Gyllenhaal, O.; Ehrsson, H. *J. Chromatogr.* **1975**, *107*, 327.
Hasinski, S. *J. Chromatogr.* **1976**, *119*, 207.
Otto, S.; Beutel, P.; Drescher, N.; Huber, R. *Adv. Pestic. Sci., Plenary Lect. Symp. Pap. Int. Congr. Pestic. Chem., 4th, 1978* **1979**, 551.
Patterson, P. L.; Howe, R. L.; Abu-Shumays, A. *Anal. Chem.* **1978**, *50*, 339.
Roper, R.; Ma, T. S. *Microchem. J.* **1957**, *1*, 245.
Singh-Verma, S. B.; Luib, M. *Proc. Natl. Acad. Sci. U.S.A* **1974**, *20*, 129.
Weed Science Society of America "Herbicide Handbook of the Weed Science Society of America"; Weed Science Society of America: Champaign, IL, 1974; p 47.

Received for review November 3, 1980. Accepted February 2, 1981.

Analysis of Carbofuran and Atrazine in Soil Samples

Greg W. Gorder[1] and Paul A. Dahm*

A very rapid procedure for extracting carbofuran and atrazine from soil is described. Both pesticides were extracted from soil with ethyl acetate following soil-moisture adjustment. Minimal or no effects were caused by soil type, pH, and pesticide concentration. When compared with other carbofuran and atrazine extraction techniques, the ethyl acetate technique was equally effective, less time consuming, and permitted extraction of larger soil samples. Extraction of whole samples was preferred over subsampling for carbofuran analyses, because of very uneven distribution of that chemical in soil samples. Carbofuran and atrazine in extracts were measured without extract concentration or cleanup, by a gas–liquid chromatograph (GLC) with a thermionic specific detector. This technique was corroborated by high-pressure liquid chromatography. Atrazine, carbofuran, and several of their metabolites were separated by GLC with an Apiezon N liquid phase. It was the only one of nine liquid phases tested that adequately separated those compounds.

Two pesticides commonly used on corn in the Midwest are atrazine, for control of annual broad-leaved weeds and some grasses, and carbofuran, for control of northern, *Diabrotica longicornis* (Say), and western, *D. virgifera* LeConte, corn rootworm larvae. Frequently, these two chemicals are applied to the same fields, so it would be useful to have procedures for analyzing both compounds simultaneously.

There are numerous extraction procedures that have been used successfully for each of these pesticides. Many of the extraction procedures are similar, so it is likely that a single procedure would effectively extract both pesticides. We hoped to develop an extraction procedure for both pesticides that would work efficiently on the large numbers of samples and large quantities of soil that must be extracted for pesticide residue work. We hoped to eliminate time-consuming steps such as refluxing, filtration, centrifugation, solvent partitioning, solvent evaporation, and extract cleanup. One technique that had the potential of meeting these criteria was shaking the soil with ethyl acetate. Kadoum and Mock (1978) showed that ethyl acetate extracted both carbofuran and atrazine from freshly treated samples, but their technique included two solvent evaporation steps and an extract cleanup step. Gorder et al. (1980) described two techniques for extracting carbofuran from soil. One of those techniques utilized ethyl acetate and had no time-consuming evaporation or cleanup steps prior to measurement of carbofuran by gas–liquid chromatography (GLC) with a thermionic specific detector (TSD).

This paper describes the extension of that methodology to soils containing both atrazine and carbofuran. The procedure required for analysis of both pesticides is described in detail and corroborated with a second analytical procedure. The extraction procedure was tested for the influences of a variety of factors, and work with freshly treated laboratory soils (Kadoum and Mock, 1978; Gorder et al., 1980) was extended to aged residues and field residues. Procedures widely accepted for extraction of carbofuran (Cook, 1973) and atrazine (Tweedy and Kahrs, 1978) were used for comparative extraction of aged and field residues.

MATERIALS AND METHODS

Analytical Chemicals. Analytical-grade 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (deethylated atrazine), and 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (deisopropylated atrazine) were received gratis from CIBA-GEIGY Corp., Greensboro, NC. Commercial-grade atrazine (AAtrex 4L) was purchased. Analytical, technical, and commercial (Furadan 10G) grades of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran) and analytical-grade 2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate (3-hydroxycarbofuran) and 2,3-dihydro-2,2-dimethyl-2-oxo-7-benzofuranyl methylcarbamate (3-oxocarbofuran) were received gratis from FMC Corp., Middleport, NY.

