level determinations, made these methods useful for the analysis of the large numbers of samples generated by the cow feeding study for which the methods were developed.

ACKNOWLEDGMENT

The author gratefully acknowledges the useful comments and suggestions of his colleagues during the course of this work, in particular, Douglas W. Bristol for his helpful discussions.

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Received for review September 17, 1979. Accepted March 14, 1980.

Determination of Terbutryn and Its Degradation Products in Water, Sediments, Aquatic Plants, and Fish

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A method is described for the determination of terbutryn [2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine] and several of its degradation products, N-deethylated terbutryn (DET), hydroxyterbutryn (HT), N-deethylated hydroxyterbutryn (DEHT), 2-(*tert*-butylamino)-4-(ethylamino)-*s*-triazine (EBT), and 2-(*tert*-butylamino)-4-amino-*s*-triazine (ABT), in samples from the aquatic environment. Water samples were extracted with dichloromethane, while sediment, aquatic plants, and fish tissues were extracted with aqueous acetonitrile. The hydroxytriazines were isolated by chromatography on a cation-exchange resin and cleanup on alumina and by high-pressure liquid chromatography. Recoveries of terbutryn, DET, HT, DEHT, ABT, and EBT from water (0.5–50 μg/L) ranged from 76 to 120%. Terbutryn and DET recoveries from sediment, plant material, and fish tissues (0.05–0.9 μg/g) ranged from 74 to 106%. HT and DEHT recoveries from sediment, plant material, and fish tissue (0.1–1.23 μg/g) ranged from 62 to 124%.

Terbutryn (2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine) is registered for control of broadleaf weeds in cereals in the United States (W.S.S.A., 1978), and over the past 10 years, terbutryn has been tested in several countries as an aquatic herbicide under the trade name Clarosan (Ciba-Geigy Ltd. Trademark). It is used for the control of floating and submerged aquatic plants and algae at concentrations of 100 μg/L. There are relatively few published reports on the degradation of terbutryn in the environment, but hydroxytriazines, N-deethylated triazines, and photoproducts have been reported as terbutryn decomposition products (Ciba-Geigy Ltd., 1978; Burkhard and Guth, 1976). In order to monitor the appearance of terbutryn degradation products as well as the disappearance of the parent compound in farm ponds (Muir et al., 1980), an analytical method was required that could be applied to both terbutryn and its more polar breakdown products.

Methods have been described for the analysis of terbutryn in water (Byast and Cotterhill, 1975) and in crops and soils (Ramsteiner et al., 1974; Tweedy and Kahrs, 1978). The analysis of triazine herbicide residues in fish (Hesselberg and Johnson, 1973) and fish eggs (Sirons, 1978a) has also been reported. Ramsteiner and Hormann (1979) have described a method for the determination of hydroxytriazines in plant material by use of high-pressure liquid chromatography (LC). With some modifications, these procedures appeared suitable for the analysis of terbutryn in samples from aquatic ecosystems. In the present work, procedures are described for the analysis of terbutryn in pond water, sediment, cattails (*Typha* sp.), and fish, and their application to the determination of N-deethylated terbutryn (DET), hydroxyterbutryn (HT), N-deethylated hydroxyterbutryn (DEHT), 2-(*tert*-butylamino)-4-(ethylamino)-*s*-triazine (EBT) and 2-(*tert*-bu-

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0021-8561/80/1428-0714$01.00/0

tylamino)-4-amino-*s*-triazine (ABT) is investigated.

