The Metabolism of 2-Chloro-1-(2',4'-dichlorophenyl)vinyl Diethyl Phosphate (Chlorfenvinphos) in the Dog and Rat

BY D. H. HUTSON, D. A. A. AKINTONWA AND D. E. HATHWAY

Tunstall Laboratory, 'Shell' Research Ltd., Sittingbourne, Kent

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1. A single oral dose of [^{14}C]Chlorfenvinphos to rats is quantitatively eliminated in 4 days. Rats do not show a sex difference in the elimination pattern and show only a small degree of biological variation in the total excretion data. Of the label 87.2% is excreted in the urine (67.5% in the first day after dosage), 11.2% in the faeces and 1.4% in the expired gases; less than 0.9% of ^{14}C is present in the gut and contents after 4 days. 2. After oral administration of [^{14}C]Chlorfenvinphos to dogs, 94.0% (91.8–97.6%) of the ^{14}C is excreted in the urine and faeces during 4 days. Dogs do not show a sex difference in the pattern of elimination, and excretion of radioactivity in the urine is very rapid: 86.0% of ^{14}C during 0–24 hr. 3. Chlorfenvinphos is completely metabolized in rats and dogs: unchanged Chlorfenvinphos is absent from the urine and from the carcass, when elimination is complete. In rats, 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate accounts for 32.3% of a dose of Chlorfenvinphos, [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid for 41.0%, 2,4-dichloromandelic acid for 7.0%, 2,4-dichlorophenylethanediol glucuronide for 2.6% and 2,4-dichlorohippuric acid for 4.3%; in dogs, 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate accounts for 69.6%, [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid for 3.6%, 2,4-dichloromandelic acid for 13.4% and 2,4-dichlorophenylethanediol glucuronide for 2.7%. 4. Dogs and rats show a species difference in the rate of excretion of ^{14}C in the urine, and in the proportions of the metabolites, with the exception of 2,4-dichlorophenylethanediol glucuronide, that are excreted in the urine. Alternative explanations for the latter species difference are suggested. 5. 2-Chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate and 2,4-dichlorophenacyl chloride probably lie on the main metabolic pathway of Chlorfenvinphos, since, in common with that insecticide, they give rise to [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid and 2,4-dichloromandelic acid as major metabolites in the urine. 6. The proposed scheme for the metabolism of Chlorfenvinphos represents a detoxication mechanism.

2-Chloro-1-(2',4'-dichlorophenyl)vinyl diethyl phosphate (Chlorfenvinphos) (I) (also known as compound 4072 and SD7859) belongs to the valuable vinyl phosphate series of insecticides that, in general, are highly toxic to insects and much less toxic to mammals. Chlorfenvinphos is a promising

agent for ectoparasitic control (Graham, 1961; Thompson, 1965; Wood, Page, Brown, Smith & Ferguson, 1965), seed dressing and soil treatment. These agricultural uses make a thorough understanding of the mammalian metabolism of Chlorfenvinphos desirable. The design of previous metabolic investigations (Chamberlain & Hopkins, 1962) would not have revealed the fate of the carbon 'skeleton' of the insecticide; this is held to be important.

MATERIALS

All melting points, which were determined on a Kofler block, are uncorrected.

2-Chloro-1-(2',4'-dichlorophenyl)vinyl diethyl phosphate (Chlorfenvinphos). Chlorfenvinphos was synthesized by

the reaction of triethyl phosphite with 2,4-dichlorophenacylidene dichloride (Lichtenthaler, 1961; Perkow, Ullrich & Meyer, 1952; Gilbert, Otto & Rumanowski, 1961). Technical Chlorfenvinphos (obtained from Shell Development Co., New York, N.Y., U.S.A.), which consisted of 90% of the β -isomer, 6% of the α -isomer, 3% of mixed ketones and 1% of water, was purified by column chromatography on silicic acid. (The β -isomer of Chlorfenvinphos is defined as the geometrical isomer in which the chlorine atom on C-2 of the vinyl group and the 2,4-dichlorophenyl substituent on C-1 are in the *trans* orientation). Authentic Chlorfenvinphos eluted with *n*-hexane-acetone (9:1, v/v) was shown by thin-layer chromatography (for details see the Methods section) to contain only a trace of α -isomer. Chlorfenvinphos is a high-boiling liquid, b.p. 333–338°/0.5 mm., m.p. –2° to –9°.

2-Chloro-1-(2',4'-dichlorophenyl)[¹⁴C]vinyl diethyl phosphate ([¹⁴C]Chlorfenvinphos). We are indebted to Shell Development Co., Modesto, Calif., U.S.A., for synthesizing the radioactive compound, which was made by the method referred to for the unlabelled compound but with 2,4-dichlorophenyl [¹⁴C]acylidene dichloride. [¹⁴C]Chlorfenvinphos (2.8 μ C/mg.) was purified on thin-layer plates of Kieselgel G run with benzene containing 4% (v/v) of methanol. The compound migrated as two spots: 95% of the β -isomer and 5% of the α -isomer. The ¹⁴C purity of the isomeric mixture exceeded 99.9%.

2-Chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate. Chlorfenvinphos (57 g.) was refluxed for 4 hr. with aq. 30% (v/v) H₂SO₄ (280 ml.), and the product was extracted with chloroform and crystallized from hexane to yield crude material (14 g.). Three recrystallizations from ether-hexane gave 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate as small prisms (11.2 g.), m.p. 108–109° (Found: C, 36.0; H, 3.0; Cl, 32.3; P, 9.2. C₁₀H₁₀Cl₂O₄P requires: C, 36.2; H, 3.0; Cl, 32.1; P, 9.3%). (The mass spectrometer showed that the parent ion occurred at *m/e* 330 and 332. Calc. for C₁₀H₁₀Cl₂O₄P: mol. wt. 331.5).

Sodium 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl phosphate. The preceding hydrogen phosphate (2 g.) was neutralized with 0.1N-NaOH to pH 7.0. The aqueous solution was extracted twice with ether to remove traces of any unchanged Chlorfenvinphos. This step was very important because the sodium salt was intended for toxicity studies and on account of the toxicity of the unchanged insecticide. Sodium 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl phosphate was crystallized from acetone-hexane, forming fine needles (1.5 g.), m.p. > 350° (Found: C, 33.1; H, 2.8; Cl, 29.1; Na, 6.6; P, 8.8. C₁₀H₉Cl₂O₄NaP requires: C, 33.9; H, 2.6; Cl, 30.1; Na, 6.5; P, 8.8%).

