Table V. Time of Death of Rats Treated Orally with Different Amounts of *O,O*-Dimethyl *S*-Propyl Phosphorothioate

dose, mg/g	no. of rats treated	time, days, of occurrence of death 1	2	3	4–25	total rats killed	% killed
7.5	5					0	0
10	4		1	2		3	75
15	5	2	1			3	60
18	4	4				4	100
20	5	4	1			5	100

and Järv (1978). A cholinergic mechanism for poisoning by *O,O*-diethyl *S*-alkyl phosphorothioates cannot be ruled out, and it is possible that both cholinergic and noncholinergic mechanisms contribute to intoxication by these esters.

Further work with trialkyl phosphorothioates is in progress.

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Methoxychlor Metabolism in Goats

Kenneth L. Davison,* Vernon J. Feil, and CaroleJean H. Lamoureux

Methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane] was given orally to two lactating goats. Goat 66 received 1 g (582 μCi) of methoxychlor, and goat 69 received 200 mg (545 μCi). At the end of 3 days, recovery of ^{14}C in feces, urine, and milk was 68%, 27%, and 0.065%, respectively, for goat 66 and 40%, 58%, and a trace, respectively, for goat 69. Seventeen metabolites plus methoxychlor were isolated from urine and feces and identified by GC–MS. Fecal and some urinary metabolites were demethylated, dechlorinated, or dehydrochlorinated products. Most urinary metabolites were completely demethylated and conjugated with glucuronic acid. Ring hydroxylation occurred in one urinary metabolite. The predominant metabolites were 4,4′-substituted dichloroethanes, and no completely dechlorinated products or acids were identified.

Methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane] is an insecticide used to control flies on livestock and in farm buildings. It has other insecticidal uses, but for this paper we are concerned with its direct contact by farm animals.

The metabolism or distribution of [^{14}C]methoxychlor has not been studied in farm animals. Kapoor et al. (1970) reported five metabolites of [^{14}C]methoxychlor in urine and feces of mice. Woodward et al. (1948) observed no methoxychlor or bis(4-methoxyphenyl)acetic acid in urine of rats fed methoxychlor, and Weikel (1957) observed that ^{14}C was eliminated predominantly in feces of rats given [^{14}C]methoxychlor intravenously.

We report the metabolism of [^{14}C]methoxychlor by lactating goats.

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MATERIALS AND METHODS

Animals. Goat 66 (weight 86 kg, 5 years old, and in first month of lactation) was given 1 g of 4,4′-methoxychlor and 582 μCi of [^{14}C]methoxychlor. Goat 69 (weight 56 kg, 4 years old, and in sixth month of lactation) was given 200 mg of 4,4′-methoxychlor and 545 μCi of [^{14}C]methoxychlor. The goats were placed in metabolism stalls (Robbins and Bakke, 1967) and given the methoxychlor orally in gelatin capsules. Milk, urine, and feces were collected for 3 days. Urine was collected directly from the bladder through a latex catheter, which emptied into a covered stainless steel pan. The goats were killed after 3 days, various tissues were sampled, and then their carcasses were ground and sampled. Tissues, milk, and feces were lyophilized and stored in glass jars. Urine was stored in glass jars at 3 °C.

[^{14}C]Methoxychlor. [*ring*-U-^{14}C]Methoxychlor was obtained from New England Nuclear, Boston, MA 02118. The specific activity was 9.03 mCi/mM. Radiochemical purity was determined by isotope dilution in ether–hexane and ethanol (four recrystallizations in each solvent system); purity was >98%. Purity was also determined by GC–

radioactive monitor (GC–RAM), and the impurity was determined to be 1,1-dichloro-2,2-bis(4-methoxyphenyl)-ethane by GC–mass spectrometry (GC–MS).

Methoxychlor. Unlabeled methoxychlor was purified by repeated recrystallization from 95% ethanol, mp 86–89 °C. Purity was greater than 99%, based on GC peak areas.

Dose Preparation. For each goat, dry methoxychlor was weighed and divided among three or four gelatin capsules. [^{14}C]Methoxychlor was dissolved in a small amount of ethanol and assayed, and an appropriate amount was added to each of the capsules. Then, about 4 mL of corn oil was added to each capsule. The capsules were sealed and refrigerated overnight before use.

Carbon-14 Analysis. Fresh milk, blood, and lyophilized materials were oxidized in a Packard Model 306 oxidizer and assayed for ^{14}C in a liquid scintillation spectrometer equipped with an external standard. Urine was assayed in Insta-Gel (Packard Instrument Co., Inc., Downers Grove, IL) scintillator solution. Water or organic solvent extracts were assayed in Insta-Gel or toluene scintillator solutions as appropriate.

Apparatus. Column chromatographic effluents were monitored continuously with a Picker Nuclear Scinti/Flow or a Nuclear-Chicago Chroma/Cell by using cerium-activated silicate glass beads with 2.5% natural lithium as a scintillator. Gas–liquid chromatographic (GC) separations were done with a Barber-Colman Series 5000 gas chromatograph or a Varian 3700 interfaced to a Packard RAM equipped with effluent splitters so that simultaneous flame ionization and ^{14}C detection could be done. Columns were 2 mm i.d. × 1 or 2 m during GC with the Barber-Colman unit or 2 mm i.d. × 2 m during GC with the Varian unit. The column packings were a 3% SP 2100 Supelcoport (Supelco, Inc., Bellefonte, PA), 2 mm i.d. × 1 m, and a 3% SP 2250 Supelcoport, 2 mm i.d. × 2 m. Mass spectra were obtained with a Varian 3700 gas chromatograph interfaced through a jet separator to a Varian 112S mass spectrometer equipped with a Varian SS-200 data system. High-pressure liquid chromatography (HPLC) was performed on a Waters Associates instrument by using a Radial-PAK C_{18} cartridge eluted with acetonitrile and water at 2 mL/min.

