Metabolism and Excretion of Tetrachlorvinphos in Dairy Cows

M. Humayoun Akhtar* and Thomas S. Foster

Metabolism and excretion of tetrachlorvinphos were studied in dairy cows after oral administration for 5 days, followed by no treatment for a 10-day period. Orally administered tetrachlorvinphos (50 ppm, based on average daily ration intake) was absorbed, extensively metabolized, and almost totally eliminated in 10 days following the end of treatment. Traces of unchanged insecticide were detected in urine. The metabolites identified in hydrolyzed urine were as follows: desmethyl tetrachlorvinphos (13.2%), 1-(2,4,5-trichlorophenyl)ethanol (34.8%), (2,4,5-trichlorophenyl)ethane-1,2-diol (28.1%), and 2,4,5-trichloromandelic acid (6.1%). In addition, unchanged insecticide (7.1%) as well as significant amounts of 1-(2,4,5-trichlorophenyl)ethanol (8.7%) was excreted in the feces. Neither unchanged insecticide nor metabolites could be detected in milk and various tissues from treated lactating cows.

Tetrachlorvinphos [2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate; Stirofos, Gardona, Rabon] is an important member of the vinyl organophosphate insecticides. It has been shown that manure from cattle fed tetrachlorvinphos is toxic to the larvae of horn fly and houseflies (Drummond, 1967; Miller et al., 1970; Miller and Gordon, 1972).

Studies on the effect of feeding tetrachlorvinphos to dairy cows over extended periods have been carried out (Miller and Gordon, 1973). The metabolism of the insecticide has been investigated in the dog and rat (Akintonwa and Hutson, 1967) and in a lactating dairy cow (Gutenmann et al., 1971). The major elimination route in the dog, rat, and cow was via the urine. A small amount of the insecticide was also excreted unchanged in the feces. In lactating cows, residues of the insecticide were not detected in milk (Gutenmann et al., 1971). The following is a report of the detailed study of metabolism and excretion of tetrachlorvinphos when fed at the 50-ppm level to lactating cows.

EXPERIMENTAL SECTION

Animal Treatment. Four (two Holstein, two Ayrshire) cows with a daily milk production of 7.5–16.8 kg were used in this study. They were kept in individual stalls and fed to appetite a standard ration for lactating cows for 7 days while daily feed intake was recorded. Gelatin capsules containing one-half the required 50 ppm of insecticide (based on average daily intake) were prepared.

The animals were maintained on the standard ration throughout the entire experiment. They were catheterized for urine collection 3 days prior to commencement of oral administration of the insecticide. For 2 days prior to the feeding study, feces and urine were collected on a 24-h basis, while milk was collected twice daily on a 10- and 14-h basis. During the 5-day treatment period, each cow was given daily, by means of a balling gun, two capsules containing the insecticide, one after the morning milking and one after the evening milking. Milk was collected, weighed, and subsampled in the morning and evening throughout the feeding period until termination of the experiment. The total 24-h urine and feces were collected, weighed, mixed, and subsampled during the entire experiment. Feces were collected in metal trays. All samples were stored frozen at −20 °C until analyzed.

On the first day of treatment, each cow was given a capsule after the morning milking. Urine, feces, and blood samples (from the tail vein) were collected at 3, 6, and 9 h after the oral administration.

The animals were slaughtered at random at various times during the experiment, and samples of omental fat, kidney, heart, and liver were removed and stored at −20 °C until analyzed. In addition, each cow was examined at slaughter for pathological and histological indications.

Chemicals. Pesticide and reagent grade solvents were used. Analytically pure samples of tetrachlorvinphos (I), desmethyl tetrachlorvinphos (II), 2,4,5-trichlorophenacyl chloride (III), (2,4,5-trichlorophenyl)acetophenone (IV), 1-(2,4,5-trichlorophenyl)ethanol (V), 2-chloro-1-(2,4,5-trichlorophenyl)ethanol (VI), (2,4,5-trichlorophenyl)ethane-1,2-diol (VII), 2,4,5-trichloromandelic acid (VIII), and 2,4,5-trichlorophenacyl alcohol were available from previous studies (Akhtar, 1977; Akhtar and Foster, 1977). Bovine liver β-glucuronidase (500 units/mL) was obtained from Sigma Chemical Co., St. Louis, MO.

Methods of Extraction and Isolation. The methods used to extract and isolate unchanged tetrachlorvinphos from milk, urine, feces, and blood are presented in Figure 1 and from omental fat, heart, kidney, and liver in Figure 2. The methods for extraction and isolation of metabolites are shown in Figure 3. Recoveries by these techniques are presented in Table I.

Several types of homogenizers were used to blend the various samples. These included Waring Blendor (Sargent-Welch Scientific Co., Toronto, Canada), Super Dispax Homogenizing Mill (Sargent-Welch Scientific Co.), and VirTis "45" Macro Homogenizer (Academy Instruments Inc., Scarborough, Ontario, Canada).