2,4-Dichlorophenyl chloride. Chlorfenvinphos (56 g.) was refluxed with aq. 30% (v/v) H₂SO₄ (280 ml.) for 24 hr., when the product was extracted with chloroform and crystallized twice from hexane to give 2,4-dichlorophenyl chloride in the form of thick plates (28.9 g.), m.p. 53–54° (Found: C, 42.9; H, 2.2; Cl, 47.1. C₆H₃Cl₂O requires: C, 43.0; H, 2.3; Cl, 47.6%).

2,4-Dichlorophenyl [¹⁴C]acyl chloride. When [¹⁴C]Chlorfenvinphos (20 μ C) was hydrolysed under the same conditions as the unlabelled insecticide, there was quantitative conversion into 2,4-dichlorophenyl [¹⁴C]acyl chloride, which was recovered from the condenser. The steam-distillate migrated as a single substance on thin-layer plates of

Kieselgel G run with benzene. The ¹⁴C purity of this compound exceeded 99.9%.

2-Chloro-1-(2',4'-dichlorophenyl)ethanol. NaBH₄ (100 mg.) was added to a methanolic solution (2.0 ml.) of 2,4-dichlorophenacyl chloride (500 mg.), and after 15 min. the concentrated solution was partitioned between water and ether. The ether-soluble fraction was crystallized twice from hexane. 2-Chloro-1-(2',4'-dichlorophenyl)ethanol formed prisms (366 mg.), m.p. 50–50.5° (Found: C, 42.4; H, 3.3; Cl, 47.0. C₈H₇Cl₂O requires: C, 42.6; H, 3.1; Cl, 47.2%). Similar reduction of the ¹⁴C-labelled ketone yielded 2-chloro-1-(2',4'-dichlorophenyl)[¹⁴C]ethanol, which was purified on thin-layer plates of Kieselgel G run with benzene containing 4% (v/v) of methanol. The ¹⁴C purity of the compound exceeded 99.9%.

2,4-Dichloromandelic acid. 2,4-Dichlorophenacylidene dichloride (4 g.) was shaken with aq. 10% (w/v) NaOH (20 ml.) for 30 min. and the acidified reaction mixture extracted from ether. The ether-soluble oil was crystallized twice from benzene and dried under reduced pressure over paraffin wax. 2,4-Dichloromandelic acid crystallized as small plates (2.7 g.), m.p. 119–121° (Found: C, 43.5; H, 2.9; Cl, 32.0. Calc. for C₈H₆Cl₂O₃: C, 43.5; H, 2.7; Cl, 32.1%) [Newman, Fones & Renoll (1947) quote m.p. 119.5–120.5° for this compound].

2,4-Dichlorophenacyl benzoate. An acetone solution (120 ml.) of 2,4-dichlorophenacyl chloride (2.88 g.) was refluxed with potassium benzoate (4.8 g.) for 48 hr. and the concentrated solution then partitioned between ether and water. The ether-soluble product was crystallized from light petroleum (b.p. 40–60°), decolorized in ethanol (animal charcoal) and recrystallized three times from hexane. 2,4-Dichlorophenacyl benzoate formed rhombs (2 g.), m.p. 69° (Found: C, 58.3; H, 3.1; Cl, 22.8. C₁₅H₁₀Cl₂O₃ requires: C, 58.3; H, 3.3; Cl, 22.9%).

2,4-Dichlorophenylethanediol. Reduction of 2,4-dichlorophenacyl benzoate (1.3 g.) in methanolic solution with NaBH₄ (0.4 g.) presumably gave 2-(2',4'-dichlorophenyl)-2-hydroxyethyl benzoate. On evaporation of the reaction mixture, however, the benzoate grouping was hydrolysed and 2,4-dichlorophenylethanediol was extracted from the acidified reaction mixture (pH 2) with ether. Evaporation of the solvent yielded a syrup, which crystallized with difficulty from ether-hexane. Recrystallization from ether-hexane yielded platelets (848 mg.) of 2,4-dichlorophenylethanediol, m.p. 58–59° (Found: C, 46.8; H, 3.9; Cl, 34.2. C₈H₈Cl₂O₂ requires: C, 46.4; H, 3.9; Cl, 34.3%).

1-(2',4'-Dichlorophenyl)ethanol and its 2,4-dichlorobenzoyl ester derivative. 2,4-Dichloroacetophenone was reduced in methanolic solution with NaBH₄. The reaction solution and purified product were examined by thin-layer chromatography on plates of Kieselgel G with ether-hexane-acetic acid (60:40:1, by vol.) as solvent. Pure product was apparently obtained quantitatively, but it could not be induced to crystallize [Koton, Samsonova & Florinskii (1952) give b.p. 126–128°/7 mm.]. The dried syrup was dissolved in anhydrous pyridine (2 ml.) and esterified by refluxing with excess of 2,4-dichlorobenzoyl chloride in benzene (10 ml.) for 5 min. The reaction mixture was poured into water and extracted with ether. The ether solution was successively extracted with *n*-HCl, aq. 2% (w/v) K₂CO₃ and water, and the concentrated solution crystallized twice from methanol. 1-(2',4'-Dichlorophenyl)ethyl 2,4-dichlorobenzoate formed aggregates of micro-crystals (2.3 g.), m.p. 63°

(Found: C, 49.8; H, 3.0; Cl, 39.1. $C_{15}H_{10}Cl_4O_2$ requires: C, 49.5; H, 2.8; Cl, 38.8%).

2,4-Dichlorohippuric acid. To an aqueous solution (10 ml.) of glycine (1.5 g.) was added 2,4-dichlorobenzoyl chloride (4.2 g.), followed by the gradual addition of aq. 10% (w/v) K_2CO_3 with shaking until the solution remained alkaline. The mixture was then diluted, acidified and extracted with ether. Crude product was crystallized twice from aqueous ethanol. 2,4-Dichlorohippuric acid formed felted needles (1.9 g.), m.p. 174–175° (Found: C, 43.7; H, 2.8; Cl, 28.4; N, 5.6. $C_9H_7Cl_2NO_3$ requires: C, 43.6; H, 2.8; Cl, 28.6; N, 5.6%).

