appeared to improve the cleanup of the aqueous phase, resulting in fewer interferences in fish and cattail chromatograms.

The recovery of [^{14}C]terbutryn from sediment in a sediment–water incubation system was investigated in order to estimate more reliably the actual recovery of terbutryn from a field-treated sample. After 1 month of incubation, recoveries of radioactivity ranged from 78.8 to 115.2% of the total radioactivity that was determined by combustion of wet (unextracted) sediment (averge terbutryn concentration about 1 μg/g). After two months of incubation, recoveries of [^{14}C]terbutryn residues ranged from 67.5 to 82.2%, with 6.6 to 12.6% of the radioactivity in the aerobic incubation and from 1.4 to 1.9% in the anaerobic incubation remaining on the sediment after extraction. Recoveries of the radioactivity were lower from the sediment of higher organic matter content especially after 1 month of incubation. The use of 1% concentrated NH_4OH in the aqueous acetonitrile (Hance and Chesters, 1970) as the second extraction solvent recovered an additional 3–8% of the radioactivity. Methanol or aqueous methanol has been recommended for extraction of triazine herbicides from field-treated soils (Ramsteiner et al., 1974; Mattson et al., 1970; Purkayastha and Cochrane, 1973). Prolonged exposure of methylthio-*s*-triazines to methanol can result in substitution of a methylthio group by a methoxy group (Sirons, 1978b); thus the use of methanol was avoided for the extraction of terbutryn in the present work.

Autoradiography indicated that 80–90% of the radioactivity was in the form of terbutryn. DET was the major degradation product, while HT and several unidentified spots were also detected. The proportion of radioactivity in the form of degradation products was lower in the anaerobic than aerobic incubations.

The relatively efficient recovery of [^{14}C]terbutryn residues from sediments and fish, which were fortified under conditions similar to field exposure, indicates that the method should be useful for the determination of terbutryn and DET in aquatic systems. HT and DEHT could be determined easily in water extracts by use of LC, but results with sediment, cattail, and fish extracts were semiquantitative, despite the use of HC as an internal standard to correct for losses to the CH_2Cl_2 phase.

ACKNOWLEDGMENT

The technical assistance of M. Pitze was much appreciated. The author also wishes to thank Ciba-Geigy Canada Ltd. for providing analytical standards of terbutryn, [^{14}C]terbutryn and all of the degradation products.

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Received for review August 27, 1979. Accepted February 13, 1980.

Direct Analysis of Carbofuran and 3-Hydroxycarbofuran in Rape Plants by Reverse-Phase High-Pressure Liquid Chromatography

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Rape plants spiked with both carbofuran and 3-hydroxycarbofuran were digested in acid solution and extracted with methylene chloride. The concentrated methylene chloride extract was passed through a cleanup column that contained silica gel and carbon–attaclay. The eluant of the first cleanup column was concentrated and passed through a silica gel column, and fractions containing carbofuran and 3-hydroxycarbofuran were collected and evaporated separately prior to the addition of internal standard. Carbofuran and 3-hydroxycarbofuran were measured separately by reverse-phase high-pressure liquid chromatography with an aqueous methanol mobile phase. Detection was carried out at 280 nm for carbofuran, 3-hydroxycarbofuran, and internal standards. The detection limit in rape plants studied was about 0.2 ppm for both carbofuran and 3-hydroxycarbofuran. Recoveries averaged 81.3% for carbofuran and 82.7% for 3-hydroxycarbofuran in the 0.2–1.0-ppm range.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is an insecticide widely used for controlling flea beetles [*Phyllotreta cruciferae* (Goeze), *P. striolata* (*F.*)] on rapeseed crops (*Brassica napus L.* and *B. campestris L.*) in Western Canada. Carbofuran is applied as either an in-furrow granular treatment or a foliar treatment. An initial step to studying the uptake by rape seedlings of carbofuran from in-furrow application was

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0021-8561/80/1428-0719$01.00/0

development of a suitable method for analysis of carbofuran and 3-hydroxycarbofuran in rapeseed foliage.

