highly polar, one or more of which may have been acidic in nature. Oxidation of both *syn*- and *anti*-12-hydroxy-endrin to the same ketone (Bedford and Harrod, 1973) suggests U-3 is not *syn*-12-hydroxyendrin. Since CI–MS data provided evidence that all of the chlorines were intact on an uncaged molecule, it would seem reasonable that U-3 may be a cyclic hemiketal (9) (Figure 7), which would produce water-soluble and acidic materials upon CrO_3 oxidation.

The recent report that *anti*-12-hydroxyendrin is the least toxic of the known metabolites of endrin in rats suggests that rapid production and excretion of this metabolite by the resistant strain represent a major contribution to the resistance phenomenon (Bedford et al., 1975). Other contributions to resistance are indicated by an apparent accumulation of endrin in the susceptible strain which retains 25% more of the administered dose of [14C]endrin than the resistant strain (Petrella et al., 1975). Rapid excretion of *anti*-12-hydroxyendrin as a metabolite of endrin by the resistant strain suggests that a major contributing factor to resistance is the rapid conversion of endrin to a less toxic derivative which may be readily excreted. The most toxic metabolite of endrin is 12-ketoendrin (Bedford et al., 1975). Although excretion of this metabolite by either resistant or susceptible strains was not observed, 12-ketoendrin has been described as a predominant tissue metabolite (Baldwin et al., 1970; Bedford et al., 1975). The availability of 12-hydroxyendrin for production of a more toxic metabolite, i.e., 12-keto-endrin, would therefore be greatly reduced in the resistant as compared with the susceptible strains.

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Fate of Methoprene (Isopropyl (2*E*,4*E*)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate) in Rats

David R. Hawkins,* Karin T. Weston, Leslie F. Chasseaud, and Eric R. Franklin

The metabolic fate of methoprene, isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, a new insect growth regulator (Altosid), has been studied in rats. After oral administration of [5-14C]methoprene, means of 19.6 and 18.0% of the dose were excreted in the urine and feces, respectively, during 5 days. During the same time, 38.8% was excreted in the expired air as $14CO_2$ and, after 5 days, a mean of 17.2% was retained in the body. Most of the residual radioactivity was present in the fat (8.5% equivalents) and muscle (2.2%) and concentrations were highest in the liver (84.5 ppm), kidneys (29 ppm), lungs (26 ppm), and fat (36.5 ppm). Whole-body autoradiographs showed extensive distribution of radioactivity and particularly notable was the high concentration in the adrenal cortex. No methoprene was detected unchanged in bile or urine and about 12 radioactive components could be detected in urine, none of which corresponded to the authentic reference compounds, which would arise by simple ester hydrolysis and/or O-demethylation. The available evidence indicated that the metabolites probably arose from β-oxidation of methoprene.

Methoprene (1, isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate; trademark, Altosid) is a member of a potent new class of insect growth regulators (IGR's) with juvenile hormone activity (Henrick et al., 1973) which is in commercial use as a mosquito and horn fly larvicide. The compound is relatively nontoxic to mammals and no effects have been observed at doses of >34 600 mg/kg to rats. Studies on the environmental degradation of methoprene by plants (Quistad and Staiger, 1974), aquatic microorganisms (Schooley et al., 1975a), photooxidation (Quistad et al., 1975a), and soil (Schooley

et al., 1975b) have shown that it is nonpersistent and rapidly degraded. Some animal metabolism studies have been performed in the guinea pig, steer, and cow (Quistad et al., 1975b,c; Chamberlain et al., 1975) and complementary to these investigations we now report the fate of methoprene in rats.

MATERIALS AND METHODS

(2*E*,4*E*)-[5-14C]Methoprene (1), synthesized as described previously (Schooley et al., 1975a), was supplied by the Zoecon Corporation (Palo Alto, Calif.). The specific activity was 3.11 mCi/mmol and the radiochemical purity was greater than 99%. Authentic reference compounds (2–4) listed below (see Table I) 7-methoxycitronellic acid were synthesized by the Chemical Department, Zoecon Research.

