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A simple and fast extraction method for organochlorine pesticides and polychlorinated biphenyls in small volumes of avian serum

Sarah E. Sundberg [1], J. Jackson Ellington *, John J. Evans [2]

National Exposure Research Laboratory, U.S. Environmental Protection Agency, 960 College Station Road, Athens GA 30605, USA

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Abstract

A solid-phase extraction (SPE) method was developed using 8 M urea to desorb and extract organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) from avian serum for analysis by capillary gas chromatography with electron capture detection (GC–ECD). The analytes were efficiently extracted from the denatured serum–lipoprotein–analyte complex by one passage through an Oasis® hydrophilic–lipophilic-balanced (HLB) SPE cartridge. No further clean-up was necessary, the entire extraction procedure and GC–ECD analysis can be accomplished in less than 3 h. Serum volumes ranged from 100 μL to 1 mL with absolute recoveries of 90–101% for PCBs and 74% to 101% for the OC pesticides.
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1. Introduction

Solid-phase extraction (SPE) is a simple and efficient alternative to conventional methods, such as liquid–liquid extraction (LLE) for extraction of organochlorine pesticide (OC) pesticides and polychlorinated biphenyls (PCBs) from serum [1–3]. The most commonly used SPE sorbents are C_{18} [1,4–6] and styrene–divinylbenzene copolymer [2]. The usual sample pretreatment is denaturation of the serum protein with either organic solvents (methanol, acetonitrile) or acids (acetic, formic, trichloroacetic, or perchloric) prior to aspiration of the liquid fraction through the SPE cartridge [1,5]. If precipitates are formed during sample pretreatment, they are removed by centrifugation prior to application of the serum supernatant to the SPE cartridge. The addition of organic solvents or acids is intended to desorb serum-bound analytes. It has been shown that at 40 mg/mL of protein the binding of *p*,*p*′-DDT, *p*,*p*′-DDE, dieldrin, and lindane was ≥74% [7]. The SPE eluent must often be cleaned up to remove natural components of serum that may be retained by the SPE cartridge during the extraction step and that would interfere with the chromatography and detection of the analytes. Clean-up of the SPE cartridge eluent usually involves exposure of the eluent to concentrated sulfuric acid [3,5] or passage of the SPE extract through a NH_2 column [8] or a silica column [3,6]. Destruction of the lipids directly on a styrene–divinylbenzene SPE cartridge with concentrated sulfuric acid has also been reported [2].

It was recently reported that water:1-propanol (85:15, v/v) denatured serum proteins and sufficiently diluted the sample so that flow through the C_{18} SPE cartridge was not restricted [9]. However, several aspirations of the denatured serum through the SPE cartridge were required to achieve acceptable recoveries of the fortified OC pesticides (88–115%) and PCBs (99–120%). The SPE eluent also required clean-up on a florisil–silica gel column treated with sulfuric acid before quantification of the analytes by capillary gas chromatography with electron capture detection (GC–ECD) [9].

The use of Oasis® hydrophilic–lipophilic-balanced (HLB) sorbent cartridges for extraction of organophosphorous pesticides (OPs) [10] and PCBs [3] from serum has been reported. The Oasis® cartridges yielded higher recoveries of the 29 OP pesticides (80–113%) compared to C_{18} cartridges and analysis by gas chromatography coupled to mass spectrometry eliminated the necessity of eluent clean-up. However, prior to

* Corresponding author. Tel.: +1 706 355 8204; fax: +1 706 355 8202.
E-mail address: ellington.jackson@epa.gov (J.J. Ellington).
[1] Present address: Clemson Institute of Environmental Toxicology, Clemson University, Pendleton, SC, USA.
[2] Senior Service America Inc.

