Effects of Organochlorine Contaminants on Loggerhead Sea Turtle Immunity: Comparison of a Correlative Field Study and In Vitro Exposure Experiments

Jennifer M. Keller,1,2 Patricia D. McClellan-Green,1,3 John R. Kucklick,2 Deborah E. Keil,4* and Margie M. Peden-Adams4,5,6,7

1Nicholas School of the Environment and Earth Sciences, Coastal Systems Science and Policy, and Integrated Toxicology Program, Duke University, Beaufort, North Carolina, USA; 2National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, South Carolina, USA; 3Department of Environmental and Molecular Toxicology, and Center for Marine Science and Technologies, North Carolina State University, Morehead City, North Carolina, USA; 4Marine Biomedicine and Environmental Science Center, and 5Department of Pediatrics, Medical University of South Carolina, Charleston, South Carolina, USA; 6Grice Marine Laboratory, College of Charleston, Charleston, South Carolina, USA; 7Mystic Aquarium and Institute for Exploration, Mystic, Connecticut, USA

Several laboratory and field studies indicate that organochlorine contaminants (OCs), such as polychlorinated biphenyls (PCBs) and pesticides, modulate immune responses in rodents, wildlife, and humans. In the present study we examined the effects of OCs on immunity in free-ranging loggerhead sea turtles (Caretta caretta). Mitogen-induced lymphocyte proliferation responses, lysozyme activity, and OC concentrations were measured from blood samples. Mitogens chosen in the lymphocyte proliferation assay were phytohemagglutinin (PHA) and concanavalin A (ConA) for T-lymphocyte stimulation, and lipopolysaccharide (LPS) and phorbol 12,13-dibutyrate (PDB) for B-lymphocyte stimulation. Lysozyme activity was significantly and negatively correlated with whole-blood concentrations of 4,4´-dichlorodiphenyldichloroethylene (4,4´-DDE) and the sum of chlordanes. Lymphocyte proliferation responses stimulated by PHA, LPS, and PDB were significantly and positively correlated with concentrations of the sum of PCBs measured in whole blood. LPS- and PDB-induced proliferation were also significantly and positively correlated with 4,4´-DDE blood concentrations. These correlative observations in free-ranging turtles suggest that current, chronic exposure to OCs may suppress innate immunity and enhance certain lymphocyte functions of loggerhead sea turtles. To further test this hypothesis, lymphocyte proliferation was measured after in vitro exposure of peripheral blood leukocytes from 16 turtles to Aroclor 1254 (0–13.5 µg/mL) or 4,4´-DDE (0–13.4 µg/mL). Both contaminants increased PHA- and PDB-induced proliferation at concentrations below those that affected cell viability. Moreover, the concentrations that enhanced PDB-induced proliferation in vitro were similar to concentrations measured in turtles with the highest proliferative responses. The similarities between the in vitro experiments and the correlative field study suggest that OC exposure modulates immunity in loggerhead turtles. Key words: DDT, immunotoxicity, organochlorine contaminants, organochlorine pesticides, PCBs, persistent organic pollutants, polychlorinated biphenyls, reptile. Environ Health Perspect 114:70–76 (2006). doi:10.1289/ehp.8143 available via http://dx.doi.org [Online 21 September 2005]

Environmental contaminants, such as organochlorine contaminants (OCs), have been shown to affect the immune functions of animals exposed in the laboratory (Harper et al. 1993; Ross et al. 1996; Segre et al. 2002; Silkworth et al. 1984; Smialowicz et al. 1989; Smits et al. 2002; Wu et al. 1999). These experiments have substantiated relationships observed between OC concentrations and immunocommodation in free-ranging wildlife (Grazman and Fox 2001; Lahvis et al. 1995; reviewed by Keller et al. 2000). OCs, such as polychlorinated biphenyls (PCBs), 4,4´-dichlorodiphenyldichloroethylene (4,4´-DDE), chlordanes, and other pesticides, have been recently documented in blood and adipose tissue of loggerhead sea turtles (Caretta caretta) from North Carolina (Keller et al. 2004a). Although the concentrations were low relative to other wildlife species that feed at higher trophic levels, the concentrations significantly correlated with several health indicators, including white blood cell counts and some plasma chemistry measurements (Keller et al. 2004c). The effects of environmental contaminants on functional aspects of sea turtle immunity, however, have not yet been addressed in any published study.

