River otters as biomonitors for organochlorine pesticides, PCBs, and PBDEs in Illinois

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Abstract

The North American river otter (Lontra canadensis) is a biomonitor for organohalogenated compounds (OHCs) associated with a wide range of deleterious health effects in wildlife and humans. We determined concentrations of twenty OHCs in livers of 23 river otters salvaged by the Illinois Department of Natural Resources from 2009 to 2011, determined sex-dependent distribution of OHCs, and compared our results to the reported concentrations of four OHCs in Illinois river otters from 1984 to 1989. Since these contaminants have been banned for over 30 years, we predicted smaller mean concentrations than those previously reported in Illinois otters. We detected eleven of twenty OHCs; PCBs (polychlorinated biphenyls), dieldrin, and 4,4′-DDE (dichlorodiphenyldichloroethylene) were present in the greatest mean concentrations. We report the largest mean concentration of dieldrin to date in the liver of North American river otters (mean: 174, range: 14.4–128 000 ppb; p < 0.05). Mean concentrations of dieldrin were greater than those detected in females (mean: 851; range: 30–130 ppb; p < 0.01). OCP concentrations were significantly higher in males (mean: 851; range: 30–130 ppb; p < 0.01). Our results suggest OHC exposure remains a concern. Future research in Illinois should focus on evaluating OHC exposures, particularly dieldrin, at the watershed level.

1. Introduction

Organohalogenated compounds (OHCs) are of global concern due to their environmental persistence, bioaccumulative potential, and adverse effects on humans and wildlife (Bernanke and Köhler, 2008; Mnif et al., 2011). OHCs include three industrial chemical groups, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), as well as organochlorine pesticides (OCPs). OCPs are further divided into five groups: dichlorodiphenyltrichloroethane (DDT) and its analogs, isomers of benzene hexachloride (BHC), cyclodiene insecticides (including heptachlor, chlordane, and aldrin), caged structures (e.g. mirex and chlordane), and toxaphene (Smith, 1991).

Cyclodiene insecticides were applied in Illinois cornfields from 1953 until their use was banned in 1978. Peak use occurred in 1967 when approximately 5.6 out of 10 million acres of corn soil in Illinois were treated. Of the OCPs applied to Illinois soil, aldrin was applied extensively; an estimated 44.9 million acres of corn soil were treated between 1956 and 1977 (Steffey et al., 1984). The main epoxide of aldrin is dieldrin (Koerner et al., 1999). Havera and Duzan (1986) reported dieldrin to be the contaminant posing the greatest threat to the health of birds of prey in Illinois between 1966 and 1981. The authors found that dieldrin concentrations in the brains of five out of 57 raptors approached or exceeded the diagnostic lethal levels of 4000–5000 parts per billion (ppb).

As apex consumers in the aquatic ecosystem of Illinois, the North American river otter (Lontra canadensis) is vulnerable to the...
biomagnification of OHCS. Thus, otters serve as biomonitors (organisms that contain information on the quantitative aspects of the quality of the environment, Markert et al., 2003) of wildlife exposure to persisting OH concentrations. Otters also serve as local biomonitors for human health, as OHCS that bioaccumulate in river otters have also been detected in humans who consumed contaminated fish (Swain, 1991; Jacobson and Jacobson, 1996).

It is unknown if river otters exhibit sex-dependent accumulation patterns of OHCS, although it has been reported in marine mammals that consume large amount of fish (Hutchinson and Simmons, 1994; Bernt et al., 1999). For example, male seals accumulate OHCS while females lose part of their OH burden through placental transfer or lactation, resulting in lower accumulations in tissues. Differences in OH concentrations between sexes have also been reported in walrus, beluga, and porpoise (Norstrom and Muir, 1994).

Concentrations of four OHs in river otters collected from Northwest Illinois between 1984 and 1989 were reported by Halbrook et al. (1996). At the time of their study, the population of river otters was estimated at fewer than 100 in the state (Anderson, 1982). Following the successful translocation of 346 wild river otters to Illinois between 1994 and 1997, the population of river otters in the state of Illinois has recovered to its historical statewide distribution (Bluett et al., 2004). We expected concentrations of OHs in river otters salvaged from 2009 to 2011 to be lower compared to the levels detected 20–25 years ago, since use of these compounds has been banned.