MATERIALS AND METHODS

Analytical Standards. Terbutryn, DET [2-amino-4-(*tert*-butylamino)-6-(methylthio)-*s*-triazine], HT [2-(*tert*-butylamino)-4-(ethylamino)-6-hydroxy-*s*-triazine], DEHT [2-amino-4-(*tert*-butylamino)-6-hydroxy-*s*-triazine], EBT, and ABT (all >99% pure) were obtained from Ciba-Geigy Canada Ltd. Hydroxycyprazine [2-hydroxy-4-(cyclopropylamino)-6-(isopropylamino)-*s*-triazine] was obtained from the Agriculture Canada, Pesticide Standards Laboratory, Ottawa. Hydroxytriazines were dissolved in methanol (10 mg/100 mL) with a few drops of acetic acid to aid dissolution. Terbutryn and DET were dissolved in ethyl acetate and EBT and ABT in methanol, all at concentrations of 10 mg/100 mL. Working standards were prepared for LC (5–10 μg/mL) in methanol and for gas–liquid chromatography (GLC) in ethyl acetate (1–4 μg/mL). Radiolabeled terbutryn (^{14}C-ring-labeled; sp act., 5 μCi/mg), obtained from Ciba-Geigy Canada Ltd., was dissolved in acetone (1.0 μCi/mL, 0.20 mg/mL).

GLC and LC Conditions. GLC was performed on a Tracor 560 and a Perkin-Elmer 900 GC equipped with alkali-flame (rubidium silicate bead) detectors; column: 1.8 m × 4 mm i.d. glass packed with 3% Carbowax 20M on Chromosorb W-HP (80/100 mesh) operated at 220 °C, or with 3% OV-17 on Chromosorb W-HP operated at 210 °C. Detector and inlet oven temperatures (°C) were 250 and 230, respectively. Gas flows (mL/min) were as follows: helium (carrier), 30; hydrogen, 3.0; air, 100–120, on both instruments.

LC was performed on a Waters Model 4000A pump and Model 440 absorbance detector (254-nm wavelength); columns: 25 cm × 4.6 mm i.d., containing Lichrosorb Si 100 (5 μm) and Lichrosorb KAT (10 μm). A Valco six-port injection valve with 20- and 100-μL sample loops was used. Solvent systems were (1) 5% methanol in CH_2Cl_2, containing 0.05 M propionic acid with the Si 100 column, flow rate, 1.5 mL/min, and (2) 10% water in acetonitrile, containing 0.001 M ammonium acetate with KAT, flow rate, 1.5 mL/min.

Retention times and concentrations of the triazine compounds were calculated from injection of working standard solutions and comparison of peak heights, for both GLC and LC analysis.

Reagents. All solvents were distilled in glass (Caledon Laboratories, Georgetown, Ontario). Water was deionized, glass distilled. Alumina was Woelm Basic and Acid, both activated at 120 °C overnight and then deactivated with water (15% by weight) before use. The ion-exchange resin [AG50W × 4 (100–200 and 200–400 mesh), Bio-Rad Laboratories] was prepared for use by washing several times with water.

Fortification Procedures. (*a*) *Substrates.* Three sediments were used for fortification studies. Sediments 1 and 2 were collected from two farm pond bottoms by using an Eckman dredge and consisted of 75 and 77% clay, 23 and 24% silt, and 1.9 and 3.7% organic carbon, respectively. Sediment 3, collected from the Red River, contained 48% clay, 45% silt, 7% sand, and 2.3% organic carbon. The pH of the sediments ranged from 7.6 to 7.8. Pond water had a pH of 8.2; total suspended solids, 5 mg/L; chlorophyll a, 24.2 μg/L. Cattails (*Typha* sp.) were obtained from untreated ponds and cut above the rhizome. The emergent portion of the plant was used for fortification. Fish were rainbow trout (*Salmo gairdneri*), weighing 10–20 g.

