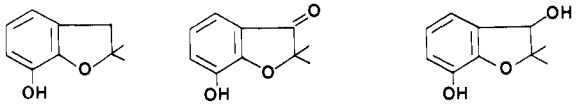
Determination of the Phenolic Metabolites of Carbofuran in Plant and Animal Matrices by Gas Chromatography of Their 2,4-Dinitrophenyl Ether Derivatives

Ronald F. Cook,* John E. Jackson,[1] John M. Shuttleworth, Oliver H. Fullmer, and Glenn H. Fujie

A gas chromatographic procedure suitable for the determination of the phenolic metabolite residues of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) in potatoes, milk, cow tissue, poultry tissue, and eggs is described. The method involved release of the conjugated phenolic residues by acid hydrolysis, ethoxylation of one of the phenolic metabolites, separation of the phenols from possible carbamate residues, and then formation of the 2,4-dinitrophenyl ether derivatives. A carbon clean-up column was required for potatoes and a Florisil clean-up column for eggs and poultry tissue. No clean-up column was used in the milk and cow tissue procedure. Detection of the derivatized phenols was accomplished by gas chromatography using a nitrogen-specific detection system. Method sensitivity achieved for each phenol component was 0.10 ppm for potatoes, 0.02 ppm for milk, 0.05 ppm for tissue, and 0.05 ppm for eggs. Recovery for the phenolic metabolites averaged 69% over all the matrices analyzed.

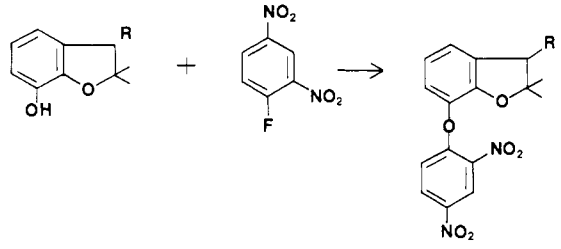
Investigations by Metcalf et al. (1968) on plants and animals, Ivie and Dorough (1968) on a lactating cow, Hicks et al. (1970) on poultry, Knaak et al. (1970) on plants, and Knaak et al. (1970) on the dairy cow have clearly shown the phenolic metabolites of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) to be 2,3-dihydro-2,2-dimethyl-7-benzofuranol (I, hereafter designated 7-phenol), 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol (II, hereafter designated 3-keto-7-phenol), and 2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol (III, hereafter designated 3-hydroxy-7-phenol). These phenolic



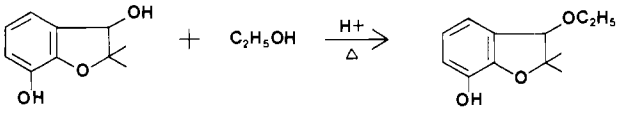
I II III

metabolites are produced in plant or animal systems as oxidation and/or hydrolysis products of the parent carbofuran moiety or its carbamate metabolites. The phenolic residues occur as water-soluble conjugates bound at the 3 position and/or 7 position of the benzofuran ring system. Determination of the phenols by gas chromatography required the release of the conjugated forms to an organo-extractable species, followed by derivatization to produce a form detectable at very low levels of sensitivity.

The acid hydrolysis technique as described by Cook et al. (1969) was found suitable for the release of the conjugated phenols. Reaction of the phenols with 1-fluoro-2,4-dinitrobenzene was developed as a route to produce the detectable form. Selection of the dinitrophenyl ether



(DNPE) derivative was based on a number of considerations such as the relatively simple reaction conditions, the stability of the resulting derivative, and the chromatographic characteristics of the derivative. The presence of the two nitro groups made the derivatives detectable by either nitrogen-specific or electron-capture gas chromatographic detectors. Our residue work was limited to the nitrogen-specific system. Quantitative conversion of the intact 7-phenol and 3-keto-7-phenol to the DNPE derivatives was easily achieved using the Cohen et al. (1969) procedure. However, conversion of 3-hydroxy-7-phenol to a DNPE derivative under the above conditions was not adequate. Satisfactory derivatization of the 3-hydroxy-7-phenol to a monosubstituted DNPE could only be routinely achieved after "protecting" the 3 position. This was accomplished by reacting the 3-hydroxy-7-phenol with ethanol to selectively form 3-ethoxy-7-phenol.