The objectives of this study were to (1) determine the concentrations of twenty OHs in the livers of Illinois river otters from samples collected between 2009 and 2011, (2) determine the sex-dependent distribution of OHs, and (3) compare the OHs concentrations for heptachlor epoxide (HE), dieldrin, (DDE), and PCBs between this study and those reported between 1984 and 1989 in Illinois river otters.

2. Materials and methods

2.1. Necropsy evaluation

Carcasses of 23 incidentally killed river otters salvaged by the Illinois Department of Natural Resources were submitted for necropsy to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine at the University of Illinois at Urbana-Champaign. The originating locations for twenty of the 23 otters were known (Fig. 1). Otters were sexed and evaluated for general body condition including skin coat, extent of musculature and adipose tissue signs, and significant gross lesions. Endocrine and reproductive organs were measured and weighed. These organs included thyroid glands, adrenal glands, ovaries, and testes. When samples were available, we used three different proxies for age: tooth cementum analysis (n = 12), body mass (n = 23), and relative testes weight (n = 11). Canine teeth were submitted to Matson’s Laboratory (Mills, MT, USA) for age estimation based on the cementum analysis.

2.2. Chemical analysis

Liver samples collected during necropsy were submitted for OH analysis to the Michigan State University Diagnostic Center for Population and Animal Health in East Lansing, MI. Samples were analyzed for twenty OHs belonging to four groups: (1) the industrial chemicals PCBs and PBDEs; (2) the DDT related compounds, 4,4′-dichlorodiphenyltrichloroethane (4,4′-DDT), 4,4′-dichlorodiphenyldichloroethane (4,4′-DDE), 4,4′-dichlorodiphenylchloroethylene (4,4′-DDE); (3) the cyclodiene insecticides, heptachlor, heptachlor epoxide (HE), trans-nonachlor, oxychlordane, α-chlordane, γ-chlordane, and endosulam, endosulfane sulfate (ES), dieldrin, aldrin, endrin, and (4) benzenehexachloride (BHC) and its group of isomers, α-BHC, β-BHC, δ-BHC, and γ-BHC. Tissue samples were extracted as follows: a 5 g sample was mixed with 10 g diatomaceous earth and ground with mortar and pestle. The mixture was transferred to a 33 ml stainless steel cell, topped off with loose diatomaceous earth and loaded into an ASE 200 Extractor ( Dionex, a subsidiary of Thermo Scientific, Sunnyvale, CA). Extraction was performed with a timed pre-programmed combination of temperature and pressure using hexane:acetone, 9:1 (v/v). Extracts contained in glass collection vials were concentrated to approximately 1 ml with a stream of nitrogen gas and applied to silica gel columns. We used a lower detection limit (LDL) of 1.0 μg kg−1 wet wt (ppb) for PBDE and 0.4 ppb for all other OHs.

Analyses were performed on Varian (now part of Agilent Technologies, Santa Clara, CA) 3400 gas chromatographs (GC), each equipped with an electron capture detector (ECD). Both instruments were operated with helium carrier gas and nitrogen as ECD makeup gas. One instrument was equipped with a DB-1701 column (Agilent), and is referred to as 1701-GC in this paper; its column dimensions were 15 m length × 0.22 mm ID with a 0.25 μm film thickness. The 1701-GC was run with the following temperature settings: (1) injector 250 °C; (2) detector 300 °C; (3) oven program: 150 °C initial, held for 0.5 min, then increasing at 5 °C/min to 280 °C final temperature, which was held for 15 min (total run time, 38 min). The second instrument was equipped with a DB-608 column (Agilent), and is referred to as 608-GC in this paper; column dimensions were 30 m length × 0.32 mm ID with a 0.5 μm film thickness. The 608-GC was run with the following temperature settings: (1) injector 250 C; (2) detector 300 C; (3) oven program: 150 C initial, held for 0.5 min, then increasing at 12 C/min to 280 C final temperature, was held for 20 min (total run time, 31.3 min). Both instruments were run in splitless mode from 0.01 to 0.8 min. Positive results found on the 1701-GC in comparison to standards from Supelco, Inc. (now Sigma-Aldrich, St. Louis, Mo.) were confirmed on the 608-GC.

