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Method for the Simultaneous Extraction and Analysis of Two Current Use Pesticides, Atrazine and Lambda-Cyhalothrin, in Sediment and Aquatic Plants

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It has been estimated that 375,000 tons of pesticides are used for agriculture annually in the Midwest area of the United States, where approximately 65% of this total is used for production of corn and soybean crops (Clark *et al.,* 1999). Within the Mississippi River Basin, greater than 100,000 tons of herbicides are used annually (Clark *et al.,* 1999). Triazine herbicides (e.g. atrazine) and synthetic pyrethroids insecticides (e.g. λ-cyhalothrin) are two classes of pesticides currently used in this agricultural area. These two types of pesticides are very dissimilar, due to their differences in structural and physical properties. A major difference between these pesticides is polarity, where triazines are relatively more polar than pyrethroids.

With demand for more rapid extractions and multiresidue pesticide analyses, analytical methods for environmental matrices, such as soil and plants, have been developed using new technologies (Kahn, 1995; Sánchez-Brunete *et al., 1998).* For example, traditional extraction methods, such as soxhlet and sonication, are being replaced with solid-phase extraction (SPE), solid-phase microextraction (SPME) and supercritical fluid extraction (SFE) techniques because they are less time consuming and require lower volumes of organic solvents (Hengel *et al.,* 1997; Miege and Dugay, 1998; Camel, 1998). In some cases, newer analytical equipment is not available, therefore older methods using traditional equipment must be reevaluated.

The following study presents the development of a rapid and sensitive gas chromatographic method using sonication for extraction of atrazine and λ–cyhalothrin in sediment and aquatic macrophytes. This method requires lower solvent volumes and reduced sample weights relative to the traditional EPA sonication method.

In addition to method development, a storage stability test was performed to determine acceptable storage times for natural water samples containing pesticide residues. Natural water samples were fortified with atrazine, λ-cyhalothrin, and metolachlor, a chloroacetamide herbicide. These three pesticides represent three of the major classes of pesticides used extensively in row crop production in the area of the lower Mississippi River Basin known as the Mississippi Delta.

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MATERIALS AND METHODS

Atrazine [2-chloro-4-ethylamine-6-isopropylamino-l,3,5-triazine], λ-cyhalothrin [*(RS)*-alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2,-dimethyl-cyclo- propanecarboxylate] and metolachlor [2-chloro-6’-ethyl-*N*-(2-methoxy- 1 -methylethyl)-acet-*o*-toluidide were obtained from USEPA (Research Triangle Park, NC). Chemical structures and physical properties are presented Figure 1 and Table1, respectively.

Sediment and aquatic plant samples were collected from an agricultural drainage ditch located in one of the Mississippi Delta Management System Evaluation Areas (MDMSEA) (Beasley Lake Watershed, Sunflower County, Mississippi). Samples were wrapped in solvent washed foil, transported to the laboratory on ice and placed in the fumehood to dry.

Individual dried sediment and plant samples were ground using a Wiley-mill fitted with a size lo-mesh screen, mixed and placed in solvent washed erlenmeyer flasks equipped with foil caps. Triplicate samples of 1000 mg of dry ground sediment were placed in separate 50 mL glass centrifuge tubes followed by the addition of 7 mL of ethyl acetate. A similar method was followed for plants with the exception that plant samples were prewetted with 1 mL of ultrapure water prior to the addition of ethyl acetate. The mixture was sonicated (Sonics GE600 sonicator) for 1 min in pulse mode using an 80% duty cycle. Following sonication, the mixture was centrifuged on high (- 2000-2500 rpm) using an IEC HN-S Centrifuge with a 4 place rotor (4 x 50 mL). The solvent layer was transferred into another 50 mL centrifuge tube. The extraction was repeated and the solvent layer was combined with the previous extract. The extract was concentrated to near dryness under a stream of UHP nitrogen using a nitrogen evaporator (N-EVAP, Organomation) and solvent exchanged into hexane (0.5 mL). These methods were derived from methods developed by O'Neal *et al.* (1996) for the extraction of pesticides and PCBs from aquatic organisms.

For comparison of extraction methods, triplicate sediment samples were extracted using Soxhlet extraction with ethyl acetate (DIG). Equal amounts of sediment (1000 mg) were ground with sodium sulfate and extracted for 6 hours. The extract was rotary evaporated to approximately 2 mL and further concentrated under a stream of UHP nitrogen to a volume of 1mL.

