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Tetrachlorvinphos Metabolism in Laying Hens

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Tetrachlorvinphos metabolism was studied, with the aid of a ^{14}C-labeled compound, in laying hens fed 50 ppm of the insecticide. Approximately 71% of the radioactivity was eliminated in 24 h in excreta. After the final dose, an additional 1.3 and 2.6% of total ^{14}C was excreted during the next 3 and 7 days. Eggs laid within 24 h of the treatment contained radioactivity. ^{14}C was also detected in tissues and organs at an insecticide equivalent in the ppm range in kidney, liver, and abdominal fat but in the ppb range elsewhere. After dosing was discontinued, the ^{14}C content of excreta, eggs, tissues, and organs gradually decreased. Compounds identified in excreta were desmethyl tetrachlorvinphos (25%), 2,4,5-trichloromandelic acid (30.5%), and tetrachlorvinphos (1.0%). During the treatment period, all tissues examined contained small amounts of the insecticide, but only traces were detected in tissues and organs from hens killed 7 days after the last dose.

The use of pesticides in poultry and animal production has increased considerably in recent years. Therefore, considerable effort is being made to ensure that edible tissues, organs, milk, and eggs marketed for human consumption are free of pesticide residues.

It is well documented that, on ingestion, a pesticide is absorbed from the gastrointestinal tract into the blood or lymph and thus distributed to various tissues, organs, eggs, milk, etc. and finally eliminated by excretion in feces and urine or excreta (Loomis, 1968). During this cycle, the pesticide may face a variety of biochemical attacks which could result in the formation of products or metabolites which are different in nature and behavior than the original insecticide.

Tetrachlorvinphos (Rabon, Stirofos, etc.) is an organophosphate insecticide which has shown great promise in the control of ectoparasites in poultry. Previous studies on the fate of tetrachlorvinphos fed to laying hens indicated only a very limited transfer of residues to tissues and eggs (Sherman and Herrick, 1971; Wasti and Shaw, 1971; Yadava and Shaw, 1970). A sufficient amount of the insecticide was reported to be eliminated in the excreta of hens to control ectoparasites (Wasti et al., 1970). Residues of the insecticide were detected in body fat and egg yolk of hens after their dust boxes and litter were treated with a tetrachlorvinphos formulation (Ivey et al., 1969).

The following is a report detailing the metabolism and distribution of tetrachlorvinphos and metabolites in various tissues, organs, and eggs of laying hens which had free access for 7 consecutive days to a standard laying ration fortified with the insecticide at the 50-ppm level and given a daily oral dose of [^{14}C]tetrachlorvinphos during this period.

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EXPERIMENTAL SECTION

Chemicals. Pesticide-grade solvents (Caledon Laboratories Ltd., Georgetown, Ontario, Canada) were used as received. Esterification reagents, 14% (w/v) BF_3–MeOH and 10% (w/v) BCl_3–2-chloroethanol, were obtained from Applied Science Laboratories, State College, PA. Bovine liver β-glucuronidase (5000 Sigma Units/mL) was purchased from Sigma Chemical Co., St. Louis, MO. Pure samples of ^{14}C-labeled and unlabeled tetrachlorvinphos (I), desmethyl tetrachlorvinphos (II), 2,4,5-trichlorophenacyl chloride (III), 2,4,5-trichloroacetophenone (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), and 2,4,5-trichloromandelic acid (VIII) and its methyl ester were available from a previous study (Akhtar and Foster, 1977).

Chicken Treatment and Collection of Tissues. Eight Single Comb White Leghorn hens, approximately 1.5 years old and at 70% production, were kept in individual laying cages as described previously (Foster et al., 1972). They were fed a standard laying ration for 7 days while the daily feed intake was recorded. This was followed by a 7-day period during which the hens were fed the standard laying ration containing 50 ppm of tetrachlorvinphos and were given a daily oral dose (1.86×10^5 dpm, 450 μg) of radiolabeled [*vinyl*-^{14}C]tetrachlorvinphos in Risella oil (Shell Canada Ltd.). This was followed by a further 7-day period during which the hens were fed the standard laying ration. In both latter periods, records were kept of daily feed intake.

Excreta and eggs were collected on 24-h basis. Eggs were stored at 4 °C and excreta at −20 °C in closed glass jars

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prior to freeze-drying. Both freeze-dried eggs and excreta were stored in screw-capped jars under N_2 at 4 °C until analyzed.

