Metabolism of [^{14}C]Dichloran (2,6-Dichloro-4-Nitroaniline) in the Lactating Goat

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After oral treatment of a lactating goat with eight doses of [^{14}C]dichloran at the rate of 11.1 mg kg^{-1} dose^{-1} at intervals of 12 h just after milking, approximately 29, 48, and 0.3% of the administered radioactivity was excreted in urine, feces, and milk, respectively (12.5% of the dose was in the gastrointestinal tract). Fractionation and characterization of metabolites from milk showed that the nitro group of dichloran was reduced to give 2,6-dichloro-1,4-benzenediamine, which was acetylated to give the metabolite 3,5-dichloro-4-aminoacetanilide (0.5%). The presence of 4′-amino-3′-chloro-5′-(methylsulfonyl)acetanilide (6–15%) suggests a pathway with the displacement of chlorine by methionine. The proposed structure for the major metabolite (25–50%), (3-amino-3-carboxypropyl)[5-(acetylamino)-2-amino-3-chlorophenyl]methylsulfonium chloride, was deduced from the fragments obtained by direct mass spectrometry and from chlorobenzothiazoles generated from acid hydrolysis.

The metabolism of 2,6-dichloro-4-nitroaniline (DCNA, dichloran, Botran, The Upjohn Co.) in the rat occurs by replacement of nitro group with hydroxyl, which is then conjugated with sulfate and glucuronide (Eberts, 1967; Mate et al., 1967). However, in soils and probably in the anaerobic environment the nitro group of DCNA is reduced to an amino group, which in turn in acetylated (Van Alfen and Kosuge, 1974, 1976). We observed a new pathway of metabolism of dichloran in the goat involving substitution of chlorine with sulfur-containing compounds.

This report describes the tissue residues and principal DCNA metabolites in milk. This report also shows evidence for the absence of 3,3′,5,5′-tetrachloro-4,4′-diaminoazobenzene in milk.

EXPERIMENTAL SECTION

Animal Treatment. After 11 days of acclimation two open lactating goats were treated with eight doses of nonlabeled dichloran at the rate of 12.5 mg kg^{-1} dose^{-1} orally at intervals of 12 h just after milking. One of the high milk producing goats was moved into a metabolism stall, and 9 days after the withdrawal from the nonlabeled dichloran, the goat was orally dosed at the rate of 11.1 mg kg^{-1} dose^{-1} every 12 h just after milking with eight [^{14}C]-dichloran doses. The animal was killed 6 h after the last treatment, and the tissues were removed and analyzed for radioactive residues.

Sample Collection and Radioactive Determination. Samples of blood via jugular venipuncture, milk by hand stripping, urine as a runoff from the fecal tray, and feces were collected every 12 h just before treatment. Urine and milk aliquots (0.9 g) were counted in 15 mL of Instagel (Packard Instrument Co.). Blood and feces aliquots were weighed into combusto-cones (Packard Instrument Co.), dried, and combusted to radioactive carbon dioxide in a biological oxidizer (Packard Instrument Co.). Samples of tissues, liver, kidney, and heart were ground and Homogenized with water, and aliquots were combusted as described for blood and feces. Fat was homogenized in hexane, and aliquots were counted in Instagel. Triplicate samples were counted in a Packard Tricarb scintillation spectrometer and dpm computed after adding [^{14}C]toluene as the internal standard.

Compounds. The compounds are numbered as in Table I. The source of authentic standards is also listed. The structures of certain compounds tagged with footnote *a* in Table I in this study were based on spectral characterization only. Compound IV is the proposed structure and was the major metabolite in both milk and urine.

Table I. List of Compounds Discussed in This Paper

no.	compound
I[a]	2,6-dichloro-4-nitroaniline
II[a]	2,6-dichloro-1,4-benzenediamine
III[a]	4′-amino-3′,5′-dichloroacetanilide
IV[b]	(3-amino-3-carboxypropyl)[5-(acetylamino)-2-amino-3-chlorophenyl]methylsulfonium chloride
V[b]	4′-amino-3′-chloro-5′-(methylsulfonyl)acetanilide
VI[b]	4′-amino-3-chloro-5′-mercaptoacetanilide
VII[b]	6-acetamido-4-chlorobenzothiazole
VIII[b]	4′-amino-3′-chloro-5′-(methylthio)acetanilide
IX[b]	3′,3‴-dithiobis[4′-amino-5′-chloroacetanilide]
X[b]	6-amino-4-chlorobenzothiazole
XI[b]	6-amino-4-chloro-2-ethylbenzothiazole
XII[a]	2′,6′-dichloro-4-nitroacetanilide
XIII[c]	4-amino-3,5-dichlorophenol
XIV[a]	4′-amino-2′,6′-dichloroacetanilide
XV[a]	3,3′,5,5′-tetrachloro-4,4′-diaminoazobenzene

[a] The Upjohn Co. [b] Structure based on limited spectral characterization. [c] Aldrich Chemical Co.

