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Methods for the Determination of Total Terbufos- [Phosphorodithioic Acid, *S*-(*tert*-Butylthio)methyl *O,O*-Diethyl Ester] Related Residues in Dermal Exposure Pads and Air-Collection Tubes and Related Alkyl Phosphate Metabolites in Urine

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Analytical methods are described that were used to estimate dermal and respiratory exposure of farmers to terbufos [phosphorodithioic acid, *S*-(*tert*-butylthio)methyl *O,O*-diethyl ester] while planting corn and applying COUNTER (American Cyanamid Co.) 15-G systemic insecticide–nematicide. Total terbufos-related compounds were extracted with acetone from both the gauze dermal patches and the XAD-2 resin used in air-collection tubes. These compounds were all oxidized with *m*-chloroperbenzoic acid to terbufos oxygen analogue sulfone, which was analyzed with a gas chromatograph equipped with a flame photometric detector. For the dermal pads, the validated limit of sensitivity was 0.2 μg/pad (5 ng/cm^2) and for the air samples it was 0.25 μg/tube. Recoveries from the dermal exposure pads ranged from 73 to 100% at fortification levels of 5–500 ng/cm^2, while recoveries from the air tubes ranged from 77 to 125% at levels of 0.4–500 ng/L. Terbufos-related alkyl phosphate esters in urine were determined by derivatization with pentafluorobenzyl bromide and gas chromatographic analysis using a flame photometric detector. Average recoveries of the three alkyl phosphates ranged from 85 to 113% at concentrations of 0.1–1.0 μg/ml.

COUNTER systemic insecticide–nematicide is an organophosphorus compound that has been commercially used on corn since 1975 to control a wide range of soil insects and nematodes. For corn use, it is formulated on montmorillonite clay and contains 15% active ingredient [terbufos: phosphorodithioic acid, *S*-(*tert*-butylthio)methyl *O,O*-diethyl ester]. To determine the level of exposure to farm workers loading and applying COUNTER 15-G at planting time, analytical methods were needed to estimate respiratory and dermal exposures and the degree of absorption by the farmer's body. Air-collection tubes filled with XAD-2 resin were used to estimate respiratory exposure while gauze pads were employed for dermal exposure. Urinary alkyl phosphate analysis was used to obtain an indication of absorption of the chemical.

Residue methods for the determination of total terbufos-related residues in various crops have previously been reported by Orloski (1980). These methods employed an oxidative step that would convert terbufos and its five potential, toxic metabolites (terbufos sulfoxide, terbufos sulfone, terbufoxon, terbufoxon sulfoxide, terbufoxon sulfone) to terbufoxon sulfone, which is determined gas chromatographically to give a total residue concentration. During loading and application of COUNTER 15-G, worker exposure would expect to be only to terbufos itself. However, to ensure a complete exposure appraisal, the dermal pads and air-collection tubes were analyzed for total terbufos-related compounds. Recoveries to validate the methods for pads and tubes were run with both terbufos and terbufos sulfoxide. Terbufos sulfoxide is the first oxidative soil metabolite and a representative of the other potential metabolites.

In addition to describing the methods used to estimate dermal and respiratory exposure, this paper also describes a method for analyzing urine for the presence of the diethyl phosphate hydrolytic metabolites. These analyses would give an indication of the absorption of terbufos due to the farm worker's total exposure.

EXPERIMENTAL PROCEDURES

Reagents. All solvents used were Burdick and Jacksons's distilled in glass brand. The oxidant, *m*-chloroperbenzoic acid, was purchased from Aldrich Chemical Co. A 10% solution of oxidant was prepared immediately before use by dissolving 1.0 g in 10 mL of methylene chloride. A 10% solution of polyethylene glycol 400 (PEG-400), USP, was prepared by diluting 1 mL of PEG-400 to 10 mL with acetone. Saturated solutions of sodium

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sulfite and sodium bicarbonate (both reagent grade) were made with distilled water.

Pentafluorobenzyl bromide was used as received from Regis Chemical Co. for derivatizing the alkyl phosphates during the urine analysis. *Caution*! Extreme care should be taken when using this derivatizing reagent since it is a potent lachrymator. All work, including disposal of derivatized samples, must be done in a hood. Rubber gloves and ventless goggles should be worn whenever this reagent is handled.

XAD-2 resin, obtained from Applied Science Laboratory, Inc., was used as received to fill the air-collection tubes. The GC packings (3% OV-210 and 5% Carbowax 20M on 80/100 mesh Supelcoport) were obtained from Supelco, Inc.