The column used in the cleanup of extracts of feces was a disposable Pasteur capillary pipet (22.9 cm) containing a cotton plug, 4 cm of deactivated Florisil (10% H_2O), and 2 cm of 25% cellulose in decolorizing carbon—in that order—which was washed with dichloromethane (4 mL) just prior to use. An aliquot of the extract was placed on the column and eluted with dichloromethane (8 mL).

When methylation was carried out prior to analysis by gas chromatography, ethereal diazomethane was prepared by treating nitrosomethylurea with cold aqueous 50% potassium hydroxide (Schultz et al., 1971). Sufficient diazomethane was added to the extract until the yellow color persisted. The mixture was allowed to stand at room temperature with occasional shaking for 3/4 h. Before diluting with benzene for gas chromatography, the ether and excess diazomethane were removed under a gentle stream of N_2. CAUTION: Special care must be taken in handling ethereal diazomethane solution.

Analyses. *Gas Chromatography (GC).* The organo-extractable compounds were analyzed on Packard-Becker 420 and Perkin-Elmer Sigma 1 gas chromatograph systems,

Animal Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

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Table I. Recovery of Tetrachlorvinphos and Metabolites from Biological Samples

sample	added, ppm	recovery (methods), %: ordinary extraction	hydrolysis	continuous extraction
		Tetrachlorvinphos (I)		
blood	0.1	92		
	0.05	79		
urine	0.1	91		
	0.02	87		
feces	0.1	92		
	0.02	89		
milk	0.1	102		
	0.02	89		
liver	0.1	89[a]		
	0.02	76[a]		
	0.1	65[b]		
	0.02	57[b]		
fat	0.1	88		
	0.02	85		
heart	0.1	87		
	0.02	89		
		Desmethyl Tetrachlorvinphos (II)		
urine	0.1		65-69	73-78
	0.04		69-71	74-78
liver	0.1	82-83[a]		
	0.04	78-84		
		1-(2,4,5-Trichlorophenyl)ethanol (V)		
milk	0.1	88-89		
	0.02	79-83		
urine	0.1		73-74	58-81
	0.04		67-71	60-69
feces	0.1	67-71		
	0.04	62-70		
		(2,4,5-Trichlorophenyl)ethane-1,2-diol (VII)		
urine	0.1		55-61	78-81
	0.05		49-58	73-77
		2,4,5-Trichloromandelic acid (VIII)		
urine	0.1		67-73	73-78
	0.05		54-59	79-81

[a] Extractions were performed at low temperature. [b] Extractions were performed at room temperature.

each equipped with a ^{63}Ni electron capture detector (ECD). The column used was a 1.83 m × 4 mm (i.d.) glass tube packed with 3% (w/w) SE-30 on 80–100 mesh Chromosorb WHP.

Analyses and quantitation, corrected for recovery, of the residues were done at various operating conditions since no single set of conditions was found to be adequate. Compounds I and VI and the methyl ester of VIII were analyzed when the injector, column, and detector temperatures were 150, 175, and 300 °C, respectively, and had retention times of 7.9, 2.7, and 5.4 min, respectively. Under these conditions I was partially decomposed; hence it was also analyzed when the column was at 135 °C, while other operating conditions were the same as detailed. Metabolite VII was analyzed as its dimethyl derivative at a column temperature of 200 °C since it gave a poor GC response at 175 °C, while the other GC parameters were the same as described. The flow rate of the carrier gas (5% methane in argon) was 30 mL/min.

Typical gas chromatograms of extracts of urine samples for individual metabolites are shown in Figure 4. Under the GC conditions detailed above, the compounds gave a 50% full-scale deflection ($^1/_2$ fsd) as shown in the parentheses: I (850 pg), V (180 pg), methyl ester of VIII (760 pg), VII ($\simeq$14–15 ng), and its dimethyl derivative (2.5 ng). The estimated detection limits of these compounds from the biological samples are as follows: I, 0.005 ppm; V, 0.001 ppm; methyl ester of VIII, 0.01 ppm; and VII as its dimethyl derivatives, 0.01 ppm. However, these values varied greatly with the condition of the electron capture detector.

Table II. R_f Values of Reference Compounds in Various Solvent Systems

compound[a]	R_f values (solvent systems)[b]: A	B	C
I	0.32	0.77	
II			0.24
III	0.63		
IV	0.49		
V	0.67	0.95	
VI	0.58		
VII	0.05	0.20	0.81
VIII	0.0		0.43

[a] See text for the name of the compound. [b] Solvent systems: A, ethyl acetate-hexane (3:7, v/v); B, chloroform-acetic acid (99:1, v/v); C, methanol-2-propanol-acetone (1:1:8, v/v/v).

Thin-Layer Chromatography (TLC). The various extracts were also analyzed on precoated (250 μm) TLC plates (silica gel G, Fisher Scientific Co.). The R_f values of the compounds in various TLC development systems are listed in Table II. Compounds were located by spraying TLC plates with silver nitrate–2-phenoxyethanol reagent (Beynon and Wright, 1969).