Synthesis of [^{14}C]Dichloran. [^{14}C]Dichloran was synthesized by New England Nuclear (sp act. 2.99 mCi/mm). It was diluted with unlabeled dichloran to give a specific activity of 0.47 mCi/mm (5089 dpm/μg). Its purity was found to be greater than 98% by TLC on silica gel GF in the solvent system hexane–acetone (150:50).

Synthesis of 3,3′,5,5′-Tetrachloro-4,4′-diaminoazobenzene (Azo Compound). The azo compound was synthesized by the classical reductive coupling of dichloran by the procedure of Vogel (1957). Dichloran in methanol was treated with magnesium filings at reflux. On being cooled, the mixture was poured into water–ice, the pH was adjusted to 4.5, and product was collected by suction filtration. Recrystallization several times from ethanol gave the azo compound.

Instrumental Analysis. NMR spectra were recorded on a Varian XL-200 spectrometer by using 5-mm o.d. tubes containing ~0.5 mL of the appropriate deuterated solvents with Me_4Si as the internal standard. IR spectra were recorded with a Digilab Model FTS14D attached to a Perkin-Elmer 6× reflecting beam condenser. The KBR pellets were 1.5 mm thick. The GC and mass spectral data were obtained with a Hewlett-Packard 5992A GC/MS system. Direct probe mass spectra were recorded on either CEC-21-110B high-resolution mass spectrometer or Varian MAT CH_5 double-focusing high-resolution mass spectrometer.

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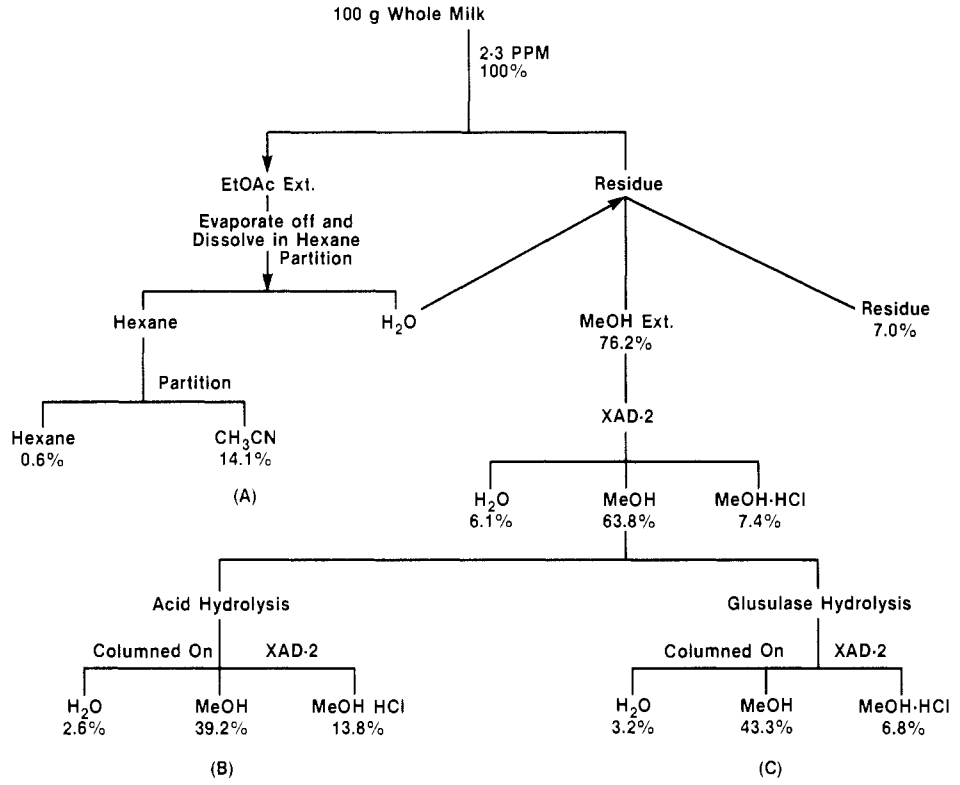

Figure 1. Partition of [^{14}C]dichloran metabolites from goat milk into nonpolar and polar fractions and fractionation of polar metabolites after acid and enzymatic hydrolysis.

