be available in the Amdro bait at a time when foraging activity was reaching its peak after the hot daytime malaise. In addition, our data indicate that the toxicant is air and heat stable; therefore, storage for long periods of time should not be detrimental. This paper was not aimed at residue analysis or to determine breakdown products; however, we can say that AC 217,300 will decompose rapidly under normal environmental conditions.

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Reaction of Trichlorfon with Diazomethane

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The reaction between trichlorfon and diazomethane was reinvestigated. Methyl trichlorfon was the initial reaction product that under the influence of excess diazomethane was transformed into (2,2-dichloro-1-methoxyethenyl)phosphonic acid dimethyl ester. A mechanism for this reaction is suggested. In addition, application of this method is explored for the estimation of trichlorfon and desmethyltrichlorfon in samples by gas chromatography.

Trichlorfon, (2,2,2-trichloro-1-hydroxyethyl)phosphonic acid dimethyl ester, has been on the market for over 25 years. Various derivatization techniques including acylation (Anderson et al., 1966), methylation with diazomethane (Zitko and Sergeant, 1977; Akhtar, 1982), decomposition at the injector block (El-Refai and Giuffrida, 1965; Anderson et al., 1966; Pieper and Richmond, 1976), alkaline conversion (Zitko and Sergeant, 1977), and trisilylation (Sergeant and Zitko, 1979; Akhtar, 1982) have been explored as a means of identification and estimation of trichlorfon in water and biological samples.

The reaction between trichlorfon and diazomethane had been reported to yield a complex mixture (Zitko and Sergeant, 1977; Akhtar, 1982) that was not considered suitable for quantitative estimation of trichlorfon by gas chromatography. However, the methylation reaction had not been thoroughly investigated as a means of an analytical method. In this report the methylation reaction has been reinvestigated to see if the methylation technique can be an effective analytical method for estimation of trichlorfon and desmethyltrichlorfon by gas chromatography.

EXPERIMENTAL SECTION

Chemicals. Glass-distilled, pesticide-grade solvents were used as received. Diazomethane was prepared by the action of 50% KOH on *N*-nitroso-*N*-methylurea in ether (Schultz et al., 1971). **Caution:** Both nitrosomethylurea and diazomethane are highly toxic chemicals. Special care must be taken in handling these chemicals. Trichlorfon (I) and desmethyltrichlorfon (IV) were available from a previous study (Akhtar, 1982).

Reaction of Trichlorfon with Diazomethane. An aliquot (100 μL, 102.5 μg) of trichlorfon in acetone solution was placed in a centrifuge tube (15 mL). Solvent was removed under a slow stream of N_2, the residue was dissolved in methanol (0.5 mL), diazomethane (2.0 mL) of known concentration was added, and the whole mixture was mixed thoroughly on a vortex mixer for ≃45 s and then allowed to stand at room temperature (20 ± 1 °C). At different time intervals, centrifuge tubes, in duplicate, were removed and excess diazomethane and ether evporated under N_2. The residues were redissolved in hexane and the solvent was again removed under N_2. The residues were redissolved and taken to volume in hexane. Aliquots were analyzed on a gas chromatograph.

Extraction of Samples and Treatment with Diazomethane. Samples were extracted as detailed previously (Zitko and Sergeant, 1977; Akhtar, 1982). Extracts were dried over anhydrous $MgSO_4$, filtered, concentrated, and transferred into a centrifuge tube (15 mL), solvent was removed under N_2, and the residue was dissolved in 0.5 mL of methanol, treated with diazomethane (2 mL, concentration 20 mg/mL), and then allowed to stand overnight (≃16 h) at room temperature. After usual workup as detailed above, aliquots were analyzed on a GC.

