29
Spleen	0.18	0.15	0.17	0.17	0.19	0.17	0.18	0.18
Stomach and contents	0.08	0.08	0.08	0.08	0.23	0.20	0.33	0.25
Testes	0.10	0.07	0.08	0.08	-	-	-	-
Thyroid gland	0.16	0.14	0.15	0.15	0.42	0.19	0.21	0.27
Uterus	-	-	-	-	0.22	0.09	0.20	0.17
Whole-blood	0.11	0.09	0.10	0.10	0.14	0.11	0.14	0.13
Plasma	0.08	0.08	0.08	0.08	0.13	0.09	0.13	0.12

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 28

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 0.5 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	161♂	162♂	163♂	Mean (♂)	164♀	165♀	166♀	Mean (♀)
Adrenal glands	0.31	0.35	0.44	0.37	0.39	0.67	0.56	0.54
Bone	0.15	0.20	0.20	0.18	0.15	0.21	0.18	0.18
Bone marrow	0.23	0.26	0.30	0.26	0.30	0.41	0.45	0.39
Brain	0.09	0.11	0.13	0.11	0.11	0.22	0.18	0.17
Fat	0.13	0.14	0.22	0.16	0.13	0.16	0.13	0.14
Heart	0.39	0.44	0.45	0.43	0.40	0.48	0.45	0.44
Intestines and contents	0.97	0.79	1.31	1.02	0.83	0.77	0.97	0.86
Kidneys	2.72	3.43	3.64	3.26	3.40	2.80	3.23	3.14
Liver	1.36	1.37	1.48	1.40	1.28	1.70	1.52	1.50
Lungs	0.47	0.56	0.57	0.53	0.59	0.78	0.74	0.70
Muscle	0.17	0.19	0.22	0.19	0.19	0.28	0.26	0.24
Ovaries	-	-	-	-	0.41	0.52	0.51	0.48
Pancreas	0.42	0.57	0.44	0.48	0.43	0.71	0.53	0.56
Skin	0.33	0.35	0.43	0.37	0.42	0.47	0.47	0.45
Spleen	0.25	0.28	0.34	0.29	0.30	0.44	0.38	0.37
Stomach and contents	27.5	19.5	25.4	24.1	23.5	27.4	18.2	23.0
Testes	0.23	0.28	0.34	0.28	-	-	-	-
Thyroid gland	0.33	0.32	0.33	0.33	0.36	0.50	0.41	0.42
Uterus	-	-	-	-	0.38	0.46	0.51	0.45
Whole-blood	0.76	0.79	0.76	0.77	0.76	0.80	0.78	0.78
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 29

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 2 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	167♂	168♂	169♂	Mean (♂)	170♀	171♀	172♀	Mean (♀)
Adrenal glands	0.44	0.44	0.82	0.57	0.86	0.58	0.60	0.68
Bone	0.30	0.29	0.28	0.29	0.23	0.58	0.26	0.36
Bone marrow	0.38	0.38	0.41	0.39	0.46	0.52	0.51	0.50
Brain	0.17	0.18	0.18	0.18	0.28	0.23	0.28	0.26
Fat	0.21	0.22	1.35	0.59	0.27	0.18	0.22	0.22
Heart	0.60	0.56	0.57	0.58	0.66	0.62	0.72	0.67
Intestines and contents	1.87	2.14	2.56	2.19	1.83	3.08	2.78	2.56
Kidneys	3.02	2.63	4.45	3.37	2.92	3.18	2.78	2.96
Liver	2.83	2.47	3.08	2.79	2.76	2.68	3.24	2.89
Lungs	0.76	0.84	0.76	0.79	1.12	0.84	0.95	0.97
Muscle	0.27	0.27	0.28	0.27	0.33	0.32	0.36	0.34
Ovaries	-	-	-	-	0.64	0.58	0.65	0.62
Pancreas	0.63	0.64	1.33	0.87	0.74	0.81	0.82	0.79
Skin	0.45	0.45	0.50	0.47	0.55	0.55	0.67	0.59
Spleen	0.40	0.41	0.47	0.43	0.53	0.48	0.54	0.52
Stomach and contents	38.1	30.1	46.7	38.3	38.2	15.6	44.4	32.7
Testes	0.40	0.47	0.74	0.54	-	-	-	-
Thyroid gland	0.48	0.39	0.42	0.43	0.73	0.57	0.51	0.60
Uterus	-	-	-	-	0.64	0.66	0.67	0.66
Whole-blood	0.77	0.66	0.78	0.74	0.79	0.78	0.79	0.79
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 30

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 48 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	173♂	174♂	175♂	Mean (♂)	176♀	177♀	178♀	Mean (♀)
Adrenal glands	2.16	1.81	2.26	2.08	2.33	2.19	1.68	2.07
Bone	0.98	0.96	1.08	1.01	1.09	1.45	0.84	1.13
Bone marrow	2.50	2.00	2.56	2.35	2.29	2.49	1.99	2.26
Brain	0.55	0.40	0.45	0.47	0.46	0.46	0.45	0.46
Fat	0.80	0.80	0.77	0.79	0.55	0.64	0.47	0.55
Heart	1.35	1.08	1.41	1.28	1.07	1.15	0.99	1.07
Intestines and contents	2.09	2.11	2.31	2.17	7.51	6.84	6.46	6.94
Kidneys	3.94	3.51	3.57	3.67	3.07	3.20	2.83	3.03
Liver	6.80	6.58	6.25	6.54	5.40	5.88	5.18	5.49
Lungs	2.17	1.75	1.90	1.94	1.59	1.86	1.67	1.71
Muscle	0.90	0.76	0.86	0.84	0.76	0.67	0.66	0.70
Ovaries	-	-	-	-	1.59	1.46	1.42	1.49
Pancreas	3.83	3.07	3.37	3.42	4.25	3.74	3.44	3.81
Skin	1.68	1.70	1.62	1.67	1.97	3.62	2.35	2.65
Spleen	2.22	1.78	2.13	2.04	1.50	1.84	1.39	1.58
Stomach and contents	0.98	0.94	1.00	0.97	1.80	2.24	2.58	2.21
Testes	1.25	0.86	1.02	1.04	-	-	-	-
Thyroid gland	1.89	1.66	1.80	1.78	3.27	2.10	1.61	2.33
Uterus	-	-	-	-	1.72	0.97	1.58	1.42
Whole-blood	1.38	1.10	1.18	1.22	1.08	1.22	1.09	1.13
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 31

Concentrations of radioactivity in the tissues of rats sacrificed 0.5 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	179♂	180♂	181♂	Mean (♂)	182♀	183♀	184♀	Mean (♀)
Adrenal glands	2.95	2.83	5.31	3.70	6.64	6.03	6.36	6.34
Bone	1.72	1.90	1.90	1.84	2.45	2.25	2.10	2.27
Bone marrow	2.60	2.60	3.29	2.83	4.56	3.95	4.37	4.29
Brain	1.15	0.99	2.29	1.48	2.38	2.06	2.32	2.25
Fat	1.51	1.34	1.84	1.56	1.52	1.76	1.50	1.59
Heart	3.64	3.67	3.68	3.66	4.67	4.74	4.68	4.70
Intestines and contents	5.23	7.25	15.7	9.39	9.66	14.1	8.55	10.8
Kidneys	26.2	20.8	16.1	21.0	30.2	31.5	22.5	28.1
Liver	11.8	13.9	12.6	12.8	11.5	14.6	13.5	13.2
Lungs	4.71	4.51	5.59	4.94	7.19	7.10	6.71	7.00
Muscle	1.74	1.65	2.44	1.94	2.85	2.77	3.04	2.89
Ovaries	-	-	-	-	5.46	5.23	4.70	5.13
Pancreas	4.59	5.12	6.36	5.36	7.06	7.87	7.35	7.43
Skin	2.89	3.19	3.19	3.09	5.16	4.71	4.79	4.89
Spleen	2.63	2.80	3.70	3.04	4.25	4.55	4.43	4.41
Stomach and contents	144	273	334	250	159	346	214	240
Testes	2.24	1.98	3.64	2.62	-	-	-	-
Thyroid gland	3.68	2.89	3.87	3.48	4.94	4.56	4.38	4.63
Uterus	-	-	-	-	4.98	5.19	5.13	5.10
Whole-blood	5.97	5.96	5.16	5.70	7.46	8.17	6.79	7.47
Plasma	7.20	7.23	5.18	6.54	9.05	10.1	7.74	8.96

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 32

Concentrations of radioactivity in the tissues of rats sacrificed 2 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	185♂	186♂	187♂	Mean (♂)	188♀	189♀	190♀	Mean (♀)
Adrenal glands	5.37	2.93	2.21	3.50	3.05	2.46	3.16	2.89
Bone	2.41	1.36	1.13	1.63	1.17	0.99	1.13	1.10
Bone marrow	4.34	2.52	1.91	2.92	2.24	2.01	2.40	2.22
Brain	3.03	1.45	1.09	1.86	1.14	0.99	1.20	1.11
Fat	1.79	1.14	0.95	1.29	0.68	0.77	0.84	0.76
Heart	4.77	2.58	1.99	3.11	2.14	1.91	2.21	2.09
Intestines and contents	9.22	7.05	6.35	7.54	9.11	7.43	8.82	8.45
Kidneys	33.2	13.4	9.75	18.8	7.88	5.30	10.9	8.03
Liver	19.0	11.7	9.46	13.4	8.98	7.99	8.39	8.45
Lungs	6.35	4.28	3.35	4.66	3.31	2.65	3.26	3.07
Muscle	2.82	1.64	1.26	1.91	1.36	1.12	1.22	1.23
Ovaries	-	-	-	-	2.30	1.95	2.31	2.19
Pancreas	6.75	4.18	4.34	5.09	4.72	5.09	4.42	4.74
Skin	4.07	2.33	1.81	2.74	2.56	1.98	2.49	2.34
Spleen	4.80	2.65	2.11	3.19	2.38	2.07	2.44	2.30
Stomach and contents	38.2	87.9	127	84.4	70.2	48.1	83.3	67.2
Testes	4.98	2.71	2.13	3.27	-	-	-	-
Thyroid gland	5.50	2.57	2.15	3.41	2.78	2.09	3.16	2.68
Uterus	-	-	-	-	2.49	1.86	2.27	2.21
Whole-blood	6.63	3.32	2.54	4.16	2.65	2.10	2.56	2.44
Plasma	6.95	3.49	2.64	4.36	2.81	2.24	2.61	2.55

