Science of the Total Environment 408 (2010) 2394–2401

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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. Holem [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

ARTICLE INFO

Article history:
Received 27 October 2009
Received in revised form 29 January 2010
Accepted 2 February 2010
Available online 6 March 2010

Keywords:
PCDD/Fs
Dioxin-like PCBs
Trophic transfer
Stable isotope
Bioaccumulation

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 ΣPCDD/Fs (20–440 pg/g ww (wet weight)), ΣPCBs (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 ΣPCDD/Fs. Lipid content and trophic level seemed to be dominant factors determining accumulation of ΣPCB and TEQs, but negative correlations between trophic status and concentrations of ΣPCBs 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.

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

* Corresponding author. Toxicology Center, University of Saskatchewan, 44 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5B3. Tel.: +1 306 966 4978; fax: +1 306 966 4796.
E-mail address: yi.wan@usask.ca (Y. Wan).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.02.003

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 tropho-dynamics 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 dolomieui*, $n=58$), largemouth bass (*Micropterus salmoides*, $n=20$), channel catfish (*Ictalurus 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 is an area of concern identified by the United States Environmental Protection Agency (U.S. EPA, 1995). 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-Root®, 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 I-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 I-Chem® (Rockwood, TN USA) 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 ^{13}C-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 ^{13}C-PCDDs, 10 ^{13}C-PCDFs and 12 ^{13}C-dl-PCBs, and 1 ml was added into each sample with concentrations of 2 ng/ml, 4 ng/ml and 2 ng/ml for ^{13}C-labeled tetra- to hepta-CDD/Fs, ^{13}C-OCDD/F and ^{13}C-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 column 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 PCDDs 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, MD). A split/splitless 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 converted carbon and nitrogen in the samples to N_2 and CO_2. N_2 and CO_2 were separated on a gas chromatography column and were analyzed using a Thermo Finnigan® Flash 1112 EA coupled to a Thermo Finnigan® Delta Plus XL® via a Conflo III® interface. Stable isotope values were calculated as below (Eqs. (1) and (2)).

$$\delta^{15}N = \left(\left(\left({}^{15}N/{}^{14}N\,sample\right)/\left({}^{15}N/{}^{14}N\,standard\right)\right)-1\right)\times 1000\,(‰) \quad (1)$$

$$\delta^{13}C = \left(\left(\left({}^{13}C/{}^{12}C\,sample\right)/\left({}^{13}C/{}^{12}C\,standard\right)\right)-1\right)\times 1000\,(‰). \quad (2)$$

The ^{15}N/^{14}N and ^{13}C/^{12}C standard values were based on atmospheric N_2 (air) and Vienna Peedee belemnite (VPDB), respectively.

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

The QA/AC 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, 17–197% 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 TeCDD/Fs, PeCDD/Fs, HxCDD/Fs and HpCDD/Fs, 0.4 pg/g ww for OCDD and OCDF, 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: $d^{13}C = -32.14 \pm 0.03$‰ VPDB ($n = 12$), which is acceptable compared to the value of $d^{13}C = -32.15 \pm 0.10$‰ 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-NO3, an intermediate international standard, gave the following result during calibration of the in-house standards: $d^{15}N = 3.96 \pm 0.08$‰ AIR ($n = 8$), which is acceptable compared to the value of $d^{15}N = 4.7 \pm 0.2$‰ 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

(DL) or ProUCL values were compared and no significantly different results were obtained for ANOVA, PCA or MLR analyses. Thus, the statistical analyses reported in this paper were based on ProUCL values.

The relative toxic potencies of dioxins measured 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, $\sum$PCDD/Fs and ΣPCB data are reported on a concentration basis (pg/g, ww). The concentration 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 = $1.1\times10^2\pm$ 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 PCDFs were greater than those of PCDDs in all fish species, and the PCDFs/PCDDs concentration ratio in northern pike (19.5 ± 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 congener patterns are discussed elsewhere (Holem 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 (8.1–42%), 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 1
Mean ± SD and range of concentrations of PCDD/Fs (pg/g ww), PCBs (ng/g ww), TEQs (pg/g ww), and biological parameters of fishes collected from the Saginaw and Tittabawassee Rivers, *n* is the sample number for each species.

