Associations between Organochlorine Contaminant Concentrations and Clinical Health Parameters in Loggerhead Sea Turtles from North Carolina, USA

Jennifer M. Keller,[1,2] John R. Kucklick,[2] M. Andrew Stamper,[3,] Craig A. Harms,[4] and Patricia D. McClellan-Green[1,5]*

[1]Duke University, Integrated Toxicology Program and Nicholas School of the Environment Coastal Systems Science and Policy, Beaufort, North Carolina, USA; [2]National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, South Carolina, USA; [3]New England Aquarium, Boston, Massachusetts, USA; [4]North Carolina State University, College of Veterinary Medicine, Center for Marine Sciences and Technology, Morehead City, North Carolina, USA; [5]North Carolina State University, Department of Environmental and Molecular Toxicology, Raleigh, North Carolina, USA

Widespread and persistent organochlorine (OC) contaminants, such as polychlorinated biphenyls (PCBs) and pesticides, are known to have broad-ranging toxicities in wildlife. In this study we investigated, for the first time, their possible health effects on loggerhead sea turtles (*Caretta caretta*). Nonlethal fat biopsies and blood samples were collected from live turtles for OC contaminant analysis, and concentrations were compared with clinical health assessment data, including hematology, plasma chemistry, and body condition. Concentrations of total PCBs (ΣPCBs), ΣDDTs, Σchlordanes, dieldrin, and mirex were determined in 44 fat biopsies and 48 blood samples. Blood concentrations of Σchlordanes were negatively correlated with red blood cell counts, hemoglobin, and hematocrit, indicative of anemia. Positive correlations were observed between most classes of OC contaminants and white blood cell counts and between mirex and ΣTCDD-like PCB concentrations and the heterophil:lymphocyte ratio, suggesting modulation of the immune system. All classes of OCs in the blood except dieldrin were correlated positively with aspartate aminotransferase (AST) activity, indicating possible hepatocellular damage. Mirex and ΣTCDD-like PCB blood concentrations were negatively correlated with alkaline phosphatase (ALP) activity. Significant correlations to levels of certain OC contaminant classes also suggested possible alteration of protein (↑ blood urea nitrogen, ↓ albumin:globulin ratio), carbohydrate (↓ glucose), and ion (↑ sodium, ↓ magnesium) regulation. These correlations suggest that OC contaminants may be affecting the health of loggerhead sea turtles even though sea turtles accumulate lower concentrations of OCs compared with other wildlife. *Key words:* health assessment, hematology, organochlorine contaminants, PCBs, persistent organic pollutants, pesticides, plasma chemistries, polychlorinated biphenyls, reptile, white blood cell counts, wildlife. *Environ Health Perspect* 112:1074–1079 (2004). doi:10.1289/ehp.6923 available via *http://dx.doi.org/* [Online 21 April 2004]

It has been well established that organochlorine (OC) compounds, including polychlorinated biphenyls (PCBs) and OC pesticides, bioaccumulate in animal tissues and cause hepatotoxicity, wasting, immunotoxicity, developmental abnormalities, and reproductive toxicity along with endocrine disruption, neurobehavioral effects, and population declines (Fox 2001; Safe 1993). OC compounds have been detected in tissues of threatened and endangered sea turtles [reviewed by Pugh and Becker (2001)], and recently, we measured OC concentrations in the tissues of live, juvenile loggerhead sea turtles (*Caretta caretta*) from North Carolina (Keller et al. 2004). PCB concentrations in these loggerhead turtles were similar to those of alligators from Florida lakes (Guillette et al. 1999) and of the general human population of North America (Feeley 1995) but were much lower than those found in snapping turtles, Caspian terns, and bottlenose dolphins (de Solla et al. 1998; Grasman and Fox 2001; Lahvis et al. 1995). Although concentrations of organic contaminants have been assessed in sea turtles, health effects from these exposures remain undocumented.

In both the hospital and the veterinary clinic, assessment of clinical health parameters provides a first line of patient health evaluation. Clinical health assessments typically include a physical examination and measurements of hematology and clinical blood chemistry values. Common parameters include total and differential counts of blood cells, activities of plasma enzymes, and concentrations of plasma proteins, glucose, and electrolytes. OC contaminants have been shown to alter hematology and blood chemistry values in laboratory-exposed animals and environmentally exposed humans and wildlife (Grasman et al. 2000b; Lawton et al. 1985; McConnell 1985). For example, in humans, an elevation in blood aspartate aminotransferase (AST) activity is a sensitive indicator of liver damage due to PCB exposure (Feeley 1995). Likewise, incidences of liver necrosis and blood AST activity were both increased in rats and American kestrels exposed to PCBs (Bruckner et al. 1973; Hoffman et al. 1996).

Potential immunotoxicity of OCs has been illustrated by changes in white blood cell (WBC) counts in both laboratory-exposed animals such as mice and seals (de Swart et al. 1995; Segre et al. 2002), as well as environmentally exposed wildlife and humans (Grasman et al. 1996; Lawton et al. 1985). An increase in the heterophil:lymphocyte ratio has also been shown to be an indicator of general stress in chickens (Gross and Seigel 1983) and of disease in sea turtles (Aguirre et al. 1995; Cray et al. 2001; Work et al. 2001). Moreover, increases in this ratio correlate with dioxin toxicity equivalents (TEQs) in juvenile Caspian terns and herring gulls (Grasman et al. 1996, 2000b).

General health assessments have been performed on some select populations of sea turtles (George 1997), and values for WBC counts and clinical chemistry parameters have been reported for loggerhead sea turtles along the East Coast of the United States (Bolten et al. 1992; George 1997; Lutz and Dunbar-Cooper 1987). Seasonal changes have been observed in some parameters, such as osmotic pressure and urea, but other parameters remain relatively constant throughout the year, including glucose and hematocrit (HCT) (Bolten et al. 1992). Moreover, Harms et al. (2002) followed health parameters of injured and sick loggerhead turtles as

Address correspondence to J.M. Keller, National Institute of Standards and Technology, Hollings Marine Laboratory, 331 Fort Johnson Rd., Charleston, SC 29412 USA. Telephone: (843) 762-8863. Fax: (843) 762-8742. E-mail: jennifer.keller@noaa.gov

*Current address: Disney's Epcot The Living Seas, Lake Buena Vista, Florida, USA.