Described in this paper is a sensitive residue procedure capable of determining the three phenolic metabolites of carbofuran in potatoes, milk, tissue, and eggs. The general method should also be readily adaptable to a wide variety of other agricultural commodities.

EXPERIMENTAL SECTION

Apparatus. The following apparatuses were used: (1) high-speed Waring blender, 1500-mL capacity blending jar; (2) round-bottom flask, 1000 mL, with 50/50 neck opening, and 50/50 to 24/40 neck reducing adapter; (3) liquid chromatographic columns, 20 mm i.d. × 400 mm and 15 mm i.d. × 600 mm; (4) Kuderna-Danish Evaporative concentrator, 500-mL and 1000-mL capacity; receiver ampules, 10-mL capacity; (5) gas chromatograph, Micro-Tek MT 220 (or equivalent) equipped with a Dohrmann microcoulometric nitrogen detection system (Model S-200 pyrolysis furnace, Model C-200-A microcoulometer and Model T-400-H nitrogen detector) and a 1.0-mV recorder or a Micro-Tek MT-220 (or equivalent) equipped with a Coulson conductivity detector system. (Note: Pyrex glass injection port liners containing a small plug of glass wool were used to provide an off column injection system.)

Reagents. The following reagents were used: (1) hydrochloric acid, concentrated, reagent grade; (2) solvents:

Research and Development Department, FMC Corporation, Agricultural Chemical Division, Middleport, New York 14105 (R.F.C., J.M.S.) and Research and Development Department, FMC Corporation, Agricultural Chemical Division, Richmond, California 94804 (O.H.F., G.H.F.).

[1]Present address: Purdue University, Indiana State Chemist Office, West Lafayette, Ind. 47907.

redistilled reagent grade or pesticide quality methylene chloride, diethyl ether, ethyl acetate, hexane, acetone, and absolute ethanol; (3) Nuchar C-190N, carbon decolorizing (Fisher Scientific); (4) aluminum oxide, neutral grade, 80–200 mesh (Woelon W-200, super grade I), deactivated to 0.5% moisture; (5) 1-fluoro-2,4-dinitrobenzene, reagent grade redistilled 126–127 °C @ 0.05 mmHg; (6) sodium lauryl sulfate, reagent grade; (7) sodium phosphate, reagent grade; (8) sodium sulfate, anhydrous granular (Matheson, Coleman and Bell); (9) analytical standards, Residue Laboratory, Agricultural Chemical Division, FMC Corporation, Middleport, N.Y. 14105.

Solutions. The following solutions were used: (1) pH 11.0 buffer; 8.2 mL of 0.1 N NaOH and 100 mL of 0.05 M sodium phosphate diluted to 200 mL with distilled water; (2) reactant, 1 g of 1-fluoro-2,4-dinitrobenzene in 100 mL of acetone; (3) coagulating solution, 4 g of sodium lauryl sulfate in 100 mL of distilled water.

Procedure. *Potatoes. Sample Preparation.* Chop or blend sufficient sample to provide a homogeneous sample. Place chopped sample in polyethylene bags and store in a freezer (−20 °C) until needed for analysis.

Extraction. Place a 100-g sample into a 1000-mL round-bottom flask containing a magnetic stirring bar or boiling chips. Add 600 mL of 0.25 N hydrochloric acid, connect flask to a water-cooled Liebig condenser, and heat to reflux temperature. (Note: The addition of Dow Corning antifoam may be necessary.) Maintain reflux temperature for 1 h with constant stirring. Following acid digestion, filter hot hydrolysate through glass wool into a 2000-mL Erlenmeyer flask, rinse boiling flask and glass wool with an additional 300 mL of 0.25 N hydrochloric acid. Place filtered hydrolysate in a freezer and cool to near freezing. Transfer a 50-g aliquot of hydrolysate to a 1000-mL separatory funnel, add 5 mL of the 4% sodium lauryl sulfate coagulating solution, and shake briefly. Extract solution with three 300-mL portions of a 75/25 v/v methylene chloride–ethyl acetate mixture. Combine extracts and dry over anhydrous sodium sulfate.

