

Organochlorine Contaminants in Loggerhead Sea Turtle Blood: Extraction Techniques and Distribution Among Plasma and Red Blood Cells

J. M. Keller,¹ J. R. Kucklick,² P. D. McClellan-Green^{1,3}

¹ Integrated Toxicology Program and Nicholas School of the Environment and Earth Sciences, Duke University, Division of Coastal Systems Science and Policy, 135 Duke Marine Lab Road, Beaufort, North Carolina 28516, USA

² Hollings Marine Laboratory, National Institute of Standards and Technology, Charleston, South Carolina 29412, USA

³ Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, North Carolina 27695, USA

Received: 26 November 2002/Accepted: 7 June 2003

Abstract. Few studies have described the organochlorine (OC) contaminant concentrations found in sea turtle tissues. These studies have relied on the opportunistic sampling of either eggs or tissues from stranded carcasses. In this study, the use of whole blood samples as well as both blood components (plasma and red blood cells) were examined as a non-destructive alternative for monitoring OCs in free-ranging loggerhead sea turtles (*Caretta caretta*). Blood samples were collected from juvenile loggerhead sea turtles ($n = 12$) captured in Core Sound, North Carolina, USA and analyzed for 55 polychlorinated biphenyl (PCB) congeners and 24 OC pesticides by gas chromatography with electron capture detection and mass spectrometry. Using pooled loggerhead sea turtle whole blood, three different liquid:liquid extraction techniques were compared. Results were similar in terms of recovery of internal standards, lipids, and OC concentrations. An extraction technique, employing formic acid and 1:1 methyl-*tert*-butyl-ether:hexane, was found to be satisfactory. This method was applied to the extraction of OCs from whole blood, plasma, and red blood cell (RBC) samples from five loggerhead sea turtles. Plasma contained the highest OC concentrations on a wet mass basis, followed by whole blood and RBCs. The majority of each OC compound was found in the plasma rather than the RBCs, suggesting that OC compounds preferentially partition into the plasma. On average (SD), 89.4% (3.1%) of total PCBs, 83.4% (11.9%) of total chlordanes, 74.3% (15.1%) of mirex, 72.6% (4.8%) of total DDTs, and 80.1% (16.6%) of dieldrin were found in the plasma. The concentrations of total PCBs, mirex, total chlordanes, and total DDTs measured in both components of the blood significantly correlated to those in whole blood. These are the first reported OC concentrations in

sea turtle blood. They were found to be similar to previously reported levels in blood components of humans and of reptiles from relatively clean sites, but lower than those measured in blood of fish-eating birds and marine mammals. The results indicate that blood, preferably plasma, can be used to detect and monitor OC contaminants in loggerhead sea turtles.

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides, such as DDT, have contaminated the global environment (Iwata *et al.* 1994). Once widely used in industry and agriculture, most of these compounds were banned from use in developed countries starting in the 1970s mainly because of their detrimental effects on wildlife and concerns regarding human health effects. However, OC contaminants continue to be detected in environmental samples and animal tissues due to their persistence, their ability to bioaccumulate through the food chain, and their continued use in some underdeveloped countries (i.e.; Iwata *et al.* 1994; Letcher *et al.* 1995; Rybitski *et al.* 1995).

Because of their toxicity and environmental persistence, it is important that OC contaminants be monitored in wildlife populations. This is especially critical for species that are threatened with extinction, as these compounds may produce detrimental health effects and contribute to population declines in these vulnerable species. All species of sea turtles are listed as threatened or endangered under the U.S. Endangered Species Act or the Convention of International Trade of Endangered Species (Pritchard 1997). There is little information available regarding the levels of contaminants in sea turtle tissues, and currently no long-term monitoring projects are in place for these animals (for review see Pugh and Becker 2001).

OC contaminants are generally measured in fatty tissues since they are highly lipophilic. In previous studies, fatty tissues from sea turtles, such as fat, liver, muscle, and eggs have been collected opportunistically from either unhatched eggs or stranded turtle carcasses in order to measure OCs (Corsolini *et al.* 2000; Rybitski *et al.* 1995; Cobb and Wood 1997; McKenzie *et al.* 1999). Recently, however, researchers

Disclaimer: Certain commercial equipment or instruments are identified in the paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the NIST nor does it imply that the equipment or instruments are the best available for the purpose.

Correspondence to: P. McClellan-Green; email: pmccllell@duke.edu

have started to use blood in wildlife monitoring studies (Henny and Meeker 1981; Jenssen *et al.* 1994; Elliott and Norstrom 1998; Reddy *et al.* 1998; Bishop and Rouse 2000; Sandau *et al.* 2000). Blood offers several benefits over traditional tissue sampling. It can be collected easily and relatively nondestructively from free-ranging populations and facilitates the repeated collection of larger numbers of samples, which improves both the monitoring of OC levels and the assessment of toxicological effects. Blood OC concentrations have been used as a surrogate for concentrations in fatty tissues. For example, blood OC concentrations were significantly correlated to concentrations measured in the blubber of marine mammals, in brains of birds, and in fat biopsies of humans (Reddy *et al.* 1998; Henny and Meeker 1981; Mes 1992). However, it is difficult to compare the results of these past studies because they differ in their choice of extraction techniques or blood components used in the analysis of contaminants.

Prior studies describing OCs in blood have employed everything from whole blood to any one of its components. Whole blood can be separated into a liquid component (plasma or serum) and a packed red blood cell (RBC) component by simple centrifugation. Serum is collected after the blood is allowed to clot, while plasma is collected from blood that is mixed with an anticoagulant, so it retains fibrinogen and other clotting factors. The liquid components of blood contain lipoproteins and other proteins, such as albumin, which are known to bind and transport OC contaminants (Norén *et al.* 1999). OCs also binds to membranes and hemoglobin in the RBC component (Moss and Hathway 1964). The goals of our study were to determine whether OCs could be reliably detected in the blood of loggerhead sea turtles (*Caretta caretta*), to describe an effective method for extracting OCs from their whole blood, and to establish the physical partitioning of OCs within their blood.

Materials and Methods

Samples

Twelve juvenile loggerhead sea turtles were captured between June 1998 and July 2001 as bycatch in the pound-net fishery in Core Sound, North Carolina, USA. Seven of the turtles were female, three were male, and two were of unknown sex. Turtles ranged in size from 62.1 cm to 79.6 cm straight carapace length (notch to tip). Based on their size, these turtles may be estimated at somewhere between 10 and 30 years of age (Snover 2002). Two 10-mL blood samples were collected within 15 min of capture from the dorsocervical sinus of each turtle using double-ended needles and blood collection tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). The first sample, taken for whole blood, was kept on ice until frozen at -20°C . The second blood sample from each turtle was centrifuged at $400 \times g$ for 5 min to separate the plasma and RBCs. The outside of each blood tube was marked at the meniscus of the plasma and at the top of the packed RBCs. The plasma was transferred to a hexane-rinsed glass vial using hexane-rinsed glass transfer pipettes. The plasma and remaining RBCs were frozen at -20°C . Once the RBCs were removed from the original tubes for contaminant analysis, the volume of total blood and RBCs were determined. Packed cell volume (PCV) was estimated as the volume of packed RBCs divided by the total blood volume.

Blood Extraction Techniques

Three different extraction techniques (A, B, and C) were compared to determine the relative efficiency of extracting OC contaminants from loggerhead sea turtle whole blood (Figure 1). Blood pooled from seven juvenile loggerhead sea turtles was sonicated and divided into nine 5-g aliquots. Three replicate blood tubes and a blank tube containing 5-mL deionized water were then assigned to each extraction technique (A, B, or C). An internal standard solution (0.2 g) of isooctane was added to each tube resulting in the addition of 35 ng of each of the following compounds: 4,4'-DDT- d_8 , 4,4'-DDE- d_8 , 4,4'-DDD- d_8 , Endosulfan 1- d_4 , PCB 103, and PCB 198. Tubes were mixed well and were allowed to equilibrate on ice for at least 2 h.