The hens were slaughtered in duplicate, at random, at various times during the experiment, and heart, kidney, liver, lungs, gizzard, oviduct, crop, and intestines plus samples of leg and breast muscle removed and any gross anomalies noted. These were freeze-dried, the radioactivity was determined, and the samples were stored at 4 °C until further analyses. Samples of abdominal fat were removed, the radioactivity was measured, and the remainder was frozen and stored at −20 °C until further analyses.

Measurement of Radioactivity. The radioactivity of freeze-dried excreta, eggs, tissues, and organs were determined by combustion in a Packard Tri-Carb sample oxidizer, Model 306, and liquid scintillation counting in a Packard Tri-Carb liquid scintillation spectrometer, Model 3320, using an external standard and correcting the data for quenching.

Extraction Methods. The following procedures were used to extract and isolate possible metabolites etc. from eggs, tissues, and organs prior to further analyses.

(*a*) *Excreta.* Distilled water (25 mL) was added to freeze-dried excreta (1 g) in an Erlenmeyer flask (50 mL) and the suspension was acidified to pH 2 with 10% (v/v) aqueous HCl. The flask was stoppered and the contents were stirred for 2 h and filtered through a Buchner funnel under vacuum. The residue was air-dried and retained. The filtrate was extracted with ethyl ether (4 × 25 mL) in a separatory funnel (125 mL). Both the aqueous layer and the organic layer, after being dried over anhydrous Na_2SO_4, were concentrated by rotary evaporation and the final volume was adjusted to 5 mL. Radioactivity was measured in aliquots of both layers.

An aliquot (2 mL) of the organic layer was transferred to an Erlenmeyer flask (50 mL), evaporated to dryness under N_2, and then incubated with β-glucuronidase (0.5 mL) at 40 °C for 24 h. The mixture was cooled, diluted with distilled water (20 mL), and extracted with ethyl ether (3 × 15 mL). The ether extract was dried over anhydrous Na_2SO_4 and concentrated to a final volume of 5 mL. An aliquot of the ether extract was transferred to a centrifuge tube (15 mL), evaporated to dryness under N_2, and made to the required volume for GC analyses.

Portions of the ether extract of the incubation reaction (2 mL) and unincubated organic layer (1 mL) were transferred to centrifuge tubes (15 mL) and evaporated to dryness under N_2. Methanol (0.5 mL) and 14% BF_3–MeOH (4 mL) were added to these dried samples. The tubes were stoppered and heated at 60 °C for 1 h. The reaction was quenched by adding distilled water (5 mL) and the mixture was extracted with 30% (v/v) hexane–ether. The extracts were dried over anhydrous Na_2SO_4 and taken to dryness under N_2, and the residue was redissolved in benzene for GC analyses.

(*b*) *Fat and Wet Tissues.* Aliquots (15 g, wet weight) were cut into small pieces and blended with a mixture of acetone–water (1:1 v/v, 100 mL). The mixture was allowed to stand for 1 h and the turbid solution was slowly decanted into a separatory funnel (500 mL). The procedure was repeated twice more and the residue finally filtered through a Buchner funnel (a very slow, cumbersome, and tedious process). The combined extracts were passed through a glass wool plug in an open column. The resultant filtrate was treated with 10% HCl (10 mL) and extracted with dichloromethane (3 × 50 mL). The dichloromethane extract was dried by passing through a column containing anhydrous Na_2SO_4 and concentrated on a rotary evaporator (2–3 mL). This was transferred quantitatively with dichloromethane to a centrifuge tube (15 mL) and evaporated to dryness under a gentle stream of dry N_2. The residue was redissolved in benzene prior to GC analyses. (Note: analysis of these extracts frequently resulted in a reduction in the sensitivity of the detector, requiring frequent cleaning of the detector.)

(*c*) *Freeze-Dried Samples.* Aliquots (5.0 g) of freeze-dried samples suspended in methanol–water (1:1 v/v, 50 mL) were acidified to pH 2 with 10% HCl and shaken vigorously for 2 h. The mixture was filtered by suction. The residue was resuspended in the methanol–water solution (25 mL), shaken for 1 h, and filtered, and the residue set aside to dry to constant weight. The combined extracts were concentrated on a rotary evaporator at 40–42 °C to ~2–5 mL. The concentrate was transferred quantitatively by means of acetone (2 × 5 mL) to a centrifuge tube (15 mL) and further concentrated under a gentle stream of dry N_2 to 3–4 mL. The volume was now made to 5.0 mL and the solution extracted with benzene (5 × 3 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under dry N_2, and the volume was adjusted to 5.0 mL. Traces of benzene were removed from the aqueous phase and the final volume was adjusted to 5.0 mL. Duplicate aliquots (1 mL) were removed from both the organic and aqueous phases for measurement of radioactivity.