(*b*) *Nonradiolabeled Recovery Studies.* Pond water samples (1.0 L) were fortified with known volumes (0.01–1.0 mL) of stock or working standard solutions to give 0.5, 5.0, and 50 μg/L concentrations of each triazine compound. The water was shaken and allowed to stand for 1 h before extraction. Sediment samples (50 g, filtered on Whatman No. 1 paper before weighing) were fortified with known volumes of working standards to give concentrations (dry weight basis) of 0.08 and 0.8 μg/g terbutryn and DET and 1.0 and 0.1 μg/g HT and DEHT. The sediments were mixed with a spatula and allowed to stand for 1 h before extraction. Cattail samples (50-g chopped sample) were fortified to give concentrations (dry weight basis) of 0.87 and 0.99 μg/g terbutryn, 0.73 and 0.07 μg/g DET, 0.82 and 0.09 μg/g HT, and 0.86 and 0.09 μg/g DEHT, and the samples were allowed to stand for 1 h before extraction. Fish were ground (meat grinder), 5-g portions were fortified by mixing the ground tissue with known volumes of working standards in the extraction tube to give concentrations of 0.01 and 0.05 μg/g terbutryn and DET and 1.23 and 0.16 μg/g HT and DEHT, and the extracted samples were extracted immediately. All fortification levels of each compound were replicated three times with each substrate.

(*c*) [^{14}C]*Terbutryn Studies.* Incubation flasks similar to those described by Simsiman and Chesters (1976) were filled with 150 mL of pond water and 15 g of sediment (dry weight) and connected to air (aerobic) or nitrogen (anaerobic) gas lines (flow rate 1–2 mL/min), and the contents were allowed to equilibrate for 7 days. An aqueous solution containing [^{14}C]terbutryn was added to each flask to give aqueous concentrations of 1.0 μg/mL terbutryn and approximately 0.045 μCi/flask. The flasks were incubated under either aerobic or anaerobic conditions for 1 or 2 months. Three sediments described previously were used (one replicate/sediment). The contents of the flasks were filtered (Whatman No. 1) to separate the sediment phase, and the moisture content of the sediment was determined. The ^{14}C content of the unextracted sediment was determined by mixing a small sample (0.5 g, in duplicate) with Solka-Floc (0.2 g) and by combusting the sample on a Packard 306 oxidizer. Samples were counted by liquid scintillation counting on a Packard 3030 liquid scintillation counter, using an internal channels ratio method of quench correction. Sediment samples were divided into two portions and extracted in the same way as other fortified sediment samples. Aliquots of sample extracts (0.5–1.0 mL) were dissolved in PCS (Amersham, Toronto)/xylene (2:1) and counted by liquid scintillation counting as described. The remaining sample extracts were spotted on thin-layer plates (0.25-mm thickness, silica gel G), developed (15 cm) in ethyl acetate/water/formic acid (70:4:4), and exposed to X-ray film (Kodak NS-2T) for 4 weeks. The proportion of the radioactivity under each spot was obtained by scraping the plates and counting each fraction by scintillation counting.

Fish (rainbow trout) were added to an aquarium (40 L) containing [^{14}C]terbutryn (50 μg/L; 0.045 μCi/L). The fish were sampled following 2 and 48 h of exposure. The fish were rinsed with distilled water and ground. Portions (0.5–1.0 g) of the ground fish tissue were combusted on the Packard Model 306 oxidizer. The remaining sample was weighed (three replicates: 5 g/sample) and extracted in the same way as fortified fish samples. Sample extracts were assayed by liquid scintillation counting and by GLC.

Extraction Procedures. (*a*) *Water.* Pond water (0.5–1.0 L; unfiltered) was shaken three times with CH_2Cl_2 (200, 75, 75 mL) in a separatory funnel. The water was adjusted to pH 7–9 with dilute NaOH solution if necessary. The CH_2Cl_2 was drained through anhydrous Na_2SO_4 into

a flask and evaporated on a rotary evaporator (30 °C). The residue, which contained terbutryn, DET, ABT, and EBT, was transferred to a graduated test tube (15 mL, screw cap) by using ethyl acetate and was analyzed by GLC. The aqueous phase, which could contain hydroxytriazines, was placed in a rotary evaporator (40 °C) to remove excess CH_2Cl_2. The entire sample was then passed through a column of cationic exchange resin (AG50W × 4, 200/400 mesh; 10 cm × 11 mm i.d.) at 1–2 mL/min. The sample flask and column reservoir were rinsed successively with water (10 mL), methanol/water (1:1, 10 mL), and methanol/3 N NH_4OH (3:2, 5 mL), which were discarded. HT and DEHT were eluted with 40 mL of 3 N NH_4OH/methanol (2:1), which was then evaporated to dryness on a rotary evaporator (55 °C). The residue in the flask was dissolved in 3 mL of methanol and cleaned up on an alumina (acid) column.