Sea turtles face many impacts from human activity, including hunting, fisheries interactions, loss of nesting habitat due to coastal development, and anthropogenic chemical contamination. Of these threats, the effects of environmental contaminants on sea turtle health are the least understood, and few studies have addressed this potential impact (Aguirre et al. 1994; Day 2003; Heesemann et al. 2004; Keller et al. 2004c; Lutcavage et al. 1995; Peden-Adams et al. 2002, 2003; Podreka et al. 1998). All species of sea turtles found in U.S. waters are protected by the U.S. Endangered Species Act under either an endangered or threatened status (Pritchard 1997). Specifically, the loggerhead sea turtle (Caretta caretta) is protected as a threatened species, and although some populations are recovering, others may still be declining (Turtle Expert Working Group 2000). It is therefore important to understand the risk that contaminants pose to the general health and immunologic function of loggerhead sea turtles because these effects could affect the survival of their populations.

In this study we examined how OCs influence loggerhead sea turtle immune responses using the mitogen-induced lymphocyte proliferation assay and plasma lysozyme activity. The lymphocyte proliferation assay has been optimized for loggerhead and green turtles.
sea turtles (Chelonia mydas) (Keller et al. 2005; McKinney and Bentley 1985; Work et al. 2000). Lysozyme activity, a measure of innate immunity, has not previously been reported for any sea turtle species. Because lymphocyte proliferation and lysozyme activity have been shown to be altered by OC exposure in other species (Burton et al. 2002; Grasman and Fox 2001; Lahvis et al. 1995; Ross et al. 1996; Segre et al. 2002; Smits et al. 2002; Wu et al. 1999), we hypothesized that OC exposure may also modulate these immune functions in loggerhead sea turtles. If shown to be affected by OCs, these immune measurements would offer a relatively simple biomarker that requires only a nonlethal blood sample.

Materials and Methods

Sampling. All turtles used in this study were treated humanely in accordance with protocols approved by Duke University Institutional Animal Care and Use Committee (protocols A351-99-07-2, A351-99-07-3, and A206-01-07) and with required federal and state permits (U.S. Fish and Wildlife Service permits PRT-676379 and TE-676379-2, National Marine Fisheries Service permit 1245, and North Carolina Wildlife Resources Commission permits 005170 and 01ST45). Forty-eight free-ranging juvenile loggerhead sea turtles with straight carapace lengths (SCLs; measured from the nuchal notch to the most posterior marginal notch) between 45.7 and 77.3 cm were captured in offshore waters of North Carolina, in July and August 2000 and in July 2001. Most turtles appeared healthy upon visual examination, and body condition indices and plasma chemistry values were measured and reported elsewhere (Keller et al. 2004a). Lymphocyte proliferation was measured in only the 2001 samples, whereas lysozyme activity was measured in both years. Blood was collected within 10 min of capture from the dorsocervical sinus using double-ended Vacutainer needles directly into Vacutainer blood collection tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ) and kept cool until processing. One blood tube from each turtle was frozen at −20°C for contaminant analysis. Plasma from another blood tube collected from 45 of the turtles was frozen at −80°C for lysozyme activity measurements. An additional blood tube from 24 of the turtles captured only in July 2001 was processed for lymphocyte proliferation. Turtles were tagged, measured, weighed, and released near their capture location. Body condition was calculated as weight (kilograms) divided by the cube of SCL (centimeters) and multiplied by 100,000 [body condition = weight/(SCL3) × 100,000] as described by Bjornid et al. (2000). Sex of the turtles was determined by measuring plasma testosterone concentrations (Owens 1997). An additional 16 juvenile turtles (SCL ranged from 52.8 to 72.3 cm) were captured in offshore waters of South Carolina, Georgia, and northeastern Florida during June 2003 (n = 8) and June 2004 (n = 8). These turtles were randomly captured in trawl nets without turtle excluder devices at randomly selected stations using a trawl tow time of 30 min. Blood samples were collected and processed in the same manner as described above for use in in vitro exposure experiments.