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application of the OPs or PCBs to the Oasis® HLB cartridge, denaturation and precipitation of the serum proteins with acetonitrile followed by centrifugation were required for the OPs and treatment with formic acid-5% acetonitrile for the PCBs. The PCB extracts were cleaned up by passage through a multilayer column containing activated silica gel, silica gel modified with sulfuric acid and anhydrous sodium sulfate [3]. Recoveries of the PCBs were in the range 73–128%.

Described in this paper is a serum spiking procedure that does not use organic solvents, serum denaturation with urea, and a fast SPE extraction that requires no centrifugation of the serum prior to SPE and no extract clean-up before analysis of OC pesticides and PCBs by gas chromatography with electron capture detection.

2. Materials and methods

2.1. High and low level GC–ECD calibration standards

An aliquot (20 μL) of a commercial mixture of 11 OC pesticides (5–20 μg/mL, Mix B; cat. no. 32034; Restek, USA) was added to a 12-mL sample vial using a 25 μL Hamilton® syringe and the solvent evaporated by natural convection in the fume hood. Methylene chloride (1 mL) was added to yield OC pesticide concentrations of 100–400 pg/μL (High-OC, Table 1). An aliquot (100 μl) of the High-OC calibration standard was added to a 12-mL sample vial, the solvent evaporated and 1 mL methylene chloride added to yield OC pesticide concentrations of 10–40 pg/μL (Low-OC, Table 1). Similarly, PCB calibration concentrations of 100 pg/μL (High-PCB, Table 2) and 10 pg/μL (Low-PCB, Table 2) were prepared from a commercial mixture of PCB congeners (10 μg/mL, Restek, cat. no. 32290).

2.2. Serum fortification with OC pesticides and PCBs to high and low concentrations

The frozen serum (C 5405, Sigma–Aldrich, USA) was allowed to warm to room temperature and was mixed thoroughly by gentle swirling. To facilitate a one-point calibration-quantification of the OC pesticides and PCBs in the serum SPE eluents, the serum samples were fortified such that the concentrations in the SPE eluents corresponded to the High level and Low level calibration concentrations. To fortify the serum, either a 20 μL aliquot of the Mix B commercial standard, a 10 μL aliquot of the commercial PCB mix, or 100 μL aliquots of the High-OC or High-PCB calibration standards were added to the 12-mL vial. The solvent was allowed to evaporate in the fume hood, leaving the analytes deposited on the glass surface. Room temperature serum (1.0 mL) was transferred to each vial and the vials swirled gently to solubilize the deposited analytes. A magnetic stirring bar (3.2 mm × 13 mm) was added and the serum samples were stirred 25 min to allow partitioning of the analytes to serum proteins. The fortified serum aliquots (1.0 mL or 0.1 mL) were processed immediately as described in Section 2.3.

Table 1
Pesticides recovered from fortified serum that was untreated and treated with urea ($n = 3$ unless otherwise noted; average ± standard deviation)

