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Absorption, Excretion, and Metabolism of Methoprene by a Guinea Pig, a Steer, and a Cow

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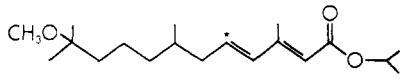
When the metabolic fate of methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) was studied in a guinea pig, a steer, and a cow, a rather large percentage of the radiolabel was incorporated in the tissues and respired by the animals. In the urine and feces, a small amount of radiolabel was metabolized into free primary metabolites, somewhat more was incorporated into simple glucuronides, and a considerable quantity of radiolabel was found in polar compounds, possibly complex conjugates or polar biochemicals. No methoprene was found in the urine, but approximately 40% of the radiolabel in feces was contributed by unmetabolized methoprene. The formation of conjugates and the metabolism of methoprene was more extensive in the steer than in the guinea pig.

Methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) (compound 1), a new insect



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growth regulator, has shown particular promise in the control of mosquitoes (Schaefer and Wilder, 1972, 1973) and horn flies (Harris et al., 1973, 1974). No toxicity to rats administered methoprene at 10,500 mg/kg was demonstrated (Siddall and Slade, 1974). Also, these workers observed no irritating effect on the conjunctiva of rabbits, and fish were not adversely affected by water containing 100 ppm. However, these insect growth regulators are so new as control agents that only a few studies have been made to determine the distribution or elimination of the materials or their metabolites in mammals. Hoffman et al. (1973) studied the metabolism (by rats) of 1-(4-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, and recently a study has been undertaken by Ivie (1974) on the metabolism of the same compound by a steer. Both these studies were conducted with the ^{14}C label within the phenoxy radical of the molecule. Also, a distribution and balance study was completed by Cline et al. (1975) with ^{3}H-labeled methoprene in white mice. Therefore, when methoprene was proposed for mosquito control in situations in which cattle might drink the treated water and when it was used successfully (at very low percentages) in mineral blocks as a feed-through-procedure for the control of horn flies (Harris et al., 1974), we undertook a study of ^{14}C-labeled methoprene (provided by Zoecon Corp.) in a guinea pig, a steer, and a cow.

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MATERIALS AND METHODS

The high-purity [^{14}C]methoprene (1) used in these studies was prepared by J. C. Leak, ICN Corp. (for details of radiosynthesis, see Schooley et al., 1975). Our most sensitive thin-layer chromatographic (TLC) system (2:1:1, benzene–pentane–methanol) indicated that the radiochemical purity of this product was 96.9%. The ^{14}C label was incorporated at the C-5 position which is important in the interpretation of the results.

In addition, Zoecon Corp. also supplied several known primary metabolites as nonradioactive standards. They were: **2,** isopropyl 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoate; **3,** 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoic acid; **4,** 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid; and **5,** 7-methoxycitronellic acid (7-methoxy-3,7-dimethyloctanoic acid). Also, a small sample of ^{14}C-labeled **4** was available as a radioactive standard, and purified methoprene was provided as a nonradioactive standard.

Treatment of Guinea Pig. The 1050-g male guinea pig was treated with 50.86 mg of the [5-^{14}C]methoprene, specific activity of 0.58 mCi/mmol, by adding the material to a no. 3 gelatin capsule conaining a small amount of powdered grain. The capsule was then held on the tip of a small rubber tube attached to a 1-ml syringe, and on compression of the syringe plunger, injected down the esophagus of the guinea pig. After treatment, the guinea pig was maintained for 24 hr in a metabolism cage (urine and feces automatically separated) and fed lettuce and fried pelleted food. The cage was completely covered with a plastic bag, and 400–500 ml of air/min was drawn through the cage and into an ethanolamine solution to remove CO_2. At the end of the test period, the guinea pig was killed and samples of muscle, fat, and blood were taken. The urine of the guinea pig was collected at irregular times during the 24 hr, and all the feces were collected at 24 hr.