Solvents. Solvent mixtures used in chromatography were as follows: solvent 1 = hexane–chloroform–methanol, 3:2:1 v/v/v (Kapoor et al., 1970); solvent 2 = diethyl ether containing 2% ethanol–hexane, 6:94 v/v (Johnson, 1965); solvent 3 = diethyl ether containing 2% ethanol–hexane, 15:85 v/v (Johnson, 1965); solvent 4 = ethanol–diethyl ether, 2:98 v/v; solvent 5 = acetonitrile–water, 1:1 v/v; solvent 6= hexane–acetone, 8:2 v/v; solvent 7 = hexane–acetone–ethanol, 79:20:1 v/v/v.

Extraction of Fecal Metabolites. Lyophilized feces were slurried with solvent and allowed to stand overnight before the solvent was filtered. The 8–24-h feces of each goat were extracted at least 3 times with each solvent, first with hexane and then with methanol. Hexane extracted 3.6% of the ^{14}C from the feces of goat 66 and 2.5% from the feces of goat 69. Methanol extracted 78% of the ^{14}C from the feces of goat 66 and 68% from the feces of goat 69. Nonextractable residues were 14% for goat 66 and 29% for goat 69. The extracts were concentrated in round-bottom flasks under vacuum.

Isolation of Fecal Metabolites. *Goat 66.* The hexane extract of goat 66 feces (Figure 1) was filtered with vacuum assistance through Filter Aid (Sargent-Welch Scientific Co., Skokie, IL). The flask and Filter Aid were washed successively with 100, 35, and 35 mL of hexane, all of which were combined (first hexane wash). The flask and Filter Aid were then washed with 67 mL of hexane (second hexane wash). Finally, residues remaining in the round-bottom flask were dissolved in diethyl ether and filtered through the Filter Aid (flask residue).

Baker 3405 silica gel columns (2 × 58 cm or 1 × 52 cm) were poured in hexane and eluted with solvent 1. LH-20 columns were 1-cm diameter and varied in length from 25 cm to 1 m. Flow rates on all columns were less than 0.5 mL/min.

Samples applied in hexane to activated Florisil (2.4 × 10 cm columns topped with 1.5 cm of anhydrous sodium sulfate) were eluted successively with 200 mL each of solvent 2, solvent 3, solvent 4, and methanol.

After column chromatography, some fractions contained abundant lipid-like material visually evident when the fractions were concentrated. These fractions were chromatographed by thin layer (TLC) on silica gel G, developed with solvent 5. ^{14}C-Labeled Material was scraped from the TLC plate and eluted with methanol and was sufficiently clean for GC–MS.

The methanol extract of goat 66 feces (Figure 1) was applied in a small amount of methanol to a G-10 column (2.4 × 25 cm) poured in water. The column was eluted first with water and then with methanol. Where pigments and metabolites cochromatographed, fractions were separated by color. The concentrated green fraction was dissolved in solvent 1 and applied to a dry silica gel column (1 × 25 cm). The column was eluted successively with solvent 6, solvent 7, solvent 1, and methanol.

A small amount of ^{14}C-labeled material eluted from G-10 was discarded because it was contained in the most intensely colored, turbid fraction near the front of the methanol eluate. Despite vigorous attempts, metabolites could not be isolated from three of the colored fractions.

Goat 69. Isolation of metabolites in excreta of goat 69 differed from that of goat 66 because goat 66 was given a larger dose and because of experience gained from goat 66. The hexane extract of goat 69 feces (Figure 1) was chromatographed on Florisil, as described for goat 66. Metabolites in the methanol eluate from Florisil separated into two incompletely resolved ^{14}C-labeled fractions (C and D) when chromatographed on LH-20, solvent 1. ^{14}C in the overlapped area was discarded.

The methanol extract of goat 69 feces (Figure 1) was cleaned up through G-10, LH-20, and silica gel columns, as described for goat 66. Metabolites were not identified in the water fraction from G-10 or in the green fraction from LH-20 despite attempts to do so.

Isolation of Urinary Metabolites. Metabolites were isolated from urine of goat 66 and goat 69 (Figure 1) via column chromatography, using Porapak Q (2.5 × 25 cm columns), DEAE-Sephadex (1 × 50 cm columns), and LH-20 (1 × 25 cm or 1 × 50 cm columns). Before use, DEAE-Sephadex (A-25; Pharmacia Fine Chemicals, Piscataway, NJ) was washed successively with several volumes of water, 1 M KBr, and water.

Urine from goat 66 was applied directly to a water-washed Porapak Q column. The column was then eluted with about 400 mL of water and 300 mL of methanol and then stripped with acetone. The unabsorbed material (bypass, Figure 1), water, and acetone fractions were discarded. Metabolites applied in water to DEAE-Sephadex columns were eluted successively with 100 mL of water, 250 mL of 1 M KBr, 400 mL of 2 M KBr, and methanol. ^{14}C eluted with 2 M KBr and methanol. Fraction II was chromatographed on LH-20 and was divided into three fractions: peak A, peak B, and an overlapped area A and B. Fraction III also was divided into three fractions: peak C, peak D, and the overlapped area C and D. In both