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 33

Concentrations of radioactivity in the tissues of rats sacrificed 48 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	191♂	192♂	193♂	Mean (♂)	194♀	195♀	196♀	Mean (♀)
Adrenal glands	0.72	0.84	0.88	0.81	1.02	1.33	1.28	1.21
Bone	0.27	0.34	0.34	0.32	0.42	0.46	0.36	0.41
Bone marrow	0.62	0.74	0.74	0.70	0.96	1.23	0.91	1.03
Brain	0.22	0.29	0.29	0.27	0.30	0.34	0.33	0.32
Fat	0.37	0.42	0.59	0.46	0.30	0.37	0.32	0.33
Heart	0.52	0.55	0.65	0.57	0.68	0.74	0.72	0.71
Intestines and contents	0.72	0.78	1.02	0.84	1.38	1.98	1.77	1.71
Kidneys	1.21	1.35	1.29	1.28	1.43	1.51	1.49	1.48
Liver	2.05	1.96	2.15	2.05	2.54	2.31	2.43	2.43
Lungs	0.65	0.84	0.82	0.77	0.90	1.01	1.00	0.97
Muscle	0.41	0.42	0.47	0.43	0.43	0.49	0.42	0.45
Ovaries	-	-	-	-	0.75	0.87	0.77	0.80
Pancreas	2.36	1.88	2.08	2.11	2.90	3.15	2.50	2.85
Skin	0.55	0.55	0.62	0.57	0.73	0.69	0.72	0.71
Spleen	0.64	0.70	0.75	0.70	0.87	0.97	0.87	0.90
Stomach and contents	1.07	0.45	0.98	0.83	1.29	1.64	1.25	1.39
Testes	0.45	0.52	0.51	0.49	-	-	-	-
Thyroid gland	0.69	0.97	0.84	0.83	1.06	1.26	1.30	1.21
Uterus	-	-	-	-	0.56	0.63	0.70	0.63
Whole-blood	0.54	0.74	0.74	0.67	0.66	0.96	0.93	0.85
Plasma	0.31	0.34	0.38	0.34	0.44	0.42	0.46	0.44

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 34

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 0.5 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	179♂	180♂	181♂	Mean (♂)	182♀	183♀	184♀	Mean (♀)
Adrenal glands	0.41	0.39	1.03	0.61	0.73	0.60	0.82	0.72
Bone	0.24	0.26	0.37	0.29	0.27	0.22	0.27	0.25
Bone marrow	0.36	0.36	0.64	0.45	0.50	0.39	0.56	0.48
Brain	0.16	0.14	0.44	0.25	0.26	0.20	0.30	0.25
Fat	0.21	0.19	0.36	0.25	0.17	0.17	0.19	0.18
Heart	0.51	0.51	0.71	0.58	0.52	0.47	0.60	0.53
Intestines and contents	0.73	1.00	3.03	1.59	1.07	1.39	1.11	1.19
Kidneys	3.64	2.87	3.11	3.21	3.33	3.11	2.91	3.12
Liver	1.64	1.93	2.43	2.00	1.27	1.45	1.75	1.49
Lungs	0.65	0.62	1.08	0.78	0.79	0.70	0.87	0.79
Muscle	0.24	0.23	0.47	0.31	0.32	0.27	0.39	0.33
Ovaries	-	-	-	-	0.60	0.52	0.61	0.58
Pancreas	0.64	0.71	1.23	0.86	0.78	0.78	0.95	0.84
Skin	0.40	0.44	0.62	0.49	0.57	0.47	0.62	0.55
Spleen	0.36	0.39	0.71	0.49	0.47	0.45	0.57	0.50
Stomach and contents	20.1	37.8	64.5	40.8	17.6	34.2	27.7	26.5
Testes	0.31	0.27	0.70	0.43	-	-	-	-
Thyroid gland	0.51	0.40	0.75	0.55	0.55	0.45	0.57	0.52
Uterus	-	-	-	-	0.55	0.51	0.66	0.57
Whole-blood	0.83	0.82	1.00	0.88	0.82	0.81	0.88	0.84
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 35

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 2 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	185♂	186♂	187♂	Mean (♂)	188♀	189♀	190♀	Mean (♀)
Adrenal glands	0.77	0.84	0.84	0.82	1.09	1.10	1.21	1.13
Bone	0.35	0.39	0.43	0.39	0.42	0.44	0.43	0.43
Bone marrow	0.62	0.72	0.72	0.69	0.80	0.90	0.92	0.87
Brain	0.44	0.42	0.41	0.42	0.41	0.44	0.46	0.44
Fat	0.26	0.33	0.36	0.32	0.24	0.34	0.32	0.30
Heart	0.69	0.74	0.75	0.73	0.76	0.85	0.85	0.82
Intestines and contents	1.33	2.02	2.41	1.92	3.25	3.32	3.38	3.32
Kidneys	4.77	3.83	3.69	4.10	2.81	2.37	4.17	3.12
Liver	2.74	3.36	3.58	3.23	3.20	3.57	3.22	3.33
Lungs	0.91	1.23	1.27	1.14	1.18	1.18	1.25	1.20
Muscle	0.41	0.47	0.48	0.45	0.48	0.50	0.47	0.48
Ovaries	-	-	-	-	0.82	0.87	0.89	0.86
Pancreas	0.97	1.20	1.64	1.27	1.68	2.27	1.70	1.88
Skin	0.58	0.67	0.69	0.65	0.91	0.89	0.95	0.92
Spleen	0.69	0.76	0.80	0.75	0.85	0.92	0.94	0.90
Stomach and contents	5.49	25.2	48.2	26.3	25.0	21.5	31.9	26.1
Testes	0.72	0.78	0.81	0.77	-	-	-	-
Thyroid gland	0.79	0.74	0.82	0.78	0.99	0.93	1.21	1.04
Uterus	-	-	-	-	0.89	0.83	0.87	0.86
Whole-blood	0.95	0.95	0.96	0.95	0.94	0.94	0.98	0.95
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 36

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 48 hours after the last of seven daily oral doses of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	191♂	192♂	193♂	Mean (♂)	194♀	195♀	196♀	Mean (♀)
Adrenal glands	2.31	2.47	2.31	2.36	2.33	3.14	2.76	2.74
Bone	0.88	1.01	0.89	0.93	0.97	1.08	0.78	0.94
Bone marrow	1.98	2.17	1.94	2.03	2.19	2.90	1.98	2.36
Brain	0.72	0.86	0.78	0.79	0.69	0.80	0.71	0.73
Fat	1.19	1.24	1.54	1.32	0.68	0.88	0.69	0.75
Heart	1.65	1.63	1.72	1.67	1.56	1.73	1.57	1.62
Intestines and contents	2.28	2.29	2.68	2.42	3.16	4.67	3.83	3.89
Kidneys	3.85	3.98	3.39	3.74	3.26	3.57	3.22	3.35
Liver	6.55	5.77	5.66	5.99	5.80	5.44	5.27	5.50
Lungs	2.09	2.48	2.17	2.25	2.05	2.38	2.16	2.20
Muscle	1.30	1.24	1.23	1.26	0.99	1.16	0.91	1.02
Ovaries	-	-	-	-	1.71	2.04	1.68	1.81
Pancreas	7.53	5.52	5.48	6.18	6.64	7.41	5.41	6.49
Skin	1.74	1.63	1.64	1.67	1.68	1.63	1.55	1.62
Spleen	2.05	2.06	1.97	2.03	1.98	2.27	1.88	2.04
Stomach and contents	3.43	1.31	2.59	2.44	2.95	3.87	2.70	3.17
Testes	1.44	1.52	1.36	1.44	-	-	-	-
Thyroid gland	2.21	2.86	2.21	2.43	2.42	2.97	2.82	2.74
Uterus	-	-	-	-	1.27	1.49	1.52	1.43
Whole-blood	1.73	2.17	1.96	1.95	1.51	2.27	2.00	1.93
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 37

Concentrations of radioactivity in the tissues of rats sacrificed 0.5 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	143♂	144♂	145♂	Mean (♂)	146♀	147♀	148♀	Mean (♀)
Adrenal glands	67.5	23.4	28.5	39.8	29.1	41.9	29.8	33.6
Bone	12.7	8.49	12.7	11.3	9.26	15.8	10.7	11.9
Bone marrow	18.5	18.7	22.1	19.8	21.0	24.6	21.1	22.2
Brain	8.81	12.1	12.1	11.0	12.6	18.5	12.8	14.6
Fat	152	10.2	13.7	58.6	7.20	10.2	7.55	8.32
Heart	24.7	19.3	32.4	25.5	25.0	32.5	24.2	27.2
Intestines and contents	40.0	57.1	34.2	43.8	59.1	82.0	82.4	74.5
Kidneys	212	92.1	179	161	131	142	109	127
Liver	69.5	65.2	84.3	73.0	55.6	68.8	53.8	59.4
Lungs	30.7	26.8	38.3	31.9	32.3	41.6	32.8	35.6
Muscle	14.3	12.8	13.9	13.7	14.5	19.7	13.8	16.0
Ovaries	-	-	-	-	27.4	33.8	27.3	29.5
Pancreas	39.5	19.9	29.1	29.5	24.2	30.4	27.3	27.3
Skin	22.3	18.8	25.3	22.1	25.8	30.4	27.9	28.0
Spleen	17.4	16.8	21.9	18.7	19.5	25.4	20.0	21.6
Stomach and contents	1100	1390	912	1130	2470	1980	2050	2170
Testes	22.1	17.9	25.6	21.9	-	-	-	-
Thyroid gland	33.1	19.0	27.7	26.6	21.3	28.1	19.5	23.0
Uterus	-	-	-	-	29.0	29.2	29.9	29.4
Whole-blood	38.5	28.0	50.8	39.1	38.0	44.3	37.3	39.9
Plasma	55.3	38.6	73.3	55.7	55.4	61.7	54.6	57.2

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 38

Concentrations of radioactivity in the tissues of rats sacrificed 2 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	149♂	150♂	151♂	Mean (♂)	152♀	153♀	154♀	Mean (♀)
Adrenal glands	80.9	7.13	113	67.0	19.1	20.1	12.0	17.1
Bone	3.37	2.86	4.46	3.56	5.70	6.37	3.97	5.35
Bone marrow	5.57	5.07	7.66	6.10	11.2	12.0	8.94	10.7
Brain	4.03	2.91	4.87	3.94	8.66	8.14	4.95	7.25
Fat	19.8	2.47	31.4	17.9	3.33	5.60	3.42	4.12
Heart	7.30	5.56	8.99	7.28	13.0	14.4	9.60	12.3
Intestines and contents	31.3	50.0	27.4	36.2	53.9	64.5	36.7	51.7
Kidneys	246	19.2	349	205	45.1	58.0	40.6	47.9
Liver	33.3	24.6	35.0	31.0	41.6	42.7	28.6	37.6
Lungs	11.5	7.74	16.3	11.8	16.9	19.0	10.7	15.5
Muscle	3.77	3.13	4.83	3.91	7.56	8.26	5.49	7.10
Ovaries	-	-	-	-	14.4	16.4	9.55	13.5
Pancreas	46.6	7.13	38.5	30.7	20.0	22.8	15.2	19.3
Skin	6.09	4.99	7.68	6.25	9.12	13.6	8.09	10.3
Spleen	13.0	4.67	16.0	11.2	11.5	12.6	8.48	10.9
Stomach and contents	768	1120	750	879	395	595	1630	873
Testes	7.52	5.85	10.0	7.79	-	-	-	-
Thyroid gland	20.1	5.36	83.7	36.4	10.2	25.8	8.10	14.7
Uterus	-	-	-	-	11.6	15.7	8.24	11.8
Whole-blood	6.38	5.82	9.14	7.11	12.3	15.9	9.85	12.7
Plasma	8.74	8.16	12.8	9.90	16.6	22.5	14.6	17.9