Soils. Soils used in these experiments were classified as Canisteo (formerly a high pH variant of Webster) and a mixture of Kenyon and Floyd. Composites of three, 10.5 cm diameter by 7.5 cm deep soil cores were tested for pH (glass electrode; soil:water ratio of 2:1), particle size (Bouyoucos, 1936) after partially destroying the organic matter with H_2O_2, and organic carbon (Mebius, 1960). The percentage of organic matter was determined by multiplying the percentage of organic carbon by 1.724 (Allison, 1965). The Canisteo soil was pH 7.8, 20% sand, 59% silt, and 21% clay and had 5.2% organic matter. The mixed Kenyon and Floyd soil was pH 6.1, 26% sand, 51% silt,

Department of Entomology, Iowa State University, Ames, Iowa 50011.
[1]Present address: Department of Entomology, Cornell University, Ithaca, NY 14853.

0021-8561/81/1429-0629$01.25/0

Table I. GLC Retention Times of Atrazine and Carbofuran

liquid phase		support		retention time, min		
material	% load	material	mesh	carbofuran	atrazine	separation
Apiezon N	10	Chromosorb W (HP)	100-120	4.06	5.90	1.84
Apiezon N	3	Chromosorb G	100-120	2.90	4.55	1.65
SE-30	3	Gas-Chrom Q	80-100	0.70	0.77	0.07
DC-200	10	Chromosorb W (HP)	80-100	2.12	2.32	0.20
OV-101	4	Gas-Chrom Q	80-100	0.90	0.90	0.00
OV-3	3	Supelcoport	100-120	1.12	1.00	0.12
QF-1	5	Gas-Chrom Q	60-80	0.87	0.60	0.27
OV-210	5	Gas-Chrom Q	80-100	1.35	1.05	0.30
Carbowax 20-M	7	Gas-Chrom Q	80-100	11.45	11.10	0.35
EGSS-X	2	Gas-Chrom Q	60-80	1.40	1.30	0.10

and 23% clay and had 3.8% organic matter. Soils that were used in laboratory experiments were not treated with carbofuran for at least 2 years and atrazine for 1 year prior to collection of the soil. Composite soil samples, consisting of six or more 10.5 cm diameter by 7.5 cm deep cores, were passed through a 2.0-mm sieve and mixed by rolling on a sheet of paper. Soil subsamples were weighed into glass containers, treated with pesticide, extracted, and analyzed as described later in this paper.

Pesticide Extraction from Soil. The ethyl acetate procedure usually was applied to ~400-g (dry weight) quantities of soil in 0.95-L glass extraction jars. Additions of 100 mL of 0.25 N HCl (~62.5 μmol of HCl/g of soil) and 200 mL of reagent-grade ethyl acetate were made to each extraction jar. The jars were sealed and placed horizontally on a Fisher-Kahn shaker (280 oscillations/min; 32-mm stroke distance) for 15 min. The ethyl acetate was removed by decanting, and the soils were extracted 2 additional times by shaking for 15 min with 150 mL of reagent-grade ethyl acetate. The three extracts of each sample were pooled, and the total volume of each pooled extract was measured. The extracts were mixed with a stirring rod, and 200 mL was transferred to 237-mL bottles, each containing 8 g of anhydrous Na_2SO_4. The remainder of each extract was discarded. The portions saved were stored at -18 °C until analyzed. When the dry weight of the soil was not determined prior to soil extraction, it was obtained by heating the jars with extracted soil at 100–110 °C for 24 h.

When smaller soil samples were extracted, the procedure was scaled down proportionally, with the exception that a total volume of at least 130 mL of ethyl acetate was always used (50, 40, and 40 mL). The addition of the 0.25 N HCl solution always brought the soil moisture level to the 35–55% range.

Extracts that also were analyzed by high-pressure liquid chromatography (LC) were evaporated to dryness, and the residues were dissolved in 5 mL of dichloromethane. The dichloromethane was cleaned on a Florisil column (Gorder et al., 1980). The ethyl acetate–hexane (35:65) eluate was evaporated to dryness, and the residues were dissolved in 5 mL of dichloromethane. Carbofuran in the dichloromethane and two additional 5-mL dichloromethane rinses of the evaporation flask were removed by adsorption when the solvents were passed through a silica SEP-PAK (Waters Associates, Inc., Milford, MA). Carbofuran was removed from the SEP-PAK with 4 mL of methanol that was used for high-pressure LC analyses.

