Fate of Tetrachlorvinphos and Its Isomer in Soluble Fraction (105000g) from Goose and Turkey Liver Homogenates

M. Humayoun Akhtar* and Thomas S. Foster

Incubation of tetrachlorvinphos [(*Z*)-2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate] with the soluble fraction (105000*g*) from goose and turkey liver produced 2,4,5-trichlorophenacyl chloride, 2,4,5-trichloroacetophenone, 1-(2,4,5-trichlorophenyl)ethanol, 2-chloro-1-(2,4,5-trichlorophenyl)ethanol, and (2,4,5-trichlorophenyl)ethanediol (goose only) via desmethyl tetrachlorvinphos. Isomerization of the insecticide to the *E* isomer occurred in glutathione-deficient enzyme systems and was found to be more pronounced in turkey liver systems. The *E* isomer was degraded by the enzyme systems at a slow rate and probably involved a P–O–vinyl hydrolytic cleavage which was catalyzed by an enzyme which did not require glutathione as a cofactor.

Studies on the fate of tetrachlorvinphos have been reported in the rat and dog (Akintonwa and Hutson, 1967) and in the dairy cow (Gutenmann et al., 1971). Extensive investigations of enzymatic degradation have been carried out with the soluble fraction (100000–105000*g*) of mammalian and avian liver. The insecticide has been shown to be dealkylated by the soluble fraction from mouse, rat, rabbit, pig (Hutson et al., 1972) and chicken, cow, sheep and pig liver preparation (Akhtar and Foster, 1977, 1979) in the presence of glutathione (GSH). It was also observed (Donninger et al., 1971; Akhtar, 1978; Akhtar and Foster, 1977, 1979) that the polar metabolite (desmethyl tetrachlorvinphos) was further metabolized by other enzyme systems to yield additional metabolites. The present report, part of a continuing study on the metabolism of tetrachlorvinphos, details the data on the fate of the insecticide and its isomer in the soluble fraction of liver homogenates from goose and turkey.

EXPERIMENTAL SECTION

Materials. Glass-distilled pesticide grade solvents were used. (*Z*)-2-Chloro-1-(2,4,5-trichlorophenyl)vinyl [^{14}C]-dimethyl phosphate (tetrachlorvinphos, IA), 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), and (2,4,5-trichlorophenyl)ethanediol (VIII) were synthesized as described earlier (Akhtar and Foster, 1977). The isomer (*E*)-2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (IB) was prepared by UV irradiation of a benzene solution of tetrachlorvinphos. Pure IB was isolated from the photolytic mixture by preparative chromatography.

Compounds IA and IB exhibit subtle differences in their physical and spectral properties. IA is a solid with a mp of 96–96.5 °C [Whetstone et al. (1966), mp 97–98 °C], whereas IB is a viscous liquid. The solid isomer has been referred to in the literature as β isomer (Whetstone et al., 1966), *cis*-Gardona (Fahey et al., 1970), and *Z* isomer Beynon et al., 1973). Compound IB has been reported as *trans*-Gardona, a low melting product (Fahey et al., 1970). The spectroscopic data on these compounds have not appeared in the literature. In this connection it is noteworthy that the first structure assignment of the solid as the β isomer was reported to have been made on the basis of its NMR and IR spectra (Whetstone et al., 1966), following methods used for the insecticide Phosdrin (Stiles et al., 1961). In the present study the NMR spectra of IA and IB were recorded in $CDCl_3$ on a Varian T-60 NMR spectrophotometer, using tetramethylsilane (Me_4Si) as the internal standard. The spectrum of IA exhibited resonance at δ 3.81 (d, 6 H, J_{P-O-CH_3} = 11.6 Hz, P–OCH_3), 6.10 (d, 1 H, $J_{P-O-C=CH}$ = 0.9 Hz, vinyl proton), and 7.6–7.75 (m, 3 H, aromatics) and that of IB had signals at δ 3.82 (d, 6 H, J_{P-O-CH_3} = 11.6 Hz, P–OCH_3), 6.62 (d, 1 H, $J_{P-O-C=CH}$ = 1.5 Hz, vinyl proton), and 7.6–7.75 (m, 3 H, aromatics).