RESULTS

Oral administration of 50 ppm of tetrachlorvinphos, based on daily food intake, to four lactating cows for 5 days, followed by no treatment for a period of 10 days, did not produce any unusual symptoms. In addition, the daily feed intake and milk production were not affected during the investigation. Furthermore, no unusual histological or pathological changes were observed.

Absorption and metabolism of the insecticide was rapid. A level of 3.4 ppm of I was reached in blood in 3 h and 1.2 ppm in 6 h and none was detected in the 9-h sample, after the first oral dose. The major portion of the absorbed material was eliminated from the body, both free and conjugated, via the kidneys in the urine (Table III). A small amount of the administered dose was also excreted unchanged in the feces.

The daily rate of excretion varied considerably among the animals. For example, the equivalent of 14–37% of the dose was excreted in the urine, while 0–2.8% was eliminated in the feces in 9 h. Excretion of I, metabolized or unchanged, was almost complete 7 days after the last treatment.

Excreted Metabolites. A comparative study of the recovery of the metabolites from biological samples by the two published procedures (Akintonwa and Hutson, 1967; Gutenmann et al., 1971) was undertaken. The recoveries of metabolites from various samples are listed in Table I. A single method was not suitable to provide reproducible and consistent results of all metabolites. For example, the direct phosphoric acid hydrolysis technique provided reproducible and consistent data only for II and V. On the other hand, the continuous extraction procedure was adequate for II, VII, and VIII. Thus, data from extraction procedures corrected for recovery are shown in Table III.

The extracts from phosphoric acid hydrolysis contained II, V, VII, and VIII (by GC and TLC). Positive identification and quantitation of II and VIII were made by means of their methyl derivatives. The yields of methylation of II and VIII were 78–102 and 75–88%, respectively. Methylation of VII could not be achieved with diazomethane. Since the methylated mixture exhibited interfering peaks

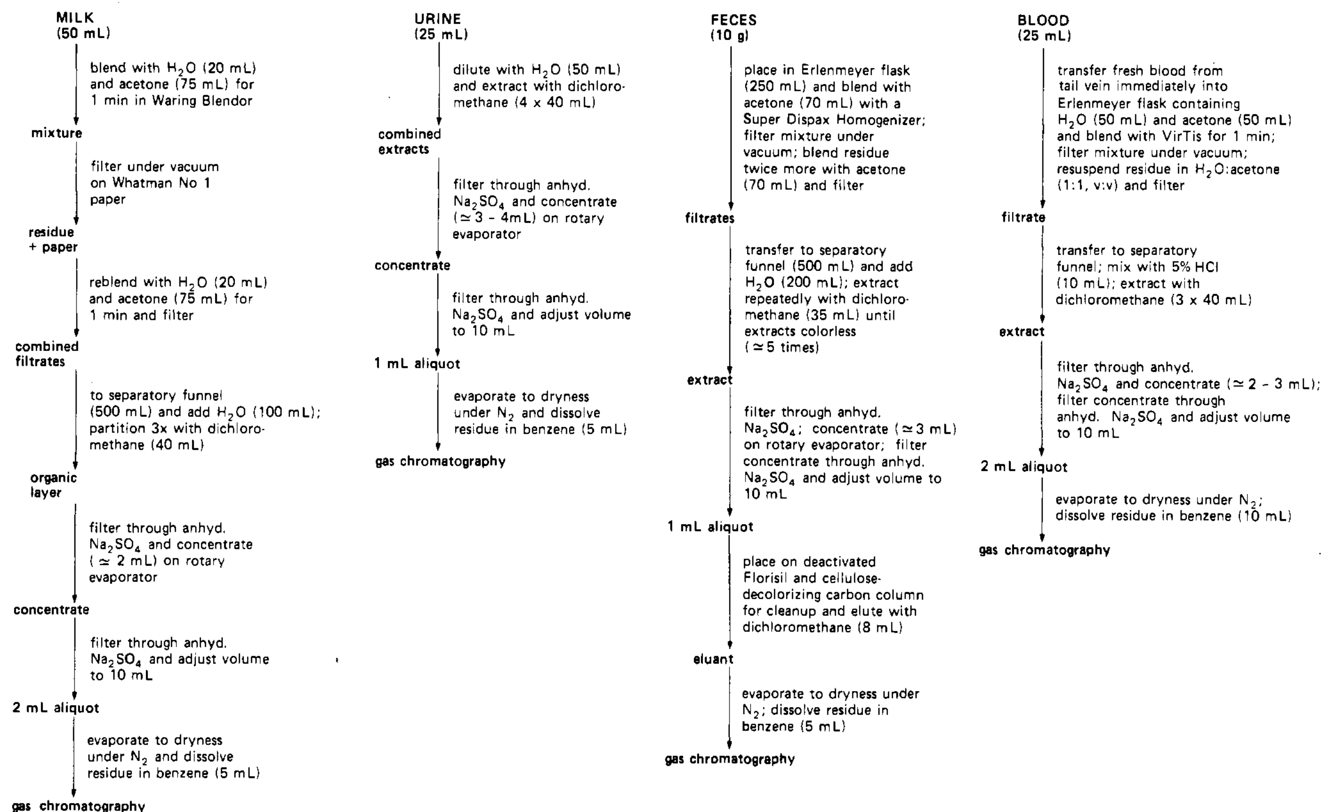

Figure 1. Techniques for extraction and isolation of tetrachlorvinphos from bovine milk, urine, feces, and blood.

