Gas-Liquid Chromatographic Determination of Iodofenphos and Several Related Compounds in Tissues and Urine of Cattle

Marcellus C. Ivey* and Delbert D. Oehler[1]

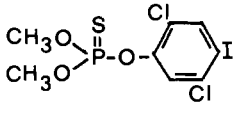
A gas chromatograph equipped with a flame photometric detector provided a highly sensitive method of determining residues of iodofenphos (CIBA C-9491) (*O*-(2,5-dichloro-4-iodophenyl) *O,O*-dimethyl phosphorothioate), its oxygen analogue, deiodinated iodofenphos, the deiodinated oxygen analogue, and ronnel in cattle tissues. Free and conjugated residues of two phenolic metabolites of iodofenphos (2,5-dichlorophenol and 2,5-dichloro-4-iodophenol) were determined after silylation by gas chromatography with an electron capture detector. With extraction and cleanup, 0.005 ppm of iodofenphos, 0.002 ppm of its photodecomposition product, 0.01 ppm of the oxygen analogue of iodofenphos, 0.004 ppm of its photodecomposition product, 0.003 ppm of ronnel, 0.01 ppm of 2,5-dichlorophenol, and 0.002 ppm of 2,5-dichloro-4-iodophenol could be detected. Recoveries of the five phosphorus compounds were 70–98%. Recoveries of the two phenolic compounds were 50–98%.

Iodofenphos (CIBA C-9491) (*O*-(2,5-dichloro-4-iodophenyl) *O,O*-dimethyl phosphorothioate) is a pesticide that effectively controls a variety of ticks on livestock (Drummond et al., 1972; Mount et al., 1971). It also controls stable flies, *Stomoxys calcitrans* (L.) (Campbell and Hermanussen, 1971). In addition, iodofenphos is a systemic insecticide that controls cattle grubs, *Hypoderma lineatum* (de Villers) (Drummond et al., 1970, 1971). A method of determining the residues of iodofenphos and its principal metabolites was therefore needed.

During the preliminary development of the analytical procedure, we found that exposure of the standard solution of iodofenphos in hexane to fluorescent light caused the development of a peak other than that for iodofenphos, which had a shorter retention time on the gas chromatogram. In further investigations, we found that iodofenphos in a hexane solution exposed to sunlight for 6–8 h completely decomposed to a product that gave only this new peak on the chromatogram. The oxygen analogue did likewise in 8–10 h of sunlight. We subsequently found that iodofenphos in carbon tetrachloride in sunlight (8–10 h) completely decomposed and produced a peak on the chromatogram with the retention time of ronnel; the exposure also produced a molecular iodine color in the carbon tetrachloride. Analytical standard solutions of the deiodinated iodofenphos and its oxygen analogue gave peaks on the chromatograms with the same retention times and response as the products that formed in sunlight from the parent compounds. Therefore, we concluded that these photodecomposition products were possible metabolites. The so-called photodecomposition products of iodofenphos and its oxygen analogue are the molecules that have lost the iodine atom in the 4-ring position. The only difference between iodofenphos and ronnel is a chlorine or iodine atom in the 4-ring position. For this reason, ronnel was included in the method. Also, in manufacture of iodofenphos, some ronnel might be formed as an impurity.

The most likely phenolic metabolites were 2,5-dichlorophenol and 2,5-dichloro-4-iodophenol. Also, subsequent treatment of an Angora goat with iodofenphos produced a small amount of 4-iodophenol only in the urine. Finally, the 2-chloro- and 5-chlorophenols were quite volatile, and the detector response was not very sensitive so their presence was not detected in the treated goat. We therefore concluded that only the two primary phenols (2,5-dichlorophenol and 2,5-dichloro-4-iodophenol) should be included in the procedure.

