Residue Determination of Dichlorvos and Related Metabolites in Animal Tissue and Fluids

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To assist in determining the fate of dichlorvos (2,2-dichlorovinyl dimethylphosphate) in animals, glc methods have been developed for measuring residues of four possible metabolites. These organic derivatives include dichloroacetaldehyde, dichloroethanol, dichloroacetic acid, and desmethyl dichlorvos. Detection limits are about 0.05–0.10 ppm.

Dichlorvos (2,2-dichlorovinyl dimethylphosphate) is used in the treatment of helminth infections in mammals. When formulated as anthelmintics (marketed by Shell Chemical Co. under brand names of ATGARD Swine, EQUIGARD Equine, and TASK Dog Anthelmintics), dichlorvos is easily administered with a wide margin of safety. Hodgson and Casida (1962) reported on the metabolism of dichlorvos in mammalian tissues, suggesting hydrolysis products of dimethylphosphate and desmethyldichlorvos, which further hydrolyzes to monomethylphosphate and finally to inorganic phosphate; the dichlorovinyl portion is converted *via* dichloroacetaldehyde (DCA) predominately to dichloroethanol (DCE) with trace amounts of dichloroacetic acid (DCAA).

To assist in determining the fate of dichlorvos in animals, analytical methods were required to determine residues of the above-mentioned organic metabolites. For selective detection of these compounds by gas chromatography, the phosphorus detector is suitable for dichlorvos; electron capture is satisfactory for dichloroacetaldehyde and derivatives of dichloroacetic acid and dichloroethanol. Stanley (1966) demonstrated the utility of diazomethane for esterifying organic acids to effect derivatives easily resolved by gas chromatography. Gutenmann *et al.* (1968) also described analyses of methyl esters in urine following acid treatment, ether extraction, and methylation. Using similar techniques, St. John and Lisk (1968a,b) determined hydrolytic metabolites of organophosphates using both methyl and ethyl esters for determining these compounds. To achieve detection of dichloroethanol at the residue level, it is necessary to convert the alcohol to its dichloroethyl trifluoroacetate derivative with trifluoroacetic anhydride. Such conversions to trifluoroacetyl derivatives have previously been adapted to the analyses of amines and amino acids. More recently, Lau and Marxmiller (1970) converted carbamic acid esters to electron-capturing derivatives through use of trifluoroacetic anhydride. This paper describes procedures for processing samples of body tissue and fluids for residue analyses of dichlorvos and four possible metabolites.

EXPERIMENTAL

Solvents and Reagents. Distilled-in-glass solvents from Burdick and Jackson Labs., Muskegon, Mich., or equivalent. Phosphotungstic acid, Mallinckrodt, analytical No. 2824. Calcium stearate, stearic acid calcium salt, practical, Matheson Coleman and Bell.

Trifluoroacetic anydride, reagent grade, Eastman No. 7386.

Nitrosomethylurea, for preparing diazomethane. Prepare 100 g in accordance with Blatt (1964) and store in a refrigerator. Alternate starting materials include Diazald or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, both available from Aldrich Chemical Co., Milwaukee, Wis.

Diazomethane, prepared from nitrosomethylurea. CAUTION: Conduct preparation and use of diazomethane in a well-ventilated hood, and wear rubber gloves. Diazomethane is a highly toxic yellow gas. The undiluted compound and concentrated solutions can explode violently, especially if impurities are present, or in contact with rough surfaces. For the preparation of 40 mequiv (approx. 1.7 g) of diazomethane, place 56 ml of ethyl ether and 18 ml of 40% KOH in a 250-ml Erlenmeyer flask. Cool the flask to ice temperature. Keep the flask cool and add in small portions with vigorous swirling 5.6 g of powdered nitrosomethylurea as rapidly as the solid dissolves (2 to 5 min). After addition is completed, decant the yellow ether layer into an ice-cold 50-ml graduated cylinder containing 5 ml of KOH pellets. The cylinder must be kept submerged in an ice bath to prevent evaporation of the reagent.

Dichloroacetaldehyde, J. T. Baker Laboratory Chemicals, Item G-904, Catalog 660. Redistil a minimum of 25 ml, taking 60% heart cut at 88–90° C (760 mm), using Claissen distillation apparatus.