Concentration of Botran in Milk. Aliquots of milk (control, fortified controls at 10–1000 ppb, and treated) were extracted with ethyl acetate. After evaporation of ethyl acetate, the residue was partitioned between hexane and acetonitrile. The acetonitrile extracts were evaporated to dryness and the residue was dissolved in ethyl acetate. Aliquots were analyzed by GC with a ^{63}Ni electron capture detector on a 3 ft (3-mm i.d.) borosilicate glass column filled with 10% OV-1 coated on Gas-Chrom Q, 100–120 mesh, with a helium flow of 40 mL/min and a purge gas argon–methane (95:5) flow rate of 110 mL/min. Column and detectors were operated at 180 and 280 °C, respectively. The peak height responses were compared with that of the 20-ppb dichloran standard.

Fractionation of Milk Metabolites. The milk was fractionated (Figure 1) and the methanol eluate from XAD-2 was hydrolyzed in 1 N HCl for 3 h under reflux as well as by glusulase (Endo lab). Hydrolysis products were fractionated on XAD-2. Fractions A–C were examined by TLC and autoradiography on silica gel GF 250 μm thick plates. The plates were developed 10 cm in hexane–ethyl acetate–methylene chloride (100:50:50). Standard reference compounds I–III and XII–XV were cochromatographed. Silica gel from the areas of samples corresponding to the above standards was scraped and counted in diotol.

Characterization of Milk Metabolites. Milk was lyophilized and extracted sequentially with hexane, methylene chloride, acetone, methanol, and methanol–water (1:1) (Figure 2). The methanol extracts were evaporated off and cleaned up on XAD-2 columns. Lactose was eluted with water, and the radioactive metabolites were eluted with methanol and methanol–HCl (99:1). Methanol eluates of XAD-2 were evaporated and dissolved in water and fractionated on DEAE-Sephadex A-25 (100 g swelled in water overnight) with a gradient of water and 1 M sodium chloride. Major metabolite (fraction D) was eluted with about 0.4 M sodium chloride. TLC and autoradiography of fraction D (Figure 2) from milk were done on silica gel GF, 250 μm in the solvent system ethyl acetate or methanol–acetic acid (99.5:0.5). TLC plates were sprayed with ninhydrin and heated for 10 min at 110 °C. Radioactive metabolites were eluted from TLC and examined by NMR, IR, GC/MS, GC/radioactive monitor, and direct probe mass spectrometry.

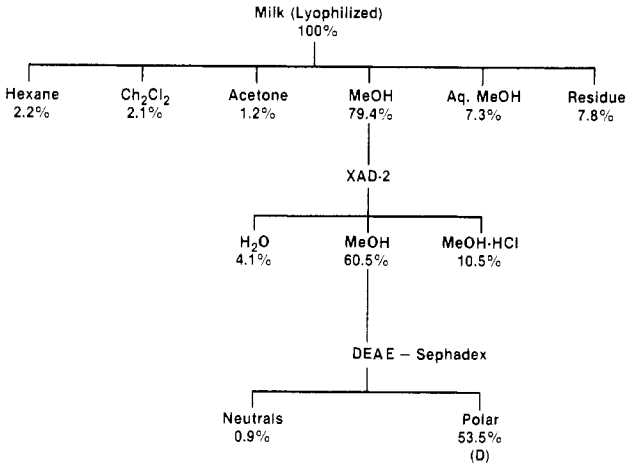

Figure 2. Extraction of [^{14}C]dichloran metabolites from lyophilized goat milk with various solvents and fractionation of polar (methanol extract) metabolites by DEAE-Sephadex chromatography.

Isolation and Characterization of Urine Metabolites. So that large quantities of the comparable milk metabolites (fraction D) could be obtained, urine was fractionated like milk (Figure 2) and fraction D from urine was examined by NMR, IR, GC/MS, TLC, etc. as described above for milk.

Fraction D isolated from urine was also hydrolyzed in 6 N HCl by refluxing for 16 h, and degradation products were extracted with ether at pH 7.0 and purified on a silica

gel 60 column by eluting with CH_2Cl_2–EtOAc (95:5). These radioactive compounds were examined by NMR, IR, and MS.

Analysis of Milk for Azo Compound. Lyophilized milk (control, fortified with 13 ppb, and treated) was extracted in hexane and partitioned with acetonitrile. The acetonitrile extract after evaporation was cleaned up on silica gel GF 60. The eluate of azo compound in hexane–CH_2Cl_2 (85:15) was evaporated off and the residue dissolved in hexane. Aliquots were analyzed by GC with a ^{63}Ni electron capture detector on a 3 ft (3-mm) borosilicate glass column filled with 10% OV-1 coated on Gas-Chrom Q, 100–120 mesh, and operated at 280 °C with a helium flow of 40 mL/min and a purge gas argon–methane (95:5) flow of 110 mL/min.