The benzene extracts were analyzed by GC after appropriate dilutions. Aliquots of benzene and aqueous phases were evaporated to dryness and treated with a fresh ethereal solution of diazomethane according to the accepted methylating procedures with diazomethane. The methylated solutions were evaporated to dryness, taken up in benzene, and analyzed by GC. **CAUTION**: extreme care must be taken while handling diazomethane (carcinogen) solutions.

Gas Chromatography (GC). A Perkin-Elmer Sigma 1 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a 1.07 m × 4 mm (i.d.) glass column packed with 3% SE-30 on Chromosorb WHP, 80–100 mesh, was used. Injector, detector, and oven temperatures were 150, 300, and 180 °C, respectively. The flow rate of the carrier gas (5% methane in argon) was 60 mL/min in total, of which 30 mL/min was as a "makeup gas".

Typical gas chromatograms of extracts of excreta are presented in Figure 1. Under the conditions for GC detailed above, the following 50% full-scale deflections ($^1/_2$ fsd) were observed (amounts and retention times, respectively, are indicated in parentheses): I (850 pg; 6.9 min), III (100 pg; 3.1 min), V (100 pg; 2.3 min), VI (150 pg; 4.3 min), dimethyl derivative of VII (195 pg; 4.6 min), methyl ester of VIII (200 pg; 5.1 min), and chloroethyl ester of VIII (210 pg; 9.7 min).

Gas Chromatography–Mass Spectrometry (GC–MS). The GC–MS analyses were performed on a Finnigan Model 3100 mass spectrometer connected to a Finnigan Model 9500 gas chromatograph by means of a jet separator. The mass spectrometer was interfaced with a Model 6100 computer-controlled data acquisition system. A 1.5 m × 4 mm i.d. glass column packed with 3% SE-30 on 80–100-mesh Chromosorb WHP was used for gas chromatographic separation. The column was run at 150 °C with a helium flow rate of 35 mL/min. The mass spectra were recorded at 70 eV.

RESULTS AND DISCUSSION

No unusual or toxicological symptoms were noted in the hens despite the consumption of this quite high level (50 ppm) of tetrachlorvinphos for 7 days. There were no

