Bioaccumulation of polychlorinated dibenzo-\(p\)-dioxins, dibenzofurans, and dioxin-like polychlorinated biphenyls in fishes from the Tittabawassee and Saginaw Rivers, Michigan, USA

Yi Wan\(^a,\)*, Paul D. Jones\(^a\), Ryan R. Holen\(^b\), Jong Seong Khim\(^c\), Hong Chang\(^a\), Denise P. Kay\(^b\), Shaun A. Roark\(^b\), John L. Newsted\(^b\), William P. Patterson\(^d\), John P. Giesy\(^a,\)\(^e,\)\(^f,\)\(^g\)

\(^a\) Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5B3
\(^b\) ENTRIX, 4295 Okemos Road, Okemos, MI 48864, USA
\(^c\) Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, South Korea
\(^d\) Saskatchewan Isotope Laboratory, Department of Geological Sciences, University of Saskatchewan, 114 Science Place, Saskatoon, Saskatchewan, Canada S7N 5E2
\(^e\) Department of Zoology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
\(^f\) Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, China
\(^g\) College of Environment, Nanjing University of Technology, Nanjing 210009, China

**Abstract**

Characterizing biological factors associated with species-specific accumulation of contaminants is one of the major focuses in ecotoxicology and environmental chemistry studies. In this study, polychlorinated dibenzo-\(p\)-dioxins (PCDDs), dibenzofurans (PCDFs), and non- and mono-ortho-substituted polychlorinated biphenyl (PCB) congeners were analyzed in various fish species from the Tittabawassee and Saginaw Rivers (12 fish species; \(n = 314\) individuals), Michigan, USA. Due to their migratory habits, greater \(\delta^{13}C\) stable isotope values were found in walleye and white sucker among 12 fish species. Meanwhile, the \(\delta^{15}N\) values indicated that the trophic status was least in carp and greatest in largemouth bass. The greatest total concentrations of dioxins were found in fishes with the lowest trophic status (carp \((n = 50)\) followed by channel catfish \((n = 49)\)), and concentrations of \(\Sigma\)PCDD/Fs (20–440 pg/g ww (wet weight)), \(\Sigma\)PCBs (16–690 ng/g ww), and TEQs (6.8–350 pg/g ww) in carp were also greater than the least mean concentrations in other fishes. Contributions of various biological factors to the species accumulation were assessed. Body weight and lipid content were found to be the most significant factors influencing accumulation of \(\Sigma\)PCDD/Fs. Lipid content and trophic level seemed to be dominant factors determining accumulation of \(\Sigma\)PCB and TEQs, but negative correlations between trophic status and concentrations of \(\Sigma\)PCBs and TEQs were observed possibly due to the great concentrations in benthivorous fishes such as carp occupying lower trophic levels. These factors can be used to predict the contaminant levels of dioxins and health risks of the fishes in the river ecosystem.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

A number of previous studies have highlighted the importance of chemical and biological factors in bioaccumulation and trophic transfer of persistent organic pollutants (POPs) in aquatic ecosystems (Fisk et al., 2001; Hop et al., 2002; Borgå et al., 2004), and the factors causing differential accumulation among species have become a major focus of ecotoxicology and environmental chemistry studies (Borgå et al., 2004). While the importance of trophic transfer and related factors in aquatic ecosystems have been well documented for organochlorine compounds, including polychlorinated biphenyls (PCBs) (Fisk et al., 2001; Hop et al., 2002; Kidd et al., 1998; Mackintosh et al., 2004), few studies have addressed the factors influencing species-specific bioaccumulation of polychlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs) in riverine ecosystems.

PCDDs, PCDFs, non- and mono-ortho-substituted PCBs are groups of classic POPs that accumulate in animal tissues due to their persistence and lipophilicity. Understanding the primary factors influencing bioaccumulation of those compounds in fishes from rivers is critical for predicting and assessing risks to upper-trophic level consumers including humans (McIntyre and Beauchamp, 2007). There is well-established evidence that trophic position and lipid content are important predictors of PCBs and other organochlorines in aquatic organisms (Kidd et al., 1998; Mackintosh et al., 2004, Walters et al., 2008). For example, previous studies of trophic transfer of dioxins in marine food webs have been conducted using the analysis of stable
isotopes of nitrogen to provide continuous trophic level estimates (Broman et al., 1992; Naito et al., 2003; Wan et al., 2005; Ruus et al., 2006). These results demonstrated that trophodynamics of dioxin-like PCBs (dl-PCBs) were similar to those of other organochlorines, but weak negative correlations were found between lipid-normalized PCDD/Fs concentrations and trophic level estimates (Naito et al., 2003; Wan et al., 2005). Thus, the factors influencing the trophic transfer of PCDD/Fs would be different from those affecting other organochlorines including PCBs. Although the above tropho-dynamic studies of dioxins were conducted for whole marine food webs from primary producers to top predators, no consistent relationships were found within more closely associated groups of organisms such as fish. Considering the ecological and economic importance of fish in riverine ecosystems and the impact of fish consumption on human health risks, studies looking for the factors associated with trophic transfer of dioxins and related compounds in fishes would be of great value for predicting risks in terms of trophic transfer to higher trophic level organisms on a site-specific basis.