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they recovered in a rehabilitation facility and found that indicators of nutrition increased, including HCT, blood urea nitrogen (BUN), and total protein. Although no studies have assessed the effects of OC contaminants on clinical health parameters in sea turtles, the effects of OC contaminants on hematologic and blood chemistry values have been investigated in one study using snapping turtles from three sites (Albers et al. 1986). Site differences were observed in OC concentrations, but no differences in blood chemistry parameters were seen that would indicate contaminant-induced physiologic impairment.

Although Albers et al. (1986) found no effects in snapping turtles, OC contaminants may affect sea turtles differently because sensitivity to contaminants can vary profoundly from one species to another. For example, the dose that kills 50% of test animals (LD_{50}) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) ranges over four orders of magnitude among six species of mammals commonly used in laboratory experiments (McConnell 1985), and sensitivity differences are expected to be even greater among wildlife species (Smith and Hall 1994). For this reason, it is important to examine the effects of OCs on sea turtles.

Because clinical measurements in other species have been shown to be altered by OC exposure, we hypothesized that they may also be modulated in loggerhead sea turtles. Because these measurements are relatively noninvasive, requiring only a blood sample, they offer a simple, nonlethal method to assess health. If shown to be affected by OC contaminants, these clinical measurements would offer a simple biomonitoring tool for risk analysis. Furthermore, all species of sea turtles are threatened or endangered, and OC contaminants may have contributed to their past and current population declines. Therefore, this study sought to determine whether associations exist between indicators of health and OC concentrations in the threatened juvenile loggerhead sea turtle.

Materials and Methods

Turtles. Forty-eight live, free-ranging, juvenile loggerhead sea turtles with straight carapace lengths (SCLs) between 46 cm and 77 cm were collected as bycatch from a pound-net fishery located in Core Sound, North Carolina (between the northernmost site, 34° 52.71' N, 76° 18.94' W, and the southernmost site, 34° 49.68' N, 76° 22.95' W) during two summer sampling periods (31 July–11 August 2000, and 13–20 July 2001). Water temperatures during these captures ranged from 24.0°C to 28.2°C. Blood samples were collected from all turtles, and biopsies of subcutaneous fat were collected from 44 of the turtles as described elsewhere (Keller et al. 2004). The sex of 42 turtles was determined definitively by laparoscopy. The sex of the remaining 6 turtles was confidently determined by plasma testosterone concentrations (Braun-McNeill et al., in press).

Contaminant analysis. PCB and OC pesticide concentrations and lipid content were previously determined in the whole blood samples and fat biopsies of 44 of the turtles captured in the summers of 2000 and 2001 (Keller et al. 2004). Whole blood samples from an additional 4 turtles captured in July 2001 were analyzed using identical methods, which have been described in detail by Keller et al. (2004). Briefly, blood was extracted by liquid:liquid extraction, and fat samples were extracted using pressurized fluid extraction. Lipids were determined gravimetrically and then removed from the extracts by alumina columns for blood and gel permeation chromatography for fat. Each extract was separated into two fractions using an aminopropylsilane column (fraction 1 contained PCBs, hexachlorobenzene, 4,4′-DDE, 2,4′-DDE, and mirex; fraction 2 contained mainly pesticides). Both fractions of fat extracts and fraction 1 of blood extracts were analyzed on a gas chromatograph with dual microelectron capture detectors. Fraction 2 of blood extracts was analyzed on a gas chromatograph mass spectrometer operating in electron-impact

Table 1. Morphometrics, hematology, and plasma chemistries for juvenile loggerhead sea turtles captured in the summers of 2000 and 2001 from Core Sound, North Carolina.

Parameter	No.	Mean ± SD	Range	Measurements from previous studies[a]
General				
SCL (cm)	48	62.0 ± 7.0	45.7–77.3	
Weight (kg)	45	35.3 ± 10.4	14.4–56.6	
Body condition	45	14.8 ± 1.5	11.4–20.9	
Sex ratio (F:M)	48	34:14		
Hematology				
RBCs (10^6/μL)	14	0.410 ± 0.098	0.275–0.615	
HGB (g/dL)	14	9.82 ± 1.46	7–12	
HCT (%)	14	31.5 ± 4.3	23–38	Range, 29–35.5
Total WBCs[b]	14	14.8 ± 4.0	5.8–20.72	
Estimated WBCs (10^3/μL)[b]	28	13.3 ± 5.3	7.0–25.5[c]	Range, 11.0–11.2
Heterophils[b]	13	4.3 ± 2.5	1.3–8.2	Median, 4.780
Lymphocytes[b]	13	9.5 ± 2.5	4.6–15.0	Median, 5.175
Monocytes[b]	13	ND	ND	Median, 0.390
Eosinophils[b]	13	1.1 ± 0.9	0.14–2.7	
Azurophils[b]	13	0.8 ± 0.4	0.17–1.5	
Basophils[b]	13	0.03 ± 0.11	0–0.38	
Heterophils:Lymphocytes[b]	13	0.5 ± 0.4	0.1–1.4	
Carbohydrate and protein homeostasis				
Glucose (mg/dL)	40	109 ± 18	76–143	Range, 79.6–107
Protein (g/dL)	40	4.0 ± 0.8	2.4–5.9	Range, 3–4.5
Albumin (g/dL)	40	1.1 ± 0.2	< 1.0–1.5	Range, 0.6–1.8
Globulin (g/dL)	40	2.9 ± 0.7	1.5–4.5	Range, 2.8–3.2
Albumin:globulin	40	0.40 ± 0.08	0.23–0.60	
BUN (mg/dL)	40	101 ± 40	25–197	Range, 49–122
Uric acid (mg/dL)	40	0.8 ± 0.7	0.3–3.4	Range, 0.7–2.1
Creatinine (mg/dL)	40	< 0.1	< 0.1–0.1	Mean, 0.2
Bilirubin (mg/dL)	40	< 0.1	< 0.1–0.2	Mean, 0.04
Enzymes				
AST (U/L)	40	229 ± 59	128–355	Range, 178–285
ALP (U/L)	40	23 ± 14	9–74	Range, 14–53
LDH (U/L)	40	182 ± 102	60–465	Range, 128–310
CPK (U/L)	26	1,243 ± 1,167	281–5,667	Range, 853–1,680
GGT (U/L)	40	< 3	< 3–4	
Ions				
Calcium (mg/dL)	40	8.4 ± 1.2	5.5–11.4	Range, 1.08–7.7
Phosphorous (mg/dL)	40	7.0 ± 1.0	5.2–9.1	Range, 5.9–8.6
Calcium:phosphorous	40	1.2 ± 0.2	0.7–2.0	Median, 0.76
Sodium (mmol/L)	40	158 ± 2	154–164	Range, 143.1–162
Potassium (mmol/L)	40	4.5 ± 0.5	3.1–5.6	Range, 3.6–5.8
Sodium:potassium	40	35.6 ± 3.7	28.2–50	
Chloride (mmol/L)	40	117 ± 4	110–125	Range, 112–117.6
Magnesium (mg/dL)	40	5.3 ± 0.7	3.9–6.7	Range, 2.38–4.0
Anion gap	40	16.1 ± 5.5	6.2–30.2	

