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Comparison of Pressurized Liquid Extraction and Matrix Solid Phase Dispersion for the Measurement of Semi-Volatile Organic Compound Accumulation in Tadpoles

Kerri Stanley[†], Staci Massey Simonich^{†,‡,*}, David Bradford[§], Carlos Davidson^{||}, and Nita Tallent-Halsell[§]

[†]Department of Environmental and Molecular Toxicology, Oregon State University, 1007 ALS, Corvallis Oregon, 97331

[‡]Department of Chemistry, Oregon State University, Corvallis Oregon

[§]U.S. Environmental Protection Agency, National Exposure Research Laboratory, Landscape Ecology Branch, P.O. Box 93478, Las Vegas Nevada, 89193

^{||}Department of Environmental Studies, San Francisco State University, 1600 Holloway Avenue, San Francisco California, 94132

Abstract

Analytical methods capable of trace measurement of semi-volatile organic compounds (SOCs) are necessary to assess the exposure of tadpoles to contaminants as a result of long-range and regional atmospheric transport and deposition. The following study compares the results of two analytical methods, one using pressurized liquid extraction (PLE) and the other using matrix solid phase dispersion (MSPD), for the trace measurement of over 70 SOC, including current-use pesticides, in tadpole tissue. The MSPD method resulted in improved SOC recoveries and precision compared to the PLE method. The MSPD method also required less time, consumed less solvent, and resulted in the measurement of a greater number of SOC than the PLE method.

Keywords

tadpoles; semi-volatile organic compounds; pesticides; pressurized liquid extraction; matrix phase solid dispersion

Introduction

Declines in amphibian species have been reported worldwide [1–4]. Several factors have been suggested to be responsible for these declines, including climate change, ultraviolet radiation, habitat destruction, introduced species, disease, and contaminants [5–9]. While multiple factors are likely responsible for the declines, among contaminants, pesticide exposure has been suggested to be important [5, 10–20].

*Corresponding Author: Staci Massey Simonich, Telephone: 1-541-737-9194, Fax: 1-541-737-0497, staci.simonich@oregonstate.edu, Address: Department of Environmental and Molecular Toxicology, Oregon State University, 1007 ALS, Corvallis Oregon, 97331.

Many pesticides are semi-volatile organic compounds (SOCs), and undergo both long-range and regional atmospheric transport and deposition to remote ecosystems [21–26]. Recently, Hageman et al. and Usenko et al. have shown that regional agricultural sources are responsible for a significant portion of the pesticide deposition in remote U.S. mountain ecosystems [22, 25]. Previous studies have linked atmospheric transport and deposition of pesticides in remote areas of the Sierra Nevada Mountains to their proximity to the intensely agricultural Central Valley of California [19, 20, 22, 26–29].

Exposure of amphibians to pesticides and other SOCs occurs in low elevation ecosystems near sources, in high elevation ecosystems, and in other remote ecosystems. Previous studies on amphibian SOC body burdens have focused on measuring a fairly limited number of pesticides in tadpole or frog tissue [17, 19, 20, 27–34]. However, amphibians are likely exposed to a far greater array of pesticides. For example, over 500 different pesticides were applied in 2006 in California alone [35] (http://www.cdpr.ca.gov/docs/pur/pur06rep/06_pur.htm).

In the present study, two analytical methods were compared for the trace measurement of over 70 SOCs, including current-use pesticides and their degradation products, in tadpole tissue. One method used pressurized liquid extraction (PLE) (referred to as the “PLE Method”) and was similar to a PLE method developed for measuring SOCs in fish with a moderate to high lipid content (0.71 – 18 %) [36]. The second method used matrix solid phase dispersion (MSPD) (referred to as the “MSPD Method”). MSPD has been used for the measurement of SOCs in food products, as well as animal samples, including tadpoles and frogs, and is a relatively simple method for the extraction of SOCs from samples with a low to moderate fat content [32, 37, 38]. Because tadpoles have a relatively low lipid content (0.01 – 3.3 %) (unpublished data), MSPD was evaluated as a potential extraction method. In order to assess the current state of tadpole exposure to pesticides at low concentration, the objectives of this research were to develop and validate an analytical method to identify and quantify low concentrations of current-use and historic-use pesticides in tadpole tissue.