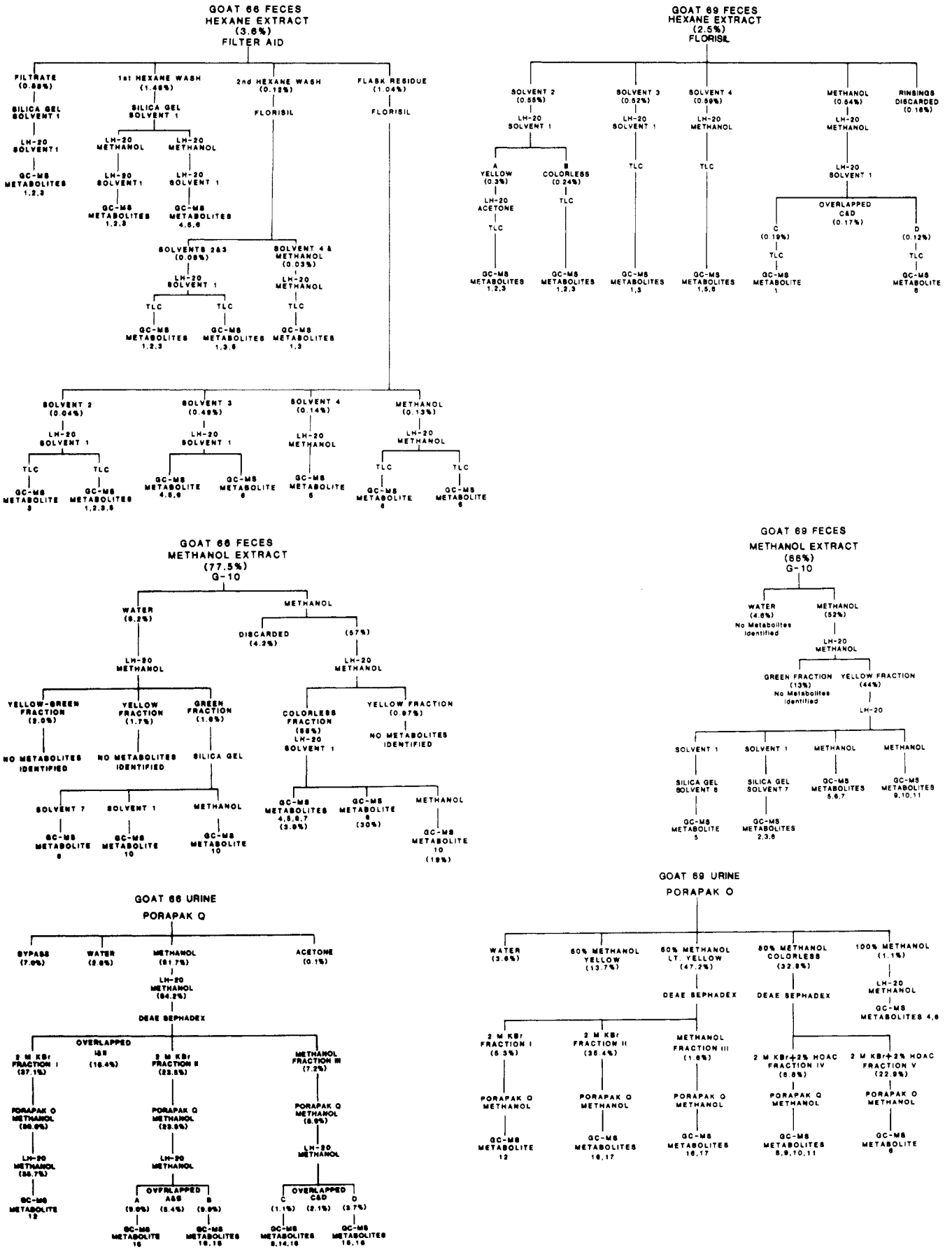

Figure 1. Isolation of metabolites from feces and urine. Percentages in parentheses are based on the total ^{14}C present in the feces or in the urine.

cases, ^{14}C in the overlapped areas represented 23% of the activity applied to the LH-20 columns.

Urine from goat 69 was applied to Porapak Q, as for goat 66, but this Porapak Q column was eluted with various mixtures of methanol and water. Most ^{14}C and color eluted with 50% methanol. The most intensely colored area was collected separately to facilitate cleanup.

^{14}C eluted from Porapak Q with 80% methanol was applied in water to DEAE-Sephadex, and the DEAE-Sephadex column was eluted first with water, then with 1 and 2 M KBr in water containing 2% glacial acetic acid, and finally with methanol.

Identification of Metabolites. After cleanup by column chromatography, most samples were derivatized with bis(trimethylsilyl)trifluoroacetamide containing 1% chlorotrimethylsilane (BSTFA). The samples were then analyzed by GC–RAM to locate areas containing ^{14}C as an aid in interpreting subsequent GC–MS. The metabolites were identified by interpretation of the mass spectra (Figures 2 and 3) and by comparing the spectra to those of authentic compounds.

Metabolite 1 was methoxychlor. Syntheses of metabolites 2, 4, 5, and 8 have previously been reported (Kapoor et al., 1970; Lamoureux and Feil, 1980). Syntheses and NMR data are reported for metabolites 3–11 (syntheses reported in this paper for 4 and 5 differ from those previously reported). The mass spectra of the metabolites and the synthetic compounds or their Me_3Si derivatives were identical. The mass spectra of metabolites 4 and 5 have been previously reported but are also included in this paper as the Me_3Si derivatives (Lamoureux and Feil, 1980).

Synthesis of 1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethene (Metabolite 2). Synthesis and spectra were the same as for compound 34 in Lamoureux and Feil (1980).

Synthesis of 1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethane (Metabolite 3). An aqueous solution of methoxychlor was refluxed with aluminum amalgam for 3 h (Inoi et al., 1962). Most of the ethanol was removed, and the remaining solution was extracted with ether. The ether layer was dried over magnesium sulfate, and the solvent was removed. The product was purified by recrystallization from ethanol–water, mp 102–110 °C. Gas chromatography indicated a purity of 95%. The NMR was obtained on a sample trapped from the GC: NMR (acetone-d_6) δ 3.76 (s, OCH_3), 4.55 (d, CH, J = 10 Hz), 6.86 (d, $CHCl_2$, J = 10 Hz), 6.87 (d, ArH, J = 8.8 Hz), 7.40 (d, ArH, J = 8.8 Hz). MS was identical with that of metabolite 3 (Figure 2).

