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Solid phase extraction/gas chromatography/ electron capture detector method for the determination of organochlorine pesticides in wildlife and wildlife food sources

A gas chromatographic method for the analysis of 10 organochlorine pesticides in a variety of biological matrices was developed. Sample preparation involved an acetonitrile extraction, followed by a solid phase extraction clean up using C18-florisil cartridges in tandem. The pesticides were quantified by gas chromatography with an electron capture detector. Method limits of detection ranged from 1.1–2.6 ng/g. The mean recovery and standard deviation for the ten pesticides in fortified deer muscle was 94.7% ± 7.9. Recoveries for individual analytes ranged from 83.6 to 105%. While the method was developed and validated using deer muscle, it was successfully applied to quantify these analytes in insects, bird eggs, calf liver, beef brain, boar, deer, elk, alligator, mussels, oysters, clams, crab, mahi mahi, and tobacco.

Key Words: Solid phase extraction; Dieldrin; Organochlorine pesticides; Capillary gas chromatography; Animal tissue

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1 Introduction

The Rocky Mountain Arsenal (RMA) has been designated as a National Wildlife Refuge and is currently being remediated for public use. As part of the remediation and restoration process, biomonitoring of the many wildlife species is ongoing. In support of this effort, methodology for the quantification of organochlorine pesticides (OCPs) in animal tissues was developed by our laboratory. The methodology for the quantification of ten OCPs was developed and validated in deer muscle. However, as a variety of tissues in multiple wildlife species are monitored as part of the remediation procedure, we demonstrated the applicability of this method for the quantification of the OCPs of concern in crickets, worms, fish eggs, bird eggs, bird liver, and beef brain. Unfortunately, as organochlorine contamination is still fairly widespread, we also demonstrated the applicability of the method to oysters, clams, mussels, mahi mahi, crab, elk, feral swine, alligator, cigars, and tobacco.

Historically, the analysis of pesticides in animal tissues has focused on livestock to be used for human consumption [1–6]. Some work had been done to determine pesticides in suspected animal poisonings which required a screening for organochlorine, organophosphate, and carbamate pesticides in a single matrix [7–10]. This paper reports the development of an analytical method that can be used to quantify OCPs in a variety of environmental matrices.

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2 Materials and methods

2.1 Equipment

A Hewlett-Packard (HP), Palo Alto, CA, Model 5890 Series II gas chromatograph equipped with electronic pressure control, dual electron capture detectors, and dual 7673A autosamplers was used to quantify organochlorine pesticides in animal tissue extracts. Solid phase extraction columns (SPE) containing one gram C_{18} (end capped) 6-mL reservoir, one gram florisil in a 3-mL reservoir, adapter with 70-mL reservoir, and Vacmaster (tm) sample processing stations were obtained from Jones Chromatography, Lakewood, CO. Gas Chromatography (GC) expendables including inlet liners, silanized glass wool, and gold inlet seals were from Restek Corporation, Bellefonte, PA.

2.2 Chemicals

Organochlorine pesticide standards (≥99% pure) for lindane, aldrin, isodrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, dieldrin, *p,p'*-DDE, endrin, and *p,p'*-DDT were obtained from Chem Service Inc., West Chester, PA

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and Supelco in Bellefonte, PA. Ether, anhydrous 99+%, and benzene were from Aldrich Chemical Co., Milwaukee, WI. Pesticide residue grade hexane was from Burdick & Jackson, Muskegon, MI. Acetone and acetonitrile were from Fisher Scientific, Pittsburgh, PA. Control deer muscle was supplied by the Rocky Mountain Arsenal.

2.3 Standard preparation

A concentrated mixed standard containing lindane, aldrin, isodrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *p,p'*-DDE, dieldrin, endrin and *p,p'*-DDT was prepared by combining aliquots of each standard solution and diluting with acetone to give a final concentration of 10.0 μg/mL. The concentrated mixed standard was diluted in acetone to prepare the diluted mixed standard fortification solutions. The concentrations of analytes in this solutions was 1.00 μg/mL. A calibration curve containing analyte concentrations of 25.0, 50.0, 100, 250, and 500 μg/L was prepared from the 10.0 μg/mL standard and diluted with hexane. A degradation standard solution was prepared with endrin (100 μg/mL) and *p,p'*-DDT (200 μg/mL) in hexane.