Species	*n*	Lipid (%)	Body weight (g)	δ^{15}N	δ^{13}C	ΣPCDDs	ΣPCDFs	Σdl-PCBs	ΣPCBs	ΣTEQs
BC	9	3.25 ± 1.41	217 ± 88	15.85 ± 1.56	−25.79 ± 1.16	1.4 ± 1.1	6.3 ± 3.5	14 ± 8.1	21 ± 12	3.4 ± 3.0
BG	12	2.59 ± 2.58	124 ± 32	15.98 ± 0.39	−26.42 ± 1.84	0.7 ± 0.4	2.6 ± 1.5	14 ± 13	25 ± 19	1.7 ± 1.5
CA	50	7.25 ± 6.08	2848 ± 963	12.63 ± 0.88	−26.18 ± 1.81	25 ± 26	81 ± 70	130 ± 120	190 ± 180	54 ± 59
CC	49	5.67 ± 2.65	1026 ± 567	14.01 ± 0.54	−24.13 ± 1.31	11 ± 7.6	35 ± 34	82 ± 60	130 ± 91	34 ± 28
FD	20	3.07 ± 1.68	969 ± 411	14.47 ± 1.34	−23.89 ± 2.20	0.7 ± 0.4	3.2 ± 2.4	17 ± 17	32 ± 33	3.7 ± 4.0
LB	20	2.08 ± 0.60	891 ± 146	16.91 ± 1.16	−26.17 ± 0.54	1.7 ± 0.9	5.4 ± 3.3	25 ± 20	38 ± 28	8.1 ± 8.3
NP	12	1.77 ± 0.95	2425 ± 1900	16.09 ± 1.28	−25.08 ± 1.09	2.1 ± 1.0	43 ± 28	23 ± 16	36 ± 25	13 ± 11
SU	10	1.96 ± 0.47	137 ± 45	15.62 ± 0.61	−28.08 ± 1.16	1.1 ± 0.5	3.8 ± 1.6	38 ± 26	56 ± 38	7.3 ± 6.5
SB	58	2.88 ± 1.76	894 ± 257	16.07 ± 0.75	−25.00 ± 0.93	2.6 ± 2.1	12 ± 10	25 ± 23	41 ± 37	7.6 ± 6.0
WA	44	3.07 ± 1.90	1474 ± 661	14.94 ± 1.44	−22.34 ± 0.47	0.9 ± 0.4	5.4 ± 3.4	40 ± 43	85 ± 110	3.5 ± 2.7
WB	15	5.98 ± 3.07	391 ± 172	16.30 ± 0.65	−24.90 ± 1.01	1.8 ± 1.1	20 ± 10	49 ± 33	82 ± 54	7.3 ± 2.9
WS	15	4.43 ± 1.27	1030 ± 218	12.03 ± 0.86	−23.26 ± 1.20	1.1 ± 0.7	12 ± 6.4	22 ± 9.0	41 ± 20	5.5 ± 7.7

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.