Synthesis of 1,1,1-Trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane (Metabolite 4). Phenol, 0.5 g, and 0.5 g of 2,2,2-trichloro-1-(4-methoxyphenyl)ethanol (Lamoureux and Feil, 1980) were combined with 20 mL of anhydrous hydrogen fluoride. The reaction was stirred at room temperature for 20 h, and then ether was added. This solution was washed with water, and the ether layer was dried over magnesium sulfate. The solution was filtered and concentrated by distillation. The resulting oil was chromatographed on silica gel with $MeCl_2$. The first colored band contained the product. The desired compound was purified by HPLC using 60% CH_3CN and 40% H_2O: NMR ($CDCl_3$) δ 3.79 (s, OCH_3), 5.15 (s, CH), 6.83 (d, HOArH, J = 8.8 Hz), 6.91 (d, CH_3OArH, J = 8.8 Hz), 7.55 (d, HOArH, J = 8.8 Hz), 7.64 (d, CH_3OArH, J = 8.8 Hz), 8.44 (s, OH); MS m/z (rel intensity) 330 (3 Cl, 2.1, $M^{+\cdot}$), 295 (0.7, M – Cl), 260 (1.8, M – 2Cl), 213 (100, M – CCl_3), 198 (2.4), 181 (3.1), 169 (3.2), 152 (2.8).

Synthesis of 1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (Metabolite 5). 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethene, 1.0 g, was reacted with diazomethane solution at room temperature. The progress of the reaction was monitored by reacting a small sample with BSTFA and analyzing by GC and GC–MS. More diazomethane was added, and the reaction was continued until about half of the starting material had reacted. The dimethoxy compound was removed by extracting an ether solution of the reaction products with 2.5 N NaOH, acidifying the base extracts, and extracting with ether. The extracts were dried over $MgSO_4$, the solvent was removed, and the residue was chromatographed on a silica gel column. The desired compound eluted with $MeCl_2$ and was recrystallized from ether–hexane: mp 115–119 °C; NMR ($CDCl_3$) δ 3.80 (s, $ArOCH_3$), 4.87 (s, ArOH), 6.78 (d, HOArH, J = 8.75 Hz), 6.85 (d, CH_3OArH, J = 8.8 Hz), 7.16 (d, HOArH, J = 8.75 Hz), 7.21 (d, CH_3OArH, J = 8.8 Hz); MS m/z (rel intensity) 294 (2 Cl, 100, $M^{+\cdot}$), 259 (12, M – Cl), 244 (2.5, M – CH_3Cl), 224 (74.6, M – 2Cl), 209 (16.4), 181 (16.9), 152 (19.6).

Synthesis of 1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane (Metabolite 6). 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethane (metabolite 10), 50 mg, was reacted with diazomethane for 2 h at room temperature, and the solvent was allowed to evaporate overnight. The residue was dissolved in acetonitrile and assayed by HPLC with a gradient of 45–90% CH_3CN. Three major peaks eluted: starting material, product, and 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane. The desired compound was collected for NMR and MS data: NMR (acetone-d_6) δ 3.75 (s, OCH_3), 4.50 (d, CH, J = 9.7 Hz), 6.78 (d, ArOH, J = 8.75 Hz), 6.83 (d, $CHCl_2$, J = 9.7 Hz), 6.87 (d, $ArOCH_3$, J = 8.8 Hz), 7.31 (d, ArOH, J = 8.75 Hz), 7.40 (d, $ArOCH_3$, J = 8.8 Hz); MS m/z (rel intensity) 296 (2 Cl, 2.31, $M^{+\cdot}$), 261 (0.43, M – Cl), 226 (2.0, M – 2Cl), 213 (100, M – $CHCl_2$), 197 (1.44), 181 (4.61), 169 (7.14), 152 (4.78).

Synthesis of 1-Chloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (Metabolite 7). 1-Chloro-2,2-bis(4-hydroxyphenyl)ethene (metabolite 11) was derivatized with diazomethane at room temperature. The reaction progress was monitored by HPLC and was allowed to proceed until the 1-chloro-2,2-bis(4-hydroxyphenyl) had disappeared. Two major peaks resulted: the desired compound (I) and 1-chloro-2,2-bis(4-methoxyphenyl)ethene (II). Both peaks were trapped for NMR and MS: NMR ($CDCl_3$) δ (I) (cis/trans mixture) 3.80 (s, OCH_3), 3.84 (s, OCH_3), 5.0 (s, OH), 6.42 (s, CH), 6.7–7.3 (complex multiplet that is a composite of the aromatic patterns of metabolite 11 and compound II); NMR ($CDCl_3$) δ (II) 3.80 (s, OCH_3), 3.84 (s, OCH_3), 6.43 (s, CH), 6.82 (d, ArH, J = 8.75 Hz), 6.92 (d, ArH, J = 8.75 Hz), 7.14 (d, ArH, J = 8.75 Hz), 7.27 (d, ArH, J = 8.75 Hz); MS m/z (rel intensity) (I) 260 (1 Cl, 100, $M^{+\cdot}$), 245 (4.8, M – CH_3), 225 (52.0, M – Cl), 211 (17.2), 210 (20.8, M – CH_3Cl); MS m/z (rel intensity) (II) 274 (1 Cl, 100, $M^{+\cdot}$), 259 (14.0, M – CH_3), 239 (57.6, M – Cl), 225 (25.5), 224 (25.3, M – CH_3Cl).

Synthesis of 1,1,1-Trichloro-2,2-bis(4-hydroxyphenyl)ethane (Metabolite 8). 1,1,1-Trichloro-2,2-bis(4-hydroxyphenyl)ethane was prepared as described by Kapoor et al. (1970). The product was purified with column chromatography by using silica gel and $MeCl_2$ and recrystallized from $MeCl_2$: mp 201–202 °C; NMR (acetone-d_6) δ 5.1 (s, CH), 6.82 (d, ArH, J = 8.7 Hz), 7.54 (d, ArH, J = 8.7 Hz), 8.44 (s, ArOH); MS m/z (rel intensity) 316 (3 Cl, 4.4, $M^{+\cdot}$), 246 (2.3, M–2Cl), 199 (100, M–CCl_3), 181 (2.4), 152 (1.4).