2.4 Sample fortification

Control tissue was fortified with the high and low mixed standard fortification solutions. For method validation, control tissue was fortified at 6 levels: 50, 100, 250, 380, and 500 ng/g of each compound. The blank was fortified at 250 ng/g with only the surrogate (lindane). Lindane was chosen as a surrogate because of its similarity to the analytes of interest and because there was no prior use of lindane on the Rocky Mountain Arsenal site.

2.5 Sample preparation

Sample homogenization varied depending on the type of sample. Egg samples were easily blended with a spatula. Many of the tissues could be homogenized in a blender. Samples too small for a blender were placed in a steel mill, frozen with liquid nitrogen, then pounded to a fine powder with a blunt steel rod. As summarized in **Figure 1**, a 1.00 gram aliquot of homogeneous tissue was transferred to a 25-mL glass tube, fortified, and allowed to equilibrate for one hour. The analytes were extracted from the tissue using three 5-mL aliquots of acetonitrile which were vortex mixed, sonicated for 10 min, mechanically shaken on high for 10 min, and centrifuged for 2 min (~1 400 × *g*). The resulting supernatants were carefully transferred to an Erlenmeyer flask containing 100 mL of deionized water. This solution was swirled to mix then eluted through a C_{18} (end-capped) SPE column that had been conditioned with 6 mL of acetonitrile followed by 6 mL of deionized water. The SPE column was equipped with a 70-mL reservoir adapter and the extract loaded onto the column using a low vacuum (−0.1 bar) to maintain a flow of about 1 drop/s. The final amount of solvent in the column was removed under a gentle vacuum then the column allowed to dry for 30 min under full vacuum. The eluate was collected and disposed of in hazardous waste. The manifold and needles were cleaned and with acetonitrile and the florisil SPE columns were conditioned with 6 mL of hexane. The dried C_{18} SPE column was attached to the top of the florisil SPE column and a calibrated evaporation tube was placed in the manifold. The analytes were eluted with 20 mL of a 5:3:2 hexane:benzene:ether solution using 4 separate aliquots (4 × 5 mL per sample). When the columns were fully saturated with the first aliquot, the stopcock was closed for a 60 s soak then elution resumed. The final amount of solvent was eluted off the column with a gentle vacuum (−0.05 to −0.1 bar). The extracts were concentrated to less than 1.00 mL under a gentle stream of nitrogen in a fume hood, equilibrated to room temperature, and brought to a final volume of 1.00 mL with hexane. The samples were then capped, vortex mixed, and transferred to GC vials for quantification of OCPs via gas chromatographic analysis.

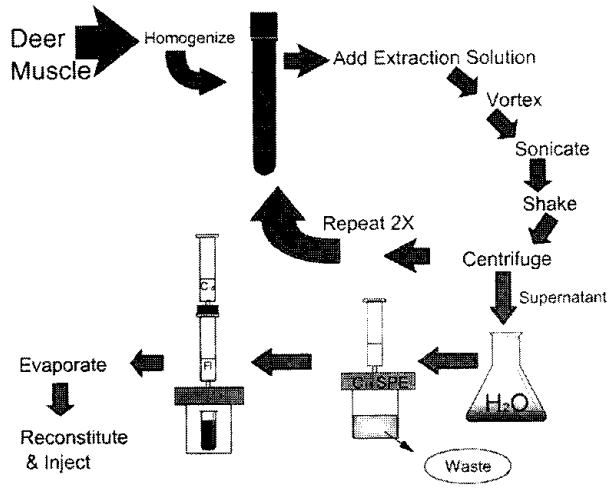


Figure 1. Flow chart of procedure.

2.6 Gas chromatography

The inlet temperature was 250°C and the detector temperature was 350°C. The GC parameters were controlled utilizing HP ChemStation software and a HP Vectra XM Series 3 computer. The carrier gas was helium (3 cm/s) and the make-up gas was argon/methane (60 mL/min). The quantitation column was a 30 m × 0.25 mm ID fused silica, HP-5 cross linked 5% phenyl methyl-silicone stationary phase, 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA). The confirmation column was a

30 m × 0.25 mm ID fused silica DB-17 cross linked 50% phenyl methyl-siloxane stationary phase, 0.15 μm film thickness (J & W Scientific, Folsom, CA).