The Saginaw and Tittabawassee Rivers have both been impacted by historical industrial activities. Several studies have shown the presence of dioxins, furans, PCBs, and other chemicals in the rivers, with furans being the dominant class of contaminants in the Tittabawassee River, while the Saginaw River is characterized by elevated PCBs (Hilscherova et al., 2003; Kannan et al., 2008). Dioxins and furans have been shown to mainly originate from a mixture of chlorophenol and other chlorinated compound production, and multiple sources of PCBs exist in the watershed, such as discharges from historical industrial operations, effluents of wastewater treatment plants, etc. (Hilscherova et al., 2003; Kannan et al., 2008). To provide additional data for human health risk assessment, 7 PCDDs, 10 PCDFs and 12 non- and, mono-ortho-substituted PCBs were measured in twelve fish species (n = 314 individuals) collected from the Tittabawassee and Saginaw Rivers. The trophic status and nutrition origin of these fishes were evaluated by stable nitrogen and carbon isotope analysis. This study aimed to determine species-specific accumulation characteristics by addressing the relationships between biological factors, such as trophic level, body size and lipid content, and measured concentrations of dioxin-like chemicals in various fish species in aquatic ecosystems.

2. Materials and methods

2.1. Fish species

The fishes studied were those that are preferentially harvested by anglers and the selection of species was based on the information from creel surveys conducted by state and federal agencies as well as local fisheries experts (Michigan DNRE reports; Michigan Fish contaminant monitoring program online database). The fishes investigated included walleye (Sander vitreus, n = 44), white sucker (Catostomus commersoni, n = 15), white bass (Morone chrysops, n = 15), smallmouth bass (Micropterus dolomieu, n = 58), largemouth bass (Micropterus salmoides, n = 20), channel catfish (Lctalurus punctatus, n = 49), carp (Cyprinus carpio, n = 50), freshwater drum (Aplodinotus grunniens, n = 20), black crappie (Pomoxis nigromaculatus, n = 9), northern pike (Esox lucius, n = 12) bluegill (Lepomis macrochirus, n = 12), green sunfish (Lepomis cyanellus) and pumpkinseed (Lepomis gibbosus) (n = 10 for both of the last two species, Supplemental material Fig. 1, and Supplemental material Table 1). White sucker, white bass and walleye (migratory species) were collected April 2 – 3, 2007, and the other species were collected May 7 – 29, 2007.

2.2. Sample collection

The Saginaw River and Bay receive discharges from 87 industrial facilities and 127 wastewater treatment plants, and the Tittabawassee River is the largest tributary to the Saginaw River (Hilscherova et al., 2003). Pollution with PCDD/Fs in this area is one of the major chemical concerns identified by the Michigan Department of Public Health (Michigan DNRE reports). Sampling locations (six reaches) were selected based on the factors including, but not limited to, the presence of desirable habitat for fish, angler access, and geographic landmarks such as bridges to serve as location boundaries. Fish were collected, using standard electro-fishing equipment (Smith-Ro®t®u™, Vancouver, WA, USA) and techniques, from six distinct river reaches, four in the Tittabawassee River and two in the Saginaw River (Supplemental material Fig. 1 and Supplemental material Table 1). Upon collection, all fish were transferred to clean coolers containing ice and were transported to a field laboratory for further processing. All fish were evaluated for general health and overt abnormalities, measured, weighed, and photographed. The edible tissue (fillets) was then removed from each fish using methods outlined in U.S. EPA protocol (EPA 823-B-00-007, 2000). Briefly, the rib cage bones and skin were removed from each fillet and the lateral line was kept intact (i.e., not trimmed). Fillets were then deposited into chemically-clean foil or l-Chem jars and frozen at -20 °C. Fish tissues were homogenized using freeze-fracturing techniques, i.e., individual fillets were placed in chemically-cleaned stainless steel food processors (Robot Coupe®) and small amounts of liquid nitrogen were added until a small granular-like consistency was reached. The homogenized fillet tissue was then deposited into chemically-clean l-Chem® jars and frozen at -20 °C until shipment to the analytical laboratories.