Synthesis of 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethene (Metabolite 9). Two methoxychlor demethylation procedures yielded 1,1-dichloro-2,2-bis(4-hydroxy-

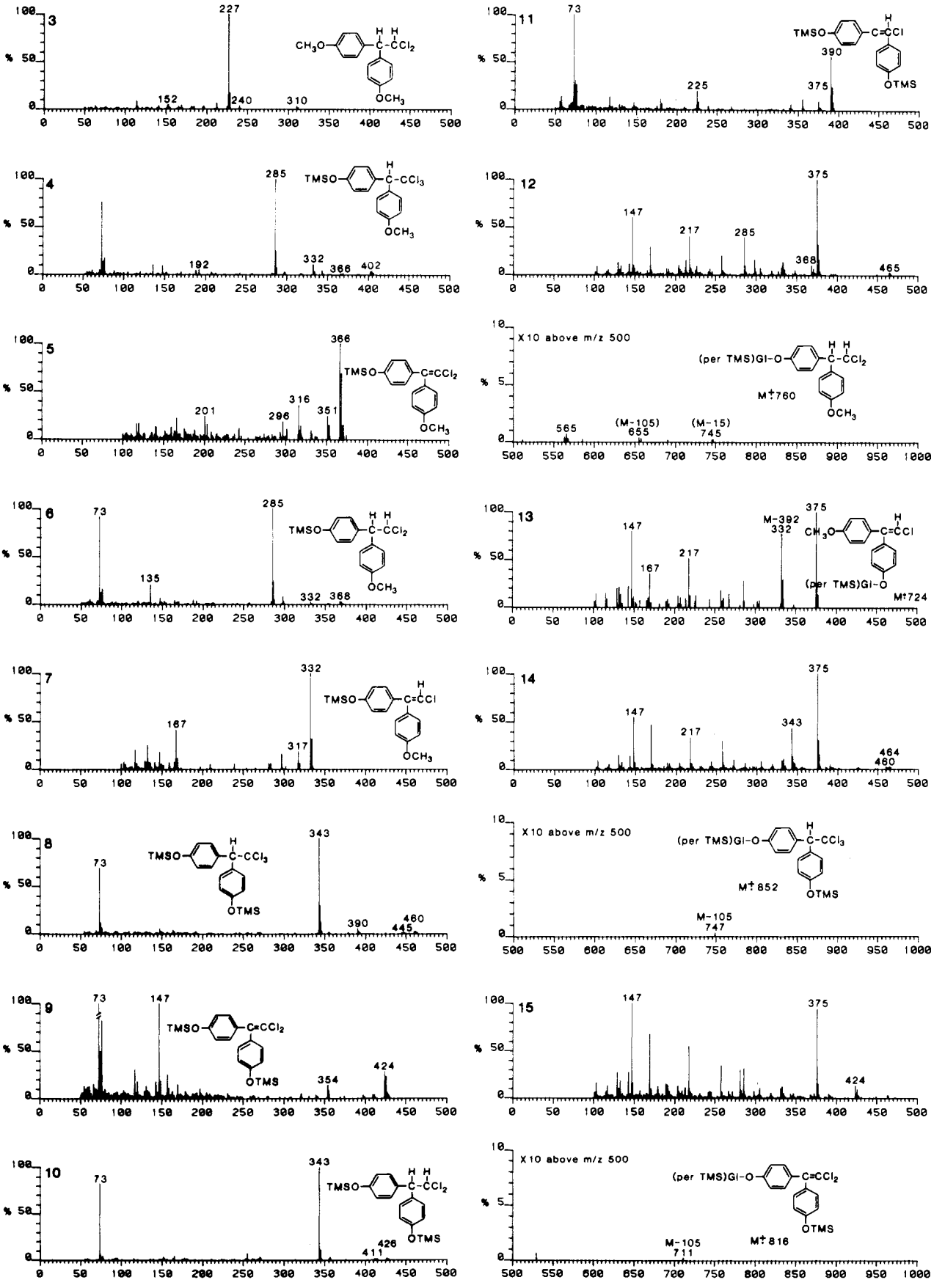

Figure 2. Mass spectra of metabolite 3 and of trimethylsilane derivatives of metabolites 4–15.

phenyl)ethene. In the first procedure, methoxychlor, 0.2 g, and 0.1 mL of boron tribromide were stirred in 20 mL of cyclohexane at room temperature for 1 h. The solution was washed with H_2O, and the organic layer was dried over $MgSO_4$, filtered, and concentrated, mp 211–214 °C.

In the second procedure, pyridine hydrochloride, 30 g, was heated to 215 °C and 5 g of methoxychlor was added. After 5 min, the solution was poured on ice. The solution was extracted with ether, and the ether layer was extracted with 2.5 N NaOH. The basic layer was acidified with