Analytical standards included the EPA CLP organochlorine pesticide mix (Sigma-Aldrich, St. Louis, MO; 2000 μg/ml; each component in hexane:toluene, 1:1; components included aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC (lindane), α-chlordane, γ-chlordane, 4,4′-DDT, 4,4′-DDE, 4,4′-DDD, dieldrin, endosulfane,
endosulfane sulfate, endrin, heptachlor and heptachlor epoxide, trans-nonachlor and oxychlordane were purchased as 100 μg/ml solutions in methanol (Chem Service, West Chester, PA). The PCB standard was Aroclor 1260 (1000 μg/ml in isooctane) from Sigma. PCBs were analyzed in comparison to a standard of Aroclor 1260 CAS# 11096-82-5 with focus on the nine most clearly characterized, i.e. well separated, peaks in the mixture of up to 209 possible PCB congeners. Specific structural assignments have not been made to these nine congeners. PBDE-99 (2,2,4,4′, 5-penta-bromodiphenyl ether) was selected as a sentinel compound for the PBDE class, owing to its consistent presence in biological samples (Nurvell et al., 2005; Basu et al., 2007) PBDE-99 was available from Sigma as a 50 μg/ml solution in isooctane.

The extraction and analytical method was based on the report by Price et al. (1986). Validation statistics in that report indicated relative standard deviations (percent RSDs) ranging from 2.2 percent to 8.2 percent for chlorinated pesticide quantitations and 2.4 percent for the same in PCBs based on the Aroclor 1260, and precision has been confirmed to be at least as good in the method reported here. Recovery was determined from blank liver samples spiked at 0.2 ppm chlorinated pesticides, PCBs and PBDE-99. Average tissue recovery for all chlorinated compounds except PCBs were 70.6 percent ± 9.2 percent to one standard deviation as determined on the 1701-GC, and 67.4 percent ± 17.2 percent on the 608-GC. PCB recoveries were 61.6 ± 12.6 percent on the 1701-GC and 70.1 percent ± 13.5 percent on the 608-GC which provided sufficient sensitivity to follow the presence of both chlorinated pesticides and PCBs in the examined population. This method has over many years successfully revealed and measured chlorinated pesticides and PCBs in a wide variety of animal matrices, including fat, brain, tissue, milk, feed, serum/plasma and blood. The 1701-GC and 608-GC retention times were reproducible from run to run with standard deviations ranging from 0.005 to 0.007 min as were typical linear standard curves with coefficient of determination, R2, of 0.999 or greater.

2.3. Statistical analyses

We tested the assumptions for normality of the OHC concentrations in males and females using the Shapiro–Wilk test. Significantly non-normal (p < 0.05) data were normalized. Only the distribution for oxychlordane was normal. We normalized heptachlor epoxide, trans-nonachlor, 4,4′-DDE, and dieldrin concentrations with a square root transformation, and PCB concentrations with a log transformation. When normalization was not possible, we used Welch’s rank sum test. We used ANOVAs to test the null hypothesis that mean concentrations of OHCs did not differ by sex. Otters without detectable concentrations were assigned a value of one half of the detection limit. hen analytes were detected at a frequency of greater than 50 percent, means were calculated using the assigned and detected concentrations for all 23 otters.

The raw data for the four OHCs detected by Halbrook et al. (1996) in eight Illinois river otters (HE, Dieldrin, DDE, and PCBs) were not available. In order to evaluate the differences in the mean concentrations of four OHCs between Illinois river otters (HE, Dieldrin, DDE, and PCBs) between the two studies (Table 2). We report the largest mean concentration of dieldrin (174 ppb; range: 14.4–534) yet reported in livers of the North American river otter (Elliot et al., 1999; Grove and Henny, 2008; Somers et al., 1987; Stansley et al., 2010). Recent studies on organohalogenated contaminants in L. canadensis in the United States report mean concentrations of 7.83 ppb dieldrin (range: 0.22–179) in New Jersey (Stansley et al., 2010) and means of 2.29 ppb dieldrin (range: 0.12–52.3) and 3.49 ppb dieldrin (range: 0.25–267) in juvenile and adult otters, respectively, from Oregon and Washington between 1994 and 1999 (Grove and Henny, 2008). These reports are considerably lower than our reported concentrations of dieldrin in Illinois river otters.

