

# Bioaccumulation and Spatiotemporal Trends of Polyhalogenated Carbazoles in Great Lakes Fish from 2004 to 2016

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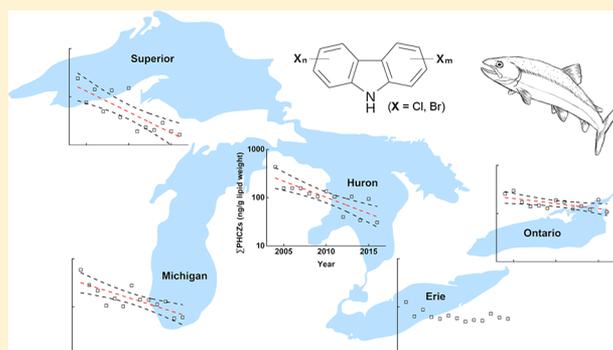
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## Supporting Information

**ABSTRACT:** Polyhalogenated carbazoles (PHCZs) were recently discovered in Great Lakes sediment and other aquatic systems. However, knowledge about their bioaccumulation and potential risks to fish and wildlife remains very limited. The present study investigated PHCZs in Great Lakes lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*; Lake Erie only) composites collected between 2004 and 2016. Median concentrations of  $\Sigma$ PHCZs by lake ranged from 54.7 to 154 ng/g lipid weight or lw (6.8–28.0 ng/g wet weight). Dominant congeners included 3,6-dichlorocarbazole, 1,3,6-tribromocarbazole, and 1,3,6,8-tetrachlorocarbazole. The highest  $\Sigma$ PHCZs concentrations were found in Lakes Michigan and Ontario fish, followed by Lake Huron, whereas Lakes Erie and Superior fish contained the lowest concentrations. Congener profiles of PHCZs also exhibited spatial variations. After age normalization to minimize fish age influence on bioaccumulation rates, fish  $\Sigma$ PHCZs' concentrations declined significantly over time in all lakes except Lake Erie, with slopes ranging from  $-10.24\%$  to  $-3.85\%$  per year. The median toxic equivalent (TEQ) of PHCZs due to their dioxin-like activity was determined to range from 8.7 to 25.7 pg/g lw in Great Lakes fish. This study provides the first insight into the bioaccumulation and spatiotemporal trends of PHCZs in Great Lakes and suggests the need for further research on this group of chemicals.



## INTRODUCTION

The Great Lakes of North America form the largest group of freshwater lakes on Earth, home to millions of people and numerous fish and wildlife. Among a number of environmental and ecological factors, chemical pollution remains a continuous and long-term threat to the Great Lakes ecosystems. Human activities generate large amounts of a wide range of pollutants. These are subsequently released into water systems via point or nonpoint discharges, including municipal and industrial wastewaters, urban and agricultural runoff, air deposition, and many others.<sup>1–3</sup> Some of these pollutants are persistent, bioaccumulative, and toxic (PBT) and may accumulate in sediment and aquatic organisms, producing long-term impacts on water quality and ecosystem/human health. Well-known PBTs, such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), have been investigated by long-term environmental monitoring programs, such as the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) supported by the United States Environmental Protection Agency (U.S. EPA).<sup>4–8</sup> However, many contaminants of

emerging concern (CECs) are still poorly understood in terms of their occurrence, distribution, sources, and potential impacts on Great Lakes ecosystems. The effort of addressing CECs complements existing monitoring programs for legacy contaminants and extends our knowledge about the problems the Great Lakes are facing.

Polyhalogenated carbazoles (PHCZs) are a potential group of CECs that possess the same base structure and a complexity of halogenation patterns. Recently, chlorinated, brominated, and mixed halogenated (Cl, Br, I) carbazoles were reported in Great Lakes sediment.<sup>9,10</sup> Median concentrations of PHCZs ranged from 22.6 to 122 ng/g dry weight (dw) in sediment collected from Lakes Michigan (LM), Superior (LS), and Huron (LH).<sup>9</sup> It was estimated that over 3000 tonnes of PHCZs were accumulated in the sediment of these three lakes,

