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).

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

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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

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Hardwicke Chemical Co. Pesticide-grade solvents were used for all residue work.

Adult largemouth bass (*Micropterus salmoides*) representing a desirable fish species were exposed to 1 mg/L Thanite in 100-L polyethylene tanks containing 18 °C spring water to which slaked lime had been added to give a total hardness of 20 mg/L as $CaCO_3$ and a pH of 7.0. Adult carp (*Cyprinus carpio*) representing an undesirable fish species were exposed to 1 mg/L Thanite in 100-L polyethylene tanks containing 12 °C well water having a total hardness of 230 mg/L as $CaCO_3$ and a pH of 7.8. Fish were removed from the Thanite solution after selected exposure times and placed in Thanite-free, flowing water. Fish muscle samples (three fish per sample) were taken at selected intervals during the withdrawal period.

Thanite was applied to 0.1-acre (0.04-ha) ponds by the procedures of Burress et al. (1976). Temperature, hardness, and pH ranges were 18–20.5 °C, 2–4 mg/L as $CaCO_3$, and 5.7–6.5, respectively. Samples of water, algae, and soil were collected at 1–7-day intervals for 28 days and analyzed for residues of Thanite and possible metabolites.

Muscle fillets were ground with dry ice by the method of Benville and Tindle (1970) and extracted by the column extraction procedure of Hesselberg and Johnson 1972); hexane–ethyl ether (75:25) was used as the extracting solvent. Soil and algae were mixed with sodium sulfate and placed in a column. The column was eluted with 200 mL of hexane–ethyl ether (75:25). The extracts were concentrated by evaporation in a warm water bath with a stream of dry air. Blood plasma samples were diluted 10 times with water, and gallbladder bile samples were diluted 50 times with water. The diluted samples were extracted 3 times with hexane–ethyl ether (75:25). The extracts were concentrated by evaporation in a warm water bath with a stream of dry air.

Analyses of the extracts for residues of Thanite were carried out with a Tracor 220 gas chromatograph equipped with flame photometric detection in sulfur mode. The operating conditions were as follows: 100 cm × 4 mm i.d. U-shaped glass column packed with 10% OV-101 on 80–100-mesh Chromasorb W-HP; nitrogen carrier gas flow 100 mL/min; column temperature 200 °C; detector temperature 200 °C; injector temperature 220 °C; electrometer setting 1.6×10^{-9} A full scale; 1-mV recorder with 1.27 cm/min chart speed.

An apparent metabolite was observed during analyses of muscle tissue from largemouth bass and carp that had been exposed to Thanite. For isolation of enough of the metabolite for mass spectral analysis, 12 carp weighing an average of 1480 g were exposed to 3 mg/L Thanite for 4 h. Muscle fillets were ground and extracted as above. The hexane–ether was evaporated to near dryness, taken up in hexane, and partitioned into acetonitrile. The acetonitrile was evaporated to near dryness and taken up in ethyl acetate–toluene (75:25). Lipids in this extract were removed by using an automated gel permeation chromatography (GPC) unit equipped with a column containing Biobeads (SX-2) and a solvent system of ethyl acetate–toluene (75:25). The pumping rate was 4 mL/min. Timed GPC cycles were set for a 25-min dump, 25-min collection, and 1-min wash. Each of the collected fractions of the samples was concentrated and reprocessed with the GPC unit a second time for additional cleanup. Final sample fractions were pooled, concentrated by evaporation, and analyzed by gas chromotography–mass spectroscopy (GC–MS).

We obtained mass spectra of the metabolite by using a Perkin-Elmer Model 270-B gas chromatograph–mass spectrometer (GC–MS) with a 3.6 m × 2 mm glass column packed with 3% OV-7 on Chromasorb W-HP (80–100 mesh). The GC–MS system was interfaced with a PDP-12 LPD computer. The column temperature was programmed from 100 to 240 °C at 5 °C/min with 20 psig of helium as the carrier. Spectral scans were initiated and acquired under computer program control every 4 s; the scan duration was 4 s.

Isobornyl α-(methylthio)acetate was prepared in high yield and excellent purity by reacting isobornyl chloroacetate with an excess of freshly prepared sodium methylmercaptide dissolved in methanol. The reaction mixture was stirred at room temperature for 30 min before it was quenched with water. Extraction of the reaction mixture with petroleum ether was followed by rotary evaporation of the solvent and gas chromatographic analysis.

Table I. Residue Concentrations of the Metabolite of Thanite in Muscle Tissue from Largemouth Bass Exposed to 1 mg/L Thanite for 1 h in Limed Spring Water at 20 °C

withdrawal time, h	residue concn, μg/g[a]
0	0.39 ± 0.032 (3)
2	0.16 ± 0.045 (3)
4	0.12 ± 0.11 (3)
24	ND
48	ND
control	ND

[a] Mean ± SD; number of samples is in parentheses. ND = nondetectable (limit of detection 0.01 μg/g).

Table II. Residue Concentrations of the Metabolite of Thanite in Muscle, Blood Plasma, and Gallbladder Bile of Carp Exposed to 1 mg/L Thanite for 8 h in Well Water at 12 °C

withdrawal time, h	residue concn in[a]		
	muscle, μg/g	plasma, μg/mL	bile, μg/mL
0	0.73 ± 0.380 (5)	0.19 ± 0.070 (4)	0.21 ± 0.11 (5)
24	0.068 ± 0.025 (5)	0.029 ± 0.005 (5)	ND
48	0.028 ± 0.011 (5)	ND	ND

[a] Mean ± SD; number of samples is in parentheses. ND = nondetectable (limit of detection 0.01 μg/g or 0.01 μg/mL).

