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Analysis of Chlorothalonil and Three Degradates in Sediment and Soil

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A method has been developed for the simultaneous extraction of chlorothalonil and three of its degradates (4-hydroxy-2,5,6-trichloroisophthalonitrile, 1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene, and 1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene) from soils and sediments; the compounds were extracted using sonication with acetone and isolation of the parent compound and matrix interferences from the degradates by solid phase extraction (SPE). The chlorothalonil fraction underwent further coextracted matrix interference removal with Florisil. The degradates were derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and chlorotrimethylsilane (TMCS). All compounds were analyzed by gas chromatography–mass spectrometry (GC-MS). Recoveries on a spiked (20 and 200 μg kg^{-1}) sediment ranged from 80% to 91% with calculated limits of detection of 1−5 μg kg^{-1} dry weight sediment. An additional 20 sediment samples were collected in watersheds from the Southeastern United States where chlorothalonil is used widely on peanuts and other crops. None of the target compounds were detected. Laboratory fortified recoveries of chlorothalonil and its degradates in these environmental sediment samples ranged from 75% to 89%.

KEYWORDS: Chlorothalonil; degradates; soil; sediment; analytical method; GC-MS

INTRODUCTION

Chlorothalonil (CHT, 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile, **Figure 1**) is a nonsystemic fungicide applied to a variety of crops including peanuts, potatoes, other fruits and vegetables and is also used on turf (*1*). CHT has been registered in the United States since 1966 (*1*), currently it ranks 13th in pesticide usage with approximately 4 to 5 million kg active ingredient applied to crops in 2001 (*2*). The transport and toxicity of CHT is of concern, especially in aquatic systems since it is considered "very highly toxic" to fish and invertebrates with acute toxicity levels of 10–80 μg L^{-1} (*3, 4*).

Although CHT is only moderately hydrophobic (log K_{oc} 3–3.8) (*3, 5*), a large fraction (65–95%) can associate with particulate matter (*6*). CHT degrades to several different compounds in water and soil; degradation half-lives (DT_{50}) of CHT in soil range from 5 to 36 days (*5*). The major degradate is 4-hydroxy-2,5,6-trichloroisophthalonitrile (compound **II**, **Figure 1**) (*7–9*), which can form via hydrolysis (*10*), photolysis (*11*) or microbial degradation (*1*). Compound **II** has a log K_{oc} of 2.7 (*12*), while not as hydrophobic as CHT it has the potential to move from the water and associate with sediment. In certain soils sorption of compound **II** can be greater than CHT (*13*), especially in soils with a higher percentage or organic carbon (*12*). Additionally, compound **II** has been found to be more persistent than CHT (*9*). Two other degradates, 1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene and 1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene (compounds **III** and **IV**, respectively, **Figure 1**), have also been found associated with soils (*7, 14*). Concentrations of CHT and its degradates in soils have been reported to range from 100 to 10 000 μg kg^{-1} (*7, 9, 14*). Toxicity information is only available for one degradate, compound **II**, which is considered "slightly toxic" to fish and invertebrates (*1*).

Many published methods target CHT but relatively few include its degradates. Two methods used high pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS; ref *9*) or HPLC with photodiode array detection (HPLC-PDA; ref *12*) for the detection of CHT and compound **II** in soil following extraction with acetone or dichloromethane, respectively. Gas chromatography (GC) methods have targeted all three degradates after extraction with acetone or acidified acetone (*7, 14*). Compounds **II**, **III**, and **IV** do not chromatograph well using GC because their hydroxy and amide functional groups (**Figure 1**) interact with the GC column phase, resulting in poor peak shape and high detection limits (*7*). To gain better sensitivity the degradates can be derivatized prior to analysis by GC. Researchers have tried methylating the hydroxy-containing compounds with diazomethane (*15*) but found that diazomethane can also create compound **II** from CHT (*16*). Putnam et al. (*14*) used iodoethane to analyze the degradates with hydroxy functional groups (compounds **II** and **III**) via gas chromatography–mass spectrometry (GC-MS). However, the amide functional groups cannot be ethylated and compound **IV** had to be analyzed using a separate instrument (*14*). The Putnam (*14*) method gave