Materials and Methods

Chemicals and materials

In the summer of 1999, Pacific chorus frog (*Pseudacris regilla*) and Cascades frog (*Rana cascadae*) tadpoles were collected from lakes, ponds, and creeks in the Cascade Mountain Range in Northern California. In the summer of 2003, *P. regilla* tadpoles were collected from several lakes in Sequoia and Kings Canyon National Park. Tadpoles from both regions were pooled and used for analytical method development and validation.

Tadpoles were placed in cryovials and in liquid nitrogen or on dry ice after collection and during shipment and were stored at –20°C to –80°C until analysis. A liquid nitrogen – cooled mortar, CoorsTek 99.5% alumina pestles (100 mm) and sodium sulfate (Na₂SO₄) was purchased from VWR (West Chester, PA, USA). Octadecylsilyl (C₁₈) (bulk sorbent), empty 60 ml solid phase extraction (SPE) columns, and silica SPE columns (Mega Bond-elut 20 g) were purchased from Varian, Inc. (Palo Alto, CA, USA). Non-labeled SOC standards (Table 1) were purchased from Chemical Services (West Chester, PA, USA),

Restek (Bellefonte, PA, USA), Sigma-Aldrich (St. Louis, MO, USA), and AccuStandard (New Haven, CT, USA), or obtained from the U.S. Environmental Protection Agency repository [39]. Isotopically labeled chemical standards, including 24 surrogate standards, were purchased from CDN Isotopes (Pointe-Claire, QC, Canada) and Cambridge Isotope Laboratories (Andover, MA, USA) and used for quantification [39]. All chemical standards were stored at 4°C until use. Optima grade solvents (acetonitrile, dichloromethane, hexane, and ethyl acetate) were purchased from Fisher Scientific (Fairlawn, NJ, USA).

Pressurized Liquid Extraction (PLE) Method

The PLE method was used to extract SOC_s from tadpole tissue as described in Ackerman et al. 2008 for extracting SOC_s from fish tissue [36]. Briefly, 2 grams of frozen, ground tadpole tissue was further ground with 65 g Na₂SO₄ (enough to fill the PLE cell) and the mixture was packed into a 66 ml PLE cell (Dionex, Salt Lake City, UT, USA). In the case of SOC spike and recovery experiments, non-labeled SOC standards (Table 1) were added to the ground sample at the top of the PLE cell prior to extraction to assess SOC recoveries over the entire analytical method. In order to measure and subtract the background SOC concentration in the tadpole tissue (tissue blanks) used in the spike and recovery experiments, the isotopically labeled surrogates were added to the ground sample at the top of the PLE cell prior to extraction. Lab blank experiments consisted of 65 g Na₂SO₄ without tadpole tissue packed into the PLE cell and spiked with the isotopically labeled surrogates at the top of the PLE cell prior to extraction. The standards, both non-labeled and labeled, were spiked at approximately 150 ng and the PLE conditions used dichloromethane (DCM) at 100°C, 1500 psi, 2 cycles of 5 min, and 150% flush volume [36] (see Table 2 for PLE method details). Additional Na₂SO₄ was added to the extracts to remove any remaining water. The extracts were reduced in volume (TurboVap II, Caliper Life Sciences, Hopkinton, MA, USA; 12 psi N₂, 30 ° C), solvent exchanged to hexane, purified with silica gel, solvent exchanged to DCM and further purified using gel permeation chromatography (Waters, Milford, MA, USA) [36].