The remaining OHCs in the present study were detec ted at mean concentrations similar to or less than in previous studies. For example, the mean concentration of total PCBs (653 ppb; 30–3450 ppb) are slightly greater but have a smaller range of variation than the concentrations of total PCBs (540 ppb; 105–13,600 ppb) reported by Stansley et al. (2010). While our study is the second to report concentrations of total PBDEs in the livers of North American river otters. We detected a lower mean concentration and range (1.1 ppb; LDL–2.0 ppb) than previously reported (10.5 ppb; 10.1–35.3 ppb) (Stansley et al., 2010). While it is unclear whether this concentration can be associated with adverse health effects in otters, potential health risks associated with PBDE exposure include thyroid hormone disruption, neurological effects, and cancer in laboratory animals (McDonald, 2002).

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4. Discussion

4.1. Chemical analysis

We detected 11 of 20 contaminants and 12 out of 23 compounds detected in Illinois river otters (HE, Dieldrin, DDE, and PCBs) between the two studies (Table 2). We report the largest mean concentration of dieldrin (174 ppb; range: 14.4–534) yet reported in livers of the North American river otter (Elliot et al., 1999; Grove and Henny, 2008; Somers et al., 1987; Stansley et al., 2010). Recent studies on organohalogenated contaminants in L. canadensis in the United States report mean concentrations of 7.83 ppb dieldrin (range: 0.22–179) in New Jersey (Stansley et al., 2010) and means of 2.29 ppb dieldrin (range: 0.12–52.3) and 3.49 ppb dieldrin (range: 0.25–267) in juvenile and adult otters, respectively, from Oregon and Washington between 1994 and 1999 (Grove and Henny, 2008). These reports are considerably lower than our reported concentrations of dieldrin in Illinois river otters.

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The mean concentration of dieldrin in our study was only exceeded by the mean concentration of PCBs. This is not in accordance with previous studies of OHCs in river otters, which reported lower concentrations for dieldrin at lower concentrations relative to other OHCs. For example, Stansley et al. (2010) reported greater concentrations of PCBs, PBDEs, oxychlordane, DDE, and DDD than dieldrin in the livers of river otters collected from 2005 to 2007 in New Jersey. This comparatively greater concentration of dieldrin in Illinois river otters in relation to other OHCs is consistent with the historical use of aldrin in the United States (dieldrin is the primary breakdown product of aldrin). In 1966, states outside the Midwest accounted for only 2.7 percent of the aldrin used on corn (Jorgenson, 2001). With the exception of Halbrook et al. (1996), previous studies of OHCs in river otters have been outside of the Midwestern United States. The comparatively high concentration of dieldrin found in Illinois river otters in our study is also consistent with findings of The National Water Quality Assessment Program (NAWQA) that reported the highest concentration of dieldrin in fish tissue from the Sangamon River in Illinois (Groschen et al., 2013). Guidelines for dieldrin concentrations in aquatic life were exceeded in 30 percent of the samples from this basin and every concentration of dieldrin detected in this basin was in the highest ten percent of the national NAWQA results.

Although the mean concentration of dieldrin reported in this study is consistent with the historical use of aldrin, our finding with regard to dieldrin remains concerning. Findings of studies on the health effects of dieldrin have been inconsistent, although a number of deleterious health effects have been associated with
from 1984 to 1989. The present study collected from 2009 to 2011 and the study of Halbrook et al. collected sublethal concentrations of dieldrin (Jorgenson, 2001). Singh et al. percent con