Contaminant analysis. Concentrations of OCs, including 55 PCB congeners and 24 pesticides, were determined in whole blood of the North Carolina turtles and are reported elsewhere (Keller et al. 2004a). Briefly, samples were amended with internal standards and extracted with organic solvents. After lipid content was determined gravimetrically, biologic molecules of large molecular weight were removed from the extracts using alumina columns. Compounds were quantified using gas chromatography with electron capture and mass spectrometry detection. Analytical blanks and standard reference materials from the National Institute of Standards and Technology were analyzed with each batch of samples. The blood lipid content did not correlate to blood OC concentrations (Keller JM, unpublished data); therefore, the blood concentrations were calculated based on the wet mass of blood extracted (nanograms per gram wet mass). OC concentrations that were below the detection limit were estimated at half the detection limit for correlations. The detection limits were calculated as the amount (nanograms) of compound in the most dilute calibration standard solution yielding a signal significantly different from the noise, divided by the grams of tissue extracted.

Lysozyme activity. We measured lysozyme activity using slight modifications of a standard turbidity assay as previously described by Demers and Bayne (1997). A 1 mg/mL stock solution of hen egg lysozyme (HEL; Sigma, St. Louis, MO) was prepared in 0.1 M phosphate buffer (pH 5.9), and aliquots were frozen until use. A solution of Micrococcus lyodeikiticus (Sigma) was prepared fresh daily by dissolving 50 mg of the lyophilized cells in 100 mL 0.1 M phosphate buffer (pH 5.9). HEL was serially diluted in phosphate buffer to produce a standard curve of 40, 20, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0.1 µg/mL. Aliquots of each concentration (25 µL/well) were added to a 96-well plate in triplicate. For each sample, 25 µL of test plasma was added in quadruplicate to the plate. The solution of M. lyodeikiticus (175 µL/well) was quickly added to three sample wells and to each of the standard wells. The fourth well containing plasma received 175 µL phosphate buffer and served as a blank. Plates were assessed for absorbance at 450 nm with a spectrophotometer (SpectraCount; Packard, Meridian, CT) immediately (T0) and again after 5 min (T5). Absorbance unit (AU) values at T5 were subtracted from AU values at T0 to determine the change in absorbance. The AU value for the blank sample well was subtracted from the average of the triplicate sample wells to compensate for any hemolysis in the samples. The resultant AU value was converted to HEL concentration (micrograms per microliter) via linear regression of the standard curve.

Mitogen-induced lymphocyte proliferation. Lymphocyte proliferation assay for correlations with OCs. Lymphocyte proliferation responses have been reported elsewhere (Keller et al. 2005). Rather than a density gradient method, peripheral blood leukocytes (PBLs) were collected from the buffy layer within 36 hr of blood collection using a slow-spin technique (42 × g for 25 min) as described in detail by Keller et al. (2005). No density gradient method is available to obtain a pure isolation of loggerhead lymphocytes (Harms et al. 2000). Cells were rinsed once with cell culture media composed of RPMI 1640 media (Mediatech, Inc., Herndon, VA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 1% (vol/vol) 100× solution of nonessential amino acids (Gibco, Grand Island, NY), 1 mM sodium pyruvate (Gibco), 10 mM HEPES (Mediatech), 50 IU/mL penicillin, and 50 µg/mL streptomycin (Mediatech) and initially brought to pH 6.9.