The oven temperature program for quantitation and confirmation was as follows: 50°C for 0.25 min, 60 °/min to 100°C hold for 0 min, 30°/min to 190°C hold for 2 min, 10°/min to 230°C hold for 13 min, 60°/min to 300°C hold for 10 min. The electronic pressure program for the quantitation column maintained the pressure at 16 psi, the confirmation column inlet pressure was held at 80 psi for 2 min and then 16 psi for the duration of the run. A double tapered 4 mm ID liner was used for the quantitation column, while a single taper 4 mm ID inlet liner packed with deactivated glass wool was used on the confirmation column. Both columns had a 1 μL injection volume.

2.7 Method validation

Detector linearity was determined by linear regression analysis of 5 point calibration curves (response versus concentration) for each analyte. After achieving $r^2 \geqq 0.99$, linear regression equations were used to quantify analytes in samples. Fortified tissue samples (6 levels including a blank) were prepared using the above fortification procedure, analyzed by GC and percent recoveries determined for each analyte at each fortification level on two consecutive days [11]. Method limits of detection (MLOD) were calculated from the 50 ng/g fortified deer muscle and control chromatograms. MLODs were calculated as the quantity of analyte required to give a response of 3 times the baseline noise at the expected retention time of the analyte in the chromatogram of a non-fortified tissue extract.

2.8 Quality control

To assure consistent instrument performance, prior to the GC analysis of any samples, the degradation standard containing 100 μg/L endrin and 200 μg/L *p,p'*-DDT was analyzed. For analysis to proceed, analyte degradation had to be ≦ 20% for each compound and ≦ 30% for both compounds. Having met the degradation criteria, a 250 μg/L instrument check standard was analyzed at the beginning, after every ten samples, and at the end of each analytical run. The magnitude of response for each analyte was required to match the response for the same compound in the 250 μg/L standard in the calibration curve by ± 25%. Additionally, retention matches of ± 0.05 min were required.

Surrogate recoveries were used to monitor method performance in every sample. In addition to analysis on the quantitation column, extracts of all tissue samples found to contain any of the target organochlorine pesticides were confirmed by GC analysis on the confirmation column.

3 Results and discussion

3.1 Chromatography

Control deer muscle obtained from the Rocky Mountain Arsenal was used for method development, validation, and quality control samples. Control deer muscle proved to be acceptable for these purposes as indicated by the lack of chromatographic responses at the retention times for the analytes of interest as shown in **Figure 2**.

3.2 Method validation

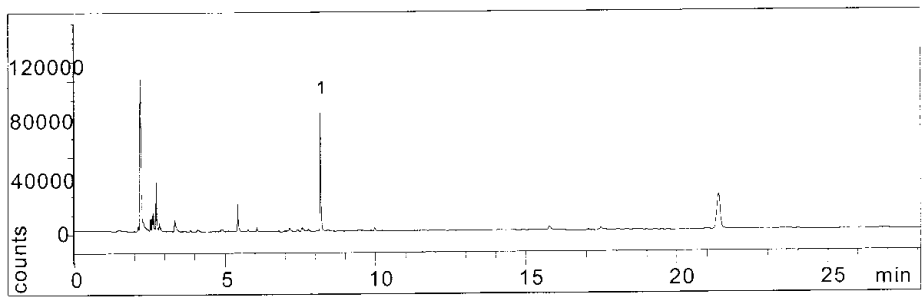
The results of the method validation experiments are presented in **Table 1**. For dieldrin, the analyte of primary concern, the mean recovery was 98.9% and the standard deviation was 4.3%. Mean recoveries of the other analytes of interest, aldrin, endrin, *p,p'*-DDT, and *p,p'*-DDE, were 83.6 ± 6.1, 101 ± 3.5%, 93.4 ± 3.7%, and 95.4 ± 4.9%, respectively. Mean recoveries of lindane, the compound

Table 1. Method validation mean% recoveries in deer muscle.