Abbreviations: ALP, alkaline phosphatase; CPK, creatine phosphokinase; F, female; GGT, gamma glutamyl transferase; HGB, hemoglobin; LDH, lactate dehydrogenase; M, male; ND, none detected; RBCs, red blood cells.
[a]Range of means or medians compiled from Bolten et al. (1992; Na heparin data only from autoanalyzer A); George (1997); Harms et al. (2002; medians from turtles that had been in rehabilitation; these data do not include the initial values upon admission); Lutz and Dunbar-Cooper (1987; turtles captured in July 1980 only); and A. Segars et al. (unpublished data). [b]Total WBC counts in year 2000 were performed using Natt-Herrick solution; estimated WBC and differential counts were performed using blood smears (units = 10^3/μL blood). [c]Two turtles were not included in the range because their WBC counts were undetectable; either the counts were too low or the blood smears were too difficult to read.

mode and using selected ion monitoring. Total (Σ) TCDD-like PCB concentrations were calculated by adding the concentrations of four PCB congeners [PCBs 105, 118, 156, and 157; International Union of Pure and Applied Chemistry (IUPAC) numbers] that were measured from the 12 congeners identified by Ahlborg et al. (1994) as having dioxin-like activity.

Health assessment. Turtles were examined for external injuries and obvious signs of illness (i.e., emaciation, lethargy). Body condition was calculated as turtle mass (kilograms) divided by the cubed SCL (centimeters) and multiplied by 100,000 (kilograms per cubic centimeter × 100,000) (Bjorndal et al. 2000). Blood samples for hematology and plasma chemistry were collected in sodium heparin tubes (Monoject, Sherwood Medical, St. Louis, MO; or Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ) within 15 min of capture, were kept cool on ice or in a refrigerator, and were processed within 6 hr of blood collection. To maximize consistency, hematology was performed by a single technician familiar with sea turtle hematology, and a single reference laboratory at the College of Veterinary Medicine at North Carolina State University was used for plasma chemistries. Table 1 lists the parameters measured.

Hematology. Hematologic examination was performed on 14 of the 21 turtles captured in the summer of 2000. Natt-Herrick solution and Neubauer counting chambers (American Optical Corp., Buffalo, NY) were used to obtain total WBC counts and red blood cell (RBC) counts on all 14 turtles. At a magnification of 40×, all nine squares of the chamber were counted for WBCs and five small squares of the center large squares were counted for the RBCs. Differential counts were performed on 13 of the turtles using Wright-Geimsa–stained thin blood smears. Heterophils, lymphocytes, monocytes, eosinophils, azurophils, and basophils were differentiated out of 100 cells counted. Estimated total WBC counts were performed using blood smears from 8 of the turtles captured in 2000 and from 20 of the turtles captured in 2001. The total count of leukocytes from 10 fields at a magnification of 40× was divided by 10 and multiplied by 1,700.

HCT and hemoglobin (HGB) concentrations were determined on 14 of the turtles from 2000. HCTs were obtained by measuring packed cell percentage through the use of microhematocrit capillary tubes (Fisherbrand, Houston, TX). Tubes were spun for 5 min in a centrifuge (Clay Adams Readacrit; Becton, Dickinson and Company, Parsippany, NJ). HCTs were read on a Critocaps Micro-Hematocrit Capillary Tube Reader (Oxford Labware, St. Louis, MO). HGB was measured by colorimetric analysis with the use of hemolysis sticks and a BMS handheld hemoglobinometer (Omron Healthcare Inc., Vernon Hills, IL).

Plasma chemistry. Plasma chemistry values were determined for 14 of the 21 turtles captured in 2000 and 26 of the 27 turtles captured in 2001. Plasma was stored at –70°C or colder until analysis was completed within 10 days. We used automated bichromatic spectrophotometry (Roche/Hitachi 912 Clinical Chemistry System, Roche Diagnostics, Indianapolis, IN) to measure glucose, total protein, albumin, BUN, uric acid, AST, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), calcium, phosphorus, magnesium, creatinine, bilirubin, alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT). An ion-selective electrode on the Roche/Hitachi 912 analyzer measured sodium, potassium, and chloride. Globulin concentrations were determined by subtracting albumin from total protein.

Statistics. The OC concentrations were not normally distributed even after log transformation; therefore we used nonparametric tests (Systat 8.0 software; SPSS, Inc., Chicago, IL). Each health assessment parameter was compared with lipid-normalized contaminant concentrations using the Spearman rank correlation test.

Results

All but one turtle captured in the summers of 2000 and 2001 appeared healthy upon initial external exam. This debilitated turtle (turtle 1328) was extremely emaciated and lethargic. Its neck, shoulder, and inguinal regions showed profound signs of emaciation. All turtles, except for turtle 1328, were active and swimming normally. Only minor and common external wounds, such as bruising and scute erosions, were observed, with the exception of one animal that had a major puncture wound to the throat. One apparently healthy turtle died after the laparoscopic procedure, and subsequent histopathologic examination showed extensive parasitic spirorchid trematode egg mass granulomas in its brain, thyroid, and adrenals.

The morphometric and health assessment data are presented in Table 1. The mean values obtained in the present study were very similar to means or medians previously reported for loggerhead turtles along the southeast coast of the United States (Table 1). These comparisons suggest that the health parameters of these free-ranging loggerhead turtles from North Carolina are generally within ranges typically observed.