Apparatus. A Tracor Model 550 gas chromatograph equipped with a flame photometric detector (526-nm phosphorus filter) was employed for all of the analyses. For the analysis of alkyl phosphate urinary metabolites, a 184 cm × 2 mm i.d. glass column was packed with 3% OV-210 on 80/100 mesh Supelcoport. The oven temperature was 150 °C, and the helium flow rate was 15 mL/min. For total terbufos-related residues the column was a 92 cm × 2 mm i.d. glass column packed with 3% OV-210 on 80/100 mesh Supelcoport. Vapor-phase deposition of Carbowax 20M was made to this column as described by Ives and Guiffrida (1970). The oven temperature was 180 °C, and the helium flow rate was 30 mL/min. The remainder of the chromatographic conditions were identical for both types of assays: inlet, 220 °C; outlet, 240 °C; detector, 220 °C; air flow, 100 mL/min; hydrogen flow rate, 150 mL/min.

For air samples, a stainless-steel tube (155 mm × 8 mm i.d.) was packed with 1.7 g of XAD-2 resin. Plugs of glass wool were inserted at each end to hold the resin in the tube, and parafilm was used to seal the ends until use. MSA portable pumps (Model S, Precision Scientific) were connected to the sample tubes by use of TYGON tubing. The flow rates were calibrated to pull 1.5 L of air/min through the resin.

The dermal exposure patches were constructed as suggested by Durham and Wolfe (1962) using 4 × 4 in. squares of 16 layers of nonsterilized absorbent gauze (Curity 1473), 3 MM Whatman chromatography paper (previously extracted with acetone), and glassine weighing paper (Schleider & Schnell). The pads were stapled on all four sides and 2-in.-wide masking tape was used to tape the edges so as to leave a gauze exposure surface of exactly 2.5 × 2.5 in. (40.3-cm^2 area). The glassine paper served as the back of the pad or skin side.

Additional equipment, used for urinary alkyl phosphate analysis, included an International Model CL clinical centrifuge and a Supelco Inc. Blok heater and 5-mL graduated Reacti-Vials. Polypropylene plastic bottles were used for urine collection and storage.

Analysis of Air-Collection Tubes. To determine respiratory exposure, air was pulled through a stainless-steel tube filled with XAD-2 resin. These tubes were designed to measure respiratory exposure by collecting dust and vapors in the farmer's breathing zone. At the end of the exposure period the tubes were sealed with parafilm at each end and stored in a freezer at −25 °C until analyzed.

For analysis, the contents (resin plus glass wool) of the air-collection tube were transferred to a 15 mm i.d. chromatographic column (Kontes Glass, 250-mL reservoir) with a glass wool plug at the bottom. The contents in the column were pressed down lightly with a glass rod. The empty collection tube was then rinsed with 25 mL of acetone into the chromatographic column. The column stopcock was opened, and the eluate was collected in an evaporation flask. An additional 75 mL of acetone was added to the column, and the eluate was collected with the first 25-mL rinse. One drop of a 10% PEG-400 "keeper" solution was added to the evaporation flask, and the solvent was evaporated just to dryness on a rotary vacuum evaporator and a water bath kept at 35 °C.

To determine total terbufos-related compounds, oxidation was performed by adding 20 mL of methylene chloride and 1 mL of freshly prepared *m*-chloroperbenzoic acid reagent to the extract. After 15 min, the methylene chloride was gently shaken for 15 s each time with successive 25-mL washes of a saturated sodium sulfite solution, a saturated sodium bicarbonate solution, and two distilled water washes. The methylene chloride layer was then evaporated to dryness and the residue dissolved in 1.0 mL of acetone for GC analysis using the 92 cm × 2 mm i.d. 3% OV-210 column (Carbowax treated).

A GC "standard" was prepared by oxidizing a 20-μg amount of terbufos as described above and diluting the final solution to 20 mL with acetone to give a concentration equivalent to 1 μg/mL. The retention time for the oxidized product, terbufoxon sulfone, was approximately 2 min with the chromatographic conditions described. Alternate 5-μL injections of oxidized standard and sample were made, and the average response of the standard injected before and after a sample was used for quantitation. If the sample peak went off scale, appropriate dilutions were made to bring the peak on scale.

Analysis of Dermal Exposure Pads. A total of 12 gauze exposure patches were attached both inside and outside of each farmer's coveralls to measure potential dermal exposure. Immediately after the exposure period, the pads were removed from the clothing and stored in a freezer at −25 °C until analyzed.