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R¹O \ $\parallel$ OR²
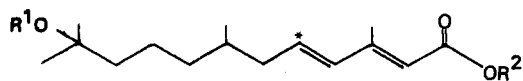
1 R¹ = CH₃   R² = (CH₃)₂CH

2 R¹ = H   R² = (CH₃)₂CH

3 R¹ = CH₃   R² = H

4 R¹ = H   R² = H

*carbon-14

Animal Experiments. Adult Ash Wistar rats (body weight 200 ± 10 g; from Charles River, Manston, Kent, U.K.) were used for all experiments. A nominal dose level of 25 mg/kg was used and animals were dosed orally with [5-^{14}C]methoprene (0.5 mg; ca. 5 μCi) and unlabeled methoprene (14.5 mg) dissolved in 70% (v/v) aqueous ethanol (0.5 ml).

For the excretion–retention studies eight rats, four of each sex, were administered [5-^{14}C]methoprene and housed singly in glass metabolism cages with free access to food and water. Urine, into receivers cooled in solid CO_2, and feces were collected at 24-h intervals, and all excreta were stored at -20 °C. The expired air from each animal was trapped in 20% (v/v) ethanolamine-2-ethoxyethanol. After 5 days, the animals were killed and the carcasses stored at -20 °C. All cages were washed out with water at the end of the experiments.

The bile ducts of three rats were cannulated with 0-0 gauge nylon catheters under halothane–oxygen anesthesia. Immediately after recovery, [5-^{14}C]methoprene was administered by oral intubation. The rats were kept in restraining cages and supplied with food and glucose/saline solution ad libitum. To prevent hypothermia, which decreases bile flow (Klaassen, 1971), the animals were kept warm using an infrared lamp. Bile, urine, and feces were collected for 48 h and then the animals were sacrificed and the gastrointestinal tracts removed and stored together with the corresponding carcasses at -20 °C.

For the plasma kinetics, six rats, three of each sex, were administered [5-^{14}C]methoprene and blood samples were taken from the tail veins at various times after dosing, into heparinized tubes. Plasma was separated from cells by centrifugation.

For tissue distribution studies, eight male rats were administered [5-^{14}C]methoprene and each animal sacrificed at a different time after administration. Various organs were dissected out and stored at -20 °C. Additional male rats were administered [5-^{14}C]methoprene and each animal was sacrificed at different times by asphyxiation with CO_2. The animals were rapidly frozen to -70 °C in an acetone–solid CO_2 mixture and then cut through the midline with a jigsaw. The carcasses were embedded in 2% (w/v) aqueous carboxymethylcellulose and mounted onto the precooled stage of a Leitz base-sledge microtome at -16 °C in a cryostat. Saggital sections of 20 μm thickness were taken at different levels to include the major organs (Ullberg, 1954). The sections were freeze-dried for 40 min in a Model EF2 freeze-drier and apposition autoradiographs were developed after 28 days exposure of Kodak Kodirex x-ray film.

Measurement of Radioactivity. Radioactivity was measured in a Philips liquid scintillation counter with automatic quench correction by external standard channels ratio (Kobayashi and Maudsley, 1969).

Feces, gastrointestinal tracts, livers, and carcasses were extracted by maceration in methanol and after centrifu-
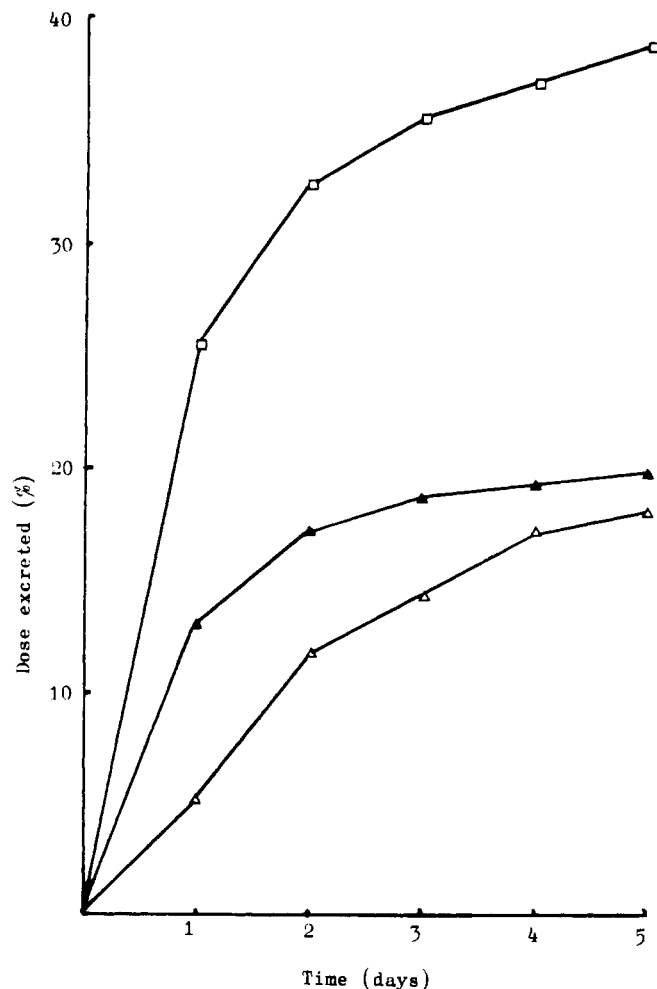

