Metabolism of Trichlorfon in Animals and Plants

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The fate of ^{32}P-labeled trichlorfon in lygus bug adults, *Lygus hesperus* Knight, tobacco budworm larvae, *Heliothis virescens* F., green lacewing larvae, *Chrysopa carnea* Stephens, white rats, and cotton plants was investigated. Differences in the susceptibility to trichlorfon among the insect species were influenced primarily by the rates at which the insecticide was absorbed. After treatment with trichlorfon, small amounts of dichlorvos, a highly toxic derivative of trichlorfon, were detected in the extracts of insects and plants, but not in the urine of rats. Both trichlorfon and dichlorvos were metabolized rapidly in all biological materials. Three previously unreported glycosidic conjugates, important in the detoxification of trichlorfon, were detected. One conjugate was a glucuronide found only in the urine of rats. The others were glucosides; one occurred in all insects and plants, and the other was found only in lygus adults.

Trichlorfon [dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate] is an important insecticide that is selectively nontoxic to mammals and comparatively nontoxic to several important beneficial insects (Lingren and Ridgway, 1967; Reynolds *et al.*, 1960). Although studied extensively by several investigators, the mode of action and metabolic fate of trichlorfon in living organisms remain uncertain. It is well established that trichlorfon rearranges readily, via dehydrochlorination, in slightly acidic, neutral, and alkaline media to form the highly toxic phosphate derivative, dichlorvos (2,2-dichlorovinyl dimethyl phosphate) (Barthel *et al.*, 1955; Lorenz *et al.*, 1955; Mattson *et al.*, 1955). Certain investigators (Arthur and Casida, 1957; Hassan *et al.*, 1965) have suggested that trichlorfon is a direct inhibitor of acetylcholinesterase (AChE), while others (Metcalf *et al.*, 1959; Miyamoto, 1959) have presented evidence that the compound is a poor inhibitor *per se* and owes its in vivo and in vitro toxicity to the formation of dichlorvos. Miyamoto (1959) also concluded that the formation of dichlorvos in different biological systems was not necessarily the result of a dehydrochlorinating enzyme, but probably could occur spontaneously under normal physiological conditions.

After studies of the metabolism of ^{32}P-labeled trichlorfon in a lactating cow, Robbins *et al.* (1956) suggested that the major metabolic pathway probably involved hydrolysis of the *O*-methyl ester linkage or modification of the trichloroethanol moiety of the molecule; rupture of the P—C bond was considered a minor reaction. However, Arthur and Casida (1957) found that 63% of a dose of trichlorfon injected into a dog was converted to a conjugate of trichloroethanol and glucuronic acid, and concluded that hydrolysis of the P—C bond was a major metabolic event. In addition, these authors found no evidence for the in vivo formation of dichlorvos in the dog or in several species of insects. In comparable studies with houseflies, *Musca domestica* L., Metcalf *et al.* (1959) found definite evidence of the in vivo conversion of trichlorfon to dichlorvos, and also suggested that a direct rupture of the phosphonate bond might not occur.

Miyamoto (1961) failed to detect any evidence of trichloroethanol or chloral in rabbits after intravenous injection with trichlorfon, and suggested that the splitting of the P—C linkage probably was a difficult reaction in mammals. He also reported that the oral administration of trichlorfon to rabbits was followed by the rapid excretion in urine of at least two glucuronides ($D \cdot G_s$ and $D \cdot G_f$), both apparently containing phosphorus and glucuronic acid in an equimolar ratio. In subsequent work, Miyamoto (1968) purified one of the glucuronides ($D \cdot G_f$) and tentatively identified it as a conjugate of *O*-demethyl dichlorvos (2,2-dichlorovinyl methyl phosphate) and glucuronic acid.