Both IA and IB used in the present study were of high purity and were not contaminated with each other as evidenced by TLC. The two isomers afforded identical mass spectral fragmentation patterns.

Enzyme Preparation and Incubation Systems. Chinese geese (1 year, female) and commercial white broiler turkeys (9 months, female) were killed by cervical dislocation, and the livers were removed immediately and homogenized (8 g/40 mL) in ice-cold 0.134 M phosphate buffer (pH 7.4) in a glass–Teflon homogenizer as described earlier (Akhtar and Foster, 1977).

GSH-deficient enzyme preparation was obtained as follows: solid ammonium sulfate (56.1 g) was added to the fresh soluble fraction (100 mL) to achieve 80% saturation (Green and Hughes, 1955). The mixture was centrifuged at 4 °C for 10 min at 10000 rpm. The resultant supernatant was discarded and the pellet was dissolved in 0.134 M phosphate buffer, pH 7.4 (100 mL), and dialyzed against the same buffer for 3 h to remove traces of ammonium sulfate. This preparation was designated as $(NH_4)_2SO_4$ fractionated enzyme preparation.

A mixture consisting of enzyme preparation (4.5 mL), 0.134 M phosphate buffer (0.5 mL), pH 7.4, and substrate IA or IB (122 μg, 3.3×10^{-7} mol) was incubated at 37.5 °C for specified times under nitrogen. Acetone was added to stop the reaction.

Incubation mixtures were extracted with acetone, concentrated, and extracted with benzene (Akhtar and Foster, 1977). The volume of both the benzene and aqueous phases was adjusted to 5 mL.

Measurement of Radioactivity. The radioactivity in duplicate aliquots (1 mL) of each phase was determined in a Packard Model 3320 Tri-Carb liquid scintillation counter, using an external standard and correcting the data for quenching. Benzene extracts were assayed in a scintillation solution containing 2,5-diphenyloxazole (PPO) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in toluene (5 g, 5 mg, 1000 mL), whereas the radioactivity in aqueous fractions was measured in PCS (Amersham/Searle), a xylene surfactant liquid scintillation cocktail.

Thin-Layer Chromatography (TLC). Silica gel linear-K preadsorbent (0.25 mm thick) TLC plates (Whatman) were spotted with aliquots (1 mL, concentrated with

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

0021-8561/80/1428-0693$01.00/0 © 1980 American Chemical Society

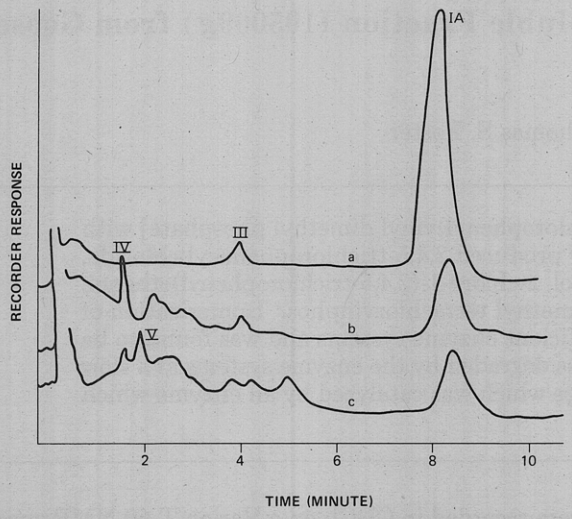

Figure 1. Gas chromatograms of benzene extracts of incubation media carried out in vitro: (a) 0 min, (b) 20 min, goose, (c) 20 min, turkey liver.

nitrogen) of benzene extracts and developed in an ethyl acetate/hexane (3:7) system. The R_f values of IA, IB, III, IV, V, VI, and VIII were 0.24, 0.19, 0.57, 0.67, 0.45, 0.38, and 0.09, respectively. The metabolites were detected by (i) viewing under UV light (254 nm) and (ii) autoradiography. To obtain autoradiographs we placed the plates on X-ray films for 21 days. Silica gel corresponding to radioactive and UV spots was scraped off the plates and eluted with solvent(s). The radioactivity of eluates was measured, and spectroscopy data were recorded for positive identification.