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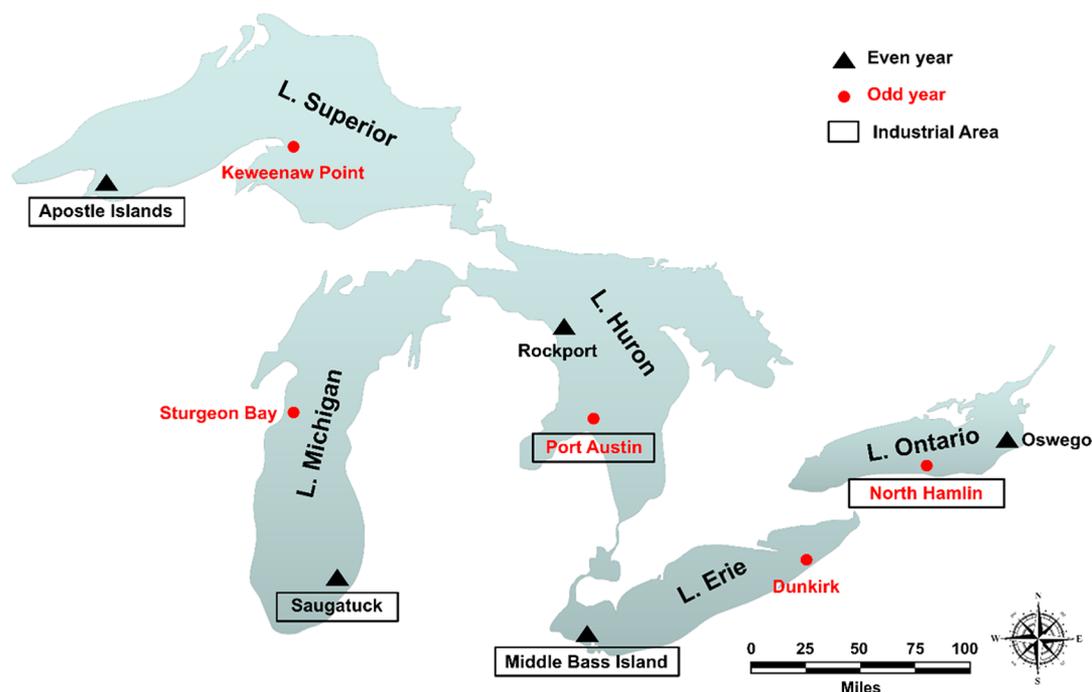


Figure 1. Map of fish sampling sites in the Great Lakes.

significantly greater than those of PCBs and BDE-209.<sup>9,10</sup> PHCZs were also reported in sediment from the San Francisco Bay (U.S.), the Saginaw River basin (U.S.), streams and rivers across southern Ontario (Canada), the North Sea Estuary (Germany), the Lippe River (Germany), the industrial coastal area of Kavala City (Greece), and Lake Tai (China), suggesting a broad distribution in global aquatic systems.<sup>11–18</sup>

Laboratory *in vivo* and *in vitro* studies reported a variety of toxic effects for PHCZs, including developmental, cardiotoxic, reproductive, and, importantly, dioxin-like effects.<sup>13,19–22</sup> However, knowledge about their sources remains fragmented and sometimes controversial.<sup>9,18,23–25</sup> Both natural and anthropogenic sources have been suggested for selected PHCZs. Halogenated indigo dyes were suggested as potential sources of 1,3,6,8-tetrabromocarbazole (1368-BCZ) and 1,8-dibromo-3,6-dichlorocarbazole (18-B-36-CCZ), as well as some other PHCZ congeners, which may be present as impurities in dye formulations.<sup>23</sup> Natural origins are also possible for some PHCZ congeners or their precursors. Selected PHCZs can be produced through enzymatic synthesis of bromo- and chlorocarbazoles by chloroperoxidase from marine fungus *Caldariomyces fumago*.<sup>26</sup> However, to date, confirmative evidence on congener-specific sources is lacking.

The recent discovery of PHCZs in sediment has initiated a need to understand the bioaccumulation, environmental distribution, and fate of these chemicals to evaluate the potential risks PHCZs pose to Great Lakes fish and wildlife. To fill some of the priority knowledge gaps, specific objectives of the present study were to investigate (1) the concentrations and compositions of PHCZs in Great Lakes fish, (2) spatial variations in PHCZ concentrations and compositional patterns among the five lakes, (3) temporal changes of PHCZ contamination from 2004 to 2016, and (4) estimated toxicity of PHCZs based on the toxic equivalent approach. Our work presents the first insight into the bioaccumulation and spatiotemporal distributions of PHCZs in Great Lakes ecosystems.