Table III. Metabolites of Tetrachlorvinphos Excreted in the Urine of Treated Cows at Various Time Intervals

	metabolites[a]			
time	II	V (free and conjugated)	VII (conjugated)	VIII
3 h	4.2	0.7	0.2	
6 h	4.4	8.1	2.1	0.2
9 h	7.1	12.4	5.0	0.6
1 day	8.8	26.7	5.2	0.9
2 days	6.3	27.2	13.7	1.5
3 days	6.5	33.5	18.2	1.8
4 days	5.3	31.8	19.4	4.5
5 days[b]	3.9	30.3	21.2	5.1
6 days	4.1	34.2	19.3	5.2
7 days	5.5	35.5	21.4	5.3
8 days[c]	7.0	35.7	20.7	5.4
9 days	8.5	37.8	22.5	5.6
10 days	9.5	38.4	25.4	5.9
11 days[d]	10.5	34.7	26.7	6.1
12 days	11.5	34.7	28.0	6.1
13 days	12.5	34.8	28.1	6.1
14 days	13.2	34.8	28.1	6.1
15 days[e]	13.4	34.8	28.1	6.1

[a] Cumulative data expressed as percent of the total administered dose at that point. Where applicable the data are corrected for percent recovery and are the average of all animals. [b] Cow slaughtered 6 h after the final oral dose. [c] Cow slaughtered 3 days after final oral dose. [d] Cow slaughtered 6 days after final oral dose. [e] Cow slaughtered 10 days after the final oral dose.

very close to that for V, the quantitation of V was made from nonmethylated extracts.

The extracts from continuous ether extraction contained II, V, and VIII in addition to large amounts of a chlorinated compound, which was nonlabile on TLC plates in the most polar solvent used (solvent C). In order to ascertain the identity of this compound, it was extracted from the plate with methanol–ether (1:1, v/v), and after concentration, the extract was incubated with β-glucuronidase (see details in Figure 3). The extract of the incubation mixture was shown by GC and TLC to contain only V and VII. Therefore, a portion of the continuous ether extracts was routinely incubated with β-glucuronidase to liberate V and VII from their conjugates prior to GC analyses. Structural confirmation and quantitation of the excreted metabolites were carried out by GC analyses. The extracts of the phosphoric acid hydrolyses served as a guide for the analysis of the complex mixture. The ether extracts were analyzed directly for free V and VII. For II and VIII, the extracts were treated with fresh diazomethane to produce GC-labile methyl derivatives. The extracts were hydrolyzed with β-glucuronidase in order to hydrolyze conjugates into products that could be identified by GC analysis. As indicated previously, VII exhibited a poor response under the GC conditions used. In order to obtain a better estimate of the concentration of VII, methylation by various methylating agents was investigated. When methylation was attempted with sodium hydride–methyl iodide or sodium hydride–dimethyl sulfoxide, complex mixtures resulted. However, treatment of VII with excess BF_3–methanol resulted in a dimethyl derivative (identified by mass spectral data) in 75–80% yield and a small amount of unidentified product.

Table III records the data on the distribution of metabolites in the urine. During the first 3 h after oral administration, the major metabolite excreted was II. But at the end of the sixth and ninth hour, the urine contained more V and VII and less II. A pattern for the distribution of metabolites was established on the third and fourth day after the treatment and continued till the end of the experiment. The major metabolites excreted in the urine were V and VII (free and conjugated) in addition to small amounts of II and VIII. Metabolites III, IV, and VI were not detected in the urine.