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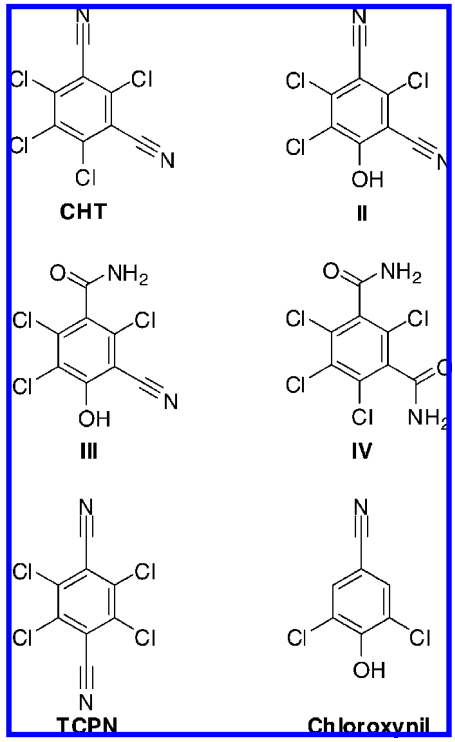

Figure 1. Structures of chlorothalonil (CHT), its degradates 4-hydroxy-2,5,6-trichloroisophthalonitrile (**II**), 1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene (**III**), and 1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene (**IV**), and the two surrogates, 2,3,5,6-tetrachloroisophthalonitrile (TCPN) and chloroxynil.

detection limits capable of measuring environmental concentrations for CHT and the three degradates (2.5 to 25 μg kg^{-1}), but the drawback is that it relied on three different extractions and two instruments.

Even though CHT has been in use for over 60 years, a method is not available to analyze for the parent and its three major degradates in a single extraction at environmentally relevant concentrations. This paper provides a method for the analysis of CHT and its three degradates in sediment and soils by GC-MS. The method uses a single extraction, followed by solid-phase extraction (SPE) cartridge cleanup which separates the parent and matrix interferences from the degradation products into two separate fractions. The CHT fraction undergoes further coextracted matrix interference removal on a Florisil column. The degradates were derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and chlorotrimethylsilane (TMCS), which derivatizes both the hydroxy and amide functional groups for greater sensitivity and resolution by GC-MS analysis.

MATERIALS AND METHODS

Reference Standards. CHT (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile, 99% pure) was obtained from Chem Service (West Chester, PA). Compound **II** (4-hydroxy-2,5,6-trichloroisophthalonitrile, 99% pure) was obtained from the EPA pesticide repository (Ft. Meade, MD). Compounds **III** (1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene, 95%) and **IV** (1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene, 93%) were synthesized via the method of Rouchaud et al. (*7*). Synthesized degradate purity was determined by HPLC-PDA (200–400 nm).

The surrogates used were TCPN [2,3,5,6-tetrachloroisophthalonitrile] (Sigma Aldrich; St. Louis, MO) and chloroxynil [3,5-dichloro-4-hydroxybenzonitrile] (Riedel-de Haën; Hanover, Germany); structures are shown in **Figure 1**. Internal standards, added to the final eluent to correct for GC injection volume, consisted of the polycyclic aromatic hydrocarbons (PAHs) d_{10}-acenaphthene and d_{10}-pyrene, and were obtained from Cambridge Isotope Laboratories (Andover, MA).

Extraction and Clean Up. Sediment used in the method development was from an agricultural drain in the Central Valley of California (1.6% organic carbon). Wet sediment was weighed (10 g dry weight) into a beaker and then spiked with the surrogates and CHT plus degradates for method validation (carrier solvent was methanol <200 μL). The samples were allowed to equilibrate from one hour (at room temperature) to one week (at - 20 °C) before being extracted twice with 25 mL of acetone in a sonicator (Branson 5200; Danbury, CT) for 30 min. After sonication, the samples were filtered through a 0.7 μm glass fiber filter (Whatman; Florham Park, NJ). The acetone was poured through ~20 g of sodium sulfate and reduced under nitrogen at 45 °C using a Zymark Turbovap II (Hopinkton, MD) to 0.5 mL.