Viable PBLs were counted by trypan blue exclusion using light microscopy. Although this technique cannot distinguish sea turtle lymphocytes from thrombocytes or other small PBLs (Work et al. 1998), thrombocytes are known to aggregate. The aggregating cells were not counted in order to decrease the chance of counting these nontarget cells. There is no evidence in the literature that thrombocytes would proliferate in the presence of a mitogen; furthermore the use of a stimulation index (SI) should account for any potential, although unexpected, background proliferation of any other PBL type.

We split the cell suspension into two tubes and diluted in two different media compositions; media 1, as described above, or media 2, which differed only by the FBS manufacturer (BioWhittaker, Walkersville, MD). Cells were plated at 1.8 × 10^6 cells/well into 96-well plates. We used phytohemagglutinin P (PHA) and concanavalin A (ConA) as T-lymphocyte mitogens, and lipopolysaccharide (LPS) and phorbol 12,13-dibutyrate (PDB) as B-lymphocyte mitogens. PDB has previously been shown to stimulate avian B lymphocytes (Scott and Savage 1996). ConA from Jack bean type IV-S
(C5275, Sigma) and LPS from Escherichia coli serotype 0111:B4 (L2630, Sigma) were diluted in media 1. PHA (Amersham Pharmacia Biotech Inc., Piscataway, NJ), ConA type IV from jack bean (Canavalia ensiformis) (C2010, Sigma), and LPS from E. coli serotype 0127:B8 (L3129, Sigma) were diluted in media 2. PDB (Sigma) was tested in both media types. Cells were tested in triplicate for each unstimulated control (containing only media 1 or 2, respectively) and each mitogen concentration with final volumes at 200 µL/well for mitogens in culture wells and 5 µL/well of these substocks was divided by counts per minute of cells exposed to PHA plus 5 ng/mL 4,4'-DDE divided by counts per minute of cells exposed to only 5 ng/mL 4,4'-DDE. This SI value was then normalized to the turtle's non-DMSO SI (counts per minute of cells exposed to PHA divided by counts per minute of cells exposed to only media).

Statistics. We used nonparametric Spearman rank correlations because contaminant concentrations did not fit a normal distribution before or after transformation. We used these correlations to examine the relationship of lymphocyte proliferation and lysozyme activity with OC concentrations determined in

Table 1. Mitogen-induced lymphocyte proliferation of loggerhead sea turtles and Spearman rank correlations between lymphocyte proliferation responses and OC concentration measured in whole blood.