Analysis of Reaction Mixtures. The reaction mixtures were analyzed on a Perkin-Elmer gas chromatograph equipped with a 1.07 m × 4 mm (i.d.) galss column packed with 3% SE-30 on Chromosorb WHP, 80–100 mesh, and an electron capture detector (^{63}Ni). Other operating temperatures were 175, 150, and 400 °C for injector, oven, and

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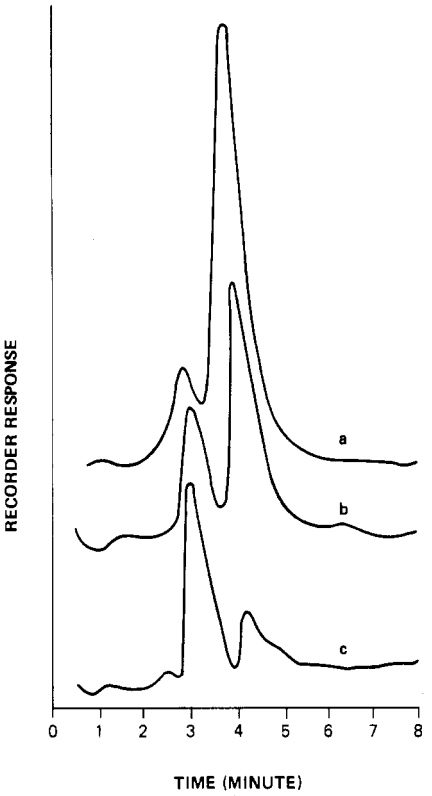

Figure 1. Chromatograms of hexane-soluble products of a reaction between trichlorfon and diazomethane carried out with different concentrations of diazomethane for 30 min: (a) 6 mg/mL; (b) 9 mg/mL; (c) 15 mg/mL. The concentration of diazomethane was estimated by assuming a 70% yield from the precursor. The reaction mixture consisted of 102.6 μg of trichlorfon, 0.5 mL of methanol, and 2 mL of ethereal diazomethane.

detector, respectively. The high detector temperature of 400 °C was necessary for good sensitivity. The carrier gas was 5% methane in argon, and the flow rate was 30 mL/min both through the column and as the makeup gas.

Identification of Products. The reaction products were identified on a GC–MS system consisting of a Varian 3700 GC connected to a ZAB-2F high-resolution mass spectrometer. The GC was equipped with 1.85 m × 4 mm (i.d.) glass column packed with 3% SE-30, operated at 150 °C, and a flame ionization detector (FID). The mass spectrometer was run at a resolution of 10 000.

RESULTS AND DISCUSSION

The reaction between trichlorfon and diazomethane produced two hexane-soluble products, while trichlorfon itself is very poorly soluble in hexane. The proportion of the two products varied at different diazomethane concentrations and is shown in Figure 1. At low concentration of diazomethane, a compound with retention time of 4.28 min (II) was the major product (Figure 1a). However, as the concentration of diazomethane was increased, the amount of a compound with a retention time of 3.17 min (III) gradually increased. Thus, the production of III was dependent on the concentration of diazomethane; i.e., the higher the concentration of diazomethane, the greater the yield of III.

The identity of products II and III was established by GC–MS. Compound II (retention time 4.28 min) was identified as (2,2,2-trichloro-1-methoxyethyl)phosphonic acid dimethyl ester (M_r 270). The mass spectrum did not exhibit a molecular ion peak. It had peaks at m/e 205 (weak) with an isotope pattern for two chlorine atoms $[Cl_2C{=}CH(OCH_3)P(OCH_3)OH]^{+\cdot}$, a strong peak at m/e 161 $[Cl_3C{-}CH(OCH_3)]^{+\cdot}$, and a strong peak at m/e 126 $[Cl_2C{=}CH(OCH_3)]^{+\cdot}$. This spectrum was identical with that published by Zitko and Sergeant (1977) for "methyltrichlorfon".