Fractionation of Radioactivity from Tissues. Fat was extracted in boiling hexane and partitioned with acetonitrile. The acetonitrile extract was examined by TLC and radioautography on 250 μm thick silica gel GF plates in hexane–acetone (150:50). Compounds I and III were cochromatographed. Radioactive spots from TLC plates were eluted with ethyl acetate and examined by GC/MS with a 6 ft (3-mm i.d.) borosilicate glass column filled with 1% SE 30 coated on Gas-Chrom Q, 100–120 mesh, and at a helium flow of 12 mL/min. GC/MS chromatograms and the fragmentation pattern were compared with those of standard compounds I and III.

Liver and kidney were extracted 3 times with (1:1) chloroform–methanol and extracts after evaporation were partitioned between ethyl acetate and water. The ethyl acetate extracts were examined by TLC and radioautography as described under fat. The ethyl acetate extracts were also purified on a silica gel 60 column (230–400 mesh), and the $CHCl_2$–EtOAc (50:50) eluate was examined by GC/MS as described under fat.

The aqueous phase after evaporation was cleaned on XAD-2 column. Methanol and methanol–HCl (99:1) eluates were examined by TLC as described for fat. Aliquots of methanol and metanol–HCl (99:1) eluates after evaporation were hydrolyzed by sulfatase and glucuronidase, and the liberated aglycons were examined by TLC as described above.

Radioactivity from liver was also fractionated into 2% perchloric acid (soluble), lipids, RNA, DNA, proteins, and sulfurated glycosaminoglycans by the procedure of Krowke et al. (1971). Pure DNA strands were also isolated from liver by the phenol extraction procedure of Irving and Veazey (1968). Soluble proteins (after centrifuging off liver homogenates nuclei, mitochondria, and microsomes) were hydrolyzed in 6 N HCl at 110 °C for 48 h in evacuated tubes by the procedure of Moore & Stein (1963). The residue after removal of HCl was dissolved in water (pH 7), and the samples were purified on an XAD-2 column. Methanol and methanol–HCl (99:1) eluates were examined by TLC in hexane–EtOAc–CH_2Cl_2 (100:50:50) or EtOAc. Reference compounds I–III and XIII were cochromatographed.

RESULTS AND DISCUSSION

Excretion and Tissue Residues. Approximately 29% of the administered radioactivity was excreted in urine and 48% in feces (Figure 3). Another 12.5% of the total administered radioactive dose (equivalent to one dose) was still present in the gastrointestinal tract at the time of slaughter 6 h posttreatment. The milk, which was of most interest, contained only 0.3% of the dose. Residues (^{14}C-equivalent dichloran) determined after 12 h of each dose in both milk (~3 ppm) and blood (1 ppm) plateaued after three to four doses. Liver, kidney, fat, heart, and muscle

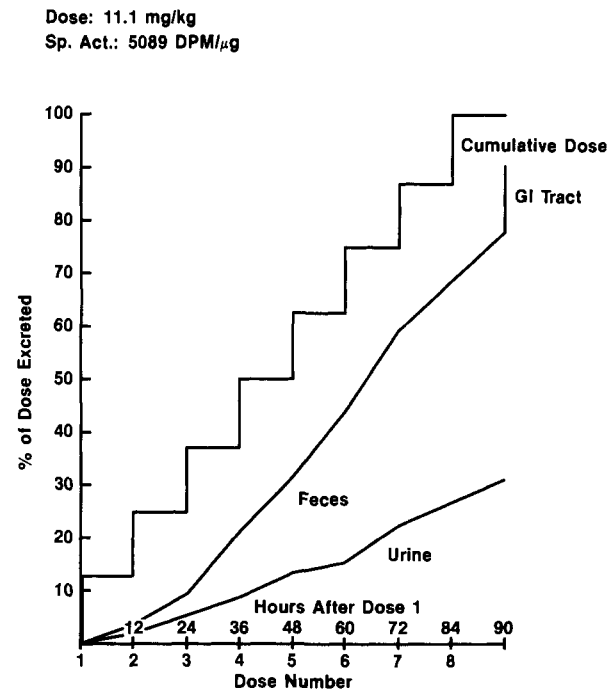

Figure 3. Excretion of radioactivity in urine and feces (excretion in milk was 0.3% of the dose).

Table II. TLC of Different Fractions

compound no.	R_f	% of milk radioactivity in fraction A	B	C
XV	0.67	0.6	0	0
I	0.63	6.3	1.3	1.8
XIII	0.55	0.1	0.4	0
II	0.35	0	4.3	0
XII	0.25	0.1	4.1	0.1
III	0.17	0.5	2.8	1.6
XIV	0.08	1.5	2.6	11.0
origin	0	5.2	23.6	28.9

contained residues equivalent to 50, 12, 4, 2, and 1 ppm of [^{14}C]dichloran, respectively. The high residue levels in liver and kidney are reasonable since one dose was still in the gastrointestinal tract at slaughter.