Pesticide	Low-OC (pg/μL)	Pesticide recovered from 1 mL fortified serum	
		Untreated (%)	Treated (%)
Alpha-BHC	10	98.2 ± 21.2	89.7 ± 9.0
Beta-BHC	20	100.8 ± 10.1	100.9 ± 5.7
Delta-BHC	20	97.7 ± 8.0	94.7 ± 5.7
Aldrin*	20	42.2 ± 3.8	83.4 ± 7.1
Gamma-chlordane*	20	41.4 ± 1.3	75.4 ± 6.7
Alpha-chlordane*	20	50.4 ± 1.2	81.3 ± 8.0
p,p-DDE*	20	47.0 ± 4.8	75.3 ± 8.2
Endrin	20	102.9 ± 3.3	107.3 ± 11.4
p,p′-DDD*	40	76.3 ± 3.5	90.2 ± 11.1
Endosulfan sulfate	40	77.6 ± 3.6	73.8 ± 9.0
Endrin ketone	40	104.7 ± 4.4	93.8 ± 10.3
Pesticide	**High-OC (pg/μL)**	**Pesticide recovered from 1 mL fortified serum**	
		Untreated (%)	Treated (%)
Alpha-BHC	100	109.0 ± 9.2	96.7 ± 13.7
Beta-BHC*	200	86.7 ± 4.7	98.8 ± 7.6
Delta-BHC	200	98.2 ± 4.4	96.8 ± 5.9
Aldrin*	200	21.6 ± 4.6	75.4 ± 5.0
Gamma-chlordane*	200	23.7 ± 3.5	82.3 ± 4.0
Alpha-chlordane*	200	22.5 ± 3.2	77.5 ± 3.6
p,p-DDE*	200	25.6 ± 5.3	76.1 ± 3.2
Endrin*	200	60.0 ± 3.4	108.4 ± 7.9
p,p′-DDD*	400	45.4 ± 4.2	94.9 ± 5.6
Endosulfan sulfate*	400	69.0 ± 4.5	91.5 ± 3.7
Endrin ketone*	400	83.9 ± 3.9	99.8 ± 6.6
Pesticide	**High-OC (pg/μL)**	**Pesticide recovered from 0.1 mL fortified serum**	
		Untreated (%)	Treated (%)
Alpha-BHC*	100	54.2 ± 3.2	65.4 ± 6.0
Beta-BHC	200	89.5 ± 5.0	93.1 ± 5.4
Delta-BHC*	200	88.6 ± 8.0	98.0 ± 3.2
Aldrin*	200	50.2 ± 5.4	62.1 ± 1.1
Gamma-chlordane*	200	71.8 ± 6.7	81.4 ± 4.3
Alpha-chlordane*	200	62.7 ± 4.9	76.3 ± 4.9
p,p-DDE*	200	56.1 ± 6.0	68.3 ± 3.5
Endrin*	200	149.3 ± 20.8	175.0 ± 6.3
p,p′-DDD*	400	79.7 ± 11.8	100.5 ± 2.4
Endosulfan sulfate	400	85.9 ± 6.6	96.1 ± 3.5
Endrin ketone*	400	92.3 ± 9.8	108.4 ± 3.1

The percent recoveries of pesticides marked with an * are significantly higher ($\alpha \leq 0.05$) in treated than those of the untreated serum as determined by SNK, Tukey's, and Duncan's tests.

2.3. Solid-phase extraction procedure for 1 mL and 0.1-mL serum samples

2.3.1. Procedure for 1-mL samples

After 25 min stirring, half the fortified serum samples (1 mL) were amended with 500 mg solid urea (~8 M) and all the samples (urea-treated and -untreated) were stirred gently for an additional 25 min. The serum samples were applied to 30 mg (1 cc) Oasis® HLB cartridges (Waters Corporation, USA) mounted in a vacuum manifold (J.T. Baker, USA). Before application of

Table 2
PCBs recovered from fortified serum that was untreated and treated with urea ($n = 3$ unless otherwise noted; average ± standard deviation)

PCB	Low-PCB (pg/μL)	PCB recovered from 1 mL fortified serum	
		Untreated[a] (%)	Treated (%)
PCB 28[b]	10	81.2 ± 1.2	83.0 ± 2.4
PCB 52[c]	10	45.7 ± 0.8	79.6 ± 1.1
PCB 101[d]	10	29.8 ± 6.1	60.7 ± 0.5
PCB 138[e]	10	18.0 ± 1.4	41.7 ± 2.8
PCB 153[f]	10	16.8 ± 0.7	46.8 ± 0.5
PCB 180[g]	10	16.2 ± 1.6	36.4 ± 1.1
PCB	High-PCB (pg/μL)	PCB recovered from 1 mL fortified serum	
		Untreated[a] (%)	Treated[a] (%)
PCB 28	100	82.7 ± 2.6	88.7 ± 0.3
PCB 52	100	61.4 ± 6.2	81.9 ± 1.5
PCB 101	100	38.7 ± 4.2	70.0 ± 0.2
PCB 138	100	25.6 ± 2.8	58.0 ± 1.0
PCB 153	100	28.6 ± 8.4	64.3 ± 3.4
PCB 180	100	25.7 ± 1.0	53.0 ± 4.6
PCB	High-PCB (pg/μL)	PCB recovered from 0.1 mL fortified serum	
		Untreated (%)	Treated[a] (%)
PCB 28	100	86.8 ± 4.4	98.2 ± 15.6
PCB 52	100	62.9 ± 2.0	92.0 ± 11.2
PCB 101	100	56.0 ± 3.1	90.6 ± 10.2
PCB 138	100	52.4 ± 1.1	101.2 ± 9.0
PCB 153	100	35.5 ± 1.3	90.1 ± 8.2
PCB 180	100	24.6 ± 1.4	89.5 ± 6.4