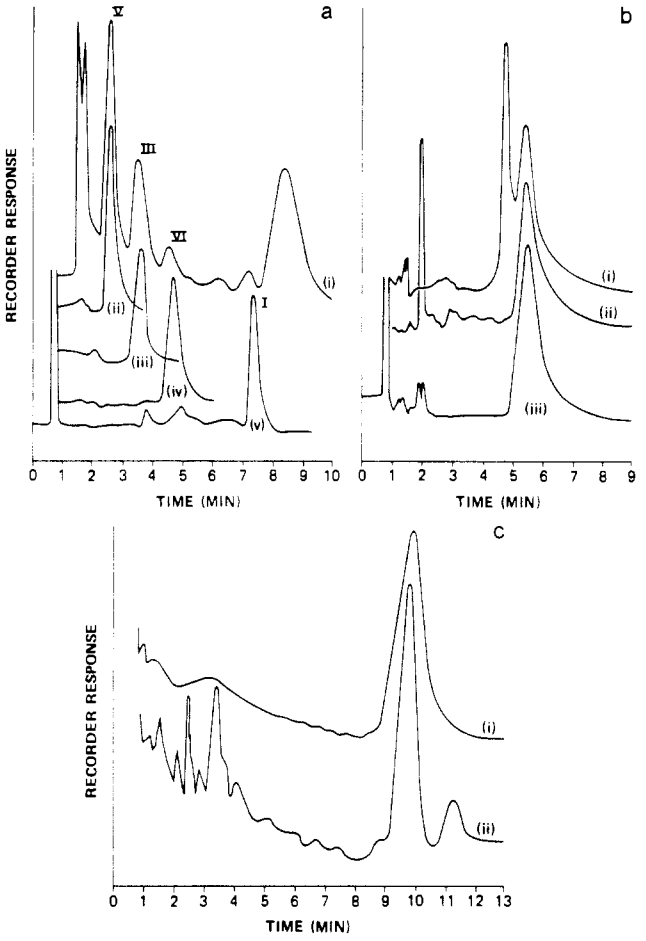

Figure 1. Gas chromatograms of the standards and the extracts of excreta. (a) (i) Organic extracts; (ii) V, 60 pg; (iii) III, 35 pg; (iv) VI, 41 pg; (v) I, 350 pg. (b) (i) Mixture of dimethyl derivative of VII and methyl ester of VIII; (ii) after methylation with BF_3–MeOH of organic extracts; (iii) methyl ester of VIII (ethereal diazomethane; same compound is also formed with BF_3–MeOH). (c) (i) Chloroethyl ester of VIII; (ii) after transesterification of organic extract.

changes in egg production, body weight, or feed consumption associated with this level of pesticide intake. In addition, none of the tissues and organs from the treated hens showed any gross histological or pathological changes.

Elimination and Retention of ^{14}C. The amount of ^{14}C which appeared in the excreta of laying hens as a result of a daily oral dose of [^{14}C]tetrachlorvinphos for 7 days is shown in Table I. In 24 h after the initial dose, an average of 66.8% of the administered radioactivity appeared in the excreta. After 7 daily doses, the cumulative excretion had increased to 75.0% (73.8 to 76.9% from day 2 to day 7 inclusive) of the total dose. Thereafter radioactivity continued to appear in the excreta and was still detected on the seventh day after the final ^{14}C dose, at which time the cumulative total had increased to 76.7%.

This rapid rate of elimination of [^{14}C]tetrachlorvinphos orally administered to hens is similar to that observed with rats and a dog (Akintonwa and Hutson, 1967) and lactating cows (Gutenmann et al., 1971; Akhtar and Foster, 1980).

Radioactivity was also detected in eggs. Data for two hens which were maintained and which consistently laid eggs during the entire period studied are presented in Figure 2. Radioactivity was detected in aliquots of freeze-dried whole eggs which had been laid within 24 h after treatment began, which amounted to less than 1% (0.85–0.97%) of the administered dose. The highest radioactivity levels were detected in eggs laid on the eighth

Table I. Elimination of Radioactivity in Chicken Excreta[a]

day	% of cumulative ^{14}C administered[b]
1	66.8 ± 5.5 (7)
2	74.1 ± 3.8 (8)
3	75.4 ± 3.3 (8)
4	73.8 ± 2.8 (6)
5	75.5 ± 2.4 (6)
6	76.9 ± 1.8 (6)
7	75.0 ± 1.0 (5)
8	75.1 ± 0.9 (4)
9	75.6 ± 0.9 (4)
10	76.1 ± 1.7 (2)
11	76.3 ± 1.8 (2)
12	76.4 ± 1.9 (2)
13	76.6 ± 1.9 (2)
14	76.7 ± 2.0 (2)

[a] Laying hens received a daily oral dose of [*vinyl*-^{14}C]-tetrachlorvinphos on days 1–7 inclusive (186 227 dpm/day). [b] Radioactivity, measured on duplicate aliquots of freeze-dried excreta by combustion, trapping $^{14}CO_2$, determined by scintillation counting, and corrected for counter efficiency and quenching, is shown as mean ± SEM with the number of hens indicated in parentheses.