Contaminant concentrations detected in the blood samples and fat biopsies from the 44 turtles have been reported elsewhere on a lipid basis (Keller et al. 2004). In that study, the concentrations in the two tissues were significantly correlated with each other, and no differences were observed between males and females. The concentrations on a wet mass basis are shown in Table 2 for Σchlordanes, dieldrin, mirex, ΣDDTs, ΣPCBs, ΣTCDD-like PCBs, and ΣOCs in the 44 fat biopsies and 48 blood samples. Almost all of the contaminants measured in the turtle tissues were intercorrelated. For example, adipose concentrations of ΣPCBs were significantly correlated with adipose concentrations of ΣDDTs [Spearman rank correlation coefficient (r_s) = 0.679], with oxychlordane (r_s = 0.720), and with mirex (r_s = 0.710) concentrations (all p-values < 0.05). These intercorrelations of complex mixtures make it difficult to discern which compound may be responsible for possible health effects.

We observed several significant correlations between contaminant concentrations and indicators of poor or altered health. The Spearman rank correlation coefficients are presented for only those health indicators that significantly correlated with concentrations of at least one contaminant class (Table 3). No contaminant concentration was correlated with total WBCs counted by the Natt-Herrick method, possibly due to the small sample size (n = 14). However, all of the major groups of contaminants, including Σchlordanes, mirex, ΣDDTs, ΣPCBs, ΣTCDD-like PCBs, and ΣOCs, were correlated with the total WBC counts that were estimated by blood smears (Figure 1A). Increasing blood concentrations of Σchlordanes and mirex were significantly correlated with fewer lymphocytes. ΣDDTs and

Table 2. Concentrations on a wet mass basis of OC contaminants in 44 fat biopsies and 48 blood samples from juvenile loggerhead sea turtles captured in the summers of 2000 and 2001 from Core Sound, North Carolina.

	Adipose (ng/g wet mass)		Blood sample (pg/g wet mass)	
Contaminant	Mean ± SD	Range	Mean ± SD	Range
ΣChlordanes	26.9 ± 21.3	< LOD–87.8	225 ± 201	< LOD–988
Dieldrin	4.89 ± 4.06	< LOD–16.7	60.8 ± 141	< LOD–952
Mirex	4.41 ± 4.17	< LOD–18.8	44.5 ± 70.8	< LOD–296
ΣDDTs	67.0 ± 68.7	< LOD–287	649 ± 685	< LOD–3,800
ΣPCBs	256 ± 269	7.99–1,360	5,560 ± 5,280	121–23,900
ΣTCDD-like PCBs	29.4 ± 30.6	1.32–169	395 ± 426	< LOD–2,010
ΣOCs	366 ± 353	9.38–1,680	6,550 ± 6,140	168–29,100

LOD, limit of detection (1 ng/g wet mass for adipose; 10 pg/g wet mass for blood). ΣChlordanes, sum of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane; ΣDDTs, sum of 4,4´-DDE, 4,4´-DDD, and 2,4´-DDT. ΣPCBs, sum of PCB congeners 8, 18, 28, 49, 52, 56, 63, 66, 70 and 76, 74, 87 and 81, 92, 95, 99, 101 and 90, 104, 105, 107, 110, 118, 128, 138, 146, 149, 151 and 82, 153, 154, 156, 157, 158, 163, 170, 174, 180, 183, 187, 193, 194, 195, 201, 206, and 209 (IUPAC numbers); ΣTCDD-like PCBs, sum of four PCB congeners (105, 118, 156, and 157) that were measured in this study and that were classified by Ahlborg et al. (1994) in a group of 12 congeners as having dioxin-like activity; ΣOCs, sum of all classes of OC contaminants.

ΣOCs in both blood and adipose and ΣPCBs in adipose positively correlated with eosinophils. Increasing adipose concentrations of mirex and ΣTCDD-like PCBs correlated with an elevation in the heterophil:lymphocyte ratio (Figure 1B). Blood concentrations of certain OC pesticides and ΣTCDD-like PCBs were negatively correlated with RBC counts, HCT, and HGB.

Indicators of nutritional status and homeostasis of proteins and glucose were significantly correlated with certain contaminants (Table 3). Body condition was negatively correlated with dieldrin in the blood. Glucose concentrations were negatively correlated with adipose concentrations of dieldrin and ΣDDTs. The ratio of albumin to globulin was negatively correlated with concentrations of Σchlordanes in blood and adipose and ΣTCDD-like PCBs in blood. BUN concentrations were positively correlated with concentrations of most OC classes measured in blood (Figure 1C).

Activities of three enzymes correlated with OC concentrations (Table 3). AST activity was positively correlated with most of the OC compounds in the blood and adipose (Figure 1D). ALP activity was negatively correlated with mirex concentrations in blood and adipose and with ΣTCDD-like PCBs in blood. GGT activity was negatively correlated with blood concentrations of dieldrin.

We noted few significant correlations between electrolyte levels and contaminant concentrations (Table 3). However, sodium concentrations were positively correlated with blood concentrations of mirex and ΣTCDD-like PCBs. Magnesium concentrations were negatively correlated with ΣDDT, ΣPCB, ΣTCDD-like PCBs, and ΣOC concentrations in the blood.

Discussion

In this study we sought to determine whether associations exist between OC concentrations and noninvasive indicators of health in loggerhead sea turtles. Several significant correlations were in fact observed. Preliminary data from an ongoing and parallel study corroborate these findings (Peden-Adams et al. 2002): in juvenile loggerhead sea turtles captured in offshore waters of South Carolina, Georgia, and Florida, significant correlations were observed between ΣPCB concentrations and increased BUN concentrations and a decreased albumin:globulin ratio. Similar correlations were seen in the present study, although not necessarily with ΣPCB concentrations.

The correlations observed in the present study are supported by a large number of previous field studies as well as experimental laboratory studies in a variety of species. For example, in loggerhead turtles, we observed positive correlations between OC concentrations and WBC counts, as well as the ratio of heterophils to lymphocytes. It has been well established that OCs affect immune cells and immune function in laboratory-exposed animals (Bruckner et al. 1973; Hoffman et al. 1996; Segre et al. 2002; Smits et al. 2002). Furthermore, associations have been documented between OC concentrations, such as TEQs, ΣPCBs, and DDE, and an elevation in the heterophil:lymphocyte ratio in juvenile herring gulls (Grasman et al. 2000b) and Caspian terns from the Great Lakes (Grasman et al. 1996). Male American kestrels experimentally exposed to PCBs exhibited increased WBC counts (Smits et al. 2002). The findings from these previous studies are similar to the correlations observed in the loggerhead sea turtles. Additional evidence of immune modulation is provided by significant positive correlations between mitogen-induced lymphocyte proliferation responses and OC concentrations in these same loggerhead turtles (Keller et al. 2002). Therefore, it seems rational that the correlations we observed in the present study may indicate modulation of the loggerhead immune system by OC contaminants.