2.3. Sample preparation

PCDD/Fs and PCBs were analyzed by isotope dilution following EPA methods 1613 and 1668, respectively (EPA Method 1613, EPA Method 1668). Samples (approximately 10–20 g wet weight (ww)) were mixed with anhydrous sodium sulfate and allowed to dry for 12–24 h. The samples were fortified with a mixture of 13C-labeled PCDDs, PCDFs and non- and mono-ortho-substituted PCB surrogates (Wellington Laboratories, Guelph, ON), and were then Soxhlet extracted with 400 ml of 1:1 hexane/dichloromethane for 16 h. The surrogate mixtures contained 7 13C-PCDDs, 10 13C-PCDFs and 12 13C-dl-PCBs, and 1 ml was added into each sample with concentrations of 2 ng/ml, 4 ng/ml and 2 ng/ml for 13C-labeled tetra- to hepta-CDD/Fs, 13C-OCDD/F and 13C-dl-PCBs, respectively. Extracts were rotary evaporated to constant weight at 35 °C, and the lipid content of each extract was determined gravimetrically. Extracts were then dissolved in 100 ml hexane, and treated with 20 ml of concentrated sulfuric acid three times in a separatory funnel. The retained upper hexane layer was then rinsed with two 20 ml aliquots of nanopure water before being dried by passage through anhydrous sodium sulfate. The extract was then concentrated to approximately 2 ml and sequentially subjected to silica gel, neutral alumina, and activated carbon-impregnated silica gel chromatography as described in the EPA methods (EPA Method 1613, 1994; EPA Method 1668, 1999). The silica gel packed glass column was packed with 2 g of silica gel, 2 g of silica gel impregnated with sulfuric acid (60% w/w), and 2 g of silica gel, in that order. After application of the sample the column was eluted with 150 ml hexane. The hexane eluate was concentrated and passed through a neutral alumina column (4 g sodium sulfate, 4 g neutral alumina, and 4 g sodium sulfate), eluted with 20 ml hexane and then with 25 ml 60% dichloromethane in hexane. The second fraction was concentrated and passed through an activated carbon-impregnated silica gel column (in the order of 0.5 g of activated carbon dispersed silica gel), and eluted with 100 ml of hexane, 100 ml 20% dichloromethane in hexane and 100 ml toluene. Two different final extracts were prepared for analysis of PCDD/Fs and dl-PCBs. The final eluent of
the carbon column was concentrated and fortified with $^{13}$C-1,3,6,8-TeCDF and $^{13}$C-1,2,3,4,6,8,9-HpCDF (Wellington Laboratories, Guelph, ON) for analysis of PCDs and PCDFs. The first fractions from the alumina column and all the early fractions of the carbon columns were combined and fortified with $^{13}$C-PCB 52, $^{13}$C-PCB 101, and $^{13}$C-PCB 138 (Wellington Laboratories, Guelph, ON) for analysis of dioxin-like PCBs.

2.4. Instrumental conditions

Identification and quantification of PCDD/Fs and dioxin-like PCBs were performed using a Hewlett-Packard 5890 series high-resolution gas chromatograph interfaced with a Micromass® Autospec® high-resolution mass spectrometer (HRGC–HRMS) (Micromass®, Beverly, MA). A split/spillless injector was used in splitless mode. Chromatographic separation was achieved on a DB-5MS fused silica capillary column (60 m length, 0.25 mm ID, 0.1 μm film thickness, Agilent, CA). After a 30 s hold at 110 °C the column oven was programmed to 180 °C at a rate of 22 °C/min, then to 235 °C at 2 °C/min and was held there for 4 min before rising at a rate of 8 °C/min to 330 °C where it was held for 3 min. PCB congeners were separated on the same column. After an initial hold of 2 min the oven temperature was raised to 150 °C at a rate of 15 °C/min, then to 270 °C at a rate of 3 °C/min, and then at 40 °C/min to 300 °C followed by a final 5 min hold. The mass spectrometer was operated in a Selected Ion-Monitoring (SIM) mode. The resolution for all reference gas peaks in all time windows was more than 10,000 for PCDD/Fs, and more than 8000 for PCB congeners. The injector temperature was held at 285 °C and the ion source was kept at 285 °C. The electron-impact ionization energy was 37 eV and the ion current was 750 μA.

2.5. Stable isotope analysis

Stable carbon and nitrogen isotope analyses were conducted for 98 individual fish selected from all locations, and fish size was selected to be within the similar range for each species (Supplemental material Table 2). Samples were homogenized by an analytical mill and then freeze-dried. Approximately 1 g of each dry sample was combined with 12 ml of methanol and was allowed to stand for 12 h to reduce variability due to isotopically lighter lipids. After 12 ml of methanol was removed, the samples were rinsed with 10 ml of methanol twice, and then dried at 80 °C for 4 h. Subsequently, the samples were acidified to remove carbonate materials and were then homogenized and loaded into tin capsules, and combusted at 1000 °C where organic materials were oxidized to carbon dioxide, various nitrogen bearing gases, and water. The carrier gas was passed through a reduction furnace packed with elemental copper and a water trap, which separated the gases, and water. The carrier gas was passed through a reduction furnace packed with elemental copper and a water trap, which separated the gases, and water. The carrier gas was passed through a reduction furnace packed with elemental copper and a water trap, which separated the gases, and water.

The $^{12}$C/$^{13}$C and $^{15}$N/$^{14}$N standard values were based on atmospheric $N_2$ (air) and Vienna Peedee belemnite (VPDB), respectively. $^{13}$C and $^{15}$N measurements are 0.1‰ and 0.4‰, respectively ($n = 38$), and data was blank corrected.

2.6. Quality assurance and quality control (QA/QC)

The QA/QC was conducted following EPA methods (EPA Method 1613, 1994; EPA Method 1668, 1999). Concentrations of all congeners were quantified by the internal standard isotope-dilution method using mean relative response factors determined from standard calibration runs. All equipment rinses were carried out with acetone and hexane to avoid sample contamination and a laboratory blank, a matrix spike and a certified reference material (Wellington Laboratories Inc., Guelph ON) were incorporated in the analytical procedure for every batch of 20 samples. Recoveries of $^{13}$C-labeled PCDD/Fs and dioxin-like PCBs internal standards were within ranges specified by the EPA methods, 17–19% and 25–125% for $^{13}$C-labeled PCDD/Fs and dl-PCBs, respectively (EPA Method 1613, 1994; EPA Method 1668, 1999). The detection limits are sample specific but were generally around 0.2 pg/g ww for TeCDF/Fs, PeCDF/Fs, HxCDD/Fs and HpCDF/Fs, 0.4 pg/g ww for OCDD and OCFD, and 1 pg/g ww for PCBs.