The acetone fraction was brought up to 100 mL in water and acidified with 100 μL of glacial acetic acid. The sample was pumped under positive pressure through a precleaned Oasis HLB extraction cartridge (6 cc, 200 g; Waters Corporation, Milford, MA). The HLB extraction cartridge was precleaned with 5 mL of dichloromethane, 5 mL of acetonitrile and 5 mL of deionized water. The sample was loaded onto the cartridge at 5 mL/min. The cartridge was dried under vacuum for 15 min. The first fraction, containing CHT, TCPN and coextracted matrix interferences, was eluted with 10 mL of dichloromethane. The second fraction, containing the degradates and chloroxynil, was eluted with 10 mL of 0.75% ammonium hydroxide in acetonitrile.

The dichloromethane extracts were concentrated to 0.5 mL under a gentle stream of nitrogen (N-evap; Organomation Associates, Berlin, MA) and exchanged to 0.5 mL of hexane for further cleanup on a Florisil column (60–100 mesh, Fisher Scientific; Fairlawn, NJ). The Florisil was activated by baking it at 550 °C for 8 h and deactivated with 10% (by volume) deionized water. The Florisil (10 g) was packed into a column and rinsed with 60 mL of hexane and then the sample was loaded onto the column. The CHT was eluted with 75 mL of 60:40 dichloromethane/hexane. The eluent was reduced to 0.5 mL under nitrogen, exchanged into ethyl acetate and brought to a final volume of 200 μL. Finally, 40 μL of the internal standards (10 ng μL^{-1} PAHs) were added.

Derivatization. The acetonitrile fraction was reduced to 100 μL under a gentle stream of nitrogen. Internal standards were added (60 μL of 10 ng uL^{-1} PAHs) prior to derivatization. The fraction was derivatized with 200 μL of a mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and chlorotrimethylsilane (TMCS) (99:1, v/v) (Supelco; Bellafonte, PA) in a closed vessel at 70 °C for 1 h. Upon removal from the oven the extracts were injected onto the GC-MS.

GC-MS Analysis. Injections were made onto a Varian Saturn 2000 (Walnut Creek, CA) gas chromatograph-ion-trap mass spectrometer. The injector was held at 275 °C and 1 μL injections were made in splitless mode with a 50 psi pressure pulse for 1 min. The flow of helium through a GC column was held constant at 1 mL/min. The oven program was 80 °C for 1 min, ramped at 10 °C/min to 300 °C and then held for 5 min. The analytical column was an Agilent (Palo Alto, CA) DB-5 ms 30 m length × 0.25 mm ID × 0.25 μm phase thickness. The temperature of the transfer line from the GC to the MS was 280 °C and of the ion trap of the MS was 220 °C. The MS was operated in electron ionization (EI) mode with an emission current of 45 μA with a multiplier offset of 300 V for the target compounds (emission current was reduced to 15 μA and no offset for the internal standards). Data were collected in the selected ion storage (SIS) mode. Major fragments and ions monitored can be found in **Table 1**.

Limit of Detection. The limit of detection (LOD) was established as the amount of analyte in the spiked sample that produced a signal greater than three times the background signal and the limit of quantitation (LOQ) was the amount of analyte that produced a signal greater than ten times the background signal (*17*). The fortification levels, detection and quantitation limits are shown in **Table 2**.

RESULTS AND DISCUSSION

Extraction Procedure. The extraction of compounds **II**, **III**, and **IV** from soil has been previously done with acetone (*9, 14*) or acidified acetone (*7, 14*). For our studies, acceptable recoveries were found with the acetone extraction and therefore the acidified acetone was not used. Two sonication extractions

Table 1. Retention Times, Molecular Weight, Mass Spectral Fragments and Selected Ion Scanning (SIS) Monitoring Ranges for CHT, Degradates and Surrogates Analyzed by GC-MS

compound	t_R[a] (min)	MW	MW of silyl derivative	fragments *m/z* (relative intensity)	SIS range
chloroxynil	9.6	187	259	244 (100), 246 (76)	240–255
CHT	11.6	266		264 (54), 266 (100), 268 (51)	260–275
TCPN	12.0	266		264 (51), 266 (100), 268 (49)	260–275
II	13.3	248	320	303 (73), 305 (100), 307 (36)	302–311
III	17.0	266	410	393 (76), 395 (100), 397 (36)	390–400
IV	19.4	302	446	429 (79), 431 (100), 433 (45)	428–438

[a] See GC-MS Analysis (Material and Methods) for experimental conditions.