All extracted sediment and plant samples were subject to silica gel clean-up prior to analysis. A glass micro-column (7 mm i.d.) was fitted with a glass wool plug and 5 cm silica gel (60-100 mesh) activated at 200°C was poured into the column. Approximately 5 mm of sodium sulfate was added to the top of the silica. The column was pre-wetted with 3 mL of hexane and the eluent was discarded. When the hexane reached the top of the sodium sulfate, the concentrated sample extract (0.5 mL) was loaded onto the column with three rinses of hexane, allowing each rinse to sink onto the column. The column was eluted as follows: A) 5 mL of hexane, and B) 10 mL of 10% acetone in hexane. Fraction A was discarded and

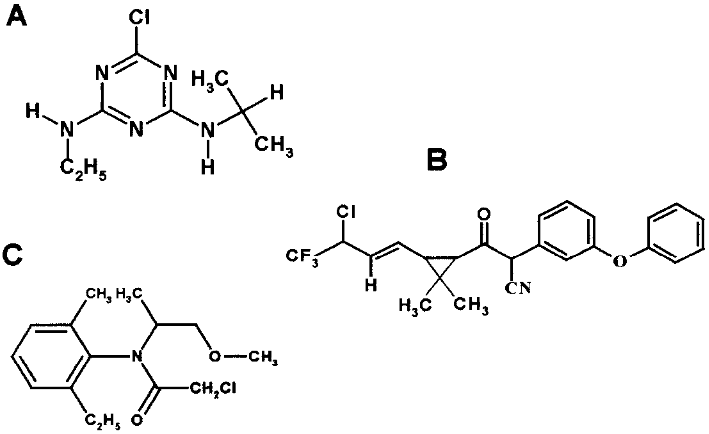

Figure 1. Chemical structures of chlorinated pesticides: A - Atrazine; B - λ-Cyhalothrin; C - Metolachlor.

Table 1. Physical properties of pesticides studied (ARS Pesticide Database, 1995).

Parameter	**Atrazine**	**λ-cyhalothrin**	**Metolachlor**
Molecular wt	215.7	449.9	283.8
Water Solubility (mg/L @ 20°C)	30	0.005	500
Vapour Pressure (mPa @ 20°C)	$4.0E^{-02}$	$2.0E^{-03}$	1.7
Henry's Law (Pa m^3/mol @ 25°C)	$2.48E^{-04}$	$1.80E^{-02}$	$2.44E^{-03}$
Log K_{ow} (@ 20°C)	2.6	7.0	3.1
Log K_{oc}	$1.5E^{02}$	$1.8E^{05}$	$2.5E^{02}$

the hexane reached the top of the sodium sulfate, the concentrated sample extract (0.5 mL) was loaded onto the column with three rinses of hexane, allowing each rinse to sink onto the column. The column was eluted as follows: A) 5 mL of hexane, and B) 10 mL of 10% acetone in hexane. Fraction A was discarded and

Fraction B containing atrazine and λ-cyhalothrin was collected in a 12 mL glass centrifuge tube, concentrated under a stream of UHP nitrogen to a final volume of 1 mL.

A water sample storage stability study was performed in a manner similar to the methods described previously by Hengel *et al.* (1998). Briefly, water samples collected from Beasley Lake and an agricultural drainage ditch were transferred into separate 125 mL amber glass bottles and fortified with atrazine and metolachlor at the 50 ng/rnL level and λ-cyhalothrin at the 100 ng/mL level and then stored at 4°C (n = 18 per sample type). Three samples for each water type were selected randomly and transferred into individual 200 mL glass jars in addition to 500 mg of KCl and 25 mL of ethyl acetate. Each sample was extracted using sonication and transferred into a 250 mL separatory funnel. After discarding the water layer, the organic layer was transferred through sodium sulfate and collected in a 125 mL erlenmeyer flask. The extract was concentrated under a stream of UHP nitrogen to approximately 2 mL, transferred to a 12 mL centrifuge tube and further concentrated to a final volume of 1 mL.