Methods were developed for determining residues of iodofenphos (I), the oxygen analogue of iodofenphos (II), deiodinated iodofenphos (III), the deiodinated oxygen analogue (IV), ronnel (V), and two possible phenolic metabolites—2,5-dichlorophenol (VI) and 2,5-dichloro-4-iodophenol (VII)—in the body tissues of cattle. These



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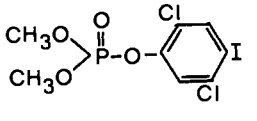

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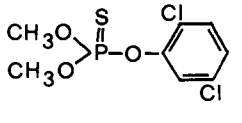

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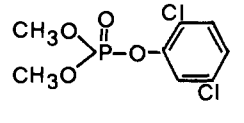

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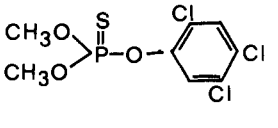

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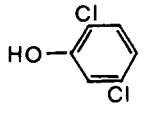

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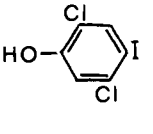

(VII)

methods make use of a gas chromatograph equipped with either a flame photometric detector operating in the phosphorus mode (for determining residues of the

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phosphorus compounds) or an electron capture (EC) detector (for determining the phenolic metabolites after silylation).

EXPERIMENTAL SECTION

Reagents and Equipment. All solvents were redistilled in glass. The adsorbents used were Florisil, as received plus 10% water added and allowed to equilibrate, and Woelm acid washed alumina as received. The chromatographic columns were Kontes technical glassware drawing No. 11416-B with 24/40 joints and Mohr burets. The gas chromatographs were a Micro-Tek Model 160 or equivalent equipped with a Tracor flame photometric detector operating in the phosphorus mode and a Beckman GC-5 or equivalent equipped with an EC detector.

Gas Chromatography. For the compounds containing phosphorus, a borosilicate glass column, 4 mm i.d. × 1.22 m, filled with 5% OV-1 coated Gas-Chrom Q, 80–100 mesh, was used. Carrier gas was prepurified nitrogen adjusted to a flow rate of 90 ml/min (exhaust). The column was heated isothermally to 205 °C, the injector to 260 °C, and the detector to 170 °C. Hydrogen and oxygen flowing to the detector were adjusted to 200 and 27 ml/min, respectively. At these conditions, the retention times for the photodecomposition product of iodofenphos, ronnel, and iodofenphos were about 53 s, 1.4 min, and 3 min, respectively; those for the photodecomposition product of the oxygen analogue and the oxygen analogue were about 48 s and 2.4 min, respectively. The two groups of materials would not separate on the gas chromatograph, but this was not critical since they were completely separated on the cleanup column.

For the phenolic metabolites, a stainless steel column, $^1/_8$ in. i.d. × 6 ft, filled with 5% DC-200 coated Gas-Chrom Q, 80–100 mesh, was used. Carrier gas was helium adjusted to a flow rate of 40 ml/min (exhaust). The column was heated isothermally to 145 °C, the injector to 210 °C, and the detector to 260 °C. Gases flowing to the detector were discharge helium at 70 ml/min and carbon dioxide at 8–10 ml/min. (The electrons of the EC detector are produced by an electrical current in a carbon dioxide–helium atmosphere.) At these conditions, the retention times for 2,5-dichlorophenol and 2,5-dichloro-4-iodophenol were about 2.08 and 11.28 min, respectively.

Extraction of Phosphorus Compounds from Fat. A 20-g sample was blended in a Waring Blendor with 50–60 g of anhydrous sodium sulfate and 125 ml of hexane. The mixture was transferred to a 600-ml beaker and heated on a hot plate or steam bath to near boiling; it was then transferred onto a folded filter paper and filtered into a 500-ml Erlenmeyer flask. The blender, beaker, and filter were washed with an additional 150–200 ml of hot hexane. A Snyder column was attached to the flask, and the extract was concentrated by distillation to 150 ml, cooled to room temperature, and transferred to a 500-ml separatory funnel by using 50 ml of hexane to make the transfer. After 100 ml of hexane was added to a second separatory funnel, the fat solution was extracted four times with 50-ml portions of acetonitrile; each time the acetonitrile was drained into the second separatory funnel and shaken with the hexane. The acetonitrile extracts were combined in a 300-ml Erlenmeyer flask and concentrated to a volume of 10 ml by distillation through a Snyder column. Seventy-five milliliters of hexane was added through the Snyder column and again concentrated to about 5 ml to remove the acetonitrile. The flask was stoppered and held for the chromatographic cleanup column. For fat samples, the hexane solutions remaining after the acetonitrile extraction were transferred into a tared flask, the solvent was evaporated, and the fatty residue was weighed and recorded as the weight of sample; thus, we could base the analysis on extracted fat.

