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Accumulation of Polychlorinated Biphenyls (PCBs) and Evaluation of Hematological and Immunological Effects of PCB Exposure on Turtles

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Abstract Concentrations of total polychlorinated biphenyls (PCBs), Aroclor 1260, and 26 congeners were measured in liver, fat, and eggs of red-eared slider turtles (*Trachemys scripta elegans*) collected from ponds near or on the Paducah Gaseous Diffusion Plant (PGDP), Kentucky, USA. Concentrations of total PCBs (wet mass) ranged from 0.002 to 0.480 mg/kg, 0.028 to 0.839 mg/kg, and 0.001 to 0.011 mg/kg in liver, fat, and eggs, respectively. Concentrations of Arochlor 1260 did not exceed 0.430, 0.419, and 0.007 mg/kg in liver, fat, and eggs, respectively. Exposure to PCBs in red-eared sliders collected from the PGDP is characterized by low concentrations of moderately chlorinated mono-ortho and di-ortho congeners (PCB 153, 180, and 118). Although PCB concentrations measured in the current study were low, chronic exposure to PCBs may have altered hematology and immunity of the turtles examined. Total white blood cell count and number of heterophils were negatively correlated with concentrations of total PCBs and Arochlor 1260, respectively. However, disease and other contaminants in the study area may influence the results. Because little is known regarding the influence of PCBs on hematology and immune function in turtles, additional study is needed to better evaluate results observed in the current study.

Keywords Polychlorinated biphenyls · Arochlor 1260 · Paducah Gaseous Diffusion Plant · Red-eared slider turtle · Total white blood cell count · Heterophil

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Polychlorinated biphenyls (PCBs) are a group of man-made chlorine-containing chemicals that are persistent in the environment and bioaccumulate via the food chains. In wildlife, acute toxicity and sublethal effects may occur due to PCB exposure. While many studies have demonstrated adverse effects of PCBs on birds and mammals, toxicological effects of PCBs are less understood in reptiles. Several studies on turtles have reported that PCBs can impair survival (Eisenreich et al. 2009) and reproduction (Bergeron et al. 1994) and alter immune function (Keller et al. 2004b).

Polychlorinated biphenyls have been detected in wildlife inhabiting ponds on and near the Paducah Gaseous Diffusion Plant (PGDP). Concentrations of Aroclor 1245 and total PCBs in fish tissue collected during 1989 from Little Bayou Creek, which flows along the eastern edge of the PGDP, were 2.196 and 4.843 mg/kg, respectively. A more recent study conducted by DeGarady and Halbrook (2003) reported that concentrations of Aroclor 1260 ranged from 0.151 to 4.470 mg/kg ww in various anuran species collected from PGDP outfalls. Because PCBs may pose a risk to aquatic biota inhabiting the PGDP area, studies are needed to assess current PCB exposure and its potential effects on aquatic organisms. In the current study, red-eared slider turtles (*Trachemys scripta elegans*) were used to determine PCB contamination in aquatic ecosystems associated with plant operation and to evaluate the effects of PCBs on hematological and immune function in aquatic turtles.

Materials and Methods

The study site and sampling method have been previously described (Yu et al. 2011; Fig. 1). Liver samples from 11 turtles and fat samples from 8 turtles were collected from

ponds near the plant in 2008 for PCB analysis. An additional 8 liver samples were collected in 2010 from turtles captured at Pond C616, which is located on the PGDP within the security fence (Fig. 1). Turtle eggs were collected during 2008. Two eggs were randomly chosen from each clutch of 5 female red-eared slider turtles for PCB analysis. Blood was collected and examined for hematocrit, total and differential white blood cell (WBC) count, and heterophil to lymphocyte ratio (HL ratio). Phytohemagglutinin (PHA) skin test was also conducted to determine stimulation index (PHA SI), which is an indication of T-cell mediated immunity. Trapping, blood collection, and determination of hematological and immunological parameters have been described in detail in Yu et al. (2011). Hematology and PHA SI were only determined in turtles collected in 2008.