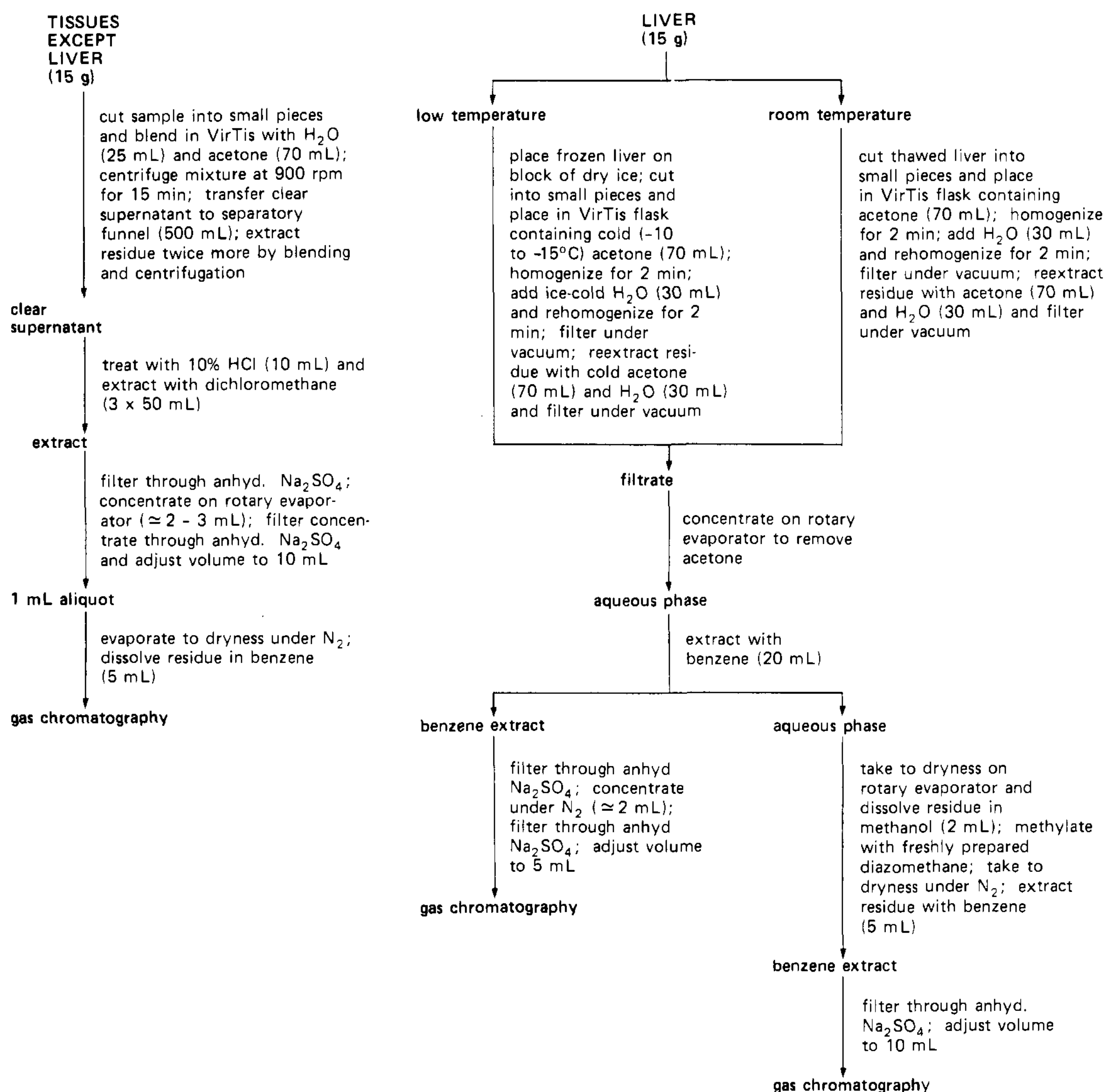

Figure 2. Techniques for extraction and isolation of tetrachlorvinphos from bovine kidney, heart, and liver.

Animals continued to excrete metabolites in the urine after the treatment had stopped. Metabolites V, VII, and VIII were detected in the urine 7 days after discontinuation of treatment, but were not found on the tenth day. Furthermore, metabolite II continued to be excreted in the urine till the last day of the experiment, which remains unexplained.

The major products in the feces were unchanged I and V. The formation of V in stomach was probably not due to a biological reaction since no degradation of I took place when incubation with rumen fluids (Gutenmann et al., 1971). In the present study, incubation of 24.4 ppm of I with filtered rumen juice for 90 min resulted in 88–91% recovery of unchanged I. However, prolonged incubation (≃24–30 h) caused 20–25% degradation of I, and V was detected in the extracts. This degradation was not investigated further.

The identifiable products excreted within 10 days after the last dose accounted for 92.9% of the total administered dose (one animal). The amount of excretion of the total administered dose varied among the animals and ranged between 70.4 and 92.9%. Although the animals showed variation in the amount of the excreted materials, the nature of the metabolites was common.

Metabolites in Milk and Tissues. The insecticide and metabolites were not detected in milk samples (<0.01 ppm), nor did they tend to accumulate in the tissues examined. In this respect, they were not detected in samples collected on the third, sixth, and tenth day after the last dose with exception of traces of V in the liver of the cow slaughtered on the third day. However, appreciable amounts of the insecticide and metabolites were detected in kidney and liver samples of a cow slaughtered 6 h after the final dose on the last day of the treatment (Table IV).

Table IV. **Residues of Tetrachlorvinphos and Metabolites in Blood and Tissues of Treated Lactating Cows**

	residues of compound in sample at various times (ppm)[a]			
sample	5th (day)	7th (day)	11th (day)	15th (day)
liver	<0.1 (I)[b]	nd[c]	nd	nd
liver	2.6 (II)	nd	nd	nd
liver	6.8 (V)	Tr (V)	nd	nd
kidney	1.2 (V)	nd	nd	nd
fat	sl[d]	nd	nd	nd
heart	1.6 (V)	nd	nd	nd

[a] Ppm are reported on the basis of wet tissue weights. [b] Number in the parentheses refers to the compound. [c] Not detected. [d] Sample lost.