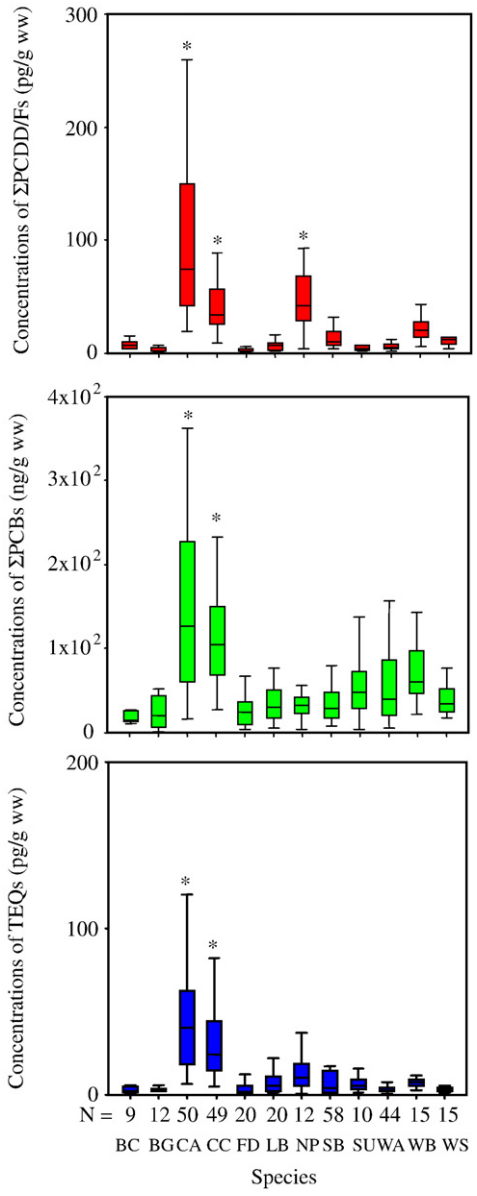

Fig. 1. Concentrations of ΣPCDD/Fs, ΣPCBs, and TEQs in twelve fish species collected from the Tittabawassee and Saginaw Rivers. WA = walleye, WS = white sucker, WB = white bass, SB = smallmouth bass, LB = largemouth bass, CC = channel catfish, CA = carp, FD = freshwater drum, BC = black crappie, NP = northern pike, BG = bluegill, and SU = green sunfish and pumpkinseed, *N* is the sample number for each species. *: significantly greater than other species.

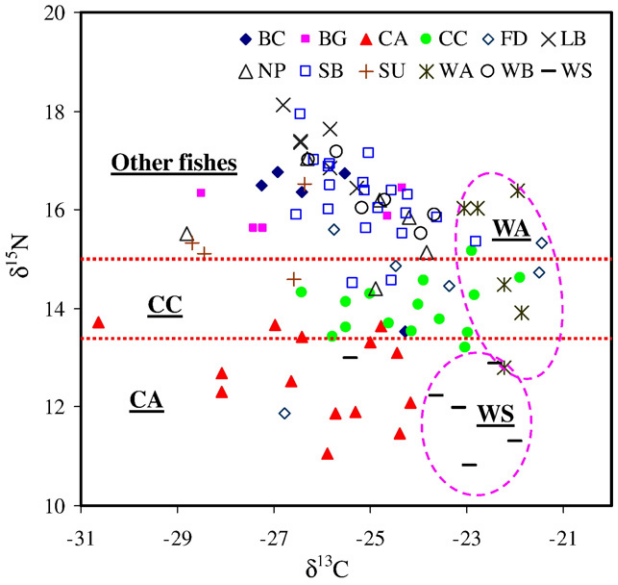

Fig. 2. δ^{15}N and δ^{13}C values of fishes collected from the Tittabawassee and Saginaw Rivers. WA = walleye, WS = white sucker, WB = white bass, SB = smallmouth bass, LB = largemouth bass, CC = channel catfish, CA = carp, FD = freshwater drum, BC = black crappie, NP = northern pike, BG = bluegill, and SU = green sunfish and pumpkinseed. Dashed line circled the species excluded in the MLR analysis due to different stable ^{13}C values.

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 δ^{13}C 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 δ^{13}C 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 δ^{15}N values (ANOVA, $p<0.05$), which live for extended periods in the investigated areas. The greater δ^{13}C 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 fish considered to be 'permanently' inhabiting the Tittabawassee and Saginaw Rivers. The δ^{13}C 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. δ^{15}N 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 ($\delta^{15}N = 12.63 \pm 0.88$), followed by channel catfish ($\delta^{15}N = 14.01 \pm 0.54$), 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 δ^{15}N 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 ΣPCDD/Fs, ΣPCBs, 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 ΣPCDD/Fs, ΣPCBs, 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 ΣPCDD/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 TEQs/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,