<table>
<thead>
<tr>
<th>Medium type</th>
<th>Mitogen</th>
<th>Type</th>
<th>Concentration (µg/mL)</th>
<th>Day</th>
<th>Mean SI (SE)</th>
<th>Sample size</th>
<th>Spearman correlation coefficient (r_s [p-value]) between lymphocyte proliferation and OCs in whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCBs, 4,4'-DDE, Chlordanes</td>
</tr>
<tr>
<td>1</td>
<td>ConA</td>
<td>C5275</td>
<td>20</td>
<td>4</td>
<td>3.94 (0.95)</td>
<td>19</td>
<td>-0.132 (0.591) -0.035 (0.989) -0.065 (0.792)</td>
</tr>
<tr>
<td></td>
<td>ConA</td>
<td>C5275</td>
<td>20</td>
<td>5</td>
<td>2.47 (0.52)</td>
<td>24</td>
<td>-0.073 (0.735) -0.020 (0.926) -0.084 (0.698)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>(L2630)</td>
<td>10</td>
<td>4</td>
<td>3.41 (0.52)</td>
<td>19</td>
<td>0.528 (0.020) 0.495 (0.031) 0.484 (0.036)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>(L2630)</td>
<td>10</td>
<td>5</td>
<td>3.01 (0.47)</td>
<td>24</td>
<td>0.249 (0.241) 0.170 (0.426) 0.157 (0.463)</td>
</tr>
<tr>
<td></td>
<td>PDB</td>
<td>(P1269)</td>
<td>0.2</td>
<td>4</td>
<td>4.52 (1.23)</td>
<td>19</td>
<td>0.074 (0.764) 0.063 (0.797) 0.036 (0.897)</td>
</tr>
<tr>
<td></td>
<td>PDB</td>
<td>(P1269)</td>
<td>0.2</td>
<td>5</td>
<td>2.84 (0.55)</td>
<td>24</td>
<td>0.113 (0.000) 0.121 (0.054) 0.159 (0.458)</td>
</tr>
<tr>
<td>2</td>
<td>PHA</td>
<td></td>
<td>5</td>
<td>5</td>
<td>114 (65)</td>
<td>17</td>
<td>0.596 (0.012) 0.431 (0.084) 0.434 (0.082)</td>
</tr>
<tr>
<td></td>
<td>ConA</td>
<td>C2010</td>
<td>10</td>
<td>5</td>
<td>29.1 (13.5)</td>
<td>17</td>
<td>0.020 (0.941) 0.020 (0.941) 0.020 (0.993)</td>
</tr>
<tr>
<td></td>
<td>ConA</td>
<td>C2010</td>
<td>20</td>
<td>5</td>
<td>1.89 (0.37)</td>
<td>17</td>
<td>0.370 (0.293) 0.370 (0.293) 0.406 (0.244)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>(L3129)</td>
<td>2.5</td>
<td>5</td>
<td>3.56 (0.70)</td>
<td>24</td>
<td>-0.111 (0.605) -0.118 (0.582) -0.151 (0.480)</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>(L3129)</td>
<td>6.0</td>
<td>5</td>
<td>2.01 (0.31)</td>
<td>17</td>
<td>0.385 (0.127) 0.201 (0.438) 0.243 (0.348)</td>
</tr>
<tr>
<td></td>
<td>PDB</td>
<td>(P1269)</td>
<td>0.2</td>
<td>5</td>
<td>1.00 (0.29)</td>
<td>17</td>
<td>0.196 (0.074) 0.103 (0.694) 0.226 (0.394)</td>
</tr>
<tr>
<td></td>
<td>PDB</td>
<td>(P1269)</td>
<td>0.4</td>
<td>5</td>
<td>6.08 (1.73)</td>
<td>17</td>
<td>0.015 (0.955) -0.005 (0.985) 0.012 (0.963)</td>
</tr>
<tr>
<td></td>
<td>PDB</td>
<td>(P1269)</td>
<td>0.8</td>
<td>5</td>
<td>4.10 (1.28)</td>
<td>17</td>
<td>0.564 (0.018) 0.507 (0.038) 0.476 (0.054)</td>
</tr>
</tbody>
</table>

*Media types are described in “Materials and Methods” and differ only by the manufacturer of FBS. Catalog numbers for mitogens purchased from Sigma are shown in parentheses; PHA was purchased from Amersham Pharmacia Biotech. Duration of mitogen exposure indicating when 3H-thymidine was added (96 hr or 120 hr, respectively). SI = cpm of mitogen-stimulated cells/cpm of unstimulated cells. Data from Keller et al. (2005). *p < 0.05.
the blood (nanograms per gram wet mass). The proportional responses of the in vitro exposure experiments were not normally distributed, so the data were log-transformed. Analysis of variance (ANOVA) with a Dunnet’s multiple comparison test was used to compare the responses at each contaminant concentration to the DMSO control. All statistical analyses were performed using JMP 4.0.2 (SAS Institute Inc., Cary, NC).