2,4-Dichlorobenzyl alcohol. 2,4-Dichlorobenzaldehyde was reduced in methanolic solution with $NaBH_4$. 2,4-Dichlorobenzyl alcohol crystallized from *n*-hexane as needles, m.p. 56–57° [Métayer & Dat-Xuong (1954) gave m.p. 56–57° for this product].

The following compounds were purchased (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) and checked for purity by thin-layer chromatography: 2,4-dichlorobenzaldehyde (m.p. 72°), 2,4-dichlorobenzoic acid (m.p. 165°) and 2,4-dichlorobenzoyl chloride (b.p. 150°/34 mm.).

Both 2,4-dichloroacetophenone (m.p. 33–34°) and 2,4-dichlorophenacylidene dichloride (b.p. 355°/760 mm. and 95–98°/0.02 mm.) were supplied by our colleagues at Schloss Birlinghoven, Shell Grundlagenforschung G.m.b.H., bei Siegburg, Germany, and also at Woodstock Agricultural Research Centre, Shell Research Ltd., Sittingbourne, Kent.

Bovine liver β -glucuronidase. This enzyme (1 g./560 000 units; Fishman, Springer & Brunetti, 1948) was obtained from the Sigma Chemical Co., St Louis 18, Mo., U.S.A.

Reagents and solvents. All reagents and solvents were of AnalaR grade.

METHODS

Experiments with animals. Young adult rats (approx. 2 months old; 150–200 g. body wt.) were used (Porton strain maintained as a specific pathogen-free colony in this Laboratory). For the metabolism experiments the animals were kept in all-glass metabolism cages under the conditions described by Wright, Akintonwa, Crowne & Hathway (1965). Compounds dissolved in olive oil were administered by stomach tube to rats that had been starved for 24 hr., and for the excretion-retention study six male and six female animals were each given a single oral dose (2 mg./kg. body wt.) of [^{14}C]Chlorfenvinphos (3.0 μ C/mg.) in this way. Unrestricted food and water were supplied, the urine and faeces were collected daily and stored at –30° and the exhaled gases were monitored for $^{14}CO_2$. These rats were killed after 4 days, when the entire alimentary canal was removed, and the alimentary canal, skin plus hair and carcass and remaining viscera were examined for radioactivity. For the identification of ^{14}C -labelled metabolites the urine of these treated animals was combined with the 48 hr. urine of seven rats dosed with 3.0 μ C of [^{14}C]Chlorfenvinphos (5.5 mg./kg.).

In a further experiment with rats a solution of 2,4-dichlorophenyl [^{14}C]acyl chloride (0.4 mg.; 3.5 μ C/mg.) in olive oil (1 ml.) was administered to each of two rats and the urine and faeces were collected daily for 2 days.

Adult hounds (7–15 kg. body wt.) were used (beagles maintained for 4–5 years as a closed colony in this Laboratory). For metabolism experiments two animals of each sex were kept in stainless-steel metabolism cages under the

conditions described by Wright *et al.* (1965). After being starved for 24 hr. each dog swallowed a gelatin capsule containing a solution of [^{14}C]Chlorfenvinphos (8 μ C; 0.3 mg./kg.) in olive oil (1 ml.). Unrestricted food and water were supplied and the urine and faeces were collected daily for 4 days.

In another experiment an adult female dog was given a gelatin capsule containing a solution of [^{14}C]Chlorfenvinphos (7.3 μ C; 2.6 mg.) in olive oil (1 ml.) and then further gelatin capsules containing in all 5.525 g. of undiluted Chlorfenvinphos. Measurement of radioactivity in the urine showed that 24.4% of the ^{14}C was excreted during 0–6 hr., 35.5% during 6–24 hr. and 2.0% during 24–48 hr. The 48 hr. faeces contained 35% of the ^{14}C , largely as unabsorbed Chlorfenvinphos. The urine from this animal was used for isolation of 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate (for details see below).

In another experiment a dog was given a single intravenous injection of 1.0 ml. of a solution of [^{14}C]Chlorfenvinphos (4.5 mg.; 3.0 μ C/mg.) in Infonutrol, and the urine and faeces were collected daily for 2 days.

Further experiments with dogs were concerned with the metabolic fate of possible Chlorfenvinphos metabolites. Thus 2-chloro-1-(2',4'-dichlorophenyl)[^{14}C]vinyl ethyl hydrogen phosphate (1.2 mg.; 3 μ C/mg.), which has been isolated from the urine of a dog dosed with [^{14}C]Chlorfenvinphos, was administered in 1 ml. of ethanol contained in a gelatin capsule to a dog. Another dog was given a gelatin capsule containing a solution of 2-chloro-1-(2',4'-dichlorophenyl)[^{14}C]ethanol (0.78 mg.; 3.4 μ C/mg.) in olive oil (1 ml.). In both experiments ^{14}C -labelled metabolites in the urine were identified.

Extraction of Chlorfenvinphos metabolites from the treated animals. In each of the experiments with animals described, except the two experiments involving withdrawal of blood and another experiment in which the isolation of 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate from dog urine was the objective, solvent extracts of the pooled urines from all the animals collectively or the single animal, as the case may be, were made by continuous extraction at pH 1.9 in a Schacherl apparatus with ether and ether-ethanol (3:1, v/v). Approx. 83% of the ^{14}C was recovered by ether extraction and a further 12% by the mixed solvents; in each case the extracts were combined. Evaporations were carried out in O_2 -free N_2 under reduced pressure below 35°.

Isolation of 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate from the urine of a dog dosed with Chlorfenvinphos. The urine from this animal (see under Experiments with animals) was extracted as described, and metabolite E was detected at R_F 0.7 on paper chromatograms by using phenoxyethanol– $AgNO_3$ and the molybdate reagent for phosphate-containing compounds as location reagents. The metabolite could not be crystallized from the extract, and the concentrated solution was accordingly dissolved in water. To concentrate the acidic components in the urine extract, the resulting solution was percolated (0.5 ml./min.) through a column (bed volume, 63 ml.; height/diameter ratio 10:1) of analytical-grade Amberlite IR-45 (HCO_3^- form; 100–200 mesh) (British Drug Houses Ltd., Poole, Dorset) anion-exchange resin. The column was treated with 2 bed volumes of water and attempts were made to elute the phosphate-containing metabolite by using increasing concentrations of aqueous formic acid. In fact, the desired metabolite appeared in the

eluate when 2N-HCl was used as eluent. 2-Chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate was extracted with ether and the concentrated solution recrystallized several times from ethanol-*n*-hexane mixture as described above. 2-Chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate formed prisms (210 mg.), m.p. 108–109° and mixed m.p. 108–109° with an authentic specimen (of m.p. 108–109°).