Extraction techniques were chosen based upon published methods and personal communications. Technique A was used by the National Institute of Standards and Technology (NIST) to determine the certified values for NIST Standard Reference Material (SRM) 1589a (PCBs, Pesticides, and Dioxins/Furans in Human Serum) and was similar to another published method (Dr. Michele Schantz, personal communication; Hovander *et al.* 2000). Technique A (Figure 1) was performed by adding 5 mL of hexane-rinsed 98% formic acid to each blood tube followed immediately by the addition of 5 mL of 1:1 (v/v) methyl-*tert*-butyl ether (MTBE):hexane. These tubes were shaken by vortex for 2 min. Technique B (Figure 1) was modified from a protocol developed for measuring dioxins in human whole blood (Dr. David J. Brown, personal communication). These tubes (technique B) received 25 mL of acetone and were mixed by shaking. Then, they received 1 mL 98% formic acid and were mixed by inversion. These tubes were extracted by the addition of 10 mL 1:1 MTBE:hexane. Technique C (Figure 1) was a method reported in a study measuring OCs in Antarctic seabird blood (van den Brink *et al.* 1998). This technique employed 25 mL acetone followed by an addition of 10 mL of 1:1 MTBE:hexane.

Emulsions that formed from each extraction technique were broken by adding another 5 mL of 1:1 MTBE:hexane to the tubes. Organic layers from all extraction tubes were transferred to clean tubes and the blood samples were re-extracted with 10 mL 1:1 MTBE:hexane. Organic layers were combined with the previous extracts and a third extraction was performed. For the third extraction, tubes in technique A received 10 mL of hexane, while those in techniques B and C received 10 mL of 1:1 MTBE:hexane. The organic layers were combined with the previous extracts. As each organic layer from techniques B and C was transferred, they were washed three times with 5 mL of aqueous 1% KCl (mass:volume) to remove water. The combined final extracts were reduced by evaporation with purified N_2 (Turbovap II, Zymark, Hopkinton, MA).

Lipid content was determined gravimetrically for all blood samples and NIST SRM 1589a. Approximately 5% to 10% of each extract by weight was removed and transferred to a tared aluminum weighing boat. The solvent in the weighing boat was allowed to evaporate at room temperature for 6 h to 12 h. The mass of dried lipid residue was measured to the nearest 0.0001 mg.

Four calibration solutions were prepared from 5 NIST SRMs: NIST SRM 2261 (Chlorinated Pesticides in Hexane), NIST SRM 2275 (Chlorinated Pesticides in Hexane II), NIST SRM 2262 (Chlorinated Biphenyl Congeners in 2,2,4-trimethylpentane), NIST SRM 2274 (PCB Congener Solution II), and a solution containing 14 additional PCB congeners. The calibration curve ranged from approximately 50 pg to 3500 pg. An internal standard solution of isooctane was added to each calibration solution resulting in the addition of approximately 35 ng of each of the following compounds: 4,4'-DDT- d_8 , 4,4'-DDE- d_8 , Endosulfan I- d_4 , PCB 103 and PCB 198. The calibration solutions were not extracted.

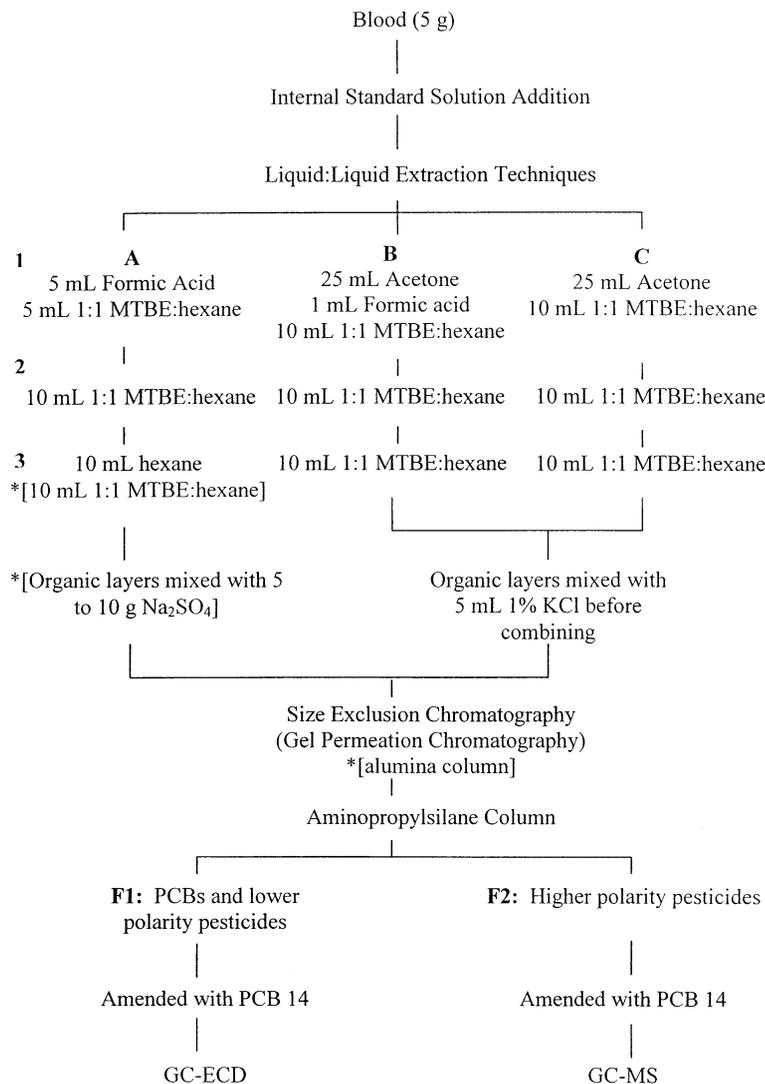


Fig. 1. Analytical scheme for determining PCBs and Organochlorine pesticides in loggerhead sea turtle blood. Original scheme was used for pooled loggerhead sea turtle blood to determine differences among the three extraction techniques (A, B, and C). *Shown in brackets are modifications made to technique A for extracting human serum (NIST SRM 1589a) and sea turtle plasma, red blood cells, and whole blood

Sample Clean-Up

High molecular mass compounds in the blood extracts were removed by gel permeation chromatography (GPC) on a 600-mm \times 25-mm (10 μ m particle size with 100 Å diameter pores) PLGel column (Polymer Labs, Amherst, MA) using CH_2Cl_2 according to Kucklick *et al.* (2002). Following separation, sample extracts were reduced in volume to approximately 0.5 mL using the Turbovap and the solvent was changed to hexane.

The sample extracts and the calibration solutions were fractionated into relatively lower and higher polarity fractions (F1 and F2, respectively) using a semi-preparative aminopropylsilane column (μ Bondapak NH_2 , Waters) (Kucklick *et al.* 2002). Each fraction was amended with 5 ng PCB 14 prior to analysis in order to calculate percent recovery of internal standards. Compounds contained in F1 included PCBs, heptachlor, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, hexachlorobenzene (HCB), aldrin, mirex, and oxychlordane. Analytes in F2 included 4,4'-DDT, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, α -, β -, and γ -hexachlorocyclohexane (HCH), heptachlor epoxide, 2,4'-DDD, 4,4'-DDD, dieldrin, endrin, endosulfans I and II, and endosulfan sulfate.