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 39

Concentrations of radioactivity in the tissues of rats sacrificed 48 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	155♂	156♂	157♂	Mean (♂)	158♀	159♀	160♀	Mean (♀)
Adrenal glands	2.13	2.35	2.68	2.39	4.06	2.52	2.39	2.99
Bone	0.77	0.83	1.00	0.87	1.27	0.96	0.69	0.97
Bone marrow	2.03	1.99	2.43	2.15	3.75	3.01	2.26	3.01
Brain	0.46	0.46	0.60	0.51	0.76	0.52	0.45	0.58
Fat	0.91	0.78	1.06	0.92	0.84	0.67	0.59	0.70
Heart	1.09	0.96	1.31	1.12	1.72	1.30	0.92	1.31
Intestines and contents	1.59	1.68	2.41	1.89	5.74	3.35	2.81	3.97
Kidneys	2.50	2.40	3.13	2.68	4.25	3.49	2.59	3.44
Liver	4.24	4.22	4.92	4.46	5.92	5.54	3.89	5.12
Lungs	1.32	1.24	1.56	1.37	2.30	1.73	1.36	1.80
Muscle	0.74	0.68	0.95	0.79	1.14	0.77	0.65	0.85
Ovaries	-	-	-	-	2.06	1.66	1.29	1.67
Pancreas	6.58	4.46	11.5	7.51	10.2	8.03	5.14	7.79
Skin	1.58	1.27	1.63	1.49	1.46	1.13	0.95	1.18
Spleen	1.52	1.45	1.85	1.61	2.15	1.63	1.37	1.72
Stomach and contents	1.27	1.34	1.36	1.32	2.99	3.13	2.43	2.85
Testes	0.90	0.83	1.02	0.92	-	-	-	-
Thyroid gland	2.02	1.74	2.23	2.00	3.07	2.11	2.79	2.66
Uterus	-	-	-	-	2.50	2.10	1.67	2.09
Whole-blood	1.23	1.03	1.30	1.19	1.94	1.42	1.21	1.52
Plasma	0.88	0.74	0.91	0.84	1.50	1.23	1.02	1.52

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 40

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 0.5 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	143♂	144♂	145♂	Mean (♂)	146♀	147♀	148♀	Mean (♀)
Adrenal glands	1.22	0.61	0.39	0.74	0.53	0.68	0.55	0.59
Bone	0.23	0.22	0.17	0.21	0.17	0.26	0.20	0.21
Bone marrow	0.34	0.48	0.30	0.37	0.38	0.40	0.39	0.39
Brain	0.16	0.31	0.17	0.21	0.23	0.30	0.23	0.25
Fat	2.75	0.26	0.19	1.07	0.13	0.17	0.14	0.15
Heart	0.45	0.50	0.44	0.46	0.45	0.53	0.44	0.47
Intestines and contents	0.72	1.48	0.47	0.89	1.07	1.33	1.51	1.30
Kidneys	3.84	2.38	2.44	2.89	2.36	2.30	1.99	2.22
Liver	1.26	1.69	1.15	1.37	1.00	1.11	0.99	1.03
Lungs	0.56	0.69	0.52	0.59	0.58	0.67	0.60	0.62
Muscle	0.26	0.33	0.19	0.26	0.26	0.32	0.25	0.28
Ovaries	-	-	-	-	0.49	0.55	0.50	0.51
Pancreas	0.72	0.52	0.40	0.55	0.44	0.49	0.50	0.48
Skin	0.40	0.49	0.35	0.41	0.47	0.49	0.51	0.49
Spleen	0.31	0.43	0.30	0.35	0.35	0.41	0.37	0.38
Stomach and contents	19.9	36.1	12.4	22.8	44.6	32.1	37.6	38.1
Testes	0.40	0.46	0.35	0.40	-	-	-	-
Thyroid gland	0.60	0.49	0.38	0.49	0.39	0.46	0.36	0.40
Uterus	-	-	-	-	0.52	0.47	0.55	0.51
Whole-blood	0.70	0.73	0.69	0.71	0.69	0.72	0.68	0.70
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 41

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 2 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	149♂	150♂	151♂	Mean (♂)	152♀	153♀	154♀	Mean (♀)
Adrenal glands	9.25	0.87	8.81	6.31	1.15	0.90	0.82	0.96
Bone	0.38	0.35	0.35	0.36	0.34	0.28	0.27	0.30
Bone marrow	0.64	0.62	0.60	0.62	0.67	0.54	0.61	0.61
Brain	0.46	0.36	0.38	0.40	0.52	0.36	0.34	0.41
Fat	2.26	0.30	2.44	1.67	0.20	0.25	0.23	0.23
Heart	0.84	0.68	0.70	0.74	0.78	0.64	0.66	0.69
Intestines and contents	3.58	6.12	2.14	3.95	3.24	2.87	2.50	2.87
Kidneys	28.1	2.35	27.2	19.2	2.71	2.58	2.77	2.69
Liver	3.81	3.02	2.73	3.19	2.50	1.90	1.95	2.12
Lungs	1.32	0.95	1.27	1.18	1.01	0.85	0.73	0.86
Muscle	0.43	0.38	0.38	0.40	0.45	0.37	0.37	0.40
Ovaries	-	-	-	-	0.86	0.73	0.65	0.75
Pancreas	5.33	0.87	3.00	3.07	1.20	1.01	1.04	1.08
Skin	0.70	0.61	0.60	0.64	0.55	0.60	0.55	0.57
Spleen	1.49	0.57	1.24	1.10	0.69	0.56	0.58	0.61
Stomach and contents	87.8	137	58.5	94.4	23.7	26.5	112	54.1
Testes	0.86	0.72	0.78	0.79	-	-	-	-
Thyroid gland	2.29	0.66	6.53	3.16	0.62	1.15	0.55	0.77
Uterus	-	-	-	-	0.70	0.70	0.56	0.65
Whole-blood	0.73	0.71	0.71	0.72	0.74	0.71	0.67	0.71
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 42

Ratios of the tissue to plasma concentrations of radioactivity in rats sacrificed 48 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	155♂	156♂	157♂	Mean (♂)	158♀	159♀	160♀	Mean (♀)
Adrenal glands	2.43	3.17	2.93	2.84	2.71	2.05	2.35	2.37
Bone	0.87	1.12	1.09	1.03	0.85	0.78	0.68	0.77
Bone marrow	2.31	2.68	2.66	2.55	2.50	2.44	2.22	2.39
Brain	0.52	0.62	0.65	0.60	0.51	0.42	0.44	0.46
Fat	1.04	1.05	1.16	1.08	0.56	0.55	0.58	0.56
Heart	1.24	1.29	1.43	1.32	1.15	1.05	0.91	1.04
Intestines and contents	1.81	2.27	2.64	2.24	3.84	2.72	2.76	3.11
Kidneys	2.84	3.24	3.42	3.17	2.84	2.84	2.55	2.74
Liver	4.83	5.68	5.38	5.30	3.95	4.50	3.83	4.09
Lungs	1.50	1.67	1.71	1.63	1.54	1.41	1.34	1.43
Muscle	0.84	0.91	1.04	0.93	0.76	0.62	0.64	0.67
Ovaries	-	-	-	-	1.37	1.35	1.26	1.33
Pancreas	7.49	6.01	12.6	8.70	6.84	6.53	5.05	6.14
Skin	1.80	1.71	1.78	1.76	0.97	0.92	0.93	0.94
Spleen	1.73	1.95	2.03	1.90	1.43	1.33	1.35	1.37
Stomach and contents	1.44	1.81	1.48	1.58	2.00	2.54	2.39	2.31
Testes	1.02	1.12	1.12	1.09	-	-	-	-
Thyroid gland	2.30	2.34	2.44	2.36	2.05	1.71	2.75	2.17
Uterus	-	-	-	-	1.67	1.71	1.65	1.68
Whole-blood	1.40	1.39	1.42	1.40	1.29	1.15	1.19	1.21
Plasma	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX 13

(continued)

TABLE 43

Concentrations of radioactivity in the tissues of male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	201♂	202♂	203♂	204♂	205♂	Mean
Adrenal glands	0.18	0.07	0.09	0.09	0.12	0.11
Bone	0.09	0.07	0.07	0.07	0.07	0.07
Bone marrow	0.09	<0.05	<0.06	<0.07	<0.07	0.07
Brain	0.05	0.02	0.04	0.05	0.03	0.04
Fat	0.07	0.05	0.06	0.06	0.04	0.06
Heart	0.10	0.07	0.07	0.07	0.07	0.08
Intestines and contents	0.06	0.03	0.03	0.03	0.03	0.04
Kidneys	0.20	0.12	0.14	0.12	0.15	0.15
Liver	0.36	0.19	0.19	0.21	0.24	0.24
Lungs	0.14	0.09	0.08	0.08	0.09	0.10
Muscle	0.09	0.05	0.05	0.05	0.06	0.06
Pancreas	0.27	0.16	0.19	0.17	0.18	0.19
Skin	0.12	0.08	0.07	0.08	0.09	0.09
Spleen	0.12	0.06	0.07	0.07	0.09	0.08
Stomach and contents	0.07	0.02	0.02	0.02	0.03	0.03
Testes	0.09	0.05	0.06	0.05	0.06	0.06
Thyroid gland	<0.15	<0.18	<0.10	<0.15	<0.11	<0.14
Whole-blood	0.09	0.04	0.05	0.05	0.06	0.06
Plasma	0.05	0.03	0.03	0.03	0.03	0.03

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 44

Concentrations of radioactivity in the tissues of female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	206♀	207♀	208♀	209♀	210♀	Mean
Adrenal glands	0.10	0.11	0.12	0.14	0.14	0.12
Bone	0.07	0.07	0.08	0.07	0.08	0.07
Bone marrow	0.07	<0.12	<0.08	<0.13	0.12	0.10
Brain	0.03	0.05	0.06	0.06	0.06	0.05
Fat	0.04	0.06	0.06	0.10	0.12	0.08
Heart	0.07	0.10	0.11	0.12	0.12	0.10
Intestines and contents	0.06	0.07	0.07	0.08	0.09	0.07
Kidneys	0.14	0.18	0.19	0.22	0.22	0.19
Liver	0.26	0.26	0.22	0.33	0.32	0.28
Lungs	0.10	0.13	0.13	0.15	0.17	0.14
Muscle	0.04	0.07	0.07	0.08	0.08	0.07
Ovaries	0.08	0.08	0.08	0.08	0.10	0.08
Pancreas	0.19	0.25	0.26	0.35	0.23	0.26
Skin	0.08	0.10	0.10	0.09	0.17	0.11
Spleen	0.08	0.10	0.11	0.11	0.12	0.10
Stomach and contents	0.06	0.05	0.05	0.17	0.13	0.09
Thyroid gland	<0.15	<0.14	<0.10	0.14	0.14	0.13
Uterus	0.07	0.11	0.12	0.10	0.13	0.11
Whole-blood	0.07	0.07	0.08	0.11	0.10	0.09
Plasma	0.04	0.04	0.04	0.05	0.05	0.04

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 45

Concentrations of radioactivity in the tissues of pre-treated* male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	21♂	22♂	23♂	24♂	25♂	Mean
Adrenal glands	0.20	0.16	0.27	0.19	0.20	0.20
Bone	0.05	0.05	0.05	0.06	0.05	0.05
Bone marrow	0.25	0.14	0.17	0.12	0.34	0.20
Brain	0.06	0.04	0.07	0.06	0.07	0.06
Fat	0.03	0.03	0.03	0.04	0.03	0.03
Heart	0.12	0.09	0.13	0.11	0.12	0.11
Intestines and contents	0.08	0.08	0.06	0.07	0.06	0.07
Kidneys	0.27	0.21	0.26	0.27	0.27	0.26
Liver	0.35	0.33	0.34	0.33	0.34	0.34
Lungs	0.15	0.12	0.17	0.14	0.16	0.15
Muscle	0.07	0.06	0.09	0.08	0.08	0.08
Pancreas	0.20	0.14	0.21	0.20	0.18	0.19
Skin	0.10	0.10	0.12	0.11	0.12	0.11
Spleen	0.15	0.11	0.16	0.15	0.14	0.14
Stomach and contents	0.16	0.11	0.12	0.16	0.13	0.14
Testes	0.10	0.11	0.11	0.10	0.10	0.10
Thyroid gland	0.15	<0.20	0.19	<0.17	0.18	0.18
Whole-blood	0.12	0.08	0.14	0.10	0.12	0.11
Plasma	0.06	0.05	0.06	0.06	0.06	0.06