Paper chromatography. Metabolites in the solvent extracts of urine from treated animals were separated by descending paper chromatography on Whatman no. 1 filter-paper sheets at 29°. Table 3 shows a typical separation of the ether-soluble ¹⁴C-labelled metabolites from urine. Whatman no. 3MM paper was used for preparative paper chromatography. Butan-1-ol-2N-NH₃ (1:1, v/v; top phase) was invariably used as developing solvent. The dried papers were scanned for radioactivity. Similar chromatograms were also run with mixtures of synthetic reference compounds, which were located with spray reagents. Phenoxy-ethanol-AgNO₃ (Mitchell, 1958) was used for the detection of chlorine-containing compounds. Phosphate-containing compounds were detected with acidic ammonium molybdate (Burrows, Grylls & Harrison, 1952). Chlorofenvinphos and the phenacyl chloride were detected by spraying with aq. N-KOH and subsequently heating to 120°. The fluorescence of aromatic compounds in ultraviolet light was used as another means of location.

Thin-layer chromatography. This was carried out on Kieselgel G (E. Merck A.-G. Darmstadt, Germany) as described by Wright *et al.* (1965). The ¹⁴C-labelled metabolites were detected by scanning for radioactivity. For the identification of ¹⁴C-labelled aglycones from the [¹⁴C]-glucuronide conjugates in urine, unlabelled synthetic reference compounds and ¹⁴C-labelled aglycones were applied to the same origin on the thin-layer plate to ensure exact co-chromatography. The developing solvents were benzene (solvent *a*) and benzene containing 4% (v/v) of methanol (solvent *b*).

High-voltage electrophoresis. Electrophoresis was carried out on strips of Whatman no. 1 paper at 100 v/cm. with 0.05 M-sodium phosphate buffer, pH 7.5. D-Glucose was used as a non-migrating marker for the correction of electro-osmotic-flow errors and benzoic acid was used as the standard migrating marker (Bourne, Hutson & Weigel, 1960). In this buffer all metabolites migrated towards the anode (*M*_{benzoic acid} values 0.5–1.0), indicating that every metabolite was acidic. Electrophoresis in formic acid-acetic acid buffer, pH 2 (Hathway, Mallinson & Akintonwa, 1965), did not cause any of the metabolites to migrate towards the cathode, indicating the absence of positively charged centres from the molecules. After electrophoresis, the paper strips, which were air-dried to remove most of the liquid and subsequently dried at 80° for 10 min., were scanned for radioactive zones.

Measurement of radioactivity. A Tri-Carb spectrometer (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.) was used for the measurement of ¹⁴C by liquid-scintillation counting. Measurement of radioactivity in urine, faeces, gut and content, carcass plus viscera, skin plus hair and of the ¹⁴CO₂ in the expired gases were made by the methods described by Wright *et al.* (1965). Zones of radioactivity on paper chromatograms and paper electrophoretograms were scanned with the Nuclear-Chicago Actigraph II equipped with a gas-flow cell utilizing a helium+*n*-butane (98.5:1.5)

mixture. Thin-layer plates were scanned for radioactive zones with a Desaga radiochromatogram scanner equipped with a gas-flow cell utilizing an argon+methane (9:1, v/v) mixture.

Enzymic hydrolysis of metabolites. The ¹⁴C-labelled metabolites were dissolved in 0.2 M-sodium acetate, pH 5.0 (2 ml.), and incubated with β-glucuronidase (1120 units) at 37° for 18 hr. Neutral products were extracted with ether; unhydrolysed starting materials were extracted with ether after acidification of the reaction mixture to pH 2. Control digests were prepared similarly and heated at 96° for 10 min. before addition of metabolite. In every case starting material was recovered quantitatively from the digests containing inactivated enzyme. D-Glucuronic acid could not be identified because of the very small quantities of metabolites used.

Confirmatory isotope-dilution analysis. In general, a known weight of synthetic carrier compound was added to a sample of the initial urine extract in methanolic solution. The mixture was then left overnight, when carrier compound was recovered by crystallization and recrystallized until three consecutive samples possessed constant specific activity. The solvent utilized for crystallization was the one employed for the isolation of the crystalline model compound (for details see the Materials section). An exception to the above technique was the method for metabolite B from rat urine. Radiochemically pure metabolite was hydrolysed by β-glucuronidase and the aglycone diluted with 1-(2',4'-dichlorophenyl) vinyl ethanol. The mixture was dried and esterified with 2,4-dichlorobenzoyl chloride (see the Materials section) to yield 1-(2',4'-dichlorophenyl)-ethyl 2,4-dichlorobenzoate, m.p. 63°. The ester was recrystallized to constant specific activity. Identifications and measurements made by isotope-dilution analysis are in good agreement with those found by chromatography and co-chromatography of the ¹⁴C-labelled metabolites and of the corresponding hydrolysis products with unlabelled synthetic reference compounds. Thus paper chromatograms run with the solvent of choice efficiently separated individual ¹⁴C-labelled components.

RESULTS

Excretion and retention of radioactivity in rats dosed with [¹⁴C]Chlorfenvinphos. After oral treatment of six young adult male and six young adult female rats with [¹⁴C]Chlorfenvinphos, the major eliminative route was via the kidneys and the remaining radioactivity was almost entirely eliminated by the faecal route; 1.1–1.6% of the dose appeared in the expired gases during 96 hr. A single dose of [¹⁴C]Chlorfenvinphos is quantitatively eliminated from rats in 4 days: 100.8% (98.7–107.0%) is excreted, these values including the contents of the gut at 96 hr. Only traces of ¹⁴C were found in the carcass and viscera remaining after removal of the gastro-intestinal tract of animals dosed with [¹⁴C]Chlorfenvinphos (Table 1).

