

oil, however, has significant miscibility in both solvents.

The solubility data indicated that only very narrow temperature and solubility ranges were studied and were correlated by an equation of the form $S = A \exp(KT)$ instead of the usual $S = A \exp[-\Delta H/(RT)]$ where T is the absolute temperature and A and K are constants. The heat of solution (or mixing) was found from a plot of $\ln S$ against $1/T$.

Again, the solubility of palm oil in *n*-hexane and petroleum spirit was found to be comparable and quite high. Heats of solution (or mixing) are also comparable and are seen from Table II to be very small.

CONCLUSION

The solubility of palm oil in *n*-hexane and petroleum ether appears reasonable and does not show unusual phenomena. UV-visible spectrophotometry can be used to monitor the concentration of palm oil in a primary solvent leaching scheme for extracting oil from the fruit. More work needs to be done to determine the rate and efficiency of primary extraction of palm oil from the fruit using these solvents.

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Methoxychlor Metabolism in Goats. 2. Metabolites in Bile and Movement through Skin

[¹⁴C]Methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane] was given orally to a bile-cannulated goat and via skin surface to two other goats. At the end of 3 days, recovery of ¹⁴C in bile, feces, and urine was 7.8%, 35.2%, and 44.4%, respectively, for the bile-cannulated goat given 1 g of methoxychlor (518 μCi). Seven metabolites plus methoxychlor were isolated from the bile and identified by GC-mass spectrometry. These were demethylated, dechlorinated, and dehydrochlorinated products. When 200 mg (545 μCi) of methoxychlor was applied to the skin of each of the two goats, 85% and 76% of the ¹⁴C were recovered in the skin 3 days later. Small amounts of ¹⁴C were recovered in muscle, adipose tissue, liver, and kidneys, and small amounts of ¹⁴C were excreted in the urine and feces.

Although methoxychlor is a relatively old insecticide, it is still frequently used in formulations for home gardeners, for control of certain insects on crops and livestock, and for fly control in farm buildings. Knowledge of the metabolic fate of methoxychlor in farm animals enables one to make better decisions regarding use of the compound. Previously, we reported isolation and identification of methoxychlor metabolites in urine and feces of lactating goats (Davison et al., 1982). We now report isolation and identification of methoxychlor metabolites in goat bile and movement of [¹⁴C]methoxychlor through skin.

MATERIALS AND METHODS

Animals. A castrated male goat (goat 104, weight 39 kg, 1 year old) was anesthetized with halothane. A polyethylene cannula (PE 240 polyethylene tubing 1.67 mm i.d. × 2.42 mm o.d.; Clay Adams, Parsippany, NJ) was then inserted into the bile duct between the liver and the union of the bile duct with the pancreatic duct. The cannula was brought outside the body through a stab in the right flank. The goat was restrained in a metabolism stall (Robbins

and Bakke, 1967), and bile was collected in a graduated cylinder. Ten days after surgery, the goat was given 1 g of 4,4'-methoxychlor, including 518 μCi of [¹⁴C]methoxychlor, bile was collected for 72 h, and the goat was killed.

Bile was not infused into goat 104 to replace that removed. The goat was given a trace-mineralized salt in addition to hay and water ad libitum.

Hair was clipped from an area about 12 cm² on the backs of two castrated male goats (goat 100, weight 63 kg, 2 years old, and goat 105, weight 46 kg, 1 year old). An area 10 cm² was marked within the clipped area, and 200 mg of 4,4'-methoxychlor, including 545 μCi of [¹⁴C]methoxychlor, was applied in 1 mL of dichloromethane. Urine and feces were collected for 72 h from the goats in metabolism stalls. Then, the goats were killed. Various tissues as well as treated and untreated skin patches were collected.

Methoxychlor and [¹⁴C]Methoxychlor. 4,4'-Methoxychlor and [*ring*-U-¹⁴C]methoxychlor have been described (Davison et al., 1982).