2,2-Dichloroethanol, Aldrich Chemical Co., Item D6180-6, Catalog 14. Convert reagent grade DCE to the *N*-methylcarbamate with methylisocyanate and purify by fractional crystallization at low temperature. Hydrolyze with concentrated hydrochloric acid back to DCE. Fractionally distil with a Nester/Faust Annular distillation apparatus.

Dichloroacetic acid, Aldrich Chemical Co., Item D5470-2, Catalog 14, purity of 97%+.

Desmethyldichlorvos, purity of 97%+, Shell Chemical Co., Agricultural Div., San Ramon, Calif.

2,2-Dichlorovinyl dimethylphosphate (dichlorvos), purity of 97%+, Shell Chemical Co., Agricultural Div., San Ramon, Calif.

Glc Apparatus. Gas–liquid chromatograph capable of operation up to at least 200° C equipped with a phosphorus detector and/or an electron capture detector and provided with an inlet system having removable liners of Pyrex, vycor, quartz, or platinum. The Hy-Fi Model 600 or 1200 Chromatograph manufactured by Varian Aerograph, Walnut

Biological Sciences Research Center, Shell Development Co., P.O. Box 4248, Modesto, California 95352

Table I. Reference Glc Operating Parameters

	Dichloroacetaldehyde	Fluorinated dichloroethanol	Methylated dichloroacetic acid	Dichlorvos and methylated desmethyldichlorvos
Detector	Electron capture	Electron capture	Electron capture	Phosphorus (Cs Br)
Column	2.5-ft × 1/8-in. stainless steel	8-ft × 1/8-in. stainless steel	2.5-ft × 1/8 in. stainless steel	3-ft × 1/8-in. glass
Liquid phase	5% OV-17	20% QF-1	None	1:1 mix of 2% Reoplex 400 and 10% QF-1
Solid support	Chromosorb 101, 80/100	Gas Chrom Q, 80/100	Chromosorb 101, 80/100	Gas Chrom Q, 80/100
Carrier gas	N, 45 ml/min	N, 40 ml/min	N, 60 ml/min	He, 40 ml/min
Column temperature	150° C	125° C	190° C	145° C
Inlet temperature	180° C	160° C	210° C	170° C
Detector temperature	150° C	155° C	190° C	145° C
Approx. retention time	1 min	2.5 min	3.5 min	1.5 min
Typical response, % of full scale	0.02 ng = 20%	0.08 ng = 45%	0.2 ng = 40%	0.4 ng = 40%

Table II. Typical Standard Solutions for Glc Analysis

	Dichlorvos	DCA	DCE	DCAA	Desmethyl-dichlorvos
Concentrate standard, γ/ml	5000	1000	1000	4000	3000
solvent	Acetone	Hexane	Benzene	Ethyl ether	0.16 N H_2SO_4 in acetone
Subconcentrate standard, γ/ml	250	100	100	200	150
solvent	Acetone	Hexane	1:1 ethyl ether and benzene	Ethyl ether	Acetone
Stock for spiking rec. samples, γ/ml	10	10	10	10	10
solvent	MeOH or H_2O	Acetone	Acetone	MeOH or H_2O	MeOH or H_2O
Stock for calibration and/or derivatization, γ/ml	10	10	10	10	10
solvent	Ethyl acetate	Hexane	1:1 ethyl ether and benzene	Ethyl ether	0.08 N H_2SO_4 in ethyl ether
Solutions for derivatization, γ/ml			0.2, 0.4, 0.8	0.5, 1.0, 2.0	0.5, 1.0, 1.5
solvent			1:1 ethyl ether and benzene	Ethyl ether	Ethyl ether
Dilution during derivatization			1/10	1/10	1/5
Solutions for glc calibration, γ/ml	0.1, 0.2, 0.3	0.005, 0.01, 0.02	0.02, 0.04, 0.08	0.05, 0.1, 0.2	0.1, 0.2, 0.3
solvent	Ethyl acetate	Ethyl ether	Ethyl ether	Ethyl ether	Ethyl acetate

Creek, Calif., is satisfactory. High quality flow controllers are required to control air to ±0.1 ml/min and hydrogen to ±0.01 ml/min to the glc unit equipped with the phosphorus detector. Aerograph recommends their Model 403 dual flow controller.