The percent recoveries of PCBs marked with an * are significantly higher ($\alpha \leq 0.05$) than those of the untreated serum as determined by SNK, Tukey's, and Duncan's tests.
[a] $n = 2$.
[b] 2,4,4′-Trichlorobiphenyl.
[c] 2,2′,5,5′-Tetrachlorobiphenyl.
[d] 2,2′,4,5,5′-Pentachlorobiphenyl.
[e] 2,2′,3,4,4′,5′-Hexachlorobiphenyl.
[f] 2,2′,4,4′,5,5′-Hexachlorobiphenyl.
[g] 2,2′,3,4,4′,5,5′-Heptachlorobiphenyl.

serum samples, the HLB cartridges were cleaned and conditioned with three 1 mL volumes of methanol followed by four 1 mL volumes of Nanopure® (18 MΩ cm) water. Urea-treated and untreated serum passed through the cartridge by gravity flow. The serum sample vials were rinsed with four 1 mL aliquots of Nanopure® water, and each aliquot was applied to the corresponding HLB cartridge by gravity flow. The cartridges were rinsed with three 1 mL portions of Nanopure® water and dried for 10 min under gentle vacuum (∼13 mmHg). The sorbed OC pesticides and PCBs were eluted with 1 mL of methylene chloride (Burdick and Jackson, GC^2, USA) by gravity flow into 2-mL gas chromatography (GC) sample vials placed in the vacuum manifold. Gentle vacuum was then used to elute the residual methylene chloride from the cartridges. The volumes of methylene chloride were adjusted to 1.0 mL in the GC sample vials using supplemental methylene chloride with 1000-μL Hamilton® syringes.

2.3.2. Procedure for 0.1-mL samples

To determine the extraction efficiency from 0.1 mL volumes of fortified serum, 0.9 mL of water were added to 0.1 mL to yield a final volume of 1.0 mL. Half of these fortified serum samples were amended with 500 mg solid urea (∼8 M). Extraction procedures for the 0.1-mL serum samples were the same as for the 1.0-mL serum samples previously described. The methylene chloride eluent was evaporated to less than 100 μL and the final volume for GC–ECD analysis was adjusted to 0.1 mL using a 100 μL Hamilton® syringe and transferred to a 250 μL insert in a 2-mL GC screw cap sample vial.

2.4. Mass balance determination

To check the efficiency of the solulibization of the OC pesticides and PCBs from the glass surface by serum lipoproteins, the empty 12-mL sample vials from Section 2.3 (urea-treated and untreated samples) were dried with nitrogen gas, followed by the addition of 0.1 mL methylene chloride and the vials capped. After 5 min, the methylene chloride extracts were collected in a 100 μL Hamilton® syringe, brought to 0.1 mL volume with supplemental methylene chloride and transferred to a 250 μL insert in a 2-mL GC screw cap sample vial. The OC pesticides and PCBs in the HLB extracts and the samples vial rinses were separated and quantified by GC–ECD analysis based on area response of the High and Low calibration standards.

To determine residual levels of OC pesticides and PCBs in the serum after passage through the HLB cartridge, the urea-treated and untreated serum wastes and the three 1 mL aqueous washes for each HLB cartridge were combined and processed as described in Section 2.3.