Gas Chromatography (GC). Analyses of benzene extracts were carried out with a Packard-Becker 420 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was a glass tube (1 m × 4 mm i.d.) packed with 3% SE-30 on 80–100 mesh Chromosorb WHP. The operating temperatures for injector, column, and detector were 135, 130, and 295 °C, respectively; 5% methane/argon (column flow 30 mL/min, purge 15 mL/min) was the carrier gas. The relative retention times for IA, IB, III, IV, V, VI, and VIII were 1.0, 1.0, 0.33, 0.22, 0.30, 0.43 and 0.55, respectively.

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 glass column, 1.52 m × 4 mm (i.d.), packed with 3% SE-30 on 80-100 mesh Chromosorb WHP was used for gas chromatographic separation. The column was run at 175 °C with a helium flow rate of 35 mL/min. The mass spectra were recorded at 70 eV.

The mass spectrum of VIII was recorded by direct probe technique at 70 eV.

RESULTS

When IA was incubated with the 105000*g* supernatant of goose and turkey liver, it was converted into a water-soluble product. The rate of metabolism in goose preparations was slower than that in preparations from turkey. GC analyses indicated that in 20 min 71–73% of IA was metabolized in the case of turkey preparations, whereas only 60–63% was metabolized in those from geese (Figure 1).

Metabolite II was the only compound detected and identified in the aqueous phases of the incubation mixtures. Its identity was established by comparing the analytical data of its methyl derivative with those of IA, following the published procedure of Akhtar and Foster (1979).

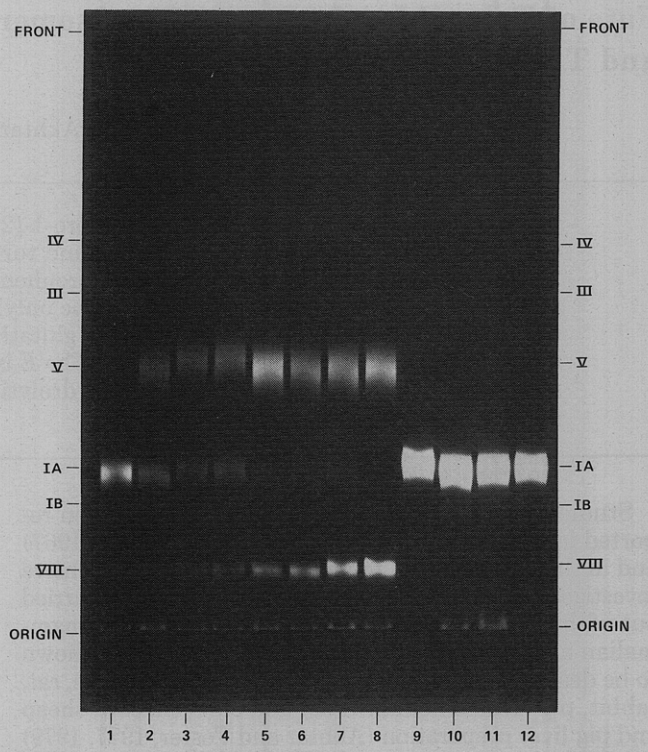

Figure 2. Autoradiograph of benzene-soluble ^{14}C products from in vitro incubation of IA with goose liver prepartion under various conditions. No. 1–8 are the extracts of incubation mixtures containing 122 μg of [^{14}C]IA (3.3×10^{-7} mol), 4.5 mL of enzyme preparation, and 0.5 mL of 0.134 M phosphate buffer (pH 7.4) which were incubated at 37.5 °C for the time intervals (1) 10, (2) 20, (3) 30, (4) 40, (5) 60, (6) 90, (7) 120, and (8) 180 min. No. 9 is [^{14}C]IA. No. 10–12 are the extracts of incubation mixtures containing 122 μg of [^{14}C]IA (3.3×10^{-7} mol), 4.5 mL of enzyme preparation [$(NH_4)_2SO_4$ (80% saturated) fractionated preparation], and 0.5 mL of buffer solution incubated at 37.5 °C for (10) 60, (11) 90, and (12) 120 min.

Benzene extracts of the incubation mixtures contained III, IV, V, VI, and VIII, depending upon the length of the incubation, as shown by TLC and GC.