(*b*) *Sediment.* Pond sediment (30–50 g wet weight) was refluxed (16 h) with acetonitrile/water (4:1, 150 mL). A portion (10 g) was dried (100 °C, 24 h in vacuo) to determine the moisture content. The refluxed mixture was cooled and filtered on a Buchner funnel. The residuum was transferred to a centrifuge tube (stainless steel Sorvall No. 522) and shaken (20 min) with acetonitrile/water/concentrated NH_4OH (80:20:1, 100 mL). The mixture was centrifuged (1000*g*, 10 min), and the combined acetonitrile extracts were evaporated to a final volume of about 20 mL on a rotary evaporator (40–45 °C). The aqueous residue was diluted to 50 mL with water in a separatory funnel. A known volume of internal standard solution (hydroxycyprazine, 10 μg/mL), usually 1.0 mL, was added and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 extracts containing terbutryn and DET were dried with Na_2SO_4, evaporated just to dryness, and cleaned up on alumina (basic). The aqueous phase containing HT and DEHT was evaporated to remove CH_2Cl_2, diluted with methanol (water/methanol, 4:1), and passed through a resin column (AG50W × 4, 100/200 mesh; 1–2 mL/min). The column was washed and eluted as described for the extraction of water, and the column eluate was cleaned up on alumina (acid).

(*c*) *Aquatic Plants.* Cattails (50-g chopped sample) were extracted by blending (Waring blender) with acetonitrile/water (95:5) (200 mL) at low speed (10 min). The moisture content of a portion of the unextracted sample was also determined (100 °C in vacuo). The macerate was filtered and the residuum was blended with 150 mL of acetonitrile/0.01 N HCl (95:5). The combined filtrates were evaporated on a rotary evaporator (40–45 °C) to remove acetonitrile and immediately diluted to 50 mL with water to avoid hydrolysis of methylthio-*s*-triazines at acid pH. The internal standard was added and the aqueous phase was extracted as described for sediments. The aqueous phase was neutralized before passing through the cation-exchange resin column.

(*d*) *Fish Tissue.* Ground whole fish (5 g) was homogenized (1 min) with acetonitrile/water (95:5, 30 mL) by using a Polytron homogenizer (Brinkmann). The mixture was centrifuged (700*g*, 5 min), and the supernatant was decanted into a separatory funnel. The residuum was reextracted with acetonitrile/0.01 N HCl (95:5, 30 mL) and the mixture was centrifuged. The combined supernatants were extracted with 60 mL of hexane, and the hexane was discarded. The acetonitrile extract was evaporated, and the aqueous residue was extracted to recover terbutryn and DET (CH_2Cl_2 phase) as well as HT and DEHT (aqueous phase) as described for aquatic plants.

Table I. Recoveries of Terbutryn and Degradation Products from Fortified Pond Water

	recov[a] (%) at each concn (μg/L)		
compound	0.5	5.0	50.0
terbutryn	108.0 ± 5.6	101.1 ± 2.3	83.1 ± 4.1
DET	76.9 ± 7.8	90.1 ± 12.0	96.1 ± 11.8
HT[b]	79.7 ± 8.0	88.7 ± 4.9	81.5 ± 4.5
DEHT	90.2 ± 14.6	92.1 ± 6.6	103.4 ± 2.8
EBT	120.1 ± 3.8	90.4 ± 6.1	105.8 ± 5.6
ABT	121.7 ± 7.0	83.8 ± 9.2	91.8 ± 14.5

[a] Average of three or more replicates for each compound with standard deviations. [b] No correction using internal standard.