Ethoxylation. Concentrate extract to approximately 10 mL using a steam bath. (Note: Use of rotary evaporators may result in severe loss of 7-phenol.) Add 40 mL of absolute ethanol and reconcentrate to 10 mL. Transfer concentrate to a 100-mL round-bottom flask, add four drops of concentrated hydrochloric acid, and reflux for 0.5 h using a heating mantle and water-cooled condenser. After refluxing, cool to room temperature and transfer the ethanolic solution to a 500-mL separatory funnel. Rinse the reaction flask and combine with the above solution to a total of 150 mL with 0.25 N hydrochloric acid.

Extract the combined solution with three 100-mL portions of methylene chloride. Combine extracts and dry over anhydrous sodium sulfate.

Phenol–Carbamate Separation. Transfer the dried methylene chloride solution to a 500-mL separatory funnel. Add 75 mL of cold 0.25 N sodium hydroxide and shake for 30 s. (Note: Draw off bottom solvent layer promptly to avoid any possible carbamate hydrolysis.) Repeat washing of the extract using a second portion of cold base. Discard the washed organic phase. Combine aqueous washes and acidify with 15 mL of concentrated hydrochloric acid. Extract acid solution with three 100-mL portions of methylene chloride. Combine and dry over sodium sulfate.

Derivatization. Filter dried extract into a 500-mL round-bottomed flask, add 100 mL of acetone, 25 mL of pH 11.0 phosphate buffer, 1 mL of 1% 1-fluoro-2,4-dinitrobenzene in acetone and boiling chips. Stopper and shake for 30 s. Attach 3-ball Snyder column and concentrate in a steam bath at 90–95 °C. Continue heating for 0.5 h after all solvent has been removed. Following reaction, transfer the warm reaction mixture to a 60-mL separatory funnel and extract with two 20-mL volumes of distilled hexane. Dry combined extracts over sodium sulfate. Filter out the sodium sulfate and rinse it with an additional 20 mL of hexane. Concentrate the combined hexane filtrates to 5 mL.

Column Cleanup. Prepare a cleanup column using the following procedure. Add a glass wool plug to the bottom of a 15 mm × 600 mm liquid chromatographic column. Slurry 2 g of Nuchar C-190N in 50 mL of hexane and pour into the column. Allow the C-190N to pack tightly using suction provided by a water aspirator through a 250-mL filter flask. Maintain the solvent level above the packed C-190N layer and add another glass wool plug. Drain the solvent level to the top of the glass wool plug. Discard the washings collected in the 250-mL filter flask during column preparation.

Add the concentrated hexane extract (5 mL) from above to the C-109N column. Rinse the Erlenmeyer flask with 5 mL of hexane and add to the column. Collection of the sample begins immediately using suction. When the solvent level reaches the top glass wool plug, add 75 mL of methylene chloride. Continue the collection until the column drains dry.

Concentrate the combined hexane and methylene chloride fractions to ca. 5 mL in the filter flask on a water bath.

Gas Chromatographic Analysis. Transfer concentrated column eluent to a 13-mL graduated centrifuge tube. Using a gentle stream of dry nitrogen, further concentrate sample to less than 2 mL. Dilute the sample to a final volume of exactly 2.0 mL with hexane. Inject a suitable aliquot (equivalent to injecting 50–100 ng of derivative) into a gas chromatograph equipped with a nitrogen specific detection system. The following conditions were used with the Dohrmann Microcoulometric detection system in the reductive nitrogen mode: injection port temperature: 275 °C; column: 3 ft × $^1/_4$ in. o.d. aluminum tubing packed with 10% OV-3 on 80/100 mesh Chromosorb W-HP; column temperature: 220 °C; transfer line temperature: 260 °C; pryolysis furnace: inlet, 260 °C; center, 800 °C; outlet, 450 °C; carrier gas flow: 300 mL/min hydrogen; reactant flow: 200 mL/min hydrogen; auxillary flow: 50 mL/min hydrogen; gain setting: high; high gain setting: 950–970; bias setting: 100 mV; range (attenuation): 200 ohms; chart speed: 0.5 in./min.