Tissue samples collected in 2008 from ponds near the PGDP were extracted at the Cooperative Wildlife Research Laboratory (CWRL), Southern Illinois University Carbondale (SIUC), while samples collected in 2010 from Pond C616 were extracted by the Mississippi State Chemical Laboratory, Mississippi State University. All samples were analyzed for total PCBs, Aroclor 1260, and 26 PCB congeners by the Mississippi State Chemical Laboratory. Congeners measured in the current study were (IUPAC number): 8, 18, 28, 44, 52, 66, 77, 81,101, 105, 114, 118,

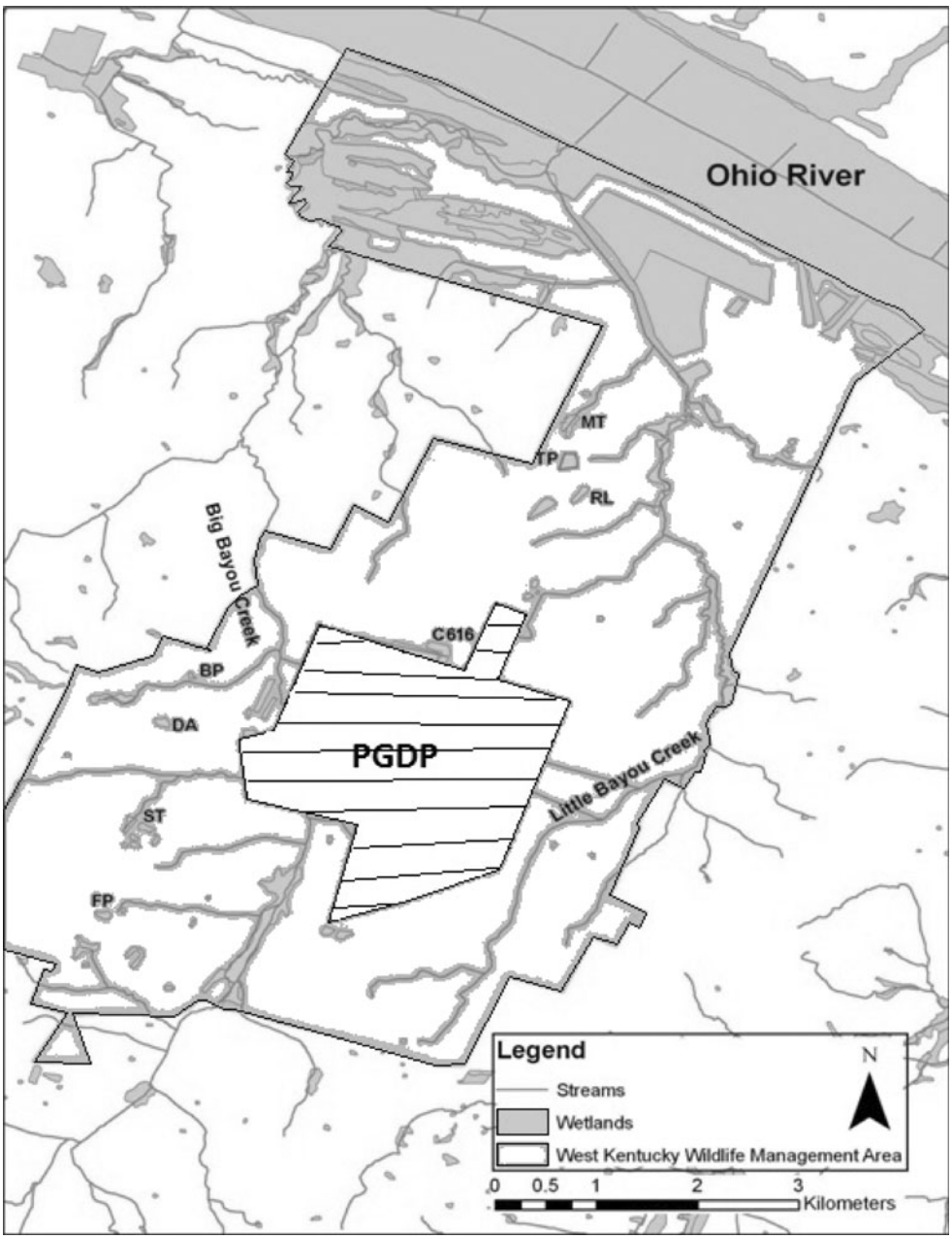

Fig. 1 Map of study ponds near and on the Paducah Gaseous Diffusion Plant, Kentucky, USA. *MT* Metzger Pond, *TP* Teal Pond, *RL* Rock Levee Pond, *BP* Beaver Pond, *DA* Disabled Access Pond, *ST* Stick Pond, *FP* Fireplug Pond, C616 Pond