Figure 1. Mean cumulative excretion of radioactivity by rats (n = 8) after an oral dose of [5-^{14}C]methoprene: (▲) urine; (△) feces; (□) CO_2.

gation radioactivity was measured in samples of both the extracts and residue. Urine and plasma samples, solvent extracts (from feces, gastrointestinal tracts, livers, and carcasses), contents of expired air traps, and cage washings were mixed with a toluene–Triton X-100 based scintillator system (Patterson and Greene, 1965). Samples of residues remaining after solvent extractions and tissues or samples of tissues were burned in oxygen using an Oxymat Automatic Sample Oxidiser, the $^{14}CO_2$ being trapped in a β-phenethylamine-based scintillator system (Dobbs, 1963). The limit of detection of radioactivity in tissues and plasma was about 0.5 ppm.

Thin-Layer Chromatography (TLC). TLC was carried out on prelayered Kieselgel F₂₅₄ plates of 0.25-mm layer thickness. The developing solvents were: (a) hexane–ether–acetic acid (50:50:1, v/v); (b) benzene–ethyl acetate–acetic acid (100:50:5, v/v); (c) butan-1-ol–acetic acid–water (2:1:1, v/v); (d) chloroform–ethanol–acetic acid (94:4:2, v/v). ^{14}C-Labeled metabolites were detected by autoradiography using Kodak Kodirex x-ray film. Non-radioactive reference compounds were detected by their quenching of fluorescence in the UV at 254 nm or by exposure to iodine vapor. The R_f values of reference compounds are given in Table I. Radioactivity adsorbed on silica gel was measured by suspending the silica directly in a water–toluene–Triton X-100 based scintillator gel (Chasseaud et al., 1972).