With the instrument equilibrated under the above conditions, a retention time of 4.2 min for 7-phenol derivative, 5.2 min for 3-keto-7-phenol derivative, and 6.5 min for 3-hydroxy-7-phenol derivative should be obtained.

Milk and Cow Muscle, Liver, or Kidney. Place a 100-g sample of milk or 50 g of minced tissue in a Waring blender jar. Blend milk once with 500 mL of acetone for 3 min or blend tissues once with 250 mL of acetone for 3 min. Filter the milk or tissue mixture through a Büchner funnel using No. 1 filter paper into a 1000-mL round-bottom flask. Retain the filtrate. Return the filter paper with the collected tissue residue to the blending jar; add 250 mL of distilled acetone. Reblend the paper and tissue, refilter, and combine the filtrates. Add 150 mL of 0.375 N hydrochloric acid and concentrate until all solvent is removed. Continue heating for an additional 15 min. Place flask in a freezer (−10 °C) until ice crystals begin to form in the acid solution. Remove from freezer and quickly filter through glass wool into a 500-mL separatory funnel to

remove any oils or waxes which have solidified during cooling. Rinse flask and glass wool with an additional 100 mL of cold 0.25 N hydrochloric acid. Extract with three 200-mL portions of the 75:25 v/v methylene chloride–ethyl acetate mixture. Combine extracts, dry over sodium sulfate, and concentrate in a Kuderna-Danish evaporator to approximately 10 mL. Add 40 mL of absolute ethanol and continue as described for potatoes. (Note: A cleanup column is *not* required for milk or cow tissues. For these samples the derivatization extract can be concentrated and injected directly using the analysis conditions listed for potatoes.)

Eggs and Poultry Muscle. Hydrolyze 50 g of eggs or minced tissue as described for potatoes. After refluxing, add dropwise to the hot hydrolysate 75 mL of 4% sodium lauryl sulfate coagulating solution for eggs and 50 mL for tissue. Cool to almost freezing and filter through a Büchner funnel lined with 11.0-cm Sharkskin filter paper. Rinse filter cake with an additional 300 mL of 0.25 N hydrochloric acid. Transfer filtrate to a 2000-mL separatory funnel. To the filtrate add 4 mL or 25 mL of sodium lauryl sulfate to eggs or tissue, respectively. Extract with three 600-mL portions of the 75:25 v/v methylene chloride–diethyl ether mixture. Combine the extracts and dry over sodium sulfate. Continue the method as described for potatoes from ethoxylation through derivatization. The dried hexane extracts should then be applied to the following column cleanup.

Column Cleanup. Plug a chromatographic column with glass wool. Add 10 g of Al_2O_3 (0.5% H_2O, neutral grade) and pack with a second glass wool plug. Wet column with 20 mL of hexane, followed by addition of dried sample extract. Allow hexane to elute to top of glass wool plug and add 150 mL of hexane–ethyl acetate mixture (80:20 v/v). Elute to dryness. Concentrate column eluent to approximately 10 mL and transfer to a graduated centrifuge tube. Concentrate to exactly 2 mL.

Gas Chromatographic Analysis. Inject a suitable aliquot (e.g., 5 to 10 μL) into a gas chromatograph equipped with a nitrogen specific detector. The following conditions were used with a Coulson conductivity detection system mounted on a Micro-Tek MT 220 gas chromatograph: injection port temperature: 275 °C; column: 3 ft × $^1/_4$ in. o.d. aluminum tubing packed with 10% OV-3 on 80/100 mesh Chromosorb W-HP; column temperature: 205 °C; transfer line temperature: 250 °C; furnace temperature: 800 °C; carrier gas flow: 260 mL/min hydrogen; volts: 30; attenuation: ×1.

