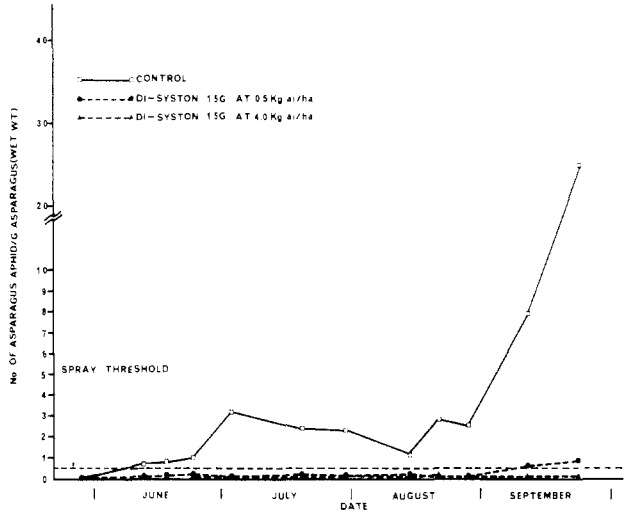

Figure 1. Population of *Brachycolus asparagi* in plots treated with Di-Syston 15 G at 0.5 and 4.0 kg of a.i./ha and in the control.

were detected in samples from plots treated at 0.5 kg of a.i./ha, and the limit of detection was 2 ppb for disulfoton and its toxic metabolites. Although the ferns collected at the end of the previous season from plots treated at 4.0 kg of a.i/ha had contained 17.1 ppm of total residue, the marketable spears in spring contained only about 0.01 ppm of the oxygen analogue sulfone.

In-furrow application of disulfoton at 0.5 or 4.0 kg of a.i./ha gave excellent control of the European asparagus aphid. The population of the pest in the treated plots remained below the spray threshold of 0.5 aphid/g of tissue during most of the growing season whereas that in the control consistently was above the spray threshold and exploded in late September when the ferns started to senesce (Figure 1). Although the aphid population in plots treated at 0.5 kg of a.i./ha was slightly higher than the spray threshold by late September, a chemical spray was not necessary because it was already near the end of the growing season. The damage indices were 0.10 and 0.07, respectively, for plots treated at 0.5 and 4.0 kg of a.i./ha, indicating that little damage occurred in the plants. By comparsion, asparagus in the control suffered from moderate to very heavy damage as evidenced by the damage index of 3.05. Based on the residue and efficacy data obtained in our studies, it is apparent that the biological activity of disulfoton against the European asparagus aphid resulted from the toxic sulfoxides and sulfones, derived from the oxidation of the parent compound.

Registry No. DSO, 2497-07-6; DSO_2, 2497-06-5; DOASO, 5286-73-7; $DOASO_2$, 4891-54-7; disulfoton, 298-04-4.

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Determination of Carbosulfan and Carbofuran Residues in Plants, Soil, and Water by Gas Chromatography

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Analytical procedures are described for the determination of carbosulfan and carbofuran residues in various crops, in soil, and in water. The quantitative methods involve extraction of residues using hexane–2-propanol or methanol–buffered water followed by column cleanup using Florisil, gel permeation, and Darco–Attaclay plus aluminum oxide, each column alone or in combination with each other. Residues of carbosulfan and carbofuran are detected by gas–liquid chromatography using a nitrogen-selective detector. Good recoveries are achieved with check samples fortified at the 0.05-ppm level with crops and soil and at the 0.01-ppm level with water.

Carbosulfan, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamino)thio]methylcarbamate (Figure 1), is a carbofuran derivative being developed as a pesticide by FMC Corp. Carbosulfan, as the parent compound, and carbofuran, as a major metabolite, have been reported in soil (Clay et al., 1980) and in cotton and corn (Umetsu et al., 1979, 1980) following treatment with carbosulfan. Analytical procedures were developed by FMC Corp. that are capable of determining the residues of carbosulfan and carbofuran in the same sample extract.