Contaminant Analysis

OC compounds contained in F1 were identified by gas chromatography (GC) with dual micro-electron capture detectors (ECD) (Hewlett Packard 6890; Kucklick *et al.* 2002). Organochlorines were separated using a 60-m, 0.25-mm interior diameter, 0.25- μ m film thickness, 5% phenyl methyl-poly-siloxane capillary column (DB-5 column, J&W Scientific, Folsom, CA) and a 60-m, 0.25-mm interior diameter, 0.25- μ m film thickness proprietary phase capillary column (DB-XLB column, J&W Scientific, Folsom, CA). The injector and detector temperatures were 220°C and 325°C, respectively. The carrier and makeup gasses were H_2 (constant velocity of 30 cm/s) and N_2 (30 mL/min), respectively. Samples were injected into the GC (2 μ L, splitless injection), and the oven was programmed for 90°C initially (1 min hold) to 170°C at 18°C/min, then 1°C/min to 260°C then ramped to 300°C at 1.5°C/min (107 min run time). The coplanar PCB congeners (PCB 77, PCB 126, and PCB 169) were not targeted for analysis, because the levels of these compounds in sea turtle blood would most likely be below the limits of detection (10 pg/g wet mass).

OC compounds in F2 were determined using a Hewlett Packard 6890/5973 GC-MS operating in the electron-impact (EI) mode and

using selected ion monitoring (SIM) programs targeting only the analytes in this fraction. By quantifying the F2 compounds using GC-MS, the signal to noise ratio was greatly improved, which was not a problem with the F1, resulting in a more reliable quantification of the compounds. Samples were injected (2 μ L) onto a 60-m, 0.25-mm interior diameter, 0.25- μ m film thickness, 5% phenyl methyl-poly-siloxane capillary column (DB-5 MS, J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a constant flow rate of 30 cm/s. The initial column temperature was 60°C; the temperature was then ramped to 170°C at 25°C/min, then to 200°C at 1°C/min, then to 240°C at 2°C/min. The final ramp brought the temperature to 300°C at 10°C/min, with a 10 min hold. The ions used to determine the analytes are given in Table 1.

The amount of each compound in the unknowns was calculated using the mass of PCB 14 added and the slope and intercept of the four calibration solutions. PCB 14 was used to calculate percent recovery of internal standards and all values were corrected with those recovery estimates. The average recovery of PCB 103 and PCB 198 was used to correct all analytes in F1, except for 2,4'-DDE, 4,4'-DDE, and 2,4'-DDT where the percent recovery of 4,4'-DDE- d_8 was used. When the values from the GC-ECD were similar between columns, they were averaged. In cases where the values from the two columns disagreed, the smaller value of the two was reported. In F2, all compounds were corrected for the percent recovery of 4,4'-DDD- d_8 , except 4,4'-DDT which was corrected using the recovery of 4,4'-DDT- d_8 .

Analysis of NIST SRM 1589a (Human Serum) and Plasma, Red Blood Cells, and Whole Blood from Individual Turtles

Five bottles of NIST SRM 1589a (PCBs, Pesticides, and Dioxins/Furans in Human Serum) were analyzed for OC contaminants. Each bottle of freeze-dried serum was reconstituted with water according to the instructions in the Certificate of Analysis. Briefly, 10.0 mL of deionized water was added to each bottle. The bottle were mixed periodically over 1 h at room temperature.

OC contaminants were analyzed in plasma, RBCs, and whole blood samples from five individual loggerhead sea turtles (three females and two males) all captured in July 2001. Analytical conditions for the NIST SRM 1589a and the turtle samples were identical to those described above except for the following modifications (Figure 1). Extraction technique A was used to extract approximately 10 g of reconstituted human serum, 3 g of turtle plasma, 3 g turtle RBCs, and 4 g turtle whole blood. Approximately 3 ng of each internal standard compound was added to these samples prior to extraction. The tubes were mixed by sonication for 15 min and were allowed to equilibrate at room temperature for 2 h. In order to reduce emulsions, the tubes were centrifuged at 1500 rpm for 5 min, and the third re-extraction used 10 mL 1:1 MTBE:hexane instead of hexane. Remaining water was removed from the combined organic extracts with 5 g to 10 g of anhydrous Na_2SO_4 . Sample clean-up was performed using alumina columns prepared using the methods described in Holden and Marsden (1969). Briefly, alumina was activated by heating to 800°C for 4 h and then deactivated by adding 5% H_2O . Above glass wool, 2 g of alumina was dry packed into a glass pipette and topped with Na_2SO_4 . Hexane (30 mL) was used to elute the OC compounds from the column. After fractioning each sample and calibration solution on the aminopropylsilane column, approximately 5 ng of PCB 14 was amended to each fraction.

The percentage of each compound that was distributed into the two components of whole blood (plasma or RBCs) was calculated using the following equation:

$$\% \text{ in component}_1 = (C_1P_1) / [(C_1P_1) + (C_2P_2)] * 100$$

Table 1. Target ions for GC-MS used to identify compounds in fraction 2

| Compound | Major ion | Minor ion |
|--|-----------|-----------|
| PCB 14 | 222 | — |
| Σ HCHs | 219 | 217 |
| 4,4'-DDT | 235 | 237 |
| 4,4'-DDT- d_8 | 243 | 245 |
| 2,4'-DDD | 235 | 237 |
| 4,4'-DDD | 235 | 237 |
| 4,4'-DDD- d_8 | 243 | 245 |
| heptachlor epoxide | 353 | 355 |
| <i>trans</i> - and <i>cis</i> -chlordane | 373 | 375 |
| <i>trans</i> - and <i>cis</i> -nonachlor | 409 | 407 |
| dieldrin | 263 | 265 |

where C_1 is the concentration of a compound in blood component₁ (either plasma or RBCs) on a wet mass basis, P_1 is the proportion of whole blood consisting of component₁ on a volume basis (calculated by PCV), and C_2 and P_2 are the same as above for component₂ of the blood.

Lab blanks (5 mL deionized water) and field blanks were processed with each sample batch. Field blanks were 5 mL deionized water pulled through a Vacutainer needle directly into a Vacutainer tube, both of which were previously taken into the field.

Statistics

All statistical tests were performed using Systat 8.0 (SPSS, Inc, Chicago, IL). Differences in percent recovery of internal standards, percent lipid, and final contaminant concentrations among the three extraction techniques were analyzed using analysis of variance (ANOVA) and the Tukey multiple comparison test. Pearson correlations were calculated to compare the contaminant concentrations between the different blood components.

Results

The three extraction techniques resulted in similar percent recovery of the internal standards and resulted in similar lipid values from the pooled loggerhead sea turtle whole blood (Table 2). Significant differences were noted for only PCB 198 in which technique A extracted less than techniques B and C. Percent lipid determined in the pooled blood did not differ among the extraction techniques.