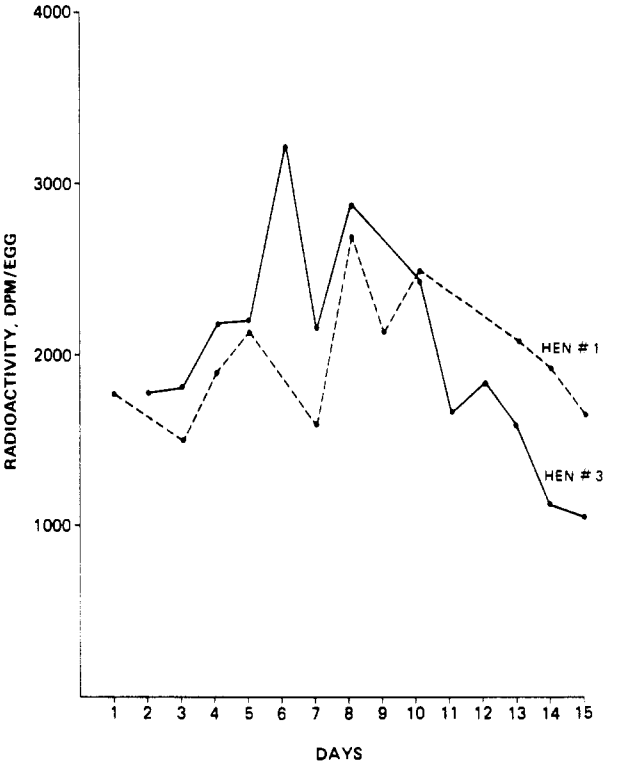

Figure 2. Radiocarbon residues in eggs up to 7 days after seven daily oral administrations of [^{14}C]tetrachlorvinphos.

day (the day after the final dose). However, radioactivity was still detected in eggs on the seventh day after treatment had been discontinued but the amount decreased daily. From 1.6 to 2.0% of the total radioactivity administered was eliminated in the eggs.

Yadava and Shaw (1970) reported that residues of I (Rabon) appeared in the yolk of eggs 2 days after the hens had been given 25, 50, 100, and 200 mg/kg of body weight of the insecticide. However, no residues were detected 5 days after the final treatment.

The data in Table II indicate the amount of radioactivity at various time intervals which was measured in several organs and tissues. This data was used in conjunction with the observed feed intake to calculate an estimate of tetrachlorvinphos equivalent residues in these organs and

Table II. Radioactivity and Estimated Tetrachlorvinphos Equivalents in Tissues and Organs of Chickens Treated Orally with [^{14}C]Tetrachlorvinphos and Fed the Insecticide at a Level of 50 ppm

sample	radioactivity[a] at various times[b]			
	4th day	8th day	10th day	15th day
abdominal fat	68 (0.94)	132 (2.39)	109 (1.75)	29 (0.91)
blood	10 (0.12)	29 (0.48)	35 (0.48)	12 (0.34)
crop	132 (1.73)	125 (2.15)	110 (1.92)	14 (0.42)
gizzard	19 (0.20)	24 (0.41)	21 (0.33)	9 (0.25)
heart	17 (0.18)	36 (0.61)	21 (0.33)	9 (0.26)
intestine	29 (0.30)	41 (0.67)	21 (0.33)	8 (0.24)
kidney	106 (1.17)	202 (3.84)	85 (1.35)	21 (0.64)
breast muscle	7 (0.09)	18 (0.30)	15 (0.19)	4 (0.05)
leg muscle	7 (0.09)	24 (0.40)	14 (0.16)	4 (0.06)
liver	22 (0.38)	63 (1.06)	38 (0.59)	9 (0.28)
lung	19 (0.20)	32 (0.51)	15 (0.22)	9 (0.23)
oviduct	14 (0.13)	25 (0.40)	12 (0.20)	5 (0.10)

[a] Radioactivity data, corrected for quenching, etc., are reported as dpm/g of wet tissue and are the average of two hens. The data in parentheses are the estimated tetrachlorvinphos equivalents in ppm calculated on the basis of radioactivity data and feed intake and are the average of two birds. [b] Fourth day means that the hens were killed 26 h after the 3rd daily oral dose of [^{14}C]tetrachlorvinphos; 8th day means that the hens were killed 26 h after the 7th and final dose; 10th day means that the hens were killed 74 h after the final dose; 15th day means that the hens were killed 194 h after the final dose.

tissues. The highest levels of radioactivity and thus the highest estimated tetrachlorvinphos equivalents were detected in abdominal fat, crop, kidney, and liver. Radioactivity was detected in all organs and tissues from hens killed 26 h after 3 daily oral doses of the ^{14}C-labeled insecticide and reached a maximum 26 h after the seventh and final oral dose. Radioactivity levels were decreasing by 74 h after the final dose and although still detectable were considerably decreased by 194 h after the final dose. These observations are similar, in part, to those reported by Yadava and Shaw (1970), who detected tetrachlorvinphos residues in the fat from hens fed the insecticide at a level of 50 mg/kg of body weight and killed 24 h later. However, they did not detect residues in liver and leg and breast muscle. In addition, Yadava and Shaw (1970) were unable to detect tetrachlorvinphos residues in the fat from hens 7 days after they had been fed the insecticide at levels of 50, 100, and 200 mg/kg of body weight for 7 days.

A total of 87.3% of the total radioactivity administered was accounted for in the excreta, eggs, tissues, organs, and remainder of carcass from one hen which was carried through the entire 7-day treatment period and killed 7 days later.