Various studies (Umetsu et al., 1979, 1980; Clay et al., 1980) have shown that carbosulfan can be extracted from soil and crop samples by using organic solvents. The same studies showed that carbosulfan, when exposed to acidic media, was unstable and decomposed to carbofuran.

Methanol has been successfully used in a metabolism study (Knaak et al., 1970) to quantitatively extract carbofuran from bean plants. Chloroform was used to extract carbofuran in an apple, potato, and sugar beet residue study (Butler and McDonough, 1968). Investigations in this laboratory showed the hexane–2-propanol (IPA) and methanol–pH 8 buffer solutions to be as efficient to extract carbofuran from various matrices.

The analytical procedure was, therefore, designed to use organic solvents to extract carbosulfan, as the parent chemical, and carbofuran, as a major metabolite, from

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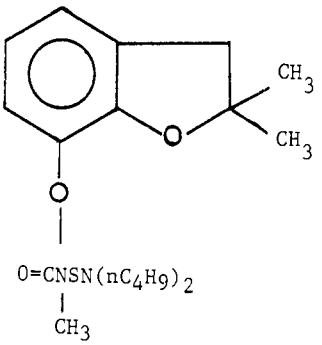

Figure 1. Carbosulfan.

samples without an acidic media that would decompose carbosulfan. The Darco–Attaclay plus aluminum oxide and Florisil cleanup columns were then taken from typical carbofuran procedures (Cook et al., 1969; Nelsen and Cook, 1980). Crops with a high oil content required additional cleanup using gel permeation or the classical hexane–acetonitrile partition prior to the above columns.

EXPERIMENTAL SECTION

Reagents. All solvents were pesticide quality from Burdick and Jackson Laboratories, Inc. (or their equivalent). Florisil was PR grade, 60–100 mesh, Floridin Co., deactivated with 3.5% water. A mixture of Darco–Attaclay was made by blending 1 part of Darco G60, ICI Americas, Inc., to 5 parts of Attaclay (hydrated aluminum magnesium silicate), free flowing powder, Attapulgus Clay Co. Aluminum oxide was Bio-Rad AG-4, 100–200 mesh, Bio-Rad Laboratories, deactivated with 4% water. The GPC column was packed with Bio-Beads S-X3, 200–400 mesh, Bio-Rad Laboratories.

Apparatus. A Hewlett-Packard 5840A gas chromatograph equipped with a nitrogen-selective detector and an automatic liquid sampler (Model 7671A or 7672A) was used. A 122 cm × 2 mm i.d. glass column packed with Chromosorb W-HP, 100–120 mesh, coated with 2% OV-101 was used to perform the separation. Prior to packing, the glass column was silanized by using the Leibrand and Dunham (1973) procedure except that hexamethyldisilazane was used instead of dimethyldichlorosilane. The inlet and detector temperatures were 275 and 300 °C, respectively. For carbosulfan analyses the column oven temperature was 210 °C, and for carbofuran it was 155 °C. Helium was used as a carrier gas with a flow rate of 45 mL/min. The detector gases were air with a 75 mL/min flow and a mixture of 8% hydrogen–92% helium with a flow rate of 40 mL/min.

For GPC analyses, an Autoprep 1001, Analytical Biochemistry Laboratories, Inc., was used. The column was 2.5 cm × 30 cm packed with 50 g of Bio-Beads S-X3 and compressed to a bed length of 27 cm by using Kontes Organic Solvent plunger assemblies. A 1:3 (v/v) mixture of hexane–ethyl acetate was the eluant, and the flow rate was 4 mL/min. The automatic operating parameters for the dump, collect, and wash cycles were 18, 9, and 4 min, respectively.