Fifty-five PCB congeners and 24 OC pesticides were targeted for analysis. Twenty-four PCBs and seven pesticides were detected in the pooled loggerhead sea turtle whole blood (Table 3). Figure 2 shows a representative chromatogram of loggerhead sea turtle whole blood. Each of the extraction techniques resulted in the detection of the major compounds. Few differences in OC concentrations were observed among the techniques. Technique A resulted in the detection of slightly higher concentrations of PCB 99, PCB 158, PCB 163, dieldrin, *trans*-nonachlor, and total chlordanes than technique C. Extraction technique A resulted in the detection of lower concentrations of PCB 206 than techniques B or C. Although some significant differences were evident, the three techniques

Table 2. Mean (SD) percent recovery of internal standards and percent lipid content extracted from pooled whole blood of juvenile loggerhead sea turtles extracted with three different techniques

| | Technique A % recovery (SD) | Technique B % recovery (SD) | Technique C % recovery (SD) | Differences ^{a,b} |
|---------------------------------|--------------------------------|--------------------------------|--------------------------------|----------------------------|
| PCB 103 | 41.1 (5.5) | 44.2 (3.6) | 46.6 (1.8) | NS |
| PCB 198 | 43.6 (5.9) | 52.4 (1.9) | 58.4 (3.4) | A < B < C |
| 4,4'-DDE- <i>d</i> ₈ | 42.8 (6.3) | 46.8 (2.1) | 51.2 (3.3) | NS |
| 4,4'-DDD- <i>d</i> ₈ | 49.1 (1.6) | 46.2 (0.9) | 49.6 (6.6) | NS |
| 4,4'-DDT- <i>d</i> ₈ | 59.8 (2.9) | 60.3 (3.0) | 60.8 (10.0) | NS |
| Percent lipid | 0.200 (0.076) | 0.267 (0.058) | 0.175 (0.096) | NS |

^a Differences were determined by the Tukey Multiple Comparison Test ($p \leq 0.05$).

^b NS, not significant.

Three replicates of pooled blood were extracted with each technique.

Table 3. Mean (SD) organochlorine concentrations (pg/g wet mass) in pooled whole blood of seven juvenile loggerhead sea turtles extracted with three different techniques

| | Technique A (pg/g wet mass) | Technique B (pg/g wet mass) | Technique C (pg/g wet mass) | Differences ^{a,b} |
|--------------------------|--------------------------------|--------------------------------|--------------------------------|----------------------------|
| PCB 66 | 48.3 (6.0) | 44.1 (6.7) | 39.2 (3.0) | NS |
| PCB 99 | 457 (19) | 430 (8) | 406 (13) | A > C |
| PCB 105 | 152 (14) | 135 (7) | 130 (9) | NS |
| PCB 118 | 392 (24) | 385 (15) | 367 (14) | NS |
| PCB 128 | 102 (9) | 96.3 (3.4) | 94.6 (6.7) | NS |
| PCB 138 | 2630 (53) | 2560 (10) | 2520 (76) | NS |
| PCB 146 | 342 (11) | 333 (3) | 325 (5) | NS |
| PCB 149 | 19.3 (2.2) | 21.5 (5.8) | 20.4 (2.9) | NS |
| PCB 153 | 2110 (190) | 2130 (197) | 2090 (144) | NS |
| PCB 156 | 49.4 (6.3) | 49.7 (4.2) | 43.7 (2.3) | NS |
| PCB 157 | 16.3 (3.7) | 13.2 (2.3) | 8.07 (7.15) | NS |
| PCB 158 | 73.7 (6.4) | 63.9 (8.2) | 56.3 (1.0) | A > C |
| PCB 163 | 329 (11) | 312 (2) | 302 (6) | A > C |
| PCB 170 | 136 (11) | 136 (5) | 130 (3) | NS |
| PCB 174 | 13.1 (12.3) | <10 | <10 | |
| PCB 180 | 762 (10) | 821 (41) | 805 (29) | NS |
| PCB 183 | 187 (6) | 195 (8) | 188 (7) | NS |
| PCB 187 | 639 (18) | 657 (10) | 644 (23) | NS |
| PCB 193 | 213 (6) | 220 (11) | 211 (8) | NS |
| PCB 194 | 56.2 (4.1) | 61.9 (1.9) | 64.2 (4.0) | NS |
| PCB 195 | 33.1 (4.9) | 34.4 (1.5) | 31.3 (0.9) | NS |
| PCB 201 | 41.0 (4.6) | 39.9 (2.1) | 34.2 (2.7) | NS |
| PCB 206 | 93.0 (1.8) | 103 (4) | 104 (4) | A < B = C |
| PCB 209 | 25.6 (2.8) | 27.8 (1.7) | 26.0 (1.0) | NS |
| Total PCBs | 8920 (369) | 8870 (272) | 8640 (304) | NS |
| mirex | 34.5 (5.7) | 33.2 (2.4) | 30.8 (3.1) | NS |
| dieldrin | 72.8 (7.2) | 66.4 (12.5) | 46.8 (6.2) | A > C |
| <i>trans</i> -chlordanes | 45.0 (11.8) | 35.1 (1.7) | 36.2 (0.7) | NS |
| oxychlordanes | 63.9 (7.2) | 58.6 (5.8) | 53.6 (2.6) | NS |
| <i>trans</i> -nonachlor | 120 (0) | 112 (4) | 106 (7) | A > C |
| <i>cis</i> -nonachlor | 36.5 (4.8) | 34.4 (1.9) | 29.7 (3.1) | NS |
| Total chlordanes | 265 (17) | 240 (6) | 225 (9) | A > C |
| 4,4'-DDE | 579 (33) | 583 (45) | 568 (10) | NS |

^a Differences were determined by the Tukey Multiple Comparison Test ($p \leq 0.05$).

^b NS, not significant.

were generally similar, at most differing by only 24% for some compounds.

In order to validate the analytical method, OC contaminants were determined from five bottles of NIST SRM 1589a (Human Serum) using extraction technique A. The OC concentrations were compared to the certified and reference values

(Table 4). The results differed from the mean certified values by less than 30% for all compounds, except PCB 118, PCB 206, PCB 209, and *trans*-nonachlor. Measured concentrations for all compounds differed from the mean reference values by less than 40%, except PCB 74, mirex, and 2,4'-DDE, which substantially differed from the reference values. Interfering,