Matrix Solid Phase Dispersion (MSPD) Method

The ground tadpole tissue (2 g) was further ground with C₁₈ and Na₂SO₄ in proportions of 1:5:17.5 by weight, respectively. The tadpole to C₁₈ ratio was similar to a previously published MSPD method [38] and the Na₂SO₄ ratio was adjusted so that the mixture filled the solid phase extraction column within approximately 2 cm of the top of the column. This tadpole mixture was packed into a 60 ml solid phase extraction column containing 30 g Na₂SO₄. In the case of SOC spike and recovery experiments, non-labeled SOC standards (Table 1) were added to the tadpole mixture on the top of the MSPD column to assess SOC recoveries over the entire analytical method. Tissue blanks and lab blanks were analyzed as described in the PLE method, by spiking the isotopically labeled surrogates on the top of the MSPD column prior to extraction. The standards, both non-labeled and labeled, were spiked at approximately 150 ng. The MSPD column containing the ground tadpole sample, was placed on a vacuum manifold (Supelco, Bellefonte, PA, USA), a vacuum was applied, and the sample was eluted with 300 ml acetonitrile, followed by 100 ml DCM at a flow rate of approximately 25 ml/min (see Table 2 for MSPD method details). The DCM fraction was reduced and stored as an archive fraction. To determine if additional SOC_s were eluted from

the MSPD column with the DCM, this fraction was analyzed and contained no spiked SOC. Acetonitrile was chosen as the MSPD column elution solvent because of its ability to simultaneously elute SOC with a wide range of polarities. The acetonitrile fraction was reduced to 0.5 ml using a TurboVap II (12 psi N₂, 30 ° C), approximately 1.0 ml hexane was added, and silica cleanup was performed. The 20 g silica solid phase extraction column was preconditioned as described in [36] and the SOC were eluted from the column using 100 ml ethyl acetate. Different silica column elution solvents were tested and it was determined that ethyl acetate successfully eluted the target SOC with minimal co-elution of matrix interferences.

Instrumental Analysis

Just prior to instrumental analysis, the triplicate recovery extracts were reduced and spiked with the isotopically labeled surrogates and internal standards to assess spiked SOC recoveries over the entire method. In the case of the tissue and lab blanks, the internal standards were spiked into the extract just prior to instrumental analysis.

Semi-volatile organic compounds were identified and quantified using an Agilent 6890 gas chromatograph (Santa Clara, USA) coupled to an Agilent 5973N mass selective detector. Briefly, 1 µl of the extract was injected using an HP 7683 autosampler, a pulsed splitless injection was performed, and 30 m × 0.25 mm inner diameter × 0.25 µm film thickness DB-5 column (J&W Scientific, Palo Alto, CA, USA) was used for separation of the SOC [39]. Standard calibration curves were prepared prior to instrumental analysis of samples. Selective ion monitoring mode was used to identify and quantify the SOC. Either electron impact ionization or electron capture negative ionization was used based on the mode of ionization with the lowest instrumental detection limit for a given SOC [39].

For quality assurance and quality control, one lab blank was included with each batch of samples. Calibration curves were monitored throughout using check standards run for every 3 to 4 samples. Ion abundances were considered a match if they were within ± 20% of the standard or National Institute of Standards and Technology mass spectra library. A signal to noise ratio of 3:1 was used in identification of target analytes and retention times were monitored such that identified target analytes matched check standards within ± 0.05 minutes. Sample specific estimated detection limits were calculated using Environmental Protection Agency method 8280A [40] (Table 3). The instrumental limits of detection, ions monitored, and gas chromatograph oven parameters for electron impact mode and negative chemical ionization mode have previously been published [39].

Statistical Analysis

Average analyte recoveries were compared using a two-sided, two-sample t-test in SPLUS (version 8.0). A *p* value < 0.01 was considered significant. Individual SOC average recoveries greater than 180 % or less than 20 % were excluded from statistical analysis, including average and standard deviation calculations, as these recoveries were outside the acceptable range.