Metabolites in Excreta. Extraction of freeze-dried excreta by the procedure detailed above resulted in 14–26% of the total radioactive dose in the aqueous phase and 30–44% in the organic phase. The remaining 18–35% could not be extracted even after adjustment of the pH of the extracting solutions and therefore remains unidentified. Recovery of ^{14}C from freeze-dried excreta fortified with a mixture of ^{14}C-labeled I, II, V, and VIII prior to freeze-drying was 70–85%. No studies of the recoveries of the individual compounds were performed.

The autoradiograph of a TLC plate of the aqueous phase, after concentration and development in methanol–2-propanol–acetone (1:1:8 v/v/v), exhibited a single broad radioactive band with an R_f identical with that of compound II. Extracts of this band were treated with diazomethane, and the TLC and GC data of the resultant compound were compared with similar data for compound I. Methylation of II with freshly prepared diazomethane produced 77–82% of I. Therefore, it was concluded that the ^{14}C in aqueous extracts of freeze-dried excreta was compound II.

GC analysis of the organic extracts of freeze-dried chicken excreta revealed peaks due to I, III, V, and VI (Figure 1a) which were identified and quantitated by comparison with data for authentic standards. However, only 3–5% of the estimated amounts of these compounds could be accounted for by GC analyses. TLC analysis of the extracts (hexane–ethyl acetate, 3:7 v/v) indicated spots corresponding to I, III, V, and VI as well as a diffuse region near the origin. In a previous study, a similar region had been shown to be the conjugates of V and VII (Akhtar and Foster, 1980). However, when a portion of the extract was incubated with β-glucuronidase, there was no appreciable increase in the amount of V. When the resultant incubation extract was treated with 14% BF_3–MeOH as described previously (Akhtar and Foster, 1980), GC analysis did not reveal a peak due to the dimethyl derivative of VII. However, there was a major peak with a retention time of 5.1 min (Figure 1b) which was identified as the methyl ester of VIII which could be produced in a yield of 80–82%.

Methylation of the organic extracts with freshly prepared diazomethane also resulted in the methyl ester of VIII. However, this technique also produced interfering peaks. Therefore, in all future analyses, the ether extracts were first incubated with β-glucuronidase in order to hydrolyze any conjugates of V and VII, if present, followed by treatment with 14% BF_3–MeOH. Extracts of the resultant methyl derivative could be analyzed without further cleanup (Figure 1b).

Positive identification of the major peak was accomplished by cochromatography with the authentic methyl ester of VIII (Akhtar and Foster, 1977). GC–MS of the peak exhibited a weak molecular ion peak at m/e 268 ($M^{+\cdot}$) with a distinctive pattern for three chlorine atoms. In addition, the mass spectrum also revealed a strong peak at m/e 209 [$(M - COOCH_3)^{+\cdot}$]. This spectrum was identical with that recorded for methyl-2,4,5-trichloromandelic acid.

Further confirmation of the structure of the compound in the major peak was obtained by using the transesterification technique of Darbre (1977). The methylated extract was treated with 10% BCl_3–2-chloroethanol for 2 h at 50 °C. GC analysis of the extract was now found to contain a major component with a peak at a retention time of 9.7 min. No peak due to the methyl ester of VIII was now observed (Figure 1c). The GC data were identical with those of an authentic sample of the 2-chloroethyl ester of VIII which was obtained by reacting authentic VIII with 10% BCl_3–2-chloroethanol. The GC–MS of the authentic 2-chloroethyl ester of VIII had a weak molecular ion peak at m/e 316 ($M^{+\cdot}$) with a four chlorine atom pattern. The base peak was at m/e 209 [$(M - COOCH_2 - CH_2 - Cl)^{+\cdot}$].