Compound	50	100	250	380	500 ppb	Grand Mean	Std. Dev.
Lindane	98.3	101	103	99.6	96.4	99.6	2.5
Aldrin	73.9	86.9	90.2	84.8	82.5	83.6	6.1
Isodrin	83.6	98.9	97.9	92.6	89.3	92.4	6.3
Heptachlor epoxide	89.8	96.6	98.0	91.7	91.4	93.5	3.6
trans-Chlordane	75.0	88.2	88.3	84.4	83.4	83.8	5.4
cis-Chlordane	101	111	109	102	101	105	4.6
p,p'-DDE	87.8	98.5	101	95.7	94.5	95.4	4.9
Dieldrin	97.5	104	103	95.8	94.3	98.9	4.3
Endrin	99.0	104	106	99.3	97.5	101	3.5
p,p'-DDT	89.0	95.4	98.7	92.5	91.8	93.4	3.7

Note: Mean % recoveries calculated from quantitative and confirmation columns on two separate days ($n = 4$). The total mean recovery for all compounds equals 94.7 ± 7.9% ($n = 50$).

Control Deer Muscle:



50 ng/g Fortified Deer Muscle:

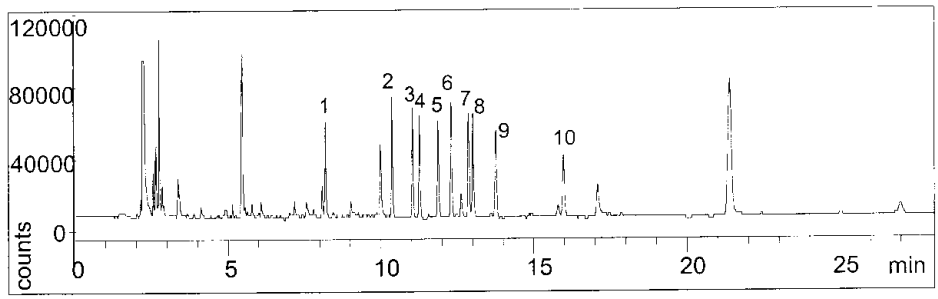


Figure 2. Chromatograms. a) control deer muscle; b) 50 ng/g fortified deer muscle. **(1)** lindane (surrogate), **(2)** aldrin, **(3)** isodrin, **(4)** heptachlor epoxide, **(5)** *trans*-chlordane, **(6)** *cis*-chlordane, **(7)** *p,p'*-DDE, **(8)** dieldrin, **(9)** endrin and **(10)** *p,p'*-DDT.

Table 2. Method limits of detection in deer muscle.

Compound	Mean MLOD (ng/g)
Lindane	Not calculated – Surrogate spiked in control samples
Aldrin	1.1
Isodrin	2.3
Heptachlor epoxide	2.6
trans-Chlordane	1.7
cis-Chlordane	1.9
p,p'-DDE	1.3
Dieldrin	1.8
Endrin	2.0
p,p'-DDT	2.5

Note: MLOD calculated using a control and a tissue sample fortified at 50 ng/g, assayed on 2 separate days on the quantitation and confirmation column ($n = 4$).

Table 3. Mean (X_3)% recovery and standard deviation in crickets and worms.

Compound	Crickets	Worms
Lindane	83.9 ± 8.5	104 ± 4.0
Aldrin	60.7 ± 3.4	66.9 ± 9.9
Isodrin	78.5 ± 6.4	86.1 ± 8.8
Heptachlor epoxide	108 ± 5.3	118 ± 6.0
trans-Chlordane	79.0 ± 4.5	83.6 ± 8.3
cis-Chlordane	72.1 ± 4.6	100 ± 11
p,p'-DDE	59.9 ± 6.7	57.6 ± 10
Dieldrin	114 ± 5.9	107 ± 5.5
Endrin	130 ± 7.2	131 ± 7.6
p,p'-DDT	66.0 ± 7.7	62.0 ± 11
Overall mean (X_{10})	85.2 ± 24	91.6 ± 25

Note: Three replicate samples for each matrix was fortified at 250 ng/g, results shown are from the quantitation column.

added to all samples as a surrogate was 99.6 ± 2.5%. This is similar to the 94.7 ± 6.9% mean recovery for all compounds, indicating the suitability of lindane as a surrogate standard for these analyses. MLODs as calculated during method validation are presented in **Table 2**. The MLOD for dieldrin was 1.8 ng/g. The MLODs for the other analytes of primary concern ranged from 1.1 ng/g for aldrin to 2.6 ng/g for heptachlor epoxide.