Cleanup Procedures. (*a*) *CH_2Cl_2 Extracts.* A small chromatographic column (8 cm × 6 mm i.d. with 6 cm × 12 mm i.d. reservoir) was packed with 2.0 g of deactivated alumina (basic), topped with 3–5 mm of Na_2SO_4, and prewashed with 3 mL of hexane/ethyl acetate (2:1). The residue from CH_2Cl_2 extraction, which contained terbutryn and DET, was dissolved in 1.0 mL of hexane/ethyl acetate (2:1) solvent system and transferred to the column. The flask was rinsed with 2 × 0.5 mL of solvent system, and the rinsings were added to the column. Terbutryn and DET were eluted with 5.0 mL of hexane/ethyl acetate (2:1). The initial 1.0 mL of column eluate was discarded, and the remaining 6.0 mL was collected in a graduated test tube and evaporated under a stream of dry air to 1.0-mL volume for GLC analysis of terbutryn and DET.

(*b*) *Cation-Exchange Resin Eluates.* A small column (described previously) was packed with 2.0 g of deactivated alumina (acid), topped with Na_2SO_4 3–5 mm, and prewashed with methanol. The residue in the evaporation flask, containing HT and DEHT, was dissolved in methanol (3.0 mL) by swirling the flask in an ultrasonic bath. The methanol was transferred to the column, and the flask was rinsed with additional methanol (2 × 1.0 mL). The column was washed with a further 1.0 mL of methanol and then with 7.5 mL of methanol containing 0.2 M propionic acid. The initial 6.0 mL of column eluate was discarded and the acidified methanol extract containing hydroxytriazines was collected in a graduated test tube. The eluate was evaporated to dryness and dissolved in LC mobile phase for determination of HT and DEHT.

(*c*) *LC Cleanup.* Extracts of cattails, fish, and sediments (high organic matter content) were often unsuitable for LC analysis of hydroxytriazines with a Lichrosorb Si 100 column, and further cleanup by LC on a Lichrosorb KAT column was then carried out. The sample extracts (acid alumina eluates) were dissolved in 0.2–0.5 mL of mobile phase solvent (acetonitrile/water, 9:1) (0.001 M ammonium acetate) and centrifuged (700*g*) to remove suspended particles. An aliquot (usually 100 μL) of the supernatant was injected (100-μL sample loop), and 2.0 mL of mobile phase solvent was collected in a test tube, starting at the time of initial appearance of the HT peak. The elution time of HT was standardized by injection of standards. The eluate was evaporated to dryness and dissolved in methanol for LC analysis of HT and DEHT on a Si 100 column. The Lichrosorb KAT column was protected by use of a guard column (4 cm × 4.6 mm i.d.) containing additional KAT (10 μm).

RESULTS AND DISCUSSION

The recovery of terbutryn and its degradation products from fortified pond water samples is shown in Table I. Recoveries ranged from 76 to 121% for all compounds over the 100-fold range of concentration that was investigated. Figure 1 shows a gas chromatogram of a water extract from

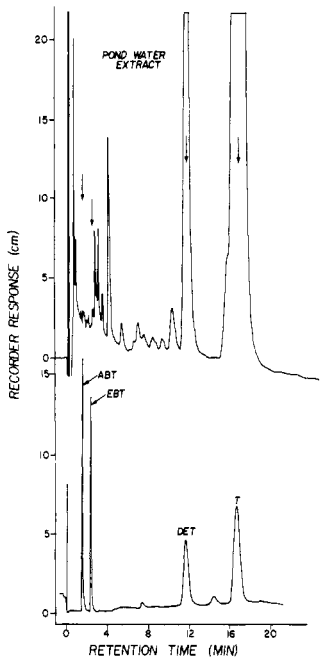

Figure 1. Gas chromatograms of terbutryn, DET, ABT, and EBT and a water extract of a treated pond (3 weeks after treatment at 100 μg/L); 5 μL/0.10 mL sample volume injected, using 3% OV-17 column at 170 °C; attenuation ×8, alkali-flame detector.