An autoradiograph of a TLC plate for the benzene extracts from incubation with goose liver preparation is shown in Figure 2. Numbers 1–8 represent the extracts of incubations carried out in the presence of added GSH. It can be seen that the concentration of IA (R_f 0.25, no. 9) decreased as the reaction progressed, while that of compounds with R_f values of 0.45 and 0.09 increased. The radioactive regions corresponding to these major metabolites for each time period were removed and extracted with benzene. The radioactivity was measured, and the structures were confirmed by spectroscopic data.

The identity of the compound with an R_f value of 0.45 was established as V by comparing TLC R_f retention value, GC retention time, and GC–MS data with those of similar data from an authentic standard of V.

The mass spectrum (direct probe) of the compound with an R_f value of 0.09 exhibited major peaks at m/e 240, 209, 181, 145, and 109. In addition, the compound gave a poor chromatographic response under the GC conditions described. These properties were also found for authentic (2,4,5-trichlorophenyl)ethanediol (VIII) which required a large quantity (≃12–15 ng) for 50% full-scale deflection. Hence, the structure of the compound with an R_f value of

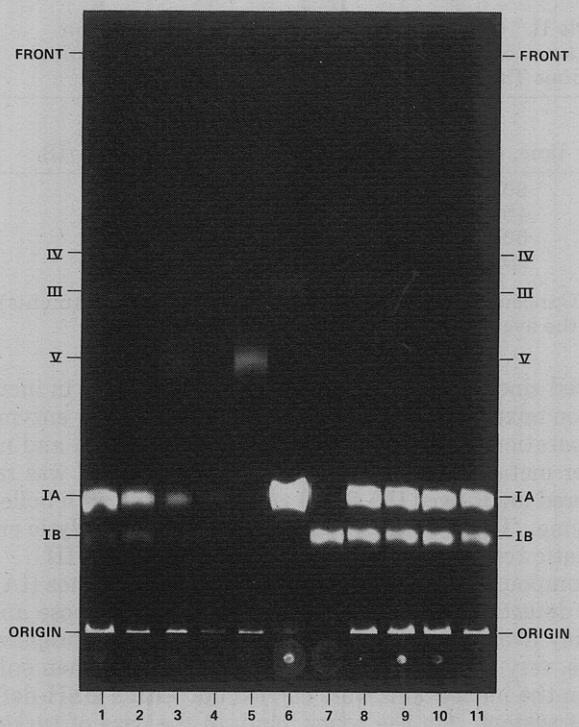

Figure 3. Autoradiograph of benzene-soluble ^{14}C products from in vitro incubations of IA with turkey liver preparations. No. 1–5 are the extracts of incubation mixtures containing 122 μg of [^{14}C]IA (3.3×10^{-7} mol), 4.5 mL of enzyme preparation, and 0.5 mL of 0.134 M phosphate buffer (pH 7.4) which were incubated at 37.5 °C for (1) 10, (2) 20, (3) 30, (4) 40, and (5) 60 min. No. 6 and 7 are IA and IB, respectively. No. 8–11 are extracts of incubation mixtures containing 122 μg of [^{14}C]IA (3.3×10^{-7} mol), 4.5 mL of enzyme preparation [$(NH_4)_2SO_4$ fractionated], and 0.5 mL of buffer solution incubated at 37.5 °C for (8) 20, (9) 40, (10) 60, and (11) 90 min.

0.09 has tentatively been assigned as VIII.

In Figure 2, 10–12 are autoradiographs of the benzene extracts from incubation of IA with a GSH-deficient enzyme preparation. The majority of the radioactivity was associated with unchanged IA. This observation was confirmed by GC analyses of the extracts. Thus, very little IA was metabolized when incubated with enzyme preparations which did not contain GSH. Radioactive regions due to III and V were also noted at R_f 0.57 and 0.45, respectively. In addition, a radioactive region at R_f 0.19 was also observed which was identified as IB by virtue of an identical R_f value with that of an authentic standard of IB.

Figure 3 is an autoradiograph of the TLC plate of benzene extracts from the incubation mixtures of IA with turkey liver enzyme preparation. Again, it is evident that the concentration of IA (R_f 0.24) decreased with increase in incubation time (no. 1–5) until finally IA could not be detected in the extracts of 60-min incubation.