2.5. Storage stability study of HLB-sorbed pesticides

Serum samples in triplicate were fortified at High and Low levels with OC pesticides, treated with urea, passed through the HLB cartridges by gravity flow, and the cartridges washed with water and dried by gentle vacuum as described in Section 2.3. One High-OC and one Low-OC sample cartridge was eluted with methylene chloride immediately (time zero). A second replicate set was eluted with methylene chloride after 14 days of storage at approximately 4 °C, and a third set after 21 days. The OC pesticides were quantified in the methylene chloride extracts to determine the effect of storage time on recovery (Fig. 2).

2.6. Gas chromatography

The extract (1 μL) was injected in splitless mode onto a HP 5890 Series II gas chromatograph (Agilent Technologies, USA) controlled by Agilent Chemstation software and equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film thickness CLPesticides II column (Restek, USA). Helium was used as a carrier gas at a constant 1 mL/min flow with nitrogen as a make-up gas (35 mL/min) through the ECD held at 325 °C. The following oven temperature program was used: initial temperature 100 °C for 1 min then 4 °C/min to 250 °C and held for 2 min. The inlet temperature was 250 °C.

2.7. Statistical analysis

The software package of the Statistical Analysis System Institute Inc., Version 8.02 was used to perform all statistical analyses. Analyses of variance (ANOVA) with serum treatment as class variables for each pesticide and PCB were performed on the data.

The following tests were used for comparisons of the percent recovery means: Student–Newman–Keuls (SNK) test, Tukey's test, and Duncan's multiple-range test, all with $\alpha \leq 0.05$.

3. Results and discussion

Fortification of serum samples with OC pesticides and PCBs is conventionally accomplished by first preparing concentrated stock solutions of known concentrations using solvents, such as methanol, acetone, hexane, or isooctane [2,6,11]. Serum samples are then fortified to final concentrations by adding 5–100 μL aliquots of the stock solution to 1–5 mL of serum with the added solvent often reaching 5% by volume. The presence of solvent could interfere with binding of the analytes to serum protein (high fortification recoveries) or retention of the analytes on the SPE cartridge (low fortification recoveries). Reported in this paper is the fortification of serum samples without solvent. Completeness of solubilization from the glass after 25 min was confirmed by a time study up to 24 h. At both High and Low serum fortification levels residual OC pesticides or PCBs found in the methylene chloride rinse of the sample vial after removal of the fortified serum ranged from non detectable to 1.2% for PCB 180 in a High PCB fortified sample.

To determine the extraction efficiency of 30 mg Oasis® HLB cartridges, 1-mL serum samples, fortified with Low-OC pesticides, were acidified with 20 μL of concentrated phosphoric acid following methods outlined by Waters Oasis® Application Handbook [12]. The serum was loaded on pre-cleaned cartridges by gravity flow and the retained pesticides eluted with 1 mL of methanol. After observing less than 20% recovery of many of the pesticides in the methanol, the cartridges were eluted in sequence with 1 mL of acetonitrile and 1 mL of methylene chloride. Additional recovery of pesticides was determined in both eluents. The fortification and recovery experiments were repeated and the cartridges eluted with two 1 mL volumes of methylene chloride. No pesticides were observed in the second 1 mL of methylene chloride. However, the recoveries of aldrin, *p*,*p*′-DDE, and gamma- and alpha-chlordane were <30%. Because only trace levels (<2%) of a few OC pesticides were detected in the methylene chloride rinses, the analytes unaccounted for were most likely bound to serum components.

A recent C_{18} SPE cartridge method for organochlorine pollutants in human serum, evaluated protein denaturation (unfolding) methods that used acids (formic acid, trichloroacetic acid, perchloric acid), organic solvents (methanol, acetonitrile) and zinc salt [5]. The best recoveries were obtained when 1 mL of serum was mixed with 1 mL of formic acid plus 5% acetonitrile and 0.2% triethylamine, and the serum aspirated through the cartridge at a slow flow. However, elimination of lipid interferences required a sulfuric acid wash of the cartridge eluent prior to analysis.