Glc columns and operating parameters are chosen which achieve optimum balance between sensitivity and degree of resolution with good symmetrical peaks emerging with reasonable retention times. Retention times are relative but not necessarily optimum, since variation will occur due to aged columns and to differences of uniformity in packed columns. Due to the number of variables involved, the operating conditions listed in Table I should serve only as a guide.

Preparation of Standard Curves. Prepare concentrated and stock solutions of standards in accordance with Table II. Solutions of dichlorvos and dichloroacetaldehyde (DCA) are diluted directly for glc calibration with no further treatment. Dichloroethanol (DCE), dichloroacetic acid (DCAA), and desmethyldichlorvos solutions for derivatization are processed to effect glc calibration standards as follows.

DCE. Using the 0.2, 0.4, and 0.8 γ/ml ether/benzene solutions, pipette 5-ml aliquots of each concentration into separate 25-ml graduated mixing cylinders. To each sample add 0.2 ml of trifluoroacetic anhydride, cap with glass stopper, shake, and allow to react for 2 hr at room temperature. After this reaction period add 15 ml of distilled water and shake vigorously. Transfer the mixtures into separate 50-ml separatory funnels, allow the layers to separate, and drain off the aqueous phase. Wash the organic phase one more time with 15 ml of distilled water, discarding the aqueous washings. Dilute each standard 1/10 with ethyl ether and dry with anhydrous Na_2SO_4. The concentrations of standards in each of the organic solutions is now 0.02, 0.04 and 0.08 μg per ml, respectively.

DCAA. Using the 0.5, 1.0, and 2.0 γ/ml ether solutions, pipette 1 ml of each into separate 10-ml volumetric flasks. Add about 0.4 ml of diazomethane solution to each flask, allow to stand for 5–10 min, and then apply gentle heat until the yellow color dissipates. Dilute to the mark with ethyl ether and mix to effect dilutions of methylated DCAA of 0.05, 0.10, and 0.20 γ/ml.

Desmethyldichlorvos. Using the 0.5, 1.0, and 1.5 γ/ml ether solutions (with 0.004, 0.008, and 0.012 N H_2SO_4, respectively), pipette 1 ml of each into separate 5-ml volumetric flasks. Add about 0.4 ml of diazomethane as discussed in the

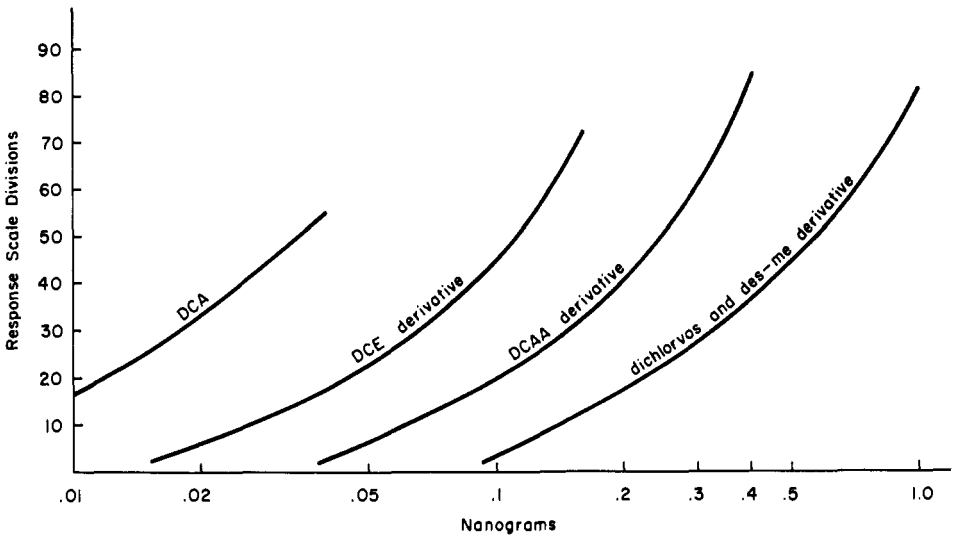

Figure 1. Relative glc responses of dichlorvos and metabolites

previous paragraph. After the color dissipates, dilute to the mark with ethyl acetate and mix to effect dilutions of methylated desmethyldichlorvos of 0.1, 0.2, and 0.3 γ/ml.