Comparison of mean concentrations in parts per billion wet weight (ppb) and 95

Table 2

<table>
<thead>
<tr>
<th>OHC</th>
<th>All otters</th>
<th>Males (n=15)</th>
<th>Females (n=8)</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>d0</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>PCB</td>
<td>653 ± 811</td>
<td>15</td>
<td>851 ± 924</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>174 ± 123</td>
<td>15</td>
<td>200 ± 135</td>
</tr>
<tr>
<td>4,4′-DDD</td>
<td>117 ± 103</td>
<td>15</td>
<td>125 ± 97</td>
</tr>
<tr>
<td>trans-Nonachlor</td>
<td>15 ± 66.5</td>
<td>15</td>
<td>70.5 ± 66.8</td>
</tr>
<tr>
<td>Oxychlorane</td>
<td>66.2 ± 48.6</td>
<td>15</td>
<td>65.3 ± 43.3</td>
</tr>
<tr>
<td>HE</td>
<td>29.6 ± 25.4</td>
<td>14</td>
<td>27.9 ± 24.8</td>
</tr>
<tr>
<td>4,4′-DDD</td>
<td>119 ± 27.4</td>
<td>9</td>
<td>10.4 ± 21.8</td>
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<td>PBDE</td>
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<tr>
<td>α-Chlordane</td>
<td>2</td>
<td></td>
<td>&lt; LDL–1.4</td>
</tr>
<tr>
<td>Endrin</td>
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<td></td>
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<tr>
<td>ES</td>
<td>1</td>
<td></td>
<td>&lt; LDL–1.9</td>
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* Significance level p < 0.05.

Table 2 Comparison of mean concentrations in parts per billion wet weight (ppb) and 95 percent confidence intervals of four OHCs in Illinois river otters between the present study collected from 2009 to 2011 and the study of Halbrook et al. collected from 1984 to 1989.

sublethal concentrations of dieldrin (Jorgenson, 2001). Singh et al. (2012) identified dieldrin as a pesticide associated with the risk of Alzheimer’s disease in the north Indian population. Hoyer et al. (1998) found that women with concentrations of dieldrin in their blood had a 200 percent increased risk of developing breast cancer, while Xu et al. (2010) did not find positive association between serum concentrations of dieldrin and breast cancer prevalence, they did report dieldrin to be significantly associated with the prevalence of prostate cancer. There is also evidence that exposure to OCPs including dieldrin may increase the risk of Parkinson’s disease (Van der Mark et al., 2012).

Dieldrin also acts as a developmental neurotoxicant. The central nervous system is a critical target for dieldrin in humans. Current evidence suggests that this OHC alters dopamine levels, induces mitochondrial dysfunction, protein aggregation, and activates a series of cell death signaling molecules in the brain (Kanthasamy et al., 2005). During early stages of fetal development, exposure to developmental neurotoxicants such as dieldrin can cause brain injury at doses considerably lower than doses that influence adult brain function (Grandjean and Landrigan, 2006). There are currently no fish consumption guidelines with respect to dieldrin in Illinois (IDNR, 2012).

4.2. Mean concentrations by sex and age

One other study has evaluated sex-dependent distributions of OHCs in the North American river otter (Stansley et al., 2010). Those authors did not detect sex-related differences in tissue accumulations of OHCs in river otters collected in New Jersey from 2005 to 2007. To the contrary, we detected significantly higher concentrations of PCBs in males than in females, a finding consistent with studies of sex-dependent distributions of PCBs in other carnivores, such as seals and red foxes (Bernt et al., 1999; Dip et al., 2003). The observed differences by sex may be attributable to larger home ranges of males (e.g. Gorman et al., 2006), differences in diet between the sexes (Blundell et al., 2002), or transfer of the compounds from mothers to young via pregnancy and lactation as has been documented in other species (Bytingsvik et al., 2012). The same factors may have contributed to our finding of a greater proportion of males compared to females with detectable concentrations of DDD and PBDEs. Since canine teeth for cementum analysis were not available for all individuals in our sample we were unable to determine if the proportion of adult males affected the gender comparison. Furthermore, no studies have been published on the movements of river otters in Illinois watersheds to determine what the difference between the sexes may be in terms of exposure to environmental pollutants. One weakness of this study was the incomplete information on the age of the otters in our sample; while we used three proxies for age, a future study based on known age is warranted.