The 0.25 N HCl reflux extraction procedure (Cook, 1973) was compared to the ethyl acetate procedure for carbofuran extraction. This procedure was changed by substituting an extract cleanup procedure modified from Nelsen and Cook (1980) for the Nuchar-attaclay column described. The modified cleanup procedure was done in a 19.5 by 2.3 cm glass column packed with successive layers of 1 cm of sea sand, 10 cm (16.5 g) of Florisil deactivated to 2.5% moisture, and 0.5 cm of sea sand. The column was prewetted with hexane, and the sample was transferred to the column with 10 mL of ethyl acetate–hexane (1:9). An additional 140 mL of ethyl acetate–hexane (1:9) was passed through the column and discarded. The sample flask was rinsed with 10 mL of ethyl acetate–hexane (3:7) that was used with an additional 140 mL of ethyl acetate–hexane (3:7) for carbofuran elution from the column. Atrazine was not found in these extracts, apparently due to destruction by acid hydrolysis.

The acetonitrile–water (9:1) reflux extraction procedure (Tweedy and Kahrs, 1978) was compared to the ethyl acetate procedure for both atrazine and carbofuran extraction. This procedure is described for *s*-triazine herbicides and probably would not work for carbofuran due to the extract cleanup procedures involved. The cleanup steps were not used in this study, so information on both atrazine and carbofuran was obtained. There was no difficulty measuring either atrazine or carbofuran in the uncleaned extracts, but long retention time contaminants made analysis of these extracts very slow.

Gas–Liquid Chromatography (GLC). The analyses were done on a Varian Model 3700 GLC with a 200 °C injector port, 250 °C detector, 175 °C column oven, 28 mL/min nitrogen carrier gas flow, 6 mL/min hydrogen gas flow, 160 mL/min air flow, 4 TSD bias voltage, and 3.90 TSD bead current. GLC voltage attenuations between 1×10^{-12} and 8×10^{-12} A/mV were used. Peak heights were used for all determinations.

Tests of various lengths and diameters of glass columns deactivated with Sylon-CT showed that carbofuran peaks were greatly reduced or absent when columns larger than 95 cm by 2 mm i.d. were used. Although it might have been possible to overcome this effect by silylation of the packing material, all additional tests were run with 95 cm by 2 mm i.d. columns. Apiezon N was the only liquid phase that provided acceptable separation between atrazine and carbofuran (Table I). Chromosorb W (HP) was a better support than Chromosorb G, because it produced columns with more theoretical plates. Apiezon N was coated on both of these supports by fluidized drying (Leibrand and Dunham, 1973). The short retention times that occurred with many of the packings were lengthened at lower column temperatures and slower carrier-gas flow rates, but resolution between atrazine and carbofuran was not improved significantly. The retention times on the Apiezon N column and minimum detectable quantities of carbofuran, atrazine, and some of their metabolites are shown in Table II.

Chromatograms obtained from ethyl acetate extracts of treated and untreated soils are shown in Figure 1. Although it was not confirmed by other techniques, it is possible that the unknown peak consisted of deethylated and (or) deisopropylated atrazine. The Apiezon N column

Table II. Retention Times of Atrazine, Carbofuran, and Some of Their Metabolites [95 cm by 2 mm i.d. Glass Column Packed with 10% Apiezon N on 100–120-Mesh Chromosorb W (HP)] and Their Minimum Detectable Quantities by Using a GLC with a TSD

compound	retention time, min	min detectable quantity, pg[a]
deisopropylated atrazine	3.20	60
deethylated atrazine	3.42	200
carbofuran	4.06	120
3-oxocarbofuran	5.45	200
atrazine	5.90	60
3-hydroxycarbofuran	7.48	300

[a] Twice the noise level at a voltage attenuation of 1×10^{-12} A/mV. Minimum detectable quantities were about one-third the values listed when determined on a new bead by using a bead current of 2.50.

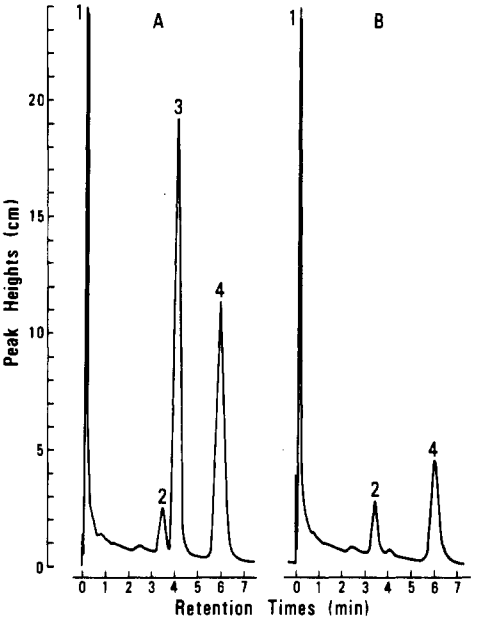

Figure 1. GLC chromatograms obtained at a voltage attenuation of 8×10^{-12} A/mV by using a TSD with a new bead operated at a bead current of 2.50. (A) 1 μL of extract of Canisteo soil treated with 4.8 ppm of carbofuran and 1.0 ppm of atrazine; 1 = ethyl acetate, 2 = unknown, 3 = 3.83 ng of carbofuran, and 4 = 0.95 ng of atrazine. (B) 1 μL of extract of untreated Canisteo soil; 1 = ethyl acetate, 2 = unknown, and 4 = 0.34 ng of atrazine.

would no longer separate carbofuran from the unknown peak or the two atrazine metabolites after use, followed by storage at room temperature without nitrogen gas flowing through it. For that reason, it was necessary to pack a new column every time the column was removed from the GLC oven.