**Results**

**Organochlorine concentrations.** OC concentrations in samples from turtles captured in 2000 and 2001 have been reported elsewhere on both a wet-mass and a lipid-normalized basis (Keller et al. 2004a). For the purpose of comparing immune function and OC concentrations, only samples from turtles assessed for lymphocyte proliferation in July 2001 are described here ($n = 27$). The mean ($\pm$ SE) concentrations of $\Sigma$PCBs (the sum of PCBs), $4,4´$-DDE, and $\Sigma$Chlordanes (the sum of chlordanes) were $6.25 \pm 1.14$ ng/g wet mass, $0.721 \pm 0.152$ ng/g wet mass, and $0.253 \pm 0.048$ ng/g wet mass, respectively. The concentrations of these compounds in this group of turtles were not significantly different from turtles sampled in 2000 (Mann-Whitney $t$-test; all $p$-values $> 0.05$).

**Plasma lysozyme activity.** We measured lysozyme activity in plasma samples from 45 animals that were assessed for OC concentrations. Lysozyme activity was $6.58 \pm 0.58$ µg HEL/µL (mean $\pm$ SE) with a range of 1.94–25.2 µg HEL/µL. The Spearman correlation coefficients, $r_S$ ($p$-values) for lysozyme versus $\Sigma$PCBs, $4,4´$-DDE, and $\Sigma$Chlordanes were $-0.269 (0.074)$, $-0.310 (0.038)$, and $-0.368 (0.013)$, respectively. All slopes were negative, and correlations with $4,4´$-DDE and $\Sigma$Chlordanes were statistically significant at $\alpha = 0.05$ (Figure 1). Lysozyme activity did not differ between gender and did not correlate to body condition or plasma testosterone levels.

**Lymphocyte proliferation: correlations with blood OC concentrations.** The mean lymphocyte proliferation responses, reported elsewhere (Keller et al. 2005), are tabulated along with the correlative results between lymphocyte proliferation and OC concentrations in Table 1. Proliferation in this data set did not differ between sexes and did not correlate with testosterone concentrations (data not shown). Body condition correlated with lymphocyte proliferation only in media type 1 when stimulated with 0.2 µg/mL PDB for 5 days ($r_S = 0.528; p = 0.012$).

Some proliferative responses were significantly correlated with blood OC concentrations. Lymphocyte proliferation stimulated by 4 days of exposure to 10 µg/mL LPS was significantly correlated with blood concentrations of $\Sigma$PCBs, $4,4´$-DDE, and $\Sigma$Chlordanes. PHA-induced proliferation (5 µg/mL PHA) correlated with blood concentrations of $\Sigma$PCBs. Proliferation stimulated by 0.8 µg/mL PDB was also positively correlated with $\Sigma$PCB and $4,4´$-DDE concentrations. ConA stimulation, however, did not correlate with any contaminant. All statistically significant correlations between OC concentrations and lymphocyte proliferation had positive slopes, indicating that turtles with higher contaminant levels exhibited elevated lymphocyte proliferation responses (Figures 2 and 3). Correlations were also examined between lymphocyte proliferation and OC concentrations calculated on a lipid-normalized basis (data not shown). These correlations were very similar to those shown in Table 1.

**Lymphocyte proliferation: in vitro exposure experiments.** PBls from 16 turtles were exposed to increasing concentrations of Aroclor 1254 (0–13.5 µg/mL) or $4,4´$-DDE (0–13.4 µg/mL). Cell viability was measured for 8 of these turtles after 5 days of contaminant exposure, examining only cells not stimulated with the mitogen (data not shown). Viability was unaffected by concentrations of $\leq 1,000$ ng/mL of either contaminant. Both contaminants significantly decreased cell viability at concentrations of $\geq 7,500$ ng/mL. Therefore, lymphocyte proliferation responses at these higher concentrations are not reported.