## MATERIALS AND METHODS

**Chemicals and Reagents.** The reference standards of 3-chlorocarbazole (3-CCZ), 3-bromocarbazole (3-BCZ), 3,6-dichlorocarbazole (36-CCZ), 2,7-dibromocarbazole (27-BCZ), 3,6-dibromocarbazole (36-BCZ), 1,3,6-tribromocarbazole (136-BCZ), 1-bromo-3,6-dichlorocarbazole (1-B-36-CCZ), 18-B-36-CCZ, 1,3,6,8-tetrachlorocarbazole (1368-CCZ), 2,3,6,7-tetrachlorocarbazole (2367-CCZ), and 1368-BCZ were purchased from Wellington Laboratories (Guelph, ON, Canada), Sigma-Aldrich (St. Louis, Missouri), or the Florida Center for Heterocyclic Compounds of the University of Florida (Gainesville, FL). Their purities were all greater than 98%. The reference surrogate standards, 4'-fluoro-2,3,3',4,5,6-hexabromodiphenyl ether (F-BDE160) and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB-204), as well as internal standards, 3'-fluoro-2,2',4,4',5,6'-hexabromodiphenyl ether (F-BDE154) and deca-chlorodiphenyl ether (DCDE), were purchased from AccuStandard (New Haven, CT). Diatomaceous earth, sodium sulfate (10–60 mesh), and high performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific (Hanover Park, IL). Isolute silica sorbent was purchased from Biotage Inc. (Charlotte, NC) and kept at 130 °C. Styrene divinylbenzene beads (3% cross-linkage, 40–80 μm bead size, ≤2000 MW limit) for gel permeation chromatography (GPC) were purchased from Bio-Rad (Hercules, CA).

**Samples.** Archived lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*) samples collected through the GLFMSP were analyzed for PHCZs. These same samples have been used by the GLFMSP to assess contamination status of many different pollutants, including mercury, PCBs, PBDEs, and organochlorine pesticides.<sup>2,6,27</sup> The fish sampling protocol has been described elsewhere in detail.<sup>28–30</sup> Briefly, lake trout and walleye (Lake Erie or LE only) were collected at one of the two sites (industrial or nonindustrial) in alternative years from each lake (Figure 1). Each year, 50 individual fish with similar sizes (600–700 mm in length for lake trout and 400–500 mm

for walleye) were collected from each site. The Great Lakes monitoring sites have a distance to the closest hatchery site of approximately <2–91 km (Apostle, 49 km; Keweenaw Point, 91 km; Sturgeon Bay, 7 km; Saugatuck, < 2 km; Port Austin, 17 km; Rockport, 19 km; Middle Bass Island, 10 km; Dunkirk, < 2 km; Oswego, < 2 km; North Hamlin, 23 km).<sup>31</sup> Lake trout movement studies revealed that lake trout usually occupied an area within 80 km of the tagging sites.<sup>32,33</sup> Therefore, it is likely that both hatchery fish (usually identified by fin clip or wire tags) and wild fish were collected. Figure S1 in the Supporting Information indicates that there is no systematic trend in the percentage of hatchery fish collected at either site in each of the lakes by the GLFMSP. Because of a similar diet between hatchery and wild lake trout, the mix of hatchery and wild trout did not produce a significant influence on the measured POPs concentrations.<sup>34</sup> Fish sex was not particularly taken into account when fish composites were made, although each composite was made up of roughly half male and half female fish. Concentration data of PCBs in LE and LS fish revealed that fish of the same age caught recently had lower concentrations than fish caught previously regardless of breeding status (Figure S2). This suggests the breeding status alone cannot explain temporal variations of contaminant concentrations. However, it should be noted that fish sex could affect the bioaccumulation through metabolic difference and maternal transfer,<sup>35–37</sup> but this potential influence was not measurable in the present study.

Fish ages were determined using multiple approaches, including otoliths, fin clips, coded wire tags (CWT), scales, and maxillae by the homogenization lab. The maxillae estimation method developed by the Michigan Department of Natural Resources (MDNR) has been used as a new aging technique to replace the traditional otolith age approach since 2012.<sup>38</sup> This change increased the speed of fish age estimation and allowed samples to be composited according to a known age instead of an assumed age based on length. The measured ages before and after this change were combined for age normalization of measured PHCZ concentrations, as there was no significant difference between maxillary age (new method) and otolith age (old method; ANOVA;  $p = 0.12$ ).