DISCUSSION

The data indicate that oral consumption of tetrachlorvinphos by lactating cows is followed by absorption through the gastrointestinal tract, metabolism, and elim-

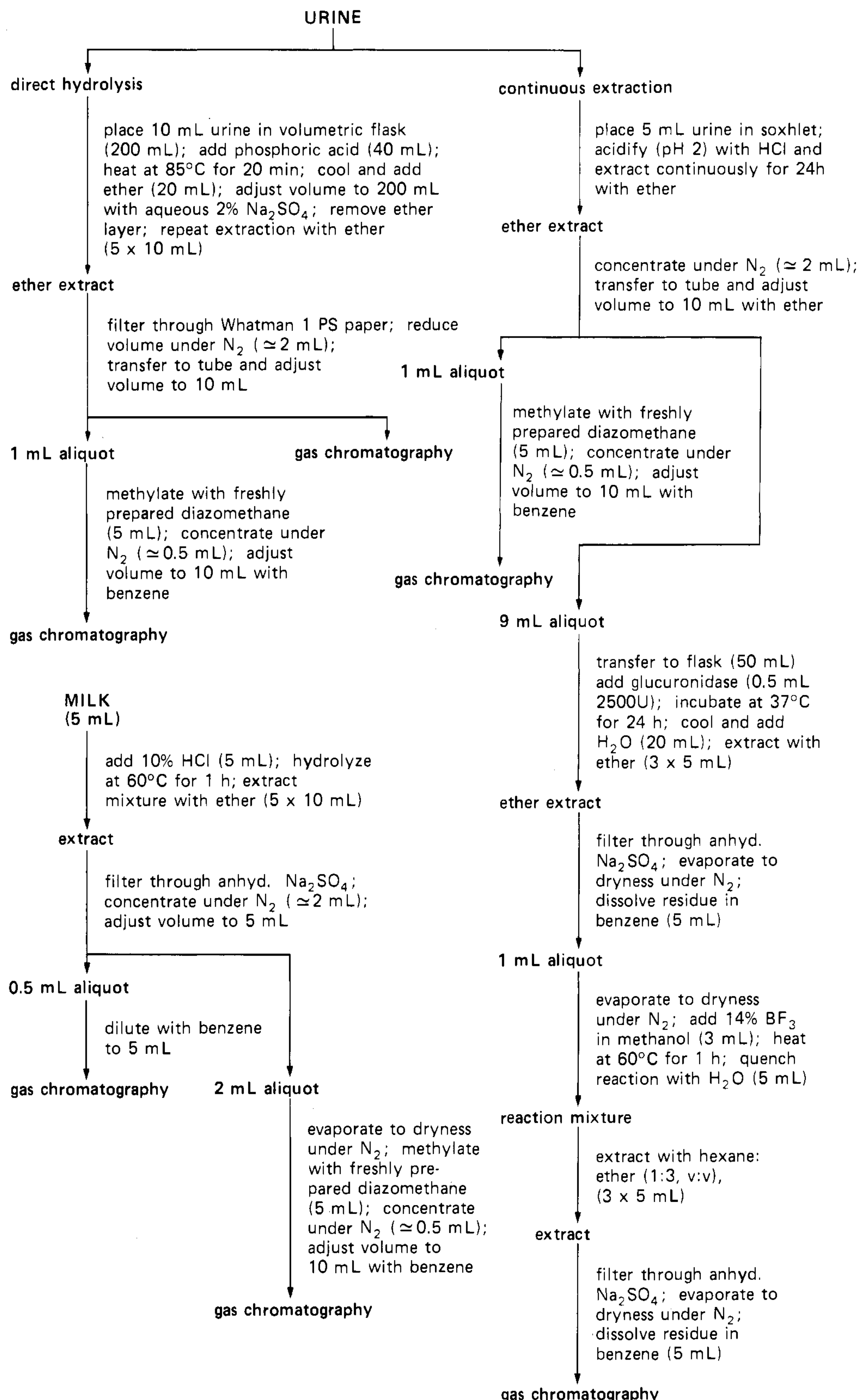

Figure 3. Techniques for extraction and isolation of metabolites from bovine urine and milk.

ination via urine and feces. Only traces of unaltered insecticide in the urine suggest that the insecticide is almost completely metabolized to more polar metabolites before excretion. Small amounts of unchanged insecticide were also eliminated in the feces.