Extraction of Phosphorus Compounds from Muscle, Heart, Liver, Kidney, Spleen, and Brain. A 20-g sample of muscle, heart, liver, kidney, spleen, or brain was blended with 125 ml of acetone. The mixture was transferred to a 600-ml beaker and filtered through a folded filter paper into a 500-ml Erlenmeyer flask. The blender, beaker, and filter were washed with another 100–125 ml of acetone. The filter paper and mass were returned to the blender, extracted with hexane, and filtered into a 300-ml Erlenmeyer flask. The acetone extract was concentrated to a volume of about 20–30 ml by distillation through a Snyder column and cooled to room temperature. The column was removed, and the corresponding hexane extract was combined with the acetone extract, the column was attached to the flask, and the extract was again concentrated by distillation to about 50 ml, cooled to room temperature, and transferred to a 500-ml separatory funnel by using 100 ml of hexane to make the transfer. After 100 ml of hexane was added to a second separatory funnel, any water present in the first funnel was drained through the second funnel and discarded. The tissue extract was partitioned with acetonitrile, and the acetonitrile was removed as described for fat.

Cleanup of Phosphorus Extracts from All Tissues. The cleanup column was prepared by adding, in order, a plug of glass wool, 2 cm of anhydrous sodium sulfate, 20 g of Florisil, 2 cm of sodium sulfate, and a plug of glass wool. The Florisil was packed by attaching the column to an aspirator and tapping the column gently on a table top until no more settling occurred. The column was prewashed with 50 ml of hexane. The sample was transferred to the column by using 50 ml of hexane in 15- to 20-ml aliquots to complete the transfer. The receiver was changed, and the iodofenphos, its photodecomposition product, and ronnel were eluted with 230 ml of hexane at a flow rate of about 200 ml/h. The receiver was changed again, the column was washed with 100 ml of dichloromethane–hexane (1:1), and the solvent was discarded. The receiver was changed again, and the iodofenphos oxygen analogue and its photodecomposition product were eluted with 115 ml of dichloromethane. (NOTE: Each lot of Florisil must be calibrated to determine the correct volume of eluting solvents.) Snyder columns were attached to the receivers containing the eluates, and the solvents were concentrated by distillation to about 5 ml. Then 25 ml of hexane was added through the Snyder column to the receiver containing dichloromethane and again concentrated to about 5 ml (to remove the last traces of dichloromethane). The extracts were transferred to 10-ml volumetric flasks with hexane and made to volume. Gas chromatographic determination of the residues present was conducted by injecting 5 μl into the instrument. Dilutions of the samples were made if necessary. Iodofenphos, its photodecomposition product, and ronnel were determined by comparing peak heights with those obtained with an injection of a standard solution of approximately the same concentrations. The same procedure was used to determine the oxygen analogue and its photodecomposition product. Peak heights were proportional to the amounts of solute if they were injected in the same volume of solvent.

Extraction of Free Phenols from Fat. A 20-g sample was blended with 100 ml of hexane and 50–60 g of anhydrous sodium sulfate. The mixture was transferred to a 400-ml beaker and filtered, by use of an aspirator, into

Table I. Percent Recovery of Iodofenphos (0.2 ppm), Ronnel (0.08 ppm), Photodecomposition Product (PDP)-Iodofenphos (0.06 ppm), Photodecomposition Product of Iodofenphos Oxygen Analogue ($PDPO_2A$) (0.05 ppm), and the Oxygen Analogue of Iodofenphos (0.25 ppm) from Various Tissues[a]