The desmethyl converts quantitatively to dichlorvos following acidification and methylation with diazomethane. Thus, dichlorvos is convenient to use as a reference standard; however, the acid and methylation treatments effect enhancement of response to the glc. The quantitation is constant providing the reference standards of dichlorvos are subjected to the same treatment.

Inject 2 μl of the calibration standards into the appropriate glc apparatus (Table I) and measure the peak height resulting from elution of the compound. Strict observance of the peak front and retention times is essential. During routine analyses, it is preferred to inject a standard after every third sample injection to insure the integrity of the sample analysis. Typical calibration curves and relative glc responses are shown in Figure 1.

Extraction. DICHLORVOS. Lean Tissue. Lean tissue samples are frozen and partially thawed to facilitate cutting into small pieces without the loss of blood and other fluids. Place 20 g of the tissue segments into a semimicro Waring blender cup with 20 g of granular sodium sulfate. Add 50 ml of ethyl acetate and blend at low speed for 1 min. Dry a portion of the extract with additional sodium sulfate and store in the refrigerator. Analyze the extract (2 g of tissue/5 ml of solvent) with the glc phosphorus detector with no further cleanup.

Fat Tissue. To 10 g of representative fat sample in a 250-ml Erlenmeyer flask, add 10 ml of saturated Na_2SO_4 solution and 100 ml of ethyl ether. Add two boiling chips, attach a 3-ball Snyder column with a 2 ft straight-tube extension, and obtain a gross weight of the entire apparatus, including contents. Reflux gently on the steam bath for 10 to 15 min until visible fat is all liquified. At the end of the reflux period, add an amount of ethyl ether equal to the weight of the solvent lost during refluxing (refer to original gross weight). Mix the contents with gentle shaking and allow to stand for phase separation. Decant most of the ether extract (0.1 g/ml) to a 100-ml graduated cylinder, add anhydrous Na_2SO_4 to dry, stopper, shake thoroughly, and allow to stand for several minutes prior to processing for the determination of dichlorvos. Transfer an 80-ml aliquot to a 250-ml Erlenmeyer flask and remove all of the ether by evaporation, leaving an oily residue. Add 30 ml of hexane-saturated acetonitrile, 2 g of calcium stearate, stopper, and shake vigorously. Decant through filter paper a 15-ml aliquot (4 g) into a 20-ml graduated centrifuge tube. Add about 5–10 ml of hexane, stopper, and mix well. Allow the phases to separate and then withdraw and discard the upper hexane layer, using a 10-ml syringe. Repeat the hexane washing twice more. Concentrate the acetonitrile to about 1 ml under a jet of dry air. Add about 10 ml of ethyl acetate, mix, and repeat the blowdown concentration. Repeat the last step once more to completely exchange the solvent from acetonitrile to ethyl acetate. Adjust the final volume to 4 ml (1 g/ml) with ethyl acetate and mix for glc analysis.

Blood. Add 2 ml of heparinized blood to a mixture of 2 ml of water and 2 ml of absolute ethanol in a 25-ml mixing cylinder and mix. Add 10 ml of ethyl acetate and shake thoroughly. The blood/water/alcohol mixture must be ex-

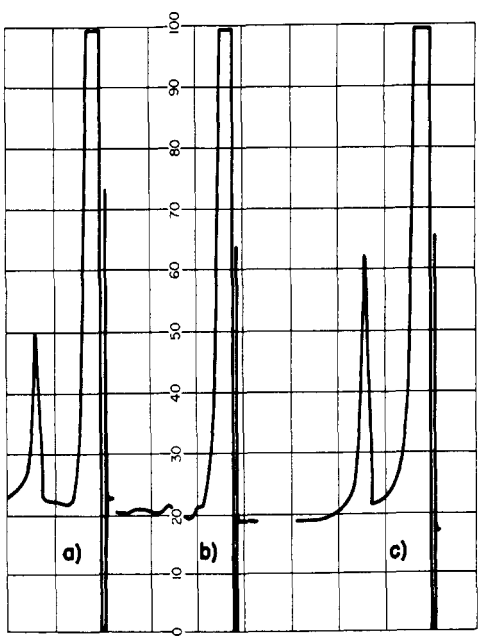

Figure 2. Typical dichlorvos gas chromatograms. (a) 0.2 ng of dichlorvos. (b) 2.0 mg of check pig brain. (c) 2.0 mg of check pig brain plus recovery of 0.2 ppm dichlorvos