The autoradiograph of benzene extracts of incubations for up to 40 min (no. 1–4) also showed a distinct radioactive region around R_f 0.19 as well as regions due to III, IV, and V. The compound had an identical R_f value with that of authentic IB (no. 7, Figure 3). The radioactive region between 0.16 and 0.20 was scraped off the plate, extracted with benzene, and analyzed by GC–MS. The mass spectra of the compound exhibited a molecular ion peak at m/e 364 and had a fragmentation pattern identical with that recorded for authentic IB (see Experimental Section for spectroscopic data).

In Figure 3, 8–11 are autoradiographs of a TLC plate of benzene extracts of incubation mixtures of IA with

Table I. Effect of Reduced Glutathione (GSH) on the Yield of Metabolites

	time, min	concentration, %[a] goose[b] metabolite V	goose[b] metabolite VIII	turkey[c] metabolite V	turkey[c] metabolite VI
IA + enzyme[d]	90	24.7	5.2	33.5	1.4
	120	32.3	7.9	45.9	2.1
	180	40.1	9.6	54.8	2.4
IA + enzyme[d] + GSH (8.7×10^{-6} mol)	90	32.3	Tr[e]	36.5	Tr[e]
	120	44.1	1.9	58.6	Tr[e]
IA + enzyme[d] + GSH (3.5×10^{-5} mol)	120	49.5	*f*	58.8	*f*
	180	65.6	*f*	68.4	*f*

[a] The percentage of the theoretical yield of the metabolites is the average concentration of duplicate incubations. [b] Metabolite VI was not detected. [c] Metabolite VIII was not detected. [d] Fresh enzyme preparation. [e] Trace. [f] Not detected.

GSH-deficient enzyme preparations. In each extract, most of the radioactivity was associated with compounds with R_f values of 0.24 and 0.19. These compounds were identified as IA and IB, respectively, by comparison of TLC retention values and GC–MS data. Under the GC conditions described above, both IA and IB had identical retention times. Hence, the GC of these extracts exhibited only one major peak due to unchanged IA. GC and autoradiographic data clearly indicate that IA was not efficiently metabolized, but isomerized in GSH-deficient turkey liver enzyme systems.

The fate of IB with enzyme prepartions from both geese and turkeys was also investigated. Very little IB was metabolized (10–15% in 3 h) when it was incubated with soluble fraction. The rate of degradation was independent of GSH concentration in the incubation medium. Under the described incubation conditions, no desmethyl tetrachlorvinphos (II) was detected in the aqueous phases of the incubation mixtures. Metabolites V and VIII were detected in benzene extracts of incubation with goose preparations. In addition, traces of IA were detected in benzene extracts of incubation mixtures of IB with GSH-deficient turkey liver enzyme preparations.

When VI was incubated with goose liver soluble fraction, VIII was produced, and no other products were detected by GC or TLC. The addition or removal of GSH from the incubation media had very little effect on the rate of transformation. Compound VIII was not formed when VI was incubated with boiled enzyme.

DISCUSSION

The above results indicate metabolic pathways for IA in the soluble fraction from geese and turkeys (Figure 4). Compound IA was rapidly metabolized to a water-soluble compound II when incubated with enzyme preparation. Metabolite II was efficiently further metabolized to other organo-extractable products. Formation of II, III, IV, V, and VI is represented by routes "a", "b", "c", "d", and "e", respectively (Figure 4). At the end of 1 h of incubation, compounds III and IV were no longer detected in the benzene extracts, which indicates their transitory position in the metabolic pathways.

The differences in the enzymatic activities of the two species (geese and turkey) resulted in the differences in the nature and the proportions of the metabolites formed. Thus, after 3 h of incubation, turkey liver preparations yielded compound V and a small amount of VI as the major (>95%) metabolites, whereas, under the same conditions, goose liver soluble fractions produced compounds V and VIII in a ratio of 4:1 on the basis of the measured