Results are expressed as mg dimethoate equivalents/kg

* rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

APPENDIX 13

(continued)

TABLE 46

Concentrations of radioactivity in the tissues of pre-treated* female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	26♀	27♀	28♀	29♀	30♀	Mean
Adrenal glands	0.24	0.24	0.16	0.26	0.29	0.24
Bone	0.06	0.05	0.03	0.05	0.06	0.05
Bone marrow	<0.23	0.14	0.11	0.17	0.17	0.16
Brain	0.07	0.07	0.05	0.06	0.09	0.07
Fat	0.04	0.04	0.02	0.03	0.05	0.04
Heart	0.14	0.13	0.10	0.11	0.17	0.13
Intestines and contents	0.11	0.10	0.08	0.11	0.12	0.10
Kidneys	0.30	0.26	0.19	0.24	0.33	0.26
Liver	0.32	0.37	0.29	0.32	0.41	0.34
Lungs	0.19	0.17	0.13	0.16	0.22	0.17
Muscle	0.07	0.08	0.06	0.07	0.11	0.08
Ovaries	0.14	0.14	0.11	0.15	0.18	0.14
Pancreas	0.49	0.40	0.23	0.27	0.48	0.37
Skin	0.14	0.12	1.73	0.09	0.12	0.44
Spleen	0.17	0.17	0.12	0.14	0.20	0.16
Stomach and contents	0.14	0.13	0.10	0.13	0.20	0.14
Thyroid gland	0.19	0.21	<0.23	<0.23	0.29	0.23
Uterus	0.12	0.14	0.09	0.11	0.13	0.12
Whole-blood	0.13	0.14	0.09	0.11	0.21	0.14
Plasma	0.08	0.07	0.06	0.07	0.09	0.07

Results are expressed as mg dimethoate equivalents/kg

* rats received a single oral 10 mg/kg bodyweight non-radiolabelled dose once daily for 14 consecutive days prior to administration of the radioactive dose

APPENDIX 13

(continued)

TABLE 47

Concentrations of radioactivity in the tissues of male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	1♂	3♂	4♂	5♂	198♂	Mean
Adrenal glands	2.46	2.49	1.97	1.73	1.52	2.03
Bone	0.46	0.52	0.32	0.38	0.36	0.41
Bone marrow	2.65	4.48	2.33	4.70	0.71	2.97
Brain	0.47	0.52	0.25	0.35	0.44	0.41
Fat	0.71	1.07	0.49	0.51	0.82	0.72
Heart	0.97	1.08	0.58	0.70	0.84	0.83
Intestines and contents	0.60	0.50	0.43	0.31	0.49	0.47
Kidneys	1.83	1.92	1.13	1.35	1.77	1.60
Liver	2.41	2.56	1.85	1.84	2.16	2.16
Lungs	1.05	1.19	0.59	0.78	0.94	0.91
Muscle	0.77	0.76	0.45	0.58	0.68	0.65
Pancreas	6.44	7.27	3.45	4.39	4.22	5.15
Skin	0.87	0.87	0.52	0.62	0.95	0.77
Spleen	1.03	1.09	0.61	0.71	0.89	0.87
Stomach and contents	0.34	0.42	0.22	0.24	0.26	0.30
Testes	0.74	0.77	0.42	0.56	0.64	0.63
Thyroid gland	3.63	4.60	6.48	6.23	4.98	5.18
Whole-blood	0.85	1.04	0.56	0.67	0.84	0.79
Plasma	0.43	0.44	0.29	0.30	0.36	0.36

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 48

Concentrations of radioactivity in the tissues of female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	6♀	8♀	9♀	199♀	200♀	Mean
Adrenal glands	2.23	1.62	2.11	2.65	1.43	2.01
Bone	0.32	0.42	0.47	0.33	0.30	0.37
Bone marrow	3.59	1.25	1.58	0.93	0.87	1.64
Brain	0.36	0.45	0.67	0.60	0.44	0.50
Fat	0.41	0.74	0.47	0.40	0.40	0.48
Heart	0.85	1.04	1.26	1.19	0.99	1.07
Intestines and contents	0.44	0.70	0.71	0.68	0.98	0.70
Kidneys	1.46	1.52	1.93	2.24	1.58	1.75
Liver	1.99	2.01	2.19	2.41	1.95	2.11
Lungs	0.91	1.20	1.43	1.32	1.11	1.19
Muscle	0.44	0.65	0.93	0.76	0.62	0.68
Ovaries	0.92	1.02	1.21	1.07	0.94	1.03
Pancreas	4.23	6.42	6.54	4.66	11.3	6.63
Skin	0.55	0.80	0.90	1.01	0.85	0.82
Spleen	0.93	1.12	1.45	1.32	1.03	1.17
Stomach and contents	0.25	0.47	0.56	0.27	0.88	0.49
Thyroid gland	2.72	2.03	2.58	1.37	1.24	1.99
Uterus	0.58	0.71	0.79	0.92	0.79	0.76
Whole-blood	0.86	1.12	1.32	1.49	1.14	1.19
Plasma	0.43	0.50	0.58	0.61	0.49	0.52

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 49

Concentrations of radioactivity in the tissues of male rats sacrificed 120 hours after a single intravenous dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	31♂	32♂	33♂	34♂	35♂	Mean
Adrenal glands	0.11	0.11	0.11	0.09	0.11	0.11
Bone	0.03	0.03	0.02	<0.02	0.04	0.03
Bone marrow	0.06	0.07	0.05	0.05	0.06	0.06
Brain	0.03	0.03	0.03	0.02	0.03	0.03
Fat	0.04	0.05	0.04	0.04	0.05	0.04
Heart	0.06	0.08	0.06	0.05	0.07	0.06
Intestines and contents	0.04	0.02	0.04	0.03	0.03	0.03
Kidneys	0.12	0.15	0.13	0.09	0.14	0.13
Liver	0.19	0.24	0.19	0.18	0.20	0.20
Lungs	0.08	0.09	0.08	0.06	0.09	0.08
Muscle	0.05	0.06	0.05	0.04	0.06	0.05
Pancreas	0.16	0.18	0.16	0.12	0.21	0.17
Skin	0.08	0.08	0.07	0.06	0.08	0.07
Spleen	0.06	0.07	0.06	0.05	0.07	0.06
Stomach and contents	0.04	0.03	0.04	0.02	0.02	0.03
Testes	0.05	0.06	0.05	0.03	0.06	0.05
Thyroid gland	<0.14	<0.11	<0.15	<0.10	<0.08	<0.12
Whole-blood	0.06	0.07	0.06	0.06	0.07	0.06
Plasma	0.03	0.03	0.03	0.03	0.03	0.03

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 50

Concentrations of radioactivity in the tissues of female rats sacrificed 120 hours after a single intravenous dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	36♀	37♀	38♀	39♀	40♀	Mean
Adrenal glands	0.13	0.11	0.14	0.08	0.11	0.11
Bone	0.02	0.02	0.02	0.02	0.02	0.02
Bone marrow	0.08	<0.06	0.08	<0.06	0.06	0.07
Brain	0.04	0.03	0.04	0.02	0.03	0.03
Fat	0.02	0.04	0.04	0.04	0.03	0.03
Heart	0.08	0.06	0.08	0.06	0.06	0.07
Intestines and contents	0.05	0.05	0.05	0.07	0.12	0.07
Kidneys	0.13	0.14	0.15	0.11	0.14	0.13
Liver	0.20	0.22	0.19	0.17	0.20	0.20
Lungs	0.09	0.09	0.10	0.07	0.09	0.09
Muscle	0.06	0.05	0.06	0.04	0.04	0.05
Ovaries	0.07	0.06	0.09	0.06	0.07	0.07
Pancreas	0.25	0.61	0.19	0.25	0.16	0.29
Skin	0.06	0.06	0.07	0.06	0.06	0.06
Spleen	0.08	0.07	0.09	0.05	0.07	0.07
Stomach and contents	0.03	0.04	0.06	0.09	0.11	0.07
Thyroid gland	<0.08	<0.14	<0.10	<0.14	<0.11	<0.11
Uterus	0.08	0.05	0.08	0.05	0.05	0.06
Whole-blood	0.07	0.07	0.08	0.06	0.07	0.07
Plasma	0.04	0.04	0.04	0.03	0.03	0.04

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 51

Concentrations of radioactivity in the tissues of male rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	41♂	42♂	43♂	44♂	45♂	Mean
Adrenal glands	<0.04	<0.29	<0.03	<0.03	<0.04	<0.09
Bone	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Bone marrow	<0.04	<0.07	NS	<0.05	<0.05	<0.05
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Heart	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Intestines and contents	0.01	<0.01	<0.01	<0.01	<0.01	0.01
Kidneys	0.02	<0.01	<0.01	<0.01	0.01	0.01
Liver	0.04	0.02	<0.01	0.01	0.03	0.02
Lungs	0.01	<0.01	<0.01	<0.01	0.01	0.01
Muscle	<0.01	<0.01	<0.01	<0.01	0.01	0.01
Pancreas	0.02	0.02	0.01	0.02	0.02	0.02
Skin	0.04	0.02	<0.02	<0.01	0.02	0.02
Spleen	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Stomach and contents	<0.02	<0.02	<0.02	<0.02	<0.01	<0.02
Testes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid gland	<0.16	<0.11	<0.11	<0.21	<0.15	<0.15
Whole-blood	0.01	<0.01	<0.01	<0.01	0.01	0.01
Plasma	0.01	<0.01	<0.01	<0.01	<0.01	0.01

Results are expressed as mg dimethoate equivalents/kg
NS no sample

APPENDIX 13

(continued)

TABLE 52

Concentrations of radioactivity in the tissues of female rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	46♀	47♀	48♀	49♀	50♀	Mean
Adrenal glands	<0.03	<0.03	<0.03	<0.02	<0.02	<0.03
Bone	<0.02	0.02	<0.02	<0.02	<0.02	0.02
Bone marrow	<0.06	<0.04	<0.07	<0.07	<0.10	<0.07
Brain	0.01	<0.01	0.02	<0.01	<0.01	0.01
Fat	<0.02	<0.01	<0.02	<0.01	<0.02	<0.02
Heart	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Intestines and contents	0.10	0.04	0.01	0.01	<0.01	0.03
Kidneys	0.02	0.01	0.02	0.02	0.01	0.02
Liver	0.03	0.03	0.03	0.03	0.02	0.03
Lungs	<0.01	0.04	<0.01	<0.01	<0.01	0.02
Muscle	0.01	0.06	<0.01	<0.01	<0.01	0.02
Ovaries	<0.02	<0.02	<0.02	<0.02	<0.01	<0.02
Pancreas	0.03	0.02	0.03	0.04	0.02	0.03
Skin	<0.02	<0.01	<0.02	0.10	0.44	0.12
Spleen	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Stomach and contents	0.11	0.03	0.02	<0.02	<0.02	0.04
Thyroid gland	<0.13	<0.18	<0.10	<0.13	<0.14	<0.14
Uterus	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Whole-blood	0.01	0.01	0.01	0.01	0.01	0.01
Plasma	0.01	<0.01	0.01	<0.01	<0.01	0.01