Carbofuran and its main metabolite, 3-hydroxycarbofuran, have been determined by gas chromatography either directly (Cook et al., 1969; Williams and Brown, 1973) or by prior derivatization (Wong and Fisher, 1975; Lau and Marxmiller, 1970; Seiber, 1972). Recently an analytical method was developed for carbofuran, nonconjugated 3-hydroxycarbofuran, and 3-ketocarbofuran, using a silica column and a mobile phase of trimethylpentane–2-propanol in a high-pressure liquid chromatography with an ultraviolet (UV) absorption detector (Lawrence and Leduc, 1977).

Radiotracer studies on aged corn by Cook et al. (1969) indicated that 80–90% of the carbamate residues found were either 3-hydroxycarbofuran or 3-hydroxycarbofuran glycoside. Investigations by Dorough (1968) and Knaak (1970) on plants showed up to 97% of the 3-hydroxycarbofuran conjugated.

There are no published reports on the quantitative determination of carbofuran and 3-hydroxycarbofuran in rape plants, using reverse-phase liquid chromatography or, for that matter, other chromatographic techniques. This paper describes a method suitable for determining carbofuran and 3-hydroxycarbofuran in rape plants.

EXPERIMENTAL SECTION

Apparatus. The high-pressure liquid chromatography (LC) system was a Spectra-Physics Model 3500 B and a LDC Spectro Monitor I Model 1201 UV detector which was connected to a Perkin-Elmer Model 56 strip chart recorder with a 10-mV span. The chromatograph column (3 mm i.d. × 25 cm, stainless steel) was packed in our laboratory with Spherisorb ODS (5 μm) by a slurry technique (Bakalyar et al., 1976).

Samples were injected onto the column via a Valco sample-loop (10 μL) injector. The mobile phase consisted of methanol in distilled water at a concentration 20–30% isocratic. The flow rate was 1.0 mL/min for all analyses. UV wavelength was 280 nm and sensitivity was 0.04 or 0.08 aufs, depending on the peak size.

Reagents. Solvents were glass-distilled residue-free grade except mobile phase methanol, which was ACS reagent grade (Fisher Scientific Co.), and the distilled water, which was distilled in a metal Barnstead still. Stock solutions of carbofuran (99.2% purity) and 3-hydroxycarbofuran (99% purity; both from FMC Corporation, Agricultural Chemical Division, Middleport, NY) were prepared in methanol. Acetophenone (BDH Chemical Ltd.) and propiophenone (Eastman Kodak Co.) were prepared in acetonitrile for use as the internal standards. Rape plants were collected from both greenhouse and field plantings. In addition, the following reagents were used: sodium lauryl sulfate, reagent grade; hydrochloric acid, ACS reagent; decolorizing carbon, Nuchar S-N (Fisher Scientific Co.), used as received; attaclay (Minerals and Chemicals Corporation of America, Philadelphia, PA), used as received; silica gel (100–200 mesh, Davison grade 923), used as received.

Procedure. *Extraction.* Fifty grams of chopped rape plants fortified with carbofuran and 3-hydroxycarbofuran was placed in a 500-mL blender cup with 250 mL of distilled water and 50 mL of methylene chloride and blended at medium speed on a Virtis homogenizer for 2 min. The macerate was transferred to a 1-L round-bottom flask with the aid of 250 mL of distilled water and 50 mL of methylene chloride, and 11 mL of 12 N hydrochloric acid was added. The acidified macerate was refluxed with magnetic stirring for 1 h and then cooled to room temperature. After the macerate was cooled, the condenser was washed down with 50 mL of 0.25 N hydrochloric acid and the acid digested sample was placed in a refrigerator (about 4 °C) overnight or in the freezer until ice just started to form. The sample was filtered through a glass wool pad on a Büchner funnel with suction and rinsed with 50 mL of 0.25 N hydrochloric acid and twice with 50 mL of methylene chloride. Ten milliliters of 4% sodium lauryl sulfate solution was added to the filtrate and mixed well, and the mixture was refiltered through a glass wool pad on a Büchner funnel. The precipitate on the glass wool pad was rinsed with 50 mL of 0.25 N hydrochloric acid and this was run into the filtrate. The filtrate was transferred to a 1-L separatory funnel and extracted with 100, 200, and 200 mL of methylene chloride, with vigorous shaking for 2 min each time. Combined methylene chloride extracts were dried over about 50 g of anhydrous sodium sulfate and filtered through filter paper on a Büchner funnel into a 1-L round-bottom flask. The drying flask and sodium sulfate were washed three times with 25 mL of methylene chloride. The filtrate was evaporated to about 25 mL on a vacuum rotary evaporator at water-bath temperature ≤30 °C (all evaporations were done in this manner), and 25 mL of hexane was added and mixed well.