Treatment of Steer. The 277-kg Hereford steer was treated with 2.0009 g (3.55 mCi) of the 5-^{14}C-labeled methoprene, specific activity of 0.58 mCi/mmol, by adding the

material to a 0.25-oz gelatin capsule containing a small amount of cracked grain. The capsule was then administered orally by using a balling gun. The steer was held in a metabolism room, and standard methods were used to collect all the urine and feces. Blood samples were taken at regular times after treatment. Also, the air within the room was sampled with an ethanolamine absorber every 3 hr (1-hr period) for the first 48 hr of the test, then every 6 hr for the next 48 hr, and finally once a day for the remainder of the test, to determine whether $^{14}CO_2$ was expired. Air conditioning was used to maintain a temperature range of 17–32° and a relative humidity of 42–84% during the test period. At 2 weeks after treatment, the steer, weighing 288 kg, was slaughtered by electrocution. Samples of tissues were removed for analysis of radioactive residues; weights of organs and various parts of the steer were recorded so radioactive residues could be calculated.

Treatment of Cow. The 4-year-old, 338-kg pregnant lactating Jersey cow, a low producer (slightly less than 2 gal of milk/day), was administered orally a smaller quantity of [5-^{14}C]methoprene than the steer, 0.2076 g (0.74 mCi, specific activity 1.11 mCi/mmol).

The ^{14}C-labeled material lost through respiration by the cow was determined by fitting a plexiglass box at intervals over the cow's head. The box was prepared according to the specifications of Robbins and Bakke (1967) as modified by Rumsey (1969). However, we changed the valving system slightly by placing the valves at the end of the system, and installing a pump of larger capacity, one capable of moving 163 l. of air/min (Gast Mfg. Co.). The gases were drawn from the box by the pump, and the flowmeters and valves were used to divert 1% of the total flow through two traps, each containing a mixture of ethanolamine and 2-methoxyethanol (1:7). The efficiency of this system was pretested by placing a known quantity of ^{14}C-labeled Na_2CO_3 in the box, closing the openings, adding 1 *N* H_2SO_4 to the beaker of Na_2CO_3, and measuring the recovery of CO_2 at the 1% setting of the flowmeters and valves. The recovery was 100% of the expected value for the 1% setting.

After treatment, the gases expired by the cow were trapped continuously for the first 4 hr, with the trapping solution being changed each hour; then the gases were trapped for 1 hr every fourth hr for the next 3 days. On days 4 through 7, measurements were made during every 12th hr. The radioactivity of the trapping solution was determined by pipetting 2 ml into Insta-gel (Packard), followed by liquid scintillation counting. The resulting counts were plotted on graph paper so we had hourly values for the 7 days of the test. On graphical integration the total amount of expired $^{14}CO_2$ could be determined.

Temperature within the metabolism room containing the cow was held at a constant 22–23°; temperature within the plexiglass box was 23.5–27°. The respiratory rate was usually 19–24 respirations/min and rarely as high as 33 respirations/min.

All the urine and feces were collected separately and the cow was handmilked at approximately 12-hr intervals. Collected milk and urine were treated with 1 ml of 38% formaldehyde/500 ml. Blood samples were taken at 6- and 48-hr posttreatment and at slaughter. The cow was fed cane hay and a complete dairy feed but consumed very little; water consumption was less than 1 gal/day. Milk production dropped from 2 gal/day to slightly less than 1 gal/day. At slaughter (7 days posttreatment) the cow weighed 291 kg. Tissues were taken to determine radioactive residues; weights of organs and various parts of the body were determined for calculation of total radioactive residue.

Radioassay and Chromatography. Thin-layer chromatography was used to separate the methoprene and metabolites in urine and extracts. Brinkman 0.25-mm silica gel F_{254} precoated chromatoplates were used for most separations, but occasionally Analtech or plates that we prepared were used to check results. Each sample of radioactive material was always developed in three or four systems on separate plates as follows: acetonitrile–water (80:20, v/v); hexane–ethyl acetate (100:15, v/v); benzene–ethyl acetate–acetic acid (100:50:5, v/v); and benzene–pentane–methanol (2:1:1, v/v). In this way, errors caused by overlapping of radioactive compounds could be eliminated. The nonradioactive standards were applied to each plate as side markers, and compounds **1**, **2**, **3**, and **4** could be located because of the quenching of the fluorescence in the silica gel 254 under uv light. Standard **5** was located by spraying a portion of the plate with ceric ammonium sulfate solution (1.0%). The cholesterol standard was located by spraying a portion of the plate with 2.0% antimony trichloride in 6 *N* HCl and then heating the treated area to 100°; the cholesterol appeared as a lavender spot.