After a study of the metabolism of ^{32}P-labeled trichlorfon by *Prodenia litura* F. larvae, Zayed and Hassan (1965) reported that most (91%) of the radioactivity associated with products of decomposition occurred as *O*-demethyl trichlorfon [methyl 2,2,2-trichloro-1-hydroxyethyl) phosphonate] and that 9% was methyl phosphate produced by cleavage of the P—C bond of *O*-demethyl trichlorfon. These authors did not report the detection of any dichlorvos. Also, in other in vitro studies of the metabolism of ^{32}P-labeled trichlorfon by homogenates of the brains of white rats, Hassan *et al.* (1965) decided that the main metabolic pathway involved the hydrolysis of methyl ester linkages and consequent formation of *O*-demethyl trichlorfon and 2,2,2-trichloro-1-hydroxyethylphosphonic acid. When rats were treated with an intraperitoneal injection of trichlorfon labeled with ^{14}C in the two methyl groups, Hassan and Zayed (1965) found that hydrolyses of P—C and P—O—CH_3 linkages apparently were equally important in the detoxification of the compound.

The present paper reports the results of studies of the factors influencing the selectivity of the trichlorfon and gives additional information on the metabolism of the compound in animals and plants.

MATERIALS AND METHODS

Chemicals. Two separate batches of ^{32}P-labeled trichlorfon (initial specific activity of 10 mc. per mmole) were obtained from the Nuclear-Chicago Corp., Des Plaines, Ill., and purified (99%) on a silica gel column by stepwise elution with different mixtures of *n*-hexane and chloroform. Pure samples of trichlorfon and *O*-demethyl trichlorfon were provided by the Chemagro Corp., Kansas City, Mo. The dichlorvos was prepared by treating radioactive or nonradioactive trichlorfon with equimolar concentrations of sodium hydroxide and purified by partitioning between *n*-hexane and water and by column chromatography as described above. The sodium salt of *O*-demethyl dichlorvos was prepared by refluxing equimolar concentrations of sodium iodide and dichlorvos for 5 minutes in dry acetone. The radiolabeled sodium salt of *O*-demethyl dichlorvos was prepared in the

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Table I. Topical Toxicity and AntiChE Activity of Trichlorfon and Dichlorvos

	LD_{50} (Mg. per Kg.) after 48 Hours			
	Green lacewing 3rd instar	Tobacco budworm 2nd instar	Lygus bug adult	pI_{50} Bovine AChE
Trichlorfon	20,000.0	77.0	1.6	5.2
Dichlorvos	200.0	63.0	0.8	6.3

same manner from ^{32}P-labeled dichlorvos. In addition, radiolabeled dimethyl phosphate was obtained by the alkaline hydrolysis of ^{32}P-labeled dichlorvos.

Purified bovine erythrocyte AChE, acetylcholine bromide, β-glucosidase (emulsin, from almonds), and β-glucuronidase (from bovine liver) were purchased from the Sigma Chemical Co., St. Louis, Mo.

Biological Materials and Their Treatment. The insect species tested included adult (6 to 8 mg.) lygus bugs, *Lygus hesperus* Knight, second instar (20 to 25 mg.) and fifth instar (650 to 750 mg.) tobacco budworm larvae, *Heliothis virescens* F., selected at random from laboratory colonies that are all susceptible to insecticides, and third instar (4 to 6 mg.) green lacewing larvae, *Chrysopa carnea* Stephens, purchased from Vitova, Inc., Rialto, Calif., and fed eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). A micrometer-driven syringe, calibrated to deliver 1 μl. of an acetone solution of the appropriate chemical, was used to treat insects. Those used for the studies of toxicity and of absorption and metabolism were anesthetized lightly with CO_2 and then treated topically on the dorsal surface of the body. Oral doses were administered to fifth instar tobacco budworms with a blunt-tipped hypodermic needle (Bull and Lindquist, 1966). Treated insects were confined individually in ventilated glass containers; only those used in the studies of toxicity were fed during the test periods.

Female white rats (200 to 250 grams, Sprague-Dawley, Inc., Madison, Wis.) were treated with intraperitoneal injections of aqueous solutions (0.5 ml.) of the desired chemicals and then held in metabolism cages equipped to separate urine and feces. The animals were provided water but no food during the 16-hour test periods.

Cotton plants of the Deltapine Smoothleaf variety were grown in a greenhouse and treated by injecting aqueous solutions of chemicals into the petioles of individual, fully expanded leaves (Bull *et al.*, 1967).