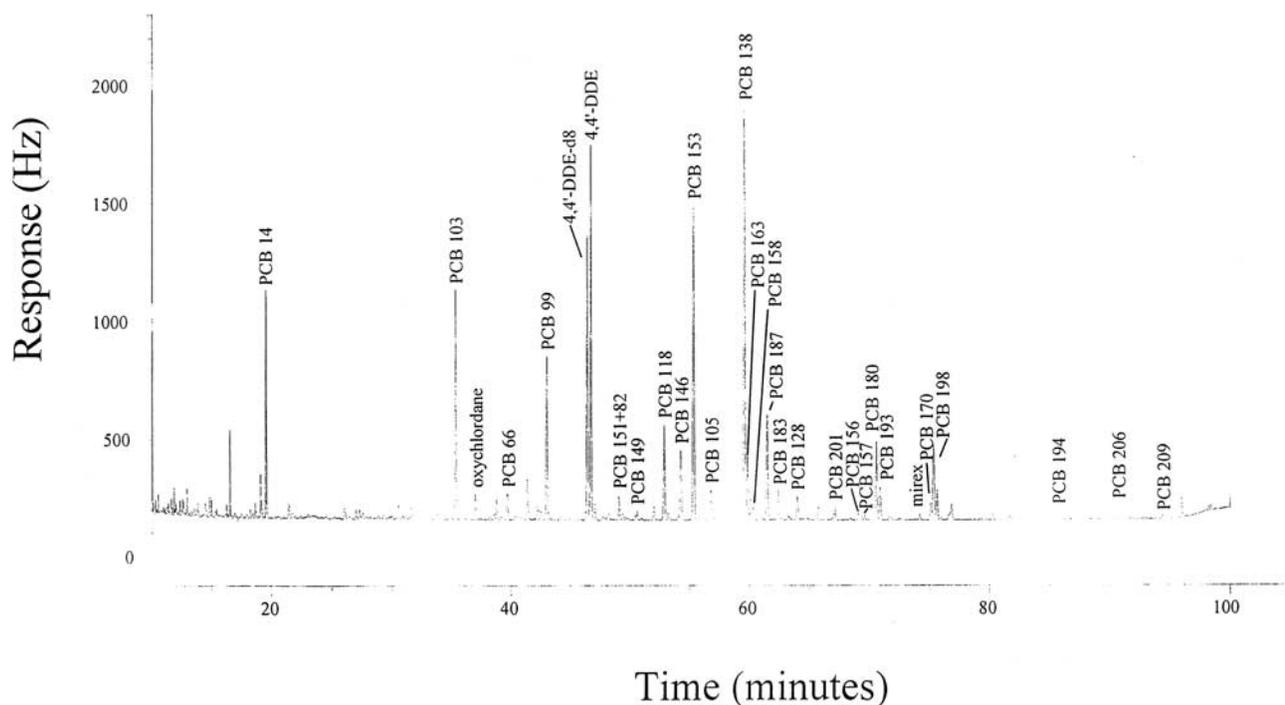


Fig. 2. Representative chromatogram of PCBs and organochlorine pesticides in fraction 1 of a sample of loggerhead sea turtle whole blood. The blood was extracted using technique A and analyzed as described in the methods section using GC-ECD with a 60 m, 0.25 mm interior diameter, 0.25 μm film thickness proprietary phase capillary column (DB-XLB column, J&W Scientific, Folsom, CA)

unknown compounds may have artificially increased the measured concentrations of PCB 118, PCB 206, PCB 209, *trans*-nonachlor, and mirex, while the reason for the lower observed concentrations of PCB 74 and 2,4'-DDE is unknown. The observed concentrations of 4,4'-DDT closely matched the reference value for NIST SRM 1589a; however, the GC-MS peak for 4,4'-DDT in the turtle samples was abnormally shaped, had a much smaller peak area, and did not consist of the proper target ions. The 4,4'-DDT concentrations, therefore, were considered below the limit of detection and not reported for the turtle samples nor were they included in the calculation of total DDTs for the turtle samples.

PCB and pesticide concentrations were compared between both components of the blood (plasma and RBCs) and whole blood from five individual loggerhead sea turtles (Table 5). Generally, plasma contained the highest concentrations of OCs on a wet mass basis, followed by whole blood and RBCs. In the turtles with lower levels of OCs, several compounds were below the limits of detection in the RBC component. As indicated by the large standard deviations, the blood OC concentrations varied greatly among individual turtles, suggesting that some turtles were exposed to or accumulated higher levels than others.

The partitioning of OCs was calculated between plasma and RBCs. Estimated packed cell volume (PCV) averaged 35.2% and ranged from 22.7% to 40.7%. Using these values we calculated the percentage of OC masses that were distributed between the plasma and RBCs. Plasma contained a larger percentage of OC contaminants than the RBC component (Figure 3). Plasma comprised 65% of loggerhead sea turtle blood and contained 81% to 95% of the individual congeners of PCBs

and chlordanes. The plasma also contained approximately 80% of the total masses of dieldrin and 75% of mirex and DDTs found in whole blood. Therefore, the majority of OC compounds preferentially partitioned into the plasma even though no differences in lipid content were observed between plasma, RBC, or whole blood. When comparing the OC concentrations measured in the two components to each other or to whole blood, significant correlations were observed for most compounds (Table 6). Dieldrin, however, did not correlate between the whole blood and the two components. The lack of correlation for dieldrin was most likely due to the fact that many of the samples were below the limit of detection.

Discussion

This study is the first to report OC concentrations in sea turtle blood and to provide comparative methods for extracting OC contaminants from their blood¹. Additionally, this study provides the first comparison of the distribution of OC contaminants among the plasma and cellular components of whole blood in a reptile species.

The extraction techniques selected for this study were com-

¹Preliminary reports of this work were presented at the 21st annual Symposium on Sea Turtle Biology and Conservation, Philadelphia, Pa, February, 2001 and the 22nd annual meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, Md, November, 2001.

Table 4. Comparison of measured organochlorine concentrations (pg/g wet mass) to certified and reference values of NIST SRM 1589a Human Serum using extraction technique A

| | Certified values ^a (pg/g wet mass) | Reference values ^a (pg/g wet mass) | Measured values ^b (pg/g wet mass) |
|------------------------------------|---|---|--|
| PCB 28 | | | 20.5 (5.2) |
| PCB 66 | | | 24.9 (4.2) |
| PCB 74 | | 226 (5) | 43.3 (9.1) |
| PCB 95 | | 47 (4) | 32.4 (6.6) |
| PCB 99 | 121 (10) | | 155 (20) |
| PCB 101 + 90 | 34 (9) for 101 | | 38.5 (6.0) |
| PCB 105 | 29 (4) | | 37.2 (4.0) |
| PCB 110 | | | 25.3 (2.8) |
| PCB 118 | 119 (9) | | 162 (17) |
| PCB 138 | | | 331 (39) |
| PCB 146 | | 76 (5) | 90.0 (9.8) |
| PCB 149 | 56 (8) | | 54.0 (9.1) |
| PCB 151 + 82 | 28 (4) for 151 | | 51.9 (18.9) |
| PCB 153 | 672 (35) | | 730 (49) |
| PCB 156 | 66 (4) | | 77.2 (22.1) |
| PCB 163 | | | 125 (15) |
| PCB 170 | | 186 (4) | 186 (28) |
| PCB 180 | 483 (29) | | 487 (65) |
| PCB 183 | 65 (5) | | 73.1 (7.9) |
| PCB 187 | 172 (25) for 187 + 182 | | 212 (22) |
| PCB 193 | | | 34.8 (9.7) |
| PCB 194 | 98 (14) | | 123 (24) |
| PCB 195 | 22 (4) | | 28.7 (5.9) |
| PCB 206 | 40 (6) | | 64.0 (12.3) |
| PCB 209 | 25 (4) | | 41.3 (7.8) |
| Total PCBs | | | 3280 (137) |
| PCB 138 + 163 hexachlorobenzene | 483 (39) for 138 + 163 + 164 | | 552 (64) |
| mirex | | 49 (9) | 64.4 (11.3) |
| dieldrin | | 43 (4) | 92.0 (17.1) |
| heptachlor epoxide | 75 (9) | 73 (7) | 102 (26) |
| oxychlorodane | 157 (14) | | 84.7 (18.1) |
| <i>trans</i> -nonachlor | 169 (29) | | 112 (16) |
| <i>cis</i> -nonachlor | | | 285 (25) |
| Total chlordanes | | | 40.6 (5.4) |
| 2,4'-DDE | | 85 (5) | 438 (18) |
| 4,4'-DDE | 6600 (1000) | | 15.2 (2.6) |
| 2,4'-DDT | | | 7030 (204) |
| 4,4'-DDT | | 85 (10) | 144 (55) |
| Total DDTs | | | 81.6 (21.6) |
| | | | 7370 (133) |

^a Mean (the expected uncertainty) described in Certificate of Analysis.

^b Mean (SD) obtained from five bottles of NIST SRM 1589a.

piled from several sources (see methods section) and differed mainly in their use of formic acid. Formic acid used in techniques A and B was expected to aid in the extraction of most compounds and decrease the amount of lipid and acid-sensitive dieldrin recovered from the samples. Interestingly, lipid recovery did not differ between the three techniques, and technique A resulted in the detection of higher dieldrin concentrations than the acid-free extraction employed by technique C. These results suggest that formic acid did not negatively affect lipid determinations or acid-sensitive compounds. Based on these data and in order to remain consistent with the methods currently used for the certification of NIST SRM 1589a, technique A with slight modifications was chosen for future analyses.