The effects of in vitro exposure to Aroclor 1254 on lymphocyte proliferation responses are shown in Figure 4. All of the tested concentrations of Aroclor 1254 generally increased PHA-induced proliferation, albeit not significantly, compared with the response...
The effect of a 5-day in vitro exposure to Aroclor 1254 on loggerhead sea turtle lymphocyte proliferation (SI) responses stimulated by 5 µg/mL PHA (A) and 0.2 µg/mL PDB (B). Data are shown as mean ± SE of the percentage of the SI measured in the control (no DMSO or Aroclor 1254) for each turtle. Sample sizes are 8 or 16 depending on the treatment group. The x-axis crosses the y-axis at the percentage of the control value for the wells receiving only DMSO. The mean ± SE SI for the DMSO controls in the PHA and PDB experiments was 96.6 ± 15.0 and 172 ± 39, respectively. Vertical dashed lines indicate the range of 4,4´-DDE concentrations measured in the blood of 17 loggerhead sea turtles used in the correlative field study.

*Significantly different from the DMSO control (ANOVA with log-transformed data, Dunnett’s multiple comparison test; p < 0.05).

Figure 5. The effect of a 5-day in vitro exposure to 4,4´-DDE on loggerhead sea turtle lymphocyte proliferation (SI) responses stimulated by 5 µg/mL PHA (A) and 0.2 µg/mL PDB (B). Data are shown as mean ± SE of the percentage of the SI measured in the control (no DMSO or 4,4´-DDE) for each turtle. Sample sizes are 8 or 16 depending on the treatment group. The x-axis crosses the y-axis at the percentage of the control value for the wells receiving only DMSO. The mean ± SE SI for the DMSO controls in the PHA and PDB experiments was 96.6 ± 15.0 and 172 ± 39, respectively. Vertical dashed lines indicate the range of 4,4´-DDE concentrations measured in the blood of 17 loggerhead sea turtles used in the correlative field study.

*Significantly different from the DMSO control (ANOVA with log-transformed data, Dunnett’s multiple comparison test; p < 0.05).
necessarily a healthy outcome, because immuneenhancement can lead to autoimmune diseases and hypersensitivity (Burns et al. 1996). Any alteration of immune function, even enhancement, can be considered an adverse effect.

Admittedly, results from correlative field studies are largely circumstantial, and no causal relationship can be identified with certainty. Because intentional, experimental exposure of protected sea turtles to contaminants is not feasible, we used in vitro experiments to further investigate the immuneenhancement suggested by the correlations observed between lymphocyte proliferation and actual environmental exposure to OCs in the pilot study. Although typical dose–response curves were not always observed in the in vitro experiments, similarities between these experiments and the correlative field study were seen not only in the concentrations that produced significant results but also, more consistently, in the direction of the response. Both study components demonstrated immunoenhancement instead of suppression. Interestingly, in vitro exposure to both PCBs and 4,4´-DDE significantly enhanced PDB-induced proliferation at concentrations that are found in loggerhead sea turtle blood (vertical lines in Figures 4B and 5B). Specifically, in vitro exposure to 5 ng/mL Aroclor 1254 significantly increased PDB-induced proliferation (Figure 4B), and the two turtles with the strongest PDB-induced proliferation responses in the correlative field study had blood PDB concentrations similar to this (4.32 and 8.40 ng/g wet mass). Likewise, the one concentration of 4,4´-DDE (0.5 ng/mL) that increased PDB-induced proliferation responses in vitro (Figure 5B) is similar to the blood 4,4´-DDE concentrations measured in the top five responding turtles (0.603–1.13 ng/g wet mass) in the field study. Although these comparisons are not identical in methods (different PDB concentrations), these results, together with the general enhancement observed in Figures 4A and 5A, support the hypothesis that environmentally relevant concentrations of OCs may enhance certain loggerhead sea turtle immune responses.