Results and Discussion

Comparison of PLE Method for Fish and Tadpoles

The PLE method resulted in higher average SOC recoveries from fish tissue (54.8 ± 15.5 % [standard deviation]) than from tadpole tissue (46.8 ± 15.3 %) (ref. [36] and Table 1). This was especially true for the DDXs (DDTs, DDDs, and DDEs), and PCBs ($p < 0.01$) (Table 1). The additional SOC losses from tadpole tissue in the PLE method may have been due to higher SOC losses during extract evaporation and solvent exchanges.

The precision for the PLE method, as indicated by the percent relative standard deviations of the SOC recoveries, was higher for the fish tissue (ranged from 0.46% to 21.6%, with an average of 5.88%) than for the tadpole tissue (ranged from 17.4% to 96.9%, with an average of 34.1%) (ref. [36] and Table 1). This may also be due to additional SOC losses during tadpole extract evaporation and solvent exchange.

Comparison of PLE and MSPD Methods for Tadpoles

The MSPD method had significantly higher average SOC recoveries for tadpole tissue (80.6 ± 25.9 %) than the PLE method (46.8 ± 15.3 %) (Table 1) ($p < 0.01$). In addition, the average MSPD recoveries of organochlorine pesticides, organophosphorous pesticides, PCBs, and PAHs were significantly higher than the average PLE recoveries of these same SOC's ($p < 0.01$). However, the MSPD average recoveries for dieldrin and endrin were above the acceptable range (Table 1) and may be the result of these target analytes not behaving in the same manner as the labeled surrogate standards they were quantified against (*d*₄-endosulfan I and *d*₄-endosulfan II, respectively). The average tadpole PLE recoveries of acenaphthylene, acenaphthene, parathion, and endrin aldehyde were below the acceptable range (Table 1) and may be a result of losses during solvent evaporation.

The MSPD method also had higher precision, as indicated by the percent relative standard deviation of the SOC recoveries, (ranging from 0.86 % to 40.7 %, with an average of 11.3 %) than the PLE method (ranging from 17.4 % to 96.9 %, with an average of 34.1 %) for tadpole tissue (Table 1). Instrumental precision was assessed using replicate injections of extracts and standards on an intra- and inter-day basis for both MS ionization modes. Intra-day instrumental precision ranged from 0 % to 20.6 % relative standard deviation for extracts (all SOC's detected; $n = 20$) and 0.025 % to 13.1 % for standards (all SOC's; $n = 10$). Inter-day instrumental precision ranged from 0 % to 38.6 % relative standard deviation for extracts ($n = 20$) and 0.63 % to 15.9 % for standards ($n = 13$).

The PLE and MSPD estimated detection limits were not significantly different and ranged from 0.19 to 2900 pg/g wet weight (Table 3). Both the PLE and the MSPD methods were capable of detecting, but not quantifying, carbaryl and carbofuran. However, the MSPD method was capable of detecting 15 additional current-use pesticides and their degradation products, including the triazine herbicides, over the PLE method (Table 1). The ability to measure current-use pesticides in tadpole tissue is particularly important because some have been reported to cause sublethal effects in amphibians at low concentrations and are among the pesticides implicated in population declines [16, 18, 20, 41].

In addition to significantly higher recoveries for several SOC classes, better precision, and detection of a larger number of SOCs, the MSPD method resulted in shorter extract preparation time and less solvent consumption (Table 2). The MSPD method also resulted in reduced use of dichloromethane, a chlorinated solvent and probable human carcinogen (Table 2) [42] (<http://www.epa.gov/iris/subst/0070.htm>).

Analytical Variability vs. Tadpole SOC Concentration Variability

The MSPD method was used to measure SOC concentrations in tadpole samples collected from several site in the Cascades Mountains, California, USA. Comparisons of the relative standard deviation of intra-day injections of the same tadpole extract (injection replicates), subsamples of the same tadpole sample processed using the MSPD method (analytical replicates), and different tadpole samples collected from the same site and processed using the MSPD method (site replicates) are shown in Figure 1. For most SOCs measured in these samples, the site variability (25 to 100% average relative standard deviation) was greater than the analytical (5 to 45%) and instrumental (1 to 5%) variability. This indicates that the MSPD method is precise enough to study intra- and inter-site variability in tadpole SOC concentrations. This method will be used in future studies to understand the accumulation of SOC in tadpoles collected throughout the California Cascade and Sierra Nevada Mountains.