Figure 5 gives the tetrachlorvinphos metabolic pathways based on identified metabolites. Cows metabolized the insecticide into II (13.2%), V (free and conjugated, 34.8%), VII (conjugated, 28.1%), and VIII (6.1%). The larger amount of V as compared to II suggests that the hydrolysis at the P–O–vinyl is much more efficient than dealkylation. A similar theory was advanced by Akintonwa and Hutson (1967) to explain the high ratio of V to II in metabolic studies of the insecticide in rats. Gutenmann et al. (1971)

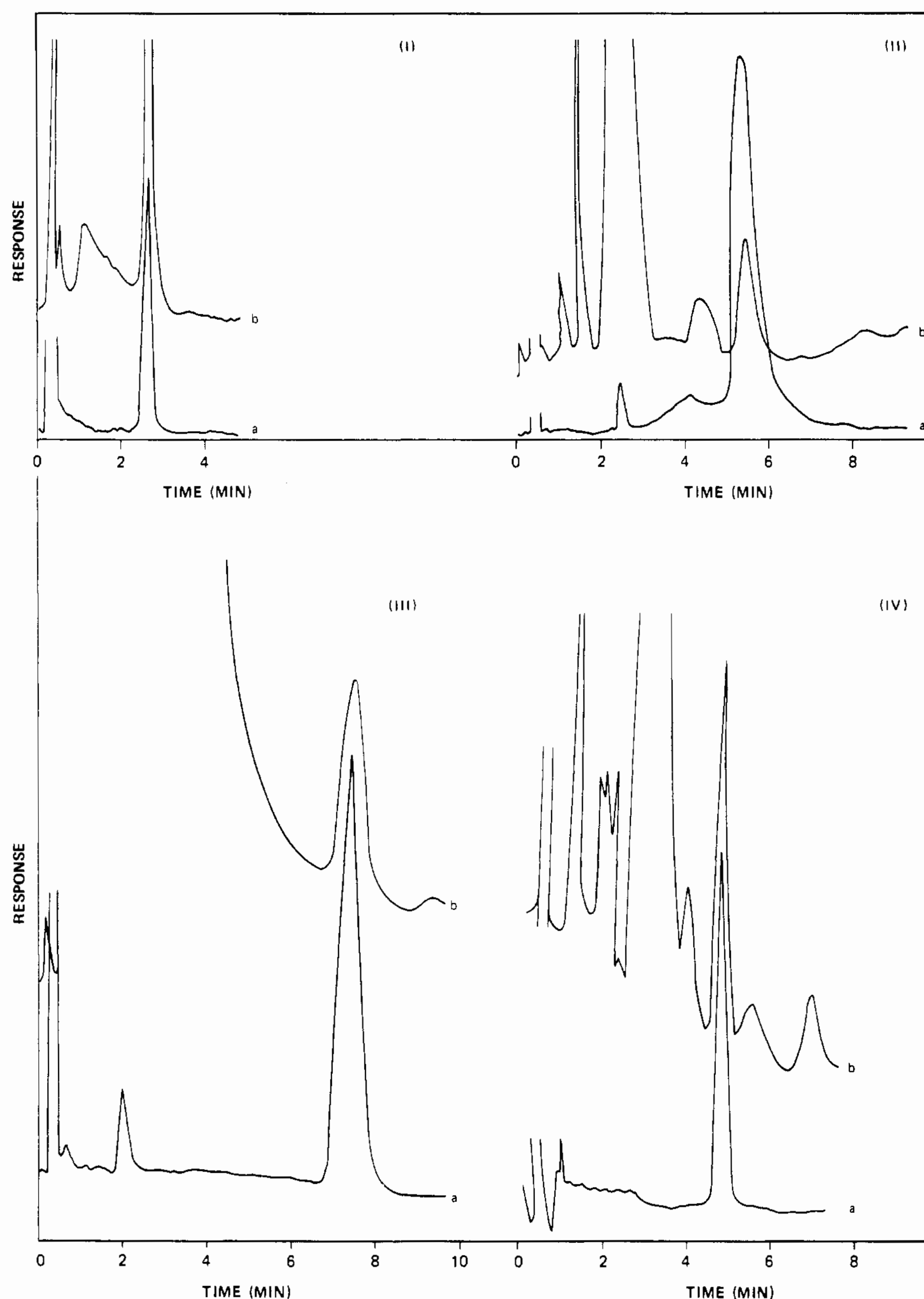

Figure 4. Gas chromatograms of the standards and the extracts of urine samples for (i) V, (ii) VIII as methyl ester, (iii) II as methyl derivative, i.e., I, and (iv) dimethyl derivative of VII. Letters a and b refer to standard and extracts, respectively. GC conditions are the same as in the text, oven temperature for i–iii and iv was 175 and 200 °C, respectively.

also observed that up to 75% of a single administered dose of tetrachlorvinphos (5 ppm on daily food intake) to a lactating cow was excreted as V in the conjugated form. In addition, Gutenmann et al. (1971) found that when I was incubated with liver preparation (10000*g*), V was the only product identified. On the basis of these data, they concluded that I was metabolized by hydrolysis.