Excretion of ¹⁴C in the urines of rats dosed with [¹⁴C]Chlorfenvinphos was rapid; 51.9–77.4% of the ¹⁴C was excreted during 0–24 hr. and 6.1–25.6% during 24–48 hr. The biological variation in the

Table 1. *Excretion and retention of radioactivity in rats after oral administration of [¹⁴C]Chlorfenvinphos*

Male and female rats (200 g. body wt.) were treated by stomach tube with single doses (2 mg./kg.) of [¹⁴C]Chlorfenvinphos (2.8 μ C/mg.) in 1.5 ml. of olive oil. This dose represents one-fifth of the LD₅₀ value, and the accuracy of measurement of ¹⁴C in body tissues was less than 0.5% of the dose, because of the low specific activity of the insecticide. The animals were grouped together, since they showed no sex difference in the rate of elimination and a comparatively small degree of biological variation in their total excretion values.

	Recovery of ¹⁴ C (% of dose)				
	Urine	Faeces	Expired gases	Gut and contents	Total
Mean	87.2	11.2	1.4	1.4 and < 0.5	100.8
± S.E.M.	1.4	1.6	0.08	0.3 —	0.7
No. of independent observations	12	12	6	6 6	12

Table 2. *Rates of urinary and faecal elimination of radioactivity in rats and dogs after oral administration of [¹⁴C]Chlorfenvinphos*

Rats were treated with [¹⁴C]Chlorfenvinphos as described in Table 1. Each dog swallowed a gelatin capsule containing a solution of [¹⁴C]Chlorfenvinphos (8 μ C; 0.3 mg./kg.) in olive oil (1.0 ml.). Dogs and rats do not show a sex difference in rate of elimination and the sexes have not therefore been treated separately.

Animal		¹⁴ C eliminated (% of dose)							
		0-24 hr.		24-48 hr.		48-72 hr.		72-96 hr.	
		Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
Rat	Mean	67.5	1.4	14.4	6.2	3.6	2.3	0.87	1.20
	± S.E.M.	2.0	0.4	1.4	1.6	0.6	0.7	0.13	0.27
	No. of independent observations	12	12	12	12	12	12	12	12
Dog	Mean	86.0	4.1	2.3	0.43	0.63	< 0.1	0.47	< 0.1
	± S.E.M.	2.0	0.8	0.5	0.10	0.22	—	0.12	—
	No. of independent observations	4	4	4	4	4	4	4	4

daily excretion values (Table 2) lies within the limits of that usually found for animals in excretion-retention studies. Slight elimination of ¹⁴C in the faeces during 24 hr. indicates that alimentary absorption was almost complete. Chlorfenvinphos and its metabolites were rapidly excreted in rats, and the variable rate of elimination of ¹⁴C in the faeces during 48-96 hr., together with the presence of ¹⁴C in the gut of the animals at 96 hr., suggested that some entero-hepatic circulation occurred in rats.

The exhalation of a small proportion of ¹⁴CO₂ from [¹⁴C]Chlorfenvinphos, in which vinyl carbon atoms were labelled with ¹⁴C, suggests that a small quantity of 2,4-dichlorobenz[¹⁴C]oic acid or a corresponding conjugate might also be present amongst the ¹⁴C-labelled metabolites in the rat.

On account of the high toxicity of Chlorfenvinphos to rats (oral LD₅₀ 10 mg./kg. body wt.), a dose level was used in the metabolic studies (2 mg./kg.)

that did not cause toxic symptoms in any of the animals during the period of the experiment. This consideration does not, however, apply to dogs, for which the insecticide was less toxic.

Excretion of radioactivity in dogs after ingestion of [¹⁴C]Chlorfenvinphos. A single dose of [¹⁴C]Chlorfenvinphos is almost entirely eliminated from dogs in 4 days: 94.0% (91.8-97.6%) is excreted. The major eliminative route was via the kidneys. Excretion of ¹⁴C in the urines of dogs dosed with [¹⁴C]Chlorfenvinphos was very rapid: 82.6-91.4% during 0-24 hr. (Table 2).

After ingestion of [¹⁴C]Chlorfenvinphos there is a significant difference in the rate of elimination of ¹⁴C in the urine of dogs and rats.

Identification of ¹⁴C-labelled metabolites in the urine of rats and dogs dosed with [¹⁴C]Chlorfenvinphos. By successive extraction at pH 1.9 of the urines of treated animals with ether and ether-ethanol mixture, 95% of the radioactivity was

Table 3. ^{14}C -labelled constituents in the urine of rats and dogs treated with $[^{14}\text{C}]$ Chlorfenvinphos

Combined urines (0–48 hr. collection) from 19 rats were extracted with ether (for details see the Methods section); the sexes were not treated separately. Butan-1-ol saturated with 2N-NH_3 was used to separate these extracts on paper chromatograms, which were subsequently scanned for ^{14}C ; the relative areas under the curves are cited. Extracts of the urines (0–24 hr. collection) from treated dogs (Table 2) were separated similarly. R_F values and relative quantities of ^{14}C are representative values for extracts of the urines from three entirely separate groups of both animal species. Zones A–E were treated individually with β -glucuronidase. Each zone was identified by its chromatographic properties and behaviour on enzymic hydrolysis. The structures of metabolites D and E in both species and of metabolite B in the rat were confirmed by isotope-dilution analysis, and the values in parentheses for the relative amounts were obtained by this technique.

Zone	R_F	Relative amounts of ^{14}C in each zone		Action of β -glucuronidase		Identity
		Rat extract	Dog extract*	Rat	Dog	
A	0.2	3	3	+	+	2,4-Dichlorophenylethanediol glucuronide
B	0.3	47	4	+	+	[1-(2',4'-Dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid
C	0.4	5	Absent	—	—	2,4-Dichlorohippuric acid
D	0.5	8 (5.2)	15 (8.6)	—	—	2,4-Dichloromandelic acid
E	0.7	37 (40.0)	78 (74.9)	—	—	2-Chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate

* These values were not altered substantially when a dog was dosed with 5.53 g. of Chlorfenvinphos; in this case the chromatograms were excised and extracted for liquid-scintillation counting.

recovered. Scanning revealed five radioactive zones on paper chromatograms of the ether-soluble material from rat urines that had been run in butan-1-ol saturated with 2N -ammonia, and on similarly developed chromatograms of the ether-soluble material from dog urines there were four radioactive zones corresponding exactly to four zones on the chromatograms of rat material (Table 3). Electrophoresis of the ether-soluble material from the urines of treated animals in phosphate buffer, pH 7.5, caused all of the ^{14}C -labelled metabolites to migrate towards the anode, and electrophoresis of this material in formic acid-acetic acid buffer, pH 2.0, confirmed that there was no migration of basic ^{14}C -labelled components towards the cathode. Hence, when animals ingested $[^{14}\text{C}]$ Chlorfenvinphos, all of the ^{14}C in the urine was due to acidic ^{14}C -labelled metabolites. In dogs and rats no unchanged Chlorfenvinphos was excreted via the kidneys and no amphoteric metabolites were present in the urine.