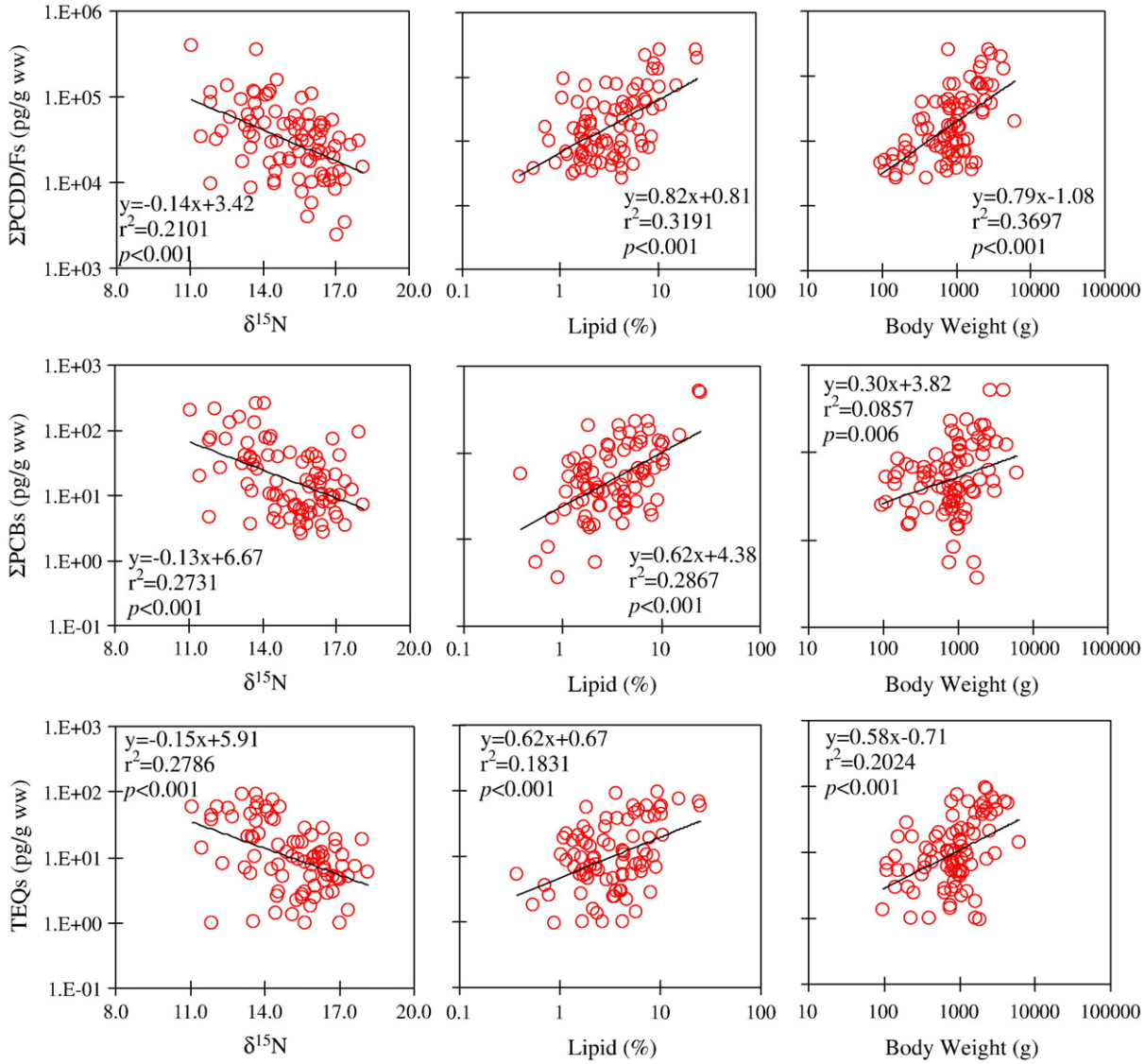

Fig. 3. Relationships between concentrations of ΣPCDD/Fs, ΣPCBs, and TEQs and biological factors including tropic level, lipid content, and body weight for all fishes excluding white sucker and walleye.