Analysis of Radioactivity in Excreta. Radioactivity was separated from urine using Amberlite XAD-2 resin by the method of Fujimoto and Haarstad (1969). Urine (20
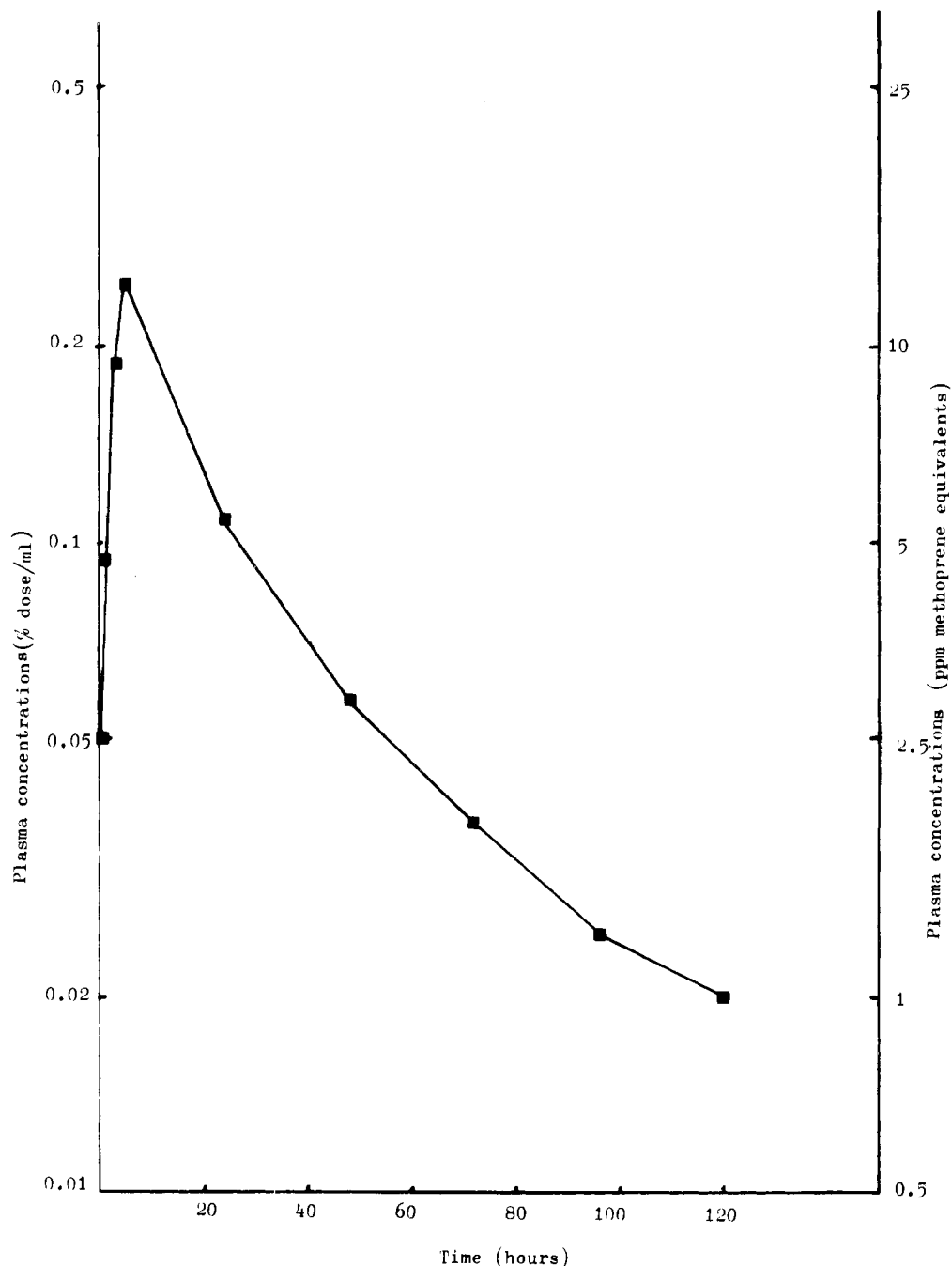
Figure 2. Mean plasma concentrations of radioactivity in rats (n = 6) after oral administration of [5-^{14}C]methoprene.

ml) was applied to columns (20 × 2 cm) of the resin and washed through with water (100 ml). The radioactivity was eluted from the column with methanol (200 ml). The methanol eluate, containing more than 90% of the applied radioactivity, was concentrated to a small volume and used directly for TLC. Aliquots of bile and fecal extracts were evaporated to dryness and the residues triturated with methanol and the resulting extracts used for TLC. Samples of urine or bile were diluted with 0.2 M sodium acetate buffer (pH 5) and incubated with excess β-glucuronidase (type H-2, Sigma Chemical Co.) at 37 °C for 18 h. Enzyme-treated samples and control samples treated similarly in the absence of enzyme were evaporated to dryness and the residues triturated with methanol to provide solutions for TLC.

RESULTS

Excretion and Retention of Radioactivity. The rates and extent of excretion of radioactivity in urine, feces, and

Table I. R_f Values of Authentic Reference Compounds on Silica Gel Thin-Layer Chromatograms

	Solvent system[a]		
Ref compd	a	b	d
Methoprene (1)	0.63	0.73	0.95
Hydroxy ester (2)	0.27	0.50	0.86
Methoxy acid (3)	0.36	0.48	0.72
Hydroxy acid (4)	0.15	0.34	0.65
Methoxycitronellic acid		0.38	
Hydroxycitronellic acid		0.27	

[a] Solvent systems are: (a) hexane-ether-acetic acid (50:50:1, v/v); (b) benzene-ethyl acetate-acetic acid (100:50:5, v/v); (d) chloroform-ethanol-acetic acid (94:4:2, v/v).