Concentration of Dichloran in Milk. Analysis of dichloran by a GLC procedure that routinely gave 80–90% recovery of fortified milk samples showed that approximately 5% of the total radioactivity in the milk was intact dichloran (~0.1 ppm).

Nature of the Milk Metabolites. Three major radioactive fractions, A–C (Figure 1), were examined by TLC. Both autoradiography and sections of the TLC plate corresponding to standard compounds (I–III and XII–XV) (Table I) showed that most of the radioactivity was at the origin, the location for polar compounds in this solvent system (Table II).

In fraction A, 6.3% of the radioactivity migrated like that for dichloran. GC/MS retention time, molecular ion, and fragmentation pattern were similar to those for dichloran. The molecular ions at m/z 206/208/210 in a 9/6/1 ratio indicated that the molecule contained two chlorine atoms. Loss of NO gave ions at m/z 176/178/180 in a 9/6/1 ratio that was characteristic of dichloran. About 0.5% of the radioactivity on the TLC plate (Table II) was similar to that of III, whose identity was confirmed by GC/MS, retention time, molecular ion, and fragmentation pattern to be similar to 4-amino-3,5-dichloroacetanilide. The molecular ions at m/z 218/220/222 with relative intensities of 9/6/1 indicated the presence of two chlorine atoms. Other ions at m/z 176/178/180 in a 9/6/1 ratio

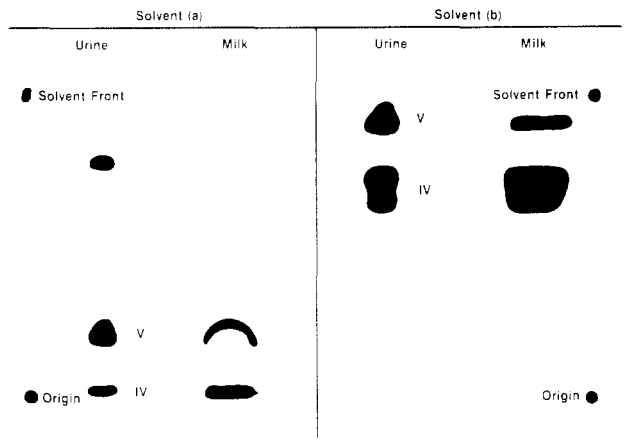

Figure 4. TLC and radioautography of major milk and urinary metabolites (silica gel GF, 250 μm thick). Plate developed 10 cm in (a) ethyl acetate and (b) MeOH–HOAc, 99.5:0.5, fraction D in Figure 2.

showed the loss of ketene and rearrangement to give rise to $NH_2(PH)Cl_2NH_2$. The rest of fraction A was diffused into several minor TLC fractions.

In fraction B, most of the radioactivity stayed at the origin in this TLC system (Table II) and only 4.3% migrated with an R_f of ~0.35, similar to that of compound II (later characterized as compound X).

In fraction C, all of the radioactivity remained at the origin in this TLC system (Table II) as highly polar compounds.

These data suggested that compound I was reduced to transient II and then acetylated to give rise to III. However, II or XIII was not found as free compounds in fraction A or as their sulfate or glucuronide conjugates in fractions B and C. Refluxing with 1 N HCl for 3 h or incubation with glucuronidase–sulfatase overnight did not produce less polar derivatives. This demonstrated that these fractions did not contain any glucuronide or sulfate conjugates.

Chlorobenzothiazoles as the Acid Hydrolysis Products of the Major Milk and Urine Metabolites. Since the polar milk metabolites were not of the sulfate or glucuronide conjugate type and since additional amounts were needed for characterization, a lyophilization and methanol extraction procedure followed by chromatography on XAD-2 and DEAE-Sephadex was employed (Figure 2). Although this procedure gave purer material, there was still an insufficient quantity of metabolites in milk for identification. Consequently urine, which contained larger amounts of the unknown metabolite (fraction D) (Figure 2), was cleaned up by this procedure and used as source of the metabolite for the characterization.

Upon hydrolysis of fraction D with 6 N HCl, a conversion product was isolated by silica gel column chromatography. It had the same R_f (0.35) as fraction B, the acid hydrolysis product of the milk metabolite on TLC plates of silica (Table II). Its mass spectrum showed molecular ions m/z at 184/186 in a 3/1 ratio (indicative of one chlorine atom) and its IR spectrum was similar to that of 6-aminobenzothiazole. Its NMR spectrum showed an aromatic doublet with protons at 6.93 and 7.06 ppm and a J value of 3 Hz, which indicated that the protons were meta to each other. A singlet at 8.75 ppm suggested that one proton was on a carbon doubly bonded to nitrogen or oxygen (D_2O had no effect). High-resolution MS gave a molecular ion at 183.9867 amu. From these spectral data the acid degradation product was assigned structure X. A lesser degradation product was observed by MS, NMR, and IR and assigned the structure of XI.