High-Pressure Liquid Chromatography (LC). Extracts and standards were injected on a Waters Associates high-pressure LC consisting of a Model U6K injector, Model 6000A pump, μBondapak C_{18} column, and Model 440 UV absorbance detector. The mobil phase was 60% methanol in water (v/v) with a flow of 1 mL/min. Detection was made at 254 nm. Peak heights were used for all determinations.

Effects of Various Factors on the Ethyl Acetate Extraction Procedure. Initial tests with the ethyl acetate extraction method gave a mean recovery of over 96% of freshly applied carbofuran (Gorder et al., 1980). This result might have been artificially high due to calculations involving the total volume of sample extract. That volume was measured before drying the extract over anhydrous Na_2SO_4 and determination of residue levels. The removal of the water might concentrate the pesticide, thus causing the volume measurement taken before drying to be too high and making it better to measure the extract volume after drying. The following experiment was done to determine if measuring the extract volume before drying gave significant errors. Four 503-g (wet weight) aliquots of Kenyon and Floyd soil were weighed into 0.95-L glass jars. Each sample was treated with 2.0 mg of carbofuran in 1.0 mL of acetone and mixed for 20 min on a jar roller. The carbofuran in these soils was 5.0 ppm on a soil dry weight basis. Two of the four samples were extracted as previously described. Extracts of the other two samples were dried with anhydrous Na_2SO_4 before measurement of the extract volume. All extracts were analyzed by GLC with a TSD.

A factorial experiment was done to determine if soil acidity and moisture levels would affect carbofuran and atrazine extraction by the ethyl acetate method. Acidity was tested by two different factors in the experiment. The actual soil pH was altered by using either acidic Kenyon and Floyd soil or basic Canisteo soil. In addition, the soil pH was lowered by adding 100 mL of moisture as 0.25 N HCl or left unaltered by adding 100 mL of moisture as glass-distilled water. The third factor tested in the experiment was the soil-moisture levels. The initial soil-moisture levels were adjusted to 5% (air-dried) or 25%. As previously mentioned, an additional 100 mL of 0.25 N HCl or water was added during the extraction, so the final soil-moisture levels were 30 or 50%. Duplicate samples of each of the eight combinations of three factors were set up by weighing the appropriate wet weight of soil into sixteen 0.95-L glass jars to make 400-g (dry weight) samples. The 16 samples were treated with 2.0 mg of carbofuran and atrazine each in 1.0 mL of acetone, mixed for 20 min on a jar roller, and extracted in the appropriate manner. An additional sample of each soil type was adjusted to the high soil-moisture level, treated with 100 mL of 0.25 N HCl, and extracted as a control without pesticide treatment. Extracts were analyzed by GLC with a TSD.

The efficiency of carbofuran and atrazine extraction from soil with ethyl acetate could be dependent on their concentration in the soil. Ten 503-g wet weight aliquots of Kenyon and Floyd soil were weighed into 0.95-L glass jars to test this possibility. Groups of two soil samples were left untreated as controls or treated with the appropriate quantity of carbofuran and atrazine in 2.5 mL of acetone to make concentrations of 0.1, 1.0, 10, and 100 ppm on a soil dry weight basis. The samples were mixed for 20 min on a jar roller prior to extraction. The extracts were analyzed by GLC with a TSD.

Pesticide Measurement by Two Techniques. An experiment was done to test the GLC technique against a second measurement technique to verify its accuracy. This experiment consisted of duplicate samples of the four combinations of Kenyon and Floyd and Canisteo soils and low and high moisture levels. The moisture levels after adjustment were 31 and 50% in Kenyon and Floyd soil and 33 and 53% in Canisteo soil. Single samples (415 g dry weight) of the four treatment combinations were treated with 2.0 mg of carbofuran and 1.0 mg of atrazine, each in 1.0 mL of methanol. The four remaining samples were left untreated to test for carbofuran initially present in the soil. The samples were mixed for 20 min on a jar roller prior to extraction. The extracts were analyzed by GLC and after additional sample cleanup by high-pressure LC.