4.3. Concentrations of HE, 4,4′-DDE, HE, and dieldrin in the present study and Halbrook et al. (1996)

Use of chlorinated pesticides has been banned in Illinois for over 30 years; we therefore expected to find less HE, PCB, DDE and dieldrin concentrations from the otters from the present study compared to otters collected in Illinois approximately 25 years ago. There was no significant difference in mean concentrations of HE, PCB, or DDE between the studies and significantly greater mean...
concentration of dieldrin in the present study. This finding is contrary to temporal trends from 1974 to 1993 in concentrations of PCBs in livers of adult herring gulls (Larus argentatus) in the Great Lakes. Authors of that study found that the bioavailability of PCBs, DDE, and dieldrin decreased at most of the sites in the study (Desforges et al., 2012). Additionally, the range of the concentrations of PCBs, dieldrin, 4,4'-DDE, and HE was greater in this study compared to the ranges reported by Halbrook et al. (1996) (Table 2).

At the time of the present study, no trapping season for river otters in Illinois was in place, limiting the available samples. Our sample size contributed to decreased statistical power and therefore a decrease in the ability to detect a difference where there was one (Zar, 1999). In spite of low power, we detected significantly greater concentrations of PCBs in males and a greater mean concentration of dieldrin in otters from the present study compared to the only report of dieldrin in otters collected from Illinois (Halbrook et al., 1996). However, differences in geographic origin of the Illinois river otters between the present study and Halbrook et al. (1996) (Fig. 1) may explain lack of differences in mean concentrations of HE, DDE, and PCBs, as well as a greater mean concentration of dieldrin. Although Halbrook et al. (1996) did not specify the geographic origin of the otters in their sample, at the time of their study, river otters were a state endangered species with small remnant populations in the northwestern corner and southern portions of Illinois. In contrast, the originating counties of the otters in our sample for which we had location-related information were in central Illinois (Fig. 1). Although the differences in OHC exposure between watersheds in Illinois are not well understood, characteristics of sampling locations have been documented to influence levels and profiles of contaminants in mustelids and OHCs in other biota (Maes et al., 2008; Kamman et al., 1999, 2002; Van den Steen et al., 2008). Continued focus on OHC concentrations in river otters inhabiting different watersheds of Illinois may help elucidate geographical differences in OHC profiles throughout different watersheds in the state.

The half-life of dieldrin in soil has been reported to range from 4.6 years to 25 years (McDougall et al., 1995; Meijer et al., 2001). Therefore, during years of peak use, the concentrations of dieldrin in Illinois soil may have been higher than the present day concentrations. Intensive aldrin and dieldrin use has been implicated in the decline of the Eurasian river otter (Lutra lutra) in England, which occurred during the mid-20th century (Jefferies and Hanson, 2000). It is unclear if dieldrin likewise hindered the recolonization of river otters in central Illinois.

Due to the lack of reported controlled or experimental studies to evaluate the effect of PCBs, PBDEs, or OCPs on the health of the North American river otter, and the lack of information on normal sizes and weight of endocrine organs, we are unable to establish the health effects of the concentrations of the OHCs detected on river otters. Reduced kit survival of mink (Neovison vison), another mustelid, has been associated with a lowest observable effect concentration (LOAEC) of 3.13 ppm PCBs wet wt. in maternal livers (Bursian et al., 2006). This concentration was exceeded by one otter in our sample. Restum et al. (1998) reported 0.98 ppm PCBs as the LOAEC in maternal livers for reduced kit body weight; this concentration was exceeded by four otters from our sample. However, the lack of controlled studies on river otters limits our ability to extrapolate effects on mink to river otters. Furthermore, opportunities for histopathological assessment of health in tissues were impacted by the frozen state of tissues prior to necropsy.

5. Conclusions

The ranges and means of OHC concentrations we detected in the North American river otter highlight the need to understand the exposure of wildlife and humans to OHCs at the watershed level in Illinois. In particular, exposure to the OHC dieldrin remains as a concern in Illinois. Future studies to compare total PCB concentrations among age and sex classes will provide insight into the possibility of maternal transfer of PCBs in Illinois otters. Future studies to evaluate the diet and home range of river otters in Illinois can also shed light on the underlying biology of the significantly greater mean concentration of PCBs we detected in male river otters.

Acknowledgments

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