The data in Table III provide much more detailed information, in comparison to those previously published by Gutenmann et al. (1971), on the in vivo metabolism of I in lactating cows treated for 5 days, followed by no treatment for a period of 10 days. Concentrations of II and other metabolites in the urine during the first 3, 6, and 9 h after oral administration are of great importance in establishing the metabolic pathways. Appearance of II as the major metabolite during the first 3 h, followed by subsequent decrease in II and increases in V and VII, strongly suggests that I initially undergoes dealkylation to II which in turn is further degraded into other metabolites. Supporting data from in vitro studies (Akhtar and Foster, 1979) demonstrated such pathways. If direct hydrolysis of I via P–O–vinyl cleavage to III had been the major route, a large amount of V and other metabolites would have been observed in the 3-h urine samples. In this connection it should be pointed out that the facile conversion of III to V via IV, in in vitro studies, is well established (Akhtar, 1979; Hutson et al., 1976).

The absence of III and IV in any of the samples is not surprising. It has been shown previously that III is very efficiently converted into a glutathione conjugate. The conjugate in turn is further metabolized to other products (Akhtar, 1979; Hutson et al., 1976). The fact that IV was not detected is also explained by the studies of Akhtar (1979), who showed that IV was efficiently reduced to V

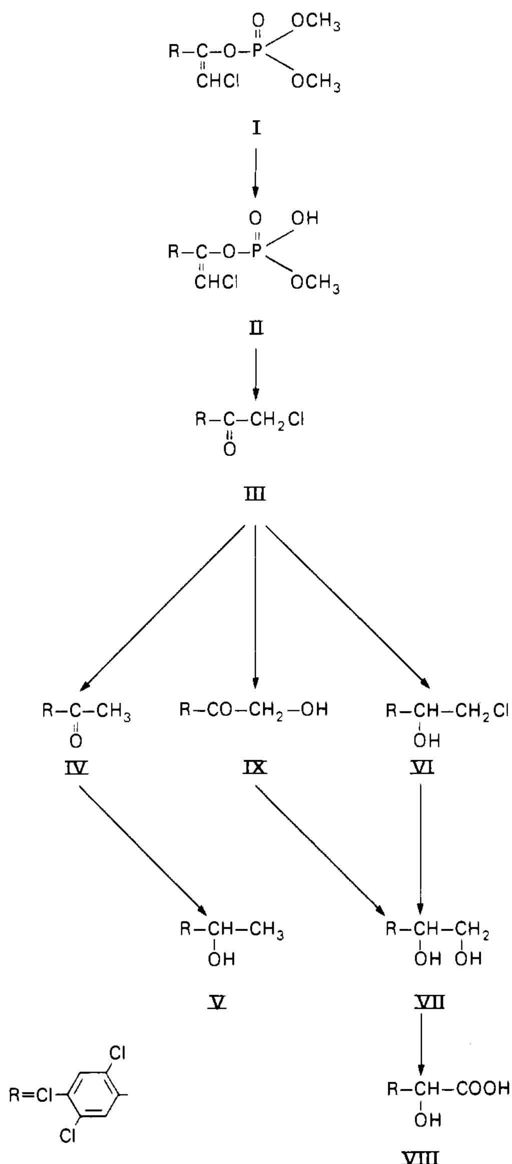

Figure 5. Metabolic pathway of tetrachlorvinphos in the lactating cow.

by the soluble fraction from liver homogenate.

Residues of VI were not detected in the current study despite the fact that they had been shown to be present in in vitro studies with the soluble fraction from bovine liver homogenates (Akhtar and Foster, 1979). This suggests that VI may have been the precursor for identifiable products VII and VIII. Akintonwa and Hutson (1967) identified the glucuronide conjugate of VII as (2,4,5-trichlorophenyl)ethanediol glucuronide as well as VIII in the urine of rats and dogs that had been fed tetrachlorvinphos. In addition, these workers also proposed IX, though not detected, as the precursor for identified products VII and VIII.

Small amounts of unchanged insecticide were eliminated in the feces. This confirms the findings of Akintonwa and Hutson (1967) in studies on the dog and rat and of Gutenmann et al. (1971) in the lactating cow. In the current study, the feces also contained V.

Residues of the insecticide and its metabolites were not detected in the milk or tissues including omental fat. Because they are highly chlorinated compounds and almost insoluble in water it had been expected that they would be lipophilic and appear in fat and milk. These data suggest that tetrachlorvinphos when fed at 50 ppm is very efficiently metabolized in the cow and readily eliminated via the urine and excreta with little or no deposit in milk, omental fat, and other tissues or organs.

The liver of a cow slaughtered 6 h after the final dose on the fifth day contained I, II, and V. When liver was extracted by the cold (−15 to −20 °C) procedure, unmetabolized I was detected. If the extraction was made at room temperature, the amount of I was considerably lower. It has been well documented that mammalian livers contain enzyme systems capable of degrading the insecticide (Akhtar and Foster, 1977, 1979; Gutenmann et al., 1971; Hutson et al., 1972).

Residues of unchanged I and V were also found in kidney and heart of the cow slaughtered 6 h after the administration of the final oral dose. Omental fat from this cow could not be analyzed because improper storage had resulted in complete deterioration of the sample.

It can be concluded that feeding tetrachlorvinphos to lactating cows does not leave residues of insecticide or metabolites in milk and certain organs and tissues. However, it is recommended that treated animals not be slaughtered until 15 days after the treatment.

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