Comparisons between Extraction Procedures. Two experiments were done comparing the ethyl acetate ex-

traction procedure to two other extraction procedures. These experiments were done with commercial pesticide formulations to make conditions realistic. The first experiment was done by extracting carbofuran and atrazine from samples collected from a field of Kenyon and Floyd soil. Carbofuran (as Furadan 10G) was applied, 11.5 g of carbofuran/100 linear m, in four 18 cm wide and 30.5 m long bands over rows of corn seeds, as described by Gorder et al. (1980). Atrazine (as AAtrex 4L) was broadcast sprayed on the soil surface at the rate of 0.224 g of atrazine/m^2 by using a Walsh sprayer (Charles City, IA). One 10.5 cm diameter by 7.5 cm deep soil core was removed from each of the four rows treated with both pesticides 48 days after treatment with carbofuran and 46 days after treatment with atrazine. The cores were kept separate, and the pesticides in each core were distributed as well as possible by passing the soil through a 2.38-mm sieve and mixing by rolling on a sheet of paper. Six 60-g (wet weight) aliquots were removed from each core sample for extraction. Two of each six soil aliquots were extracted with ethyl acetate, two with 0.25 N HCl, and two with acetonitrile–water (9:1) according to the procedures previously described. All extracts were analyzed by GLC with a TSD.

The second experiment was done with laboratory-treated soil according to a factorial design. The ethyl acetate, 0.25 N HCl, and acetonitrile–water (9:1) extraction procedures were each tested for extraction of atrazine and carbofuran as the first factor in this experiment. The second factor was either Canisteo or Kenyon and Floyd soil. The third factor was incubation of the pesticide residues for either 0 or 15 days. Six samples of each of the six combinations of the first two factors (three methods × two soil types) were set up by weighing the appropriate quantity of wet soil into 118-mL jars to provide 50 g (dry weight) of soil. Two of each group of six samples were extracted for use as untreated controls. The remaining four of each group of six samples were treated with 22.5 mg of Furadan 10G (equivalent to 2.25 mg of carbofuran) and 1.0 mL of AAtrex 4L diluted with glass-distilled water to a concentration of 2.25 mg of atrazine/mL and mixed for 20 min on a jar roller. Two of each four treated samples were brought to 50% moisture and immediately extracted in the appropriate manner. The remaining samples were maintained at 50% moisture by adding water to correct for weight loss each 5 days during the 15-day incubation period. For reduction of water loss, the latter samples were covered with Parafilm that had a central hole large enough to accommodate a pipet tip for water additions. The incubation temperature was maintained at 22–27 °C. All extracts were analyzed by GLC with a TSD.

RESULTS AND DISCUSSION

Effects of Various Factors on the Ethyl Acetate Extraction Procedure. When soil extracts were passed through anhydrous Na_2SO_4 to remove water prior to measurement of the extract volume, recovery was 97.7 ± 0.8% of applied carbofuran as opposed to 99.0 ± 1.4% when the extracts were dried in the storage bottle after volume measurement. These recoveries suggest that drying the extract in the storage bottle might give recoveries that are slightly greater than the actual recovery, but the difference is very small and not significant in this experiment ($t_{0.10}$ with 2 df).

The effects of various factors on the recovery of carbofuran and atrazine by the ethyl acetate extraction procedure are shown in Table III. Soil type had no effect on the extraction of carbofuran but might have had some effect on the extraction of atrazine. Unfortunately both soils used in this experiment contained low levels of atrazine that had carried over from treatments in previous years. Results in Table III include corrections for the initial levels of atrazine. Small errors in these correction values rather than differences in atrazine extraction might have caused the apparent difference between soils. In either case, the extraction was good in both soils and any differences were very small. Thus, the soils had no effect or a very minor effect on the extraction of carbofuran and atrazine by the ethyl acetate procedure.

Table III. Recovery of Carbofuran and Atrazine from Soil As Dependent on Soil Type, pH, and Soil Moisture; Results Are Means ± SD of Duplicate Tests

experimental factors			recovered in % of applied	
soil type	soil moisture[a]	pH adjustment[b]	carbofuran	atrazine
Kenyon and Floyd	low	none	96.1 ± 2.1	97.7 ± 1.3[c]
Kenyon and Floyd	high	none	97.9 ± 0.1	97.0 ± 0.3[c]
Kenyon and Floyd	low	acid	97.3 ± 0.4	97.1 ± 0.8[c]
Kenyon and Floyd	high	acid	98.0 ± 0.3	95.0 ± 0.3[c]
Canisteo[d]	low	none	93.3 ± 1.8[e]	99.1 ± 2.8
Canisteo	high	none	99.1 ± 1.8	99.9 ± 3.5
Canisteo[d]	low	acid	92.9 ± 2.5[e]	99.7 ± 3.0
Canisteo	high	acid	97.8 ± 0.3	103.0 ± 0.3

[a] Low = 5% moisture before pH adjustment and 30% after; high = 25% moisture before pH adjustment and 50% after. [b] None = 100 mL of glass-distilled water; acid = 100 mL of 0.25 N HCl. [c] Kenyon and Floyd soils were significantly lower than the Canisteo soils at the 5% level (F with 1 and 9 df). [d] Extracted 4 times with ethyl acetate. [e] Low-moisture Canisteo soils were significantly lower than high-moisture Canisteo soils at the 5% level (F with 1 and 4 df).