Indicators of anemia, such as decreased RBC counts, HCT, and HGB concentrations, correlated with dieldrin and Σchlordanes measured in the loggerhead blood. Previous studies have shown that OC contaminants can decrease these parameters. For example, rats and monkeys exposed to PCBs exhibited decreased RBC counts, HGB, and HCT (Arnold et al. 1993; Bruckner et al. 1973; Chu et al. 1994). Blood concentrations of PCBs in capacitor workers correlated with decreased RBC counts (Lawton et al. 1985). Likewise, TEQs and DDE concentrations in adult herring gulls from the Great Lakes were also negatively correlated with HCT (Grasman et al. 2000b). These findings suggest that OC contaminants may lead to anemia in sea turtles.

The kidneys are a well-known target for the toxic effects of PCBs, and several blood chemistry parameters, such as BUN and electrolytes, can indicate kidney dysfunction (McConnell 1985). Increased BUN concentrations, at least in mammals, suggest that the kidneys are not properly removing this nitrogenous waste product from the blood. Increased BUN concentrations have been observed in capacitor workers (Lawton et al. 1985) and cynomolgus monkeys exposed to Aroclor 1254 (Arnold et al. 1990). In turtles, however, BUN is a poor indicator of renal disease (Campbell 1996) and probably

Table 3. Spearman rank correlation coefficients between OC concentrations and health assessment data in juvenile loggerhead sea turtles captured in the summers of 2000 and 2001 from Core Sound, North Carolina.

	Sample size		ΣChlordanes		Dieldrin		Mirex		ΣDDTs		ΣPCBs		ΣTCDD-like PCBs		ΣOCs	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Estimated WBC	25	28	0.546*	0.403*	0.342	0.047	0.472*	0.397*	0.450*	0.411*	0.551*	0.418*	0.548	0.514	0.555*	0.453*
Lymphocytes	13	13	–0.214	–0.577*	–0.148	–0.192	–0.379	–0.588*	–0.088	–0.237	–0.231	–0.418	–0.302	–0.538	–0.181	–0.451
Eosinophils	13	13	0.308	0.511	0.423	0.088	0.247	0.324	0.626*	0.707*	0.599*	0.397	0.527	0.473	0.610*	0.599*
Azurophils	13	13	0.374	0.192	0.286	0.582*	0.390	–0.121	–0.104	0.138	–0.110	–0.207	0.082	–0.066	–0.110	–0.269
H:L ratio	13	13	0.421	0.269	0.269	0.382	0.606*	0.112	0.443	0.160	0.511	0.250	0.615*	0.225	0.478	0.220
RBC	14	14	–0.262	–0.611*	–0.027	–0.529	–0.289	–0.464	–0.039	–0.165	–0.002	–0.484	–0.158	–0.594*	0.004	–0.491
Hematocrit	14	14	–0.348	–0.706*	–0.192	–0.577*	–0.387	–0.412	0.014	–0.027	–0.049	–0.332	–0.181	–0.374	–0.046	–0.321
Hemoglobin	14	14	–0.317	–0.760*	–0.210	–0.630*	–0.373	–0.427	–0.125	–0.113	–0.177	–0.434	–0.286	–0.421	–0.188	–0.451
Body condition	44	45	–0.084	–0.244	–0.066	–0.310*	–0.151	–0.114	–0.025	–0.040	–0.049	–0.077	–0.105	–0.101	–0.072	–0.093
Glucose	36	39	–0.277	–0.202	–0.352*	–0.304	–0.246	–0.092	–0.475*	–0.246	–0.294	–0.161	–0.281	–0.145	–0.318	–0.177
Albumin	37	40	–0.365*	–0.210	–0.208	–0.097	–0.313	–0.315*	–0.207	–0.183	–0.269	–0.064	–0.283	–0.152	–0.295	–0.115
Albumin:globulin	37	40	–0.332*	–0.339*	–0.179	–0.170	–0.280	–0.268	–0.071	–0.182	–0.217	–0.296	–0.318	–0.370*	–0.204	–0.290
BUN	37	40	0.092	0.338*	0.091	0.055	0.073	0.242	0.229	0.463*	0.118	0.377*	0.100	0.387*	0.145	0.409*
AST	37	40	0.580*	0.604*	0.349*	0.087	0.477*	0.613*	0.261	0.456*	0.399*	0.592*	0.438*	0.597*	0.398*	0.581*
ALP	37	40	–0.325	–0.291	–0.157	0.118	–0.369*	–0.435*	–0.059	–0.158	–0.296	–0.287	–0.318	–0.342*	–0.279	–0.297
GGT	37	40	–0.019	0.086	–0.067	–0.341*	0.142	0.082	–0.216	0.090	–0.086	0.023	–0.031	0.104	–0.103	0.021
Sodium	37	40	0.241	0.296	0.138	0.130	0.183	0.323*	0.123	0.201	0.132	0.294	0.142	0.348*	0.135	0.287
Magnesium	37	40	–0.022	–0.251	–0.096	–0.248	–0.125	–0.311	–0.257	–0.344*	–0.230	–0.476*	–0.176	–0.398*	–0.224	–0.445*

Abbreviations: A, adipose; B, blood; H:L, heterophil:lymphocyte. ΣChlordanes, sum of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane; ΣDDTs, sum of 4,4´-DDE, 4,4´-DDD, and 2,4´-DDT; ΣPCBs, sum of PCB congeners 8, 18, 28, 49, 52, 56, 63, 66, 70 and 76, 74, 87 and 81, 92, 95, 99, 101 and 90, 104, 105, 107, 110, 118, 128, 138, 146, 149, 151 and 82, 153, 154, 156, 157, 158, 163, 170, 174, 180, 183, 187, 193, 194, 195, 201, 206, and 209 (IUPAC numbers); ΣTCDD-like PCBs, sum of four PCB congeners (105, 118, 156, and 157) that were measured in this study and that were classified by Ahlborg et al. (1994) in a group of 12 congeners as having dioxin-like activity; ΣOCs, sum of all classes of OC contaminants.
*Significant correlation; $p < 0.05$.

better represents nutritional status and protein metabolism. For example, BUN concentrations increased from a median of 50 mg/dL to 122 mg/dL during rehabilitation of injured or ill loggerhead sea turtles (Harms et al. 2002). The positive correlation between BUN and blood OC concentrations in the loggerhead turtles may suggest that turtles with higher BUN concentrations have been feeding recently and may have higher levels of certain blood lipids that can transport lipophilic contaminants. The fact that BUN was correlated strongly with OCs in blood rather than in adipose tissue further supports this conclusion. Future studies are needed to investigate the relationships between BUN, protein metabolism, blood lipids, and OC contaminants in sea turtles.