A GC–MS of III, the compound with a retention time of 3.17 min, exhibited major peaks at m/e 236, 234, 233, 205, and 169. The peak at m/e 234 (weak) was attributed to the parent molecular ion (M^+). The exact mass of the peak at m/e 234 was found to be 233.9629, which was 6 ppm higher than that calculated for $C_5H_9O_4PCl_2$. The $(M + 2)^+$ peak at m/e 236 had an exact mass of 235.9612. Mass of 233 may be due to loss of a hydrogen atom from the molecular ion. The peaks at m/e 205 and 169 were assigned to $[Cl_2C{=}CH_2P(O)(OCH_3)_2]^{+\cdot}$ and $[ClC{=}CHP(O)(OCH_3)]^{+\cdot}$, respectively. On the basis of the mass spectral data, the identity of III was established as (2,2-dichloro-1-methoxyethenyl)phosphonic acid dimethyl ester. The spectrum of III was identical with that recorded for authentic III prepared by the reaction of II with 20% methanolic KOH (Fricke and Georgi, 1963). An identical mass spectrum was reported for "methoxy DDVP" by Zitko and Sergeant (1977).

When Fricke and Georgi (1963) treated I with excess diazomethane, the major product (65%) was II, but III was also formed in approximately 34% yield. They also obtained III in 90% yield from a reaction between II and methanolic KOH, a dehydrochlorination step. This suggested that III was the product of a base action on II. Similarly, Zitko and Sergeant (1977) observed the production of III when I was allowed to react with diazomethane for 24 h. Although all these researchers used excess diazomethane, no explanation was advanced on its influence on the rate of production of III.

In the present study, attempts were made to remove residual alkali, if any, from the ethereal solution. The ethereal diazomethane was distilled slowly and collected over anhydrous $MgSO_4$. Reactions between this diazomethane and I did not alter the rate of production of III appreciably. Only a slight decrease in the amount of III was observed. Similarly, III was produced quantitatively when II was treated with excess diazomethane. Thus, it appears that the dehydrochlorinating agent in the reaction is diazomethane itself. This is the first example of dehydrochlorination by diazomethane. The intermediate nature of II in the reaction scheme is also established.

Distribution of II and III in hexane extracts of reactions carried out over a period from 15 min to 16 h is shown by chromatograms in Figure 2. It is evident that after 15 min, as expected, II was the major product with a small but detectable amount of III. After 90 min, the major product was III, and II was also present. Compound II could still be detected after 3 h but not after 16-h reaction. It should be pointed out that on numerous occasions, II was not detected after a 5-h reaction. The clean conversion of I, in the presence of diazomethane, to III via II provides an additional means of identification and estimation of I by GC.

In recent months, silylation has been the method of choice for the estimation of I (Sergeant and Zitko, 1979; Akhtar, 1982). A comparison of the silylation and methylation methods showed the following: silylation was efficient and reproducible when the solution to be silylated was free of water; however, traces of water produced erratic results. GC analyses were convenient when traces of the silylating reagent (Tri-Sil) and volatile silyl byproducts were removed. The presence of traces of Tri-Sil in the solution to be analyzed caused deterioration of the column and required constant "recharging" with Silyl-8. Methylation, on the other hand, was very efficient and repro-