First Column Cleanup. About 50 mL of ethyl acetate was added to a column with a glass wool plug at the bottom. Before the column was packed, 0.4 g of carbon and 6.6 g of attaclay were mixed well and washed twice with about 60 mL of ethyl acetate each time. This carbon–attaclay mixture was added to the column and packed tightly with suction. The wall of the column was rinsed with ethyl acetate, and a glass wool plug was placed on the top of the packed carbon–attaclay. Five grams of silica gel was washed twice with about 30 mL of ethyl acetate each time. This silica gel was packed in the column above the carbon–attaclay with suction, and the wall of the column was rinsed with ethyl acetate. The column was capped with a plug of glass wool. The solvent was drained from the column with suction until level with the glass wool cap. Fifty milliliters of methylene chloride–hexane mixture (1:1, v/v) was added and drained with suction to the top of the glass wool cap, and the eluate was discarded.

The receiver was changed, the dried and concentrated rape plant extract was added to the column, and the collection of the eluate was started immediately with suction. The extract was drained to the top of the glass wool cap. The flask and the wall of the column were rinsed five times with 10 mL of methylene chloride–hexane mixture (1:1, v/v) and the solvent was drained to the top of the glass wool cap; then the column was eluted with 200 mL of 20% hexane–ethyl acetate. This fraction was evaporated to about 2–3 mL on a vacuum rotary evaporator, and 30 mL of hexane was added and mixed well.

Second Column Cleanup. To a 1.2 cm i.d. × 40 cm glass column with a glass wool plug were added about 20 mL of 20% benzene–hexane, 5 g of anhydrous sodium sulfate, 5 g of silica gel (prewashed twice with about 30 mL of ethyl acetate and then washed twice with 30 mL of 20% benzene–hexane before packing), and 5 g of sodium sulfate. The sample was added to the column and drained to the top of sodium sulfate. The flask and the wall of the column were rinsed twice with 10 mL of 30% methylene chloride–hexane, and finally 50 mL of 20% benzene–hexane was passed through the column and this fraction was discarded.

The receiver was changed and carbofuran was eluted from the column with 200 mL of 15% ethyl acetate–hexane. The receiver was changed and the column was eluted

with 200 mL of 15% ethyl acetate–hexane and this fraction was discarded. The receiver was changed and 3-hydroxycarbofuran was eluted from the column with 200 mL of 30% hexane–ethyl acetate. Carbofuran and 3-hydroxycarbofuran fractions were evaporated separately on a vacuum rotary evaporator to about 2–3 mL and then were evaporated carefully to just dryness by using a gentle stream of nitrogen and a water bath at ≤40 °C. The internal standard solution and acetonitrile were then added to each fraction, filtered through a 2-μm filter, and injected into the LC.

Calculations. Before an unknown sample was analyzed, the response factors of carbofuran and 3-hydroxycarbofuran were determined separately from the chromatogram of a synthetic mixture containing known weights of the internal standard and the compound to be estimated, using the following equation:

$$f_p = (W_s/W_p)(A_p/A_s) \quad (1)$$

where f_p = response factor of pesticide to be determined, W_s and W_p = weights of internal standard and pesticide, respectively, present in the synthetic mixture, and A_s and A_p = peak areas of internal standard and pesticide, respectively.

Before the unknown sample mixture was chromatographed, a known quantity of the internal standard was added (W_s), and the sample mixture was chromatographed under the same conditions as were used for the synthetic mixture. The areas for sample component (A_p) and internal standard (A_s) were measured. The concentration of pesticide in a sample (C_p) was calculated from

$$C_p = (W_s/f_p)(A_p/A_s)(1/G) \quad (2)$$

where G is the weight of rape plant.

RESULTS AND DISCUSSION

The detector response was linear to carbofuran, 3-hydroxycarbofuran, acetophenone, and propiophenone over the range 50 to ~1550 ng/injection, with three of the four compounds having almost identical sensitivity and the fourth, propiophenone, being slightly less sensitive.