The masking tape border of each exposure patch was cut off and discarded along with the glassine paper backing. The remaining exposed gauze pad plus chromatography paper was cut into four pieces into an 8-oz bottle and shaken for 30 min with 100 mL of acetone. The acetone extract was decanted into a 250-mL evaporation flask. The extraction bottle and sample were washed with two 25-mL portions of acetone that were combined with the first extract. One drop of a 10% PEG-400 solution was added, and the acetone was evaporated just to dryness. The oxidation procedure, described for analysis of air-collection tubes, was followed to determine total terbufos-related compounds. The final residue was dissolved in 1.0 mL of acetone for GC analysis, using the same column and standard as used for the analysis of air samples.

Analysis of Urine. Urinary alkyl phosphate metabolite analysis was performed to measure the degree of absorption of terbufos by the exposed farmers. Twenty-four hour urine samples were collected from each of the farmers and stored in a freezer until analyzed. Analysis, by a method similar to that reported by Reid and Watts (1981), was performed for diethyl phosphate (DEP), diethyl phosphorothioate (DETP), and diethyl phosphorodithioate (DEDTP), potential urinary metabolites of terbufos.

After the sample was allowed to thaw, a 1-mL aliquot of the mixed urine sample was pipetted into a 15-mL centrifuge tube and 9 mL of acetonitrile was added. The sample was mixed and then centrifuged for 10 min at approximately 2500 rpm to separate the precipitated salts. The supernatant was decanted into a 5-mL Reacti-Vial, evaporating to near-dryness between 5-mL portions. A

Table I. Recovery of Total Terbufos-Related Compounds from XAD-2 Air-Collection Tubes

amt added, μg	vol of air, L	fortification level, ng/L	% recovery terbufos	% recovery terbufos sulfoxide[a]
0.25	720	0.35	77, 130	80
0.25	500	0.50	96 (78–125)[b]	
1.0	720	1.4	115	108
1.2	240	5.0	96, 112	
4.0	90	44.4		96
12.0	240	50.0	91, 95	
60.0	120	500	96 (86–111)[c]	

[a] Terbufos sulfoxide. [b] Average and range of six recoveries. [c] Average and range of seven recoveries.

Blok heater, set at 90 °C, and a stream of nitrogen were used to remove the water and evaporate the solvent to dryness. The residue was dissolved in 1.0 mL of acetonitrile, and 50 mg of anhydrous potassium carbonate and 50 μL of pentafluorobenzyl (PFB) bromide were added. The vial was capped and heated for 2 h at 90 °C. After cooling, the derivatized sample was ready for GC analysis using the 184 cm × 2 mm i.d. 3% OV-210 column.

A GC standard mixture was prepared by derivatizing 1 mL of an acetonitrile solution containing 0.2 μg/mL of each compound as described above. Alternate 5-μL injections of derivatized standard and sample were made, and the average response of the standard injected before and after a sample was used for quantitation. Retention times of the three PFB derivatives were 3.8 min for DEP, 4.4 min for DEDTP, and 5.6 min for DETP. A 10-min interval was used between injections to allow for complete elution of extraneous compounds from the urine samples, which caused erratic chromatographic degradation of the derivatives in the following standard injection when insufficient time was allowed.

RESULTS AND DISCUSSION

Recoveries performed with the air-collection tubes were conducted by adding known amounts of standard to the glass wool plug at one end of the XAD-2 tube. The air pump was connected to the other end of the tube, and the appropriate amount of air was drawn through the tube. The recoveries obtained for terbufos and terbufos sulfoxide are shown in Table I. The validated limit of sensitivity is 0.25 μg/tube or 0.35 ng/L (μg/m^3) for an 8-h collection time at 1.5 L/min. Recoveries ranged from 77 to 130% (overall average of 98%) for fortification levels of 0.35–500 ng/L. Typical chromatograms for this procedure are shown in Figure 1.

Recoveries were also conducted using a "split" collection tube where the XAD-2 resin was separated with a plug of glass wool into two equal segments. After the terbufos was added to one end of the tube and the appropriate amount of air was drawn through, the two portions of XAD-2 resin were analyzed separately. Recoveries ranged from 88 to 125% (0.5–500 ng/L; 0.25–60 μg total) in the first half of the tube with no detectable terbufos found in the second half. Stability studies were conducted at room temperature and showed that terbufos is not broken down for at least 2 weeks when added to XAD-2 resin.