Soil-moisture levels did not influence the extraction of either pesticide from Kenyon and Floyd soil but did influence extraction from Canisteo soil. The effect of soil moisture on the extraction of atrazine from Canisteo soil is not evident in Table III because the fourth extraction was totally effective. The need for the fourth extraction was evidenced by recovery of 96.3 ± 1.0% of the ethyl acetate added to the four high-moisture Canisteo samples but only 77.0 ± 0.5% of the ethyl acetate added to the four low-moisture samples. This shows that poor extraction was due largely to poor decantation of the ethyl acetate from the soil. This problem did not occur in the low-moisture Kenyon and Floyd samples, apparently due to a lower water-holding capacity in that soil. These results show that both pesticides are effectively extracted over a wide range of soil-moisture levels, but those levels must be in a range that allows easy separation of the solvent from the soil. This problem was shown in Canisteo soil at the low soil-moisture level, but it can also occur in very high-moisture soils. The addition of 100 mL of 0.25 N HCl (or water) to 400-g (dry weight) samples that contain moderate amounts of moisture has been effective for us. More moisture should be added to some air-dried soils and less to soils that are approaching saturation. A final moisture level between 35 and 50% will work for soils similar to the ones studied.

No effects occurred due to the addition of soil moisture as glass-distilled water or 0.25 N HCl. This suggests that extraction was not influenced by soil pH. In subsequent experiments we used 0.25 N HCl because it did not produce any harmful effects, and potentially it could help to extract aged residues from the soil.

Table IV. Recovery of Carbofuran and Atrazine by Different Extraction Methods from Four Kenyon and Floyd Field-Collected Soil Samples Taken 48 Days after Treatment with Carbofuran (as Furadan 10G) and 46 Days after Treatment with Atrazine (as AAtrex 4L); Results Are Means ± SD of Duplicate Tests Expressed as ppm of Pesticide on a Soil Dry Weight Basis

	ppm of pesticide				
	carbofuran extraction			atrazine extraction	
soil sample no.	ethyl acetate[a]	acetonitrile-water (9:1)[b]	0.25 N HCl[c]	ethyl acetate[a]	acetonitrile-water (9:1)[b]
1	0.54 ± 0.35	0.55 ± 0.23	0.52 ± 0.11	1.09 ± 0.03	1.06 ± 0.07
2	0.26 ± 0.10	0.32 ± 0.10	0.21 ± 0.18	1.11 ± 0.02	1.06 ± 0.01
3	0.60 ± 0.01	0.55 ± 0.11	0.35 ± 0.09	0.82 ± 0.04	0.83 ± 0.00
4	0.21 ± 0.08	0.22 ± 0.09	0.09 ± 0.04	1.28 ± 0.04	1.29 ± 0.02

[a] Method presented in this paper. [b] Method modified from Tweedy and Kahrs (1978). [c] Method modified from Cook (1973).

Table V. Recovery of Carbofuran and Atrazine by Different Extraction Methods from Two Soils, Containing 50% Soil Moisture, That Were Extracted Immediately or 15 Days after Treatment with Carbofuran (as Furadan 10G) and Atrazine (as AAtrex 4L); Results Are Means ± SD of Duplicate Tests Expressed as Percent Pesticide Recovered

		% pesticide recovered				
		carbofuran extraction[a]			atrazine extraction[a]	
incubation time, days	soil type	ethyl acetate	acetonitrile-water (9:1)	0.25 N HCl	ethyl acetate	acetonitrile-water (9:1)
0	Kenyon and Floyd	95.8 ± 7.2	82.2 ± 10.2	70.6 ± 0.8[b]	102.5 ± 1.7	90.6 ± 1.2[b]
0	Canisteo	90.5 ± 8.3	82.5 ± 0.4	83.9 ± 4.0[b]	99.1 ± 0.2	85.0 ± 0.8[b]
15	Kenyon and Floyd	3.7 ± 3.5	2.2 ± 0.2	0.8 ± 0.1	83.9 ± 3.5	84.7 ± 0.8
15	Canisteo	7.9 ± 1.0	3.9 ± 0.4	7.1 ± 4.8	89.7 ± 0.9	81.9 ± 3.1

[a] See footnotes a–c in Table IV. [b] Significantly lower recoveries at the 5% level (F with 1 and 6 df for carbofuran and 1 and 4 df for atrazine) than those for the other methods used to extract that pesticide from soils incubated for the same length of time.