Table II. Recoveries of Terbutryn and Degradation Products from Fortified Sediment Samples by GLC and LC

sediment	concn, μg/g dry wt	% recovery[a] T	% recovery[a] DET
1	0.8	97.1 ± 7.3	89.1 ± 3.5
	0.08	106.4 ± 5.0	94.8 ± 14.7
2	0.8	98.4 ± 7.3	95.1 ± 3.6
	0.08	107.3 ± 18.2	82.3 ± 5.4
3	0.8	83.2 ± 2.6	80.9 ± 9.8
	0.08	101.5 ± 9.5	87.2 ± 9.9

sediment	concn, μg/g dry wt	% recovery[a] HT[b]	% recovery[a] DEHT
1	1.0	75.4 ± 5.3	94.5 ± 0.8
	0.1	75.8 ± 11.4	81.0 ± 14.0
2	1.0	61.3 ± 0.8	71.2 ± 15.0
	0.1	74.2 ± 6.2	61.3 ± 10.2
3	1.0	91.7 ± 17.3	124.2 ± 18.4
	0.1	62.8 ± 17.6	63.7 ± 19.6

[a] Average of three replicates ± standard deviation. [b] Corrected by use of an internal standard.

a pond treated with terbutryn. In order to analyze ABT and EBT at the same time, it was necessary to lower the column temperature to 170 °C (3% OV-17) from the 210 °C temperature normally used for the analysis of terbutryn and DET. Extraction of HT and DEHT by the resin column was quantitative, and following cleanup on alumina (acid), the extracts were suitable for LC analysis on the silica column. Figure 2 shows a liquid chromatogram of the resin column eluate of a water sample from a treated pond. HT was detected (5–10 μg/L) following treatment of the pond with terbutryn at 100 μg/L (Muir et al., 1980).

Recoveries of terbutryn and its degradation products from three different fortified sediments are shown in Table II. Terbutryn and DET recoveries ranged from 80 to 107% over a tenfold concentration range that was similar to that found in treated pond samples. The different organic matter contents of the sediment appeared to have

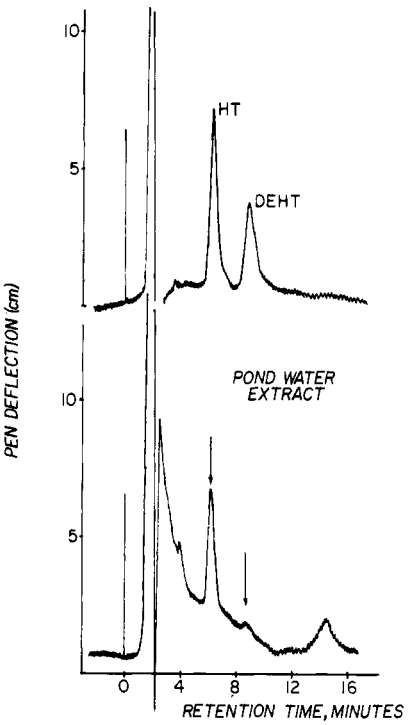

Figure 2. Typical chromatogram of HT in water samples from a pond treated with terbutryn. Column: Lichrosorb Si 100, using 4% methanol in CH_2Cl_2 containing 0.05 M propionic acid, 1.5 mL/min; 0.005 aufs, 5 μL injected.

little influence on the recovery efficiency, but at lower concentrations (<0.1 μg/g) gas chromatograms showed interfering peaks when extracts of sediment no. 2, which contained large amounts of organic detritus (3.7% organic carbon), were analyzed.