With the instrument equilibrated under the above conditions a retention time of 5.5 min for 7-phenol derivative, 6.5 min for 3-keto-7-phenol derivative, and 8.0 min for the 3-hydroxy-7-phenol derivative should be obtained.

Calculations. For the microcoulometric detector system: mathematically convert the appropriate peak areas (i.e., square inches) associated with the treated samples directly into micrograms of dinitrophenyl ether derivatives of 7-phenol, 3-keto-7-phenol, and 3-ethoxy-7-phenol by using the following equation:

$$\text{micrograms of DNP ether der.} = \frac{\text{peak area (in.}^2) \times 174.1}{\text{attenuation } (\Omega) \times \%\text{ N compound}}$$

For complete explanation and discussion of conversion formula refer to Dohrmann Instrument Company Manual on the T-400 H cell.

Correct the micrograms found for instrument efficiency (i.e., the ratio of micrograms detected to micrograms injected). Then use the following equation to calculate the ppm phenol residue in the treated samples.

$$\text{ppm phenol} = \frac{\text{corrected micrograms of DNP ether} \times C}{\text{gram sample injected} \times \text{average method recovery}}$$

where C = phenol to DNP ether molecular weight ratio of 0.498 for 7-phenol, 0.518 for 3-keto-7-phenol, and 0.480 for 3-hydroxy-7-phenol.

Prepare a standard solution containing 10 μg each of the dinitrophenyl ether derivatives of 7-phenol, 3-keto-7-phenol, and 3-hydroxy-7-phenol per milliliter of hexane. Inject appropriate aliquots (i.e., 10–15 μL) of the standard solution into the microcoulometric detection system to establish the instrument efficiency (i.e., the ratio of micrograms injected to micrograms detected). Periodically during a series of determinations, inject additional appropriately sized aliquots of the standard solution to insure that the instrument efficiency has remained reasonably constant.

Since the operating principle of the microcoulometric system allows the direct calculation of a given peak in terms of micrograms detected, establishment of a calibration curve for use in quantitating data is not required.

Calculations for the Coulson Conductivity Detector were based on peak height determinations. A linear range standard curve was established and quantitative date for "unknowns" read from the curve. Instrument sensitivity was rechecked daily and new standard curves prepared when necessary.

Preparation of Derivative Standards. Preparation of the 2,4-dinitrophenyl ethers was based on the work of Reinheimer et al. (1957). The technique was used as follows:

Derivatives of 7-Phenol and 3-Keto-7-phenol. Weigh 0.002 mol of each phenol into separate 100-mL round-bottom flasks. Add 20 mL of acetone, 0.002 mol of 1-fluoro-2,4-dinitrobenzene, and an excess of triethylamine. Reflux for 0.5 h. After reaction, evaporate to dryness and grind crystallized material with 5% hydrochloric acid, followed by 5% sodium hydroxide. Air dry solid and recrystallize from ethanol.

Derivative of 3-Hydroxy-7-phenol (3-Ethoxy-7-phenol). Weigh 0.002 mol of 3-hydroxy-7-phenol into a 100-mL round-bottom flask. Add 20 mL of absolute ethanol and an excess of concentrated HCl. Reflux for 0.5 h. Dilute solution with 250 mL of distilled water and extract with three 100-mL portions of methylene chloride. Dry extract over sodium sulfate and concentrate to near dryness. Dissolve residue in 20 mL of acetone and proceed as described above for the formation of the 7-phenol derivative.

RESULTS

Table I lists the method sensitivities achieved (0.02 to 0.20 ppm) for each of the three phenols in the commodities investigated by the derivatization procedure.

Consistent recoveries in the range of 60 to 80% were achieved for each phenol in the various samples. For potatoes the standard deviation was 8, 10, and 6%, respectively, for 7-phenol, 3-keto-7-phenol, and 3-hydroxy-7-phenol when measured as their DNPE derivatives. For raw milk the standard deviation was 5, 8, and 5%. For the cow tissue and poultry study work, the standard deviation averaged 8% for each phenol. The recoveries obtained were considered adequate, especially considering their excellent reproducibility. Average recoveries and the ranges of fortification levels for the materials studies are shown in Table II.