The mean carbofuran recoveries from soil samples treated with 0.1, 1.0, 10, or 100 ppm ranged between 96.3% (1.0 ppm) and 93.7% (10 ppm) of applied carbofuran. The results were best fitted by a line with the equation $Y = 95.4 - 0.7 \log_{10}$ (concentration in ppm). The slope of the line was not significantly different from 0 (5% level). These results show that between 0.1 and 100 ppm the concentration of carbofuran in soil has little or no effect on the percentage of carbofuran extracted with ethyl acetate.

The mean atrazine recoveries from these samples were 110.9% (0.1 ppm), 94.8% (1.0 ppm), 91.9% (10 ppm), and 91.6% (100 ppm) of applied atrazine. These results were best fitted by the second-order equation $Y = 96.4 - 10.0 \log_{10}$ (concentration in ppm) $+ 4.0 [\log_{10}$ (concentration in ppm)$]^2$. It is difficult, however, to justify this as a realistic model. Results had to be corrected for low levels of atrazine that were present in the soil prior to treatment. It is likely that this initial level of atrazine was underestimated, causing an apparent exponential increase in residue levels with decreasing atrazine concentration. The results show that the recovery of atrazine exceeded 90% at all levels between 0.1 and 100 ppm. This suggests that atrazine concentration has little or no effect on recoveries within the range tested.

Pesticide Measurement by Two Techniques. High-pressure LC confirmed the absence of carbofuran and presence of atrazine in untreated Kenyon and Floyd and Canisteo soils. High-pressure LC recovery results were corrected for losses of carbofuran that occurred in cleanup steps that were not used prior to the GLC analyses. Essentially no atrazine was lost in these steps. The carbofuran correction (1.141 × percent of applied carbofuran recovered after cleanup) was determined by the analysis of carbofuran standards before and after cleanup. Results from the four treated soil extracts showed that recoveries calculated from high-pressure LC measurements averaged 0.7 and 5.1% of applied pesticide higher than recoveries calculated from GLC measurements for carbofuran and atrazine, respectively. Neither of these deviations was significant ($t_{0.10}$ with 3 df). This result shows that GLC measurements of carbofuran and atrazine made on uncleaned sample extracts are reliable.

Comparisons between Extraction Procedures. Table IV shows that ethyl acetate extracted carbofuran and atrazine residues from field-collected soil samples treated with Furadan 10G and AAtrex 4L as well as the other procedures tested (no significant differences at $F_{0.10}$ with 2 and 12 df for carbofuran and 1 and 8 df for atrazine). The ethyl acetate procedure was preferred because it was less time consuming yet equally effective. There were large differences in carbofuran residue levels between soil samples no. 1–4 (Table IV). This type of variation resulting from uneven distribution of carbofuran in the field was previously described by Caro et al. (1973). Carbofuran also was poorly distributed within each sample as shown by mean coefficients of variation (SD/mean × 100) calculated from Table IV of 38% for carbofuran but only 3% for atrazine. This variability resulting from uneven distribution of carbofuran in field-collected soil samples could be reduced by more thorough soil mixing procedures before subsampling or entirely eliminated by extraction of whole soil samples rather than subsamples. The amount of soil extracted by the ethyl acetate procedure is more flexible than that by the other procedures. This allows the ethyl acetate procedure to be used on whole samples and makes subsampling unnecessary. The distribution of atrazine in soil was not a major problem, so either whole samples or subsamples could be analyzed when only atrazine is measured.

Table V shows that ethyl acetate and acetonitrile–water (9:1) were slightly better than 0.25 N HCl for extracting carbofuran from soil freshly treated with Furadan 10G. In addition, ethyl acetate was slightly better than aceto-

nitrile–water (9:1) for extracting atrazine from soil freshly treated with AAtrex 4L. The methods gave similar results for both atrazine and carbofuran in samples aged 15 days ($F_{0.10}$ with 2 and 6 df for carbofuran and 1 and 4 df for atrazine). The ethyl acetate procedure gave slightly better results on the extraction of fresh residues, but overall the results with all three procedures were very similar. The results in Table V also show rapid breakdown of carbofuran during the 15-day incubation in these high-moisture soils. This result is consistent with other observations that will be described in a later publication.