Analytical Procedures. The spontaneous rearrangement of trichlorfon to dichlorvos during preparation of samples was minimized by adjusting all of the distilled water used to pH 5.

The initial extracts of unabsorbed, internal, and excreted radioactivity from treated insects were made with water and acetone as described by Bull *et al.* (1967). Then the acetone was removed under vacuum from internal and fecal extracts, and the remaining aqueous portion was partitioned three times with chloroform. The chloroform fractions were combined and dried with anhydrous sodium sulfate. Urine collected from treated rats was diluted to a convenient volume with water and partitioned with chloroform as described; the nature of radioactivity in the feces was not determined. Extracts of treated cotton leaves were prepared according to the procedure described previously (Bull, 1968).

Each extract was radioassayed and then evaporated under vacuum to a small volume that was convenient for chromatography. Compounds that partitioned into the chloroform fractions were resolved by one-dimensional thin-layer chromatography (TLC) on glass plates coated (0.25 mm. thick) with silica gel G (Brinkmann Instruments, Inc., Westbury, N. Y.). Solvent mixtures used for TLC were A, 9 to 1 chloroform–methanol, or B, 1 to 1 chloroform–ethyl acetate. Compounds that remained in the aqueous fractions were resolved with one-dimensional paper chromatography (PC) by using Whatman 3-mm. paper and the ascending technique described by Bull *et al.* (1963). Solvent mixtures for PC included C, 40:9:1 acetonitrile, water, and ammonium hydroxide, and D, 12:8:6 butanol, pyridine, and water. In addition, certain of the water-soluble metabolites were chromatographed on an anion (Dowex 1-X8) exchange column (Plapp and Casida, 1958).

Tentative identifications were based primarily on the cochromatography of radioactive materials with authentic compounds after two-dimensional development with two different solvent mixtures. Radioactive areas were located by exposing the developed chromatograms to x-ray films; standards were located colorimetrically by spraying chromatograms with a 5% aqueous solution of silver nitrate or by using the phosphorus-detection reagent of Hanes and Isherwood (1949) and exposing them briefly to ultraviolet light.

Aliquots of liquid fractions and radioactive areas from chromatograms were radioassayed by liquid scintillation (Nuclear-Chicago Corp., Des Plaines, Ill.) at ambient temperature. Radioactivity that could not be extracted from certain tissues and that in the feces of treated rats were measured by counting the dried material in planchets with a thin-window gas-flow Geiger counter (Tracerlab, Inc., Waltham, Mass.). Appropriate corrections were made for radioactive decay, self-absorption, and quenching.

Estimates of the antiChE activity of trichlorfon and certain of its derivatives against bovine erythrocyte AChE were made by the colorimetric procedure of Simpson *et al.* (1964). The only change in the procedure was the use of 0.1*M* phosphate buffer at pH 7. The appropriate concentrations of potential inhibitors were preincubated with the enzyme preparation for one hour at 37° C. prior to the addition of the substrate (acetylcholine bromide).

RESULTS AND DISCUSSION

Toxicity and Anticholinesterase Activity. The results of the studies of the biological activity of trichlorfon and dichlorvos are shown in Table I. Third instar green lacewing larvae were virtually insensitive to topical doses of trichlorfon; second instar bollworms and adult lygus bugs were 260-fold and 12,500-fold, respectively, more sensitive to the compound. Dichlorvos was more toxic than trichlorfon to all test species. In contrast, to the insect toxicity data, the reported LD_{50} for intraperitoneal injections of trichlorfon and dichlorvos in white rats is 400 and 6 mg. per kg., respectively (Arthur and Casida, 1957). The antiChE evaluations with bovine erythrocyte AChE yielded pI_{50} values for trichlorfon and dichlorvos comparable to those reported by others (Arthur and Casida, 1957).