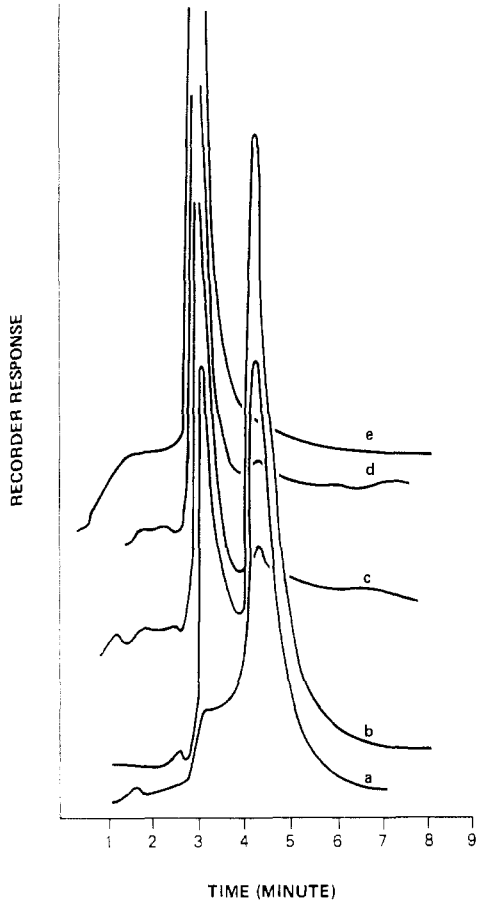

Figure 2. Chromatograms of hexane-soluble products of reactions carried out for (a) 15 min, (b) 30 min, (c) 90 min, (d) 180 min, and (e) 16 h. The reaction mixture consisted of 102.6 μg of trichlorfon, 0.5 mL of methanol, and 2 mL of ethereal diazomethane (15 mg/mL).

ducible for water-free samples. Even though traces of diazomethane lowered the sensitivity of the detector, the problems with diazomethane were not as pronounced as those observed with silylation. In general, both of these methods are efficient provided special precautions are taken to remove traces of water prior to reaction and traces of reagents prior to GC. From the simplistic point of view, the methylation technique was preferable and cheaper.

At present, the only known method for identification and estimation of desmethyltrichlorfon (IV) by GC involves methylation followed by silylation prior to analysis (Akhtar, 1982). The methylation technique, as detailed above, was extended to include detection and quantitation of IV by GC. Treatment of IV with excess diazomethane produced III in 89–92% yield and afforded a reaction mixture that was clean for GC analysis. Thus, it appears that methylation of IV prior to analysis by GC would be the preferred method for its detection and estimation.

The methylation technique was used for identification and estimation of I and IV in samples of water, buffer, and enzyme incubates. Extracts, after appropriate workup, were treated with excess concentrated diazomethane and analyzed for III by GC. Recovery of I and IV ranged

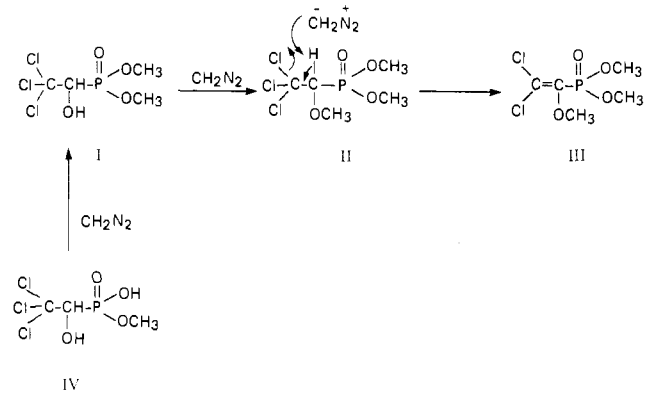

Figure 3. Reaction of trichlorfon (I) and desmethyltrichlorfon (IV) with diazomethane.

between 78 and 85% (for 0.01–0.1 ppm), which was better than those obtained by the silylation technique (Akhtar, 1982).

The reaction of excess diazomethane with I and IV produced (2,2-dichloro-1-methoxyethenyl)phosphonic acid dimethyl ester (III, methoxy-DDVP) via (2,2,2-trichloro-1-methoxyethenyl)phosphonic acid dimethyl ester (II, methyltrichlorfon). The first step is the simple methylation of hydroxy to methoxy followed by diazomethane-catalyzed dehydrochlorination to III as shown in Figure 3. Recently, Look and White (1981) demonstrated efficient dehydroclorination of 1-acyl derivatives of trichlorfon with 1,5-diazabicyclo[5.4.0]undec-5-ene (BDU). The methylation by diazomethane can be used successfully as the derivatization technique for trichlorfon (I) and desmethyltrichlorfon (IV) prior to their detection and quantitation as (2,2-dichloro-1-methoxyethenyl)phosphonic acid dimethyl ester (III) by gas chromatography.

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