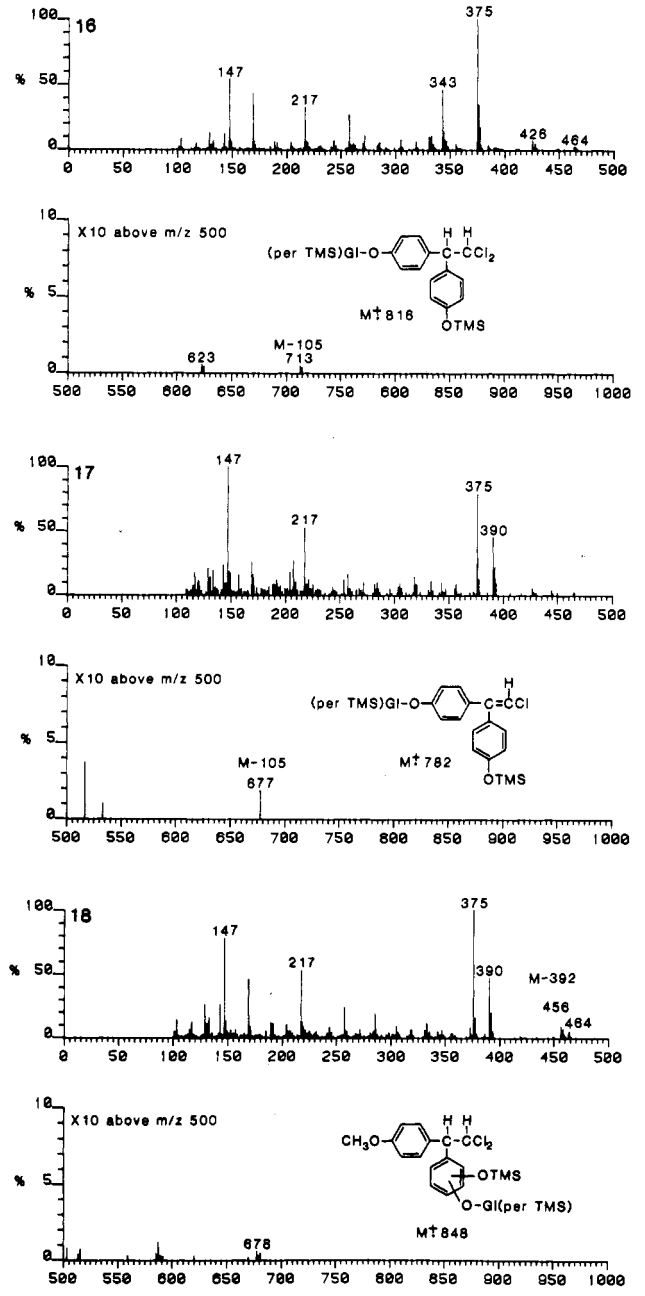

Figure 3. Mass spectra of trimethylsilane derivatives of metabolites 16–18.

concentrated HCl and extracted with ether. The ether layer was dried over $MgSO_4$, filtered, and concentrated to an oil. The product was purified by column chromatography on silica gel and EtOAc and recrystallization from ether–hexane: mp 209–212 °C; 59% yield; NMR ($CDCl_3$) δ 4.76 (s, ArOH), 6.78 (d, ArH, J = 8.75 Hz), 7.16 (d, ArH, J = 8.75 Hz); MS m/z (rel intensity) 280 (2 Cl, 90.5, $M^{+}\cdot$), 245 (13.2, M – Cl), 244 (6.9, M – HCl), 228 (2.2), 210 (100, M – 2Cl), 197 (7.2), 181 (16), 152 (15.4).

Synthesis of 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethane (Metabolite **10**). 1,1-Dichloro-2,2-bis(4-hydroxyphenyl)ethane was prepared from 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane as described for metabolite **3**. The product was recrystallized from ethanol–H_2O, mp 169–171 °C, and then from $MeCl_2$, mp 168–175 °C. GC and GC–MS indicated a mixture of two compounds: 90% of the desired compound and 10% of the starting material.

The sample was purified by HPLC using a column of C_{18} Phase Bonded Support, 200–325 mesh (Applied Science Laboratories Inc.), 1.8 × 25 cm packed in methanol.

Table I. Recovery of [^{14}C]Methoxychlor Given to Goats

item		% of dose goat 66	% of dose goat 69
adrenals		4.5×10^{-5}	3.5×10^{-5}
brain		7.1×10^{-5}	7.5×10^{-5}
gallbladder and contents		1.1×10^{-3}	1.6×10^{-4}
heart		5.5×10^{-4}	4.6×10^{-4}
kidneys		2.7×10^{-3}	7.2×10^{-4}
liver		1.2×10^{-2}	9.5×10^{-3}
ruminal contents		0.68	8.7×10^{-2}
intestinal contents		1.37	0.20
carcass		0.32	0.14
feces:	0–8 h	0.01	1.4×10^{-3}
	8–24 h	29.6	19.5
	24–32 h	15.6	12.0
	32–48 h	15.2	7.1
	48–56 h		1.3
	56–72 h	4.7	0.62
	subtotal feces	(67.5)	(40.5)
urine:	0–8 h	2.6	16.0
	8–24 h	14.6	34.6
	24–32 h	4.3	4.8
	32–48 h	4.0	2.5
	48–56 h	0.77	0.34
	56–72 h	0.81	0.24
	subtotal urine	(27.1)	(58.4)
milk:	0–8 h	1.2×10^{-2}	BD[a]
	8–24 h	2.9×10^{-2}	BD
	24–32 h	9.0×10^{-3}	BD
	32–48 h	7.2×10^{-3}	BD
	48–56 h	1.7×10^{-3}	BD
	56–72 h	5.8×10^{-3}	BD
	subtotal milk	(6.5×10^{-2})	
total recovered		94.6	99.4

[a] BD = below detection limits. Goat 66 was given 1 g of methoxychlor and goat 69 was given 200 mg. Tissues were sampled on the third day.