Extraction: High-Moisture Crops. Twenty-gram subsamples of macerated crop, e.g., green alfafa and citrus, were blended for 3 min in 100 mL of a 2:1 (v/v) mixture of hexane–IPA. The samples were filtered through Büchner funnels lined with glass fiber filter paper. The filter cakes and papers were returned to the blenders and blended 3 min with a second portion of 2:1 hexane–IPA. The samples were filtered and the blending jars and filter cakes rinsed with 50 mL of blending solvent. The combined filtrates of each sample were transferred to 500-mL separatory funnels. The samples were diluted with 100 mL of distilled water and 30 g of sodium chloride was added. The samples were shaken for 1 min and the phases were allowed to separate. The lower (aqueous) phase of each sample was transferred to a second separatory funnel and extracted with 100 mL of hexane. The aqueous phases were discarded and the hexane fractions were filtered through anhydrous sodium sulfate (which has been rinsed with blending solvent and then hexane) into graduated cylinders. The volume of each sample was adjusted to 300 mL with hexane.

Extraction: Low-Moisture Crops and Soil. Ten-gram subsamples of macerated crops, e.g., alfalfa hay and rice straw, or 20 g of soil was extracted as described previously by using a 9:1 (v/v) mixture of methanol–pH 8 buffer (buffer is 4.925 g of K_2HPO_4 plus 0.465 g of KH_2PO_4 per L). The samples were filtered, and the blending jars and filter cakes were rinsed with 50 mL of blending solvent. The volume of each sample was adjusted to 300 mL with blending solvent. Aliquots (1–5 g of crop equivalent) of sample extracts were transferred to 250-mL separatory funnels. The samples were diluted with 100 mL of distilled water and 15 g of sodium chloride was added. The samples were extracted 3 times with 50 mL of methylene chloride. Each portion of methylene chloride was filtered through anhydrous sodium sulfate into 250-mL Phillips beakers.

Extraction: High Terpene Oil Samples. Five-gram subsamples of high terpene content oils, e.g., citrus oil, were diluted to 30 mL with hexane in a 125-mL separatory funnel. The samples were extracted 3 times with 15 mL of acetonitrile, and the hexane (in which the oil was dissolved) was discarded. The acetonitrile was combined in a 250-mL separatory funnel and extracted 4 times with 100 mL of hexane. At this point, the carbosulfan was in the hexane and the carbofuran was in the acetonitrile. The hexane was used directly as is. The acetonitrile was concentrated to ca. 5 mL, transferred to 250-mL separatory funnels, and diluted with 100 mL of distilled water. The samples were extracted 3 times with 50 mL of methylene chloride and dried through anhydrous sodium sulfate as before.

Extraction: Water. One hundred gram subsamples of water were poured into a 250-mL separatory funnel, and 30 g of sodium chloride was added. The water was extracted 3 times with 50 mL of methylene chloride. Each portion of methylene chloride was filtered through anhydrous sodium sulfate into 250-mL beakers.

Concentration. Aliquots (1–5 g of crop equivalent) of sample extracts were concentrated on a steam table under a stream of dry nitrogen to ca. 3 mL. Nine drops of a 1% solution of glycerol in ethyl acetate was added to each sample as a "keeper". Before the Florisil cleanup column, samples in methylene chloride then had 10 mL of hexane added and were concentrated to ca. 3 mL. The hexane addition and concentration were repeated 2 more times until only the higher boiling hexane remained. Similarly, before the gel permeation and Darco–Attaclay cleanup columns, the same process was used with two additions of ethyl acetate instead of hexane. It was important that the samples were not concentrated to dryness and that no traces of methylene chloride remained. Samples requiring gel permeation cleanup were quantitatively transferred to 12-mL graduated centrifuge tubes, diluted to 10 mL with hexane, and centrifuged at 2500 rpm for 3 min to settle out any suspended solids.