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 53

Concentrations of radioactivity in the tissues of male rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	R51♂	R52♂	R53♂	R54♂	R55♂	Mean
Adrenal glands	<0.21	<0.19	<0.74	<0.20	<0.19	<0.31
Bone	<0.09	<0.10	<0.10	<0.10	<0.10	<0.10
Bone marrow	<0.45	<0.33	<0.70	<0.28	<1.21	<0.59
Brain	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Fat	<0.09	<0.10	<0.09	<0.09	<0.10	<0.09
Heart	<0.07	<0.07	<0.07	<0.08	<0.07	<0.07
Intestines and contents	<0.06	<0.07	<0.06	<0.07	<0.07	<0.07
Kidneys	<0.07	<0.07	<0.06	<0.07	<0.07	<0.07
Liver	<0.07	<0.07	<0.08	<0.08	<0.07	<0.07
Lungs	<0.07	<0.07	<0.06	<0.07	<0.07	<0.07
Muscle	<0.07	<0.05	<0.05	<0.05	<0.05	<0.05
Pancreas	<0.07	<0.07	<0.08	<0.07	<0.07	<0.07
Skin	<0.09	0.65	<0.09	<0.10	<0.09	0.20
Spleen	<0.07	<0.08	<0.08	<0.08	<0.08	<0.08
Stomach and contents	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
Testes	<0.05	<0.05	<0.06	<0.05	<0.05	<0.05
Thyroid gland	<0.95	<0.67	<0.93	<0.93	<0.91	<0.88
Whole-blood	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Plasma	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 13

(continued)

TABLE 54

Concentrations of radioactivity in the tissues of female rats sacrificed 120 hours after a single dermal dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	R56♀	R57♀	R58♀	R59♀	R60♀	Mean
Adrenal glands	<0.16	<0.13	<0.19	<0.16	<0.14	<0.16
Bone	<0.10	<0.10	<0.10	<0.10	<0.09	<0.10
Bone marrow	<0.69	<0.52	<0.49	<0.92	<0.51	<0.63
Brain	<0.06	0.08	<0.06	<0.06	<0.06	0.06
Fat	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Heart	<0.07	<0.07	<0.07	<0.09	<0.08	<0.08
Intestines and contents	<0.07	0.33	<0.07	0.11	<0.07	0.13
Kidneys	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
Liver	<0.08	0.11	<0.07	<0.07	<0.07	0.08
Lungs	<0.07	<0.07	<0.06	<0.07	<0.06	<0.07
Muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Ovaries	<0.11	<0.07	<0.11	<0.09	<0.11	<0.10
Pancreas	<0.07	0.08	<0.07	0.10	<0.07	0.08
Skin	<0.09	0.19	0.80	<0.09	0.32	0.30
Spleen	<0.09	<0.08	<0.08	<0.09	<0.09	<0.09
Stomach and contents	<0.08	<0.08	<0.08	<0.09	<0.08	<0.08
Thyroid gland	<0.63	<0.94	<0.64	<0.86	<0.63	<0.74
Uterus	<0.14	<0.07	<0.13	<0.10	<0.14	<0.12
Whole-blood	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Plasma	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02

Results are expressed as mg dimethoate equivalents/kg

APPENDIX 14

Data from animals not included in the main report

Shown in this appendix are data from animals in experiments 2b and 2c which were subsequently replaced by additionally dosed animals.

TABLES

1. Experiment 2c (single oral dose, 10 mg/kg bodyweight), males (11 - 15♂)
2. Experiment 2c (single oral dose, 10 mg/kg bodyweight), females (16 - 20♀)
3. Experiment 2b (single oral dose, 100 mg/kg bodyweight), rats 2♂, 7♀ and 10♀

APPENDIX 14

(continued)

TABLE 1

Excretion and retention of radioactivity in male rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	11♂	12♂	13♂	14♂	15♂	Mean	sd
Urine (hours)							
0 - 6	75.9	76.1	81.8	74.4	74.2	76.5	3.1
6 - 12	6.02	10.1	7.20	9.76	7.68	8.15	1.74
12 - 24	3.15	1.95	1.70	2.55	2.62	2.39	0.58
24 - 48	0.96	0.54	0.40	0.98	1.44	0.86	0.41
48 - 72	0.84	0.30	0.20	0.52	0.75	0.52	0.28
72 - 96	0.26	0.14	0.09	0.25	0.43	0.23	0.13
96 - 120	0.21	0.16	0.06	0.21	0.34	0.20	0.10
Cage wash	0.30	0.09	0.34	0.27	0.24	0.25	0.10
Total urine and cage wash	87.6	89.4	91.8	88.9	87.7	89.1	1.7
Expired air (hours)							
0 - 6	0.87	0.74	0.76	0.69	0.90	0.79	0.09
6 - 24	1.04	0.88	1.08	0.87	0.87	0.95	0.10
24 - 48	0.19	0.16	0.17	0.18	0.20	0.18	0.02
48 - 72	0.09	0.07	0.08	0.11	0.11	0.09	0.02
Total expired air	2.19	1.85	2.09	1.85	2.08	2.01	0.15
Total recovery	(89.8)	(91.2)	(93.9)	(90.8)	(89.8)	(91.1)	(1.7)

Results are expressed as % dose
sd standard deviation

APPENDIX 14

(continued)

TABLE 2

Excretion and retention of radioactivity in female rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 10 mg/kg bodyweight

Animal number	16♀	17♀	18♀	19♀	20♀	Mean	sd
Urine (hours)							
0 - 6	85.7	70.7	66.7	70.4	75.3	73.8	7.3
6 - 12	4.82	7.83	11.4	8.10	7.47	7.92	2.34
12 - 24	4.81	3.01	3.90	3.27	2.22	3.44	0.97
24 - 48	1.03	2.45	0.97	1.62	1.54	1.52	0.60
48 - 72	0.66	1.03	0.43	0.88	0.70	0.74	0.23
72 - 96	0.33	0.46	0.27	1.88	0.28	0.64	0.70
96 - 120	0.36	0.63	0.13	0.42	0.22	0.35	0.19
Cage wash	0.73	0.68	1.89	0.42	0.28	0.80	0.64
Total urine and cage wash	98.4	86.8	85.7	87.0	88.0	89.2	5.2
Expired air (hours)							
0 - 6	0.96	1.08	0.74	0.72	0.88	0.88	0.15
6 - 24	0.77	1.10	1.03	0.78	1.15	0.97	0.18
24 - 48	0.21	0.28	0.21	0.20	0.28	0.24	0.04
48 - 72	0.09	0.18	0.10	0.17	0.18	0.14	0.05
Total expired air	2.03	2.64	2.08	1.87	2.49	2.22	0.33
Total recovery	(100)	(89.4)	(87.8)	(88.9)	(90.5)	(91.3)	(4.9)

Results are expressed as % dose
sd standard deviation

APPENDIX 14

(continued)

TABLE 3

Excretion and retention of radioactivity in rats sacrificed 120 hours after a single oral dose of ^{14}C-dimethoate at a nominal level of 100 mg/kg bodyweight

Animal number	2♂	7♀	10♀
Urine (hours)			
0 - 6	61.3	64.7	51.9
6 - 12	18.7	5.75	17.8
12 - 24	3.44	4.42	7.56
24 - 48	2.04	3.17	4.22
48 - 72	0.67	1.31	1.16
72 - 96	0.22	0.59	0.75
96 - 120	0.24	0.56	0.41
Cage wash	0.34	0.31	0.40
Total urine and cage wash	87.0	80.8	84.2
Expired air (hours)			
0 - 24	2.23	2.16	1.57
24 - 48	0.20	0.40	0.34
48 - 72	0.10	0.18	0.18
Total expired air	2.53	2.74	2.09
Faeces (hours)			
0 - 24	1.42	0.37	0.20
24 - 48	0.16	0.32	0.34
48 - 72	0.06	0.09	0.07
72 - 96	0.04	0.05	0.07
96 - 120	0.01	0.04	0.06
Total faeces	1.69	0.87	0.74
Carcass			
GIT	0.04	0.09	0.08
Kidneys	0.01	0.02	0.01
Liver	0.10	0.12	0.10
Residual carcass	0.75	2.65	1.99
Total carcass	0.90	2.88	2.18
Total recovery	92.1	87.3	89.2

Results are expressed as % dose
GIT gastrointestinal tract and contents

APPENDIX 15

Study Protocol and Amendments

DTF/16

STUDY PROTOCOL

The Biokinetics and Metabolism of ^{14}C-Dimethoate in the Rat

Study Sponsor:	Dimethoate Task Force (Chairman: Dr W F Biegel) Selztalstrasse 151 D-6507 Ingelheim Federal Republic of Germany
Monitoring Organisation:	SCC Scientific Consulting Company Chemisch-Wissenschaftliche Beratung GmbH Hauptstr 35 D-6551 Biebelsheim Phone 06701-7874, Fax 06701-7878
Monitoring Scientist:	Dr. F. Pistel
HRC Study No:	DTF/16
Testing Facility:	Huntingdon Research Centre Ltd P O Box 2 Huntingdon Cambridgeshire PE18 6ES England
Guidelines:	OECD 417 US EPA 40 CFR Part 140, FIFRA Subdivision F, Guideline 85-1
Date:	5 January 1993

APPENDIX 15

(continued)

A PREFACE

HRC Study personnel:

Metabolism & Environmental Chemistry Group:

Head of Group:	Dr D R Hawkins
Study Director:	Dr D Kirkpatrick
Study Co-ordinator:	Mr D Shaw

Proposed Schedule

Experimental start date:	In January 1993
Experimental termination date:	by 31 December 1993
Despatch of draft final report:	By 16 March 1994

Amendment Procedures: This protocol can be amended at the discretion of the Study Director in consultation with the Study Sponsor. Detailed descriptions of all amendments will be signed by the Study Director. The amendment will be effective at the time of the Study Director's signature. The Sponsor will receive two copies; one must be signed and returned to the Study Director. The amendment will be distributed and added to all copies of the protocol.

Health and Safety: The radiolabelled test substance and samples generated during the study will be handled in accordance with HRC procedures. Radio-active waste will be disposed of according to radioactive disposal guidelines.

Routine laboratory work will be conducted in accordance with Standard Operating Procedures in effect within the Metabolism & Environmental Chemistry Group. A toxicity evaluation document (supplied by the Sponsor) will accompany the test substance. Special care should be taken as dimethoate is a cholinesterase inhibitor

APPENDIX 15

(continued)

Location of Study:	Metabolism & Environmental Chemistry Group, Building E04

B TEST & REFERENCE SUBSTANCES

Test Substance General Information:

Name:	Dimethoate.
Molecular formula:	$C_5H_{12}NO_3PS_2$
Molecular weight:	229.3
Chemical name:	O,O-Dimethyl-S-methylcarbamoylmethyl-phosphorodithioate.
CAS number:	60-51-5
Physical state (20°C):	Crystalline solid.
Water solubility:	23.8g/litre at 21°C.
Source:	Dimethoate will be supplied by the Study Sponsor.
Storage:	At 5°C in darkness.
Characterisation:	Mass spectra of the unlabelled dimethoate samples will be obtained and compared to a dimethoate reference spectrum in order to confirm the identity.