Tissue	Iodofenphos	Ronnel	PDP iodofenphos	$PDPO_2A$ iodofenphos	Oxygen analogue of iodofenphos
Fat	87	82	98	98	87
Muscle	82	83	90	76	88
Heart	91	88	98	90	75
Liver	87	84	95	87	74
Kidney	90	84	92	91	91
Spleen	90	87	94	96	93
Brain	93	89	96	89	70

[a] Control values were <0.005, 0.003, 0.002, 0.004, and 0.01 ppm, respectively, for the various compounds.

a 500-ml separatory funnel fitted with a fritted disk Buchner funnel (capacity 150 ml) containing a 1-cm layer of sodium sulfate. The blender, beaker, and funnel were washed with another 150–200 ml of hexane. The extract was partitioned with two 10-ml portions of approximately 1 N sodium hydroxide solution. The sodium hydroxide extracts were combined in a 125-ml centrifuge separatory funnel, and the fat–hexane solution was discarded. The alkali extract was washed by shaking with 25 ml of hexane and centrifuged at 1250 (250*g*) rpm for 10 min. The hexane was siphoned off and discarded. This step was repeated, and the sodium hydroxide solution was drained into a 60-ml separatory funnel. Five milliliters of water was used to rinse the funnel, and the rinse was combined with the sodium hydroxide extract. Ten milliliters of hexane was added to the funnel and then 20 ml of approximately 1 N sulfuric acid solution (the sulfuric acid solution was adjusted so that 1 ml would neutralize 1 ml of the sodium hydroxide solution to Congo Red indicator paper). The funnel was stoppered and shaken for 2 min. The aqueous phase was drained off and discarded, the hexane was transferred to a 10-ml volumetric flask containing 0.5 g of anhydrous sodium sulfate, and the flask was shaken occasionally to dry the hexane. Five milliliters was transferred to another 10-ml volumetric flask, 20 μl of *N,O*-bis(trimethylsilyl)acetamide (Supelco, Inc., Bellefonte, Pa.) was added to the flask, and the flask was stoppered tightly, shaken to mix the contents, placed in a 50–54 °C water bath for 20 min, removed, and cooled to room temperature. Five microliters of sample was injected into the gas chromatograph. If dilutions were necessary, a smaller aliquot of sample was taken and silylated. The amount of phenols present was determined by comparing peak heights with those obtained with an injection of a standard solution of approximately the same concentrations.

Extraction of Free Phenols from Muscle, Heart, Liver, Kidney, Spleen, and Brain. A 20-g sample of muscle, heart, liver, kidney, spleen, or brain was blended at low speed for 2 min with 60 ml of acetone. One hundred milliliters of hexane and 75 g of anhydrous sodium sulfate were added to the blender, and the mixture was blended for 2 additional min. The contents were transferred to a 400-ml beaker and filtered into a 500-ml Erlenmeyer flask equipped with a Buchner funnel as previously described except that the funnel contained a 2-cm layer of sodium sulfate. The blender, beaker, and funnel were washed with hexane to a total volume of 300–350 ml. A Snyder column was attached to the flask, and the extract was concentrated by distillation to about 70 ml, cooled, and filtered through a 3-cm plug of cotton (acetone extracted and dried) into a 125-ml centrifuge separatory funnel by using 15 ml of hexane to complete the transfer. If any water was present, it was drained off and discarded. The extract was partitioned with sodium hydroxide, and the procedure was continued as described for fat.

Extraction of Free Phenols from Urine. A 10-ml sample of urine was pipetted into a 60-ml separatory funnel. Twenty milliliters of dichloromethane and 3 ml of approximately 12 N sulfuric acid were added to the funnel. The funnel was stoppered, and the contents were shaken gently for about 1 min. The phases were allowed to separate, and the dichloromethane was filtered through a 2-cm plug of anhydrous sodium sulfate into a 100-ml volumetric flask. The extraction was repeated with two 20-ml and one 15-ml aliquots of dichloromethane; each time the solvent was filtered through the sodium sulfate, and the filter was washed with 5 ml of dichloromethane. The solvent was made to volume.