Recoveries of HT and DEHT (0.1–1.0 μg/g) from three different sediments ranged from 61 to 124%, and standard deviations were generally high. The HT recovery results (Table II) were adjusted by use of a hydroxycyprazine (HC) internal standard in order to correct for losses of HT (15–20%) to the CH_2Cl_2 phase. HC is separated from HT and DEHT by LC on a silica column and the HT results could be adjusted by dividing by the fraction of the HC recovered. HC has similar partitioning behavior to HT between CH_2Cl_2 and water, and since cyprazine is not used extensively, it is unlikely to be encountered in untreated pond samples. Actual recoveries of HT ranged from 40 to 60% by LC analysis which was similar to recoveries of hydroxyatrazine from soils found by GLC (after methylation) (Muir and Baker, 1978; Khan et al., 1975). A liquid chromatogram of a fortified sediment extract containing HC, HT, and DEHT is shown in Figure 3. Little interference from coextractive peaks was encountered in the analysis of HC, HT, and DEHT in most sediment extracts. The detection limit for HT and DEHT was about 0.05 μg/g (dry weight), which was less sensitive than gas chromatographic procedures (Muir and Baker, 1978; Khan and Marriage, 1977) because of the relatively low sensitivity of the UV absorbance detector to hydroxytriazines at 254 nm.

Recoveries of terbutryn and three degradation products from fortified cattail samples are shown in Table III. Terbutryn and DET gave recoveries that ranged from 87 to 94% by GLC analysis (OV-17 column). Recoveries of HT (0.09 and 0.82 μg/g) ranged from 59 to 64%, while those for DEHT were somewhat higher (56.6–82.6%). Without the use of the internal standard correction, the recoveries of HT ranged from 40 to 50%. Considerable

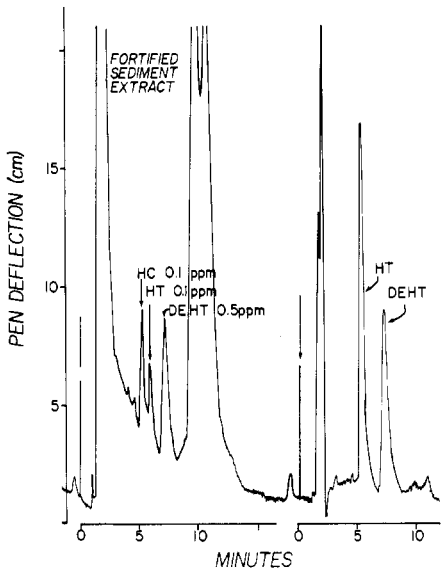

Figure 3. Chromatogram of a fortified sediment extract containing HT and DEHT (HC as internal standard): 0.1, 0.1, and 0.5 ppm, respectively. Column: Lichrosorb Si 100, 10 μL injected; conditions as in Figure 2.

Table III. Recoveries of Terbutryn and Three Degradation Products from Fortified Cattail Samples by GLC and LC

compound	fortification level, μg/g	% recov[a]
terbutryn	0.87	94.3 ± 7.0
	0.09	91.8 ± 16.6
DET	0.73	92.1 ± 10.9
	0.07	87.9 ± 5.8
HT[b]	0.82	59.5 ± 15.5
	0.09	64.2 ± 21.3
DEHT	0.86	82.6 ± 11.1
	0.09	56.6 ± 33.0

[a] Average of three replicates ± standard deviation. [b] Corrected by use of internal standard.

problems with coextractive interferences were encountered with the LC analysis of cattail extracts containing hydroxytriazines. Alumina column eluates were not suitable for direct LC analysis with the silica column. Lichrosorb KAT (a bonded cation-exchange microparticulate packing) was investigated for analysis of the hydroxytriazines but the internal standard (HC) could not be separated from HT with aqueous acetonitrile (1.0 mM ammonium acetate) and little improvement in the separation of HT and DEHT from coextractives was noted on adjustment of the ammonium acetate concentration. The similar elution times of HC, HT, and DEHT on the Lichrosorb KAT column were ideal for cleanup purposes since it was necessary to collect only a small volume of mobile phase to recover the hydroxytriazines quantitatively. Cleanup of cattail extracts by LC resulted in great improvement in the resolution of HT and DEHT as illustrated in Figure 4. Ramsteiner and Hormann (1979) found that cation-exchange resin was not sufficient to cleanup aqueous methanol extracts of plant material containing hydroxytriazines and they included XAD adsorption and gel filtration (Bio-Gel P2) steps in their cleanup procedures. They reported somewhat higher recoveries of hydroxyprometryn (76–110%; 0.1–0.5 μg/g) by use of blending with methanol combined with exhaustive hot extraction of the homogenized plant material.