Carbon isotope ratios were corrected for $^{17}$O contribution and reported in part per million notation relative to the VPDB scale. Nitrogen isotope ratios were reported in part per million notation relative to air. The standards used in the analysis were purchased from the IAEA (International Atomic Energy Agency) and the USGS (United States Geologic Survey). Carbon data was calibrated against the international standards L-SVEC ($d^{13}$C = −46.6‰ VPDB) and IAEA-Ch6 ($d^{13}$C = −10.45‰ VPDB). IAEA-Ch7, an intermediate international standard, gave the following result during calibration of the in-house standards: $d^{13}$C = −32.14 ± 0.03‰ VPDB ($n = 12$), which is acceptable compared to the value of $d^{13}$C = −32.15 ± 0.10‰ VPDB. Nitrogen data was calibrated against the international standards USGS-25 ($d^{15}$N = −30.4‰, AIR) and IAEA-305A ($d^{15}$N = 39.8‰, AIR), IAEA-N03, an intermediate international standard, gave the following result during calibration of the in-house standards: $d^{15}$N = 3.96 ± 0.08‰ AIR ($n = 8$), which is acceptable compared to the value of $d^{15}$N = 4.7 ± 0.2‰ air. Accuracies of data were monitored through routine analyses of in-house standards which are stringently calibrated against the IAEA standards mentioned above. Accuracy of $d^{13}$C and $d^{15}$N measurements are 0.1‰ and 0.4‰, respectively ($n = 38$), and data was blank corrected.

2.7. Data analysis

Differences in concentrations of ΣPCDD/Fs, ΣPCBs, and TEQs among fishes were compared using one-way Analysis of Variance (ANOVA) on log$_{10}$ transformed data (Wan et al., 2006). Levene’s test was used to check the equality of variances (the value of significance is less than 0.05). Where variances were equal, data were analyzed by the F test. Where the equality of variances could not be assumed, Welch’s and Brown–Forsythe’s robust tests were used to perform one-way ANOVA analysis (Field, 2005). Multiple paired comparisons were used to determine which means differed from one another. Tukey’s Honestly Significant Differences (HSD) was used where variances were presumed to be equal, and the Games–Howell test was used where equality of variances could not be assumed (SPSS 11, SPSS Inc., Chicago, IL). The effects of trophic level, carbon source, lipid content, and body weight on ΣPCDD/Fs, ΣPCBs, and TEQs in fishes were assessed by Multiple Linear Regression (MLR). Multicollinearity of the independent variables was not a problem given that the variance inflation factor (VIF) values were all <4 (Stine, 1995). Percent lipid, body weight, and chemical concentrations were log$_{10}$ transformed prior to analysis, and when the p value was less than 0.05, the linear regression was regarded as significant and only parameters that were significant at the 0.10 significance level were retained. SPSS 11.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

In the case of missing concentration values (e.g., less than the detection limit), ProUCL (US EPA, ProUCL 4.00.02) values were generated by a bootstrap method based on the distribution of all samples. Results of all analyses based on “0”, the actual detection limit
The relative toxic potencies of dioxins in fishes were assessed by calculating 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent concentrations (TEQs). Concentrations of TEQ were calculated as the sum of the products of the concentrations of each congener multiplied by its respective 2006 World Health Organization (WHO) Toxic Equivalency Factors (TEFs) for mammalian risk assessment (Van den Berg et al., 2006) because the fish collection and analysis were conducted with the primary purpose of estimating risk to humans who might consume the fish. Unless otherwise noted, ΣPCDD/Fs and ΣPCB data are reported on a concentration basis (pg/g, ww). The concentrations of 2,3,7,8-equivalents (TEQs) is the sum of TEQ contributed by PCDD, PCDF, and PCB congeners. The calculation details are given in the Supplemental material.

3. Results and discussion

3.1. Level differences among species

Concentrations of PCDD/Fs and PCBs measured in 12 fish species from the Tittabawassee and Saginaw Rivers (Table 1 and Fig. 1) varied greatly among species. ΣPCDD/Fs in carp (Mean ± SD = 1.1 × 10^7 ± 86 pg/g ww) was significantly greater than in other species (p < 0.01), and the next greatest concentrations were found in channel catfish (45 ± 39 pg/g ww) and northern pike (45 ± 28 pg/g ww), which were significantly greater than those in the remaining species (p < 0.01) except white bass (22 ± 11 pg/g ww). The relatively large variability observed in the reported data can be related to the considerable size range of individuals sampled which probably represent at least 2 and probably more year classes, for example the length range of carp in this study was between 339 and 778 mm. The least concentrations of ΣPCDD/Fs were measured in bluegill (3.2 ± 1.8 pg/g ww) and freshwater drum (4.0 ± 2.7 pg/g ww), both of which contained significantly less than white sucker (13 ± 6.9 pg/g ww), smallmouth bass (15 ± 11 pg/g ww), white bass, northern pike, channel catfish and carp. Concentrations of ΣPCBs ranged from 21 ± 12 ng/g ww (‘black crappie) to 190 ± 180 ng/g ww (carp) (Table 1 and Fig. 1). Concentrations of ΣPCBs in carp were significantly greater than those in other fishes (p < 0.01) except channel catfish (130 ± 91 ng/g ww) and white bass (82 ± 54 ng/g ww). While ΣPCBs in northern pike (36 ± 25 ng/g ww) were not particularly great, those in channel catfish were significantly greater than those in other fishes (p < 0.01) except carp, white bass and sunfish (56 ± 38 ng/g ww). Concentrations of TEQs in two species, carp (54 ± 59 TEQs pg/g ww) and channel catfish (34 ± 28 TEQs pg/g ww) were significantly greater than those in other species (p < 0.01), which contained concentrations from 1.7 ± 1.5 to 13 ± 11 TEQs pg/g ww.