The kidney is also responsible for ion regulation. Correlations were seen in the study between OC contaminants and increased Na and decreased Mg concentrations. In fish, chlordane exposure has been shown to increase Na and Mg concentrations (Bansel et al. 1979). Capacitor workers exposed to PCBs similarly exhibited blood osmolality values above the normal range (Lawton et al. 1985). Electrolyte balance in sea turtles is regulated not only by the kidney but also by the salt gland. The loggerhead salt gland concentrates Na 8-fold and Mg 45-fold above the concentrations in plasma and excretes the resulting fluid through ducts near the eye (Vargo et al. 1986). If the kidneys or the salt glands of sea turtles are sensitive to OCs, as has been shown in kidneys of other species (McConnell 1985), then these correlations could suggest that OCs are affecting these organs, thereby altering ion regulation in sea turtles.

The concentration of blood glucose is clearly related to nutritional status in loggerhead sea turtles (Lutcavage et al. 1995). Plasma glucose is tightly regulated by the liver and its complex interactions with the hypothalamus, pituitary, and adrenal glands. It is therefore possible that OCs may interfere with glucose regulation at multiple control points. In the present study, we observed negative correlations between glucose and adipose concentrations of dieldrin and ΣDDTs in the loggerhead turtles. OC exposure has resulted in decreased glucose concentrations in other vertebrate species, including PCB and mirex exposure in rats (Boll et al. 1998; Chu et al. 1994; Rogers et al. 1984) and chlordane exposure in mice (Khasawinah and Grutsch 1989), suggesting that OCs may be affecting glucose regulation in loggerhead turtles.

OC contaminants are also known to alter the activity of metabolic enzymes in the liver, such as phosphoenolpyruvate carboxykinase and malic enzyme, that are responsible for protein, glucose, and lipid regulation (Boll et al. 1998; Lorenzen et al. 1999), thereby altering blood concentrations of protein and glucose (McConnell 1985). In the present study, turtles with higher concentrations of OCs exhibited a decreased ratio of albumin to globulin. This response was previously observed in fish exposed to Aroclor 1254 (Camp et al. 1974). In addition, changes in these protein classes were correlated with PCB and DDE concentrations in Caspian tern and herring gull chicks from the Great Lakes (Grasman et al. 2000a).

Blood enzyme activities are useful as early warning monitors of subacute effects of contaminants on particular organs in birds and mammals (Arnold et al. 1990; Dieter et al. 1976; Feeley 1995). In the present study, AST activity was elevated and ALP activity was decreased in the loggerhead sea turtles with higher concentrations of certain OCs. Increased AST is commonly used as an indicator of hepatocellular damage in birds and mammals exposed to OCs (Arnold et al. 1990; Bruckner et al. 1973; Dieter et al. 1976; Feeley 1995). In fact, American kestrels exhibited an increase in AST activity and a decrease in ALP activity after PCB exposure (Hoffman et al. 1996), a response consistent with the correlations seen in the present study in loggerhead turtles.

It is difficult to interpret the observed correlations between OC levels and plasma enzyme activities because no previous study has determined the distribution of these enzymes among organs of sea turtles. In reptiles, the distribution of these enzymes has been assessed only in two species, the yellow rat snake (Ramsay and Dotson 1995) and the green iguana (Wagner and Wetzel 1999). AST was a major enzyme found in the snake liver, but it was also found at high concentrations in the kidney and heart. Moderate AST activity was found in all tissues examined in the iguana; therefore, the authors concluded that an increase in blood AST would not reflect damage to a specific tissue in this species (Wagner and Wetzel 1999). Based on the preponderance of experimental evidence showing that OCs produce liver damage and subsequently increase plasma AST in mammals and birds, it is plausible that the strong correlations between AST and OCs are indicative of hepatocellular damage in sea turtles. This interpretation is further supported by the lack of correlations between OC concentrations and CPK activity, an enzyme of presumed muscular origin. Hepatocellular damage is expected to result in an increase in AST but not in CPK activity (Campbell 1996). Yet, future studies should examine the distribution of these enzymes among organs of sea turtles before this interpretation could be considered conclusive.

The associations observed between OC concentrations and indicators of health in the loggerhead turtles suggest that their health is affected by these contaminants. However, it is important to note that most of the measured health indicators, even in turtles with the highest exposure, did not fall outside ranges reported previously for this species (Table 1). From this, one might conclude that the correlations are not predictive of an overt adverse effect. However, the ranges reported by past studies have examined free-ranging turtles from similar locations that had undoubtedly been exposed to ubiquitous OC contaminants. In order to define the true reference ranges for health indicators, a control population free from contaminant exposure would have to be assessed. Additionally, it is possible that adverse