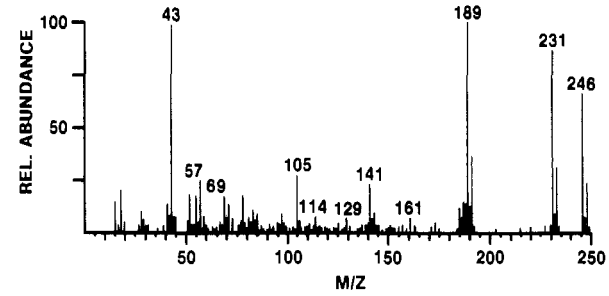

Figure 5. Direct probe mass spectra of metabolite V from urine.

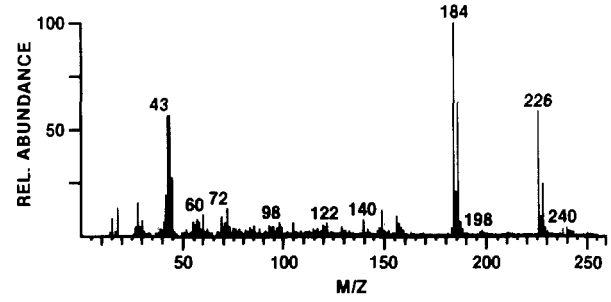

Figure 6. Direct probe mass spectra of metabolite IV from urine.

The identification of the chlorothiazoles as degradation products of acid hydrolysis was significant because these data showed that one of the chlorine has been substituted by a sulfur-containing compound. Therefore, identification of the major milk and urine metabolites was continued on the basis that a chlorine had been displaced in the metabolite originally thought to be II, R_f 0.35 (Table II).

Identification of Major Milk and Urine Metabolites. When unhydrolyzed milk and urine metabolites (fraction D, Figure 2) were chromatographed on TLC plates in two solvent systems, two metabolites were observed (Figure 4).

Compound V. The NMR of the less polar metabolite (Figure 4) in $CDCl_3$ from urine showed a singlet at 2.12 ppm with three protons (C-CH_3), a singlet at 2.90 ppm with three protons (S-CH_3), a broad singlet at 5.33 ppm with two protons (NH_2), another broad singlet at 7.28 ppm with one proton (NH), and aromatic doublets at 7.38 and 7.59 ppm (with J = 3 Hz) with one proton each.

The IR spectrum was consistent with the structural features of compound V with the following assignments: NH stretch (3430, 3300, 3170, 3110); C=O stretch (1665); C=C stretch (1600, 1475); amide II (1540); C–N stretch (1270). The presence of the sulfoxide group was confirmed with a strong peak at 1020 cm^{-1} due to a S=O stretch.

A direct probe mass spectrum (Figure 5) showed a typical *one chlorine pattern* because the molecular ions at m/z 246/248 were present in a 3/1 ratio. A loss of a CH_3 radical gave ions at m/z 231/233. A further loss of ketene gave major ions at m/z 189/191. On the basis of NMR, IR, and MS, the structure V was assigned. Similar GC/MS and its fragmentation pattern were noticed for the comparative milk metabolite. However, the level of milk metabolite was too low to obtain meaningful NMR and IR data.

Compound IV. The more polar metabolite (Figure 4) eluted from the TLC plate by methanol was prepared as a KBR pellet. Its IR spectrum indicated the presence of an amine, an amide, and an aromatic group in the molecule. However, the use of methanol as the eluting solvent resulted in weak absorption in the NH stretch region. Lack of a broad peak near 1020 cm^{-1} indicated that it was

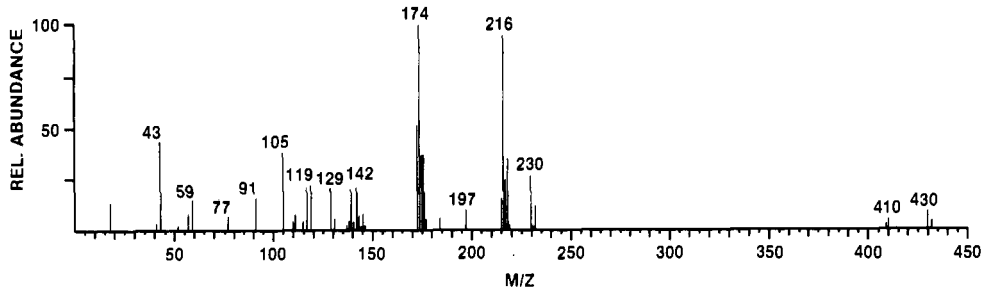

Figure 7. Direct probe mass spectra of metabolite IV from urine (sample of Figure 6 repurified and reanalyzed).