Radioactive areas on the developed plates were located by exposing the plates to nonscreen Gaevert X-ray film for periods ranging from 4 to 83 days, depending on the total amount of radioactive material on the plates. This procedure allowed for the separation of similar radioactive regions. After the films were developed, they could be rematched to the TLC plates, and the indicated areas of radioactivity could be removed and counted by liquid scintillation in a 2,5-diphenyloxazole–bis-MSB toluene solution.

The radioactivity in the urine of all three animals was determined by pipetting 1 ml or less (depending on the activity) into 15 ml of Insta-gel liquid scintillation counting medium. Also, the nature of the ^{14}C-labeled residues within the urine of the guinea pig and steer was evaluated either by applying the urine directly to the TLC plates, treating with concentrated HCl to adjust the pH to 6.5, and then applying it to the TLC plates, or separating it into fractions by Amberlite XAD-2 columns. In addition, these urine samples were treated with several glucuronidases (Sigma Chemical Co.) in acetate buffer (0.2 *M*) by the procedure described by Chamberlain and Hopkins (1973) and then examined by TLC after 24–48 hr at 36°.

The urine of guinea pigs normally has a high pH of near 10, which results in the precipitation of solid material, and makes TLC difficult; therefore, all the guinea pig urine was brought to a pH 6.5 by addition of concentrated HCl to dissolve the solids before chromatography or the Amberlite XAD-2 column was used.

The total radioactive material in the tissues and feces of all three animals was determined either by combusting 100 mg of wet or lyophilized material in the Packard 303 Tri-Carb oxidizer, trapping the ^{14}C-labeled CO_2 in ethanolamine, and counting in 2,5-diphenyloxazole–bis-MSB toluene after addition of methanol or by pipetting a 10% suspension of the feces directly into the Insta-gel. All counting was done in a Beckman LS-150 liquid scintillation counter with both internal and external standardization and channels ratio. The radioactivity in the blood was determined by pipetting 100-μl samples onto cotton balls and then combusting and collecting the CO_2 for counting. Also, the radioactivity in the milk was determined by pipetting 1 ml into Insta-gel and counting.

The feces of the guinea pig and the steer were extracted with several solvents either by mixing in a blender or by Soxhlet extraction (methanol and methyl acetate gave best results). The ^{14}C-labeled residues in the extracted feces were determined by combustion as described for the unextracted feces. The extracts were then counted or examined by TLC.

RESULTS

Guinea Pig. The total radioactive material in the blood of the guinea pig was 18.8 μg equiv of methoprene/ml of blood at 24-hr posttreatment. The material in muscle was 14.3 μg equiv/g of dried meat; this value equaled 3.31 μg

Table I. Radioactive Materials Found in Urine, Feces, and Blood of a Steer Treated Orally with [^{14}C]Methoprene

	Urine			Feces			
Hr post-treatment	μg equiv/ml	Total, mg	Cumulative % of administered dose	μg equiv/g	Total, mg	Cumulative % of administered dose	Blood, μg equiv/ml
3	20.8	11.1	0.6	0	0		0.2
6	35.4	18.4	1.5	0			0.3
12	21.4	31.5	3.1	3.7	8.5	0.4	0.7
24	23.7	70.6	6.6	8.3	36.7	2.2	2.3
36	32.6	74.1	10.3	20.7	122.9	8.3	No sample
48	24.0	69.2	13.8	21.6	142.2	15.5	4.1
72	15.1	67.7	17.2	16.0	221.6	26.6	4.5
96	7.6	35.0	19.0	6.2	78.8	30.5	4.2
120	3.5	17.2	19.9	3.7	46.2	32.8	4.1
144	1.9	8.3	20.3	2.0	21.9	33.9	3.4
168	1.1	4.8	20.5	1.4	17.4	34.8	3.3
192	3.6	14.3	21.2	0.9	14.0	35.5	3.2
216	0.6	2.3	21.3	0.7	10.0	36.0	No sample
240	0.6	2.3	21.4	1.9	25.6	37.3	2.3
264	0.4	1.4	21.5	0.5	6.4	37.6	No sample
288	0.5	1.9	21.6	0.6	7.4	38.0	2.0
312	0.4	1.7	21.7	0.6	8.4	38.4	No sample
336	0.2	0.7	21.7	0.6	7.7	38.8	2.0
	Totals	432.5			775.7		