Some previous studies reported that concentrations of dioxin-like compounds were generally within similar ranges among fishes in riverine ecosystems (Walters et al., 2008; Stachel et al., 2007; Han et al., 2007; Kay et al., 2005). However, the average concentrations of ΣPCDD/Fs, ΣPCBs, and TEQs in carp in this study were approximately 30-, 9.0- and 30-fold greater than those from the least contaminated other fishes, respectively. When concentrations in carp were normalized to lipid content, there were still 9.0 and 16-fold differences when average concentrations of TEQs and ΣPCDD/Fs were compared, while the difference for ΣPCBs were 3.2-fold. Similar concentrations of PCDD/Fs and PCBs in channel catfish and carp compared to other species (rock bass, pumpkinseed, black crappie, perch, brown bullhead, white sucker, eel, and rainbow smelt) have been observed in previous investigations in the Great lakes and their tributaries (Harless et al., 1982; Okeefe et al., 1984; Ryan et al., 1984) and these two species served as indicators of the presence of these contaminants in these areas. In marine ecosystems, species-specific accumulation characteristics for PCDD/Fs have also been reported previously in some fishes (eel, cod, sea trout, flounder, mackerel and herring) collected from Greenland fjords (Knutzen et al., 2003). Those authors attributed this observation to differential uptake and metabolism among species. No additional studies were conducted to determine reasons for differences in accumulation of persistent chemicals, although an understanding of these mechanisms is important for risk prediction and assessment for higher trophic level organisms.

3.2. Patterns of relative concentrations among species

The sums of concentrations of PCDDs were greater than those of PCDD/Fs in all fish species, and the PCDDs/PCDD/Fs concentration ratio in northern pike (195 ± 9.9, p < 0.01, ANOVA) was particularly great, compared with other fishes (3.5 ± 1.5–11.9 ± 3.8). The spatial distributions of congeners patterns are discussed elsewhere (Holm et al., submitted for publication), and no significant pattern differences were found. For all PCDD/Fs congeners, 51–95% of ΣPCDD/Fs were contributed by 2,3,7,8-TCDF (13–65%), 2,3,4,7,8-PeCDF (81–42%), 1,2,3,7,8-PeCDF (5.1–12%) and 2,3,7,8-TCDD (2.9–17%). Relatively great proportions of these four congeners have also been found in sediment from the Tittabawassee River (Kannan et al., 2008). The congener patterns of carp and channel catfish were different from other species. The proportion of 2,3,7,8-TCDF was relatively less and proportions of more chlorinated congeners (e.g., 1,2,3,4,6,7,8-HpCDD and OCDD) were relatively great. Carp and channel catfish contained significantly greater concentrations of