Carbon-14 Analysis. Lyophilized tissues and feces were combusted and assayed for ¹⁴C by liquid scintillation

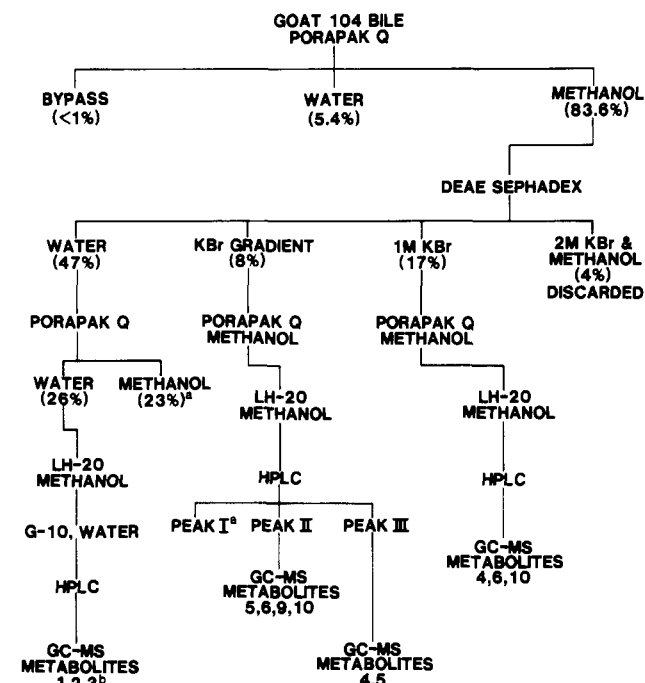


Figure 1. Isolation of metabolites from bile. The goat was given 1 g of methoxychlor orally. Bile was collected for 3 days. Percentages in parentheses are based on the total ¹⁴C present in the bile. (a) Unable to isolate metabolites from these fractions. (b) The metabolite numbering is identical with that of Davison et al. (1982).

spectrometry. Urine was assayed in Insta-Gel scintillator solution (Davison et al., 1982).

Isolation of Biliary Metabolites. Metabolites were isolated from bile via column chromatography, beginning with the addition of fresh bile to Porapak Q (Figure 1), as described for isolation of urinary metabolites by Davison et al. (1982).

Identification of Metabolites. After cleanup, most samples were derivatized with bis(trimethylsilyl)trifluoroacetamide containing 1% chlorotrimethylsilane. The samples were then analyzed by a GC equipped with a radioactive monitor to determine retention times of components containing ¹⁴C as an aid in interpreting subsequent scans from GC-mass spectrometry. Metabolites were identified by interpretation of the mass spectra and by comparing the spectra to those of authentic compounds (Davison et al., 1982).

RESULTS AND DISCUSSION

Goat 104 stopped eating after surgery. Feed was consumed voluntarily by the seventh day. During the 3-day experimental period (10th through 13th days), the average daily consumption of goat 104 was 480 g of hay, 1.6 L of water, and 10 g of salt. The average daily elimination was 152 g of fecal dry matter, 650 mL of urine, and 246 mL of bile. Body temperature never exceeded the normal range for goats. The surgical site and internal organs, including gallbladder, appeared normal when this goat was slaughtered. Goat 105, which was similar in age, sex, and size to goat 104, eliminated 182 g of fecal dry matter and 488 mL of urine per day during the 3-day experimental period.

Recovery of ¹⁴C is shown in Table I. Goat 66 of Davison et al. (1982), a lactating female which underwent no surgery, was given 1 g of methoxychlor under conditions similar to those for bile-cannulated goat 104. Recovery of ¹⁴C in gallbladder, liver, kidneys, and carcass relative to the total dose administered was small for both goats 104