Other techniques such as solid phase extraction (SPE) are available to extract OCs from blood components (Guillette *et*

al. 1999). SPE can easily extract plasma and serum samples but not coarse tissues such as whole blood or RBC. Coarser samples must first be extracted using a liquid:liquid technique prior to employing the SPE (Volz *et al.* 2001).

Regardless of which technique is employed, insufficient sample volumes or high analytical costs often limit the number of replicates that can be analyzed for each sample. When each sample can only be analyzed once, which is often the case; it is important to extract replicates of a pooled sample or a standard material in order to estimate the variation in measured contaminant concentrations. This study provides two estimates of variation, a) the standard deviation observed from the bottles of human serum (NIST SRM 1589a, Table 4) and b) the standard deviation of the three replicates of pooled loggerhead sea turtle blood for each extraction technique (Table 3). Among the three

Table 5. Organochlorine concentrations (pg/g wet mass) in different components of blood from five juvenile loggerhead sea turtles using extraction technique A

| | Whole blood (pg/g wet mass) | | Plasma (pg/g wet mass) | | Red blood cells (pg/g wet mass) | |
|-------------------------|-----------------------------|-------------|------------------------|-------------|---------------------------------|-------------|
| | Mean (SD) | Range | Mean (SD) | Range | Mean (SD) | Range |
| PCB 66 | 25.7 (27.6) | <10–56.9 | 34.3 (14.5) | 17.6–47.4 | 16.4 (11.5) | <10–31.8 |
| PCB 99 | 337 (280) | 71.2–709 | 555 (478) | 84.5–1120 | 99.1 (79.9) | 15.1–190 |
| PCB 105 | 96.4 (72.3) | 25.9–205 | 122 (82.2) | 32.1–239 | 33.5 (34.2) | <10–80.4 |
| PCB 118 | 324 (264) | 62.3–717 | 444 (327) | 87.6–872 | 136 (103) | 29.9–281 |
| PCB 128 | 69.4 (49.3) | 13.4–136 | 74.6 (54.9) | <10–138 | 28.1 (28.7) | <10–67.0 |
| PCB 138 | 1050 (863) | 271–2390 | 1550 (1140) | 407–3130 | 303 (240) | 63.8–635 |
| PCB 146 | 129 (101) | 28.8–291 | 199 (171) | 35.8–486 | 29.1 (39.6) | <10–95.8 |
| PCB 153 | 1500 (1270) | 303–3560 | 2100 (1610) | 405–4580 | 479 (394) | 64.8–1070 |
| PCB 163 | 143 (105) | 21.3–253 | 186 (131) | 26.3–353 | 67.3 (54.7) | <10–122 |
| PCB 170 | 91.5 (66.4) | 19.7–174 | 111 (66) | 31.8–177 | 37.6 (36.9) | <10–74.5 |
| PCB 180 | 509 (443) | 52.2–1190 | 678 (450) | 178–1330 | 177 (136) | 43.4–372 |
| PCB 183 | 138 (104) | 25.6–296 | 164 (119) | 25.5–339 | 46.3 (39.3) | <10–102 |
| PCB 187 + 182 | 320 (178) | 97.5–570 | 412 (265) | 105–822 | 106 (101) | <10–246 |
| PCB 193 | 114 (98) | 20.7–260 | 147 (106) | 33.7–280 | 38.4 (38.3) | <10–89.3 |
| PCB 194 | 58.2 (49.5) | <10–131 | 80.1 (43.6) | 36.5–140 | 31.4 (30.5) | <10–68.7 |
| PCB 206 | 71.0 (59.9) | <10–142 | 89.5 (61.2) | 13.5–157 | 35.3 (34.7) | <10–75.4 |
| Total PCBs ^a | 5140 (3950) | 1010–11000 | 7130 (4940) | 1540–13700 | 1720 (1350) | 231–3560 |
| mirex | 55.8 (74.8) | <10–178 | 36.5 (52.9) | <10–116 | 29.6 (47.1) | <10–108 |
| dieldrin | 45.9 (29.2) | <10–78.3 | 18.8 (19.1) | <10–44.3 | 8.90 (12.6) | <10–26.8 |
| oxychlordanes | 111 (116) | 17.7–307 | 121 (118) | 39.6–322 | 64.8 (75.9) | <10–182 |
| trans-nonachlor | 109 (60) | 44.2–176 | 103 (48) | 45.4–148 | 51.0 (52.4) | <10–116 |
| Total chlordanes | 260 (182) | 61.8–531 | 238 (155) | 98.2–485 | 135 (135) | <10–292 |
| 4,4'-DDE | 576 (305) | 194–901 | 575 (294) | 236–998 | 448 (329) | 107–941 |
| Total DDTs ^b | 583 (307) | 194–918 | 578 (294) | 236–997 | 457 (320) | 107–941 |
| % lipid | 0.341 (0.080) | 0.209–0.427 | 0.269 (0.095) | 0.166–0.426 | 0.281 (0.040) | 0.238–0.343 |

^a Totals include the major compounds listed in this table plus other minor detectable compounds.

^b In parentheses are the number of samples out of five that had detectable concentrations.

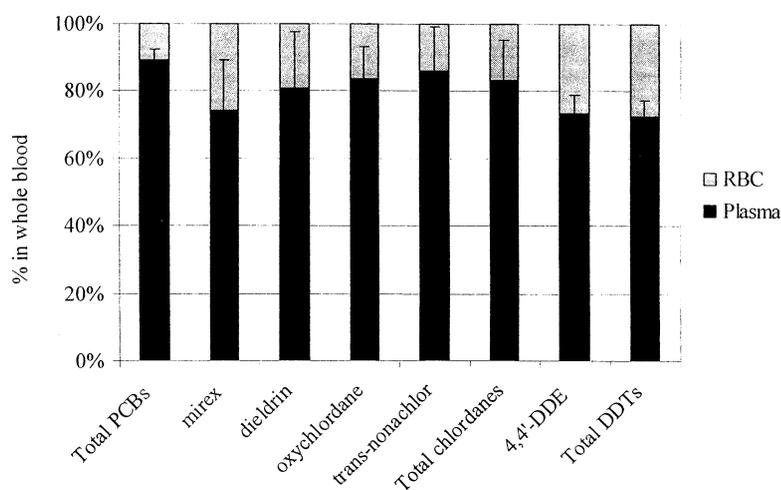


Fig. 3. Distribution of organochlorine contaminants between loggerhead sea turtle plasma and red blood cells (RBCs). Paired samples of plasma and RBCs from five loggerhead sea turtles were extracted with technique A. The percentage of each compound found in each component was calculated and averaged among the 5 turtles. The error bars indicate standard deviation

extraction techniques, the standard deviations for most compounds are similar and relatively small (Table 3). The standard deviations are also similar between loggerhead sea turtle blood (technique A) and the human serum except for those compounds that are found at a higher concentration in one sample than the other (i.e., 4,4'-DDE). These known standard deviations could provide an estimate of variation for future analyses of sea turtle blood when only one replicate can be analyzed per sample. It should also be noted that analytical error in measur-

ing OCs in sea turtle blood (see SD of the pooled turtle blood in Table 3) is much smaller than the biological variation of OCs measured among five individual turtles (Table 5).