The forces involved in the binding of pesticides to proteins have been speculated to be of both hydrophobic and hydrophilic (electrostatic) character [13]. Urea, which has a dipole-moment, denatures (unfolds) proteins by disrupting the intramolecular electrostatic interactions. Urea has also been reported to eliminate the binding of Vitamin B_{12} (cyanocobalamin) to human, dog, and rabbit serum [14] and to interfere with the binding of antithyroid drugs with serum albumin [15]. The effects of urea on proteins are mild and often reversible as shown by restoration of more than 90% activity of a urea-denatured eclosion hormone [16]. To compare acidic denaturation with neutral (urea) denaturation, triplicate sets of 1 mL avian serum samples fortified with Low-OC pesticides were denatured by treatment with either phosphoric acid (1:1, v/v), formic acid (1:1, v/v), or urea (∼8 M). A Low-OC pesticides control set (triplicate) was fortified but the serum was not denatured (untreated). The denatured samples and the untreated control samples were loaded on precleaned Oasis® HLB cartridges and extracted and analyzed as described in Sections 2.3 and 2.6, respectively. The mean recoveries of OC pesticides from the urea-treated serum samples ranged from 74 to 107% (Table 1) and recoveries for formic and phosphoric acid treated samples ranged from 35 to 184% and 18 to 94%, respectively (data not shown). The urea denaturation and extraction was repeated for 1.0-mL High-OC pesticide level fortified serum samples. The GC–ECD chromatogram for the High-OC pesticide samples had less baseline offsets and fewer interfering background peaks than the chromatograms from the formic and phosphoric acid treatments (not shown). The low background enabled quantifiable recoveries of the Low-OC pesticides and Low-PCB fortified serum samples.

As stated previously, <2% fortified OC pesticides and PCBs were recovered in the methylene chloride rinse of the sample vials. Similar deviations in response were observed when GC–ECD responses in a calibration standard in methylene chloride were compared after evaporation and reconstitution in solvent, e.g. *p*,*p*′-DDE area response decreased 1.4%. To complete a mass balance study, the residual levels of OC pesticides in the combined serum and water washes after passage through the HLB cartridge were determined. The recoveries ranged from non detectable for the three BHCs to 14% recovery of endosulfan sulfate in the untreated waste. In the urea-treated waste 10 of the pesticides were not detected while 7% of endosulfan sulfate was recovered. Apparently urea treatment completely disrupts protein binding for 10 of the 11 pesticides, but does not interfere with sorption to the HLB matrix.

In Table 1, the recoveries of aldrin, gamma- and alpha-chlordane, *p*,*p*′-DDE, and *p*,*p*′-DDD from 1.0-mL Low-OC samples were significantly ($p < 0.05$) higher when the serum was treated with urea. Interestingly, the recoveries of the BHCs, endrin, endosulfan sulfate and endrin ketone were not significantly different. In the 1.0-mL High-OC serum samples the recovery of alpha-BHC and delta-BHC was quantitative with and without urea treatment. Recovery of each of the other nine pesticides from High-OC serum was increased significantly ($p < 0.05$) by the addition of urea. The mean recoveries of aldrin,

gamma- and alpha-chlordane, and p,p'-DDE were increased from <26% to >80% by the urea.

To test the effect of sample dilution on recovery, 0.1 mL aliquots of High-OC serum were diluted with 0.9 mL of Nanopure® water, 500 mg urea added (~8 M) and the samples extracted and analyzed (Table 1). Nine of the pesticide recoveries were significantly greater in the urea-treated samples compared to the untreated (Table 1). A cause for the high recoveries of endrin in the 0.1 mL untreated and urea-treated samples was not determined. Interestingly, in the 0.1 mL untreated samples the recoveries of aldrin, gamma- and alpha-chlordane, p,p'-DDE, p,p'-DDD, and endosulfan were significantly ($p < 0.05$) greater compared to the 1.0-mL untreated samples. This suggests dilution of the fortified serum enhances sorption of OC pesticides to the Oasis® HLB sorbent.