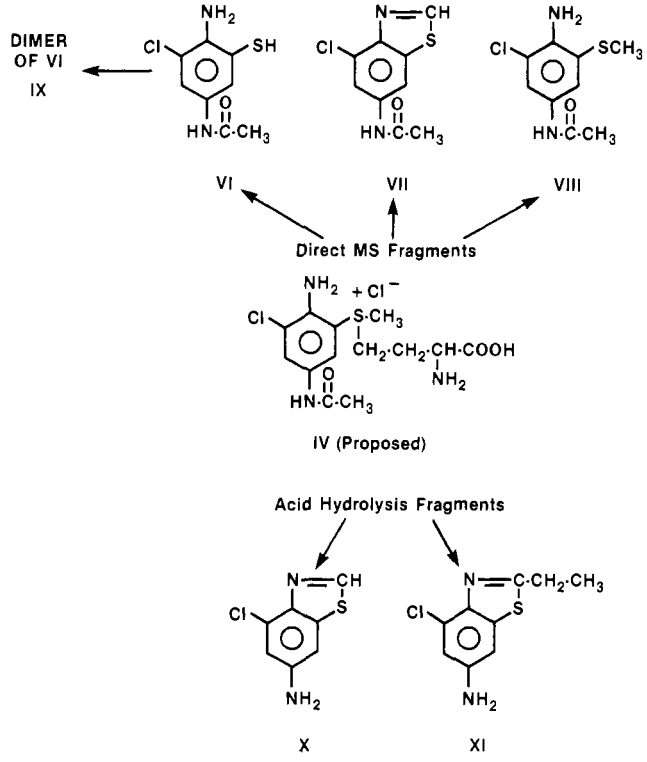

Figure 8. Basis of the structure of metabolite IV.

not a glucuronide. This metabolite also gave a positive ninhydrin test for amino acids. No meaningful NMR data were obtained because of the presence of potassium acetate and other large amounts of impurities eluted from the silica gel plate by methanol. No peaks were observed by GC/MS or GC/RAM.

Direct probe MS (Figure 6) gave molecular ions at m/z at 226/228 in a 3/1 ratio and suggested a structure with one chlorine, i.e., compound VII. A loss of ketene gave major ions at 184/186 similar to that observed for compound X. However, reanalysis of the same by direct probe MS (Figure 7) gave a major product with molecular ions at 216/218 in a 3/1 ratio, compound VI. High-resolution MS measurements by the peak-matching technique on the 216 ion gave 216.010, which was in good agreement with structure VI. Loss of ketene gave major ions at m/z 174/176.

Small amounts of compound VIII and IX were also present, ions at m/z 230/232 and 430/432. Compound IX probably is generated from the air oxidation of VI or from thermal reaction in the mass spectrometer. The differences in ions from the same sample (Figure 6 and 7) probably resulted from a difference in the source temperature of MS or degradation of the sample on storage. On the basis of the fragments observed in MS (Figures 6 and 7) and the chlorobenzothiazole generated from acid hydrolysis, the structure IV is proposed (Figure 8) for the polar metabolites eluted from the TLC plate by methanol.

Determination of the Azo Compound. When radioactive milk from the [14C]dichloran-treated goat was analyzed for the azo compound (3,3′,5,5′-tetrachloro-4,4′-diaminobenzene) by the same procedure that gave ~100%