Cleanup of Urine. A chromatographic cleanup column (Mohr buret) was prepared by adding, in order, a small plug of glass wool, 4 g of Woelm acid washed alumina, as received, and a small plug of glass wool. The column was prewashed with 10 ml of hexane, an appropriate aliquot of urine extract was transferred to the column, and gentle air pressure was used to push the solvent through. Twenty-five milliliters of hexane was passed through the column to remove the dichloromethane. Two milliliters of distilled water was added to the column and pushed into the adsorbent with air pressure. A 60-ml separatory funnel was used as the receiver, and 35 ml of hexane was used to elute the phenols. The small amount of water eluted was drained off and discarded, and the hexane was filtered through a 2-cm plug of anhydrous sodium sulfate into a 125-ml Erlenmeyer flask. A Snyder column was attached to the flask, and the solvent was concentrated by distillation to about 5 ml, cooled, transferred to a 10-ml volumetric flask by using hexane, and made to volume. An appropriate aliquot was pipetted into another 10-ml volumetric flask, and enough hexane was added to give a total volume of 5 ml. Twenty microliters of *N,O*-bis(trimethylsilyl)acetamide was added, and the quantification procedure was continued as previously described.

Extraction of Free and Alcohol–Water–Sodium Hydroxide Soluble (Conjugated) Phenols from Liver and Kidney. A 10-g sample was blended thoroughly in a stainless steel (Monel) Waring Blendor (360-ml volume) with 25 ml of an ethyl alcohol–water–sodium hydroxide solution (90 ml of ethanol–10 ml of distilled water–1 ml of 1 N sodium hydroxide solution), and filtered through a Buchner funnel containing a disk of Whatman No. 1 filter paper, by use of an aspirator, into a 300-ml Erlenmeyer flask. The blender and funnel were washed with 75 ml of alcohol–water–sodium hydroxide solution; the filter was then washed with 15 ml of ethanol–water (2:1) solution. Twenty-five milliliters of hexane was added to the flask and then 3 ml of 1 N sulfuric acid solution; the flask was stoppered tightly, by moistening the stopper with water, and the flask was placed into a 55–57 °C water bath for 1 h. The flask was removed and cooled to room

Table II. Percent Recovery of 2,5-Dichlorophenol (0.05 ppm) and 2,5-Dichloro-4-iodophenol (0.02 ppm) from Various Tissues[a]

Tissue	2,5-Dichlorophenol	2,5-Dichloro-4-iodophenol
	Free Phenols	
Fat	78	81
Muscle	79	90
Heart	84	96
Kidney	68	88
Liver	69	98
Spleen	60	87
Brain	65	97
Urine	50	89
Alcohol-Water-Sodium Hydroxide Soluble (Conjugates)		
Liver	73	81
Kidney	80	97

[a] Control values were <0.01 and 0.002 ppm, respectively, for the two compounds.

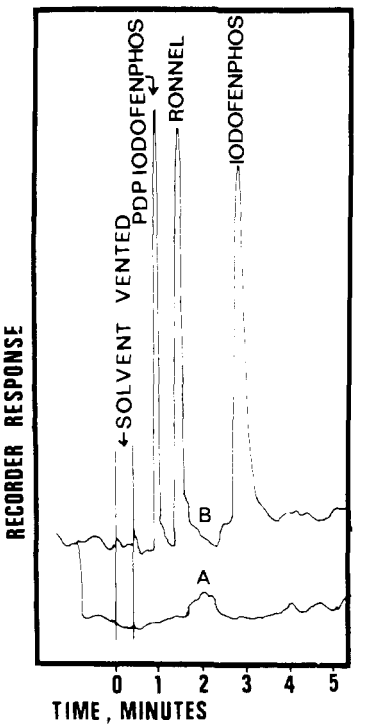

Figure 1. Chromatogram of extracts from omental fat: (A) untreated tissue; (B) tissue + 0.06, 0.08, and 0.2 ppm of the indicated compounds.