The cleanup procedure described is capable of providing samples of sufficient purity to be analyzed quantitatively by an UV absorption detector. We found that the amount of decolorizing carbon was a critical feature of the procedure. With too little carbon the cleanup was insufficient; with a little too much the recovery efficiency was reduced. The second cleanup column separated waxy compounds of rape plants from carbofuran and 3-hydroxycarbofuran. The first fraction which was eluted with 50 mL of 20% benzene–hexane contained the waxy compounds of rape plants. This fraction when chromatographed had extraneous peaks and was discarded. The second fraction which was eluted with the first 200 mL of 15% ethyl acetate–hexane contained only carbofuran and had some extraneous peaks, one of which can interfere with determination of 3-hydroxycarbofuran. For this reason carbofuran and 3-hydroxycarbofuran were collected separately. Occasionally an extraneous peak occurred near the acetophenone internal standard in the carbofuran fraction, depending on the variety and maturity of rape plants, and a propiophenone internal standard was substituted for the acetophenone. A chromatogram of a mixture of carbofuran, 3-hydroxycarbofuran, acetophenone, and propiophenone standards detected at 280 nm shows the excellent separation between all four components (Figure 1). The lower trace shows a typical chromatogram of the carbofuran fraction with acetophenone as the internal standard. This rape plant sample was spiked with carbofuran at 0.2 ppm and the final volume prior to injection was about 1 mL.

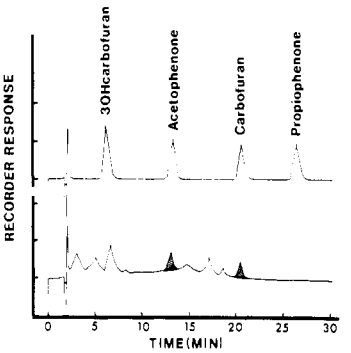

Figure 1. (Upper) Chromatogram of a mixture of 3-hydroxycarbofuran (206.0 ng), acetophenone (205.4 ng), carbofuran (223.6 ng), and propiophenone (377.6 ng). (Lower) Chromatogram of the carbofuran fraction of rape plant spiked at 0.2-ppm carbofuran on a 50-g sample. Mobile phase was 29% methanol in water, isocratic, 1.0 mL/min, and detection was at 280 nm, sensitivity 0.04 aufs.

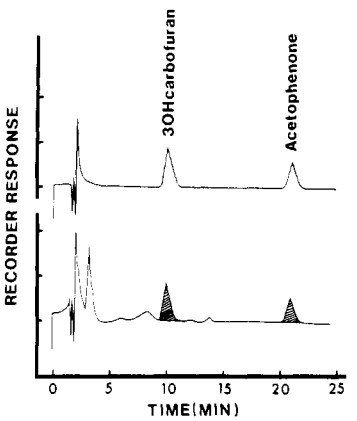

Figure 2. (Upper) Chromatogram of a mixture of 3-hydroxycarbofuran (103.0 ng) and acetophenone (102.7 ng). (Lower) Chromatogram of the 3-hydroxycarbofuran fraction of rape plant spiked at 0.2-ppm 3-hydroxycarbofuran on a 50-g sample. Mobile phase was 24% methanol in water, isocratic, 1.0 mL/min, and detection was at 280 nm, sensitivity 0.04 aufs.

Table I. Recovery of Carbofuran and 3-Hydroxycarbofuran from 50 g of Fortified Rape Plants

fortification, ppm		recov, %[a] ± SE	
carbofuran	3-hydroxycarbofuran	carbofuran	3-hydroxycarbofuran
0.22	0.21	84.6 ± 1.52	84.3 ± 5.78
0.42	0.40	81.6 ± 2.47	80.5 ± 2.30
1.12	1.03	77.7 ± 3.41	83.4 ± 1.52
	av	81.3	82.7

[a] Results are the mean percentage recovery from three replicates. SE, standard error.

The 3-hydroxycarbofuran fraction had few extraneous peaks and acetophenone could be used as the internal standard. The chromatogram (Figure 2) is for a rape plant sample spiked at the 0.2-ppm level. The minimum detectable concentrations of both carbofuran and 3-hydroxycarbofuran by this method were about 0.2 ppm, using a 50-g rape plant sample.

This method resulted in a good base line separation of carbofuran and 3-hydroxycarbofuran with reasonable recoveries at the concentrations studied (Table I). The recovery efficiencies obtained were acceptably high and consistent across the range studied in contrast to some recoveries reported by other workers (Cook et al., 1969;

Williams and Brown, 1973).