The serum fortification and SPE extraction experiments were repeated in triplicate with serum fortified with six PCB congeners at High-PCB (100 pg/μL) and Low-PCB (10 pg/μL) levels (Table 2). At Low-PCB fortification, the recovery of PCB 28 from 1.0-mL samples was 81% without urea treatment and 83% with urea treatment. The recoveries of the other five congeners were significantly ($p < 0.05$) greater in the urea-treated samples but the percent recovered decreased with increasing number of chlorines. The recovery of PCB 180 (seven chlorines) was 36%. The same decrease in recovery with increasing number of chlorines was observed with the 1.0-mL High-PCB samples. Recovery for five of the six congers recoveries was significantly ($p < 0.05$) increased by urea treatment

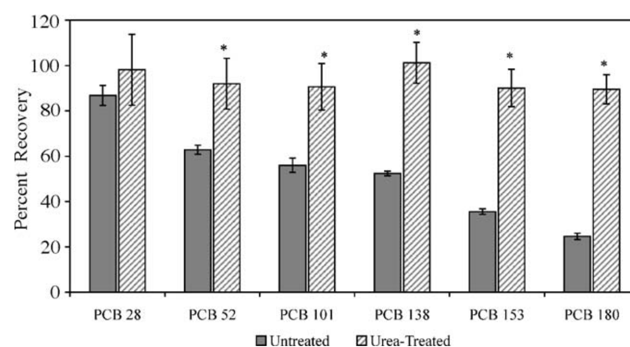

Fig. 1. Mean percent recoveries ($n = 3$, unless otherwise indicated) of 100 pg/μL PCBs in 0.1-mL serum. Vertical bars represent standard deviations. Urea treatment percent recoveries marked with an (*) are significantly higher than untreated serum as determined by SNK, Tukey's, and Duncan's tests.