Recoveries of terbutryn and three degradation products from fortified whole fish samples are given in Table IV. Terbutryn and DET recoveries ranged from 74 to 89%

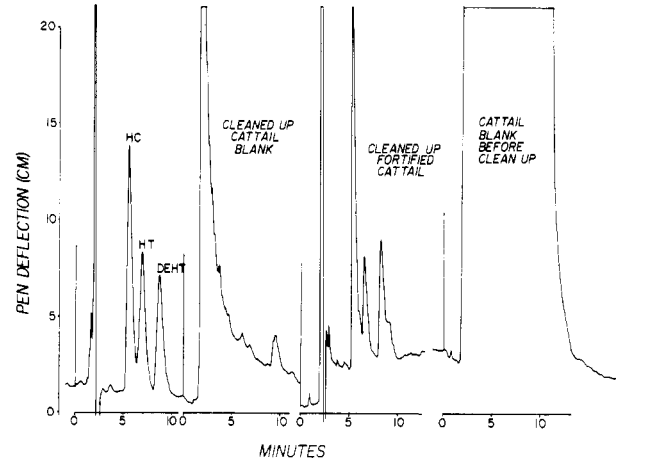

Figure 4. Chromatograms of cattail extracts containing HT and DEHT (HC as internal standard) on the Lichrosorb Si 100 column; 0.0025 aufs, 10 μL injected; conditions as in Figure 2.

Table IV. Recoveries of Terbutryn and Three Degradation Products from Fortified Whole Fish Samples by GLC and LC

compound	exposure time, h	fortification level, μg/g	% recov[a]
terbutryn		0.10	86.6 ± 8.4
		0.05	89.0 ± 14.8
DET		0.10	76.6 ± 20.9
		0.05	74.0 ± 20.8
HT[b]		1.23	71.7 ± 17.7
		0.16	67.8 ± 3.4
DEHT		1.30	70.1 ± 31.3
		0.17	[c]
[^{14}C]terbutryn	2	0.51[d]	111.1 ± 8.7
	48	1.60	70.9 ± 6.4

[a] Average of three or more replicates ± standard deviation. [b] Corrected by use of internal standard. [c] Peaks obscured at this concentration. [d] Concentrations determined by combustion of samples exposed for 2 h (five replicates) or 48 h (three replicates).

which reflected some losses (5–10%) to the hexane that was used to remove neutral lipids. Recoveries of HT ranged from 67 to 71% (0.16–1.23 μg/g), while DEHT gave similar but more variable results. Interfering coextractive materials prevented direct LC analysis of alumina eluates, and cleanup on Lichrosorb KAT was essential for the determination of HT.

The recovery of [^{14}C]terbutryn from fish is shown in Table IV. GLC analysis of fish sample extracts (48-h exposure) indicated that terbutryn represented about 80% and DET about 20% of the radioactivity that partitioned into the CH_2Cl_2 phase. The radioactivity in the aqueous phase was not identified. Lower recoveries after a 48-h exposure (to a 50 μg/L terbutryn concentration) reflected increased metabolism apparently to unextractable compounds. The use of 5% 0.01 N HCl in acetonitrile extracted an additional 2–6% of the radioactivity. Extraction of fish tissue with 10% water in acetonitrile gave greater recovery of radioactivity from the fish exposed for 48 h, but also increased the amount of coextractive interferences encountered in the analysis of sample blanks by GLC and LC. The final pH of the aqueous phase before partitioning with CH_2Cl_2 ranged from 3 to 4 in the fish extracts. Prolonged standing of the sample at this pH could hydrolyze terbutryn and DET and was avoided by immediately shaking with CH_2Cl_2. The partition at an acid pH

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