temperature; the contents were transferred to a 500-ml separatory funnel by using 75 ml of hexane and then 100 ml of sodium chloride solution (100 ml of water + 20 g of NaCl) and 100 ml of ethyl ether; and the funnel was stoppered and shaken for about 1.5 min. The aqueous phase was drained into a second funnel, and the contents were shaken gently (to prevent formation of a stable emulsion) with 150 ml of ether–hexane (1:1). The aqueous phase was drained off and discarded. The extracts were combined and washed with 100 ml of sodium chloride solution, the water phase was discarded, and the extract was filtered through a loosely packed 3-cm plug of extracted cotton into a 500-ml Erlenmeyer flask. A Snyder column was attached to the flask, and the solvent was concentrated by distillation to about 65 ml at 62–64 °C (solvent vapor temperature in the Snyder column). If necessary, 15 ml of additional hexane was added through the Snyder column and heated again to reach the desired temperature. The flask was cooled to room temperature, and the contents were transferred to a 125-ml centrifuge

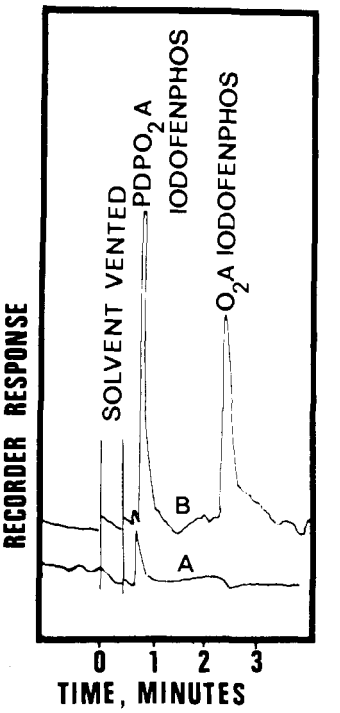

Figure 2. Chromatogram of extracts from omental fat: (A) untreated tissue; (B) tissue + 0.05 and 0.25 ppm of the indicated compounds.

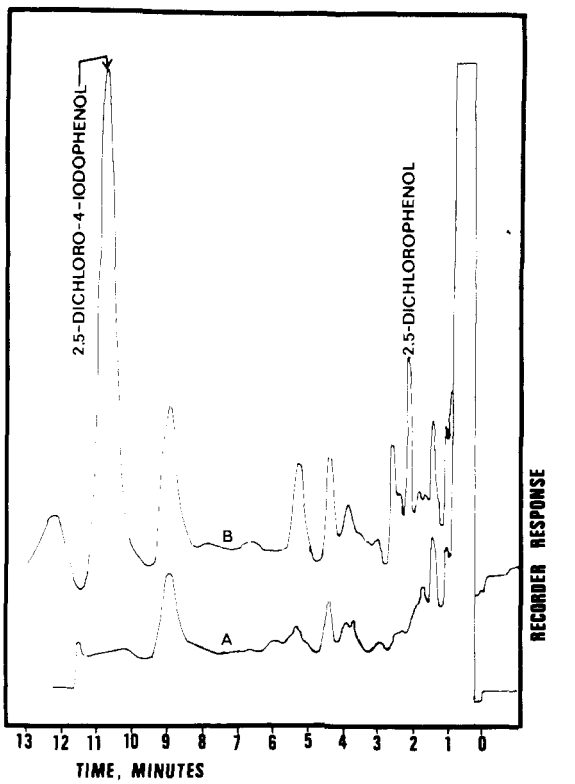

Figure 3. Chromatogram of extracts from muscle: (A) untreated tissue; (B) tissue + 0.05 and 0.02 ppm of the indicated compounds.

separatory funnel by using 15 ml of additional hexane to complete the transfer. The extract was partitioned with sodium hydroxide solution, and the procedure was continued as described for fat.