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Lipid (%)</th>
<th>Body weight (g)</th>
<th>δ13C</th>
<th>δ15N</th>
<th>ΣPCDDs</th>
<th>ΣPCDFs</th>
<th>ΣPCBs</th>
<th>ΣTEQs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>9</td>
<td>3.25 ± 1.41</td>
<td>217 ± 88</td>
<td>15.85 ± 1.56</td>
<td>−25.79 ± 1.16</td>
<td>1.4 ± 1.1</td>
<td>63.3 ± 3.5</td>
<td>14 ± 8.1</td>
<td>21 ± 12</td>
</tr>
<tr>
<td>BG</td>
<td>12</td>
<td>2.59 ± 2.58</td>
<td>124 ± 32</td>
<td>15.08 ± 0.39</td>
<td>−26.42 ± 1.84</td>
<td>0.7 ± 0.4</td>
<td>24.5 ± 1.5</td>
<td>14 ± 13</td>
<td>23 ± 19</td>
</tr>
<tr>
<td>CA</td>
<td>50</td>
<td>7.25 ± 6.08</td>
<td>2848 ± 963</td>
<td>12.63 ± 0.88</td>
<td>−26.18 ± 1.81</td>
<td>25 ± 26</td>
<td>85 ± 20</td>
<td>130 ± 120</td>
<td>190 ± 180</td>
</tr>
<tr>
<td>CC</td>
<td>50</td>
<td>5.67 ± 2.65</td>
<td>1026 ± 567</td>
<td>14.01 ± 0.54</td>
<td>−24.13 ± 1.31</td>
<td>11 ± 7.6</td>
<td>35 ± 14</td>
<td>82 ± 60</td>
<td>130 ± 91</td>
</tr>
<tr>
<td>FD</td>
<td>20</td>
<td>3.07 ± 1.68</td>
<td>969 ± 411</td>
<td>14.47 ± 1.34</td>
<td>−23.89 ± 2.20</td>
<td>0.7 ± 0.4</td>
<td>32 ± 2.4</td>
<td>17 ± 17</td>
<td>32 ± 33</td>
</tr>
<tr>
<td>LB</td>
<td>20</td>
<td>2.08 ± 0.60</td>
<td>891 ± 146</td>
<td>16.91 ± 1.16</td>
<td>−26.17 ± 0.54</td>
<td>1.7 ± 0.9</td>
<td>54 ± 3.3</td>
<td>25 ± 20</td>
<td>38 ± 28</td>
</tr>
<tr>
<td>NP</td>
<td>12</td>
<td>1.77 ± 0.95</td>
<td>2425 ± 1900</td>
<td>16.09 ± 1.28</td>
<td>−25.08 ± 1.09</td>
<td>21 ± 1.0</td>
<td>43 ± 28</td>
<td>23 ± 16</td>
<td>36 ± 25</td>
</tr>
<tr>
<td>SB</td>
<td>58</td>
<td>2.88 ± 1.76</td>
<td>894 ± 257</td>
<td>16.07 ± 0.75</td>
<td>−25.00 ± 0.93</td>
<td>2.6 ± 2.1</td>
<td>12 ± 10</td>
<td>25 ± 23</td>
<td>41 ± 37</td>
</tr>
<tr>
<td>WA</td>
<td>44</td>
<td>3.07 ± 1.90</td>
<td>1474 ± 661</td>
<td>14.94 ± 1.44</td>
<td>−22.34 ± 0.47</td>
<td>0.9 ± 0.4</td>
<td>54 ± 3.4</td>
<td>40 ± 43</td>
<td>85 ± 110</td>
</tr>
<tr>
<td>WB</td>
<td>15</td>
<td>5.98 ± 3.07</td>
<td>391 ± 172</td>
<td>16.30 ± 0.67</td>
<td>−26.01 ± 1.20</td>
<td>10 ± 1.1</td>
<td>20 ± 9</td>
<td>49 ± 33</td>
<td>82 ± 54</td>
</tr>
<tr>
<td>WS</td>
<td>15</td>
<td>4.43 ± 2.17</td>
<td>1030 ± 218</td>
<td>12.23 ± 0.86</td>
<td>−23.26 ± 1.20</td>
<td>11 ± 0.7</td>
<td>12 ± 6.4</td>
<td>22 ± 9.0</td>
<td>41 ± 20</td>
</tr>
</tbody>
</table>

BC = black crappie, BG = bluegill, CA = carp, CC = channel catfish, FD = freshwater drum, LB = largemouth bass, NP = northern pike, SU = green sunfish and pumpkinseed, SB = smallmouth bass, WA = walleye, WB = white bass, and WS = white sucker. PCDDs = polychlorinated dibenzo-p-dioxins, PCDFs = polychlorinated dibenzofurans, PCBs = polychlorinated biphenyls, TEQs = toxic equivalents.
PCDD/Fs (Table 1) and different congener profiles compared to other fishes from the Tittabawassee River. Greater than 98% of Σdl-PCBs were contributed by mono-ortho-substituted PCBs, where CB-118 was found to be the predominant congener (46–60%), followed by CB-105 (19–23%), CB-123 (5.5–11.1%) and CB-156 (4.0–12%). The congener pattern for the non-ortho-substituted PCBs was dominated by CB-77 (24–97%).

3.3. Trophic relationships among fishes

Since trophic level is an important factor influencing bioaccumulation of POPs in aquatic food webs (Fisk et al., 2001; Hop et al., 2002; Walters et al., 2008), the trophic relationships of fishes in the Tittabawassee and Saginaw Rivers were assessed by use of stable carbon and nitrogen isotope ratios (Fig. 2 and Table 1). The δ13C value is an indicator of the origin of nutrients, such that greater values are expected when progressing from riverine to lacustrine and eventually to marine ecosystems (Hobson et al., 1997). The δ13C values of walleye (−22.34±0.47) were significantly different from those of the other seven species (ANOVA, p<0.05). Those of white sucker (−23.26±1.20), a benthic species, were significantly different from another benthic fish (carp) with similar δ15N values (ANOVA, p<0.05), which live for extended periods in the investigated areas. The greater δ13C values are probably due to their habitats as these two fishes (walleye and white sucker) live in Saginaw Bay and Lake Huron during most of its lifetime and migrate into the river systems only during spawning periods (Gilbert and Williams, 2002). Due to these different stable carbon isotope values and known migratory habits, walleye and white sucker were not included in further assessments of fishes considered to be ‘permanently’ inhabiting the Tittabawassee and Saginaw Rivers. The δ13C values of another migratory fish (white bass) were not significantly different from other resident fish species and were therefore included as one of the local fishes. δ15N values ranged from 12.03±0.86 (white sucker) to 16.91±1.16 (largemouth bass) (Table 1). Stable nitrogen isotope values indicate that the trophic status of carp was the lowest (δ15N=12.63±0.88), followed by channel catfish (δ15N=14.01±0.54), which is consistent with the bottom feeding behaviors of these two species (Ryan et al., 1984; Moermond et al., 2004). The top predator among the fishes in this study was found to be the largemouth bass with a δ15N of 16.91±1.16.