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)).

$$\log(Conc.) = A_1\left(\delta^{15}N\right) + A_2\log(LP) + A_3\log(BW) + A_4\left(\delta^{13}C\right) + A_0 \quad (3)$$

where: "δ^{15}N" reflects trophic level, "LP" is the lipid percent, "BW" is the body weight, and "δ^{13}C" reflects carbon sources and fish habitat locations, concentrations were expressed on a wet weight basis. The MLR was run for ΣPCDD/Fs, ΣPCBs 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)). δ^{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.

$$\log(\Sigma PCBs) = 0.438\log(LP)(p<0.001, 28.7\%) - 0.090\delta^{15}N(p<0.001, 10.4\%) + 5.826 \quad (4)$$

$$\log(\Sigma PCDD/Fs) = 0.634\log(LP)(p<0.001, 17.7\%) + 0.646\log(BW)(p<0.001, 37.0\%) - 0.976 \quad (5)$$

$$\log(TEQs) = 0.574\log(LP)(p = 0.001, 35.1\%) - 0.093\delta^{15}N(p<0.001, 9.1\%) + 4.801. \quad (6)$$

Only lipid content and δ^{15}N (tropic indicator) were found to be important factors for ΣPCBs, and the percentage contributions were 28.7% and 10.4%, respectively. The positive relationships between lipid content and ΣPCBs 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 ΣPCDD/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 ΣPCDD/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 (Borgå 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 ΣPCBs, ΣPCDD/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 ΣPCBs and TEQs, while body size and lipid content were more important factors for describing accumulation of ΣPCDD/Fs. The percentages of variance explained for ΣPCDD/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 ΣPCBs 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

Borgå K, Fisk AT, Hoekstra PF, Muir DCG. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminant in Arctic marine food webs. Environ Toxicol Chem 2004;10:2367–85.

Broman D, Näf C, Rolff C, Zebühr Y, Fry B, Hobbie J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dixoins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the Northern Baltic. Environ Toxicol Chem 1992;11:331–45.

EPA 823-B-00-007. Guidance for assessing chemical contaminant data for use in fish advisories. Volume 1: fish sampling and analysis third edition; 2000.

EPA Method 1613. Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. U.S. Environmental Protection Agency Office of Water; 1994. October.