Possible Chlorfenvinphos metabolites were accordingly synthesized, and the chromatographic properties on paper of some of these substances were compared with those of relevant ^{14}C -labelled metabolites in the urine of treated animals.

By chromatography and co-chromatography on papers run with butan-1-ol saturated with 2N -ammonia, ^{14}C -labelled metabolite D from the urine of rats and dogs dosed with $[^{14}\text{C}]$ Chlorfenvinphos was shown to be identical with unlabelled 2,4-dichloromandelic acid (Table 3), and this assignment

of chemical structure was confirmed by isotope-dilution analysis.

Similarly, on chromatography and co-chromatography ^{14}C -labelled metabolite E from the urine of treated rats and dogs behaved identically with unlabelled 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate (the *O*-desethyl metabolite). Further, when ^{14}C -labelled metabolite was hydrolysed with aq. 30% sulphuric acid, under the conditions described in the Materials section, the single ^{14}C -labelled product of R_F 0.8 was found to be chromatographically identical with unlabelled 2,4-dichlorophenacyl chloride on thin-layer plates run with benzene. The assignment of chemical structure for ^{14}C -labelled metabolite E from treated animals was confirmed by isotope-dilution analysis, and, for a dog dosed with Chlorfenvinphos (5.33 g.), by isolation from the urine.

The relative proportions based on isotope-dilution methods of 2,4-dichloro- $[^{14}\text{C}]$ mandelic acid and 2-chloro-1-(2',4'-dichlorophenyl)- $[^{14}\text{C}]$ vinyl ethyl hydrogen phosphate in the urines of treated dogs and rats are in fair agreement with those found by the radioactive scanning of paper chromatograms (Table 3).

The possibility that some of the remaining ^{14}C -labelled metabolites in the urines of animals dosed with $[^{14}\text{C}]$ Chlorfenvinphos may have been β -D-glucuronides was tested by the action of β -glucuronidase on individual ^{14}C -labelled metabolites after their separation by chromatography on thick paper (Table 3). The chromatographic

properties on thin-layer plates of the ^{14}C -labelled products, obtained by β -glucuronidase hydrolysis of the ^{14}C -labelled metabolites A and B from treated dogs and rats (see Table 3), were compared with those of the following alcohols: 2-chloro-1-(2',4'-dichlorophenyl)ethanol, 2,4-dichlorobenzyl alcohol, 2,4-dichlorophenylethanediol and 1-(2',4'-dichlorophenyl)ethanol (Table 4). In this connexion, we failed to synthesize the easily reducible 2,4-dichlorophenacyl alcohol, which could not, however, have been a hydrolysis product of the ^{14}C -labelled metabolites A and B, since neither these compounds nor the ^{14}C -labelled aglycones to which they gave rise were reduced by sodium borohydride. Chromatography and co-chromatography on thin-layer plates run with benzene and benzene containing 4% (v/v) of methanol showed that the ^{14}C -labelled aglycones from ^{14}C -labelled metabolites A and B were identical with unlabelled 2,4-dichlorophenylethanediol and 1-(2',4'-dichlorophenyl)ethanol respectively (Table 4). The assignment of chemical structure to the ^{14}C -labelled metabolite in the urine of rats dosed with [^{14}C]Chlorfenvinphos was confirmed by isotope-dilution analysis. All of the radioactivity (99.4%) in the sample was due to 1-(2',4'-dichlorophenyl)ethanol. Thus in both species ^{14}C -labelled metabolite B was probably [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid, and ^{14}C -labelled metabolite A was one of the possible β -D-glucuronides of 2,4-dichlorophenylethanediol.

The ^{14}C -labelled metabolite C from the urine of treated rats that did not undergo hydrolysis with β -glucuronidase was shown to differ from simple acidic reference compounds by chromatography. When this metabolite was hydrolysed with 5N-hydrochloric acid at 100% for 3 hr., paper chromatography revealed that 50% of the ^{14}C was present as unchanged ^{14}C -labelled metabolite C and 50% of the ^{14}C was present as a new compound, which co-chromatographed with unlabelled 2,4-dichlorobenzoic acid (R_F 0.55 in butan-1-ol saturated with 2N-ammonia). On this evidence the chromatographic properties of the original ^{14}C -labelled metabolite C were compared

with those of unlabelled 2,4-dichlorohippuric acid. By co-chromatography on papers run with butan-1-ol saturated with 2N-ammonia and on thin-layer plates run with ether-*n*-hexane-methanol-acetic acid (60:40:10:1, by vol.) mixture the compounds proved to be identical (R_F 0.40 on thin-layer plates in the solvent specified).

In conclusion, among the metabolic products of Chlorfenvinphos in the urine of animals, the *O*-desethyl metabolite accounts for 32.3% of the dose in rats and 69.6% in dogs, [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid for 41.0% in rats and 3.6% in dogs, 2,4-dichloromandelic acid for 7.0% in rats and 13.4% in dogs, 2,4-dichlorophenylethanediol glucuronide for 2.6% in rats and 2.7% in dogs, and 2,4-dichlorohippuric acid for 4.3% in rats. In rats 1.9% of the dose is eliminated in the expired gases. Thus after administration of a single oral dose of Chlorfenvinphos rats and dogs show a significant species difference in the proportions of the metabolites, with the exception of 2,4-dichlorophenylethanediol glucuronide, that are excreted in the urine.

In the dog, the relative proportions of the metabolites in the urine are not altered substantially by increasing the size of the dose.

Metabolic fate of intravenously administered [^{14}C]Chlorfenvinphos. When a single dose of [^{14}C]Chlorfenvinphos was administered intravenously to a dog it was rapidly metabolized, and the *O*-desethyl metabolite accounted for 83% of the ^{14}C in the 48 hr. urine, 2,4-dichloromandelic acid for 9% and [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid for 8%. In comparison with the oral administration of Chlorfenvinphos in dogs, intravenous administration did not appear to alter the rate of metabolism or the approximate proportions of the major metabolites in the urine.