Absorption and Metabolism in Insects. The insects used for studies of the rate at which ^{32}P-labeled trichlorfon penetrated the cuticle were the same species and life stages that were used for toxicity determinations. Topical doses of 0.05 μg. per adult lygus bug and 0.5 μg. per green lacewing or tobacco budworm larva were used. The results revealed substantial differences between species in rate of the diminution of external radioactivity and the accumulation of internal

Table II. R_f Values of Trichlorfon and Certain of Its Phosphorus-Containing Derivatives

Compound	TLC Systems A	TLC Systems B	PC Systems C	PC Systems D
Chloroform-soluble				
Trichlorfon	0.65	0.23		
Unknown D	0.82	0.30		
Dichlorvos	0.93	0.63		
Water-soluble				
H_3PO_4			0.00	0.00
Methyl phosphate			0.04	0.05
Unknown C			0.07	—
Unknown E			0.00	0.15
Dimethyl phosphate			0.19	0.26
Unknown B			0.19	0.34
O-Demethyl trichlorfon			0.40	0.41
Unknown A			0.71	—
O-Demethyl dichlorvos			0.67	0.67

radioactivity (Figure 1)—for example, after 4 hours, the unabsorbed radioactivity on green lacewing larvae amounted to 72.5% of the dose, that on tobacco budworms was 41.0%, and lygus bugs had only 7.0%. Internal radioactivity in lygus bugs accumulated to 57.3% of the dose after only 1 hour; however, in the other two species, it never exceeded 8.0%. Although the trichlorfon was absorbed fairly rapidly by tobacco budworms, internal accumulation apparently was minimized by rapid metabolism and excretion. The low levels of radioactivity in internal extracts of green lacewing could be attributed to both poor penetration and rapid excretion.

Extracts of internal and excreted radioactivity prepared during the absorption studies were analyzed to determine the nature and relative concentrations of radioactive materials. The R_f values of the metabolites and other known compounds are listed in Table II. Results shown in Table III compare the metabolism of trichlorfon by the three insect species at 1 and 2 hours after treatment. The supporting evidence for tentative identifications and the nature of unidentified metabolites is discussed below. Of the compounds listed, only trichlorfon and dichlorvos were biologically active *per se*. Relatively large concentrations of trichlorfon and dichlorvos were detected in internal extracts, and also in the excreta of lygus bugs. Only small amounts of trichlorfon and no detectable amounts of dichlorvos were found in internal extracts of tobacco budworms or green lacewing larvae; however, both compounds appeared in the excreta of these species. Although the treated insects were handled carefully, a portion of the radioactivity reported for the excreta might have resulted from contamination of the holding containers by unabsorbed insecticide.

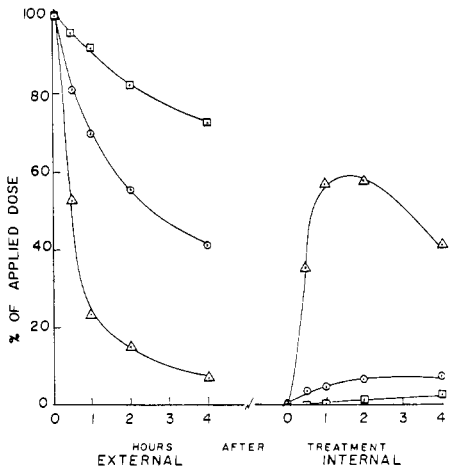

Figure 1. Radioactivity in external and internal extracts at different times after topical treatment with ^{32}P-trichlorfon

□ Green lacewing larvae
○ Third instar tobacco budworms
△ Lygus adults

The great susceptibility of lygus bugs to trichlorfon can thus be ascribed to rapid penetration through the cuticle and to internal accumulation of large concentrations of the compound and its highly toxic derivative, dichlorvos. Although the lygus bugs detoxified the absorbed insecticide rapidly, this advantage probably was nullified by the high rate of penetration. On the other hand, the great tolerance of green lacewing larvae to trichlorfon can be attributed primarily to poor penetration and failure of toxic products to accumulate. The factors influencing the relative susceptibility of second instar tobacco budworms to trichlorfon are not as clearly defined. Although trichlorfon was absorbed by the tobacco budworms at a fairly rapid rate, toxic products did not tend to accumulate internally.