RESULTS AND DISCUSSION

Recovery Experiments. *Phosphorus Compounds.* The efficiency of the overall procedure was tested by

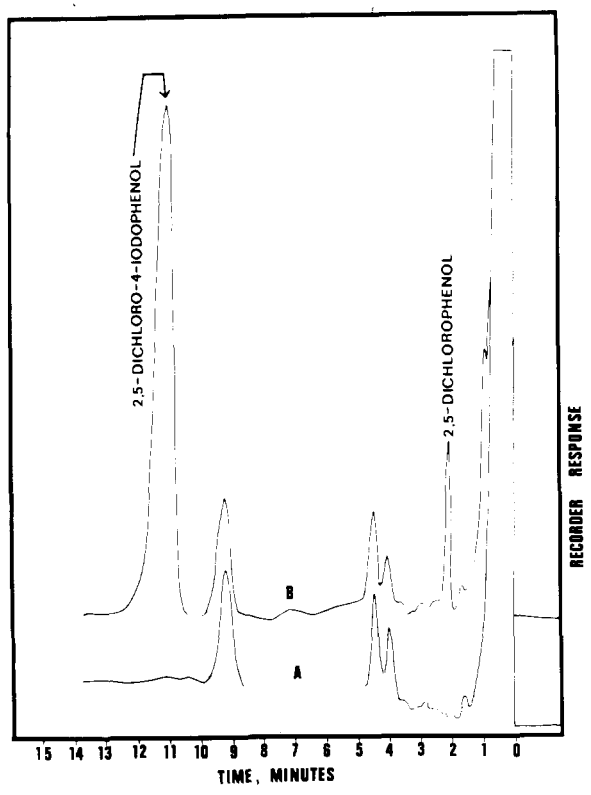

Figure 4. Chromatogram of extracts from kidney with the alcohol–water–sodium hydroxide procedure: (A) untreated tissue; (B) tissue + 0.05 and 0.02 ppm of the indicated compounds.

adding known amounts of iodofenphos, its photodecomposition product, ronnel, the oxygen analogue of iodofenphos, and its photodecomposition product to control samples of the various tissues before blending. The recovery of these compounds from fortified tissues is reported in Table I.

Phenolic Compounds. The efficiency of the procedure was tested by adding known amounts of 2,5-dichlorophenol and 2,5-dichloro-4-iodophenol to control samples of the various tissues before blending. The recovery of these compounds from fortified tissues is reported in Table II.

Figures 1 and 2 are chromatograms showing recoveries of the phosphorus compounds from fat. Figure 3 is a chromatogram showing recovery of the phenolic compounds from muscle, and Figure 4 shows recovery from kidney with the alcohol–water–sodium hydroxide procedure.

The control tissues showed no peaks at the retention times for any of the compounds. With the sample sizes and dilutions used, 0.005 ppm of iodofenphos, 0.002 ppm of its photodecomposition product, 0.003 ppm of ronnel, 0.01 ppm of the iodofenphos oxygen analogue, 0.004 ppm of its photodecomposition product, 0.01 ppm of 2,5-dichlorophenol, and 0.002 ppm of 2,5-dichloro-4-iodophenol can be detected in the various tissues.

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Photodegradation of *O*-Ethyl *O*-[4-(Methylthio)phenyl] *S*-Propyl Phosphorodithioate (BAY NTN 9306)

G. Wayne Ivie* and Don L. Bull

The ^{14}C-labeled organophosphorus insecticide, *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306), degraded rapidly when exposed to sunlight as deposits on cotton foliage, glass surfaces, or in water solution. The half-life of the compound was in each case less than 2 days. Exposure of water solutions of the insecticide to >280-nm artificial light resulted in very rapid degradation (half-life <2 h) to the same major photoproducts as were generated by exposure to sunlight. Degradation pathways included oxidation of the methylthio sulfur to sulfoxide and sulfone derivatives, hydrolysis of the phosphorus–*O*-phenyl ester, oxidative desulfuration of the P=S moiety, and undefined transformations leading to highly polar products that increased in quantity with time of exposure. When ^{14}C-labeled photoproducts of BAY NTN 9306 were orally administered to rats, they were rapidly excreted and no appreciable radiocarbon was retained in body tissues.

The organophosphorus (OP) compound *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306) has given good control of certain phytophagous insects that attack cotton and other crops; moreover, its mammalian toxicity is considerably below that of many of the OP insecticides in current use. It thus appears that the efficacy and toxicological characteristics of 9306 are such that the compound may find widespread application in a variety of insect control situations. A thorough evaluation of the metabolic and environmental behavior of 9306 is needed since the compound and its derivatives can be expected to interact with a variety of nontarget

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