Table 2. Fortification Levels, Method Recoveries and Relative Standard Deviations, Limits of Detection (LOD), and Limits of Quantitation (LOQ) for CHT and Its Degradates in Soil and Sediment (10 g Dry Weight)

compound	fortification levels[a] (μg kg^{-1})	recovery ± RSD (%)	*n*	LOD[b] (μg kg^{-1})	LOQ[c] (μg kg^{-1})
CHT	20, 200	91 ± 4	6	1	3
II	20, 200	86 ± 6	6	5	15
III	20, 200	82 ± 6	6	2	6
IV	20, 200	80 ± 5	6	2	6

[a] Three samples were fortified at the lower level (20 μg/kg) and three at the higher level (200 μg/kg) for a total of six samples. [b] Limit of detection. [c] Limit of quantitation.

were needed to recover the compound reproducibly; other methods have used anywhere from one (*14*) to three (*9*) extractions of the sample.

Elimination of Matrix Interferences. The sediment extract was reconstituted in acidified water which allowed the use of a clean up step previously developed for CHT and its degradates in water (*18*). This method was modified for sediment samples by first eluting the cartridge with dichloromethane to remove the coextracted matrix interferences (and the CHT) so the degradates can be collected in a clean fraction with basic acetonitrile. The removal of matrix greatly aided in effective derivatization of the CHT degradates with the BSTFA/TMCS.

CHT, present in the dichloromethane SPE fraction, was then separated from matrix interferences on a Florisil column. No further sulfur cleanup was needed as the samples tested for this method did not contain sulfur that interferes with the chromatogram peaks; however, if sulfur were present it can be removed with either activated copper or gel permeation chromatography.

Derivatization Method. Trimethyl silyl derivatives of compounds **II**, **III**, **IV** and chloroxynil were generated using BSTFA/TMCS (99:1, v/v) as the silylation reagent. This reagent was chosen because of its ability to react with both hydroxyl and amide functional groups. The high volatility of the reagent ensured that it did not coelute on the GC with other peaks of interest. The derivatization was carried out in acetonitrile, requiring no further exchanges of solvent. The CHT degradates were stable up to 48 h, as determined by injecting the same sample over time.

Several ratios of derivatization reagent to sample were tested. A 1:1 ratio of reagent to sample was sufficient for achieving 100% conversion of compounds in samples with no matrix interferences present. Environmental sample extracts required a 2:1 ratio of reagent to sample of BSTFA/TMCS to achieve complete derivatization because of matrix interferences present in the sample. The time and temperature necessary for complete derivatization was also higher in environmental samples. In organic solvent the compounds were completely derivatized

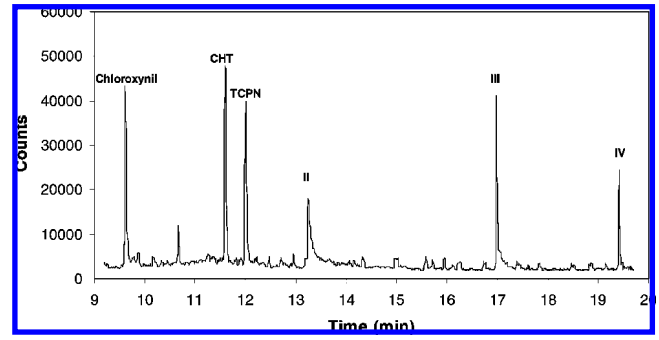

Figure 2. Chromatogram of chloroxynil, CHT, TCPN, and compounds **II**, **III**, and **IV** at ~10 ng μL^{-1}. Compounds **II**, **III**, and **IV** are the silyl derivatives. Internal standards are not shown, d_{10}-acenaphthene and d_{10}-pyrene elute at 8.1 and 14.9 min, respectively.

(double derivatives for compounds **III** and **IV**) in 5 min at room temperature, whereas the environmental sample extracts required an hour in a 70 °C oven to achieve complete derivatization (as seen by negligible increase in peak area with increased time and temperature). The stability of the degradates in the 70 °C oven was confirmed by no loss of peak area over time.