Gel Permeation Cleanup. Samples with a high vegetable oil content, e.g., rice grain, were cleaned up by gel permeation chromatography using the GPC Autoprep

Table I. Recovery of Carbosulfan and Carbofuran from Plant, Soil, and Water Samples

sample	no. of analysis	fortification, ppm	average % recovery: carbosulfan	average % recovery: carbofuran
alfalfa, green	15	0.3-15	84 ± 10	89 ± 8
alfalfa, hay	28	0.5-50	93 ± 7	94 ± 7
rice grain	16	0.05-0.50	82 ± 14	79 ± 13
citrus	15	0.05-0.20	81 ± 7	86 ± 7
citrus oil	3	0.05-4.0	80 ± 2	86 ± 13
soil	36	0.05-0.30	86 ± 10	86 ± 13
water	16	0.005-0.100	93 ± 7	96 ± 5
Brussels sprouts	3	0.05-0.20	82 ± 10	85 ± 13
cabbage	3	0.05-0.20	84 ± 9	75 ± 8
cauliflower	3	0.05-0.20	74 ± 7	80 ± 6
potatoes	4	0.05-0.50	86 ± 7	82 ± 2
corn kernals	4	0.25-1.50	94 ± 4	100 ± 8
corn forage	3	0.25-1.0	86 ± 11	100 ± 9
sugar beet roots	4	0.25-2.5	92 ± 10	96 ± 9
sugar beet tops	3	0.25-2.5	91 ± 11	90 ± 8

1001. Approximately 8 mL of sample was injected into the 5-mL sample loops with the excess being discarded to waste. The previously calibrated parameters (dump, collect, and wash times) were set and the samples were eluted, one at a time, through the gel column. The individual samples were collected in 125-mL Phillips beakers and concentrated to 2–3 mL as previously described.

Darco–Attaclay plus Aluminum Oxide Cleanup. Glass chromatographic columns 1.5 cm o.d. × 17 cm with 50-mL reservoirs were plugged with glass wool. A 2-cm layer of anhydrous sodium sulfate was added, and the column was washed with ethyl acetate. One gram of Darco–Attaclay was slurried and added to each column in ethyl acetate and then packed with positive air pressure. Two grams of aluminum oxide (4.0% water deactivated) were added dry to the column that still contained a 5-cm layer of ethyl acetate on the Darco–Attaclay. The packings were capped with a 2-cm layer of anhydrous sodium sulfate. The samples were added and rinsed on with 5 mL of ethyl acetate. Elution was with 60 mL of ethyl acetate. The samples were concentrated as described into hexane.

Florisil Cleanup. Glass chromatographic columns 1.5 cm o.d. × 22 cm with 100-mL reservoirs were plugged with glass wool and washed with ethyl acetate. Ten grams of Florisil (3.5% water deactivated) was packed dry and capped with a 2-cm layer of anhydrous sodium sulfate. The samples were added and rinsed on with 5 mL of hexane. Carbosulfan was eluted from the column with 100 mL of 9:1 (v/v) hexane–ethyl acetate. Carbofuran was eluted next with 60 mL of 7:3 (v/v) hexane–ethyl acetate. The eluants were concentrated as previously described to an appropriate volume for analysis by gas chromatography.

Gas Chromatographic Analysis. Quantitation of residues was accomplished by comparing the peak areas of responses in samples to the peak areas of responses in external standards of carbosulfan or carbofuran. Detector linearity was checked daily for each compound. Typically 0.25–4.0 ng of either compound at an attenuation of 2^3 (8) gave a linear response.

RESULTS AND DISCUSSION

Fifteen kinds of samples were subjected to the procedures. Recovery values for samples fortified with carbosulfan and carbofuran are reported in Table I. No attempts were made to determine the maximum method sensitivity of the procedure for each kind of sample. As was true with a previous method for carofuran (Nelsen and Cook, 1980), a background response in the carbofuran region of 0.02 ppm was typical and would be the limiting factor. Representative chromatograms are displayed in Figures 2 and 3.