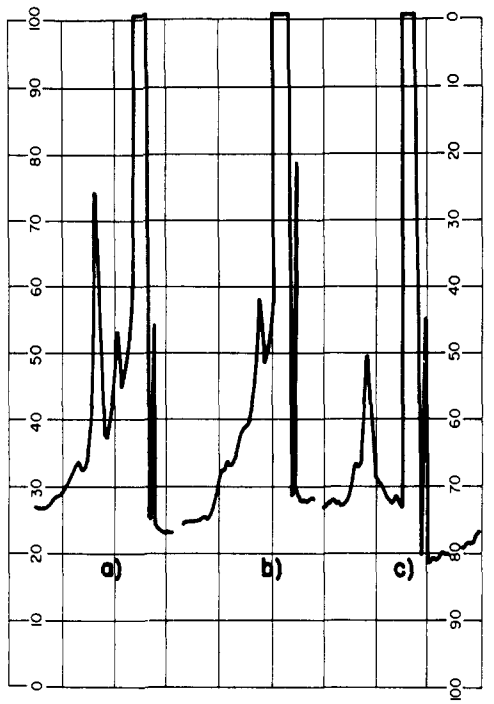

Figure 3. Typical DCA gas chromatograms. (a) 0.2 mg of check pig liver plus recovery of 0.2 ppm DCA. (b) 0.2 mg of check pig liver. (c) 0.02 ng of DCA

tracted with the 10 ml of ethyl acetate immediately upon withdrawal from the animal. Following phase separation, dry a portion of the upper phase with anhydrous sodium sulfate and store in the refrigerator. Analyze the extract (2 ml of blood/12 ml of solvent) with the glc phosphorus detector with no further cleanup.

Urine. Urine samples are extracted similarly to blood, using 10 ml of urine, 2 ml of ethanol, and 18 ml of ethyl acetate to effect 1 ml of urine per 2 ml of solvent.

DICHLOROACETALDEHYDE. Tissue and Blood. Tissue samples are frozen and partially thawed to facilitate cutting into small pieces without the loss of blood and other fluids. Place about 20 g of the tissue segments into a semimicro Waring blender cup with about 20 g of pulverized Dry Ice. Macerate to a fine particle size and allow the Dry Ice to evaporate completely.

Weigh a 10-g sample of the macerated tissue into a 250-ml nalgene centrifuge bottle. Add 25 ml of saturated sodium sulfate solution, 10 g of anhydrous granular sodium sulfate, and 5 ml of concentrated sulfuric acid, in that order, with mixing in between additions. Allow the sample to stand at room temperature for 10 min and add 70 ml of ethyl ether and shake vigorously for 2 min. Process 10-g samples of blood in the same manner.

Centrifuge the sample for 3 min at 1800 rpm and decant the liquid phases into a 250-ml separatory funnel. To the solid material remaining in the bottle add 30 ml of ethyl ether and drain the aqueous phase from the separatory funnel back into the plastic bottle and shake vigorously for 2 min. Centrifuge the sample at 1800 rpm for 3 min. Transfer the ethyl ether extract remaining in the separatory funnel to a 100-ml volumetric mixing cylinder. Decant the liquid phase in the plastic bottle into the empty separatory funnel, discard the aqueous phase, and add the ethyl ether phase to the mixing cylinder. Rinse the separatory funnel with sufficient ethyl ether to bring the total ethyl ether phase to 100 ml. Add 20 g of granular anhydrous Na_2SO_4 to the ethyl ether, shake vigorously, and allow to stand for 20 min. Mix 2 g of calcium stearate with a 30-ml aliquot of the dried ethyl ether extract, equivalent to 3 g of original sample, and shake well. This solution (0.1 g/ml) is now ready for glc analysis after centrifuging for 3 min at 1800 rpm.