OC contaminants were detectable in both components of the blood (plasma and RBCs) as well as whole blood. The OC concentrations measured in the two components correlated to those detected in whole blood and to each other for the majority of the compounds. The same relationship has been observed for total PCB concentrations between human whole blood and

Table 6. Pearson correlations of organochlorine contaminant concentrations (on a wet mass basis) between the different components of loggerhead sea turtle blood

| | Whole blood vs. Plasma | | | | Whole blood vs. red blood cells | | | | Plasma vs. red blood cells | | | |
|-------------------------|------------------------|----------|-------|-----------|---------------------------------|----------|-------|-----------|----------------------------|----------|-------|-----------|
| | R ² | <i>p</i> | slope | intercept | R ² | <i>p</i> | slope | intercept | R ² | <i>p</i> | slope | intercept |
| Total PCBs | 0.985 | 0.002 | 0.789 | -481 | 0.930 | 0.022 | 2.89 | 152 | 0.944 | 0.016 | 0.271 | -206 |
| mirex | 0.983 | 0.003 | 1.43 | 0.657 | 0.894 | 0.041 | 1.63 | 3.78 | 0.827 | 0.084 | 0.868 | -1.58 |
| dieldrin | 0.535 | 0.352 | 0.899 | 28.0 | 0.558 | 0.328 | 1.62 | 26.5 | 0.917 | 0.028 | 0.530 | 1.44 |
| oxychlordane | 0.989 | 0.001 | 0.987 | -8.77 | 0.960 | 0.010 | 1.53 | 9.87 | 0.972 | 0.005 | 0.620 | -9.24 |
| <i>trans</i> -nonachlor | 0.982 | 0.003 | 1.22 | -16.2 | 0.873 | 0.053 | 1.20 | 45.0 | 0.922 | 0.026 | 0.983 | -47.2 |
| Total chlordanes | 0.969 | 0.007 | 1.16 | -16.6 | 0.936 | 0.019 | 1.28 | 85.7 | 0.977 | 0.004 | 0.795 | -53.0 |
| 4,4'-DDE | 0.955 | 0.011 | 0.990 | 6.53 | 0.955 | 0.011 | 0.794 | 220 | 0.908 | 0.033 | 0.756 | 13.7 |
| Total DDTs | 0.957 | 0.011 | 1.01 | 0.883 | 0.956 | 0.011 | 0.805 | 214 | 0.902 | 0.036 | 0.735 | 32.7 |

plasma (Sandau *et al.* 2000) and for chlordanes, DDE, and total PCB concentrations between polar bear plasma and RBCs (Bernhoft *et al.* 1997).

OC contaminants preferentially partitioned into the liquid component of sea turtle blood, which is similar to observations in mammalian studies. Harbor seal blood is made up of approximately 33% serum and 66% RBCs, but 41% of PCBs that were measured in whole blood were found in the serum, suggesting that substantial amounts of OC compounds bind to non-cellular molecules (Boon *et al.* 1987). Matthews and others (1984) injected Sprague-Dawley rats with eight PCB congeners to investigate the distribution of the PCBs between the plasma and RBC components. The larger proportion of each PCB congener (52% to 83%) was found in the plasma. Similarly, the majority of hexa- to octa-chlorinated PCBs, *trans*-nonachlor, and DDTs were found in human serum or plasma rather than the RBC component (Mes *et al.* 1992). OC contaminants are known to associate with plasma proteins, such as lipoproteins and albumin (Norén *et al.* 1999). This could explain the partitioning of OCs into the plasma.

The results observed in this study with sea turtle blood suggest that any blood component may be used to measure OCs, but that the plasma component offers significant advantages. Plasma contained the highest concentrations of OCs, therefore allowing a better chance at detection of compounds present. Operationally, plasma is easier to use as it resulted in the formation of fewer emulsions during the liquid:liquid extraction, and it can be passed directly through a solid phase extraction cartridge. However, in large-scale field projects that survey sea turtle populations, collecting whole blood offers a simpler technique and minimizes the potential for contamination of the sample in the field. If feasible, the blood components can be separated by centrifugation and the tubes frozen in an upright position for later analysis. Based on observation of other frozen samples, as long as the blood is thawed in an upright position without shaking, the plasma can be removed carefully from the underlying RBCs without contamination. The plasma at the RBC interface may appear pink from hemoglobin diffusing out of ruptured RBCs, but the transfer of this interface can and should be avoided.

The blood OC concentrations (mean, and/or ranges on a wet mass basis) measured in the loggerhead sea turtles were similar to those reported for other reptile species. PCB concentrations measured in the blood of loggerhead sea turtles (mean = 5.14 ng/g; range = 1.01–11.0 ng/g) were similar to concentrations

reported in blood components of the adult northern water snakes (3–12 ng/g) from the Great Lakes and juvenile American alligators (1.54 ng/mL) from lakes in central Florida (Bishop and Rouse 2000; Guillette *et al.* 1999). However, the loggerhead blood PCB concentrations were lower than those in plasma of male snapping turtles from reference lakes (~18 ng/g) and contaminated sites (means ranged from 263.3 ng/g to 414.8 ng/g) in the Great Lakes (de Solla *et al.* 1998).

Compared to PCB concentrations in blood components of fish-eating or predatory birds, the loggerhead sea turtle has one to three orders of magnitude lower levels of PCB contaminants. For example, total PCBs in plasma of Caspian tern chicks from Lake Huron were reported to range from approximately 20 ng/g to 400 ng/g (Grasman and Fox 2001). Total PCB concentrations in the plasma of bald eagle chicks from Lake Erie ranged from 9.9 ng/g to 326 ng/g (Donaldson *et al.* 1999), and from the Pacific coast of Canada they ranged from 1.9 ng/g to 114 ng/g (Elliott and Norstrom 1998).

PCB levels in loggerhead sea turtle blood were similar to those measured in RBC from grey seal pups from Norway (1–32 ng/g; Jenssen *et al.* 1994), but substantially lower than those in blood of bottlenose dolphins from Sarasota, Florida (26.3–752 ng/g; Lahvis *et al.* 1995). Compared to humans, PCB levels in loggerhead blood were similar to the mean plasma concentration of fish-eating men from Ontario, Canada (5.5 ng/g; Kearney *et al.* 1999) and slightly lower than those seen in Canadian Inuit blood (15.2 ng/g; Sandau *et al.* 2000). Future studies should examine the relationship between blood OC concentrations and those in fatty tissues to determine whether blood can be used as a surrogate for those tissues in sea turtles and other species.

Similar comparisons can be observed for 4,4'-DDE concentrations. Loggerhead blood concentrations of 4,4'-DDE (0.576 ng/g) were similar to alligator and snapping turtles from reference sites (means ranged from 0.77–1.28 ng/mL and 0.2–0.7 ng/g, respectively), but lower than those animals from contaminated sites (7.35–17.98 ng/mL and 10.1–21.7 ng/g; Guillette *et al.* 1999; de Solla *et al.* 1998). The loggerhead had three orders of magnitude lower 4,4'-DDE concentrations than blood of snakes and red-eared slider turtles from a site in Texas (56.1–3830 ng/g; Clark *et al.* 2000). DDE concentrations in the birds and marine mammals mentioned previously were typically one to three orders of magnitude higher than those of loggerhead sea turtles. This indicates a greater variability in exposure to these contaminants from their environment.

In conclusion, OC concentrations are relatively low in sea turtle blood, but the sensitivity of sea turtles to these levels is unknown. OC contaminants were detected in loggerhead sea turtle blood components and a liquid:liquid extraction technique (technique A) employing formic acid and 1:1 MTBE:hexane was determined to be sufficient for future analyses. This analytical technique was validated using a similar tissue matrix, human serum (NIST SRM 1589a). Additionally, loggerhead sea turtle plasma was determined to contain the largest proportion of OC compounds found in the whole blood, suggesting that plasma may be the best blood component to use when monitoring OCs in sea turtles. Numerous studies, worldwide, survey sea turtle populations and many already collect blood for genetic research. This fact combined with the results and methods described in this study indicate that monitoring OCs may now be easily incorporated in these projects using non-destructive and selective blood sampling.