Metabolic fate of some possible intermediate metabolites of Chlorfenvinphos. The products of the hydrolysis *in vitro* of Chlorfenvinphos, 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate and 2,4-dichlorophenacyl chloride, may lie on the main metabolic pathway of the insecticide in animals, and to test this supposition the metabolic

Table 4 R_F values on thin-layer plates of potential aglycones from glucuronide conjugates

Solvent (a) was benzene and solvent (b) benzene-methanol (24:1, v/v) mixture. Values are given for synthetic reference compounds, and those for ^{14}C -labelled aglycones appear in parentheses.

Compound	R_F		Aglycones derived from the following metabolites
	Solvent(a)	Solvent(b)	
2-Chloro-1-(2',4'-dichlorophenyl)ethanol	0.50	0.80	
2,4-Dichlorobenzyl alcohol	0.20	0.40	
2,4-Dichlorophenylethanediol	0.05 (0.05)	0.20 (0.20)	A
1-(2',4'-Dichlorophenyl)ethanol	0.25 (0.25)	0.50 (0.50)	B

Table 5. *Rates of elimination of radioactivity in the urine of animals dosed with possible ^{14}C -labelled metabolites of Chlorfenvinphos*

Each dog swallowed a gelatin capsule containing either a solution of 2-chloro-1-(2',4'-dichlorophenyl)[^{14}C]vinyl ethyl hydrogen phosphate (3.5 μC ; 3 $\mu\text{C}/\text{mg.}$) in 1 ml. of ethanol or a solution of 2-chloro-1-(2',4'-dichlorophenyl)-[^{14}C]ethanol (2.68 μC ; 3.4 $\mu\text{C}/\text{mg.}$) in 1 ml. of olive oil. Male and female rats were treated by stomach tube with 0.4 mg. (3.5 $\mu\text{C}/\text{mg.}$) of 2,4-dichlorophenyl[^{14}C]acyl chloride in 1 ml. of olive oil.

Compounds	No. of animals	^{14}C eliminated (% of dose)		
		0-24 hr.	24-48 hr.	Total
2-Chloro-1-(2',4'-dichlorophenyl)[^{14}C]vinyl ethyl hydrogen phosphate	1 dog	70.6	15.6	86.2
2,4-Dichlorophenyl[^{14}C]acyl chloride	2 rats	42.9, 90.2	47.8, 7.7	90.7, 97.9
2-Chloro-1-(2',4'-dichlorophenyl)[^{14}C]ethanol	1 dog	49.0	49.3	98.3

products of the two hydrolysis products were investigated. A reduction product of 2,4-dichlorophenacyl chloride, 2-chloro-1-(2',4'-dichlorophenyl)-ethanol, was also tested in this way.

When 2-chloro-1-(2',4'-dichlorophenyl)[^{14}C]vinyl ethyl hydrogen phosphate was administered orally to a dog, the rate of elimination of ^{14}C in the urine (Table 5) resembled that resulting from the administration of [^{14}C]Chlorfenvinphos to rats. Examination of the ^{14}C -labelled products in the urine showed that 2,4-dichlorophenyl[^{14}C]glycollic acid (2,4-dichloro[^{14}C]mandelic acid) accounted for 40% of the ^{14}C , [1-(2',4'-dichlorophenyl)[^{14}C]ethyl β -D-glucopyranosid]uronic acid for 50% and unchanged initial substance for 10%. Thus 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate was metabolized in high proportion in the dog into an array of products rather similar to that arising from the metabolism of the insecticide in rats.

2,4-Dichlorophenyl[^{14}C]acyl chloride was also rapidly metabolized by rats, and the ^{14}C -labelled metabolites were excreted almost quantitatively via the kidneys (Table 5). Examination of appropriate urine extracts showed that 2,4-dichlorophenyl[^{14}C]glycollic acid (2,4-dichloro[^{14}C]mandelic acid) accounted for 30% of the ^{14}C in the urine, 2,4-dichlorobenzyl[^{14}C]oxygen glycine (2,4-dichloro[^{14}C]hippuric acid) for 5% and [1-(2',4'-dichlorophenyl)-[^{14}C]ethyl β -D-glucopyranosid]uronic acid for 60%. This array of metabolites resembles that afforded by rats dosed with [^{14}C]Chlorfenvinphos (Table 3), except for the absence of the *O*-desethyl phosphate.

The conclusion is drawn that the *O*-desethyl metabolite and 2,4-dichlorophenacyl chloride could lie on the main metabolic pathway of Chlorfenvinphos, since the respective feeding with these compounds yields [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid and 2,4-dichloromandelic acid as major metabolites in the urine, and these compounds are also major metabolites of Chlorfenvinphos. Further, 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate has

been isolated from among the Chlorfenvinphos metabolites *in vivo*, and 2,4-dichlorohippuric acid is a minor metabolite of both Chlorfenvinphos and 2,4-dichlorophenacyl chloride in rats.

After oral administration of 2-chloro-1-(2',4'-dichlorophenyl)[^{14}C]ethanol to a dog, 80% of the label was excreted as the corresponding ^{14}C -labelled glucuronide and 20% as 2,4-dichloro[^{14}C]mandelic acid; no other products were detected. Since 2-chloro-1-(2',4'-dichlorophenyl)ethanol glucuronide does not occur among the metabolic products of Chlorfenvinphos, it is unlikely that 2-chloro-1-(2',4'-dichlorophenyl)ethanol is a metabolite of the insecticide. The persistence of this 2-chloroethanol residue *in vivo* is noteworthy in view of the ease of transformation of 2-chloroethanols into epoxides and the reactivity of such epoxides *in vitro*.

DISCUSSION

Ingestion of Chlorfenvinphos by animals is followed by a high degree of alimentary absorption, and up to 90% of a single dose (2 mg./kg. in rats and 0.26 mg./kg. in dogs) has been accounted for in terms of metabolic products in the urine and exhaled air.