Unlabelled test substance:

There will be two samples of unlabelled dimethoate. The analytical standard will be used for radiodilution of the radiolabelled test substance and as a reference standard. Technical dimethoate will be used for the preliminary toxicity testing in animals. Specifications are as follows:

	Analytical	**Technical**
Quantity supplied:	2g	25g
Purity:	99.5%	99.1%
Identification code:	ST001-01	PR020
Batch number:	00315-00	20522-00

APPENDIX 15

(continued)

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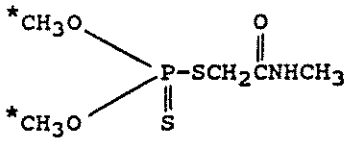
Date of receipt at HRC:	7 December 1992	7 December 1992
Expiry Date:	April 1995	May 1995

The original containers will be kept at HRC until the final report is despatched.

Labelled test substance:

Structural formula:



* Denotes position of the radiolabel

Specific activity:	To be advised.
Radiochemical purity:	To be advised. Radiochemical purity will be measured at Huntingdon Research Centre before each administration of the test substance. If the radiochemical purity is less than 98% then the need for purification will be discussed with the Study Sponsor.
Source:	^{14}C-Dimethoate will be suppled by the Study Sponsor.
Storage:	At about -20°C in the dark.
Characterisation:	^{14}C-Dimethoate will be compared to unlabelled dimethoate by mass spectrometry and TLC.
Batch number:	To be advised
Date of receipt at HRC:	To be advised (note the original container will be kept at HRC until the final report is despatched).

APPENDIX 15

(continued)

Reference Substances: For use in characterisation of metabolites, analytical reference substances may be supplied by the Study Sponsor. Their structures and code numbers will be recorded in the study raw data and will be shown in the final report. A mass spectrum will be obtained of any reference substance used for metabolite characterisation.

C STUDY OBJECTIVES

1 To assess absorption and rates and routes of excretion of radioactivity after administration of oral, intravenous and dermal doses of ^{14}C-dimethoate to male and female rats.

2 To investigate the pharmacokinetics of the radiolabel and the parent compound after oral administration of ^{14}C-dimethoate to male and female rats:

3 To investigate the distribution of the radiolabel in tissues and the rate of elimination of tissue radioactivity after single or multiple oral doses of ^{14}C-dimethoate to male and female rats.

4 To obtain information on the biotransformation of dimethoate and the influence of dose level and route of administration on this process.

D TEST ANIMALS

Species: Rat.

Sex: Male and female.

Strain: Wistar.

Source: Charles River UK Ltd
Manston Road
Margate
Kent
England

APPENDIX 15

(continued)

Rationale for selection:	Recognized by international guidelines as the recommended test system (OECD No 417 and USA EPA). Study data have to be set in relation to already available data from the same test system.
Age at Administration of Radioactive and Pretest Doses:	7-8 weeks (males) 9-10 weeks (females).
Bodyweight at Administration of Radioactive and Pretest Doses:	ca 200 g (weight range will not be greater than ± 20%, applies to first of multiple doses).
Method of Identification:	Prior to treatment the animals will be individually identified by tail mark. The study schedule number combined with the animal number will constitute a unique identification.
Acclimatisation and Certification of Health:	Animals will be received at the testing facility at least five days before the intended date of dosing. The health status of the animals will be evaluated in accordance with accepted veterinary practice. Rats considered unsuitable for health or other reasons will be discarded.
Number of Animals and Randomisation:	A minimum of 196 animals (100M, 96F) is required for the study. Additional animals may be required for pretest observations.
Selection of Animals:	Type and duration of such studies require that animals of similar age are ordered sequentially in groups prior to the experi-ments. Therefore the conventional random-isation and assignments to groups are not possible.

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(continued)

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E ANIMAL HUSBANDRY

Housing:	During acclimatisation and during pharmacokinetic experiments, rats will be housed in groups of 3 to 6 animals of the same sex in stainless steel cages with suspended mesh floors. For excretion-balance experiments the animals will be housed singly in individual glass metabowls (equipped with urine/faeces separators) from 24 hours before administration of the radiolabelled dose until the termination of the experiment.
Room Temperature:	22 ± 2°C.
Relative Humidity:	40-60%
Ventilation:	Approximately 15 air changes per hour.
Light/dark Cycle:	An alternating 12-hour light/dark cycle will be maintained.
Diet:	LAD 1, from Scientific Feeds, Croydon, UK (satisfactory certificates of analysis for "contaminants" from the manufacturer for the diet batch used, will be acceptable). Diet will be available to the animals ad libitum. Exaple certificates of analysis will be included in the final report.
Water:	Fresh drinking water (Anglian Water mains supply) will be available ad libitum. The water is analysed regularly at source for a broad range of contaminants. In view of the aims and duration of animal experimentation there is no need for further analysis. Example certificates of analysis will be included in the final report.

F DOSE SELECTION AND DOSE GROUPS

Dose levels

The following combination of dose route and level occur in the study:

Oral administration:	high dose low dose

APPENDIX 15

(continued)

Intravenous administration:	low dose
Dermal administration:	high dose low dose

It is intended that the high and low dose levels will be the same for each dose route. The levels will be determined following pretest observations of rats given oral high level doses and intravenous low level doses. The dose levels finally selected will differ by at least a factor of ten. The levels for the initial pretests will be 10 mg/kg (low) and 100 mg/kg (high).

Rationale for dose selection

The oral and dermal routes are chosen as they are expected to be the potential routes for human exposure to the test susbstance. Relevant guidelines (OECD, EPA) specify that two dose levels should be studied, a high level which causes slight toxic effects and a low level which causes no toxic effects. These dose levels will be selected using results from previously performed toxicology studies. The intravenous route of administration is requested by EPA guidelines where the water solubility of the test substance permits dosing by this route.

Dose administration

The test substance will be administered in solution in water (oral doses) isotonic saline (intravenous dose) or 2% aqueous Tween 80 (dermal doses). If the high dose level cannot be completely dissolved in water then all oral doses will be given in 1% aqueous carboxymethyl cellulose.

Dose groups

A summary of animal experimentation and dose groups is shown in the table.

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(continued)

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TABLE

Dose Route and Level	Number of Animals	Experiment Type	Experiment No
Oral, High*	2M 2F	Pretest observation (non-radioactive)	1a
Intravenous, low*	2M 2F	Pretest observation (non-radioactive)	1b
Oral, High	2M 2F	Pilot excretion-balance	2a
Oral, High	5M 5F	Excretion balance	2b
Oral, Low	5M 5F	Excretion-balance	2c
Oral, Low (pretreated)	5M 5F	Excretion-balance	2d
Intravenous, test 1 or test 2	5M 5F	Excretion-balance	2e
Dermal, Low	4M	Pretest (washing procedure)	2f
Dermal, Low	5M 5F	Excretion-balance	2g
Dermal, High	5M 5F	Excretion-balance	2h
Oral, High	5M 5F	Plasma radioactivity kinetics	3a
Oral, Low	5M 5F	Plasma radioactivity kinetics	3b
Oral, High	12M 12F	Plasma parent compound kinetics	3c
Oral, High	3M 3F	Biliary excretion	4a
Oral, Low	3M 3F	Biliary excretion	4b
Oral, Low	5M 5F	Tissue distribution, whole body autoradiography	5a
Oral, High	9M 9F	Tissue distribution single dose	5b

APPENDIX 15

(continued)

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Dose Route and Level	Number of Animals	Experiment Type	Experiment No
Oral, Low	9M 9F	Tissue distribution single dose	5c
Oral, Low	9M 9F	Tissue distribution multiple dose	5d

* If these doses are found to cause toxic effects lower doses will be tested. The numbers of animals shown represent the minimum numbers required.

G ANIMAL EXPERIMENTS

Pretest Observation of the Effect of the High Level Oral Dose and the Intravenous Low Dose

1(a) Although doses are set in relation to existing toxicity data it cannot be fully excluded that animals used for metabolism studies will show unexpected symptoms. Therefore preliminary tests with non-radiolabelled dimethoate will be performed. Four rats (2M, 2F) will receive single high (proposed) level oral doses of dimethoate and will be kept under observation for at least 24 hours. If at this dose level the animals show significant visible toxic signs then lower levels will be tested until a satisfactory level is found.

1(b) Four rats (2M, 2F) will receive single intravenous doses of dimethoate at the proposed low level. The animals will be kept under observation for up to 24 hours. If at this dose level the animals show toxic signs then lower doses will be tested as above.

Excretion and retention of radioactivity

2(a) The objective of this pilot experiment is to determine whether a five day duration will be sufficient for the main excretion experiments (2b-2c)and to determine whether expired air monitoring will be necessary for those experiments. Excreta samples produced in this experiment will be used in the development of methods for metabolite pattern analysis.

APPENDIX 15

(continued)

Excretion and retention of radioactivity (continued)

Four rats (2M, 2F) will receive single oral doses (high level) of ^{14}C-dimethoate. Urine will be collected separately from each animal into receivers cooled in solid CO_2 at 0-6, 6-12, 12-24 hours and at 24-hour intervals up to 120 hours. Faeces will be collected separately from each animal into receivers cooled in solid CO_2 at 24-hour intervals up to 120 hours.

Expired air of each animal will be monitored separately during 0-6, 6-24 and 24-48 hours and for as long afterwards as necessary. Radioactivity remaining in the total animal will be measured. The cages will be washed with water at 120 hours and the washings will be retained for radioactivity measurement.

2(b) Ten rats (5M, 5F) will receive single oral doses (high level) of ^{14}C-dimethoate. Urine, faeces and cagewash will be collected as described for experiment 2(a). Expired air will be monitored if the results of experiment 2(a) show this to be necessary. At 120 hours after dosing, the animals will be lightly anaesthetised with halothane and a blood sample will be withdrawn by cardiac puncture. The animals will then be sacrificed by cervical dislocation and the tissue samples listed below will be taken for analysis. The collection of samples and time of sacrifice will be extended to 168 hours after dosing if the results of experiment 2(a) show this to be necessary.

adrenal glands	ovaries
bone	pancreas
bone marrow	spleen
brain	skin
fat	testes
heart	thyroid gland
kidneys	uterus
liver	stomach and contents
lungs	intestinal tract and contents
muscle	blood/plasma
	remaining carcass

2(c) Ten rats (5M, 5F) will receive single oral doses (low level) of ^{14}C-dimethoate. Samples of excreta, tissues and carcasses will be collected and expired air will be monitored (if necessary) as described above (2(b)).

2(d) Ten rats (5M, 5F) will receive single oral doses (low level) of non-radiolabelled dimethoate once daily for 14 days, followed on the day 15 by a single oral dose (low level) of ^{14}C-dimethoate. Following administration of the radioactive dose, samples of excreta, tissues and carcasses will be collected and expired air monitored (if necessary) as described above (2(b)).

APPENDIX 15

(continued)

2(e) Ten rats (5M, 5F) will receive single intravenous doses (test level 1 or 2) of ^{14}C-dimethoate. Samples of excreta, tissues and carcasses will be collected and expired air monitored (if necessary) as described above (2(b)).

2(f) A preliminary experiment will be performed to confirm a procedure for removing unabsorbed dose from the skin. Four male animals will be administered a low level topical dose as described in 2(g) and after about 4 minutes the treated areas of two animals shall be washed with swabs soaked in soapy water and for the other two animals washing will be performed with swabs soaked in ethanol. The amounts of radioactivity recovered in the washes will be measured to determine the effectiveness of the washing procedure.