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Figure 1.

SOC concentration variability among tadpole samples collected from the Cascade Mountains, California, USA using the MSPD method. “Injection replicates” are intra-day injections of the same tadpole extract ($n = 9$); “analytical replicates” are subsamples of a tadpole sample processed using the MSPD method ($n = 8$); “site replicates” are different tadpole samples, collected from the same site, processed using the MSPD method ($n = 8$). “< detection limit” indicates concentrations were below the estimated detection limit in greater than 50 % of replicates. Only replicate sets with at least 50% detections are shown and values below the estimated detection limit (EDL) were substituted with $\frac{1}{2}$ EDL.

Table 1

Average semi-volatile organic compound recoveries over the entire pressurized liquid extraction (PLE) and matrix solid phase dispersion (MSPD) methods for tapoles (RSD = relative standard deviation, NR = not recovered)

	Log K _{ow}	PLE Average Recovery (% RSD)	MSPD Average Recovery (%RSD)	Log K _{ow}	PLE Average Recovery (% RSD)	MSPD Average Recovery (%RSD)
Amide Pesticides						
Alachlor	2.6	NR	76.3 (8.6)	3.2	NR	56.5 (8.1)
Acetochlor	3.0	NR	60.7 (9.7)	3.8	NR	65.3 (4.9)
Metolachlor	3.1	NR	120 (9.2)	4.6	61.3 (40.9)	142 (12.2)
Propachlor	2.4	NR	174 (12.3)	Triazine Herbicides and Metabolites		
Organochlorines Pesticides and Metabolites						
HCH, gamma	3.8	35.0 (49.3)	76.8 (8.2)	1.4	NR	91.2 (0.9)
HCH, alpha	3.8	28.0 (64.4)	71.0 (8.6)	1.8	NR	58.8 (40.7)
HCH, beta	4.0	48.4 (25.8)	87.0 (10.1)	2.3	NR	84.4 (9.6)
HCH, delta	4.1	47.8 (25.6)	84.6 (8.4)	2.2	NR	103 (3.2)
Methoxychlor	4.5	55.9 (27.3)	92.8 (14.6)	1.7	NR	103 (13.7)
Heptachlor	5.2	44.0 (47.4)	106 (6.5)	Miscellaneous Pesticides		
Heptachlor epoxide	4.6	27.0 (32.9)	48.7 (10.1)	2.6	NR	71.7 (5.9)
Hexachlorobenzene	5.5	26.2 (70.8)	64.5 (9.6)	4.3	70.5 (34.7)	152 (3.5)
Endrin	5.2	75.3 (24.2)	224 (8.0)	5.3	38.0 (58.3)	75.9 (9.0)
Endrin aldehyde	4.8	13.9 (35.9)	84.6 (14.2)	Polycyclic Aromatic Hydrocarbons		
Chlordane, trans	6.1	26.4 (30.1)	33.1 (12.5)	3.9	14.6 (102)	64.8 (4.9)
Chlordane, cis	5.9	27.7 (29.1)	35.8 (13.1)	4.0	21.0 (93.6)	65.1 (6.9)
Nonachlor, trans	6.1	26.5 (29.8)	33.1 (12.7)	4.2	19.6 (91.8)	60.0 (8.5)
Nonachlor, cis	6.1	41.5 (26.9)	50.3 (14.6)	4.5	33.2 (49.0)	76.4 (8.2)
Chlordane, oxy	5.5	27.3 (32.3)	43.4 (11.2)	4.5	23.9 (96.9)	50.4 (15.8)
Dieldrin	5.5	114 (25.4)	236 (8.4)	5.1	48.7 (20.4)	79.3 (10.5)
Aldrin	6.4	37.5 (43.3)	76.6 (8.7)	5.2	48.4 (21.6)	79.4 (12.0)
o,p'-DDT	6.5	40.6 (21.4)	56.4 (11.7)	5.7	51.4 (24.5)	106 (17.6)
o,p'-DDD	6.1	49.4 (25.5)	98.6 (18.1)	5.9	61.3 (22.8)	100 (13.4)
o,p'-DDE	5.5	45.4 (23.7)	83.7 (9.3)	6.4	63.3 (20.4)	94.0 (11.6)
				6.5	54.5 (24.6)	104 (16.5)