Table I. Recovery of ¹⁴C from Goats Given [¹⁴C]Methoxychlor^a

item	% of dose		
	goat 104	goat 100	goat 105
gallbladder	1.3 × 10 ⁻²		
kidneys	1.0 × 10 ⁻²	2.0 × 10 ⁻³	2.0 × 10 ⁻³
liver	4.4 × 10 ⁻²	5.0 × 10 ⁻³	8.0 × 10 ⁻³
rumen tissue	7.4 × 10 ⁻²		
rumen contents	2.3		
intestinal tissue	3.7 × 10 ⁻¹		
intestinal contents	11.4		
gastrointestinal tract ^b		9.5 × 10 ⁻²	2.1 × 10 ⁻¹
dosed skin patch carcass		85.0	76.0
bile: 0-8 h	0.6	3.8	5.1
8-24 h	0.9		
24-32 h	4.7		
32-48 h	1.0		
48-56 h	0.9		
56-72 h	0.2		
subtotal	0.1		
subtotal	(7.8)		
feces: 0-8 h	2.0 × 10 ⁻⁵	4.0 × 10 ⁻⁴	6.0 × 10 ⁻⁴
8-24 h	1.6	5.5 × 10 ⁻²	1.5 × 10 ⁻²
24-32 h	5.9	2.8 × 10 ⁻²	2.8 × 10 ⁻²
32-48 h	15.0	8.1 × 10 ⁻²	6.7 × 10 ⁻¹
48-56 h	5.3	10.4 × 10 ⁻²	6.5 × 10 ⁻¹
56-72 h	7.3	9.7 × 10 ⁻²	1.3 × 10 ⁻¹
subtotal	(35.2)	(3.7 × 10 ⁻¹)	(9.1 × 10 ⁻¹)
urine: 0-8 h	2.6	6.7 × 10 ⁻²	4.4 × 10 ⁻²
8-24 h	21.0	1.4 × 10 ⁻¹	1.9 × 10 ⁻¹
24-32 h	5.5	6.6 × 10 ⁻²	1.3 × 10 ⁻¹
32-48 h	8.7	1.0 × 10 ⁻¹	0.9 × 10 ⁻¹
48-56 h	2.3	4.2 × 10 ⁻²	1.6 × 10 ⁻¹
56-72 h	3.5	1.2 × 10 ⁻¹	1.1 × 10 ⁻¹
subtotal	(44.4)	(5.3 × 10 ⁻¹)	(7.2 × 10 ⁻¹)
total recovered	102.0	89.9	83.0

^a Goat 104 was given 1 g of methoxychlor orally, and goats 100 and 105 were given 200 mg of methoxychlor on their skin. Tissues and gastrointestinal contents were sampled on the third day after dosing. ^b Rumen through rectum, including contents.

and 66; however, recovery in these materials was 4-10 times higher in goat 104 than in goat 66. More ¹⁴C was recovered in gastrointestinal contents and urine from goat 104 compared to that from goat 66, and less ¹⁴C was recovered in feces from goat 104. The maximum rate of elimination of ¹⁴C in feces occurred 1 day later in goat 104 than in goat 66, but the maximum rate of elimination in bile and urine of goat 104 coincided with that in urine of goat 66. While one must be careful when making comparisons between individual animals because of individual differences, we believe that the rate of passage of materials down the gastrointestinal tract was slower in goat 104, thereby allowing for a greater absorption of the ¹⁴C. Goat 66 eliminated 437 g of fecal dry matter and 1656 mL of urine per day during the 3-day experimental period. Also, it is possible that the absence of bile in goat 104 affected the absorption of ¹⁴C from the intestine or the rate of passage of materials down the gastrointestinal tract.

Seven metabolites and methoxychlor (metabolite 1) were identified in the bile [Figure 1; the metabolite numbering follows that of Davison et al. (1982)]. The metabolites were 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene (metabolite 2), 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane (metabolite 3), 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane (metabolite 4), 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (metabolite 5), 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane

Table II. Methoxychlor Equivalents in Selected Tissues from Goats Given [^{14}C]Methoxychlor on the Skin

tissue	equiv, $\mu\text{g/g}^a$	
	goat 100	goat 105
skin ^b	0.016	0.293
muscle ^b	0.035	0.080
adipose tissue ^b	0.020	0.062
liver	0.049	0.076
kidneys	0.110	0.138
carcass	0.293	0.654

^a Values are expressed as micrograms of methoxychlor equivalents per gram of lyophilized tissue. ^b Skin was taken from the back, posterior to the area dosed; muscle was taken from the rear leg; adipose tissue was taken from the viscera. Tissues were sampled on the third day after 200 mg of methoxychlor was applied to the skin.