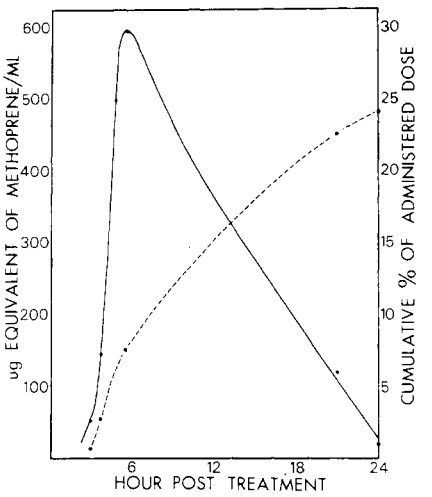

Figure 1. Radioactivity in guinea pig urine after treatment with [5-^{14}C]methoprene: (●—●) microgram equivalents of methoprene/milliliter of urine; (●---●) cumulative percent of administered dose recovered.

equiv/g of wet tissue. The fat sample had 10.95 μg equiv/g of wet tissue. The value for feces was 579 μg equiv/g of dried material, and the total recovery in the feces was 4628 μg or 9.1% of the administered dose. The peak in radioactive material in the urine occurred at 5.5 hr after treatment (Figure 1). The total recovery in the urine during the 24-hr test period was 24.0% of the administered dose. A surprisingly large amount of radioactive material was trapped in the ethanolamine through which the air from the cage was passed. A total of 8633 μg equiv was trapped in the 210 ml of ethanolamine, that is, 17.2% of the applied dose. The total recovery from all sources within 24 hr amounted to 50.3% of the administered dose.

When dried guinea pig meat was extracted (with methanol), 61.8% of the radioactivity in the sample (8.85 μg equiv/g of dried meat) was removed. Extraction of the guinea pig blood with ethyl acetate yielded only 10.6% of its radioactivity. Extraction of the feces with methanol yielded 96.0% of its radioactivity.

Steer. The highest level of radioactive material was found in the blood of the steer at 72 hr after treatment (Table I), but the peak was flat, and the 2-week level was nearly one-half the maximum value.

The peak in total radioactive material in the feces occurred at 48 hr after treatment, and 38.8% of the applied dose was recovered in the feces during the 2-week test period (Table I). A maximum of 83% of the radioactive material in the feces was extractable with methyl acetate or methanol.

The urinary excretion pattern demonstrated a poorly defined peak in radioactivity at 36 hr after treatment (Table I). Small quantities of radioactive materials were still being excreted in the urine 2 weeks after treatment. The total recovery of the applied dose in the urine was 21.6%.

A total of 60.4% of the applied dose was recovered in the urine and feces of the steer.

The sampling of the air in the metabolism room where the steer was held indicated that a peak in radioactive materials occurred at 30 hr after treatment when a maximum value of 975 μg equiv of methoprene/hr was recorded. At 96 hr after treatment the value was 352 μg equiv. The sampling technique was not designed to be quantitative, but the radioactive material collected in the samples amounted to 2.7% of the applied dose, probably only a small portion of the total radioactivity respired.

The total radioactive material found in the tissues of the steer taken at 2 weeks after treatment varied greatly (Table II). However, the indicated amounts represent any methoprene present and also, to a very large extent, all the primary metabolites of methoprene and any anabolic products synthesized by the steer from ^{14}C fragments formed during catabolism of the methoprene (Quistad et al., 1974b, 1975a,b). The bile had the highest level of radioactive materials and the tissue portion of the gall bladder the next highest. Among the conventional tissues, the liver and kidney had nearly 5 μg of total radioactive equiv/g of wet tissue. The highest fat value was that for renal fat, and all the principal meat tissues, the muscles, had less than 1 μg of total radioactive equiv/g of wet tissue.