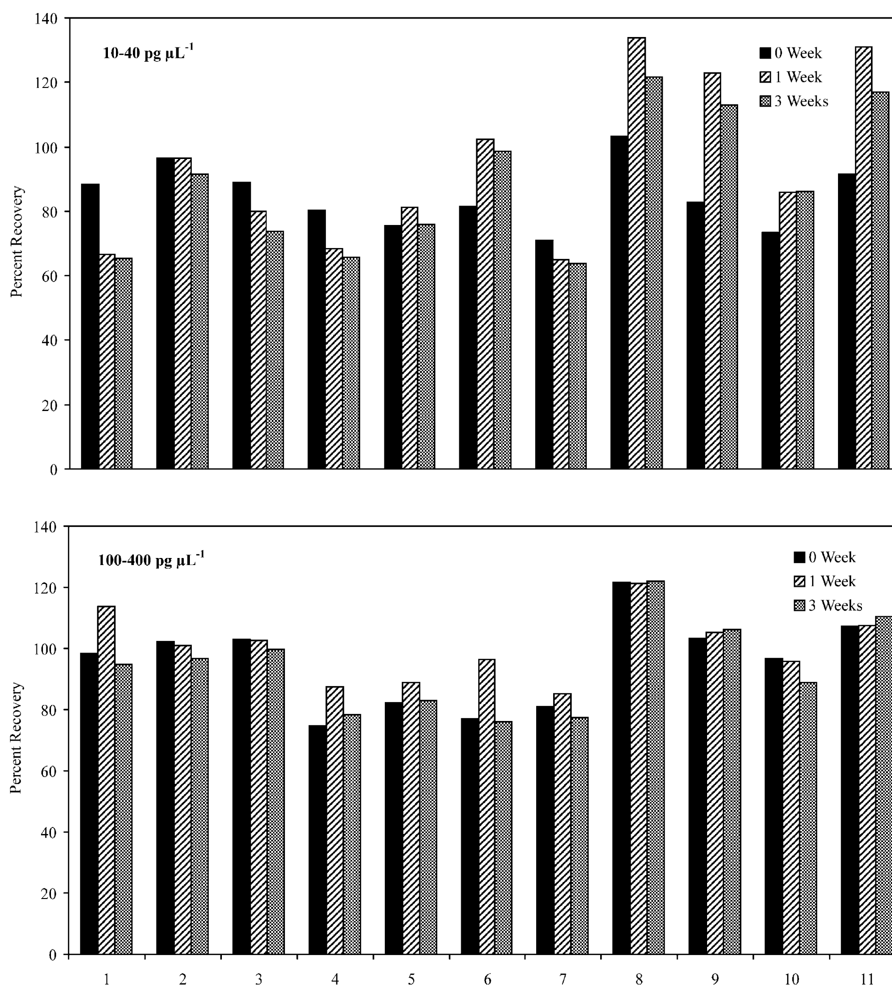

Fig. 2. Percent recoveries ($n = 1$) of 10–40 and 100–400 pg/μL pesticides in 1.0-mL serum eluted from HLB cartridge at 0, 1, and 3 weeks after drying: (1) alpha-BHC; (2) beta-BHC; (3) delta-BHC; (4) aldrin; (5) gamma-chlordane; (6) alpha-chlordane; (7) p,p-DDE; (8) endrin; (9) p,p-DDD; (10) endosulfan sulfate; (11) endrin ketone.

and recovery of PCB 180 from the urea-treated sample was 53%.

The recoveries of the six PCB congeners from the untreated 0.1-mL High-PCB serum samples that were diluted with 0.9 mL water before SPE were similar to the recoveries of the untreated 1.0-mL High-PCB and 1.0-mL Low-PCB samples. However, recovery from the diluted 0.1 mL urea-treated samples was nearly quantitative for each of the six PCB congeners (Fig. 1). The lowest recovery for the diluted urea-treated samples was PCB 180 with a recovery of $89.5 \pm 6\%$. The greatly enhanced recoveries of the PCB congeners can be attributed to both the use of urea and dilution of the serum sample, with the greatest effect due to urea.

To determine the effect of storage time on recovery, OC pesticides were eluted with methylene chloride after storage on the dried HLB cartridges for 0, 14, and 21 days at approximately 4 °C (Fig. 2). Similar recoveries of OC pesticides after 14 and 21 days indicate the sorbed OC pesticides are not degraded during HLB cartridge storage.

4. Conclusions

A simple SPE method was developed to extract OC pesticides and PCBs from avian serum. Fortification of the serum with OC pesticides and PCBs without using a solvent was accomplished by solubilizing the analytes from the glass surface after the solvent carrier was evaporated. Treatment with 8 M urea denatured serum proteins without precipitation. The use of Oasis® HLB cartridges eliminated the need for additional lipid clean-up prior to analysis. Urea efficiently desorbed the highly chlorinated PCBs from 0.1- to 1.0-mL serum. Extractions using this method were optimized with higher pesticide and PCB concentrations in serum samples of 0.1 mL. Although 1.0- and 0.1-mL sample sizes was used for method development, serum samples from wild birds with volumes as low as 20 μL have been extracted and analyzed for OC pesticides in this laboratory. The identified OCs were separated and quantified by gas chromatography interfaced with negative ion chemical ionization using selected ion monitoring [17]. The urea denaturation method is faster and is equally or more efficient than previously developed SPE and LLE methods for extracting bound OC pesticides and PCBs from serum. The stability of OC pesticides and PCBs sorbed to the HLB cartridge for at least 3 weeks should allow for interlaboratory shipment or delayed extraction of the HLB cartridge.

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