expired air are shown in Figure 1. Excretion in the urine was most rapid during the first 24 h, representing a mean of 13% increasing to 19.6% after 5 days. A mean of 11.9%
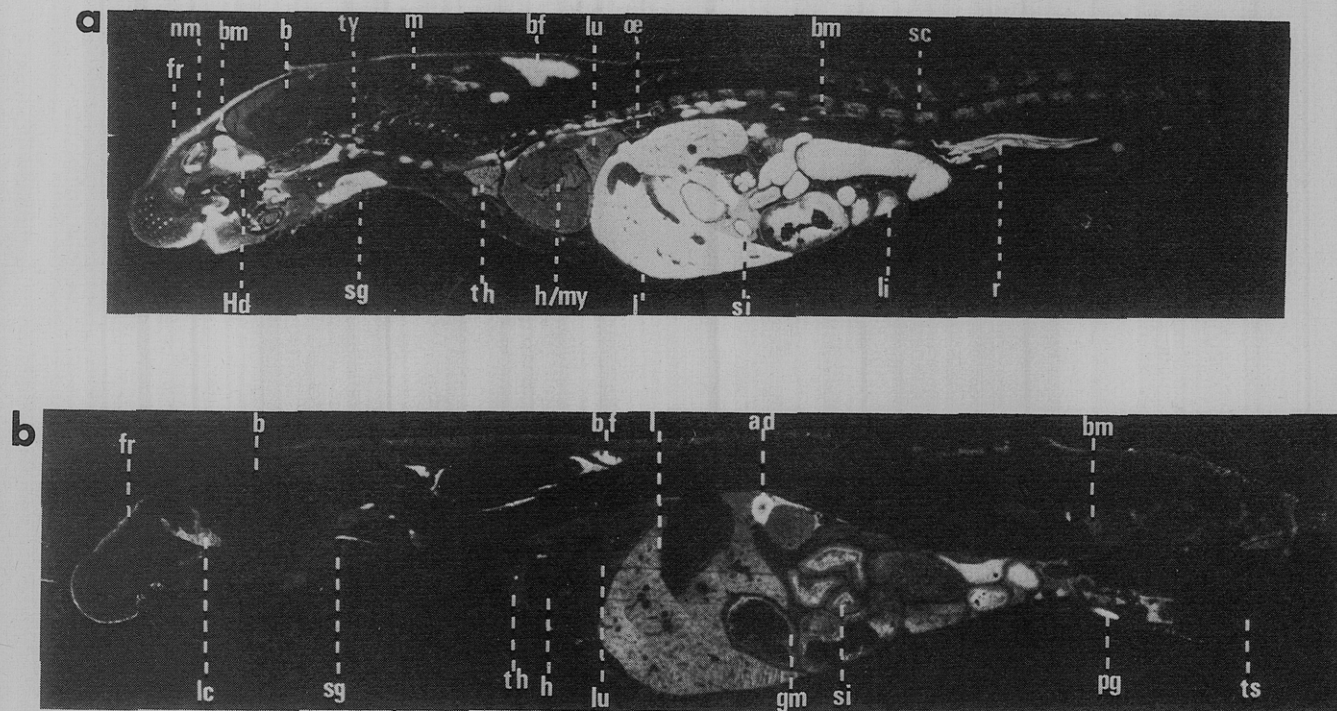
Figure 3. Whole-body autoradiographs of rats sacrificed at (a) 6 h and (b) 48 h after oral administration of [5-^{14}C]methoprene: ad, adrenal; b, brain; bf, brown fat; bm, bone marrow; fr, fur; gm, gastric mucosa, h, heart blood; Hd, Harerian gland (lachrymal); l. liver; lc, lachrymal gland; li, large intestine; lu, lung; m, muscle mass; my, myocardium; nm, nasal mucosa; oe, esophagus; p, pancreas; pg, preputial gland; r, rectum; sc, spinal cord; sg, salivary gland; si, small intestine; st, stomach; th, thymus; ts, testes; ty, thyroid.

of the dose was excreted in the feces during 48 h increasing to 18% after 5 days. There was extensive degradation of [5-^{14}C]methoprene and subsequent excretion of radioactivity as $^{14}CO_2$ in the expired air. A mean of 25.5% of the administered radioactivity was eliminated during 24 h increasing to 38.8% after 5 days. Radioactivity was still slowly being eliminated in urine, feces, and expired air at 5 days, suggesting that the radioactivity retained in the carcass (mean of 17.2%) would have been subsequently eliminated at a similar rate. The mean total recovery of radioactivity in the excreta and carcass residues was 94.1%.