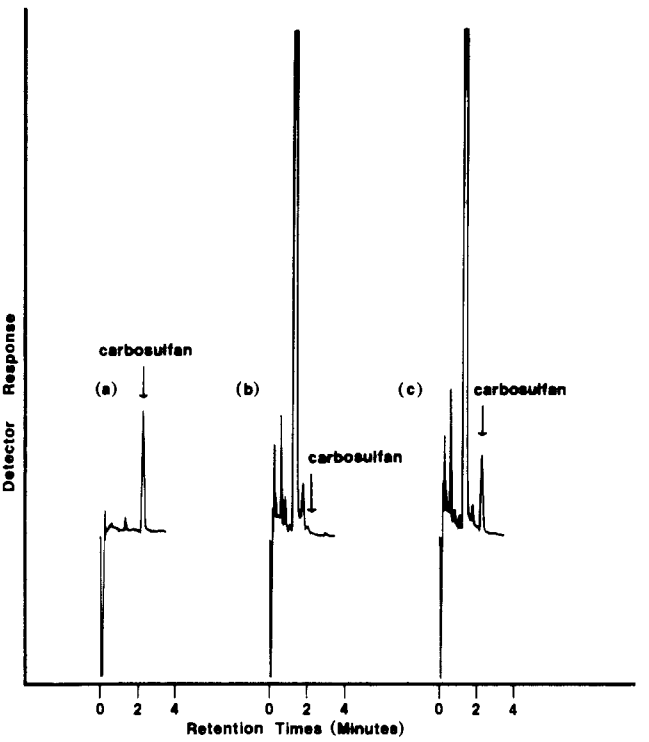

Figure 2. Typical chromatograms for the analysis of carbosulfan in citrus: (a) 1.5 ng of carbosulfan; (b) 10 mg of citrus check sample; (c) 10 mg of citrus check sample fortified with 0.05 ppm of carbosulfan.

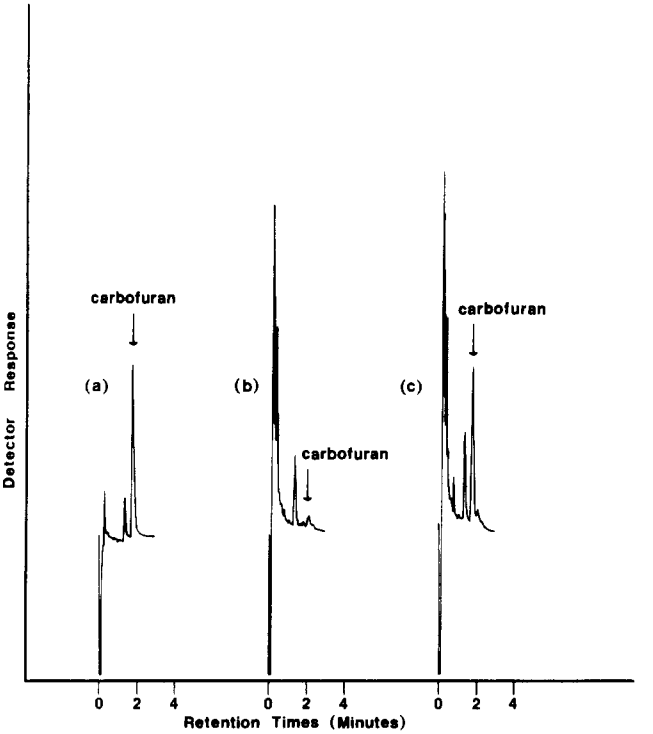

Figure 3. Typical chromatograms for the analysis of carbofuran in citrus: (a) 1 ng of carbofuran; (b) 10 mg of citrus check sample; (c) 10 mg of citrus check sample fortified with 0.10 ppm of carbofuran.

In the development process it was found that water was critical to the extraction of each compound from low-moisture crops and soil. In addition, buffering the methanol–water mixture at pH 8.0, which is similar to the work

of Umetsu et al. (1979), helped assure the stability of the carbosulfan, which is unstable and breaks down rapidly below pH 7.0. There was no evidence of decarbamylation of carbosulfan or carbofuran by the weakly basic buffer in sample extracts stored at room temperature for 1 week, which was sufficient time for analysis.

In order to ensure the stability of intact carbosulfan in soil samples, the soil samples should be frozen immediately after sampling and then kept frozen until analysis.

As additional precautions against decomposition, soil samples should not be put through the classical thawing, air-drying, and sieving process before analysis to obtain a uniform sample. The soil samples should be, however, ground and well-mixed while frozen.