2(g) Ten rats (5M, 5F) will receive single topical doses of ^{14}C-dimethoate at a low no-effect level. The backs of the rats will be shaved with an electric clipper about 24 hours before treatment and the compound applied as a solution in water containing 2% Tween 80 (0.2 ml) to an area of about 10 cm^2. The treated area will be protected with a plastic net cover attached by Sleek adhesive dressing to prevent loss and disturbance. The cover will not be in contact with the dosing area which will be open to the air. After dose application the animals will be housed in restraining cages to allow separate collection of urine and faeces. Animals will have access to food and water ad libitum. At 6 hours the cover will be removed and the treated area washed by the more effective procedure confirmed in the preliminary test (2(f)). The animals will then be housed in metabolism cages until sacrifice at 120 hours. Urine and faeces will be collected separately at 6-24 and then at 24-hour intervals. At sacrifice the treated skin and tissues listed in 2(b) will be taken from each animal.

2(h) Ten rats (5M, 5F) will receive single topical doses of ^{14}C-dimethoate at a high dose level 10-fold higher than the low level. Animals will be dosed and samples collected as described in 2(g).

Plasma Concentrations of Radioactivity (plasma kinetics)

3(a) Ten rats (5M, 5F) will receive single oral doses (high level) of ^{14}C-dimethoate. Blood samples will be taken from a tail vein into heparinised tubes at pre-dose, and at 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, and 120 hours post-dose and at further intervals if necessary, until radioactivity in plasma is near the limit of accurate measurement.

3(b) Ten rats (5M, 5F) will receive single oral doses (low level) of ^{14}C-dimethoate. Blood samples will be taken at the times listed above (3(a)).

APPENDIX 15

(continued)

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3(c) Twenty-four rats (12M, 12F) will receive single oral doses (high level) of ^{14}C-dimethoate. Groups of six rats (3M, 3F) will be sacrificed at each of four times after dosing. The sacrifice times will be based on the results of experiment 3(a) and will be agreed with the Study Sponsor prior to dosing. Immediately prior to sacrifice a blood sample (as large as possible) will be taken from each animal by cardiac puncture, and the plasma will be separated from the cells.

Biliary Excretion of Radioactivity

4(a) Six rats (3M, 3F) with cannulated bile ducts, will receive single oral doses (high level) of ^{14}C-dimethoate. Bile will be collected deep frozen at 0-3, 3-6, 6-12, 12-24 and 24-48 hours Urine and faeces will be collected at 0-24 and 24-48 hours. At 48 hours, the animals will be sacrificed by cervical dislocation and the gastro-intestinal tract, liver, and residual carcass will be taken for analysis.

4(b) Six rats (3M, 3F) with cannulated bile ducts will receive single oral doses (low level) of ^{14}C-dimethoate. Samples will be taken as described above (4(a)).

Tissue Distribution of Radioactivity

5(a) Ten rats (5M, 5F) will receive oral doses (high level) of ^{14}C-dimethoate. Pairs (1M, 1F) of rats will be sacrificed by CO_2 asphyxiation at times to be specified, but including the times used in experiment 5(b). Animals will then be subjected to whole-body autoradiography using procedures based on the work of Ullberg (Acta Radiol Suppl 118, 22). Sections will be presented at six different levels of the rat body to include as many tissues as possible. Distribution of radioactivity will be assessed by visual examination of the autoradiographs.

5(b) Eighteen rats (9M, 9F) will receive single oral doses (high level) of ^{14}C-dimethoate. Groups of six animals (3M, 3F) will be sacrificed at each of three times after dosing. These times will be based on data obtained from the plasma kinetic experiment 3(a), to correspond to the times of peak plasma concentration, approximately half of the peak concentration and a terminal time. Actual times will be advised later.

APPENDIX 15

(continued)

Immediately prior to sacrifice, a blood sample will be taken from each animal by cardiac puncture. Following sacrifice, tissue samples listed below will be removed from each carcass and taken for analysis.

adrenal glands	ovaries
bone	pancreas
bone marrow	spleen
brain	skin
fat	testes
heart	thyroid gland
kidneys	uterus
liver	stomach and cotents
lungs	intestinal tract and contents
muscle	blood/plasma
remaining carcass	

5(c) Eighteen rats (9M, 9F) will receive single oral doses (low level) of ^{14}C-dimethoate. Groups of six animals (3M, 3F) will be sacrificed at each of three times after dosing. These times will be based on the results from the plasma kinetic experiment 3(b)), to correspond to the times of peak plasma concentration, approxi-mately half of the peak concentration and a terminal time. These need not necessarily be the same as times used in experiment 5(b). Samples of blood, organs and tissues will be taken for analysis as described for experiment 5(b).

5(d) Eighteen rats (9M, 9F) will receive oral doses (low level) of ^{14}C-dimethoate once daily for seven days. Groups of six animals (3M, 3F) will be sacrificed at the same times after the final dose as used in experiment 5(c). Samples of blood, organs and tissues will be taken for analysis as described for experiment 5(b).

H SAMPLE PROCESSING & RADIOACTIVITY MEASUREMENT

Urine/Bile

Each sample will be thawed and made up to a fixed volume with distilled water in a volumetric flask. Duplicate aliquots (1 ml) will be taken for radioassay by liquid scintillation counting (LSC).

Faeces

Procedures for handling faeces will be developed using samples from the pilot excretion experiments, and will initially depend on the extent of urinary excretion of radioactivity. If significant faecal excretion of radioactivity is expected then the faeces will be extracted with organic solvents or solvent/water mixtures. Otherwise faeces may be homogenised with water and analysed by combustion.

APPENDIX 15

(continued)

Expired Air Traps

When required, the expired air from each animal separately will be drawn through two sequential traps containing a 2-ethoxy-ethanol: ethanolamine mixture (3:1, v/v). The initial volumes of trapping solvent will be 300 ml (trap 1) and 150 ml (trap 2). At the end of each collection period, the total volumes will be checked and duplicate aliquots (1 ml) taken for radioassay by LSC.

Tissues/Organs

Larger organs will be homogenised. Triplicate (where possible) portions of these homogenates, samples of muscle, fat, skin and bone and whole small organs will be either solubilised in a commercial solubiliser or combusted in an automatic sample oxidiser prior to radioassay.

Blood

(a) From excretion-balance and tissue distribution experiments

The sample will be divided. Duplicate aliquots of whole-blood (ca 0.5 ml) will be taken for radioassay by combustion/LSC. The remaining sample will be centrifuged to separate cells and duplicate aliquots of plasma (ca 0.5 ml) taken for radioassay by LSC.

(b) From plasma kinetic experiments

Plasma will be separated by centrifugation. If possible, duplicate aliquots (0.1 ml) will be taken directly for LSC.

Carcasses

These will be solubilised in a mixture of 2M aqueous sodium hydroxide solution:methanol:Triton X405 (6:3:1, v/v) at 55°C for 24 hours. Aliquots of solution (1 ml) will be neutralised with nitric acid and taken for radioassay by LSC.

APPENDIX 15

(continued)

Measurement of Radioactivity

Concentrations of radioactivity in liquid samples will be measured by liquid scintillation counting (LSC) using MI 31 Special Scintillator Cocktail (Packard Instruments Ltd). Concentrations of radioactivity in solid samples will be measured by liquid scintillation counting after solubilisation or combustion in an Automatic Sample Oxidiser (Packard Instruments Ltd). Combustion efficiencies will be determined using carbon-14 standards for sample oxidisers (Amersham International plc, Amersham, UK). Samples will generally be counted for a maximum of ten minutes or long enough to accumulate 40,000 counts (whichever is less). Quench correction will be by an automatic external standard method. Radiochemical purity measurements will be made using a Berthold (Models LB 2832, LB 2842) linear analyser linked to an IBM XT personal computer.

Storage of Samples

Plasma samples from kinetic studies will normally be analysed immediately. If storage is necessary, this will be at 1-5°C. All other samples will be stored at ca -20°C when not being analysed. Storage periods for samples requiring chromatographic analysis will be kept to the minimum possible.

Disposal of Samples

Biological samples generated during the study will be stored deep-frozen for a period of 6 months after the date of despatch of the draft final report to the Study Sponsor. Samples will be stored for additional periods of time at the request of the Study Sponsor, subject to a possible additional storage charge being levied. No samples will be discarded without permission from the study sponsor.

I INVESTIGATION OF METABOLITE PATTERNS

Summary

Suitable organic solvent extracts of pooled samples (male and female separately) of urine, faeces, bile, plasma, and liver (or other important tissues which are found to contain unusual levels of radioactivity) which contain appreciable amounts of radioactivity will be prepared. The metabolites in these extracts will be examined by chromatographic procedures and deconjugation studies. The proportions of the unchanged compound and metabolites will be measured after selection of the optimum conditions for the separation of radioactive components.

APPENDIX 15

(continued)

Samples to be Analysed

Urine

Samples collected from experiments 2(b), 2(c), 2(d), 2(e), 2(g) and 2(h) will be analysed separately. Urine collected during the various time periods will be pooled to provide representative samples.

Faeces

Faeces from experiments 2(b), 2(c), 2(d), 2(e), 2(g) and 2(h) will be analysed provided they contain an appreciable proportion of the dose. Extracts of faeces collected during various time periods will be pooled to provide representative extracts.

Bile

Bile from experiments 4(a) and 4(b) will be analysed if more than 5% dose is extracted in bile. Bile collected during the various time periods will be pooled to provide representative samples.

Tissues

Samples of liver from the peak concentration time in experiments 5(a) 5(b) and 5(c) will be analysed. Other tissues (eg fat) may be investigated if they are found to contain high concentrations of radioactivity.

Plasma

Samples from experiment 3(c) will be analysed. Samples from all time points will be analysed to determine the concentration of the parent compound present. Samples from the peak level time, and if possible the other times, will also be analysed to determine the concentrations of metabolites present.

Deconjugation Studies

The possible presence of conjugated metabolites in urine and bile will be investigated via enzymic deconjugation studies. Initially the enzyme used will be ß-glucuronidase type H1, from Helix pomatia, also containing sulphatase activity (Sigma catalogue no G0751). The use of other enzymes may be investigated at the discretion of the Study Director.

APPENDIX 15

(continued)

Characterisation of metabolites

Information on the identity of metabolites will be obtained by co-chromatographic comparison with reference compounds. If co-chromatography alone is the basis for identification then every effort will be made to obtain correspondence in two TLC and one HPLC systems. The objective of the work is to identify all metabolites which represent more than 5% dose in any sample analysed. Major metabolites in excreta will be isolated and purified where possible for examination by mass spectrometry.

QUALITY ASSURANCE AND REPORTING

Reports

A draft final report will be submitted to the Study Sponsor's co-ordinator. Following receipt of comments from the Sponsor a second draft version of the report may be submitted. The final report will include a summary of the objectives stated in the protocol and a full description of methods, results and conclusions. It will contain all pertinent data with tabular or graphic illustrations thereof, and appropriate statistical analyses.

The following specific information will be included.

1 Specific activity and radiochemical purity of the test material at the time of dosing, and the amounts administered to rats.

2 Excretion-retention balance of radioactivity, rates of excretion.

3 Plasma radioactivity concentration-time profiles, AUC's (areas under curves) and half-lives if possible. Concentrations of radioactivity in plasma, blood and tissues as weight equivalents.

4 Tabulated data relating to proportions of radiolabelled metabolites in samples analysed.

5 Representative radioscans and autoradiographs of tlc plates, or hplc chromatograms.

6 Identification of major metabolites (where achieved). Separation and purification procedures and spectral data supporting the identifications will be presented in their entirety.