	Log K _{ow}	PLE Average Recovery (% RSD)	MSPD Average Recovery (%RSD)	Log K _{ow}	PLE Average Recovery (% RSD)	MSPD Average Recovery (%RSD)
p,p'-DDT	6.9	43.6 (26.4)	69.6 (11.8)	6.5	46.1 (17.4)	73.1 (11.0)
p,p'-DDD	5.9	54.2 (19.5)	116 (9.2)	6.6	58.5 (24.6)	113 (17.6)
p,p'-DDE	6.8	46.4 (23.8)	67.2 (10.5)	6.9	57.6 (24.4)	108 (17.5)
Mirex	6.9	51.1 (26.0)	89.5 (14.2)	6.7	57.2 (24.2)	83.5 (10.6)
Organochlorine Sulfide Pesticides and Metabolites						
Endosulfan I	4.7	32.7 (30.2)	46.0 (12.7)	6.8	55.2 (25.5)	91.0 (9.7)
Endosulfan II	4.8	49.7 (26.3)	73.2 (13.3)	7.0	51.9 (24.2)	77.5 (9.6)
Endosulfan sulfate	3.7	53.1 (27.4)	58.5 (9.1)	Polychlorinated Biphenyls		
Phosphorothioate Pesticides						
Methyl parathion	2.7	36.2 (40.6)	63.5 (7.9)	6.3	45.8 (32.5)	101 (10.5)
Malathion	2.9	NR	59.9 (18.7)	6.4	47.4 (33.9)	87.3 (12.3)
Diazinon	3.7	NR	70.1 (6.0)	7.0	47.7 (35.6)	75.0 (15.3)
Parathion	3.8	19.4 (75.2)	81.6 (12.0)	6.9	50.1 (34.2)	96.4 (9.4)
Ethion	5.1	38.3 (41.4)	46.6 (17.8)	6.7	51.8 (34.1)	99.1 (10.2)
Chlorpyrifos	5.1	55.0 (36.2)	87.6 (9.5)	7.2	49.7 (36.1)	86.3 (10.4)
Ave, Min, and Max Recoveries, % RSD						
ave					46.8 (34.1)	80.6 (11.3)
max					114	236
min					14.6	33.1

Table 2

PLE and MSPD method conditions (DCM = dichlormethane, MeCN = acetonitrile, HEX = hexane, EA = ethyl acetate, SPE = solid phase extraction)

		PLE method	MSPD method
Sample Mass		2 g	2 g
Grinding Agent		Na ₂ SO ₄	Na ₂ SO ₄ , C ₁₈
	Mass	65 g	35 g, 10 g
Extraction			
	Pressure / Flow	1500 psi	25 ml/min
	Temperature	100°C	25°C
	Solvent	DCM	MeCN, DCM
	Solvent Volume	200 ml	300ml, 100 ml
Solvent Exchanges		2	0
	HEX	4 × 10 ml	
	DCM	4 × 10 ml	
Extract Purification		Silica SPE	Silica SPE
	Conditioning Solvent	HEX, EA, DCM	HEX, EA, DCM
	Solvent Volume	75 ml, 40 ml, 25 ml	75 ml, 40 ml, 25 ml
	SPE Solvent	HEX, DCM	EA
	Solvent Volume	62.5 ml, 62.5 ml	100 ml
		Gel Permeation Chromatography	
	Elution Solvent	DCM	
	Solvent Volume	200 ml	
Total Solvent Volume		745 ml	640 ml
Total DCM Volume		528 ml	125 ml
Extract Preparation Time (Set of 4 Samples)		12 hours	9.3 hours

Table 3

Tadpole semi-volatile organic compound estimated detection limits in pg/g wet weight (NR = not recovered) for the pressurized liquid extraction (PLE) and matrix solid phase dispersion (MSPD) methods.