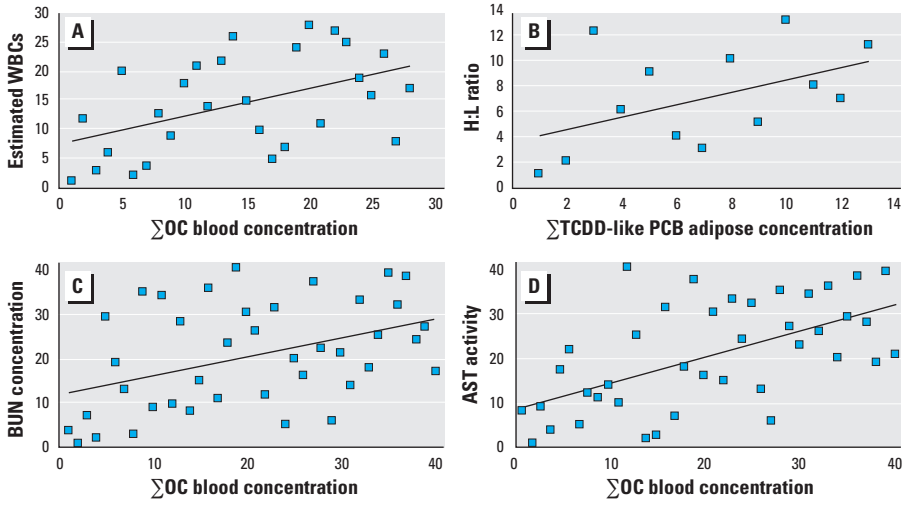

Figure 1. Selected scatter plots of ranked data depicting significant Spearman rank correlations between health indicators and OC contaminants in juvenile loggerhead sea turtles captured in the summers of 2000 and 2001 from Core Sound, North Carolina. (*A*) Ranked blood concentration of ΣOCs versus ranked total WBC count estimated from blood smears ($r_s = 0.453$; $p < 0.02$). (*B*) Ranked concentration of ΣTCDD-like PCBs measured in fat biopsies (PCB congeners 105, 118, 156, and 157) versus ranked heterophil:lymphocyte ratio ($r_s = 0.615$; $p < 0.05$). (*C*) Ranked blood concentration of ΣOCs versus ranked BUN concentration ($r_s = 0.409$; $p < 0.01$). (*D*) Ranked blood concentration of ΣOCs versus ranked AST activity ($r_s = 0.581$; $p < 0.001$).

health effects in an individual animal could occur even when its health indicators fall within the population reference range. Sea turtle physiology may be adapted to maintain homeostasis, so measurable health indicators may not change appreciably even with poor health. Moreover, multiple minor alterations could result in cumulative health impacts. The fact that significant correlations were noted even though sea turtles have OC concentrations much lower than those found in other wildlife suggests that sea turtles may be more sensitive to the health impacts of these contaminants than previously thought.

Conclusion

This study provides the first evidence, although strictly correlative, that OC contaminants may be affecting sea turtle health. Although the concentrations of OCs are relatively low compared with other species, we observed significant correlations between OC levels and health indicators for a wide variety of biologic functions, including immunity and homeostasis of proteins, carbohydrates, and ions. Studies using experimentally and environmentally exposed animals support these correlative findings, but further studies are required to determine the precise causal relationships between OC contaminants and health effects in sea turtles. Additional populations, such as those exposed to higher levels of OCs, and more sensitive life stages (i.e., embryo) should also be investigated because they may face a greater risk than juvenile turtles foraging in North Carolina waters.

References

Aguirre AA, Balazs GH, Spraker TR, Gross TS. 1995. Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. Physiol Zool 68:831–854.

Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, et al. 1994. Toxic equivalency factors for dioxin-like PCBs: report on WHO-ECEH and IPCS consultation, December 1993. Chemosphere 28:1049–1067.

Albers PH, Sileo L, Mulhern BM. 1986. Effects of environmental contaminants on snapping turtles of a tidal wetland. Arch Environ Contam Toxicol 15:39–49.

Arnold DL, Bryce F, Karpinski K, Mes J, Fernie S, Tryphonas H, et al. 1993. Toxicological consequences of Aroclor 1254 ingestion by female rhesus (*Macaca mulatta*) monkeys. Part 1B. Prebreeding phase: clinical and analytical laboratory findings. Food Chem Toxicol 31:811–824.

Arnold DL, Mes J, Bryce F, Karpinski K, Bickis MG, Zawidzka ZZ, et al. 1990. A pilot study on the effects of Aroclor 1254 ingestion by rhesus and cynomolgus monkeys as a model for human ingestion of PCBs. Food Chem Toxicol 28:847–857.

Bansel SK, Verma SR, Gupta AK, Dalela RC. 1979. Physiological dysfunction of the haemopoietic system in a fresh water teleost, *Labeo rohita*, following chronic chlordane exposure. Part II—Alterations in certain organic components and serum electrolytes. Bull Environ Contam Toxicol 22:674–680.

Bjorndal KA, Bolten AB, Chaloupka MY. 2000. Green turtle somatic growth model: evidence for density dependence. Ecol Appl 10:269–282.

Boll M, Weber LWD, Messner B, Stampfl A. 1998. Polychlorinated biphenyls affect the activities of gluconeogenic and lipogenic enzymes in rat liver: is there an interference with regulatory hormone actions? Xenobiotica 28:479–492.

Bolten AB, Jacobson ER, Bjorndal KA. 1992. Effects of anticoagulant and autoanalyzer on blood biochemical values of loggerhead sea turtles (*Caretta caretta*). Am J Vet Res 53:2224–2227.

Braun-McNeill J, Epperly SP, Owens DW, Patterson RW, Evich LT. In press. Predicting sex ratios of benthic immature sea turtles—does temperature make a difference? In: Proceedings of the Twenty-first Annual Symposium on Sea Turtle Biology and Conservation (Coyne M, compiler), 24–28 February 2001, Philadelphia, PA. NOAA Technical Memorandum. Miami:U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Southeast Fisheries Science Center of the National Marine Fisheries Service.

Bruckner JV, Khanna KL, Cornish HH. 1973. Biological responses of the rat to polychlorinated biphenyls. Toxicol Appl Pharmacol 24:434–448.

Camp BJ, Hejtmancik E, Armour C, Lewis DH. 1974. Acute effects of Aroclor 1254 (PCB) on *Ictalurus punctatus* (catfish). Bull Environ Contam Toxicol 12:204–208.

Campbell TW. 1996. Clinical pathology. In: Reptile Medicine and Surgery (Mader DR, ed). Philadelphia:W.B. Saunders Company, 248–257.

Chu I, Villeneuve DC, Yagminas A, LeCavalier P, Poon R, Feeley M, et al. 1994. Subchronic toxicity of 3,3´,4,4´,5-pentachlorobiphenyl in the rat. I. Clinical, biochemical, hematological, and histopathological changes. Fundam Appl Toxicol 22:457–468.

Cray C, Varella R, Bossart GD, Lutz P. 2001. Altered *in vitro* immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. J Zoo Wildl Med 32:436–440.

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.

de Swart RL, Ross PS, Timmerman HH, Vos HW, Reijnders PJH, Vos JG, et al. 1995. Impaired cellular immune response in harbour seals (*Phoca vitulina*) feeding on environmentally contaminated herring. Clin Exp Immunol 101:480–486.

Dieter MP, Perry MC, Mulhern BM. 1976. Lead and PCB's in canvasback ducks: relationship between enzyme levels and residues in blood. Arch Environ Contam Toxicol 5:1–13.