3.3 Method applicability to other matrices

As OCPs bioaccumulate in the food chain, the remediation procedure required that OCP levels be determined in potential wildlife food sources. Such food sources including crickets and worms were collected from the Rocky Mountain Arsenal site. To evaluate method performance, triplicate samples of crickets and worms were fortified with

Table 4. Percent recovery and standard deviations for tissue fortifications.

Compound	Deer muscle	Chicken eggs	Chicken liver	Beef brains	Crickets
Lindane	75.6 ± 8.9	89.8 ± 6.9	98.4 ± 5.2	88.2 ± 6.3	111 ± 3.0
Aldrin	70.5 ± 8.5	65.0 ± 5.4	69.3 ± 5.9	75.6 ± 4.2	62.1 ± 26
Isodrin	85.5 ± 13	79.6 ± 3.8	86.9 ± 4.8	89.3 ± 6.1	79.8 ± 26
Heptachlor epoxide	83.3 ± 13	94.7 ± 3.0	93.2 ± 4.3	90.3 ± 4.4	121 ± 4.5
trans-Chlordane	82.4 ± 17	81.4 ± 6.1	89.6 ± 6.4	83.4 ± 5.7	82.6 ± 34
cis-Chlordane	83.1 ± 12	84.4 ± 5.6	90.2 ± 18	95.5 ± 8.1	105 ± 70*
p,p'-DDE	86.7 ± 12	62.1 ± 8.1	60.9 ± 8.5	97.3 ± 11	57.9 ± 29*
Dieldrin	89.0 ± 11	96.2 ± 3.2	85.7 ± 8.5	97.5 ± 9.2	101 ± 19*
Endrin	100 ± 14	109 ± 3.9	108 ± 6.8	110 ± 7.6	119 ± 15
p,p'-DDT	94.4 ± 20	67.3 ± 8.1	79.1 ± 17	112 ± 6.6	63.9 ± 39
Overall mean (X_{10})	85.1 ± 8.5	82.9 ± 15	86.1 ± 14	93.9 ± 11	90.3 ± 24

Note: These quality control samples were fortified at 250 ng/g and extracted with actual samples on separate days. There were 11 replicates of deer muscle, 4 replicates of both chicken liver, and chicken eggs and 3 replicates of beef brains, 3 replicates of crickets. Results shown are from the quantitation column. There was one interfering peak detected in a deer muscle control sample, the concentration was 7 ng/g dieldrin, which is much lower than the method reporting limit.

* These three recoveries are effected by an interfering peak that is visible in the control and in the composite fortification for one of the three days.

Table 5. Percent recovery in animal tissues.

Matrix	Control	Fortified sample									
	LIN	LIN	ALDRN	ISODR	HPCLE	TCLDAN	CCLDAN	*p,p'*-DDE	DLDRN	ENDRN	*p,p'*-DDT
Alligator tail	95.3	82.2	75.5	79.0	86.4	81.8	81.8	81.0	90.8	91.6	85.2
Boar	98.2	88.1	84.8	87.3	96.2	89.9	89.5	87.2	97.4	103	92.2
Calf liver	95.8	82.4	67.7	76.7	81.5	65.4	74.9	75.2	83.4	82.1	80.5
Littleneck clams	96.7	75.1	71.5	74.5	85.4	82.6	82.2	88.4	88.0	94.0	88.6
Dungeness crab	102	90.8	74.9	74.2	95.8	88.7	88.5	93.1	93.0	98.8	93.5
Red deer	93.0	80.8	74.7	77.6	86.3	77.0	78.3	71.9	86.3	90.4	75.6
Elk	95.8	80.4	77.7	80.5	86.6	81.4	81.0	81.6	86.7	91.7	83.2
Mahi mahi	99.4	94.9	80.1	83.9	91.0	83.9	83.9	84.8	88.6	87.0	81.8
Mussels	92.2	87.2	67.6	76.2	95.5	75.5	76.8	52.0	97.0	99.3	56.0
Blue point oysters	105	85.1	72.4	80.0	92.9	81.9	83.8	66.3	94.2	103	72.6

Note: Recoveries were calculated from a 1.00-g control sample fortified at 250 ng/g with lindane, results shown are from the quantitation column. No organochlorine pesticide contamination was observed in any of the matrices.