DICHLOROETHANOL. Lean Tissue and Blood. Weigh 10 g of ground lean body tissue or blood into a 250-ml Erlenmeyer flask. Add 30 ml of 3 *N* sulfuric acid and allow to hydrolyze for 30 min on a wrist action shaker set at high speed. Remove the flask and add 2 g of phosphotungstic acid to the sample and shake for an additional 2 min. Transfer the mixture into a 250-ml Nalgene centrifuge bottle and centrifuge at 2000 rpm for 5 min. Filter the aqueous phase into a 50-ml graduated cylinder and measure the volume recovered. The sample weight recovered is calculated according to the tissue's water content and the amount of acid used for extraction; *e.g.*, if the tissue contains 60% water, the total aqueous portion is 36 ml (30 ml of acid plus 6 ml from 10-g sample) and each 3.6 ml is equivalent to 1 g of tissue. The Food and Drug Administration (The Analysis of Pesticide Residues, 1968) lists typical water contents.

Transfer the recovered acid extract to a clean 125-ml Erlenmeyer flask and saturate with Na_2SO_4. Add enough 50% ethyl ether in benzene solution to effect a 1 g/2 ml concentration and shake at high speed for 30 min on the wrist action shaker. Decant the ether–benzene extract into a clean flask and thoroughly dry over 10 g of anhydrous sodium sulfate for 15–20 hr. Store the extract (0.5 g/ml) in a closed container in the refrigerator until derivatized for analysis.

Fat Tissue. Following the addition of 30 ml of 3 *N* sulfuric acid to 10-g fat samples, reflux on a steam bath for about 15 min or until the fat is visibly "liquified." Remove from the heat, continue to hydrolyze for 15–20 min while shaking on a wrist action shaker, cool, add the phosphotungstic acid, and process as in the previous paragraph. Add additional phosphotungstic acid, if necessary, to effect a clear solution.

DICHLOROACETIC ACID AND DESMETHYLDICHLORVOS. Tissue. Weigh 10 g of representative tissue into a semimicro Waring blender, add 50 ml of 0.5 *N* sulfuric acid and 3 to 4 g

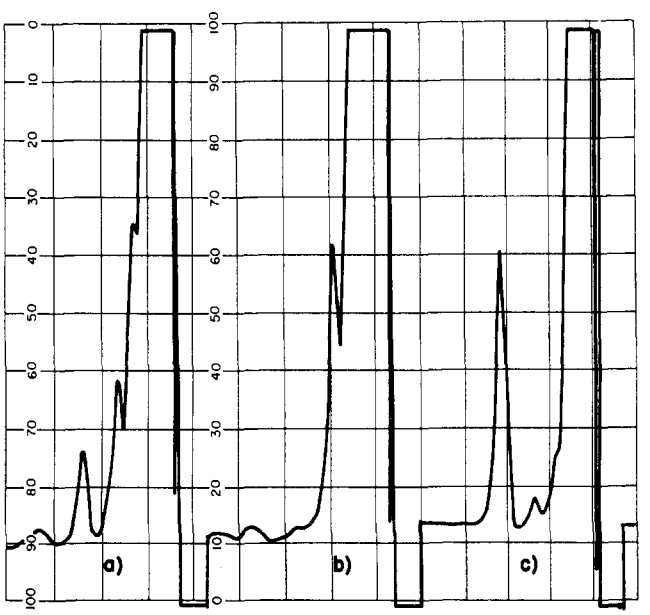

Figure 4. Typical DCE gas chromatograms. (a) 0.2 mg of check pig fat plus recovery of 0.2 ppm DCE. (b) 0.2 mg of check pig fat. (c) 0.08 ng of fluorinated DCE