Table II. Total Radioactive Materials Found in Tissues of the Steer and Cow Treated Orally with [^{14}C]Methoprene

Tissue	Steer[a]		Cow[a]	
	μg equiv of methoprene/g	Total, mg	μg equiv of methoprene/g	Total, mg
Bile	51.20	4.10	0.490	0.122
Gall bladder	20.34	0.65	0.196	0.015
Liver	4.98	17.97	0.489	2.220
Kidney	4.43	2.13	0.374	0.297
Ovaries			0.364	0.004
Lung	3.53	7.91	0.330	0.973
Adrenal	4.23	0.25	0.227	0.009
Spleen	4.12	2.33	0.264	0.180
Heart	1.91	2.11	0.154	0.420
Renal fat	3.19		0.183	
Omental fat	1.34		0.254	
Subcutaneous fat	1.43		0.167	
Bone marrow	1.48		0.185	
Skin	1.18	26.79	0.089	1.488
Brain	1.42	0.43	0.152	0.065
Pancreas			0.233	0.041
Thyroid			0.239	0.005
Fetus			0.158	2.114
Udder			0.160	1.161
Bone	0.40		0.066	
Longissimus dorsi	0.88		0.062	
Semitendinosus	0.67		0.083	
Triceps	0.53		0.077	
Tongue			0.143	0.058
Stomach			0.220	

[a] Steer dosed with 2.0009 g of [^{14}C]methoprene; cow received 0.209 g.

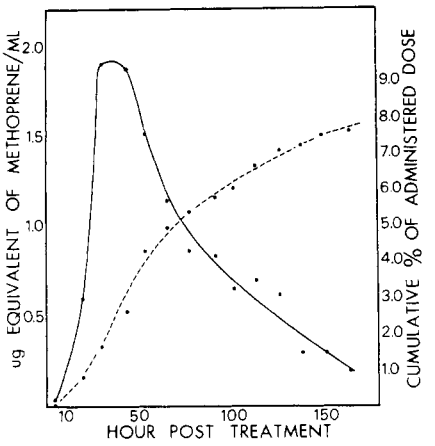

Figure 2. Radioactivity in milk of the Jersey cow after treatment with [5-^{14}C]methoprene: (●—●) microgram equivalents of methoprene/milliliter of milk; (●---●) cumulative percent of administered dose recovered.

Jersey Cow. In the test with the Jersey cow, the radioactivity in the gases collected from the plexiglass chamber peaked sharply between 24 and 28 hr after treatment when 1008 μg equiv of methoprene/hr were recovered. At 7 days, the value had dropped to 41 μg equiv/hr. The radioactivity apparently recovered from respiration of the cow as determined by graphical integration accounted for 15.1% of the applied dose.

Only two blood samples were taken while the cow was alive because we wished to reduce some of the physiological stress in the cow. The value for 6 hr was 0.062 μg equiv/ml and that for 48 hr was 0.268 μg equiv/ml. When the blood collected at slaughter was checked, the value was 0.143 μg equiv/ml.

Twenty-five tissues in addition to the blood were analyzed for radioactive content after the cow was slaughtered. Results are shown in Table II. The highest radioactivity was found in the bile, the next highest in the liver. However, the fairly high levels found in the ovaries should be noted. The value obtained for the 4-month fetus (from a sample of rump muscle) is notably higher than that for the muscles of the cow but lower than the average value of fat for the cow. All muscles of the cow had less than 0.1 ppm of total radioactive equivalents.

Peak radioactivity occurred in the milk at 30-hr posttreatment (Figure 2), but the amount present at 44 hr was nearly as high. At the end of the test (after 7 days), the amount present was only ca. 10% of the maximum value. The total recovery of radioactive material in the milk amounted to 7.6% of the applied dose.

Radioactive material in the urine of the Jersey cow reached a maximum in the sample collected between 24- and 36-hr posttreatment (Table III). By the end of the 7-day test, the rate of excretion in the urine was only ca. 1/50th of the maximum rate. A total of 19.8% of the applied dose was excreted in the urine of the Jersey cow during the 7-day test.

Because of the low intake of food by the Jersey cow, the amount of feces produced was considerably below that expected; however, the pattern of excretion of radioactive material in feces did follow a consistent curve (Table III), and the maximum occurred in the 24- to 48-hr sample. The value at 1 week was only ca. 10% of the maximum recorded. A total of 30.2% of the applied dose was excreted in the feces.