Three rats excreted a mean of 27.4% of the dose in bile during 48 h, most rapidly during the first 24 h. Assuming that the unrecovered radioactivity represented loss of $^{14}CO_2$ from absorbed radioactivity then the mean total absorption of [5-^{14}C]methoprene was at least 74%, by summation of the radioactivity excreted in the expired air, urine, and bile and the residues in the tissues excluding the gastrointestinal tract.

Plasma Concentrations of Radioactivity. The peak of the mean plasma concentrations in rats occurred at about 6 h, indicating slow absorption, and then declined slowly with a half-life of about 48 h during the period 48–120 h after dosing (Figure 2). The total amount of radioactivity in plasma at 6 h was calculated to be 1.63% of the dose by assuming a plasma volume of 6.7 ml for a 200-g rat (Schermer, 1967).

Tissue Distribution of Radioactivity. The concentrations of radioactivity in tissues of rats are shown in Table II. The highest concentrations in most tissues occurred within 6 to 12 h after dosing which period corresponded to the peak plasma concentrations. Peak concentrations in the less well-perfused tissues, such as muscle and fat, were reached at the later time. Highest concentrations occurred in liver (84.5 ppm equiv), kidneys (29 ppm equiv), lungs (26 ppm equiv), and fat (36.5 ppm equiv) and were higher than in the corresponding plasma (12.5 ppm equiv). The high concentrations in the liver,

Table II. Residues of Radioactivity in Rat Organs after an Oral Dose of [5-^{14}C]Methoprene (Results Are Expressed as ppm of Methoprene Equivalents)

Organ	Time, h							
	1	3	6	12	24	72	120	288
Plasma	4.4	7.2	10.7	12.5	7.0	2.0	1.0	<0.5
Liver	28.5	55.5	84.5	47.0	5.0	6.0	3.0	1.0
Kidneys	14.5	11.5	29.0	17.5	16.5	6.5	4.0	1.5
Lungs	6.5	9.5	26.0	25.5	18.0	5.0	3.0	1.5
Heart	9.0	9.0	9.5	3.5	9.0	3.5	2.0	1.5
Brain	3.0	3.5	4.0	3.0	1.5	1.5	1.0	1.0
Eyes	2.5	<0.5	3.0	3.5	2.0	1.0	0.5	0.5
Testes	2.0	1.0	5.0	1.0	4.5	2.0	3.2	2.0
Muscle	4.5	2.0	6.5	11.5	4.5	2.0	1.2	1.0
Fat	4.0	7.0	17.0	36.5	15.0	27.0	30.5	31.5
Adrenals	NM^a	NM	NM	NM	NM	NM	13.5	12

^{a} Not measured.

kidneys, and lungs are not surprising since these are the organs concerned with the elimination of methoprene by metabolism and biliary, urinary, and pulmonary excretion. Uptake into adipose tissue was probably due to the formation of radioactive endogenous components (e.g., cholesterol and fatty acids) produced by the incorporation of small carbon fragments into the pathways of intermediary metabolism. Most of the radioactive residues in the carcasses of rats after 5 days were present in the fat (8.5% dose) and muscle (2.2% dose).