Table I. Method Sensitivity of Three Carbofuran Phenols from Animal and Plant Material

Material	Sensitivity, ppm[a]		
	7-Phenol	3-Keto-7-phenol	3-Hydroxy-7-phenol
Potato tuber	0.10	0.10	0.10
Raw milk	0.02	0.02	0.02
Cow muscle	0.05	0.05	0.05
Cow liver	0.05	0.05	0.05
Cow kidney	0.10	0.10	0.10
Fresh eggs	0.05	0.05	0.05
Poultry muscle	0.05	0.05	0.05

[a] For qualitative purposes, detectability was one-half these values.

Figure 1 illustratets typical chromatograms of control and fortified samples of potato, milk, and cow muscle as obtained using the microcoulometric nitrogen detector. Figure 2 illustrates typical chromatograms of dinitrophenyl ether standards for both detection systems, as well as the egg and poultry muscle chromatograms using the Coulson conductivity detector.

DISCUSSION

The sample sizes and solvent volumes presented in this paper were selected so as to be compatible with the routinely achievable detector sensitivity. If detector operation warrants it, adjustments (i.e., reductions) in size and volume can be made so long as they are done proportionately.

Derivatization techniques which did not incorporate the ethoxylation step were found suitable only for the 7-phenol and the 3-keto-7-phenol. Recovery of the 3-hydroxy-7-phenol as the DNPE were consistently less than 40% in procedures not including the ethoxylation step. The major losses of the 3-hydroxy-7-phenol were attributable to the poor derivatization efficiency of the intact phenol, but other losses also occurred as a result of poor extractability from aqueous solutions and loss during column cleanup. The conversion of the 3-hydroxy-7-phenol to the 3-ethoxy-7-phenol eliminated or minimized these losses by reducing the polarity of the molecule. Structural elucidation demonstrated the ethoxylation reaction to be selective as well as quantitative at the 3 position.

Carbamate and phenolic metabolites could potentially be present in the same sample. Carbamate compounds present in the derivatization step would hydrolyze and convert to DNPE derivatives. The carbamate DNPE derivatives would interfere with the quantitative measurement of the phenolic DNPE derivatives during gas chromatographic analysis. Therefore, it is essential that the method include a step to separate the carbamates from the phenols prior to derivatization. Carbofuran and 3-ketocarbofuran residues are quantitatively separated by the base wash, but greater than 50% of the 3-hydroxycarbofuran residue partitioned into the phenol containing

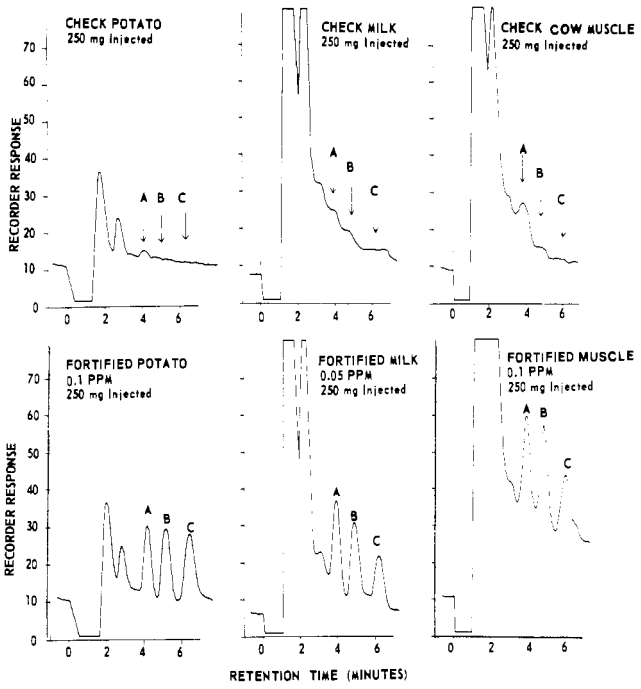


Figure 1. Typical potato, milk, and tissue chromatograms: (A) 7-phenol as a DNPE, (B) 3-keto-7-phenol as a DNPE, (C) 3-ethoxy-7-phenol as a DNPE.