	Log Kow	PLE Estimated Method Detection Limit (pg/g ww)	MSPD Estimated Method Detection Limit (pg/g ww)	Log Kow	PLE Estimated Method Detection Limit (pg/g ww)	MSPD Estimated Method Detection Limit (pg/g ww)
Amide Pesticides						
Alachlor	2.6	NR	620	3.2	NR	710
Acetochlor	3.0	NR	320	3.8	NR	150
Metolachlor	3.1	NR	240	4.6	36	39
Propachlor	2.4	NR	210	Triazine Herbicides and Metabolites		
Organochlorines Pesticides and Metabolites						
HCH, gamma	3.8	24	26	1.4	NR	2900
HCH, alpha	3.8	21	19	1.8	NR	390
HCH, beta	4.0	29	71	2.3	NR	300
HCH, delta	4.1	17	44	2.2	NR	830
Methoxychlor	4.5	17	150	1.7	NR	44
Heptachlor	5.2	108	240	Miscellaneous Pesticides		
Heptachlor epoxide	4.6	68	48	2.6	NR	620
Hexachlorobenzene	5.5	0.19	1.4	4.3	32	7.5
Endrin	5.2	800	400	5.3	4.1	11
Endrin aldehyde	4.8	140	48	Polycyclic Aromatic Hydrocarbons		
Chlordane, trans	6.1	2.7	1.1	3.9	230	160
Chlordane, cis	5.9	69	44	4.0	290	730
Nonachlor, trans	6.1	2.7	1.2	4.2	68	360
Nonachlor, cis	6.1	5.3	6.0	4.5	130	520
Chlordane, oxy	5.5	56	80	4.5	50	290
Dieldrin	5.5	260	260	5.1	66	33
Aldrin	6.4	44	160	5.2	130	120
o,p'-DDT	6.5	270	240	5.7	24	41
o,p'-DDD	6.1	190	270	5.9	26	68
o,p'-DDE	5.5	400	170	6.4	93	160
				6.5	320	110

	Log K _{ow}	PLE Estimated Method Detection Limit (pg/g ww)	MSPD Estimated Method Detection Limit (pg/g ww)	Log K _{ow}	PLE Estimated Method Detection Limit (pg/g ww)	MSPD Estimated Method Detection Limit (pg/g ww)
p,p'-DDT	6.9	63	310	6.5	180	190
p,p'-DDD	5.9	93	260	6.6	220	74
p,p'-DDE	6.8	110	250	6.9	170	130
Mirex	6.9	12	84	6.7	95	210
Organochlorine Sulfide Pesticides and Metabolites						
Endosulfan I	4.7	13	16	6.8	120	160
Endosulfan II	4.8	34	7.6	7.0	77	110
Endosulfan sulfate	3.7	2.7	9.7			
Polychlorinated Biphenyls						
				6.3	730	250
				6.4	180	710
				7.0	12	23
				6.9	11	19
				6.7	26	98
				7.2	5	3.2
				8.3	4.7	2.8
Phosphorothioate Pesticides						
Methyl parathion	2.7	1100	310			
Malathion	2.9	NR	260			
Diazinon	3.7	NR	120			
Parathion	3.8	1600	230			
Ethion	5.1	390	210			
Chlorpyrifos	5.1	6.9	22			
Ave, Min, and Max Recoveries, % RSD						
			ave		160	230
			max		1600	2900
			min		0.19	1.1