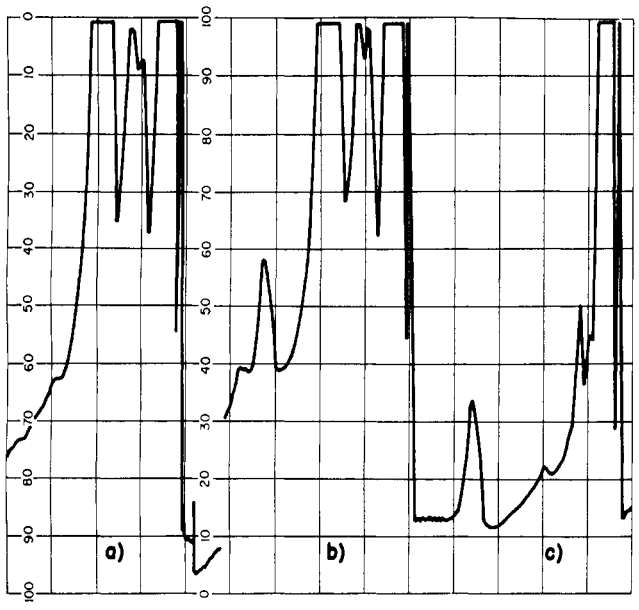

Figure 5. Typical DCAA gas chromatograms. (a) 0.4 mg of check pig kidney. (b) 0.4 mg of check pig kidney plus recovery of 0.2 ppm DCAA. (c) 0.1 ng of methylated DCAA

of phosphotungstic acid crystals. Blend at low speed for 1 to 2 min. Transfer the mixture into a 250-ml polypropylene bottle and centrifuge at about 2300 rpm for 5 min. Fat samples in blender cups are "liquified" on a steam bath before blending and cooled to room temperature before centrifuging. Filter a sufficient amount of the centrifuged solution to obtain a 5-g sample into a 125-ml Erlenmeyer flask. The aliquot is dependent on the water content of the sample, *e.g.*, if tissue contains about 60% water, the total aqueous portion is 56 ml (50 ml 0.5 *N* acid plus 6 ml from 10-g sample; thus, 28 ml are filtered). Immerse the flask into an ice bath and, while chilling the contents by swirling, slowly add and mix 5 ml of concentrated sulfuric acid. Remove the flask from the ice bath, add 15 g of sodium chloride crystals, 50 ml of ethyl ether, stopper, and shake on mechanical shaker for 15 min. Decant the ether extract (0.1 g/ml) to a graduated cylinder, add 10 g of anhydrous sodium sulfate to dry, stopper, shake thoroughly, and allow to stand for 30 min prior to processing for methylation.

Liquid Samples. For liquid samples such as blood, urine, bile, etc., add 5 g of sodium chloride to a sample aliquot (suggest 20 ml of urine or 10 ml of heparinized blood) in an Erlenmeyer flask. Blood must be extracted immediately upon withdrawal from the animal. Recovery samples are also spiked at this time. Immerse the flask into an ice bath and, while chilling the contents by swirling, slowly add and mix 5 ml of concentrated sulfuric acid. Remove the flask from the ice bath, add 5 ml of ethyl ether per gram of sample, stopper, and then shake on a wrist action shaker for 15 min. Decant and dry the ether extract (0.2 g/ml) as in the previous paragraph.

Derivatization. Dichloroacetaldehyde. Ether extracts of DCA are subjected to glc electron-capture analysis without any treatment.

Dichloroacetic Acid or Desmethyldichlorvos. To a graduated centrifuge tube, transfer an aliquot of the ether extract to obtain 3 or 4 g of sample. Urine = 0.2 g/ml × 20 ml = 4 g. Blood = 0.2 gl/ml × 20 ml = 4 g. Tissue = 0.1 g/ml × 30 ml = 3 g. Concentrate to about 1 ml by air blowdown. Rinse down the inner walls of the tube with about 1 ml of ether. Cool the tube to ice temperature and add about 0.5 ml of the diazomethane solution. If necessary, continue the addition of diazomethane until the evolution of gas ceases and/or the solution remains yellow. Allow to stand for 5–10 min and then apply gentle heat until the yellow color dissipates and the volume is reduced to about 1 ml. For desmethyldichlorvos analysis by glc phosphorus detector, adjust the volume with ethyl acetate to effect 1 g/ml. For DCAA analysis by glc electron-capture, adjust the volume with ethyl ether to effect 0.2 g/ml.