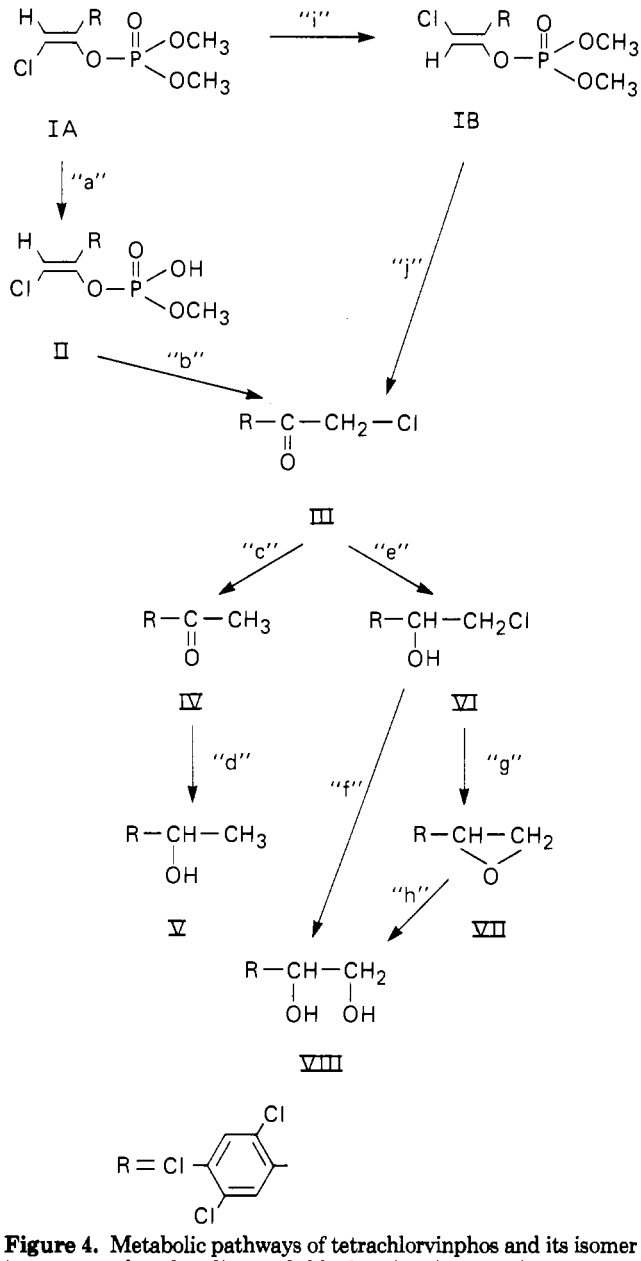

Figure 4. Metabolic pathways of tetrachlorvinphos and its isomer in goose and turkey liver soluble fraction (105000*g*).

radioactivity of each component (Table I). The proposed metabolic pathway (Figure 4) predicts that the yields of V and VIII would depend largely on the concentrations of IV and VI, respectively.

Table I records the data on the amounts of metabolites in the two enzyme systems at various time intervals. In turkey systems, V was the major metabolite with small amounts of VI. Addition of excess GSH (3.5×10^{-5} mol) to the incubation media resulted in the production of V exclusively. Goose liver systems yielded both V and VIII. Again, addition of excess GSH (3.5×10^{-5} mol) favored V exclusively at the expense of VIII. Thus in a system with high GSH content, IV would be favored over VI, while the opposite would be true in a GSH-deficient system. These data are in agreement with a previous report (Akhtar, 1979) on the conversion of III to V and VI.

Conversion of VI to VIII may occur by (i) direct hydrolysis (route "f") or (ii) formation of an epoxide (VII) and its subsequent hydrolytic cleavage to VIII (routes "g" and "h"). Under favorable conditions, such transformations are quite common. However, in the above investigations routes "g" and "h" appear less likely to have occurred since the epoxide (VII) was not detected in incubation mixtures. Incubation of VI with goose liver enzyme preparation (with or without GSH) yielded VIII and no other metabolite was detected. In addition, VI was recovered unchanged (80–85%) when incubated with boiled enzyme. These data strongly support the hydrolytic enzymatic route "h" for the conversion of VI to VIII.

Table II. Concentration of IA and IB in Incubation Mixtures of GSH-Deficient Turkey Liver Preparation at Various Time Intervals

time, min	concentration, μg^a IA	IB	ratio, IA/IB
20	87.6	27.4	3.2
40	80.9	25.7	3.15
60	68.2	23.1	2.95
90	64.02	21.2	3.02

[a] Concentrations (based on radioactivity measurements) are the average of duplicate incubations.