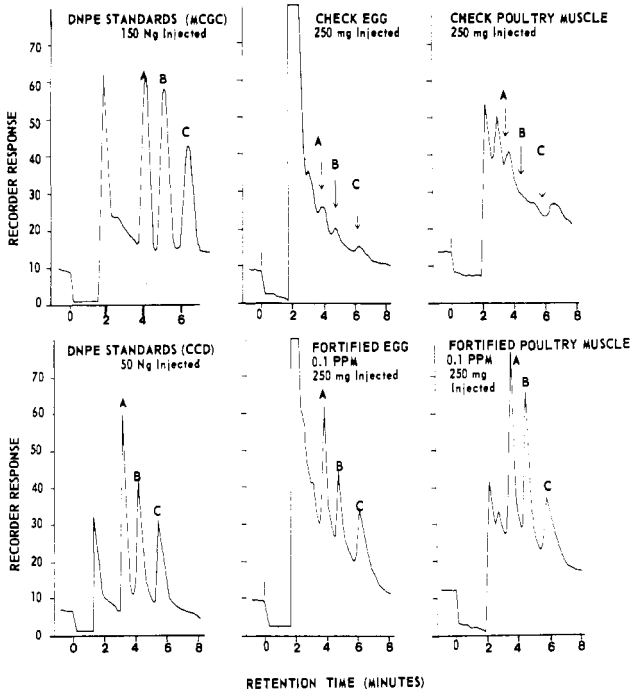


Figure 2. Typical standard, egg, and tissue chromatograms: (A) 7-phenol as a DNPE, (B) 3-keto-7-phenol as a DNPE, (C) 3-ethoxy-7-phenol as a DNPE.

Table II. Method Recovery of Three Carbofuran Phenols from Plant and Animal Samples

Sample	Fortification range	Av % recovery		
		7-Phenol	3-Keto-7-phenol	3-Hydroxy-7-phenol
Potato tubers	0.1-1.0	66	73	63
Raw milk	0.02-0.50	61	71	65
Cow muscle	0.05-0.20	65	80	71
Cow liver	0.05-0.20	56	77	67
Cow kidney	0.05-0.20	51	64	63
Fresh eggs	0.05-0.10	68	84	64
Poultry muscle	0.05-0.10	65	112	61

wash fraction. By incorporating the ethoxylation step, the 3-hydroxycarbofuran was converted to 3-ethoxycarbofuran and was then totally separable from the phenols.

The formation of the 2,4-dinitrophenyl ether derivatives with the phenols of interest was optimum over a pH range of 8 to 9. Apparent release of acidic components during the derivatization reaction step lowered the initial phosphate buffer pH from 11.0 to the optimum range. A borax buffer of pH 9.0 was also found to give equivalent results. The derivatization was also enhanced by the addition of acetone. The addition of acetone produced a homogeneous solution which allowed greater contact between the water-soluble phenols and organo-soluble 1-fluoro-2,4-dinitrobenzene. The DNPE derivative standards are stable in a pure form and may be stored intact under refrigeration for indefinite periods of time. Storage of stock solvent solutions are also known to be stable for periods exceeding 3 months when stored under refrigeration. Room temperature storage of very dilute solutions in hexane and ethyl acetate are stable for at least 2 to 3 months. The derivatives are also stable under reflux conditions for at least 1 h.

The chromatographic characteristics of the nonpolar derivatives required little prior conditioning of the gas chromatographic column. Overnight heating at 275 °C with a constant carrier gas flow was usually sufficient for conditioning.

The methods described here can be applied to the detection of carbofuran phenols in a wide variety of commodities. Applicability of the technique to other systems requiring phenol analysis should also be readily attainable.