GC-MS Analysis. The chromatogram for the compounds analyzed using full scan is shown in **Figure 2**. The derivatization allowed the compounds to be detected using GC-MS. The underivatized compounds were unable to be seen or in the case of compound **II** had a very poor response. Even with derivatization, compound **II** is still a broad peak (possibly due to degradation in the injector) but much improved over the underivatized compound. Retention times for the compounds are listed in **Table 1**.

Both the underivatized and derivatized compounds give one major ion in the mass spectra with the corresponding chlorine isotopes. Mass spectra obtained in full scan mode are shown in **Figure 3**. For compounds **II**, **III**, **IV**, and chloroxynil, the silylated derivative, mass ion (*m/z*) was not found, instead the molecular ion minus a methyl group *m/z* $[M - 15]^+$ was targeted. To increase the sensitivity of the compounds in natural samples, SIS windows were selected to include the most abundant ions (**Table 1**).

Another advantage of the extraction method presented is the ease to which it could be modified for liquid chromatography–mass spectrometry (LC-MS) (*18*). The degradates are eluted from the HLB cartridge in basic acetonitrile so this fraction could be blown down and injected onto the LC-MS without any derivatization. The fraction eluted off Florisil containing CHT would have to be exchanged to a reversed phase solvent before injection onto a LC-MS. A disadvantage to analyzing CHT and its degradates via LC-MS is that two ionization methods must be used. Atmospheric pressure chemical ionization (APCI) in negative-ion mode for CHT and compound **IV** and electrospray ionization (ESI) in negative-ion mode for compounds **II** and **III** (*18*), which would require two separate runs on the LC-MS reconfigured for the different ionization techniques.

Quantitative Analysis. The response of compounds was linear over the calibration range (0.05–10 ng μL^{-1}). Each compound was quantified using the most abundant ion and each calibration curve had a minimum of five concentration levels. Linear regression analysis by the least-squares method of peak area ratio of analyte/internal standard against different analyte concentrations for each compound gave R^2 values >0.99. In spiking studies, recoveries (**Table 2**) were greater than 80% for all compounds and relative standard deviations were less