Whole-body autoradiographs (Figure 3) showed extensive distribution of radioactivity. At 6 h, high concentrations were observed in the stomach, liver, small intestine, and brown fat and significant levels were also present in the kidneys, salivary and lachrymal glands, brain, thyroid, thymus, spleen, lungs, adrenals, myocardium, and bone marrow. After 48 h, concentrations were generally lower although high levels were still apparent in the brown fat, liver, lachrymal glands, intestines, and particularly the adrenals. Enlargement of the autoradi-
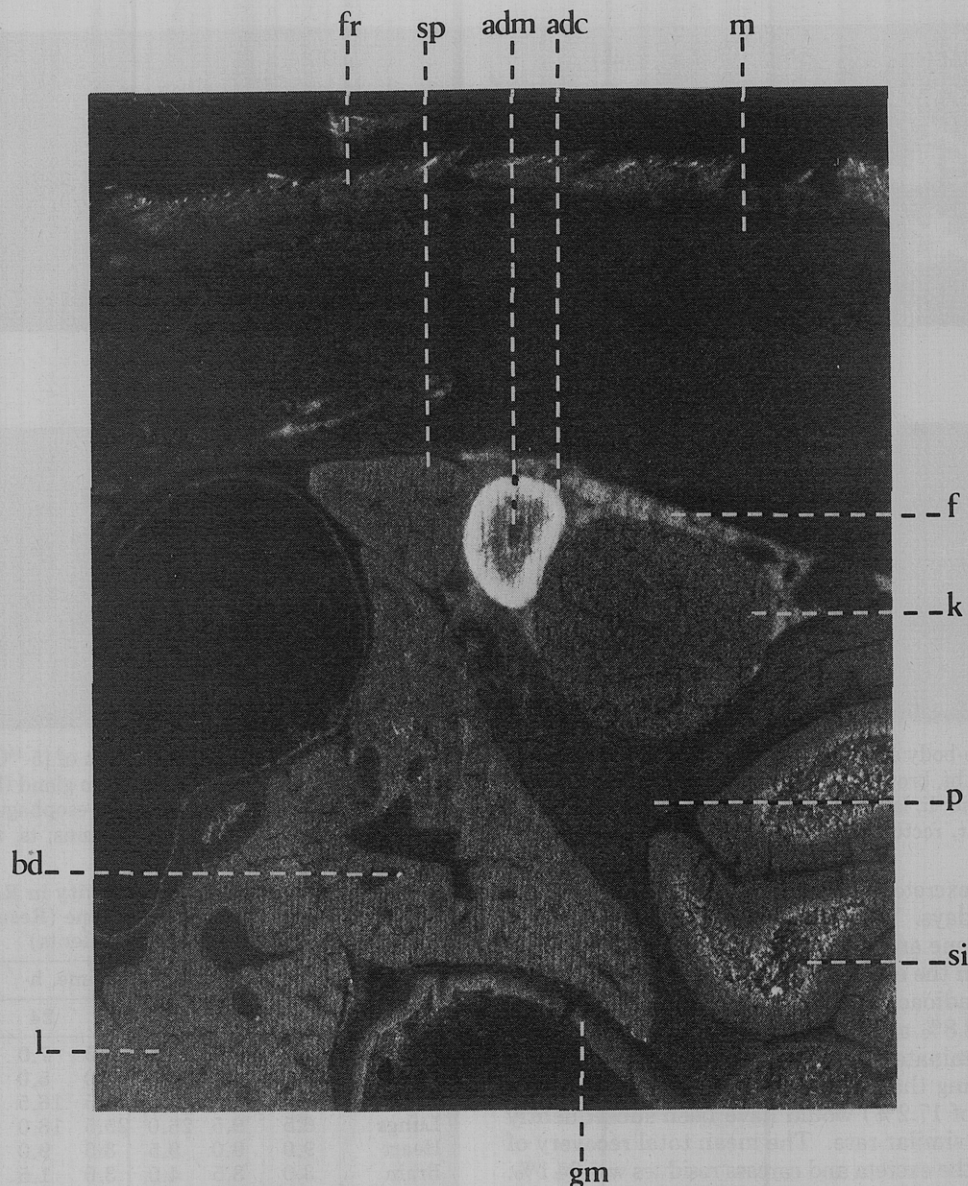
Figure 4. Enlargement of a section of the autoradiograph in Figure 3: adc, adrenal cortex; adm, adrenal medulla; bd, bile duct; f, fat; fr, fur; gm, gastric mucosa; k, kidney; l, liver; m, muscle; p, pancreas; si, small intestine; sp, spleen.

ographs (Figure 4) clearly showed that in the adrenals, the radioactivity was mainly confined to the cortex.

Radioactive Components in Excreta. The proportions of radioactive components in urine and bile extracts are shown in Table III. The major urine metabolites did not correspond to any of the available reference compounds, compared to which they were generally more polar. The best separation was achieved in solvent system b where at least 12 individual components could be detected. Incubation with β-glucuronidase/sulfatase did not significantly alter the chromatographic pattern. The major proportion of the radioactivity in bile was associated with very polar material, which was unaffected by enzyme treatment. Two major radioactive bands were separated in solvent system c, the more polar of which gave a faint purple color with ninhydrin, but no coloration with the platinic iodide reagent (Toennies and Kolb, 1951). This indicated that the metabolite could be an amino acid conjugate, although the lack of a positive result with the latter reagent suggests the absence of a glutathione or cysteine conjugate.