The column was washed thoroughly with MeOH, CH_3CN, and H_2O before being equilibrated with 25% CH_3CN. The sample was applied in CH_3CN, and the column effluent was split 8:1 for monitoring with a UV detector. After 150 mL of 25% CH_3CN, the desired compound was eluted. The purity was determined by HPLC using 45% CH_3CN in H_2O. The solvent was removed and the solid was recrystallized from $MeCl_2$: mp 173–175 °C; NMR (Me_2SO-d_6) δ 4.38 (d, CH, J = 10.06 Hz), 6.66 (d, ArH, J = 8.3 Hz), 7.07 (d, $CHCl_2$, J = 10.06 Hz), 7.28 (d, ArH, J = 8.3 Hz), 9.31 (s, ArOH); NMR ($CDCl_3$) δ 4.45 (d, CH, J = 10.0 Hz), 6.77 (d, ArOH, J = 8.8 Hz), 6.79 (d, $CHCl_2$, J = 10.0 Hz), 7.30 (d, ArOH, J = 8.8 Hz), 8.43 (s, ArOH); MS m/z (rel intensity) 282 (2 Cl, 3.43, $M^{+}\cdot$), 247 (0.57, M – Cl), 212 (1.35, M – 2Cl), 199 (100, M – $CHCl_2$), 181 (4.47), 169 (2.02), 165 (1.72), 152 (2.94).

Synthesis of 1-Chloro-2,2-bis(4-hydroxyphenyl)ethene (Metabolite **11**). 1-Chloro-2,2-bis(4-hydroxyphenyl)ethene was prepared from 0.5 g of 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane and 5 g of pyridine hydrochloride as in the procedure for metabolite **9**. The resulting oil was purified by using HPLC with a 30–100% gradient of CH_3CN and H_2O. The major peak (95%) was trapped for MS and NMR: NMR ($CDCl_3$) δ 4.77 (s, ArOH), 4.81 (s, ArOH), 6.42 (s, CH), 6.75 (d, ArH, J = 8.75 Hz), 6.84 (d, ArH, J = 7.88 Hz), 7.09 (d, ArH, J = 8.75 Hz), 7.19 (d, ArH, J = 7.88 Hz); MS m/z (rel intensity) 246 (1 Cl, 100, $M^{+}\cdot$), 231 (1.8, M – CH_3), 211 (51.7, M – Cl), 210 (25.9, M – HCl), 197 (20.4), 181 (58.0), 165 (72.9), 152 (52.4).

RESULTS AND DISCUSSION

Both goats were mature and were lactating about 1700 g/day. Total recovery of ^{14}C was 95% for goat 66 given 1 g of methoxychlor, and 99% for goat 69 given 200 mg

Table II. Methoxychlor Equivalents in Selected Tissues and Milk of Goats Given [^{14}C]Methoxychlor

item	equiv, μg/g[a] goat 66	goat 69
adipose tissue	0.03	0.019
adrenals	0.52	0.080
brain	0.03	0.006
gallbladder and contents	2.5	0.050
heart	0.05	0.012
kidneys	0.83	0.038
liver	0.34	0.038
muscle	0.03	0.008
milk	0.21	<0.001

[a] Values are expressed as micrograms of methoxychlor equivalents per gram of dry matter for tissues and per milliliter for milk. Goat 66 was given 1 g of methoxychlor and goat 69 was given 200 mg. Tissues were sampled on the third day.

of methoxychlor (Table I). A higher percentage of ^{14}C was recovered in intestinal and ruminal contents, carcass, and milk from goat 66 than from goat 69. Feces was the major route for eliminating methoxychlor and metabolites by goat 66, and urine was the major route for elimination by goat 69. These differences are likely a result of the variation in mass of the doses.

Concentration of ^{14}C (methoxychlor equivalents) per unit of tissue dry matter was greater in kidneys than in liver of goat 66 and was equal in kidneys and liver of goat 69 (Table II). Adrenals contained the highest concentration of ^{14}C of the tissues from goat 69, but all tissues from goat 69 contained quite small amounts of methoxychlor equivalents.

Maximum concentration of methoxychlor equivalents in milk occurred during the 8–24-h time period for goat 66 (Table II). For goat 69, maximum ^{14}C per milliliter of fresh milk for any time period was less than 10 cpm above background, the lowest amount that we believed to be reliably detected.

Isolation of metabolites from tissues and milk was not attempted because of the small amounts of metabolites that could be present.

Total ^{14}C extracted from feces of goat 66 with hexane and methanol was 81%. Exhaustive extraction of a sample of these feces on a Soxhlet, first with hexane and then with methanol, yielded only 86% extraction, with 14% (by combustion analysis) of the ^{14}C remaining in the residue. Treatment of this residue with HCl did not improve the extraction. Hexane and methanol extracted 70% of the ^{14}C from feces of goat 69, with 29% of the ^{14}C remaining in the residue. Exhaustive extraction of a sample of goat 69 feces with these solvents on a Soxhlet reduced the ^{14}C remaining in the residue to 25%. Thus, we believe that the metabolites identified represent 81% of the ^{14}C present in feces of goat 66 and 70% of the ^{14}C present in feces of goat 69.

There were times during column chromatographic cleanup of the metabolites that fractions, while distinct, were not sufficiently separated to quantitatively determine the proportion of ^{14}C belonging to the specific fractions. These times are indicated in Figure 1. Although we lack proof, we have no reason to believe that these effluents contained metabolites other than those identified in the adjacent fractions.

Identification of metabolites was based on the comparison of mass spectra to those of authentic compounds, comparison of relative GC retention times, and interpretation of mass spectra. Trichloroethanes, such as DDT and methoxychlor, show a loss of CCl_3 (117), and dichloroethanes, such as DDD, show a loss of $CHCl_2$ (83) from the molecular ion to give an intense ion, usually the base peak. Dichloroethenes such as DDE, however, usually yield an intense molecular ion and an M – 70 ion (loss of 2 Cl) (Sphon and Damico, 1970). This fragmentation pattern appears unaffected by aryl substituents such as OH, OCH_3, or $OSiMe_3$. The $OSiMe_3$-substituted compounds also show a loss of 15 from the molecular ion.

The mass spectra of metabolite 7 and of the synthetic cis/trans mixture of 1-chloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene were identical. This does not prove that the isolated metabolite was a cis/trans mixture because cis and trans isomers may yield identical mass spectra. Attempts to separate the cis/trans mixture (or Me_3Si derivative) by GC and HPLC failed.