Compound IB, the *E* isomer of tetrachlorvinphos (IA), was detected in incubation mixtures of both goose and turkey liver preparations. In the case of goose preparations, very small quantities were observed and then only when the incubations were carried out with a GSH-deficient system. On the other hand, in the case of turkey preparations, IB was detected even in systems containing GSH (Figure 3, no. 1 and 2). These data suggest that both goose and turkey livers contain a soluble isomerase. This is the first report of isomerization of a vinyl organophosphate by avian liver soluble fraction (105000*g*).

The formation of a significant amount of IB by GSH-deficient turkey liver preparations should be discussed further. Although no attempt was made in the present study to observe the effects of various cofactors on the yield of IB, the data do provide some information about the rate of production of IB. The data suggest that simultaneously competitive dealkylation and isomerization reactions exist when turkey liver preparation was used. In a system containing sufficient GSH, the dealkylation route predominated, whereas in a GSH-deficient system, in which the dealkylation route is inoperative, the isomerization step predominated.

The ratio of IA to IB in GSH-deficient turkey liver preparation is important in that it provides some insight on the activity of the enzyme(s) isomerase. Table II records the amounts of the ratios of IA to IB at various time intervals. The ratios appear to remain almost constant over the entire length of the incubation, while the amounts of IA and IB show a slight decrease. In order to reach a conclusion on the mechanistic nature of the transformation, the following facts were carefully considered: (i) IA and IB were not interconverted when incubated with either GSH and buffer or boiled enzyme system, and (ii) isomerization of IB to IA was insignificant when it was incubated with GSH-deficient enzyme system (Figure 5). Thus, it appears that the isomerization of IA to IB is enzymatic and irreversible in nature. The almost constant ratio of IA to IB could be explained by the concentration of the enzyme. It is, therefore, suggested that the concentration of the enzyme in the total volume of the soluble fraction which was used was just sufficient to convert approximately 25% of IA to IB. This aspect of the enzymatic behavior was not further investigated in the present study. The concept of high activity of the isomerase receives further support from the presence of a small amount (visual inspection) of IB in the benzene extracts of 10 and 20 min incubation (no. 1 and 2, Figure 3) with fresh enzyme preparation. The slight decrease in the amounts of

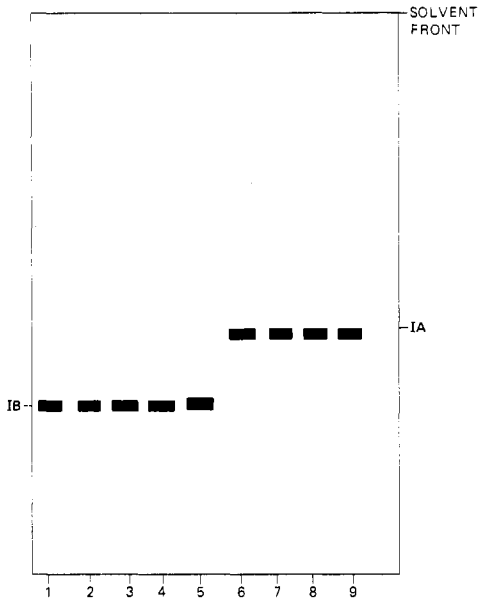

Figure 5. Thin-layer chromatograms of benzene extracts of IA and IB when incubated under different conditions. No. 1–5 represent IB: (1) 60 min and (2) 90 min incubated with buffer alone, (3) 60 min and (4) 90 min when incubated with boiled enzyme, and (5) authentic IB. No. 6–9 are the extracts of IA: (6) authentic IA, (7) 60 min incubated with buffer alone, (8) 60 min and (9) 90 min incubated with boiled enzyme.

both IA and IB in the benzene extracts of GSH-deficient enzyme systems with time suggests that both these compounds were degraded very slowly in this system. The slow rate of degradation of IA in GSH-deficient enzymatic system is well established (Akhtar and Foster, 1977, 1979).

The present study was extended to include the metabolism of IB. It should be noted that, although isomerization of IA to IB was observed on treated foliage of cabbage, rice, and apples grown under indoor conditions, and IB appears to be considerably more stable than IA (Beynon and Wright, 1969), no study of the metabolism of IB in water, soil, and mammals has been reported.