Previous studies have examined similar in vitro exposures of mammalian lymphocytes to PCBs (De Guise et al. 1998; Smithwick et al. 2003; Snyder and Valle 1991). Only one of these studies, however, tested PCB concentrations in the same range as used in the present study (Snyder and Valle 1991). Snyder and Valle (1991) also observed immunoenhancement of lymphocyte proliferation using rat splenocytes exposed to 0.01 µg/mL Aroclor 1254. The other two studies demonstrated immunosuppression, but higher exposure concentrations were used (15 µg/mL of three PCB congeners combined, De Guise et al. 1998; 10 µg/mL Aroclor 1242, Smithwick et al. 2003). These concentrations are near the high end of the concentration range used in the present study that resulted in decreased cell viability. These comparisons suggest that stimulation of lymphocyte proliferation may occur at low levels of exposure and that sea turtle lymphocytes may be more sensitive to the cytotoxic effects of OCs than are mammalian cells.

To our knowledge, no previous study examining sea turtle immunity has measured innate (nonspecific) immune functions. Circulating lysozyme is a marker of pro-inflammatory responses, has antibacterial functions, and is a measure of innate immunity (Burton et al. 2002; Weeks et al. 1992). In mammals and fish, lysozyme is secreted by neutrophils (cellular equivalents of reptilian heterophils) upon entry of foreign bacteria and lyses gram-positive bacterial cells by degrading the cell wall (Balflry and Iwama 2004; Ito et al. 1997). In fish, PCBs are known to exhibit varied effects on lysozyme activity (Burton et al. 2002; Hutchinson et al. 2003). In the present study, we observed significant negative correlations between lysozyme and both 4,4´-DDE and chlordane. These findings suggest that OCs in the blood may suppress lysozyme production or activity in sea turtles. More recently, we have also assessed lysozyme activity in a separate set of juvenile loggerhead turtle turtles captured from near shore waters of South Carolina, Georgia, and Florida and obtained similar results (Peden-Adams MM, Keller JM, unpublished data). In that study, blood chlordane, 4,4´-DDE, and Σ chlordane concentrations correlated with lysozyme activity (ρ = −0.496, −0.505, and −0.381, respectively, with ρ = 0.005, 0.004, and 0.038, respectively) measured in 30 turtles captured in July 2001. Interestingly, in that study and the present one, 4,4´-DDE exhibited very similar, statistically significant correlations. ΣDDT concentrations in human breast milk have been previously noted to correlate with lysozyme in milk (Saleh et al. 1998). Although the mechanism of decreased lysozyme is not understood, the data suggest that heterophil function is suppressed in turtles as exposure to OCs increases. Future studies should assess phagocytosis and respiratory burst to further elucidate effects of OCs on innate immune functions in sea turtles.

In a parallel study with the same sample set of loggerhead sea turtles, the ratio of heterophils to lymphocytes was significantly and positively correlated with adipose concentrations of mirex and dioxin-like PCBs (Keller et al. 2004a). An elevation in this ratio is a common response to many stressors in birds, mammals, and sea turtles (Aguirre et al. 1995; Grasman et al. 1996; Gross and Siegel 1983; Maxwell and Robertson 1998). The fact that concentrations of various OC classes in the loggerhead sea turtles were significantly correlated to four immune parameters (increased T-cell proliferation, increased B-cell proliferation, decreased lysozyme activity, and increased heterophil:lymphocyte ratio) provides additional evidence that turtles with elevated OC exposure exhibit immunomodulation.

Conclusion

Certain lymphocyte proliferation responses in loggerhead sea turtles are positively correlated with OC concentrations measured in blood, even though the concentrations in sea turtles are generally much lower than in fish-eating wildlife. In vitro exposure experiments using relevant concentrations of both PCBs and 4,4´-DDE support the correlative field observations. Another measure of immune function, lysozyme activity, is also significantly correlated with concentrations of two major classes of OCs in the blood. These results are similar to the findings of many other wildlife studies and suggest that the sea turtle immune system is modulated by environmentally relevant concentrations of OCs. Future studies could use these relatively simple immune function assays as biomonitoring tools. They should also develop and optimize additional assays, such as natural killer cell activity, in order to more completely assess the effects of environmental contaminants on the immune system of sea turtles.

**REFERENCES**


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