(metabolite 6), 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene (metabolite 9), and 1,1-dichloro-2,2-bis-(4-hydroxyphenyl)ethane (metabolite 10). These metabolites accounted for about 50% of the ^{14}C in the bile.

No attempt was made to isolate metabolites from three fractions that contained about 10% of the ^{14}C . These were the bypass and water fractions from the first Poropak Q column and the 2 M KBr fraction from the DEAE-Sephadex column (Figure 1). Despite vigorous attempts, metabolites could not be isolated from two fractions, footnoted on Figure 1, which contained about 25% of the ^{14}C . These two fractions contained abundant lipid-like material, and further attempts at purification yielded ^{14}C -labeled fractions in amounts too small for analysis.

The metabolites isolated from goat bile were dechlorinated, dehydrochlorinated, and demethylated products quite similar to those isolated from goat feces (Davison et al., 1982). Two monochloro metabolites found in feces were not found in bile. These were 1-chloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (metabolite 7) and 1-chloro-2,2-bis(4-hydroxyphenyl)ethene (metabolite 11). Glucuronide metabolites of methoxychlor were found only in the urine of the goat.

When the [^{14}C]methoxychlor was given on the skin, most of the ^{14}C remained on the skin 3 days later, and less than 1% of the ^{14}C was recovered in either feces or urine (Table I). Methoxychlor equivalents in selected tissues are shown in Table II. Muscle tissue was taken from a rear leg, and adipose tissue was taken from the viscera. The concentration of ^{14}C in the carcass, while quite low, was higher than that in the individual tissues. This is probably due to a higher concentration of ^{14}C -labeled material in the tissues immediately beneath the skin to which the methoxychlor was applied, and, in retrospect, tissue cores should probably have been taken in this area to follow the penetration of the ^{14}C . The relatively low total recovery (83%) of ^{14}C from goat 105 cannot be explained. The most likely

explanation for this low recovery is sampling error.

Goat 100 was larger than goat 105, probably accounting for the lower concentrations of ^{14}C -labeled material in tissues of goat 100 compared to those of goat 105. The percentage of lipids in lyophilized carcass was 71% for goat 100 and 52% for goat 105. The tissue residues of goats 100 and 105 are slightly higher than those of goat 69 (Davison et al., 1982), which was given 200 mg of methoxychlor orally and slaughtered 3 days later.

When used, methoxychlor is normally applied to animals as a spray or as a dust. Neither of these methods would be expected to make as positive a contact of the methoxychlor with the skin as did the method used herein, and the methoxychlor probably would be less likely to stay on the animal. Through normal use, 200 mg of methoxychlor is an amount that would likely be applied to an animal of this size (McBride and Kopp, 1982).

The data herein show that ^{14}C from skin-applied [^{14}C]methoxychlor persists on the skin, is absorbed into the animal to give small tissue residues, and is excreted in urine and feces.

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Registry No. Methoxychlor, 72-43-5; 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene, 2132-70-9; 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane, 7388-31-0; 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 28463-03-8; 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene, 75938-34-0; 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 79648-83-2; 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene, 14868-03-2; 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethane, 13005-40-8.

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Tocopherol Content of Some Southeast Asian Foods

5,7,8-Trimethyltolcol (α -tocopherol) has been assayed in a number of Southeast Asian foods. The local green vegetables are a valuable source of the vitamin, as is the widely utilized soybean.

It is regrettable that individuals in Southeast Asia expend scarce resources on purchasing vitamin E preparations when the vitamin is widely distributed in the local foods. That the distribution is wide is shown by the extant analyses. However, not all of the foodstuffs available in

the area have been analyzed; moreover, the values that do appear in the literature consist of single figures (Bunnell et al., 1965; Machlin and Brin, 1980). So that the present knowledge of vitamin E nutrition in the area could be extended, some further analyses were undertaken.