The total recovery of radioactive material in the urine, feces, and milk of the cow therefore amounted to 57.6% of

Table III. Radioactive Materials Found in Urine and Feces of a Cow Treated Orally with [^{14}C]Methoprene

	Urine			Feces		
Hr post-treatment	μg equiv/ml	Total, mg	Cumulative % of administered dose	μg equiv/g	Total, mg	Cumulative % of administered dose
6	0.87	0.77	0.4			
12	2.72	2.08	1.4			
24	4.71	7.91	5.2	1.19	5.08	2.4
36	6.38	10.73	10.4			
48	2.66	7.08	13.8	3.57	39.27	21.3
72	1.35	4.81	16.1	1.53	6.23	24.3
96	1.08	3.45	17.8	1.25	4.52	26.5
120	0.80	2.31	18.9	0.67	3.78	28.3
144	0.34	1.21	19.5	0.45	1.27	28.9
168	0.24[a]	0.64	19.8	0.39	2.76	30.2
	Totals	40.99			62.91	

[a] The average for 144–168 hr was 0.24, but the value for urine at slaughter was 0.11 μg equiv/ml.

the applied dose, a value very close to the recovery from the steer in the urine and feces. When the recovery of radioactive material from the plexiglass box is added, the total recovery for the cow was 72.7% of the applied dose.

Comparison of Metabolism of Methoprene by Guinea Pig and Steer. The metabolism of methoprene by the guinea pig and the steer appeared to differ considerably. When the urine of the two animals was examined by the various techniques, there were always higher concentrations of known metabolites in the guinea pig urine than in the steer urine (Tables IV and V). The figures given in these tables are the averages from several sample periods from 12 to 96 hr after treatment because few changes in chemical composition occurred during these hours. Where such changes were noted they are recorded. The major radioactive compounds in the steer urine remained at the origin or in the area of more polar compounds in any given chromatographic system. Treatment of the guinea pig urine with glucuronidase yielded larger quantities of primary metabolites than treatment of steer urine, probably because of a more complex nature of the glucuronides or other types of conjugates within the steer urine. The amounts of identifiable radiocompounds found in untreated steer urine were too small to establish a predominant known metabolite; however, when the steer urine was treated with glucuronidase, the benzene–ethyl acetate–acetic acid system indicated that metabolite **3** was present to a larger extent than **4**, but the benzene–pentane–methanol system indicated approximately equivalent quantities of these two compounds. In guinea pig urine treated with glucuronidase, the predominant identifiable compound was **4**, possibly because the benzene–pentane–methanol system apparently moved **3**, **4**, and **5** more completely from the bound position at the origin than did the benzene–ethyl acetate–acetic acid system. The demethylated methoprene (**2**) was not detected in enzyme-treated steer urine, but it appeared to be present in the enzyme-treated guinea pig urine by TLC. No methoprene was positively identified in either guinea pig or steer urine whether or not the urine was treated with enzyme. Also, no radiolabeled cholesterol was identified in either guinea pig or steer urine or feces. If present, this compound chromatographs between hydroxy ester (**2**) and methoprene in the benzene–pentane–methanol system.

The results obtained with the acetonitrile–water system were somewhat similar to those obtained with the benzene–ethyl acetate–acetic acid system except that a slightly larger percentage of the radioactive materials could be recognized as known compounds; however, the separation of these compounds was incomplete.

In the tables, the compounds more polar than **3** are grouped together, but four–seven separate compounds were indicated by separate radioactive areas on the chromatograms. Some were apparently conjugates with glucuronic acid as indicated by the effect of glucuronidase on the distribution of radioactive material. Others may have been complex conjugates or products of anabolic reactions having rather high polarity. Treatment of the urine with acid, buffer, sulfatase, or Amberlite column fractionation caused little change in the amounts of indicated metabolites from those recorded for the untreated urine. Sulfatase treatment increased the hydroxy acid to 1.6%.