To compare the metabolism of trichlorfon and dichlorvos, fifth instar tobacco budworms were treated orally with 1 μg. of ^{32}P-labeled toxicant. Moderate symptoms of poisoning were observed among larvae treated with dichlorvos, but the treatment with trichlorfon had no apparent adverse effect. Both compounds were detoxified rapidly, particularly dichlorvos (Table IV). Small concentrations of dichlorvos were detected in the excreta; however, the early loss of the applied radioactivity (25.6% in 1 hour) suggests that substantial portions of the compound were either lost directly from larvae by volatilization or excreted rapidly, then volatilized. The major products of decomposition formed by the metabolism of dichlorvos were dimethyl phosphate and *O*-demethyl

Table III. Relative Concentrations of Radioactive Compounds Obtained from Insects Treated Topically with ^{32}P-Labeled Trichlorfon

Compound	Lygus Bug 1 hr.	Lygus Bug 2 hr.	Tobacco Budworm 1 hr.	Tobacco Budworm 2 hr.	Green Lacewing 1 hr.	Green Lacewing 2 hr.	Lygus Bug 1 hr.	Lygus Bug 2 hr.	Tobacco Budworm 1 hr.	Tobacco Budworm 2 hr.	Green Lacewing 1 hr.	Green Lacewing 2 hr.
	% of Dose, Internal						% of Dose, Excreta					
H_3PO_4 + methyl phosphate	2.2	2.8	0.3	0.6	0.0	0.2	0.4	0.6	0.7	1.6	0.0	0.0
Unknown C	29.4	30.6	0.0	0.0	0.0	0.0	2.4	2.1	0.0	0.0	0.0	0.0
Dimethyl phosphate	1.8	2.0	0.7	1.6	0.1	0.1	0.3	0.5	6.0	10.8	0.8	1.3
O-Demethyl trichlorfon	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Unknown A	1.7	1.9	3.0	3.3	0.2	0.3	0.1	0.2	3.1	4.2	0.1	0.2
Trichlorfon	18.0	17.0	0.4	0.8	0.5	0.4	9.5	10.5	3.6	3.8	3.0	7.3
Dichlorvos	3.7	2.8	0.0	0.0	0.0	0.0	1.5	2.0	6.8	8.4	0.1	0.4

Table IV. Relative Concentrations of Radioactive Compounds Obtained from Fifth Instar Tobacco Budworms after Oral Treatment with 1 μg. of ^{32}P-Labeled Trichlorfon or Dichlorvos

Nature of Radioactivity	Trichlorfon			Dichlorvos			Trichlorfon			Dichlorvos		
	1 hr.	2 hr.	4 hr.	1 hr.	2 hr.	4 hr.	1 hr.	2 hr.	4 hr.	1 hr.	2 hr.	4 hr.
	% of Dose, Internal						% of Dose, Excreta					
H_3PO_4 + methyl phosphate	4.5	9.0	10.2	8.2	9.2	11.4	0.1	0.4	1.0	0.1	0.5	1.6
Dimethyl phosphate	18.2	20.7	16.0	25.7	31.4	24.0	0.6	2.2	8.9	0.9	1.5	8.7
O-Demethyl trichlorfon	—	—	—	—	—	—	0.1	0.2	0.4	—	—	—
Unknown A	29.8	11.3	5.8	—	—	—	6.8	17.1	23.5	—	—	—
O-Demethyl dichlorvos	—	—	—	27.2	20.9	6.0	2.5	4.8	6.2	0.8	2.4	13.2
Dichlorvos	0.8	0.1	0.0	6.5	2.0	0.0	1.3	1.8	1.8	3.0	3.1	3.1
Trichlorfon	24.5	13.5	5.0	—	—	—	2.2	3.7	4.7	—	—	—
Unextractable	7.5	10.0	11.0	2.0	2.5	2.0	—	—	—	—	—	—

Table V. Relative Concentrations of Radioactive Compounds Obtained from the Urine of White Rats Treated Intraperitoneally with ^{32}P-Labeled Samples of Trichlorfon (5 Mg.), Dichlorvos (0.5 Mg.), or *O*-Demethyl Dichlorvos (1.7 Mg.)