Gel permeation was a useful tool for separating carbosulfan from the lipids in crop samples high in vegetable oil content but was not suitable for separating carbosulfan from the terpenes comprising citrus oils. Samples, such as citrus oil, with a high terpene content required the classical hexane–acetonitrile partition.

The hexane–acetonitrile partition adequately separated the terpenes from the pesticides. As an additional separation tool, an excess of hexane in a subsequent hexane–acetonitrile partition was used. At this point, the partition of carbosulfan is favored into the hexane.

In most cases the Darco–Attaclay plus aluminum oxide cleanup columns were used as described. When more cleanup was desired, such as for dry alfafa hay and citrus oil, a 2 g plus 4 g (60-mL elution) or a 5 g plus 7 g (125-mL elution) Darco–Attaclay plus aluminum oxide column was used.

Other liquid phases used routinely in the gas chromatograph included SE-52 (2%), Super Pak 20M (Analabs), and Ultrabond (Ultra Scientific, Inc.). The best peak shape and response were achieved by using the OV-101. The SE-52, however, was especially useful with soil and field water samples in separating carbosulfan from interferences. Super Pak 20M (or Ultrabond) was useful with soil samples in separating carbofuran from interferences. The Super Pak 20M and Ultrabond could also be used to separate carbosulfan from carbofuran isothermally in a single chromatogram.

The OV-101 could also be used to separate carbosulfan from carbofuran in a single chromatogram if the columns were temperature programmed. The packing needed extra care in conditioning to ensure a minimum of column bleed and a prohibitively steep base line during analysis. The column was temperature programmed at a rate of 3 °C/min, while disconnected from the detector, from ambient to 230 °C and held at 230 °C for 24 h. In use, the column was held at 170 °C for 2 min and then programmed at a rate of 10 °C/min to 225 °C, where the temperature was held for 1.0 min.

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Registry No. Carbosulfan, 55285-14-8; carbofuran, 1563-66-2; water, 7732-18-5.

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Metabolism of [^{14}C]Fosamine Ammonium in Brush and Turf

Robert L. Chrzanowski

The metabolism of ammonium ethyl [^{14}C]carbamoylphosphonate ([^{14}C]fosamine ammonium, the active ingredient in Du Pont Krenite Brush Control Agent) was studied at 13.4 kg of a.i./ha, on a pasture area consisting of a small pin oak surrounded by grass and clover. Fosamine ammonium had an average half-life of 7 days in the pasture flora. The only metabolites found were carbamoylphosphonic acid (CPA) and carboxyphosphonic acid which reached a maximum concentration after 2 weeks and then rapidly degraded. Twelve months after treatment, no fosamine ammonium, CPA, or carboxyphosphonic acid (<0.05 ppm) was found in pasture turf.

Du Pont Krenite Brush Control Agent when applied as a foliar spray in late summer or early fall controls and/or suppresses the growth of many woody species. Except for certain pines, susceptible species show little or no effect and defoliate normally at the end of the season. The following spring, these species fail to refoliate and eventually die or are severely retarded.

The metabolism of [^{14}C]fosamine ammonium in the rat (Chrzanowski et al., 1979) and its degradation in water and soil (Han, 1979) were reported previously.

This paper describes the metabolism of [^{14}C]fosamine ammonium in pasture flora and brush oak.

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EXPERIMENTAL PROCEDURES

The methodology for analyzing radioactive samples such as liquid scintillation (LSC), combustion analysis (CA), thin-layer chromatography (TLC), X-ray autoradiography, and gas chromatography/mass spectrometry (GC/MS) was described in a previous paper (Chrzanowski et al., 1979).

Materials. Ammonium ethyl [^{14}C]carbamoylphosphonate and [^{14}C]carbamoylphosphonic acid ([^{14}C]-CPA) were obtained as described by Chrzanowski et al. (1979). Unlabeled and ^{14}C-labeled carboxyphosphonic acid

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