The apparent complete metabolism of methoprene by both the guinea pig and steer indicated by the examination of the urine was supported by the results of Amberlite XAD-2 fractionation. When steer urine was so treated, an average 75.3% of the radioactive material was eluted with methanol as a yellow fraction; this fraction contained mostly conjugates and polar compounds (98.7%). However, when the original [^{14}C]methoprene was mixed with pretreatment urine and immediately chromatographed, 77–93% of the radioactivity was eluted only after acetone was added to the column. By comparison, in urine from the treated steer, the maximum radioactivity eluted with acetone was only 0.09% of the total radioactive materials on the column from any sample. When the guinea pig urine was fractionated on the Amberlite XAD-2 column, 66.3% of the radioactivity was eluted in the yellow fraction and 0.6% in the nominally methoprene fraction. The composition of the radioactive material in the yellow fraction from both the steer and the guinea pig urine was somewhat similar. The benzene–pentane–methanol system indicated the percentages as 12.3% as **3** and **5**, 7.7% as **4**, and no **2**, cholesterol, or methoprene for the steer vs. 8.3% as **3** and **5**, 11.5% as **4**, and less than 0.1% as **2** or methoprene for the guinea pig.

TLC of the methanol extracts of the guinea pig feces in the benzene–ethyl acetate–acetic acid system showed that 78.8% of the extracted radioactivity was methoprene, 8.0–10.3% was **2** and/or **4**, and 1.7–2.9% was **3**. In the benzene–pentane–methanol system, 77.0% of the extracted radioactivity in the guinea pig feces was methoprene.

The principal extractable radioactive compound found in the steer feces was methoprene (Tables IV and V), but the composition of the radioactive material in the feces varied somewhat with the time of sampling. This effect was particularly noticeable in the relationship between **3** and **2**: we found **3** in relatively large quantities in the 24-hr sample, but small quantities were present in the later samples; the reverse was true for **2**.

Metabolite **5** co-chromatographed in some systems with

Table IV. Oral Treatment with [^{14}C]Methoprene: Average Amounts of Radioactive Compounds in the Urine and Feces Extracts of a Steer 6-72-hr Posttreatment and in Urine of a Guinea Pig 3-6-hr Posttreatment; TLC System Benzene-Ethyl Acetate-Acetic Acid (20:10:1)

	Percentages of recovered radioactivity						
	Steer					Guinea pig	
			Feces extracts				
Compound	Untreated urine	β-Glucuronidase-treated urine	24-hr methanol	36–72-hr methanol	36–72-hr MeOAc[a]	Urine at pH 6.5–6.9	H-1 glucuronidase-treated urine
Conjugates and other polar compounds	99.6[b]	87.1[c]	31.7[d]	27.5[d]	16.4[d]	96.1	76.1
Hydroxy acid (3)	0.3	8.1	24.3	4.9	2.9	3.1	15.6
Methoxycitronellic acid (5)	0	1.3	1.0	3.8	5.7		
Methoxy acid (4) and hydroxy ester (2)	0.1	3.5	16.7	23.8	23.7	0.7	8.3
Methoprene (1)	0	0	26.3	40.0	50.7	0	0
Unknown	0	0	0	0	0.7		

[a] Methyl acetate. [b] As many as six polar compounds indicated. [c] As many as five polar compounds indicated. [d] As many as four polar compounds indicated.

Table V. Oral Treatment with [^{14}C]Methoprene: Average Amounts of Radioactive Compounds in Urine of a Steer 6-72-hr Posttreatment and in Urine of a Guinea Pig 3-6-hr Posttreatment; TLC System, Benzene-Pentane-Methanol (2:1:1)

	Percentage of recovered radioactivity						
	Steer				Guinea pig		
			Feces extracts				
Compound	Normal urine	6-hr H-1 glucuronidase-treated urine	24-hr methanol	36–72-hr methanol	Urine at pH 6.5–6.9	H-1 glucuronidase-treated urine	Methanol ext. G.P. feces
Conjugates and polar compounds	97.1	85.0	24.6	14.8	98.7	17.3	6.5
Hydroxy acid (3) and methoxycitronellic acid (5)	1.5	6.2	23.7	4.8	1.3	7.4	3.4
Methoxy acid (4)	1.3	8.9	7.1	12.3	0.1	63.6	4.9
Hydroxy ester (2)	1.0	0	17.6	26.4	0	11.6	8.2
Methoprene (1)	0	0	27.0	41.7	0	0	77.0

other primary metabolites such as metabolite **3**, but we were able to effect a good separation from **3** and **4** in the benzene–ethyl acetate–acetic acid system. In normal steer urine, **5** could not be detected, but small quantities were found after enzyme treatment (Table IV); in extracts of steer feces, as much as 5.7% of the total radioactive materials co-chromatographed with an authentic standard.