The GLFMSP sampling design includes creating 10 composites (each containing five individual fish) per site per year. Since 2004, GLFMSP also combined the 10 composites to create a mega-composite per site. Only mega-composite samples were used for PHCZ analysis. While this may result in a limitation of trend analyses due to the lack of a variability assessment, the use of mega-composites allows for the investigation of contaminants of emerging concern in a cost-effective way. PHCZ concentrations in mega-composite samples were compared with other PBT concentrations in individual composite samples for age normalization (additional details are given in the Supporting Information). Fish mega-composites were stored at  $-20\text{ }^{\circ}\text{C}$  prior to chemical analysis.

**Sample Preparation.** Determination of PHCZs followed the previously reported analytical procedure with slight modifications.<sup>11,18</sup> Briefly, approximately 0.5–1 g of wet fish mega-composite was spiked with surrogate standards (FBDE-160 and PCB-204) and then subjected to accelerated solvent extraction (Dionex ASE 350, Sunnyvale, CA), employing two 5 min extraction cycles with dichloromethane (DCM) at  $100\text{ }^{\circ}\text{C}$  and 1500 psi. The extract was run through pre-cleaned sodium sulfate to remove moisture. After gravimetric determination of lipid content by using 10% of the extract, the remaining extract

was cleaned through a GPC column (length, 40 cm, diameter, 1.5 cm) packed with 6 g of styrene divinylbenzene beads in a mixture of hexane and DCM (HEX/DCM, 1:1, v/v). After the extract was loaded, the first fraction was eluted with 30 mL of HEX/DCM mixture (1:1, v/v) and discarded. Target PHCZs as well as surrogate standards were collected in the second fraction eluted with 60 mL of HEX/DCM (1:1, v/v). The latter fraction was further purified through a 2 g isolute silica solid phase extraction (SPE) cartridge preconditioned with 10 mL of hexane. After the sample was loaded, the first fraction was eluted with 3 mL of hexane and discarded as it does not contain any analyte of interest. The second fraction was eluted with 11 mL of HEX/DCM mixture (6:4, v/v) and then concentrated and transferred to a gas chromatography (GC) vial. After spiking with internal standards (FBDE-154 and DCDE), the final extract was subjected to instrumental analysis.

**Instrumental Analysis.** The determination of PHCZs was performed on an Agilent 7890B GC (Agilent Technologies, Palo Alto, CA), equipped with a 30 m HP-5MS column (0.25 mm i.d.,  $0.25\text{ }\mu\text{m}$ , J&W Scientific, Agilent Tech.) and coupled with a single quadrupole mass analyzer (Agilent 5977A MS). Congeners 3-CCZ, 36-CCZ, and 3-BCZ were detected in electron impact (EI) mode, whereas the others were analyzed in electron-capture negative ionization (ECNI) mode.<sup>18</sup> The GC injector was operated in pulsed-splitless mode and held at  $260\text{ }^{\circ}\text{C}$ . Initial oven temperature was held at  $50\text{ }^{\circ}\text{C}$  for 3 min, ramped to  $150\text{ }^{\circ}\text{C}$  at  $10\text{ }^{\circ}\text{C}/\text{min}$ , and finally increased to  $300\text{ }^{\circ}\text{C}$  at  $5\text{ }^{\circ}\text{C}/\text{min}$  (held for 10 min). Quantification of PHCZs was conducted based on each congener's characteristic ions under the selected ion monitoring (SIM) mode.<sup>11,18</sup> Our previous study confirmed a number of PHCZ congeners with unspecified halogen substitution positions (referred to as UNC<sub>s</sub>) in LM sediment.<sup>10</sup> In the lack of relevant reference standards, these UNC<sub>s</sub> were screened in fish based on the retention times and SIM ions of UNC<sub>s</sub> present in previous LM sediment extract. In general, only two UNC<sub>s</sub> were quantifiable in fish, both of which were tribromocarbazoles (referred to as Br<sub>3</sub>-CZ<sub>a</sub> and Br<sub>3</sub>-CZ<sub>b</sub>) and semiquantified using the calibration curve for 136-BCZ. The other UNC<sub>s</sub> were either not detectable or below instrumental detection limits (IDL). In particular, trichlorocarbazoles were screened, but none of the peaks possessed both the theoretical molecular ions (i.e.,  $m/z$  269 and 271) and the fragmentation pattern of a trichlorocarbazole. A laboratory synthetic study produced a mixture of halogenated carbazoles from chloroperoxidase-catalyzed halogenation of carbazole, including two trichlorocarbazoles.<sup>39</sup> The comparison with the laboratory synthesized mixture revealed no presence of trichlorocarbazoles in our samples (Figure S3). However, it cannot be entirely excluded that some of the UNC<sub>s</sub> could be removed by our sample preparation procedures. More commercial reference standards are needed to facilitate the identification and determination of additional PHCZ congeners in the environment.