Efficiency of trapping terbufos vapors by the XAD-2 collection tubes was further validated by using an apparatus similar to that described by Spencer and Cliath (1969) with their vapor pressure work. A plastic tee, was attached to the end of a saturator column filled with terbufos-coated sand. Two XAD-2 collection tubes were then connected in series followed by an MSA pump that pulled air through at 1.5 L/min. The plastic tee provided

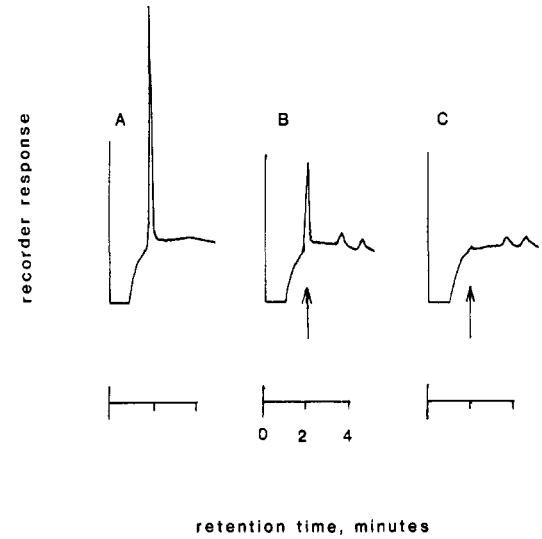

Figure 1. Typical chromatograms for determining total terbufos-related compounds in air: (A) oxidized terbufos standard, 5 ng injected; (B) control air tube, fortified at 0.35 ng/L, equivalent of 3.6 L injected, 130% recovered; (C) control air tube, equivalent of 3.6 L injected.

Table II. Recovery of Total Terbufos-Related Compounds from Dermal Exposure Pads

amt added, μg	fortification level,[a] ng/cm^2	% recovery terbufos	% recovery terbufos sulfoxide[b]	% recovery mixture[c]
0.2	5.0	96		
0.5	12.4	84, 100	90	86
0.8	20	100		
5.0	124			74, 90
20.0	496	78	73	86

[a] Exposed surface area of dermal pad was 40.3 cm^2. [b] Terbufos sulfoxide [c] A 4:1 mixture of terbufos and terbufos sulfoxide.

makeup gas to the pump since flow through the saturation column was only 3 mL/min. After a collection time of 4 h, analysis showed no detectable terbufos in the second XAD-2 collection tube (less than 0.5% of that found in the first tube).

For the analysis of total terbufos-related compounds on dermal exposure patches, recoveries of terbufos, terbufos sulfoxide, and 4:1 mixtures of the two compounds were run by adding, dropwise, an acetone solution of the standard to a dermal patch, allowing the acetone to evaporate, and analyzing the pad as previously described. Recoveries are shown in Table II and ranged from 73 to 100% (overall average of 87%) at fortification levels of 5–496 ng/cm^2. The validated sensitivity of the method is 0.2 μg/pad or 5 ng/cm^2. Typical chromatograms from these analyses are shown in Figure 2. Storage stability of the dermal pads was checked by adding a 4:1 mixture of terbufos and terbufos sulfoxide to dermal pads (at 124 ng/cm^2, 40 μg total) and storing in a freezer at −25 °C. At 2- and 8-week intervals the recoveries were 91 and 78%, respectively, showing good storage stability for at least 2 months.

Analysis of alkyl phosphate hydrolytic metabolites in urine has been widely used for monitoring exposure to organophosphorus pesticides. Most of the methods employ an alkylation reaction with a diazoalkane reagent to produce a volatile ester suitable for gas chromatographic analysis (St. John and Lisk, 1968; Shafik and Enos, 1969; Shafik et al., 1973). However, a simple and rapid method was recently published by Reid and Watts (1981) that minimizes the variability of the older methods and produces a single reaction product with DETP.

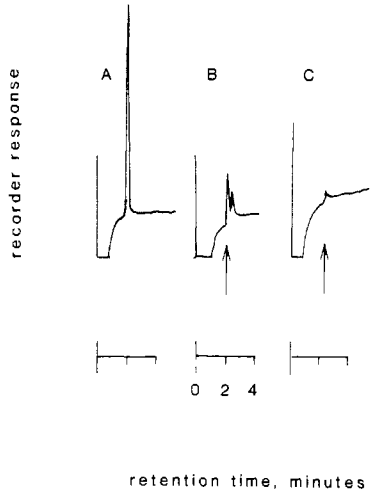

Figure 2. Typical chromatograms for determining total terbufos-related compounds on dermal pads: (A) oxidized terbufos standard, 5 ng injected; (B) control pad, fortified with 5 ng/cm^2, equivalent of 0.20 cm^2 injected, 96% recovered; (C) control pad, equivalent of 0.20 cm^2 injected.