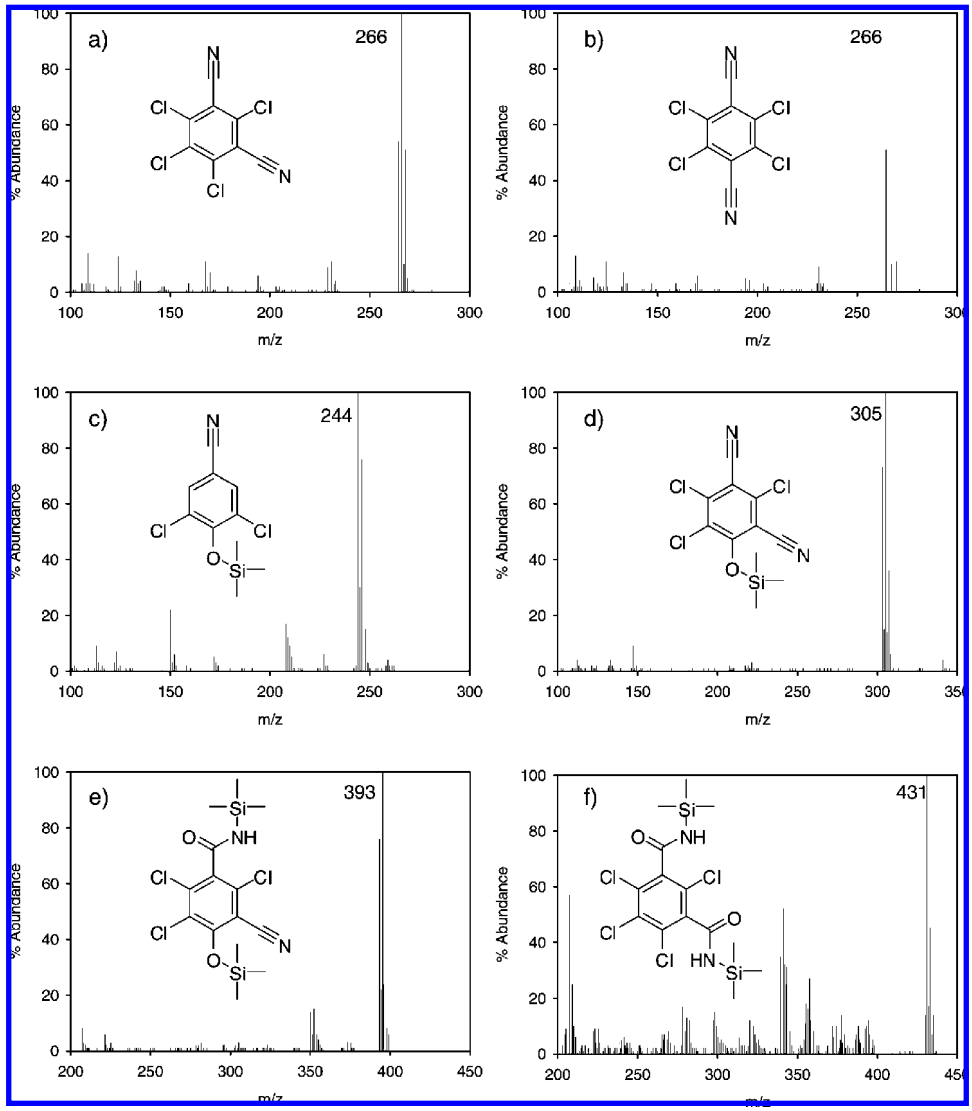

Figure 3. Mass spectra of CHT (a), the CHT surrogate TCPN (b), and the silyl derivatives of the CHT degradate surrogate chloroxynil (c) and compounds **II** (d), **III** (e), and **IV** (f).

than 6%. LODs indicate that the method is sensitive for both CHT (1 μg kg^{-1}) and compounds **II**, **III**, and **IV** (2–5 μg kg^{-1}), with LOQ ranging from 3 to 15 μg kg^{-1}. All LODs are at environmentally relevant concentrations and were similar (and slightly lower) than those given by previous researchers, 2.5−25 μg kg^{-1} (*14*).

Method Application. Twenty depositional (top 2 cm) sediment samples from Georgia, Florida and Alabama were collected in July of 2005 (for further details of sampling sites see ref *18*) and extracted for CHT and its degradates in 2006. These samples were from areas where there were known CHT applications on peanut crops during the summer months (applications are intense with approximately 4−7 applications per season). None of the samples showed detectable concentrations of CHT or its degradates. The degradation half-lives (DT_{50}) in soil are 3−36 and 6−43 days for CHT and compound **II**, respectively (*5*). If there had been recent applications some residues should remain. The lack of detection is most likely because of the unusually high amount of rainfall in the 2 weeks prior to sampling that washed the compounds away from the sites. The samples were frozen at −20 °C until analysis and extracted wet, it is unlikely that the target compounds degraded during storage. The organic carbon concentrations of the samples were 0.2−8.1%. Average recoveries (and relative standard deviation) of the surrogates were 93 ± 12% and 98% ± 7% for chloroxynil and TCPN, respectively. Matrix spikes of the samples showed acceptable recoveries at the 30 μg/kg spiking concentrations. Average recoveries (and % RSD) were 89% ± 9%, 87% ± 5%, 79% ± 8%, and 75% ± 16% for CHT, compounds **II**, **III,**and **IV**, respectively. No relationship was found between the amount of organic carbon in the sediment and the recovery of the laboratory matrix spikes.

A sensitive, precise and reproducible method was developed for the determination of CHT and three of its major degradates in soil and sediments at low concentrations (detection limits of 1−5 μg kg^{-1}). The strength of the current method is that both CHT and its degradates can be extracted in one step. After SPE separation all of the degradates are contained in one fraction. CHT and most interferences are contained in a separate fraction that is not derivatized, so concerns of parent transformation during derivatization are alleviated. This method provides a reduction in the time necessary for extraction and sample analysis of CHT and its three major degradates. By derivatization of the degradates, analysis of all compounds can be conducted using a GC-MS at environmentally relevant concentrations with spectral confirmation.

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