3.4. Effects of biological factors on bioaccumulation

Several reports have been published about the dioxins in this area (Hilscherova et al., 2003; Kannan et al., 2008), for example, the spatial distributions of PCDD/Fs in sediment have been reported for the Tittabawassee River (Hilscherova et al., 2003), and the concentrations of PCDD/Fs in sediments from upstream (tributary rivers) were found to be less than those at downstream locations. In this study, the sampling reaches were situated in the downstream locations, and spatial distributions of PCDD/Fs and dl-PCBs in fishes in this area. No
statistically significant differences were found in fishes collected from different sampling reaches in the rivers (ANOVA, \( p > 0.05 \), Supporting information Fig. 2). The influence of locations on concentrations of PCDD/Fs and dl-PCBs were be further explored by MLR analysis based on carbon isotope data reflecting the fish habitat characteristics.

Previous investigations on the biomagnification of POPs have demonstrated that organisms containing greater lipid, occupying higher trophic levels and/or being larger contain greater tissue concentrations of these chemicals (Kidd et al., 1998; Kidd et al., 2001). After lipid normalization, significant species-specific accumulation was still observed (see “Level differences among species” section). So, regression analysis was conducted to investigate the relationships between concentrations of \( \Sigma \) PCDD/Fs, \( \Sigma \) PCBs, and TEQs and biological factors (tropic level, lipid content, carbon sources, and body weight) for all fishes excluding white sucker and walleye, eliminated from consideration due to their different \( \delta^{13}C \) values. Statistically significant correlations were observed between the stable nitrogen isotope ratio (trophic level), lipid content, body weight, and tissue residue concentrations (Fig. 3). While concentrations increased with lipid content and size as in other studies (Kidd et al., 1998, 2001; Walters et al., 2008), significant negative relationships were observed between stable nitrogen isotope ratios and concentrations of \( \Sigma \) PCDD/

Fs, \( \Sigma \) PCBs, and TEQs for the fishes in this study with the correlation slopes ranging from \(-0.15\) to \(-0.13\) and \( r^2 \) values ranging from \(0.2101\) to \(0.2786\) (\( p < 0.01 \)) (Fig. 3). Trophic dilution of \( \Sigma \) PCDD/Fs has also been reported in investigations of trophic transfer of dioxins in aquatic food webs (Broman et al., 1992; Naito et al., 2003; Wan et al., 2005; Ruus et al., 2006). However, in that case the slopes of the correlations between TEQs and trophic level were zero or positive due to the relatively large trophic magnification potentials of less chlorinated congeners (Broman et al., 1992; Naito et al., 2003; Wan et al., 2005; Ruus et al., 2006). Also in those studies the concentrations of dl-PCBs generally increased significantly with trophic level (Broman et al., 1992; Naito et al., 2003; Wan et al., 2005; Ruus et al., 2006). The tropho-dynamic differences of PCDD/Fs and dl-PCBs in fishes collected from the Tittabawassee and Saginaw Rivers were mainly due to the significantly greater concentrations of PCDD/Fs and PCBs in the lower trophic level fishes (e.g., carp and channel catfish). One possible reason for this observation could be that these fishes ingest a considerable amount of sediment (Moermond et al., 2004), and the concentration of PCDD/Fs and dl-PCBs in the sediment of these rivers were in the ranges of 310–800 pg TEQ/g due to historic industrial activity (Hilscherova et al., 2003; Kannan et al., 2008). In previous tropho-dynamic studies of PCDD/Fs and PCBs in marine ecosystems,
benthic fish were seldom included in the food web and the benthic fishes accumulated directly from sediment compared to the fish occupying high trophic level. In addition to the benthic habitats, the greater lipid content and larger size of these fishes could also result in greater concentrations. 

MLR was used to further assess the effects of biological factors on accumulation of dioxins for all local fishes (Eq. (3)).

$$\log(\text{Conc.}_i) = A_1(\delta^{15}N) + A_2 \log(LP) + A_3 \log(BW) + A_4(\delta^{13}C) + A_5$$

(3)

where: $\delta^{15}N$ reflects trophic level, “LP” is the lipid percent, “BW” is the body weight, and $\delta^{13}C$ reflects carbon sources and fish habitat locations, concentrations were expressed on a wet weight basis. The MLR was run for $\Sigma$PCDD/Fs, $\Sigma$PCBs and TEQs in samples with stable isotope values (Supplemental material Table 2). Stepwise modeling was also used to remove any insignificant parameters. These analyses gave significant multiple regression models ($p<0.001$ for all models) (Eqs. (4)–(6)). $\delta^{13}C$ was not a significant factor in these equations, further indicating that spatial distribution of contaminants in these rivers was not an important factor influencing accumulation of dioxins in local fishes.

$$\log(\Sigma\text{PCBs}) = 0.438 \log(LP)(p<0.001, 28.7\%) - 0.090(\delta^{15}N)(p<0.001, 10.4\%) + 5.826$$

(4)

$$\log(\Sigma\text{PCDD} / \text{Fs}) = 0.634 \log(LP)(p<0.001, 17.7\%) + 0.646 \log(BW)(p<0.001, 37.0\%) - 0.976$$

(5)

$$\log(\text{TEQs}) = 0.574 \log(LP)(p = 0.001, 35.1\%) - 0.093(\delta^{15}N)(p<0.001, 9.1\%) + 4.801$$

(6)