The *E* isomer, IB, on incubation with enzyme preparation with or without GSH, was metabolized slowly (10–15% in 3 h by GC analyses). That GSH had very little effect on the rate of metabolism strongly favors a hydrolytic step which involves the cleavage of the P–O–vinyl bond and is catalyzed by an enzyme which does not require GSH as a cofactor (route "j"). The two geometrical isomers of 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate are metabolized by the soluble fraction by two independent routes. It should be noted that the *Z* isomer (tetrachlorvinphos, *trans*-styrene structure), in which the methyl groups are partially hindered by the chlorine atom, underwent desmethylation, whereas the *E* isomer, which has relatively free methyl groups, did not dealkylate. In this connection, it has been reported that the two isomers of mevinphos (2-carbomethoxy-1-methylvinyl phosphate) were also degraded by two different routes (Morello et al., 1968). The cis isomer, in which the methyl groups are relatively free, was dealkylated by a GSH-dependent enzyme system. On the other hand, *trans*-mevinphos, in which the methyl groups are partially masked by the carbomethoxy group, was not metabolized by dealkylation. These data are directly opposite to those obtained in the present study. In the absence of any convincing explanation as to why a molecule, which is sterically hindered, underwent desmethylation, perhaps the well-accepted structure of tetrachlorvinphos as the *Z* isomer requires reevaluation.

The observed in vitro isomerization of IA to IB in GSH-deficient liver preparation and to a limited extent in freshly prepared turkey liver soluble fraction provides a precautionary guideline. The physiological significance of such a transformation may be insignificant.

The data presented above indicate that both goose and turkey livers contain an effective GSH-dependent enzyme system(s), *S*-transferase(s), which is capable of metabolizing IA to a water-soluble compound. The soluble fraction (105000*g*) also contains enzyme systems such as hydrolase, reductive dechlorinase, and reductase. The data also indicate that the livers of both species contain an isomerase. It was also demonstrated that the turkey liver isomerase was considerably more active than that of the goose. The soluble fraction from either species did not significantly metabolize the *E* isomer.

ACKNOWLEDGMENT

The authors gratefully acknowledge the able technical assistance of N. Zabolotny and N. L. York. We also thank S. I. M. Skinner for recording GC–MS spectra.

LITERATURE CITED

Akhtar, M. H., *J. Agric. Food Chem.* **26**, 932 (1978).
Akhtar, M. H., *J. Environ. Sci. Health* B**14**, 53 (1979).
Akhtar, M. H., Foster, T. S., *J. Agric. Food Chem.* **25**, 1017 (1977).
Akhtar, M. H., Foster, T. S., *J. Agric. Food Chem.* **27**, 113 (1979).
Akintonwa, D. A. A., Hutson, D. H., *J. Agric. Food Chem.* **15**, 682 (1967).
Beynon, K. I., Wright, A. N., *J. Sci. Food Agric.* **20**, 250 (1969).
Beynon, K. I., Hutson, D. H., Wright, A. N., *Residue Rev.* **47**, 55 (1973).
Donninger, C., Nobbs, B. T., Wilson, K., *Biochem. J.* **122**, 51P (1971).
Fahey, J. E., Nelson, P. E., Ballee, D. L., *J. Agric. Food Chem.* **18**, 866 (1970).
Green, A. A., Hughes, W. L., in "Methods in Enzymology", Vol. 1, Colowick, S. P., Kaplan, N. O., Eds., Academic Press, New York, 1955, p 67.
Gutenmann, W. H., St. John, L. E., Jr., Lisk, D. J., *J. Agric. Food Chem.* **19**, 1259 (1971).
Hutson, D. H., Pickering, B. A., Donninger, C., *Biochem. J.* **127**, 285 (1972).
Morello, A., Spencer, E. Y., Vardanis, A., *Can. J. Biochem.* **46**, 885 (1968).
Stiles, A. R., Reilly, C. A., Pollard, G. R., Tieman, C. H., Ward, L. F., Jr., Phillips, D. D., Soloway, S. B., Whetstone, R. R., *J. Org. Chem.* **26**, 3960 (1961).
Whetstone, R. R., Phillips, D. D., Sun, Y. P., Ward, L. F., Shellenberger, T. E., *J. Agric. Food Chem.* **14**, 352 (1966).

Received for review August 6, 1979. Accepted March 18, 1980. Contribution No. 879.