each target analyte at 250 ng/g. Following homogenization with a blender, these samples were analyzed using the previously described methodology. The mean recovery for all the analytes in crickets was 85.2% with a standard deviation of 24% and 91.6% with a standard deviation of 25% in worms (**Table 3**). Recoveries for individual analytes are summarized in Table 3. Crickets were chosen as an insect control matrix and two samples were fortified and concurrently analyzed with each lot of insect samples. One sample, fortified with lindane, served as a blank. The other sample was fortified with all the analytes and served as a positive control. Recoveries varied from 57.9% in *p,p'*-DDE to 121% in heptachlor epoxide (**Table 4**). A large interfering peak was observed in the control chromatogram at the retention times for *cis*-chlordane, *p,p'*-DDE and dieldrin, which accounts for the high standard deviations. Three lots of insects from the RMA were extracted using the methodology described. The insects varied between grasshoppers, moths, crickets, millipedes, and worms.

Table 4 shows analyte recoveries from fortified deer muscle, chicken eggs, chicken liver, and beef brains. These were quality control samples fortified with all analytes at 250 ng/g and analyzed concurrently with actual samples. Deer muscle recoveries were slightly lower than seen in validation but still acceptable. Beef (cow) brains showed the highest overall recoveries with a mean of 93.9% and a standard deviation of 11%, the chicken liver was just lower at 86.1% and standard deviation of 14%.

3.4 Analysis of a variety of animal, fish, insect matrices, and commercial products

A wide variety of matrices were fortified with lindane and extracted using the methodology described. The lindane

Table 6. Pesticide recoveries in tobacco.

	Lindane % recovery	Contaminants found
Cigar (Honduras)		
Inner leaves:	79.7	Heptachlor epoxide confirmed @ 3.2 ng/g
Outer leaves:	80.3	*p,p'*-DDE confirmed @ 24.4 ng/g
Cigar (Dominican Republic)		
Inner leaves:	78.9	*p,p'*-DDE confirmed @ 13.4 ng/g
Outer leaves:	76.8	
Cigar (United States)		
Inner leaves:	79.5	*p,p'*-DDE confirmed @ 14.0 ng/g
Outer leaves:	76.7	*p,p'*-DDE confirmed @ 20.0 ng/g *p,p'*-DDT confirmed @ 14.0 ng/g
Leaf chewing tobacco	53.8	
Snuff chewing tobacco	94.5	

Note: Recoveries were calculated from a 1.00-g control sample fortified at 250 ng/g with lindane, results shown are from the quantitation column. The outer leaves for the Honduran cigar weighed 0.32 g which was less than the method required, calculations were performed using the actual weight. All contaminants were confirmed using a confirmation column. Reported concentrations for contaminants are from the column with the lowest contamination reported.

recoveries are shown in **Table 5**. The methodology had recoveries of approximately 92% for red deer and 102% for crabs. The methodology proved acceptable for all the matrices

3.5 Analysis of tobacco products

Since plants serve as potential wildlife food sources, we applied this methodology to tobacco, a plant matrix that we suspected would contain OCP residues. Three different cigar brands from three different countries were chosen. The outside tobacco leaves were assayed separately from the inner tobacco. A common leaf chewing tobacco and a snuff chewing tobacco were also assayed.

Each sample was fortified with lindane and extracted as per the method. Contaminants found in samples were confirmed on a confirmation column. **Table 6** shows the recoveries for lindane to be lower in the plant matrices than they were in the animal tissue. However, organochlorine pesticides were extracted from the control tobacco leaves showing *p,p'*-DDE as a major contaminant observed in all three samples along with heptachlorepoxide and *p,p'*-DDT. The results shown in Table 6 conclude that this methodology is well suited for the analysis of OCPs in plants such as tobacco.

4 Conclusions

The extraction procedure described for the simultaneous analysis of 10 OCP in animal tissue offers the following advantages: it is a simple extraction and SPE clean-up, requiring low solvent volumes with 100 mL of water and 15 mL of acetonitrile being the entire hazardous waste per sample; the method provides precise recoveries in animal tissues while being a valid screening method for insects. Knowing the quantity of OCP's that accumulate in animals and the food chain will allow for accurate assessment of clean-up for such sites as the RMA Wildlife Refuge.

Acknowledgments

Mention of commercial products is for identification only and does not constitute endorsement by the U.S. Government.

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