Acknowledgment. Partial funding for this study was provided by the Morris Animal Foundation (PMG), the Disney Wildlife Conservation Fund (PMG), and the Oak Foundation (JMK). We thank Sheryan Epperly, Joanne Braun-McNeill, and Larisa Avens for their help in obtaining samples. We also thank Drs. Michele Schantz and Paul Becker for their experienced guidance in data analysis. For their help in various aspects of this project, a warm thanks also goes to Karen Tuerk, Stacy Vander Pol, and Rebecca Pugh.

References

- Bernhoft A, Wiig Ø, Skaare JU (1997) Organochlorines in polar bears (*Ursus maritimus*) at Svalbard. *Environ Pollut* 95:159–175
- Bishop CA, Rouse JD (2000) Chlorinated hydrocarbon concentrations in plasma of the Lake Erie water snake (*Nerodia sipedon insularum*) and Northern water snake (*Nerodia sipedon sipedon*) from the Great Lakes Basin in 1998. *Arch Environ Contamin Toxicol* 39:500–505
- Boon JP, Reijnders PJH, Dols J, Wensvoort P, Hillebrand MTJ (1987) The kinetics of individual polychlorinated congeners in female harbour seals (*Phoca vitulina*), with evidence for structure-related metabolism. *Aquatic Toxicol* 10:307–324
- Clark DR Jr, Bickham JW, Baker DL, Cowman DF (2000) Environmental contaminants in Texas, USA, wetland reptiles: Evaluation using blood samples. *Environ Toxicol Chem* 19:2259–2265
- Cobb GP, Wood PD (1997) PCB concentrations in eggs and chorioallantoic membranes of loggerhead sea turtles (*Caretta caretta*) from the Cape Romain National Wildlife Refuge. *Chemosphere* 34:539–549
- Corsolini S, Aurigi S, Focardi S (2000) Presence of polychlorobiphenyls (PCBs) and coplanar congeners in the tissues of the Mediterranean loggerhead turtle *Caretta caretta*. *Mar Pollut Bull* 40:952–960
- de Solla SR, Bishop CA, Van Der Kraak G, Brooks RJ (1998) Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles (*Chelydra serpentina serpentina*) in Ontario, Canada. *Environ Health Perspect* 106:253–260
- Donaldson GM, Shutt JL, Hunter P (1999) Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. *Environ Contamin Toxicol* 36:70–80
- Elliott JE, Norstrom RJ (1998) Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. *Environ Toxicol Chem* 17:1142–1153
- Grasman KA, Fox GA (2001) Associations between altered immune function and organochlorine contamination in young Caspian terns (*Sterna caspia*) from Lake Huron, 1997–1999. *Ecotoxicology* 10:101–114
- Guillette LJ Jr, Brock JW, Rooney AA, Woodward AR (1999) Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallus size in juvenile American alligators. *Arch Environ Contamin Toxicol* 36:447–455
- Henny CJ, Meeker DL (1981) An evaluation of blood plasma for monitoring DDE in birds of prey. *Environ Pollut (Ser A)* 25:291–304
- Holden AV, Marsden K (1969) Single-stage clean-up of animal tissue extracts for organochlorine residue analysis. *J Chromatogr* 44:481–492
- Hovander L, Athanasiadou M, Asplund L, Jensen S, Wehler EK (2000) Extraction and cleanup methods for analysis of phenolic and neutral organohalogenes in plasma. *J Anal Toxicol* 24:696–703
- Iwata H, Tanabe S, Sakai N, Nishimura A, Tatsukawa R (1994) Geographical distribution of persistent organochlorines in air, water and sediments from Asia and Oceania, and their implications for global redistribution from lower latitudes. *Environ Pollut* 85:15–33
- Jenssen BM, Skaare JU, Ekker M, Vongraven D, Silverstone M (1994) Blood sampling as a non-destructive method for monitoring levels and effects of organochlorines (PCB and DDT) in seals. *Chemosphere* 28:3–10
- Kearney JP, Cole DC, Ferron LA, Weber J-P (1999) Blood PCB, *p,p'*-DDE, and mirex levels in Great Lakes fish and waterfowl consumers in two Ontario communities. *Environ Res Section A* 80:S138–S149
- Kucklick JR, Struntz WDJ, Becker PR, York GW, O'Hara TM, Bohonowych JE (2002) Persistent organochlorine pollutants in ringed seals and polar bears collected from northern Alaska. *Sci Total Environ* 287:45–59
- Lahvis GP, Wells RS, Kuehl DW, Stewart JL, Rhinehart HL, Via CS (1995) Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ Health Perspect* 103(Suppl 4):67–72
- Letcher RJ, Norstrom RJ, Bergman A (1995) Geographical distribution and identification of methyl sulfone PCB and DDE metabolites in pooled polar bear (*Ursus maritimus*) adipose tissue from western hemisphere Arctic and sub-Arctic regions. *Sci Total Environ* 161:409–420
- Matthews HB, Surlis JR, Carver JG, Anderson MW (1984) Halogenated biphenyl transport by blood components. *Fundamental Appl Toxicol* 4:420–428
- Mckenzie C, Godley BJ, Furness RW, Wells DE (1999) Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters. *Mar Environ Res* 47:117–135
- Mes J (1992) Organochlorine residues in human blood and biopsy fat and their relationship. *Bull Environ Contamin Toxicol* 48:815–820
- Mes J, Marchand L, Turton D, Lau P-Y, Ganz PR (1992) The determination of polychlorinated biphenyl congeners and other chlorinated hydrocarbon residues in human blood, serum and plasma. a comparative study. *International J Environ Anal Chem* 48:175–186
- Moss JA, Hathway DE (1964) Transport of organic compounds in the mammal. *Biochem J* 91:384–393
- Norén K, Weistrand C, Karpe F (1999) Distribution of PCB congeners,

- DDE, hexachlorobenzene, and methylsulfonyl metabolites of PCB and DDE among various fractions of human blood plasma. *Arch Environ Contam Toxicol* 37:408–414
- Pritchard PCH (1997) Evolution, phylogeny, and current status. In: Lutz PL, Musick JA (eds) *The biology of sea turtles*. CRC Press, Boca Raton, FL, pp 1–28
- Pugh RS, Becker PR (2001) Sea turtle contaminants: A review with annotated bibliography. Report #NISTIR 6700. National Institute of Standards and Technology, 144 pp.
- Reddy M, Echols S, Finklea B, Busbee D, Reif J, Ridgway S (1998) PCBs and chlorinated pesticides in clinically healthy *Tursiops truncatus*: Relationships between levels in blubber and blood. *Mar Pollut Bull* 36:892–903
- Rybitski MJ, Hale RC, Musick JA (1995) Distribution of organochlorine pollutants in Atlantic sea turtles. *Copeia* 1995:379–390
- Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ (2000) Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian Inuit. *Environ Health Perspect* 108:611–616
- Snover ML (2002) Growth and ontogeny of sea turtles using skeletochronology: Methods, validation and application to conservation. Ph.D. dissertation. Duke University, Durham, NC
- van den Brink NW, van Franeker JA, de Ruiter-Dijkman EM (1998) Fluctuating concentrations of organochlorine pollutants during a breeding season in two Antarctic seabirds: Adélie penguin and southern fulmar. *Environ Toxicol Chem* 17:702–709
- Volz SA, Johnston JJ, Griffin DL (2001) Solid phase extraction gas chromatography/electron capture detector method for the determination of organochlorine pesticides in wildlife whole blood. *J Agric Food Chem* 49:2741–2745