The excreta of the laying hens fed a standard laying ration fortified with I contained I, II, III, V, VI, and VIII. The major metabolites in the excreta from the first day of treatment until the end of the experiment were compounds II and VIII. A typical example of the distribution of I and its metabolites in excreta is shown in Table III. The data are derived from GC analyses of the aqueous and organic phases, following appropriate derivatizations where necessary, and have been corrected for recovery (80%). Autoradiographs of TLC plates of both phases showed a single radioactive region in each case. This was due to II

Table III. Distribution of Tetrachlorvinphos and Metabolites in Excreta of Laying Hens Treated Orally with [^{14}C]Tetrachlorvinphos and Fed the Insecticide at a Level of 50 ppm for Seven Days

day	feed intake, g	distribution, cumulative % of total intake[a] I	II	III	V	VI	VIII
1	51	0.88	29.4	0.25	1.00	0.50	27.1
2	90	1.00	28.9	0.25	0.88	0.25	31.8
3	75	0.88	28.9	0.19	0.63	0.31	31.4
4	64	0.88	25.9	0.19	0.57	0.25	29.3
5	66	1.00	24.9	0.19	0.63	0.19	31.6
6	73	0.88	25.1	0.13	0.63	0.19	29.6
7	80	1.00	25.0	0.19	0.57	0.25	30.5

[a] The concentration of tetrachlorvinphos and metabolites was calculated from GC analysis of aqueous and organic extracts, after appropriate derivatization, and takes into consideration the total amount of the insecticide consumed at that point in time.

in the aqueous phase and to VIII in the organic phase. The distribution pattern was similar for all the hens on the experiment. Hens continued to excrete traces of II and VIII after the treatment was discontinued.

The data in Table III indicate that a full metabolic pathway for the hen was established within 24 h of commencement of treatment. The formation of II as a metabolite was not unexpected since it has been well documented that dealkylation is the major route of detoxification in the avian liver (Akhtar and Foster, 1977). However, the small amounts of V detected in the excreta were unexpected. It has been shown previously that incubation of I with the soluble fraction (105000*g*) from both mammalian and avian liver produced V in quantitative amounts (Akhtar and Foster, 1977, 1979; Gutenmann et al., 1971). In addition, it has been shown that lactating cows which had been fed I excreted V—mostly as a conjugate—as one of the major metabolites (Akhtar and Foster, 1980).

The minute amounts of V in the excreta suggest that it may have been further metabolized. By analogy with 1-phenylethanol, a nonchloro analogue (El Masri et al., 1958), V, would be expected to undergo a biochemical transformation to produce VIII and 2,4,5-trichlorohippuric acid. When either I or V was incubated with crude chicken liver homogenate (10000*g*), VIII was one of the resultant products. It can therefore be concluded that V may be one of the precursors of VIII.

Metabolites in Tissues, Organs, and Eggs. The radioactivity in tissues, organs, and eggs was extracted by the techniques outlined above. The extracted radioactivity was fractionated into organo- and water-soluble fractions

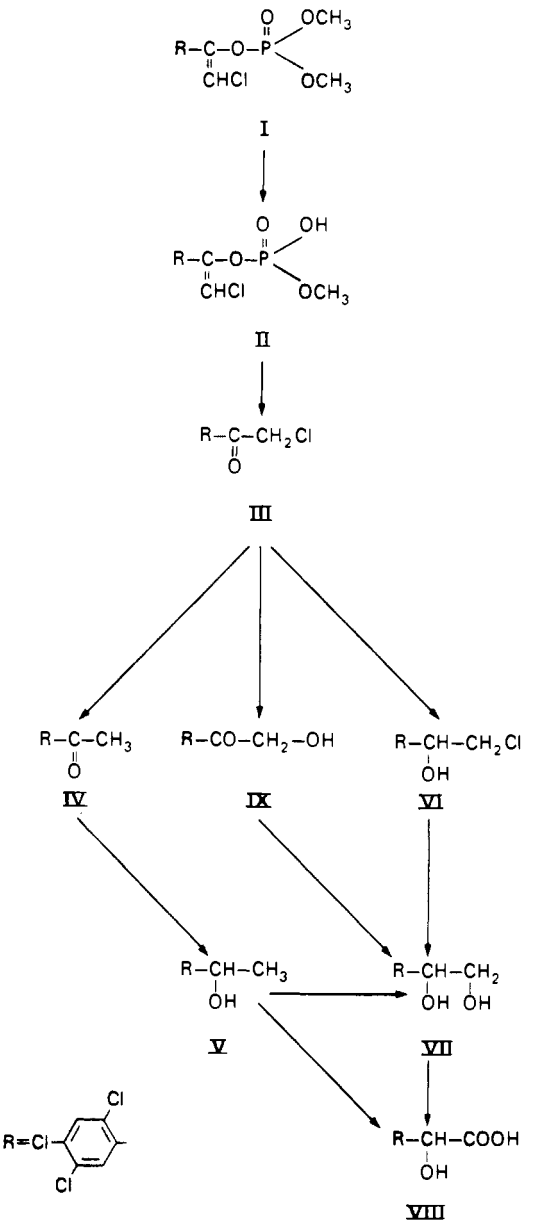

Figure 3. Proposed metabolic pathway of tetrachlorvinphos in the laying hen.