All three analytes were analyzed by gas chromatography-electron capture detection using a Tracer 540 gas chromatograph equipped with a Dynatech Precision GC-411V autosampler and a 15 m x 0.53mm i.d. J&W 30 m DB-5 (1 µm film thickness) Megabore™ column. Column oven, inlet and detector temperatures were 195°C, 240°C and 350°C, respectively. The carrier gas was UHP helium (nexAir, Memphis, TN) at 12.3 cc/min, whereas both the column makeup gas and detector purge gas were UHP nitrogen (nexAir, Memphis, TN) at 60 and 10 cc/min, respectively. Digital data were collected using a PE Nelson 2700 chromatography data system and analyzed using Turbochrom™ 4.11 software. A multi-level calibration procedure was used with standards and was updated every ninth sample. The limits of detection (LOD) for atrazine and cyhalothrin in water were 0.34 ng/rnL and 0.05 ng/mL, respectively while the LODs for sediment and plants were 12 ng/mL and 1.4 ng/mL, respectively.

Concentrations of all analytes in sediment and plant samples were calculated in units of µg/s dry weight. Mean, standard deviation and coefficient of variance about the mean for each analyte were calculated from sample replicates (n=3). Data were analyzed using a two-way analysis of variance (ANOVA) with a significance level of $P \leq 0.05$. When significant differences were detected, a Tukey's multiple comparison test was utilized to compare efficiencies of different solvents and extraction procedures.

RESULTS AND DISCUSSION

Traditionally, when methods are being developed or compared, environmental matrices are amended with the compound(s) of interest and extracted using various solvents to determine the most efficient extraction method (Snyder et *al.*, 1992; Hengel *et al.*, 1998; Babic *et al.*, 1998). Initially this method was followed, where sediment samples were fortified with atrazine and λ-cyhalothrin, made up in acetone, and rolled for 24 hrs to homogenize the sediment. Sub-samples were extracted in ethyl acetate by sonication and extraction efficiencies were calculated to be 90% and >99 % for atrazine and λ-cyhalothrin, respectively. One problem with this type of amendment method is that analytes may not mimic the process in which these compounds are associated to matrices in the aquatic environment, therefore actual extraction efficiencies may be lower in environmental samples. Thus, instead of using this traditional method, native sediments known to be recently contaminated with atrazine and λ-cyhalothrin were used for method development.

Results from native sediment and plant samples sonicated using three different solvents indicate, in both cases, that ethyl acetate was the most effective extraction solvent ($p < 0.05$)(Table 2). It was expected that the mixture of hexane and methylene chloride would be more effective at extracting these pesticides from these matrices. To further validate this method and to ensure that ethyl acetate was the more effective solvent to use, sediment and plant samples were pre-wetted with water prior to extraction. In the case of sediment, extraction efficiencies were increased for both hexane and the hexane/methylene chloride (DCM) mix, but there was no observed significant difference ($p < 0.05$) between ethyl acetate groups (Table 3). Conversely, atrazine recoveries increased significantly ($p < 0.05$) for all three extraction solvents, but there was no observed significant difference ($p < 0.05$) between their recoveries in each pre-wetted group.

Since atrazine is much more polar than λ−cyhalothrin, organic solvents were not as effective in extracting this compound from plant material (Table 2 and 3). By pre-wetting the plant samples with water prior to addition of organic solvent, atrazine extraction efficiencies increased by 6 fold. Similar results were shown by Lino and Noronha da Silveira (1997) where they used a mixture of water and organic solvents to extract a range of organochlorine pesticides. They found that their extraction efficiencies improved for more polar pesticides with the addition of water. It is thought that the addition of water to dried plant samples causes the deactivation of the cellulose active sites where these pesticides are sorbed, therefore increasing extraction efficiencies (Luke and Doose, 1983).

To further validate this method, extraction efficiencies from this procedure were compared to extraction efficiencies using a traditional soxhlet extraction. Sediments containing two levels of atrazine and λ−cyhalothrin contamination were compared using these methods. Results from the comparison show that the present sonication method, which requires considerably less organic solvent, is as effective as soxhlet extraction (Table 4).

As part of the Mississippi Delta MSEA project, routine water samples are collected from agricultural ditches, monitoring wells and lakes for pesticide

Table.2. Mean (n=3) extraction efficiencies of atrazine and λ-cyhalothrin (µg/g) in native sediment and plant samples using various organic solvents.