7 A metabolic pathway showing structures of metabolites and proposed intermediates.

The final report will also include a list of signatures of personnel conducting the experimental work, names of study co-ordinator and other scientists involved in the study and HRC's certificate of GLP compliance.

APPENDIX 15

(continued)

Retention of Data

All records relating to the study will be maintained in study note-books and supplementary files by the Department of Chemical Metabolism & Radiosynthesis. Following completion of the study, all records will be collected together and will be stored together with a copy of this protocol and the final report, in the Huntingdon Research Centre Archives, Huntingdon, Cambs, England. All records will be kept for a minimum period of five years from the date of the final report. No records will be destroyed without the Study Sponsor's written consent.

Quality Assurance

The study will be conducted in accordance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983, and subsequent amendment, Federal Register, 17 August 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris, 1982.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health and Social Security, 1986, and subsequent revision 1989.

The Quality Assurance Unit of the Huntingdon Research Centre will inspect the study, audit the final report against the raw data and include a signed statement to document dates of inspection.

APPENDIX 15

(continued)

HUNTINGDON RESEARCH CENTRE LTD
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England.
Telephone: International +44 480 890 431 : National (0480) 890431
Facsimile : International +44 480 890 693 : National (0480) 890693
Telex : 32100 HRCUK G

PROTOCOL APPROVAL

Quotation No. 03242

Schedule No. DTF/16

D Kirkpatrick - 6 January 1993
Date.

Study Director,
Huntingdon Research Centre Ltd.

D R Hawkins - 6 January 1993
Date.

For Laboratory Management,
Huntingdon Research Centre Ltd.

Dr F Pistel - 16 January 1993
Date.

Sponsor's Monitoring Scientist*
SCC Scientific Consulting Company
Chemisch-Wissenschaftl. Beratung GmbH
Hauptstr. 35, D-6551 Biebelsheim
Tel. 06701/7874 · Fax 06701/7878

* After signature, please return this page to the Study Director, HRC. The corresponding page which is included in the Protocol should also be signed and the complete document retained.

APPENDIX 15

(continued)

HRC

Huntingdon Research Centre Ltd

PROTOCOL AMENDMENT

		HRC Schedule No:	DTF/16
Amendment No:	1 (One)		
		Corrections to protocol:	✓
Date of issue of Protocol:	5 January 1993	Corrections to Amendment	×
		Addition to Protocol:	×
Test Material:	^{14}C-Dimethoate	Other:	×

Abbreviated study title:

Metabolism in the Rat

Signature of Study Director: D. Kirkpatrick 22/9/93

Signature of Sponsor: [illegible] 5/10/93

ON BEHALF OF DTF

SCC Scientific Consulting Company, Chemisch-Wissenschaftl. Beratung GmbH, Hauptstr. 32, D-55545 Bad Kreuznach, Tel. 06701/179-74, Fax 06701/179-75

To: Dr F Pistel

From: Dr D Kirkpatrick

Copies: Client(2), QA, D Roberts, D Shaw, DRH.

Reason for Amendment

1. To provide information on the labelled test substance.
2. To advise change in design of plasma kinetics experiments.
3. To advise proposed dosing schedule.

Amendment (Where appropriate put both original and revised statements)

1. Page 4 of Protocol:

   The specific activity of ^{14}C-dimethoate was measured at the Huntingdon Research Centre by HPLC. Specific activity: 32300 dpm/μg.

2. Page 12 of Protocol:

   The plasma concentrations of radioactivity (plasma kinetics) experiments will be performed as follows:

   Exp 3(a): Eighteen rats (9M, 9F) will receive single oral doses (high level) of ^{14}C-dimethoate. The animals will be divided into three groups of six (3 of each sex). Blood samples (*ca* 0.4ml) will be taken from a tail vein into heparinised tubes at the following times from each group:

   Group 1: Pre-dose, 1, 6, 48 and 120 hours
   Group 2: 0.25, 2, 12 and 72 hours
   Group 3: 0.50, 4, 24 and 96 hours

APPENDIX 15

(continued)

HRC

DTF/16

PROTOCOL AMENDMENT NO 1 - continued

Up to two additional samples (one per group 2 and 3) may be taken if necessary until the radioactivity in plasma is near the limit of accurate measurement.

Expt 3(b): Eighteen rats (9M, 9F) will receive single oral doses (low level) of ^{14}C-dimethoate. Blood samples will be taken at the times listed above (3(a)).

3. The proposed schedule for dose administration and reporting is as follows:

Expt No	Date
2a	21 September 1993
2b, 3a	13 October 1993
2c, 3b	3 November 1993
2d	1 December 1993 (radioactive dose 15 December 1993)
3c	10 January 1994
4a	24(♂) and 27(♀) January 1994
5b	24 January 1994
4b	21(♂) and 24(♀) February 1994
5a	21 February 1994
2e	23 March 1994
2f	11 April 1994
2g	4 and 5 May 1994
2h	8 and 9 June 1994
5c	4 July 1994
5d	9 August 1994 (1st dose)

Experimental termination date: By 30 November 1994
Despatch of draft final report: By 28 February 1995

APPENDIX 15

(continued)

HRC

Huntingdon Research Centre Ltd

PROTOCOL AMENDMENT

		HRC Schedule No:	DTF/16
Amendment No:	2 (Two)		
		Corrections to protocol:	✓
Date of issue of Protocol:	5 January 1993	Corrections to Amendment	×
		Addition to Protocol:	×
Test Material:	^{14}C-Dimethoate	Other:	×

Abbreviated study title:

Metabolism in the Rat

Signature of Study Director: D. Kirkpatrick 20 Jan 1994

Signature of Sponsor: Dr. F. Pistel 4 Feb 1994 ON BEHALF OF DTF

To: Dr F Pistel

From: Dr D Kirkpatrick

Copies: Client(2), QA, D Roberts, D Shaw, DRH.

SCC Scientific Consulting Company Chemisch-Wissenschaftl. Beratung GmbH Hauptstr. 68, D-55545 Bretzenheim Tel. 0671 - 75 74 - Fax 0671 / 76 73

Reason for Amendment

1. To advise minor changes in sample handling procedures.
2. To advise sampling schedule for the plasma parent compound kinetics experiment.
3. To advise sampling schedules for the tissue distribution experiments.

Amendment (Where appropriate put both original and revised statements)

1. Page 11 of Protocol:

   Faeces will not be collected deep-frozen (Note: faecal excretion of radioactivity is so low as not to warrant chromatographic analysis and faeces will be radioassayed directly following homogenisation with water).

2. The sacrifice times for experiment 3(c) will be 0.5, 2, 6 and 24 hours.

3. The sacrifice times for experiments 5(b), 5(c) and 5(d) will be 0.5, 2 and 48 hours. For experiment 5(a) (whole body autoradiography), the sacrifice times will be 0.5, 2, 6, 48 and 120 hours.

APPENDIX 15

(continued)

HRC

Huntingdon Research Centre Ltd

PROTOCOL AMENDMENT

HRC Schedule No: DTF/16

Amendment No: 3 (Three)

Date of issue of Protocol: 5 January 1993

Test Material: ^{14}C-Dimethoate

Abbreviated study title:

Metabolism in the Rat

Corrections to protocol: ✓
Corrections to Amendment (No.1): ✓
Addition to Protocol: ×
Other: ×

Signature of Study Director: D. Kirkpatrick 10 May 1994

To: Dr F Pistel

On behalf of

Signature of Sponsor: F. Pistel 20/May 1994

Dr. F. Pistel
SCC Scientific Consulting Company
Chemisch-Wissenschaftl. Beratung GmbH
Hauptstr. 35, D-55546 Biebelsheim
Tel. 06701 / 78 74 - Fax 06701 / 78 78

From: Dr D Kirkpatrick

Copies: Client(2), QA, D Roberts, D Shaw, DRH.

Reason for Amendment

To advise changes in experimental procedures and in dosing schedule for dermal application experiments.

Amendment (Where appropriate put both original and revised statements)

Page 12 of Protocol:

1. Experiment 2f: The preliminary experiment was performed at a dose level of 100 mg/kg, using 1% w/v sodium carboxymethylcellulose (CMC) as the application vehicle.

2. Experiments 2g and 2h: 1% CMC will be used as the application vehicle.

3. Experiments 2g and 2h: Swabs soaked in soapy water will be used to wash the treated areas (results from experiment 2f showed that soapy water was more effective than ethanol in removing unabsorbed dose).

Amendment No 1:

Dose administration for experiment 2h will be 2 June 1994. The results of this experiment will be reviewed by the Sponsor before proceeding with the low level dermal application experiment (2g).

APPENDIX 15

(continued)

HRC Study No.: DTF/16

HUNTINGDON RESEARCH CENTRE

PROTOCOL AMENDMENT

Amendment No.:	4	HRC Study No.:	DTF/16
Study Title:	Metabolism in the Rat		
Compound Name:	^{14}C-Dimethoate		

AUTHORISATION: (Please sign and date)

Study Director: ..... 12 Oct 1994
Dr D Kirkpatrick

Study Sponsor: ..... 17 Oct 1994
Dr F Pistel

Reason for Amendment:

To advise change in dosing schedule (described in Protocol Amendment No. 1)

Amendment:

The schedule for dose administration in the following experiments is as follows:

Expt No.	Date
5c	12 July 1994
5d	16 August 1994 (1st dose)
2g	7 November 1994

Distribution: Sponsor(2), QA, D Roberts, D Shaw, DRH

APPENDIX 15

(continued)

HRC

Huntingdon Research Centre Ltd

PROTOCOL AMENDMENT

HRC Schedule No: DTF/16

Amendment No: 5 (Five)

Date of issue of Protocol: 5 January 1993

Test Material: ^{14}C-Dimethoate

Abbreviated study title:

Metabolism in the Rat

Corrections to protocol: ✓
Corrections to Amendment (No.1): ×
Addition to Protocol: ×
Other: ×

Signature of Study Director: 12 Dec

Signature of Sponsor: ON BEHALF OF DTF 19 DEC 1994

(Dr. F. Pistel SCC Scientific Consulting Company, Chemisch-Wissenschaftl. Beratung GmbH, Hauptstr. 35, D-55546 Bad Kreuznach, Tel. 0671 / 78 74 - Fax 0671 / 78 75)

To: Dr F Pistel

From: Dr D Kirkpatrick

Copies: Client(2), QA, D Roberts, D Shaw, DRH.

Reason for Amendment

Dermal application experiments:

1) To describe the dose procedure in more detail.
2) To provide information on the sample processing of dressing and dose washes.

Amendment (Where appropriate put both original and revised statements)

Section G (Page 12)

Experiments 2(g) and 2(h): The rats were lightly anaesthetised with halothane prior to dose application.

Section H (Pages 14-15)

Soapy water dose washes will be made up to a fixed volume with water in volumetric flasks. Duplicate aliquots (1ml will be taken for radioassay by LSC.

Dressings and plastic covers will be extracted with methanol. Duplicate aliquots (1ml) will be taken for radioassay by LSC.

APPENDIX 16

Testing facility GLP certificate

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
Huntingdon Research Centre Ltd Huntingdon Cambridgeshire PE18 6ES	Analytical chemistry Clinical chemistry Environmental tox. Environmental fate Mutagenicity studies Ecosystems Phys/Chem tests Toxicology

DATE OF INSPECTION

6 March 1995

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

D.F. Moore

19/5/95.

D.F. Moore
Director
UK GLP Monitoring Authority