Summary. A very rapid and efficient procedure for extraction of carbofuran and atrazine from soil with ethyl acetate was described. The time-saving step of drying the extracts after volume measurement was satisfactory because it did not significantly influence results. Soil type, pH, and pesticide concentration had little or no influence on the extraction of carbofuran and atrazine with ethyl acetate, but crude adjustment of soil moisture was necessary. The results obtained with ethyl acetate were very similar to results obtained with other procedures, but the ethyl acetate procedure was faster and had sample size flexibility that allowed extraction of whole soil samples to overcome uneven distribution of carbofuran between subsamples. The ethyl acetate soil extraction procedure should be very effective for carbofuran and atrazine residue studies and might also be useful for a large number of other pesticides (Kadoum and Mock, 1978).

ACKNOWLEDGMENT

Special thanks are extended to Patti O'Callaghan-Diekman for helping with the high-pressure LC procedures.

LITERATURE CITED

Allison, L. E. In "Methods of Soil Analysis"; Black, C. A., Ed.; American Society of Agronomy: Madison, WI, 1965; Part 2, pp 1367–1378.
Bouyoucos, G. J. *Soil Sci.* **1936**, *42*, 225.
Caro, J. H.; Freeman, H. P.; Glotfelty, D. E.; Turner, B. C.; Edwards, W. M. *J. Agric. Food Chem.* **1973**, *21*, 1010.
Cook, R. F. *Anal. Methods Pestic. Plant Growth Regul.* **1973**, *7*, 187–210.
Gorder, G. W.; Tollefson, J. J.; Dahm, P. A. *Iowa State J. Res.* **1980**, *55*, 25.
Kadoum, A. M.; Mock, D. E. *J. Agric. Food Chem.* **1978**, *26*, 45.
Leibrand, R. J.; Dunham, L. L. *Res./Dev.* **1973**, *24*, 32.
Mebius, L. J. *Anal. Chim. Acta* **1960**, *22*, 120.
Nelsen, T. R.; Cook, R. F. *J. Agric. Food Chem.* **1980**, *28*, 98.
Tweedy, B. G.; Kahrs, R. A. *Anal. Methods Pestic. Plant Growth Regul.* **1978**, *10*, 493–521.

Received for review February 15, 1980. Revised January 21, 1981. Accepted February 24, 1981. Journal Paper No. J-9768 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2121, a contributing project to North Central Regional Project NC-96.

Residues of Isobornyl Thiocyanoacetate (Thanite) and a Metabolite in Fish and Treated Ponds

John L. Allen,* Joe B. Sills, Verdel K. Dawson, and Ronald T. Amel

Isobornyl thiocyanoacetate (Thanite) is an insecticide that induces a surfacing response in fish and therefore has been considered to have potential as a fish collecting agent. Analyses for residues of Thanite in carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) exposed to the chemical yielded only a trace of the parent compound. A metabolite, isobornyl α-(methylthio)acetate, was isolated and identified by GC–MS, and a reference standard for the metabolite was synthesized. Residues of the metabolite were present in largemouth bass muscle tissue within 1 h after exposure to Thanite. The metabolite was also observed in the muscle, blood plasma, and bile of carp. Residues of the metabolite are rapidly eliminated after the fish are transferred to Thanite-free water. Residues of Thanite in water, algae, and soil from ponds treated with Thanite declined to undetectable levels within 28 days after treatment.

Isobornyl thiocyanoacetate (Thanite) is an insecticide that has been investigated as a potential tool for the live collection of fish because it induces a surfacing response at 1.0–1.6 μL/L (Burress et al., 1976; Buckner and Perkins, 1975; Lennon et al., 1970; Lewis, 1968). A chemical that facilitated live collection would make it possible to harvest fish at low cost and would also make it feasible to recover desirable fish that are otherwise lost when ponds or lakes are treated with toxicants for the removal of undesired fish populations or when flood waters dry up.

No data are available on the residues of Thanite in fish exposed to the chemical or on the persistence of Thanite in the aquatic environment. We determined the persistence of Thanite in ponds treated with the chemical, identified a major metabolite of Thanite in fish, and studied the elimination of the metabolite after exposed fish were transferred to Thanite-free water.

MATERIALS AND METHODS

Thanite (82% isobornyl thiocyanoacetate and 18% other active terpenes) was obtained from McLaughlin Gormley King Co., and isobornyl chloroacetate was obtained from

National Fishery Research Laboratory, U.S. Fish and Wildlife Service, La Crosse, Wisconsin 54601 (J.L.A. and V.K.D.), Southeastern Fish Control Laboratory, U.S. Fish and Wildlife Service, Warm Springs, Georgia 31830 (J.B.S.), and Viterbo College, La Crosse, Wisconsin 54601 (R.T.A.).