SEDIMENT

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.8 ±(0.31)	11	0.064 ±(0.007)	11
Hexane	N.D.	-	N.D.	-
50:50 (Hex:DCM)	0.25 ±(0.04)	16	0.030 ±(0.004)	12

PLANT

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.4 ±(0.70)	29	0.51 ±(0.13)	25
Hexane	0.47 ±(0.08)	18	0.18 ±(0.03)	19
50:50 (Hex:DCM)	1.3 ±(0.56)	43	0.39 ±(0.15)	38

C.V. = coefficient of variation

Table 3. Mean (n=3) extraction efficiencies of atrazine and λ–cyhalothrin (µg/g) for native sediment and plant samples pre-wetted with water prior to the addition of various organic solvents.

pre-wetted SEDIMENT

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	2.1 ±(0.25)	12	$5.4E^{-02} \pm (6.5E^{-03})$	12
Hexane	0.76 ±(0.12)	16	$3.0E^{-03} \pm (5.1E^{-04})$	17
50:50 (Hex:DCM)	0.89 ±(0.06)	7.3	$9.0E^{-03} \pm (2.1E^{-03})$	23

pre-wetted PLANT

Solvent	Atrazine	C.V.	λ-Cyhalothrin	C.V.
Ethyl acetate	13.7 ±(2.3)	17	0.44±(0.11)	25
Hexane	13.3 ±(1.3)	10	0.20 ±(0.03)	16
50:50 (Hex:DCM)	13.1 ±(1.1)	8.7	0.28 ±(0.05)	17

Table 4. Comparison of soxhlet vs sonication extraction methods using ditch sediment contaminated with high and low concentrations of atrazine and λ–cyhalothrin (µg/g).

	Soxhlet		**Sonication**	
Sediment A	Mean (n=3)	C.V.	Mean (n=3)	C.V.
Atrazine	11.1 ±(1.04)	9.4	9.48 ±(0.81)	8.5
λ-Cyhalothrin	9.4E^{-03} ±(7.1E^{-04})	7.6	9.50E^{-02} ±(5E^{-03})	4.8
Sediment B				
Atrazine	0.23 ±(0.06)	25	0.28 ±(0.04)	14
λ-Cyhalothrin	2.6E^{-03} ±(3.9E^{-04})	15	2.5E^{-03} ±(7.3E^{-04})	29

C.V. = coefficient of variation

analysis. To ensure optimal pesticide recovery from these samples, a study was performed to determine acceptable sample storage time. The USEPA recommends a maximum storage time of 7 days for chlorinated pesticides (EPA Method 608). To determine this, water samples taken from an oxbow lake (Beasley Lake) in the Mississippi Delta region and an agricultural ditch that drams into the lake were spiked with atrazine, λ-cyhalothrin and metolachlor. For both water samples, results show that there was a rapid reduction in recovery of atrazine and λ-cyhalothrin at 1, 2, 4 and 8 days (Table 5). Hengel *et al.* (1998) reported similar data with pyrethroids, esfenvalerate and permethrin (cis and trans), using a similar test method. Results for metolachlor indicate that, over an 8 day period, recoveries were reduced by approximately 20%. These losses may be attributed to adsorption on glass (Sharom and Solomon, 1981) but it is more likely that these losses were due to adsorption to suspended solids. Both water samples contained a high amount of suspended solids (~ 400 mg/L). Other possibilities for reduction in recovery may include chemical degradation via oxidative free radicals or microbial degradation (Sharom and Solomon, 1981). These data indicate, as also reported by Hengel *et al.* (1998), the importance of immediate extraction of water samples upon returning from the field.

Overall, this research presents an easy and rapid method for the extraction and analysis of two classes of pesticides using a sonication method that requires lower solvent volumes than traditional methods. This method could be used for the extraction of other triazine herbicides and pyrethroid insecticides simultaneously, but it would be suggested that all sediment and plant samples be pre-wetted with

Table 5. Results of the pesticide stability storage test in surface waters fortified with atrazine, λ-cyhalothrin and metolachlor in surface waters.

		% Recovery (n = 3)		
Water Sample	Storage Time (days)	metolachlor	λ-cyhalothrin	atrazine
Beasley Lake	0	100 ±4	82 ±4	100 ±1
	1	87 ±6	56 ±3	79 ±7
	2	87 ±10	NA	61 ±14
	4	73 ±6	51 ±2	43 ±12
	8	83 ±4	60 ±3	47 ±7
Ditch Water	0	93 ±13	87 ±12	100 ±2
	1	78 ±3	69 ±1	100 ±7
	2	66 ±14	NA	75 ±30
	4	68 ±5	72 ±0.3	35 ±6
	8	66 ±4	54 ±5	43 ±10

water prior to the addition of solvents. Even though there was no difference in recoveries between dry and pre-wetted sediments using ethyl acetate, polar pesticides, such as other triazines, may require this pre-wetting step for optimal recoveries.

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