RESULTS AND DISCUSSION

Largemouth bass exposed to Thanite were analyzed for residues of the compound, with only a trace of the parent compound being detected by gas chromatography with flame photometric detection in the sulfur mode. However, a large peak with a retention time approximately half that of Thanite was found. The "*p* value" (Bowman and Beroza, 1965) of this metabolite of Thanite in hexane–acetonitrile (0.34) indicated that the metabolite is much less polar than Thanite, which has a *p* value of 0.08. Subsequent analyses with a nitrogen-selective detector (alkali flame) indicated that the metabolite did not contain nitrogen.

We performed GC–MS analyses by electron impact on muscle extracts of carp that had been exposed to Thanite. No parent ion was detected for the metabolite. However, a fragment *m/e* 61 ($^{+}CH_2$–S–CH_3) corresponding to a thio ether was found.

Isobornyl α-(methylthio)acetate that we synthesized yielded a product of ~90% purity. By using distillation

Table III. Residue Concentrations of Thanite in Samples from Ponds in Warm Springs, GA, Treated with 3.6, 1.2, and 0.4 mg/L Thanite

days posttreatment	sample and treatment concn								
	water, μg/mL			algae, μg/g			soil, μg/g		
	3.6	1.2	0.4	3.6	1.2	0.4	3.6	1.2	0.4
0	3.25	0.75	0.25	2.33	ND[a]	ND	1.53	0.20	ND
1	2.75	0.50	0.16	2.17	0.38	ND	0.28	0.26	ND
4	1.92	0.18	0.03	1.30		ND	0.26	ND	ND
7	1.25	0.05	ND	5.20	ND	ND	0.25	ND	ND
14	0.28	ND	ND	3.55	1.03	ND	0.07	ND	ND
21	ND		ND	ND	0.38	ND	ND	ND	ND
28	ND	ND	ND	ND	ND	ND	ND		ND
pretreatment	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] ND = nondetectable (limit of detection 0.01 μg/g or 0.01 μg/mL).

under reduced pressure, we achieved 99% purity. The infrared spectrum was very similar to that of the isobornyl chloroacetate starting material. The nuclear magnetic resonance spectrum of the synthesized metabolite differed from that of isobornyl chloroacetate in that there was an *S*-methyl absorption (three protons) at δ 2.53 and the acetate methylene absorption (two protons) was shifted upfield to δ 3.38 as a result of the *S*-methyl group replacing the chlorine. The synthesized compound, cochromatographed with the metabolite of Thanite on two GC columns (OV-101 and OV-17), showed a fragment of *m/e* 61 on GC–MS and had a *p* value (hexane–acetonitrile) identical with that of the metabolite of Thanite. This material was then used as a standard for the analysis of Thanite metabolite residues.

Recoveries of residues from pond water and fish muscle tissue spiked with 0.5 mg/L Thanite averaged 85 and 80%, respectively.

Largemouth bass exposed to 1 mg/L Thanite for 1 h contained 0.39 μg of the metabolite in 1 g of the muscle. The concentration of the metabolite decreased rapidly after the fish were placed in Thanite-free, flowing water (Table I) and was below the limit of detection (<0.01 μg/g) after 24 h.

Samples of blood plasma, gallbladder bile, and muscle of carp which had been exposed to 1 mg/L Thanite for 8 h contained no detectable residues of Thanite (Table II). However, residues of the metabolite were detected in all samples immediately after the exposure (0-h withdrawal; Table II).

Carp muscle contained the highest concentration of the metabolite at the 0-h withdrawal (0.73 μg/g), but the concentration declined to less than 4% (0.028 μg/g) of the 0-h concentration during 48 h of withdrawal. The concentration of the metabolite in blood plasma declined from 0.19 μg/mL at the 0-h withdrawal to less than the limit of detection (<0.01 μg/mL) within 48 h of withdrawal. The metabolite was detected in the 0-h withdrawal samples of gallbladder bile (0.21 μg/mL) but was below the limit of detection (<0.01 μg/mL) after 24 h of withdrawal.

Thanite is rapidly metabolized and eliminated by fish. Hunn (1972) and Lewis (1968) suggested that the thiocyanate bond of Thanite is split by the fish, thereby releasing cyanide and producing the incapacitating and toxic effects of the compound. Ohkawa et al. (1972) reported the liberation of hydrogen cyanide from Thanite by reaction of the chemical and glutathione, even in the absence of glutathione *S*-transferase. Apparently the cyanide group is released and the remaining molecular structure of the compound is rapidly methylated by fish.

Residues of Thanite were detectable in water and soil from three experimental ponds at Warm Springs, GA, up to 14 days after the treatment but not 21 days after treatment (Table III). Algae contained detectable concentrations of Thanite in the 21-day samples, but residues were below the detection limit (<0.01 μg/g) in the 28-day samples. We did not detect the metabolite of Thanite found in fish in samples of water, algae, or soil from the treated ponds.

These studies show that Thanite is rapidly metabolized and excreted by fish. Residues of the chemical do not persist in a pond environment beyond 3 weeks.

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