Compound	% of Dose, 16 Hours after Treatment		
	Trichlorfon	Dichlorvos	*O*-Demethyl dichlorvos
H_3PO_4 + methyl phosphate	0.8	9.2	43.6
Unknown E	18.4	0.0	11.2
Dimethyl phosphate	37.5	64.1	—
Unknown B	11.1	0.0	0.0
O-Demethyl trichlorfon	1.4	—	—
O-Demethyl dichlorvos	0.8	1.7	21.2
Trichlorfon	0.7	—	—

dichlorvos. Small amounts of dichlorvos were found in internal extracts and excreta of larvae that were treated with trichlorfon, but rapid degradation precluded accumulation of the material.

Metabolism in Rats. The metabolic products detected in the urine of rats after intraperitoneal injections of ^{32}P-labeled samples of trichlorfon (5 mg.), dichlorvos (0.5 mg.), or *O*-demethyl dichlorvos (1.7 mg.) are listed in Table V. The analyses were done with pooled samples of urine collected during the first 16 hours after treatment. Only trace amounts of trichlorfon and no dichlorvos were detected in the urine. Dimethyl phosphate was the major detoxification product in animals treated with trichlorfon; however, significant concentrations of two unidentified products, unknowns B and E, also were found, as were minor concentrations of *O*-demethyl derivatives and simpler products. Dimethyl phosphate was by far the predominant metabolite in the urine of rats treated with dichlorvos; only small amounts of radioactivity could be identified as *O*-demethyl dichlorvos, and no unknown products were found. An appreciable amount of the administered *O*-demethyl dichlorvos was excreted unchanged; the major metabolic products were phosphoric acid and methyl phosphate and an unidentified material that was chromatographically similar to unknown E.

Metabolism in Plants. The results of a comparative study of the metabolism of ^{32}P-labeled trichlorfon and dichlorvos in individual cotton leaves are shown in Table VI. In leaves treated with trichlorfon, the major metabolic products were unknown A and dimethyl phosphate; the parent material was essentially depleted after 2 days. The progressive loss of applied radioactivity probably could be attributed primarily to translocation of highly polar hydrolytic products to other areas in the plant. In leaves treated with dichlorvos, a great deal of the applied radioactivity could not be accounted for. Since most of this loss occurred immediately after treatment (53.6% after 1 hour), it probably was a result of volatilization of the dichlorvos from the treated leaves. The major metabolite of dichlorvos was dimethyl phosphate; only minor amounts of *O*-demethyl dichlorvos were detected.

Identification of Compounds. In the initial extracts, only two radioactive compounds were detected in the chloroform fractions. These cochromatographed in two dimensions with authentic samples of trichlorfon and dichlorvos, and, after recovery from TLC plates, gave similar I_{50} values in the antiChE assay. In addition, the product designated as trichlorfon responded positively to a modified Fujiwara test (Arthur and Casida, 1957; Fujiwara, 1916) for the trichloromethyl group; a negative reaction was obtained with the compound identified as dichlorvos.

All other radioactive materials in the initial extracts partitioned into the aqueous fraction. Following partial purifica-

Table VI. Relative Concentrations of Radioactive Compounds Obtained from Individual Cotton Leaves after Petiole Injection with 100 μg. of ^{32}P-Labeled Trichlorfon or Dichlorvos

Nature of Radioactivity	% of Dose					
	Trichlorfon			Dichlorvos		
	1 hr.	24 hr.	48 hr.	1 hr.	24 hr.	48 hr.
H_3PO_4 + methyl phosphate	0.6	6.4	7.2	0.9	1.1	2.2
Dimethyl phosphate	3.9	14.5	14.7	6.0	13.3	12.3
O-Demethyl trichlorfon	0.0	1.2	0.8	—	—	—
O-Demethyl dichlorvos	0.0	0.6	0.8	1.7	0.2	0.1
Unknown A	5.2	34.0	35.0	—	—	—
Trichlorfon	88.3	17.5	5.6	—	—	—
Dichlorvos	0.8	0.9	0.1	36.8	0.1	0.0
Unextractable	1.2	8.1	8.3	1.0	4.5	4.2
Lost	0.0	16.8	27.5	53.6	80.8	81.2