Only lipid content and $\delta^{15}N$ (trophic indicator) were found to be important factors for $\Sigma$PCBs, and the percentage contributions were 28.7% and 10.4%, respectively. The positive relationships between lipid content and $\Sigma$PCBs found in this study were consistent with earlier studies of organochlorines including PCBs in riverine (Walters et al., 2008) and marine food webs (Fisk et al., 2001; Kidd et al., 1998, 2001; Wan et al., 2005; Ruus et al., 2006). However, in the current study, significant negative correlations between concentrations of dl-PCBs and trophic status were observed for the first time. This difference could be explained by the habitat characteristic of the benthivorous fishes, occupying lower trophic levels, consequently having an extra uptake pathway (sediment ingestion), which resulted in the accumulation of greater concentrations of organochlorines directly from the sediment. Thus, the influence of the benthivorous habits of organisms to the characterization of trophic transfer should be considered in future tropho-dynamic studies of these chemicals.

Lipid content and body weight were significant predictors of $\Sigma$PCDD/Fs concentrations, explaining 17.7% and 37.0% of the variance, respectively. Previous studies looking for the tropho-dynamics of PCDD/Fs in aquatic food webs showed that lipid equivalent concentrations of $\Sigma$PCDD/Fs decreased with trophic status, but the correlations were not as significant as those for dl-PCBs (Naito et al., 2003; Wan et al., 2005). In the present study, body size followed by lipid content were more important factors influencing bioaccumulation of PCDD/Fs, while trophic level was not found to be significant in the MLR analysis. The importance of body size may be due to the fact that hydrophobic compounds (PCDD/Fs, log $K_{ow}$: 6.46–8.75, ref from Govers and Krop, 1998) are slower to partition out of lipids and so will have longer half-lives resulting in greater tissue concentrations in larger organisms (Borga et al., 2004). The mechanism is likely due to the fact that body size is related with age and the time to steady state for PCDD/Fs is generally longer than that of smaller molecules. This also suggests that big and fatty fish will accumulate greater concentrations of PCDD/Fs.

In all fishes, 29.8–57.5% of TEQs were contributed by PCDD/Fs, with 42.5–70.2% of TEQs contributed by non- and mono-ortho-substituted PCBs, especially by non-ortho-substituted PCBs. The results of the MLR analysis of TEQs suggested that lipid content and trophic status were the major predictors, explaining 35.1% and 9.1% of the variance, respectively. Lipid-normalized TEQs were reported to increase significantly with trophic level due to the large biomagnification potential of lesser chlorinated congeners and small biomagnification potential of more chlorinated congeners (Broman et al., 1992; Naito et al., 2003; Wan et al., 2005). A similar importance of lipid and trophic status was observed in fishes in the present study further supporting that these two factors are important at influencing accumulation by piscivorous wildlife.

Overall, 39.1%, 54.7% and 44.2% of the total variation in $\Sigma$PCBs, $\Sigma$PCDD/Fs and TEQs were explained in the MLR analysis. The MLR analysis also showed that lipid content and trophic status were significant predictors for accumulation of $\Sigma$PCBs and TEQs, while body size and lipid content were more important factors for describing accumulation of $\Sigma$PCDD/Fs. The percentages of variance explained for $\Sigma$PCDD/Fs and TEQs were similar to those reported in other studies of PCDD/Fs in aquatic food webs (Naito et al., 2003; Wan et al., 2005; Ruus et al., 2006), and those of $\Sigma$PCBs were relatively low compared to other studies (Naito et al., 2003; Wan et al., 2005), which suggests that other factors (such as age, sex etc.) could explain the remainder of the variability in the values.

Acknowledgments

This research was supported by a contract to ENTRIX, Inc. and an unrestricted grant from Dow Chemical Co. to Michigan State University and the University of Saskatchewan. Portions of the study were supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project # 6807). The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and State Key Laboratory in Marine Pollution, City University of Hong Kong. We thank A. McGuire for her assistance in sample preparations, thanks to P. Bradley and M. Shotwell for data validation. We also thank M. Barker, N. Hubbard, J. Matousek, and P. Bradley for their efforts in the field and laboratory; Dr D.J. Jude, Great Lakes Fishery Laboratory, University of Michigan assisted with some fish collection; J. Baker and support staff from the Michigan Department of Natural Resources for collection of some of the migratory fish as well as significant study design input. Additional thanks to M. Haamen, M. Shotwell, and K. Smyth for database maintenance and logistic support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2010.02.003.

References


Acknowledgments

This research was supported by a contract to ENTRIX, Inc. and an unrestricted grant from Dow Chemical Co. to Michigan State University and the University of Saskatchewan. Portions of the study were supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project # 6807). The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and State Key Laboratory in Marine Pollution, City University of Hong Kong. We thank A. McGuire for her assistance in sample preparations, thanks to P. Bradley and M. Shotwell for data validation. We also thank M. Barker, N. Hubbard, J. Matousek, and P. Bradley for their efforts in the field and laboratory; Dr D.J. Jude, Great Lakes Fishery Laboratory, University of Michigan assisted with some fish collection; J. Baker and support staff from the Michigan Department of Natural Resources for collection of some of the migratory fish as well as significant study design input. Additional thanks to M. Haamen, M. Shotwell, and K. Smyth for database maintenance and logistic support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2010.02.003.

References


October.