123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 195, and 206. The detection limits were 0.001 mg/L for all PCB congeners and 0.010 mg/L for both Aroclor 1260 and total PCBs. For samples extracted by the CWRL at SIUC, PCB concentrations reported by the Mississippi State Chemical Laboratory were converted from extract concentrations (mg/L) to tissue concentrations (mg/kg). Recovery was 93 % for total PCBs, 115 % for Aroclor 1260, and varied from 68 % to 131 % for different congeners. Concentrations (mg/kg) are reported on a wet mass (ww) and lipid mass basis. Moisture ranged from 57.6 % to 83.0 % for liver and 74.3 % to 78.4 % for eggs. Lipid content appeared to vary greatly among samples, ranging from 0.94 % to 28.03 % in liver and 1.49 % to 74.23 % in fat, while it was more consistent among eggs (6.60 %–7.96 %). Data were analyzed using SPSS 13.0 (SPSS Inc.). Normality and homogeneity of variances were tested and those not conforming to normality were log-transformed and re-tested. Non-parametric tests were used when necessary. Results were considered significant at $\alpha = 0.05$.

Results and Discussion

Concentrations of total PCBs and Arochlor 1260 did not exceed 0.839 mg/kg ww (29.448 mg/kg lipid mass) or 0.430 mg/kg ww (26.380 mg/kg lipid mass), respectively, in all samples (Table 1). Concentrations of Aroclor 1260 and total PCBs were significantly greater in liver tissues of turtles collected from Pond C616 on the PGDP compared with turtles collected from ponds near the PGDP combined (Mann–Whitney U test, all $p \leq 0.001$; total PCBs: 0.111 ± 0.054 vs. 0.007 ± 0.002 mg/kg ww and 7.455 ± 3.481 vs. 0.128 ± 0.048 mg/kg lipid mass; Aroclor 1260: 0.104 ± 0.048 vs. 0.004 ± 0.0005 mg/kg ww and 6.938 ± 3.106 vs. 0.081 ± 0.031 mg/kg lipid mass). Turtles from Pond C616 were expected to have greater PCB concentrations because this pond is located on the plant and is likely to receive a greater amount of contaminants relative to ponds near the plant. Total PCB concentrations measured in turtle tissues and eggs in the current study were similar to or lower than those previously reported in freshwater turtles (e.g. Bonin et al. 1995; Dabrowska et al. 2006; Kannan et al. 1998, 2000).

There appeared to be considerable variations in lipid content for fat samples among individuals (1.49 %–74.23 %). Other studies have also reported variable lipid content in adipose tissues, for example, 14.9 %–88 % in common snapping turtles (*Chelydra serpentina*) collected in Minnesota (Helwig and Hora 1983) and 25.9 %–68.2 % in loggerhead sea turtles (*Caretta caretta*) from eastern Adriatic Sea (Lazar et al. 2011). The mean lipid content in adipose tissues of snapping turtles collected from different locations in New Jersey and Maryland ranged from 13.88 % to 73.38 % (Albers et al. 1986). Keller et al. (2004a) reported lipid content from 0.255 % to 64.7 % in

Table 1 Mean concentrations (mg/kg) and range of PCBs in liver, fat, and eggs of red-eared slider turtles (*Trachemys scripta elegans*) collected in 2008 and 2010 from ponds near and on the Paducah Gaseous Diffusion Plant, Kentucky, USA