In preparing extracts of feces, we also extracted a large quantity of colored material, but when these extracts were chromatographed, the colored bands clearly visible on the plates did not match any of the radioactive areas indicated by the X-ray film though some were very near the radioactive areas. Thus, we found that none of these natural products were co-eluted with any of the primary metabolites when the proper TLC system was combined with exposure to X-ray film to locate radioactive areas.

DISCUSSION

In most balance and metabolism studies, we depend on the recovery of radioactive materials from the urine and feces to provide us with a measure of the completeness of metabolism and elimination of a compound. However, the determination of the metabolism of methoprene requires a critical analysis of the loss of radiolabel through respiration (as CO_2), the determination of the total radiochemical deposited within the tissues of the animals, and a study of the chemical composition of these radiochemical residues. In addition, small losses could occur by loss through the skin and by the escape of gases from the urine and feces.

With the guinea pig, "complete" recovery was limited by the shortness of the test; however, even in 24 hr, 17% of the radioactive material administered was recovered in the respired gases. With the steer, we sampled only the respired gases so we certainly did not recover all loss of radioactivity that might have occurred. With the Jersey cow, a more exacting procedure was employed, and this method should have given the same accuracy of recovery of radioactivity in respired gases as the standard methods used for the urine and the feces. Then since both the cow and the guinea pig show considerable loss of the label via respired gases (15%

by the cow, 17% by the guinea pig) we conclude that considerably more than 2.7% of the radiolabel was lost by the same route by the steer.

The other major loss of radiolabel for a balance study results from deposition of ^{14}C-labeled residues in tissues. The only accurate way to measure the total radioresidue would be the complete combustion of the whole animal or of a very well prepared aliquot of a homogenate of the whole animal. In the present case, such a procedure is not necessary, partly because considerable data have been gathered on the relative amounts of muscle, fat, and bone, and the weights of various tissues of domestic animals, although care should be taken to consider breed, age, sex, and physical condition in the application of these data to other animals of the group. Morrison (1954) divided cattle and other domestic animals into several groups, and by his grouping, our steer would be a "growing steer" and the cow would be a young Jersey cow. From the data we obtained on the weights of a number of tissues, our values for radioactivity of tissues, and data from Morrison (1954), Edelmann et al. (1944), and Kaneko and Cornelius (1970), we can calculate a figure for the total deposit of radioactive materials within the tissues of the steer and cow. With such methods, we found that 3.4–3.6% of the dose to the steer was located in muscles, 2.7% in fat, 0.8% in bone, 1.3% in skin, 1.3–1.9% in blood, and 1.6% within the intestine. Then with the values for the organs found in Table II, the total deposited in the tissues of the steer amounted to about 13% of the applied dose. The total accountability for the steer, exclusive of unaccounted respiratory losses and other minor losses, would be 76.3–76.8% of the applied dose. With the cow, recovery was more complete. There was 6.0% in the intestine, 4.8% in the fat, 2.8–3.0% in muscle, 0.9–1.3% in blood, and 1.1% in bone. With the amounts of radioactivity in organs (Table II), the total amount in the cow was 20.3% of the applied dose, and the total accountability came to 93% of the applied dose.

The metabolism by the steer and the cow appeared to be similar because the data for relative levels of radioactive material in the tissues were comparable although a full metabolism study of the cow was not conducted. The cow was treated at one-tenth the dose given the steer. When this difference is considered, most of the values for radioactivity in tissues (except the bile) are seen to be comparable. For instance, when the microgram equivalents found in the brain of the cow are converted to those for the brain of the steer, we obtain 1.46 μg equiv/g in comparison with the value actually found, as shown in Table II, and for the kidney, we obtain 3.60 μg equiv/g.

The metabolism of methoprene in alfalfa and rice was studied extensively (Quistad et al., 1974a), and all of the primary metabolites found in our steer and guinea pig were found in alfalfa and rice. Also, the conjugation of the various acids was demonstrated in both studies. Probably the only marked difference was the very large elimination of radiolabel through respiration of the animals; only small quantities of radiolabeled CO_2 were evolved by the plants. The schematic for the catabolism of methoprene reported in Quistad et al. (1974a) is therefore similar to the schematic that would be drawn for the steer and guinea pig.

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