ACKNOWLEDGMENT

The authors are thankful to FMC Corporation, Agricultural Chemical Division, Middleport, NY, for supplying the insecticide standards and to R. O. Vibert and J. A. Janzen for technical assistance.

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Received for review October 2, 1979. Accepted March 17, 1980. Contribution No. 742 of the Research Station, Saskatoon.

Residues of Rotenone and Rotenolone on Lettuce and Tomato Fruit after Treatment in the Field with Rotenone Formulations

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Rotenone and 6aβ,12aβ-rotenolone levels were studied as a function of time on lettuce and tomato crops treated with dust or wettable powder formulations, according to label instructions. On both crops, the dust resulted in higher levels of residue. On lettuce, the half-life of rotenone applied as a dust or wettable powder was 2.9 or 3.6 days, respectively. The corresponding half-life of rotenolone was 4.5 or 5.0 days, respectively. Fourteen days after treatment 0.2–0.3 ppm of rotenone persisted, while 0.2 ppm of rotenolone was found after 9 days. The half-life of rotenone on tomato fruit was 2.7 days for the dust and 0.9 days for wettable powder formulations. Six days after treatment with dust, 0.2 ppm of rotenone was found on tomatoes, while 0.06 ppm of rotenolone was present after 2 days. Both rotenone and rotenolone were stable to boiling in tomato homogenate.

Rotenone [1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano[3,4-*b*]furo[2,3-*h*][1]benzopyran-6(6*H*)-one] is a naturally occurring insecticide registered for use on a variety of food crops on a negligible residue basis. It is unstable in the presence of light and air, and when irradiated on leaf surfaces in the laboratory, it has been shown to degrade to a number of compounds (Cheng et al., 1972). Among the decomposition products, 6aβ,12aβ-rotenolone has been identified as the major compound formed after 4 h of exposure (Cheng et al., 1972).

In reviewing the literature of rotenone, Haley (1978) noted that rotenone has been found tumorigenic in rats but not in mice and that further studies are required to define the toxicity of rotenone and its decomposition products. Because of the potential hazard arising from agricultural use, the present study was initiated to provide residue data on rotenone and rotenolone on food crops obtained by liquid chromatographic methods. Tomato and lettuce crops were selected for study to represent two extremes with respect to surface area and thus amount of residue expected to be present. Further, two commercially available formulations were compared in terms of initial residue and persistence.

EXPERIMENTAL SECTION

Crops. Lettuce (Grand Rapids variety) and tomatoes (Ottawa 78 variety) were grown in field plots at the Ottawa Research Station, Agriculture Canada, during the summer of 1979. Lettuce was seeded in three plots, each containing two 25-ft rows. One plot served as a control, while the others were treated with dust or wettable powder formulations of rotenone. Similarly, tomatoes were grown in three plots (one control and two treatment plots), each consisting of two 30-ft rows containing 10 plants each.

Rotenone Application. The two formulations of rotenone studied were a commercially available dust containing 1% rotenone as active ingredient and a wettable powder containing 5% rotenone. Each formulation was applied at a rate as indicated by the label instructions. The dust was applied with a hand duster at a rate of 57 g/50 row ft. The wettable powder (7.8 g) was suspended in water (2 L) and applied with a Chapin no. 35 hand sprayer at a rate of 2 L/50 row ft. Both lettuce and tomatoes received three treatments of each formulation at intervals of 7 days.

Sampling. Each plot was divided into four areas and a sample was removed from each at various time intervals. Sampling was commenced as soon as the final spray treatment appeared dry on the plant. Upon receipt at the laboratory, lettuce plants were trimmed to remove roots and the leaves were rinsed with water to remove adhering soil. The leaves from several plants were homogenized in a Waring Blendor, and a sample of the homogenate was extracted immediately. Tomato fruit was not rinsed but was quartered, and representative quarters were combined and homogenized. Samples of homogenate were extracted immediately.

Analytical Method. *Materials.* Rotenone (97%) was obtained from Aldrich Chemical Co. (Canada) Ltd., Montreal, Quebec, and was used without further treatment.

Rotenolone was synthesized by aeration of an alkaline suspension of rotenone as described by Crombie and Godin

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0021-8561/80/1428-0722$01.00/0