PCB	Wet mass			Lipid mass		
	Liver	Fat	Egg	Liver	Fat	Egg
Total PCBs	0.056	0.167	0.005	3.571	3.059	0.073
	0.002–0.480	0.028–0.839	0.001–0.011	0.008–29.448	0.042–7.283	0.013–0.141
Aroclor 1260	0.051	0.095	0.004	3.297	2.065	0.055
	0.002–0.430	0.021–0.419	0.001–0.007	0.008–26.380	0.031–5.318	0.013–0.094
Congeners						
28	ND[a]	0.003	ND	ND	0.125	ND
101	ND	0.003	ND	ND	0.135	ND
		0.001–0.003			0.061–0.177	
118	0.0004	0.003	0.0004	0.003	0.003	0.006
		0.0006–0.008			0.012–0.125	
123	0.003	ND	ND	0.161	ND	ND
153	0.008	0.004	0.0004	0.595	0.075	0.006
	0.0002–0.029	0.0005–0.015	0.0002–0.0006	0.001–2.872	0.0007–0.177	0.003–0.008
170	0.007	ND	ND	0.550	ND	ND
	0.003–0.017			0.066–1.064		
180	0.013	0.004	0.0004	0.963	0.026	0.006
	0.0002–0.051	0.0005–0.010		0.001–3.129	0.0007–0.061	

[a] Not detected

loggerhead turtles captured in Core Sound, North Carolina. It is possible that some turtles did not eat and therefore lost fat reserves when kept in captivity 5–10 days (food was provided ad libitum) during egg induction and PHA skin tests (Yu et al. 2011).

Among the 26 PCB congeners evaluated in all samples, seven congeners were above detection limit including 3 mono-ortho congeners (PCB 28, 118, and 123) and 4 di-ortho congeners (PCB 101, 153, 170, and 180). The more toxic non-ortho coplanar PCB congeners were not detected in any sample. Five congeners were detected in both liver and fat samples while three were detected in egg samples (Table 1). Polychlorinated biphenyl 118 (penta-chlorobiphenyl [CB]), 153 (hexa-CB), and 180 (hepta-CB) were detected in all 3 tissues, and PCB 153 was the most abundant congener. Polychlorinated biphenyl 153 was measured in 63 % of liver samples, 75 % of fat samples, and 50 % of eggs, while PCB 180 was measured in 47 % of liver samples, 37 % of fat samples, and 10 % of eggs. Polychlorinated biphenyl 118 was detected in 50 % of fat samples but only 5 % of liver samples and 10 % of eggs. Exposure to PCBs in red-eared slider turtles is characterized by low concentrations of moderately chlorinated mono-ortho and di-ortho congeners (penta-, hexa-, and hepta-CBs). Congeners with 5–7 chlorine atoms are prevalent in the environment because they occur in greater proportions in many PCB formulations (McFarland and Clarke 1989). They are more often found in biota because they are not readily metabolized compared to less chlorinated PCBs, but also do not tightly bind to soil and sediment as do congeners with high chlorination (McFarland and Clarke 1989). Polychlorinated biphenyl 153 (di-ortho) has been reported as the dominant PCB congener in many studies because it is not as easily biotransformed as other congeners (Safe 1984). It is also the most abundant congeners detected in turtle tissues in the current study. Other occasionally detected congeners (mono-ortho PCB 28 and 123, and di-ortho PCB 101 and 170) were only detected in one type of tissue and very few samples. Concentrations of major PCB congeners measured in red-eared sliders from on and near the PGDP were similar to or lower compared with concentrations reported by previous studies (e.g. Kannan et al. 1998, 2000).

Because only a few congeners were detected in tissue samples and the concentrations were low, association between PCB concentrations and hematological and immunological parameters were only evaluated for Aroclor 1260 and total PCBs. Total WBC count was negatively correlated with lipid-normalized concentrations of total PCBs in liver (Pearson Correlation $r = -0.909$, $p = 0.033$, n = 5), and heterophil count was negatively correlated with lipid-normalized concentrations of Aroclor 1260 in liver ($r = -0.890$, $p = 0.043$, n = 5; Fig. 2). Other hematological parameters including hematocrit, number of other WBCs, HL ratio, and PHA SI were not correlated with PCB concentrations.