tion and concentration with PC, each radioactive zone isolated from different treated biological materials was tested to determine whether it included discernible conjugated materials. Each sample was incubated for 2 hours at 37° C. with β-glucuronidase and β-glucosidase (5 mg. of enzyme per ml., pH 4.4 phosphate buffer) and with 0.1 or 0.5*M* HCl, then partitioned with chloroform and analyzed. The acid treatments were ineffective in breaking the suspected conjugates, even after boiling for 5 minutes in some cases. The enzyme treatments caused no apparent change in the radioactive areas that were tentatively identified as inorganic phosphate, methyl phosphate, dimethyl phosphate, *O*-demethyl dichlorvos, and *O*-demethyl trichlorfon by cochromatography in the two PC solvent systems. The identification of dimethyl phosphate was supported by the results of column chromatography with the anion exchanger. Under the same experimental conditions, the elution profile of the metabolite was identical to that of the ^{32}P-labeled dimethyl phosphate that was prepared by the hydrolysis of dichlorvos. However, the question of whether this metabolite is produced by direct hydrolysis of the P—C bond or by hydrolysis after the rearrangement of trichlorfon to dichlorvos was not resolved.

The unknown A recovered from the plants and the unknown A recovered from insects appeared similar, since they had the same chromatographic properties in the two PC solvent systems used. This metabolite (from both sources) was not changed by β-glucuronidase; however, after incubation with β-glucosidase, 70 to 75% of the starting material was recovered as a single radioactive product (unknown D) that partitioned entirely into chloroform.

Unknown D is characterized as follows: The mobility on TLC suggests that the compound is less polar than trichlorfon, but slightly more polar than dichlorvos; it had definite antiChE activity (I_{50} $10^{-4}M$), but it could not be evaluated accurately because it is unstable in the buffer used for ChE assays; the half life at pH 7 was only 15 minutes; hydrolysis with equimolar concentrations of NaOH causes the formation of a single radioactive product, dimethyl phosphate; the reaction to the Fujiwara test is negative, which indicates that the $—CCl_3$ portion of the original trichlorfon molecule has been changed; retreatment of cotton leaves with 50 μg. of unknown D led to the formation of small concentrations (6.4% of the dose) of the original conjugate, unknown A, which suggests that the 1-hydroxy group is present and available for conjugation; and neither dichlorvos nor *O*-demethyl dichlorvos forms conjugates comparable to unknown A, thus eliminating those compounds as direct contributors to the structure. Whether unknown D is formed first from trichlorfon and then enters immediately into the conjugation reaction or is formed by the action of β-glucosidase on the conjugate has not been resolved. The properties suggest a compound comparable to trichlorfon; however, the available evidence is insufficient to allow assignment of a definite structure.

Unknown B, isolated from urine of rats after treatment with trichlorfon, was not altered by the action of β-glucosidase, but incubation for 2 hours with β-glucuronidase led to complete conversion to a chloroform-soluble form. Analysis of the chloroform fraction revealed two radioactive products; one was unknown D (57%), and the other satisfied all of the qualitative tests for trichlorfon (43%).

Unknown C, recovered from extracts of lygus bugs treated with trichlorfon, was not affected by treatment with β-glucuronidase, but was totally converted to a chloroform-soluble product by the action of β-glucosidase. A limited TLC study of this aglycone suggested that it was comparable to unknown D; however, insufficient amounts of the material were available for a careful characterization.

When the unknown E from the urine of rats injected with trichlorfon was treated with either enzyme, no chloroform-soluble radioactivity was isolated. It is possible that unknown E is a conjugate of a nontoxic metabolite with glucuronic acid or perhaps sulfuric acid; however, this was not established.

It is apparent that conjugation is an important mechanism for the detoxification of trichlorfon in animals and also in cotton plants. Since the conjugates formed resist acidic hydrolysis and are excreted rapidly by animals, potential hazards due to the toxic aglycone moieties are minimized.

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