Feeley MM. 1995. Biomarkers for Great Lakes priority contaminants: halogenated aromatic hydrocarbons. Environ Health Perspect 103(suppl 9):7–16.

Fox GA. 2001. Wildlife as sentinels of human health effects in the Great Lakes-St. Lawrence Basin. Environ Health Perspect 109(suppl 6):853–861.

George RH. 1997. Health problems and diseases of sea turtles. In: The Biology of Sea Turtles (Lutz PL, Musick JA, eds). Boca Raton, FL:CRC Press, 363–385.

Grasman KA, Armstrong M, Hammersley DL, Scanlon PF, Fox GA. 2000a. Geographic variation in blood plasma protein concentrations of young herring gulls (*Larus argentatus*) and Caspian terns (*Sterna caspia*) from the Great Lakes and Lake Winnipeg. Comp Biochem Physiol 125C:365–375.

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.

Grasman KA, Fox GA, Scanlon PF, Ludwig JP. 1996. Organochlorine-associated immunosuppression in prefledgling Caspian terns and herring gulls from the Great Lakes: an ecoepidemiological study. Environ Health Perspect 104(suppl 4):829–842.

Grasman KA, Scanlon PF, Fox GA. 2000b. Geographic variation in hematological variables in adult and prefledgling herring gulls (*Larus argentatus*) and possible associations with organochlorine exposure. Arch Environ Contam Toxicol 38:244–253.

Gross WB, Seigel HS. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis 27:972–979.

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.

Harms C, Lewbart G, Beasley J, Stamper A, Chittick B, Trogdon M. 2002. Clinical implications of hematology and plasma biochemistry values for loggerhead sea turtles undergoing rehabilitation. In: Proceedings of the Twentieth Annual Symposium on Sea Turtle Biology and Conservation (Mosier A, Foley A, Brost B, compilers), 29 February–4 March 2000, Orlando, FL. NOAA Technical Memorandum NMFS-SEFSC-477. Miami:U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Southeast Fisheries Science Center of the National Marine Fisheries Service, 190–191.

Hoffman DJ, Melancon MJ, Klein PN, Rice CP, Eisemann JD, Hines RK, et al. 1996. Developmental toxicity of PCB 126 (3,3´,4,4´,5-pentachlorobiphenyl) in nestling American kestrels (*Falco sparverius*). Fundam Appl Toxicol 34:188–200.

Keller JM, Kucklick JR, Harms CA, McClellan-Green PD. 2004. Organochlorine contaminants in sea turtles: correlations between whole blood and fat. Environ Toxicol Chem 23:726–738.

Keller JM, Kucklick JR, Peden-Adams MM, Stamper MA, McClellan-Green P. 2002. Correlations between organochlorine contaminants and health indicators in loggerhead sea turtles [Abstract]. In: Proceedings of SETAC 23rd Annual Meeting in North America, 16–20 November 2002, Salt Lake City, UT. Pensacola, FL:Society of Environmental Toxicology and Chemistry, 176.

Khasawinah AM, Grutsch JF. 1989. Chlordane: 24-month tumorigenicity and chronic toxicity test in mice. Regul Toxicol Pharmacol 10:244–254.

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):67–71.

Lawton RW, Ross MR, Feingold J, Brown JF Jr. 1985. Effects of PCB exposure on biochemical and hematological findings in capacitor workers. Environ Health Perspect 60:165–184.

Lorenzen A, Moon TW, Kennedy SW, Fox GA. 1999. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes herring gull embryos. Environ Health Perspect 107:179–186.

Lutcavage ME, Lutz PL, Bossart GD, Hudson DM. 1995. Physiologic and clinocopathologic effects of crude oil of loggerhead sea turtles. Arch Environ Contamin Toxicol 28:417–422.

Lutz PL, Dunbar-Cooper A. 1987. Variations in the blood chemistry of the loggerhead sea turtle, *Caretta caretta*. Fish Bull 85:37–43.

McConnell EE. 1985. Comparative toxicity of PCBs and related compounds in various species of animals. Environ Health Perspect 60:29–33.

Peden-Adams MM, Keller JM, Day RD, Johnson AR, EuDaly J, Keil DE, et al. 2002. Relationship of lymphoproliferation and clinical blood parameters to contaminants in loggerhead turtles [Abstract]. In: Proceedings of SETAC 23rd Annual Meeting in North America, 16–20 Nov 2002, Salt Lake City, UT. Pensacola, FL:Society of Environmental Toxicology and Chemistry, 75.

Pugh RS, Becker PR. 2001. Sea Turtle Contaminants: A Review with Annotated Bibliography. Report NISTIR 6700. Charleston, SC:National Institute of Standards and Technology.

Ramsay EC, Dotson TK. 1995. Tissue and serum enzyme activities in the yellow rat snake (*Elaphe obsolete quadrivitatta*). Am J Vet Res 56:423–428.

Rogers JM, Morelli L, Grabowski CT. 1984. Plasma glucose and protein concentrations in rat fetuses and neonates exposed to cataractogenic doses of mirex. Environ Res 34:155–161.

Safe S. 1993. Toxicology, structure–function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ Health Perspect 100:259–268.

Segre M, Arena SM, Greeley EH, Melancon MJ, Graham DA, French JB Jr. 2002. Immunological and physiological effects of chronic exposure of *Peromyscus leucopus* to Aroclor 1254 at a concentration similar to that found at contaminated sites. Toxicology 174:163–172.

Smith GJ, Hall RJ. 1994. Wildlife toxicology. In: Basic Environmental Toxicology (Cockerham LG, Shane BS, eds). Boca Raton, FL:CRC Press, 409–424.

Smits JE, Fernie KJ, Bortolotti GR, Marchant TA. 2002. Thyroid hormone suppression and cell-mediated immunomodulation in American kestrels (*Falco sparverius*) exposed to PCBs. Arch Environ Contamin Toxicol 43:338–344.

Vargo S, Lutz P, Odell D, Van Vleet E, Bossart G. 1986. Study of the Effects of Oil on Marine Turtles, Final Report. MMS 86-0070. Vienna, VA:U.S. Department of the Interior, Minerals Management Service.

Wagner RA, Wetzel R. 1999. Tissue and plasma enzyme activities in juvenile green iguanas. Am J Vet Res 60:201–203.

Work TM, Rameyer RA, Balazs GH, Cray C, Chang SP. 2001. Immune status of free-ranging green turtles with fibropapillomatosis from Hawaii. J Wildl Dis 37:574–581.