ACKNOWLEDGMENT

The authors wish to acknowledge the excellent technical assistance of M. H. Gruenauer, M. Herian, H. D. Lobbins, G. E. Lover, M. J. Sotdolka, and T. F. Uebler. The authors also wish to thank S. T. Young for his assistance in standard preparation.

LITERATURE CITED

Cohen, I. C., Norcup, J., Ruzicka, J. H. A., Wheals, B. B., *J. Chromatogr.* **44**, 251–255 (1969).

Cook, R. F., Stanovick, R. P., Cassil, C. C., *J. Agric. Food Chem.* **17**, 277–282 (1969).

Hicks, B. W., Dorough, H. W., Davis, R. B., *J. Econ. Entomol.* **63**, 1108–1111 (1970).

Ivie, G. W., Dorough, H. W., *J. Agric. Food Chem.* **16**, 849–855 (1968).

Knaak, J. B., Munger, D. M., McCarthy, J. F., *J. Agric. Food Chem.* **18**, 827–831 (1970).

Knaak, J. B., Munger, D. M., McCarthy, J. F., Satter, L. D., *J. Agric. Food Chem.* 18, 832 (1970).

Metcalf, R. L. Fukuto, T. R., Collins, C. Borck, K., Abd El-Aziz, S., Munoz, R., Cassil, C. C., *J. Agric. Food Chem.* **16**, 300–311 (1968).

Reinheimer, J. D., Douglas, J. P., Leister, H., Voelkel, M. B., *J. Org. Chem.* **22**, 1743 (1957).

Received for review January 27, 1977. Accepted April 28, 1977.

Metabolism of Tetrachlorvinphos by the Soluble Fraction (105 000*g*) from Chicken Liver Homogenates

M. Humayoun Akhtar* and Thomas S. Foster

The soluble fraction (105 000*g*) from chicken liver homogenates contains an enzyme(s) which metabolizes tetrachlorvinphos. Studies which employed the [^{14}C]vinyl-labeled insecticide indicated that the reaction was glutathione dependent. The primary step was demethylation to desmethyl tetrachlorvinphos, a water-soluble metabolite. It was shown that GSH acts as an acceptor for the transferred methyl group to form *S*-methylglutathione. The soluble fraction contains enzymes other than glutathione *S*-alkyltransferase. The polar fraction was further metabolized to benzene extractable compounds which were identified as 2,4,5-trichloroacetophenone, 1-(2,4,5-trichlorophenyl)ethanol and 2-chloro-1-(2,4,5-trichlorophenyl)ethanol.

Tetrachlorvinphos (2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate) is an important member of the vinyl phosphate insecticide family. It is used on a wide range of crops including fruit, vegetables, corn etc. for the control of corn earworm, fall armyworm, codling moth, gypsy moth etc. In the cattle industry, it is used to control external parasites such as ticks, lice, and flies and is used to control mites and lice on and around chickens.

The metabolism of tetrachlorvinphos has been investigated in the rat and dog (Akintonwa and Hutson, 1967) and in the dairy cow (Gutenmann et al., 1971). Residue studies of the insecticide in tissues and eggs of chicken have also been reported (Ivey et al., 1969; Wasti and Shaw, 1971). Residues of the insecticide were detected in the omental fat and muscle of sprayed cattle (Ivey et al., 1968). Studies have also been reported on the residues of the insecticide in milk after dermal application (Oehler et al., 1969) and after feeding trials (Miller and Gordon, 1973).

The metabolic fate of tetrachlorvinphos in supernatant (100 000*g*) from mammalian (mouse, rat, rabbit, and pig) liver has been investigated (Hutson et al., 1972). The oxidative dealkylation of the insecticide by microsomes from liver homogenates has also been studied (Donninger et al., 1972). The present studies were undertaken to obtain information on the metabolism of tetrachlorvinphos by the soluble fraction (105 000*g*) from chicken liver homogenates.

The possible reactions involved in the metabolism of tetrachlorvinphos (I) in laying hens are summarized in Figure 1. The metabolites which might be encountered

Animal Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, K1A 0C6.