by partitioning the aqueous phase with ether. Some 7–23% of the radioactivity was organosoluble, while 18–37% was found in the aqueous phase. Once again, a major portion of the radioactivity could not be extracted from the samples. Recovery of radioactivity in which a

Table IV. Tetrachlorvinphos and Metabolites in Tissues and Organs of Laying Hens Treated Orally with [^{14}C]Tetrachlorvinphos and Fed the Insecticide at a Level of 50 ppm for Seven Days

tissue[a] or organ	concentrations at various times, ppm[b] 4th day	8th day	10th day	15th day
abdominal fat	0.12 (I) 0.07 (VIII)	0.21 (I) 0.13 (VIII)	0.05 (I) tr[c] (VIII)	0.03 (I) 0.02 (VIII)
kidney	0.15 (I) 0.48 (II) 0.13 (VIII)	0.25 (I) 1.85 (II) 0.54 (VIII)	0.12 (I) 0.28 (II) 0.17 (VIII)	0.02 (I) 0.17 (II) 0.13 (VIII)
liver	tr (I) 0.17 (II) 0.11 (VIII)	0.07 (I) 0.23 (II) 0.53 (VIII)	tr (I) 0.21 (II) 0.37 (VIII)	tr (I) tr (II) 0.17 (VIII)

[a] Traces of I, II, and VIII were detected in all other tissues and organs examined. The detection limit was 0.01 ppm. [b] Concentrations expressed as ppm based on wet weights were obtained by GC analysis of extracts after appropriate derivatization and are the average of samples from two hens. [c] tr = trace (<0.01 ppm).

mixture of radiolabeled I, II, IV, and VIII were added to the freeze-dried tissues at a level of 1250 dpm/g and then extracted was 80–85%. It is concluded that the major portion of tetrachlorvinphos and metabolites in tissues, organs, and eggs are "bound residues". The nature of these residues was not investigated further.

Most of the tissues and organ samples contained only traces (<0.01 ppm) of I, II, and VIII. Only kidney, liver, and abdominal fat contained sufficient amounts to be quantitized. The data on concentrations and nature of tetrachlorvinphos and metabolites are presented in Table IV. As expected, maximum concentrations of I, II, and VIII were reached 26 h after the last oral dose of ^{14}C-labeled insecticide. The levels for individual hens were proportional to the amount of "contaminated" feed consumed. Table IV also indicates that the residues of tetrachlorvinphos and metabolites II and VIII decreased when feeding "contaminated" ration was discontinued. By the 15th day or 194 h after the final daily oral dose of radiolabeled insecticide, only traces of very low levels of I, II, and VIII could be detected.

Radioactivity in eggs was difficult to extract. Most of that which was extracted from whole freeze-dried eggs remained in the aqueous phase. Approximately 40–50% of the radioactivity was not extractable. Extracts contained only traces (<0.01 ppm) of I and traces to 0.07 ppm of II. Eggs laid 74 and 194 h after the final oral dose contained detectable ^{14}C activity; however, residues of I and II were not detected.

A pathway for the metabolism of tetrachlorvinphos in the laying hen, which is based on the metabolites which were identified in these studies, has been proposed and is shown in Figure 3, Dealkylation of I to II has been well established. Further degradation of II and III and further transformations to IV, V, and VI are also well established. As indicated elsewhere above, the metabolism of V to VIII via VI is possible. Similarly, by analogy to 2-chloro-(2,4-dichlorophenyl)ethanol, a dichloro analogue of V, VI could be metabolically transformed to VIII via VII (Hutson et al., 1967). In the present study, no residues of VII were detected in excreta, which suggests that, in the chicken, VII is completely or very effectively metabolized to VIII.

In conclusion, when laying hens receive tetrachlorvinphos via the feed, detectable amounts of the insecticide and metabolites are deposited in abdominal fat, eggs, kidney, liver, etc. during the feeding. However, when "contaminated" feed is discontinued, these residues are rapidly eliminated, leaving very little or no detectable residues 170 h later. Therefore, it is recommended that eggs and meat from hens treated with tetrachlorvinphos not be marketed for at least 15 days after the last treatment.

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