Dogs and rats show a species difference in the proportions of Chlorfenvinphos metabolites excreted in the urines of treated animals, and this might be attributed to a difference in renal clearance of 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate in the two species. Thus in the dog there might be massive renal clearance of the *O*-desethyl metabolite, whereas in the rat a lower degree of renal clearance of the *O*-desethyl metabolite might provide greater opportunity for its hydrolysis into 2,4-dichlorophenacyl chloride by route (a) (Scheme 1) and the subsequent metabolism of this product. Alternatively, the 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate produced might be excreted via the kidneys with similar efficiency in both species. The different proportions of the other metabolites in the urines of

Scheme 1. Metabolism of Chlorfenvinphos in animals.

treated animals would then appear to result from the varying extent to which 2,4-dichlorophenacyl chloride might be formed in the two species by a single-step hydrolysis of Chlorfenvinphos (route b) (Scheme 1). Present work does not permit a choice to be made between (1) a difference in renal clearance of the *O*-desethyl metabolite coupled with the two-step hydrolysis of the insecticide, and (2) a difference in output of *O*-desethyl metabolite and variable formation of 2,4-dichlorophenyl chloride by a single-step hydrolysis, and in practice the different proportions of Chlorfenvinphos metabolites in dogs and rats might be caused by differences in both mechanisms, which could be operating simultaneously.

The other metabolites for which we have to account are 1-(2',4'-dichlorophenyl)ethanol, 2,4-dichlorophenylethanedione, 2,4-dichlorohippuric acid and 2,4-dichloromandelic acid, and the expected routes of metabolism are shown in Scheme 1. Route (e) can be eliminated, because we have found that the principal metabolic product of 2-chloro-1-(2',4'-dichlorophenyl) ethanol is [2-chloro-1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid, which is neither a metabolite of 2,4-dichlorophenacyl chloride nor of Chlorfenvinphos; route (f) can similarly be eliminated, because it is known that unsubstituted phenacyl alcohol is

largely metabolized into phenacyl alcohol glucuronide (El Masri, Smith & Williams, 1958), and in our work 2,4-dichlorophenacyl alcohol glucuronide is not a metabolite of 2,4-dichlorophenacyl chloride. Route (c) is possible, since 2,4-dichloroacetophenone is an acceptable transformation product of the carbanion from 2,4-dichlorophenacyl chloride, and since [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid, which we have found among the metabolic products of Chlorfenvinphos, would be expected to be the major metabolite of 2,4-dichloroacetophenone (see Smith, Smithies & Williams, 1954). It is noteworthy that 2,4-dichloroacetophenone is one of the principal breakdown products of Chlorfenvinphos in unsterilized soils. Route (d) is possible, since by analogy with 1-phenylethanol (El Masri *et al.* 1958) 1-(2',4'-dichlorophenyl)ethanol would be expected to yield 2,4-dichlorohippuric acid and 2,4-dichloromandelic acid in the urine, and in the present work the intermediate 2,4-dichlorophenylethanedione was also detected. In this connexion ($\pm$)-phenylethanedione, when fed to rabbits, yielded hippuric acid, mandelic acid and phenylethanedione glucuronide (El Masri *et al.* 1958); mandelic acid is not metabolized in animals and man (Garry & Smith, 1940; Klingmüller & Brune, 1956; Williams, 1959).

Four further comments appear to be necessary.

The first concerns [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid. By analogy, acetophenone is reduced in rabbits to (-)-1-phenyl-ethanol, which is excreted as a glucuronide, identical with the glucuronide isolated after feeding with the (-)-carbinol (Smith *et al.* 1954). The second comment concerns the monoglucuronide of 2,4-dichlorophenylethanol, of which four isomers are possible. The glucuronide could be 1- or 2-glucuronide of the (+)- or (-)-glycol [(+)- or (-)-C₆H₃Cl₂·CH(OG)·CH₂·OH or (+)- or (-)-C₆H₃Cl₂·CH(OH)·CH₂·OG, where G is C₆H₉O₆]. At present we have no data to decide which isomer was isolated, but it is reasonably certain that the primary alcoholic group was substituted, since primary alcoholic groups are more reactive than secondary alcoholic groups in non-specific enzymic transglycosylation processes. The third remark concerns the optical nature of the 2,4-dichloro-mandelic acid, isolated as a Chlorfenvinphos metabolite, and again we have no data to discriminate between the possible isomers, though considerable racemization might be predicted (see Scheme 1). The fourth comment concerns the O-desethylation of Chlorfenvinphos. Although the di-O-methyl organophosphate triesters, especially those of thionophosphates, often yield O-demethylated products *in vivo* (Heath, 1961; Plapp & Casida, 1958), the corresponding di-O-ethyl esters less often undergo O-desethylation. However, O-(3-chloro-4-methylumbelliferone) diethyl phosphate was metabolized into the O-desethyl phosphate in rats (Krueger, Casida & Niedermeier, 1959). The dimethyl vinyl phosphate, methyl 3-(dimethoxyphosphinyloxy)crotonate, was not O-demethylated in cows (Casida, Gatterdam, Knaak Lance & Niedermeier, 1958), whereas 2,2-dichloro-vinyl dimethyl phosphate yielded the O-demethyl phosphate in mammals (Hodgson & Casida, 1962; Casida, McBride & Niedermeier, 1962). Thus when the present work was undertaken there was insufficient information available for the course of hydrolysis *in vivo* of a dialkyl vinyl phosphate, such as Chlorfenvinphos, to be predicted.

Hydrolysis of the lipid-soluble Chlorfenvinphos *in vivo* yielded two products of low toxicity: 2-chloro-1-(2',4'-dichlorophenyl)vinyl ethyl hydrogen phosphate with an oral LD₅₀ value in the rat exceeding 1000 mg./kg. body wt. and 2,4-dichlorophenacyl chloride with an oral LD₅₀ in the rat 1450 mg./kg. The former, which is hydrophilic, was excreted in the urine of animals, whereas the latter, which is lipid-soluble, was transformed by the hepatic drug-metabolizing enzymes into the

hydrophilic products [1-(2',4'-dichlorophenyl)ethyl β -D-glucopyranosid]uronic acid, 2,4-dichloro-mandelic acid (oral LD₅₀ in the rat exceeding 1000 mg./kg.), 2,4-dichlorophenylethanol glucuronide and 2,4-dichlorohippuric acid, which were also excreted in the urine. The proposed scheme for Chlorfenvinphos metabolism in animals accordingly represents a detoxication mechanism.

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