EPA Method 1668. Revision A: chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS. Environmental Protection Agency Office of Water; 1999. December.
Field AP. Discovering statistics using SPSS. second edition. London: Sage; 2005.
Fisk AT, Hobson KA, Norstrom RJ. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater Polynya marine food web. Environ Sci Technol 2001;35:732–8.
Gilbert CR, Williams JD. National Audubon Society field guide to fishes. New York: North America. Revised Edition. Alfred A. Knopf; 2002.
Govers HAJ, Krop HB. Partition constants of chlorinated dibenzofurans and dibenzo-p-dioxins. Chemosphere 1998;37:2139–52.
Han JL, Shen HT, Tie XW, Zhang WP, Zhu GN, Ren YP. Polychlorinated dibenzo-p-dioxins/furans and polychlorinated biphenyls in fresh fishes from Qiantanjiang River, China. Chemosphere 2007;68:112–9.
Harless RL, Oswald EO, Lewis RG, Dupuy AE, Mcdaniel DD, Tai H. Determination of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in fresh-water fish. Chemosphere 1982;11: 193–8.
Hilscherova K, Kannan K, Nakata H, Hanari N, Yamashita N, Bradley PW, et al. Polychlorinated dibenzo-p-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. Environ Sci Technol 2003;37:468–74.
Hobson KA, Hughes KD, Ewins PJ. Using stable-isotope analysis to identify endogenous and exogenous sources of nutrients in eggs of migratory birds: applications to Great Lakes contaminants research. Auk 1997;114:467–78.
Holem RH, Wan Y, Jones PD, Newsted JL, Roark SA, Matousek J, Kay DP, Naile J, Giesy JP. Submitted. Evaluation of 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin equivalents in edible tissues of fishes from the Tittabawassee and Saginaw Rivers, Michigan, USA. Arch Environ Con Tox, submitted for publication.
Hop H, Borga K, Gabrielsen GW, Kleivane L, Skaare JU. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. Environ Sci Technol 2002;36:2589–97.
Kannan K, Yun SH, Ostaszewski A, McCabe JM, Mackenzie-Taylor D, Taylor AB. Dioxin-like toxicity in the Saginaw River watershed: polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee rivers and Saginaw bay, Michigan, USA. Arch Environ Contam Toxicol 2008;54:9-19.
Kay DP, Blankenship AL, Coady KK, Neigh AM, Zwiernik MJ, Millsap SD, et al. Differential accumulation of polychlorinated biphenyl congeners in the aquatic food web at the Kalamazoo river superfund site, Michigan. Environ Sci Technol 2005;39:5964–74.
Kidd K, Schindler DW, Hesslein RH, Muir DCG. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. Can J Fish Aquat Sci 1998;55:869–81.
Kidd KA, Bootsma HA, Heslein RH. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environ Sci Technol 2001;35:14–20.
Knutzen J, Bjerkeng B, Næs K, Schlobach M. Polychlorinated dibenzofurans/dibenzo-p-dioxins (PCDD/PCDDs) and other dioxin-like substances in marine organisms from the Greenland fjords, S. Norway, 1975–2001: present contamination levels, trends and species specific accumulation of PCDF/PCDD congeners. Chemosphere 2003;52: 745–60.
Mackintosh CE, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou MG, et al. Distribute of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. Environ Sci Technol 2004;38:2011–20.
McIntyre JK, Beauchamp DA. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Sci Total Environ 2007;372:571–84.
Michigan DNRE reports. Great lakes creel survey program. http://www.michigan.gov/dnr/0,1607,7-153-10364_52259_10951_11301-97829-,00.html.
Michigan Fish contaminant monitoring program online database. http://www.deq.state.mi.us/fcmp/default.asp.
Moermond CTA, Roozen FCJM, Zwolsman JJG, Koelmans AA. Uptake of sediment-bound bioavailable polychlorobiphenyls by benthivorous carp (*Cyprinus carpio*). Environ Sci Technol 2004;38:4503–9.
Naito W, Jin J, Kang YS, Yamamuro M, Masunaga S, Nakanishi J. Dynamics of PCDD/DFs and coplanar-PCBs in an aquatic food chain of Tokyo Bay. Chemosphere 2003;53: 347–62.
Okeefe P, Hilker D, Meyer C, Aldous K, Shane L, Donnelly R, et al. Tetrachlorodibenzo-para-dioxin and tetrachlorodibenzofurans in Atlantic coast striped bass and in selected Hudson river fish, waterfowl and sediments. Chemosphere 1984;13: 849–60.
Ruus A, Berge JA, Bergstad OA, Knutsen JA, Hylland K. Disposition of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in two Norwegian epibenthic marine food webs. Chemosphere 2006;62:1856–68.
Ryan JJ, Lau PY, Pllon JC, Lewis D, McLeod HA, Gervals A. Incidence and levels of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in Lake Ontario commercial fish. Environ Sci Technol 1984;18:719–21.
Stachel B, Christoph EH, Götz R, Herrmann T, Krüger F, Kühn T, et al. Dioxins and dioxin-like PCBs in different fish from the river Elbe and its tributaries, Germany. J Hazard Mater 2007;148:199–209.
Stine RA. Graphical interpretation of variance inflation factors Am. Statistician 1995;49: 53–6.
U.S. EPA. Assessment of sediments in the Saginaw River Area of Concern, September 28, 1995. Prepared by Science Applications International Corporation. EPA Contract No. 68-D3-0030.
Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland M, Feeley M, et al. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 2006;93: 223–41.
Walters DM, Fritz KM, Johnson BR, Lazorchak JM, Mccormick FH. Influence of trophic position and spatial location on polychlorinated biphenyl (PCBs) bioaccumulation in a stream food web. Environ Sci Technol 2008;42:2316–22.
Wan Y, Hu JY, An W, Zhang ZB, An LH, Hattori T, et al. Congener-specific tissue distribution and hepatic sequestration of PCDD/Fs in wild herring gulls from Bohai Bay, North China: comparison to coplanar PCBs. Environ Sci Technol 2006;36: 1462–8.
Wan Y, Hu JY, Yang M, An LH, An W, Jin XH, et al. Characterization of trophic transfer for polychlorinated dibenzo-p-dioxins, dibenzofurans, non- and mono-ortho polychlorinated biphenyls in the marine food web of Bohai Bay. North China. Environ Sci Technol 2005;39:2417–25.