Identification of the glucuronide-type metabolites was based on the fragmentation pattern that is typical of these compounds (Bakke, 1976). Molecular ions were not seen; instead, M – 15 ions were the highest observed. All spectra had intense 375 ions and aglycon ions (M – 392). Because of insufficient quantity of some of the metabolites, the highest ion observed was either 375 or M – 105 or M – 90.

The fecal metabolites were demethylated, dechlorinated, and dehydrochlorinated products of methoxychlor. Hexane-extractable metabolites were predominantly dechlorinated or dehydrochlorinated products or products with only one methyl moiety removed. Methanol-extractable metabolites usually had one or both methyl moieties removed. The 4,4′-substituted dichloroethanes were the predominant metabolites in both feces and urine.

Most urinary metabolites were completely demethylated and conjugated with glucuronic acid. One glucuronic acid moiety per metabolite molecule was usual. In a few cases, however, it was suspected that the metabolite existed as a diglucuronide conjugate. Diglucuronides could not be confirmed because of inadequate volatility or stability in the gas chromatograph and mass spectrometer.

Ring hydroxylation was detected in one metabolite in the urine of goat 66, metabolite 18. It was concluded that the hydroxylation and glucuronidation were on the same ring because the NMR spectrum clearly showed the para splitting pattern characteristic of the methoxy-substituted ring. The remaining aromatic resonances could not be assigned but were compatible with a ring containing both hydroxyl and glucuronide groups.

Metabolites identified in the excreta of the two goats were similar. Any differences observed in the metabolite profile are considered too minor to be attributed to differences in the size of the dose, especially since only two goats were used. The primary reason that goat 66 was given the 1-g dose was to facilitate isolation and identification of metabolites.

Kapoor et al. (1970) reported the ethane moiety of methoxychlor to be metabolized to both a ketone and an acid by mice. However, Woodward et al. (1948) did not detect these metabolites in urine of rats, and we were unable to detect these metabolites in feces or urine of goats.

In conclusion, 17 metabolites plus methoxychlor were identified in excreta from goats. Most metabolites were present in greater amounts than the parent methoxychlor. The metabolites might be better candidates for residue monitoring than methoxychlor. However, the significance of these metabolites for residue monitoring or their potential for biological activity has not been determined.

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Determination of Free and Hydrolyzable Residues of 2,4-Dichlorophenoxyacetic Acid and 2,4-Dichlorophenol in Potatoes

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Chemical methods were developed and used to provide residue and metabolism data in support of the registered use of 2,4-dichlorophenoxyacetic acid (2,4-D) on potatoes. The free and the free plus hydrolyzable forms of trace residues of both 2,4-D and 2,4-dichlorophenol (2,4-DCP) in potato tubers and vines were determined separately by the following methods: extraction of free residues with an acidified mixture of ethyl and petroleum ethers, or after hydrolysis, extraction of the free plus acid hydrolyzable forms with benzene; partitioning cleanup into alkali and then, after acidification, into ethyl ether; separation of 2,4-DCP from 2,4-D on an acidic alumina column; methylation of 2,4-D; quantification by GLC. Residues of 2,4-D averaged 0.11 part per million (ppm), whereas residues of 2,4-DCP were less than 10% those of 2,4-D. At least 18% of the total 2,4-D found was present in conjugated form. During simulated commercial storage, 2,4-D dissipated from whole potatoes with a half-life of about 12 weeks. Residues were not concentrated in the peel of potatoes, and baking had no influence on the residue present.

A major part of the mission of our laboratory involves the development of data to support the establishment of tolerances and registration of agricultural chemicals for use on minor crops. In 1971, the federal registration covering the use of 2,4-dichlorophenoxyacetic acid (2,4-D on potatoes was in danger of cancellation for lack of adequate data. This plant growth regulator has been used as a foliar spray on potatoes for many years to intensify the skin color of red varieties and to reduce the yield of oversize tubers while increasing the yield of medium, more desirable tubers of all table stock varieties (Nelson and Nylund, 1963; Wort, 1965; Hegazy et al., 1978). To support this use, data on the resulting residues of 2,4-D and one of its possible metabolites, 2,4-dichlorophenol (2,4-DCP), had been requested by the U.S. Environmental Protection Agency. This study was organized to provide the needed data. It required methodology capable of determining separately the free and the hydrolyzable residues of both 2,4-D and 2,4-DCP at low ppb (nanograms per gram) levels in potato tubers. High sensitivity was required because potatoes, susceptible to the herbicidal effects of 2,4-D, are treated with very low levels of commercial formulations.

Bevenue et al. (1963) reported that residue levels of 2,4-D in potatoes were very low using a method that involved the direct extraction of potatoes with an acidified organic solvent mixture. Using similar methodology, Nelson et al. (1971) reported that potato samples boiled in 6 N H_2SO_4 prior to extraction gave a 2–7-fold increase in the levels of 2,4-D found over those obtained by direct extraction with acidified benzene. Similar increases resulting from hydrolysis had been observed for residues of 2,4-D in beans (Thomas et al., 1963; Crosby, 1964) and in Forage (Yip and Ney, 1966). At the time this study began, Chow et al. (1971) reported that alkaline hydrolysis of wheat that had been treated with 2-methyl-4-chlorophenoxyacetic acid gave from 3 to 7 times higher residue levels than those found by direct extraction with acidified organic solvents. These studies demonstrated that hydrolysis plays an important role in the analysis of conjugated 2,4-D residues in plants. Since that time, numerous studies have shown that 2,4-D and other phenoxyalkanoic acids are metabolized extensively (Mumma and Hamilton, 1976; Feung et al., 1976, 1978; Bristol et al., 1977; Leng, 1977; Chkanikov et al., 1976, 1977; Lokke, 1975; Steen et al., 1974; Fleeker, 1973). However, no residue methods were available which satisfied the specific requirements of this study when it was undertaken.

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