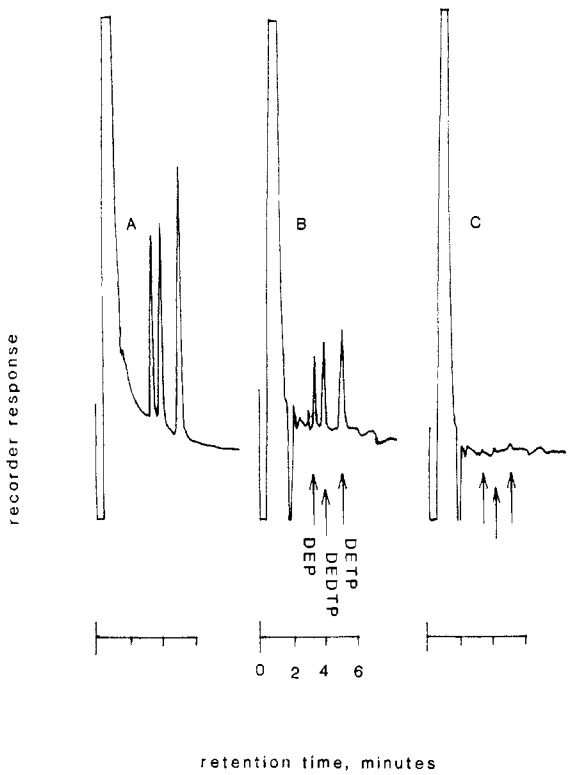

Figure 3. Typical chromatograms for determining DEP, DETP, and DEDTP residues in urine: (A) derivatized standard mixture, 1 ng of each injected; (B) control urine, fortified with 0.1 ppm each compound, equivalent of 5 mg injected, recovery of 85% for DEP, 72% for DETP, and 86% for DEDTP; (C) control urine, equivalent of 5 mg injected.

Contrary to the data shown by Reid and Watts, an OV-210 chromatographic column gave excellent separation between the PFB esters of DETP and DEDTP. With a freshly conditioned GC column, however, separation of the DEP and DETP derivatives was not complete. After several alternating injections of a derivatized urine sample and standard were made, the separation increased to that shown in Figure 3, where typical chromatograms are presented. After the initial injections of urine samples, this separation was maintained.

Table III. Recovery of Alkyl Phosphates from Urine

fortification level, μg/mL	no. of replicates	% av recovery DEP	DETP	DEDTP
0.1	5	113 (85–138)[a]	87 (66–106)	89 (83–94)
0.2	3	98 (78–119)	100 (98–104)	92 (87–98)
0.5	3	98 (76–120)	97 (93–100)	88 (82–93)
1.0	8	85 (78–90)	94 (88–99)	86 (78–95)

[a] Range of recoveries given in brackets.

Table IV. Storage Stability of DEP, DETP, and DEDTP Residues in Urine (1 ppm Fortification Level)

compd	% recovery 0 day	8 weeks: −25 °C	10 weeks RT[a]	10 weeks −25 °C
DEP	82	139	110	110
DETP	94	98	105	103
DEDTP	86	91	73	81

[a] Room temperature.

A summary of the recoveries conducted with the three potential urinary metabolites is presented in Table III. The validated sensitivity of the method is 0.1 ppm for each compound. Average recoveries over the fortification range of 0.1–1.0 ppm were 96% for DEP, 94% for DETP, and 88% for DEDTP. Urine, fortified with 1.0 ppm of each compound, was stored both at room temperature and in a freezer to determine storage stability. Results, as shown in Table IV, show that all three compounds are stable in urine for at least 10 weeks either at room temperature or at −25 °C.

These methods have been successfully used for samples from a farmer exposure study recently conducted with COUNTER 15-G. Results from this exposure study are reported in a separate paper (Devine et al., 1986).

Registry No. DEP, 598-02-7; DETP, 2465-65-8; DEDTP, 298-06-6; terbufos, 13071-79-9; pentafluorobenzyl bromide, 1765-40-8; terbufos sulfoxide, 10548-10-4.

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