Dichloroethanol. Measure 5 ml of the DCE extract (0.5 g/ml) into a 25-ml graduated mixing cylinder. Add 0.2 ml of trifluoroacetic anhydride reagent, cap the cylinder with a glass stopper, shake the contents, and allow to react at room temperature for 2 hr. Add 15 ml of distilled water and shake vigorously. Transfer the mixture into a 50-ml separatory funnel, allow the layers to separate, and drain off the aqueous phase. Wash the organic phase one more time with 15 ml of distilled water and discard the aqueous phase. Dilute 1 ml of the organic phase to 10 ml with ethyl ether to effect 0.05 g/ml for glc electron-capture analysis. Add anhydrous sodium sulfate to dry.

Sample Analysis. Inject a 2-μl aliquot of sample extract into the appropriate glc apparatus applying the same technique used for the standards. Carefully check the elution time of any peak appearing in the chromatogram to be certain that it matches that of the standard. Typical gas chromatograms of the recovery of dichlorvos and metabolites from various pig tissues are shown in Figures 2 through 6. If the resulting peak time varies by more than 2 or 3 sec, as measured from the point of injection, the analysis is suspect. When variation is noted, a standard should be injected to verify the elution time and detector response. If identification is unequivocal, measure the peak height and compare with the respective calibration curve.

In addition to nontreated check samples and recovery samples (fortified nontreated check samples), each series of samples should include a "reagent blank," which is also car-

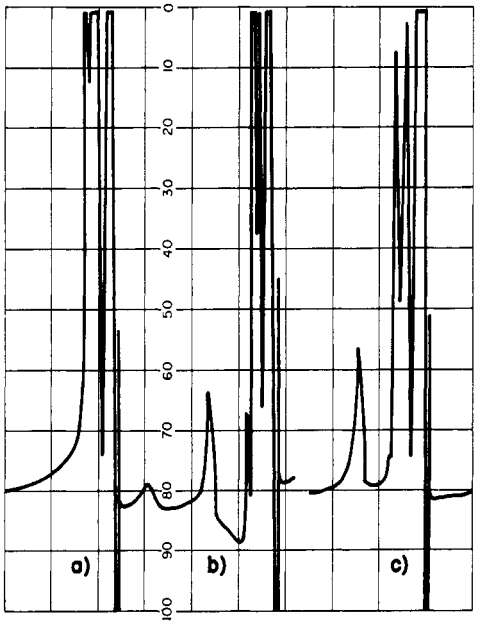

Figure 6. Typical desmethyldichlorvos gas chromatograms. (a) 1.0 mg of check pig muscle. (b) 0.2 ng of methylated desmethyldichlorvos. (c) 1.0 mg of check pig muscle plus recovery of 0.2 ppm desmethyldichlorvos

Table III. Assessment of Methods for Analysis of Pig Tissue (Brain, Liver, Muscle, Kidney)

	Dichlorvos	DCA		DCE		DCAA	Desmethyl-dichlorvos
Amount compound added, ppm	0.50	0.10	0.20	0.20	0.50	0.25	0.50
Series, number of results	10	33	10	8	15	30	18
Mean, ppm	0.46	0.071	0.172	0.20	0.47	0.25	0.44
Variance	0.00040	0.00029	0.00035	0.0035	0.0035	0.00210	0.00300
Standard deviation, ppm	0.020	0.017	0.019	0.019	0.059	0.046	0.055
Relative std. dev., % of mean	4.3	23.9	11.1	9.5	12.7	18.4	12.5
Range, ppm	0.06	0.075	0.05	0.06	0.21	0.20	0.25
Mean error, ppm	0.04	0.029	0.028	0	0.03	0	0.06
Relative error, % of true	8	29	14		6		12
95% confidence level, ± ppm	0.040	0.034	0.038	0.038	0.118	0.092	0.110

ried through from extraction to the final analysis. The background responses from the check sample and reagent blank are related to the degree of interference.

Calculate the chemical content of the sample by means of the following equation: ppm of chemical = W/S, where W = weight of chemical, in μg, found in the aliquot of the sample injected (from the calibration curve), and S = amount of the sample, in grams, represented by the aliquot injected.

ASSESSMENT OF THE METHODS

Precision and accuracy data are listed in Table III. The data are derived from recovery samples, where known amounts of chemicals were mixed with samples before extraction.

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