Environmental contaminants can suppress immune system and reduce resistance to disease. It has been reported that organochlorine (OC) compounds, including PCBs, can suppress immunity in birds and mammals. In contrast, few studies have examined how contaminants influence reptilian immune system. In the current study, total WBC and heterophil counts were negatively correlated with PCB concentrations suggesting that PCB may suppress immune function in turtles. However, a study on loggerhead sea turtles reported that estimated WBC and HL ratio were positively correlated with concentrations of total PCBs and total TCDD-like PCBs, respectively (Keller et al. 2004b). In addition, previous studies involving birds and sea turtles suggest OC compounds may suppress T-cell mediated

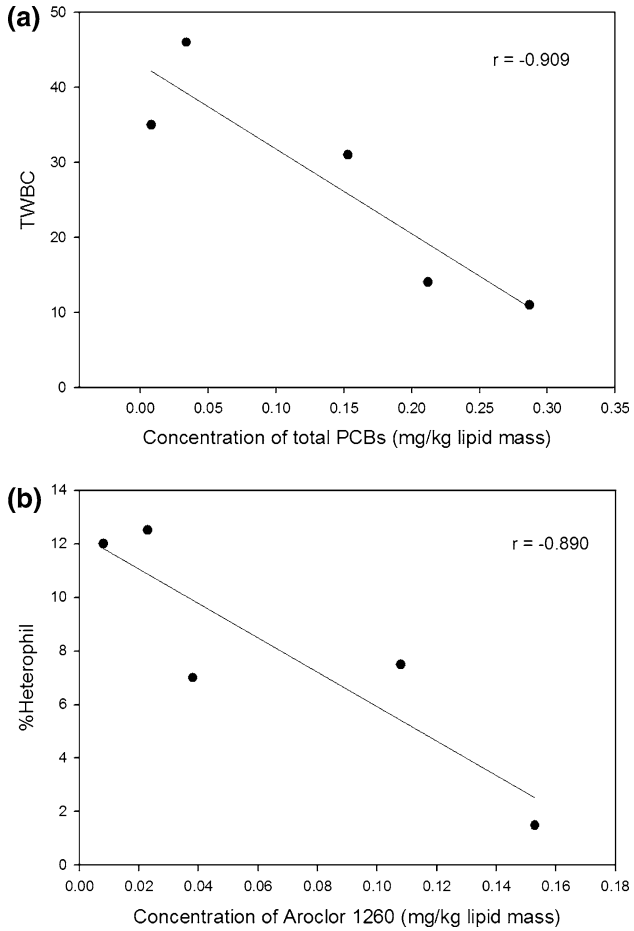

Fig. 2 **a** Correlations of total PCBs concentrations in liver tissue of red-eared slider turtles (*Trachemys scripta elegans*) collected during 2008 from ponds near the Paducah Gaseous Diffusion Plant, Kentucky, USA and total white blood cell count (TWBC; $p = 0.033$, n = 5). **b** Correlations of Aroclor 1260 concentrations in liver tissue of red-eared slider turtles (*Trachemys scripta elegans*) collected during 2008 from ponds near the Paducah Gaseous Diffusion Plant, Kentucky and percentage of heterophils ($p = 0.043$, n = 5)

immunity (Grasman et al. 1996) or enhance lymphocyte proliferation in peripheral blood (Keller et al. 2006). The inconsistencies between the current study and previous studies may be explained in several ways. First, immunological responses may differ among species. For example, Grasman et al. (1996) observed that two avian species had different relationship between the number of heterophils and lymphocytes and antibody responses; antibody response increased as the ratio of lymphocytes to heterophils increased in one species and antibody responses increased as the number of heterophils increased relative to the number of lymphocytes in another. Second, it is possible that immunological responses of aquatic turtles may differ depending on PCB concentrations. For example, low PCB concentrations may cause immunoenhancement while high concentrations cause immunosuppression (Keller et al. 2005). However, Keller et al. (2004b, 2006) measured blood PCB concentrations which can not be compared with tissue PCB concentrations measured in the current study. In addition, factors such as disease and other contaminants in the PGDP area may have affected the physiological condition of the turtles examined in the current study influencing the immunological effects observed.

In conclusions, PCB concentrations measured in red-eared slider turtles collected on or near the PGDP were low. However, chronic exposure to PCBs may have altered total WBC and heterophil counts. In addition, other confounding factors may have influenced observed immunological effects. It is still unclear how the reptilian immune system is modulated by contaminants and further study is warranted to evaluate effects of PCBs on hematology and immune function in turtles. It is also recommended that PCBs be evaluated in higher trophic level turtles (e.g. common snapping turtles) in the study area because they may have greater PCB accumulation and be at a greater risk of PCB toxicity than herbivore turtle species.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the experiments conducted in the current study comply with the current laws of the United States.

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