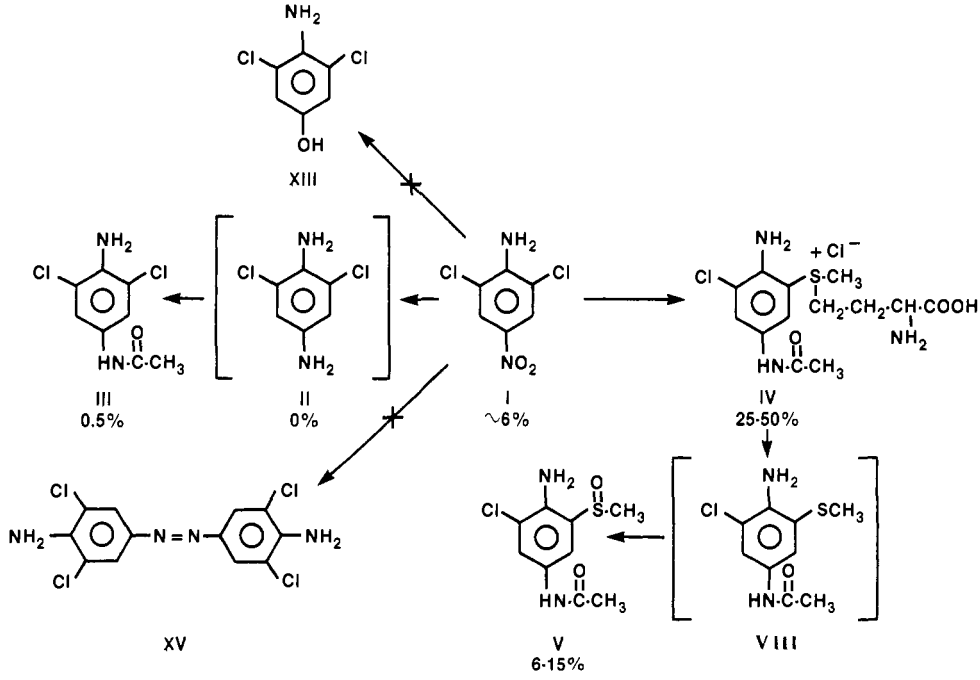

Figure 9. Metabolism scheme of dichloran in milk.

recovery for fortified milk samples, no azo compound (<0.8 ppb) was found. This is not surprising, since it has been shown that rumen fluid has a detoxification mechanism that not only destroys azo-forming systems but also destroys azo compounds already present (Katz et al., 1969).

Metabolites in Tissues. Over 90% of the radioactivity in fat was extractable with boiling hexane. Approximately 82% partitioned from hexane into acetonitrile. TLC, autoradiography, and GC/MS data confirmed the presence of intact dichloran, compound I (57.7%), and compound III (14%). The rest of the radioactivity was present as minor compounds dispersed among many fractions.

Approximately 80% of the radioactivity in kidney was extractable with chloroform–methanol (1:1) of which 15% was characterized as compound III by TLC and GC/MS, but no metabolites were found that correspond to compounds I, II, or XIII on the TLC plate even after sulfatase and glucuronidase hydrolysis. Only about 20% of the liver radioactivity was extractable with chloroform–methanol (1:1). TLC analysis of these extracts showed that 2.5% of the liver radioactivity was present as compound III. No metabolites were found corresponding to I, II, or XIII in the solvent extracts or after sulfatase and glucuronidase hydrolysis.

Radioactivity in the liver was separated into various protein fractions according to that reported by Krowke (1971), i.e., lipids as lipoproteins, 12.4%, glycoproteins as sulfurated glycosaminoglycans, 10.9%, and proteins, 33.9%. No radioactivity was found in the liver DNA fraction according to the phenol extraction procedure described by Irving and Veazcy (1968).

When the protein bound fraction in liver was hydrolyzed to the individual amino acids, only 19.6% was found in the aqueous phase, while the rest was found in the black polymeric residue resulting from the presence of carbohydrates.

CONCLUSION

The metabolism scheme (Figure 9) shows that the nitro group of dichloran was reduced and then acetylated in the ruminant goat as Van Alfen and Kosuge (1974, 1976) observed in soils and not by displacement of the nitro group by hydroxyl as Eberts (1967) and Mate et al. (1967) observed in rat urine. No azo compound (XV) was found in milk. The nucleophilic displacement of chlorine by a sulfhydryl group (methionine?) in IV was unexpected because of the ortho amine function. Since this displacement is unlikely under normal laboratory conditions, it may be enzyme mediated and probably involves a metal ion as a cofactor. Major metabolite IV may readily decompose to yield a methylthio-containing metabolite (VIII) that can be easily oxidized to V. The reaction of dichloran or metabolites with sulfhydryl groups appears to be a detoxification mechanism because of their polarity and rapid excretion in urine.

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Fiber-Reactive Insect-Proofing Agents for Wool: Phosphorus Esters of 3-(Hydroxymethyl)-4-nitrophenol

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Organophosphorus esters of some substituted (hydroxymethyl)phenols were synthesized and screened for insecticidal activity against the keratin-digesting insects *Tineola bisselliella* and *Anthrenus flavipes*. Fiber-reactive groups were attached via the hydroxymethyl substituent of the more active compounds, and these were applied to wool from a dyebath. Fiber-reactive esters of *O*-ethyl *S*-*n*-propyl *O*-[3-(hydroxymethyl)-4-nitrophenyl] phosphorothionthiolate durably protected wool at an application rate of 1.5 mg/g of wool.

Recent work in this laboratory has shown that the usefulness of insecticidal organophosphorus esters for the protection of wool from insect damage is greatly enhanced by the presence of a 2-bromoacryloyl substituent in the molecule (Jones et al., 1982). This substituent is capable of covalently binding the insecticide to the wool so that the resistance of the treated wool to insect damage is rendered durable to washing, exposure to light, and dry cleaning.

Organophosphorus esters of nitrogen heterocyclic compounds have very good insecticidal activity (Jones, 1983a), but these compounds are readily hydrolyzed during dye-

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