Most of the urine metabolites appeared to be carboxylic acids as they were converted to less polar components on

Table III. Proportions of Radioactive Components Excreted by Rats after Oral Doses of [5-¹⁴C]Methoprene[a]

Component	R_f	Urine 0–24 h	Bile 0–48 h Rat 2 (male)	Bile 0–48 h Rat 3 (male)
1	0.00	4.3	15.6	22.9
2	0.05	4.2		
3	0.10	1.4		
4	0.21	0.6	1.3	
5	0.32/0.34	0.4	2.9	1.2
6	0.44		1.7	2.2
7	0.61		0.5	0.7
8	0.67		0.7	0.7
Minor metabolites		2.3	6.4	3.5

[a] Results are expressed as a percentage of the administered dose. Quantitation in solvent system b, benzene–ethyl acetate–acetic acid (100:50:5, v/v).

treatment with diazomethane. These metabolites were not visualized on TLC by UV absorption which suggests that the unsaturated UV absorbing chromophores, such as the carbon–carbon double bonds, were not present.

DISCUSSION

Methoprene is a lipid-soluble compound which is, however, incompletely absorbed after oral administration to rats. Of the unabsorbed material, only a small part was excreted as methoprene in the feces, suggesting that the remainder was degraded by the gut flora. The absorbed compound underwent extensive biotransformation and no methoprene was excreted unchanged in urine or bile. The extensive biliary excretion of radioactivity suggested the formation of relatively high molecular weight compounds (mol wt >300) in order to produce metabolites with the physicochemical properties necessary for biliary excretion.

Considering the relative metabolic inaccessibility of the radiolabel it was unexpected that such a large proportion (30%) of the administered radioactivity was excreted as $^{14}CO_2$. The radiolabel was probably initially removed from the carbon chain as acetate via a β-oxidation process. Radiolabeled acetate may then enter the intermediary metabolic pathways and be incorporated into endogenous products. The formation of radiolabeled natural products, some with long half-lives, would account for the slow elimination of a proportion of the radioactivity. The urinary excretion rates of radioactivity following administration of compounds such as [^{14}C]glycine, sodium [^{14}C]formate, and sodium [^{14}C]acetate follow a similar pattern (Rosenblum, 1965). After an initial rapid excretion, there follows 10–15 days after administration a slow excretion phase with a half-life of about 9 days.

Some features of the autoradiographs obtained in this study were similar to those obtained by Appelgren (1967) from mice dosed parenterally with [4-^{14}C]cholesterol. High concentrations of radioactivity were observed by Appelgren in the adrenal cortex 4 days after dosing and were still detectable after 20 days and TLC of adrenal extracts suggested that almost all the radioactivity corresponded to cholesterol and its esters. Uptake of radioactivity also occurred in the bone marrow. Uptake of [^{14}C]estrone into the adrenal cortex of male mice has been observed by Ullberg (1965) whereas similar whole-body autoradiographic studies with [^{14}C]cortisone showed that radioactivity was absent from the adrenal cortex (Hanngren et al., 1964). The retention of a high concentration of radioactivity in the adrenal cortex of rats in our study is interesting. If the radioactivity in the adrenal cortex represents natural steroids, as it is recognized that the adrenals are involved in the synthesis and storage of steroids, then the high concentration of radioactivity suggests the incorporation of a methoprene metabolite into the pathways of steroid biosynthesis. Methoprene is structurally related to farnesoic acid, an intermediate in cholesterol biosynthesis, and it is possible that a more complex labeled precursor than acetate is incorporated. Some elegant studies have been carried out on the nature of tissue residues in a steer administered [5-^{14}C]meth-oprene (Quistad et al., 1974). [^{14}C]Cholesterol was identified as a major nonpolar residue and radiolabeled bile acids were detected in bile from the same animals. The distribution of carbon-14 was determined by chemical degradation which precluded the incorporation of intermediates larger than acetate. The labeling pattern strongly suggested that [2-^{14}C]acetate was the major precursor with less than 20% contribution from [1-^{14}C]acetate. The formation of [2-^{14}C]acetate could occur by an α-oxidation followed by two β-oxidations, which is typical for branched chain fatty acids. Thus, it seems likely that the same situation occurs in rats and that much of the residual radioactivity in the body after 5 days is due to endogenous components such as cholesterol, resulting from biosynthetic incorporation of [^{14}C]acetate.

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