**Quality Assurance and Control.** A number of QA/QC procedures were conducted to ensure the quality of generated data. A procedural blank was processed along with every seven samples. No PHCZs were detected in the procedural blanks. Spiking tests were conducted to evaluate the recoveries of PHCZs from sample extraction and cleanup. Target PHCZs, along with surrogate standards, were spiked into a composite of Tilapia (*Oreochromis niloticus*) filets purchased from a local supermarket and processed using the methodology described above. The Tilapia filets were tested prior to spiking

experiments and proven to be free of PHCZs. The mean ( $\pm$ standard deviation) recoveries of PHCZ congeners ranged between  $81.1 \pm 8.4\%$  and  $106.9 \pm 7.7\%$  during spiking tests ( $n = 6$ ). Replicate analysis of randomly chosen Great Lakes fish samples ( $n = 6$ ) revealed the relative standard deviations (RSDs) were less than 7.4% for the total PHCZs concentrations and ranged from 5.7% to 10.2% for individual congeners. Surrogate standards FBDE-160 (for ECNI determination) and PCB-204 (for EI determination) had mean ( $\pm$ standard deviation) recoveries of  $101.6 \pm 15.4\%$  and  $88.4 \pm 13.7\%$  in Great Lakes fish, respectively. Previous studies have demonstrated the suitability of these reference standards for PHCZ analysis in various matrixes (e.g., sediment, fish, marine mammals, and bird eggs).<sup>11,12,18</sup> The IDL of an individual PHCZ congener, defined as 5 times the standard deviation of the noise from the GC-MS determination, ranged from 0.02 to 0.26 ng/mL. The method limit of quantification (MLOQ) of each congener was assessed by multiplying a Student's *t*-value designated for a 99% confidence level with standard deviations in replicate analyses ( $n = 8$ ) of Tilapia filets.<sup>11</sup> The MLOQs were estimated to be 0.6–1.1 ng/g lw (Table S2). To date, there have been no reference data ever published for PHCZs in any standard reference materials (SRMs). Considering that PHCZ determination may be interfered with by coeluting compounds (particularly PCBs) during instrumental analysis, we employed several procedures to ensure data quality, including (1) the use of a 30-m column to reduce the possibility of coelution, (2) the use of relatively abundant fragment ions for quantification if the molecular ions of a congener resemble those of PCBs, and (3) employment of GC-triple quadrupole MS to confirm analytical results of the congeners potentially confounded by coeluting substances. Details of these procedures are given in the [Supporting Information](#).

**Age Normalization.** Bioaccumulation rates of chemicals vary with fish size and age,<sup>40–43</sup> which are largely influenced by a lake's trophic status.<sup>44–48</sup> Although the GLFMSP controls the size of the fish collected, the age of sampled fish has varied significantly over the past decade even within the same lake (particularly in LH, LM, and LS; Figure S4). This occurred likely due to changes in trophic structure caused by invasive species and eutrophication.<sup>47–53</sup> Therefore, fish age variations among lakes or over time within the same lake should be taken into account for valid determination of a contaminant's spatiotemporal distributions. For this purpose, an age normalization approach was developed based on recent Great Lakes studies to normalize measured concentrations of individual PHCZs.<sup>44–46</sup> The age normalization method is based on fish bioaccumulation patterns as described in previous work and the concentration comparability among PBT chemicals in fish.<sup>44–47</sup> The details of the development and application of the age normalization method are summarized in the [Supporting Information](#). The normalized data were used for the determination of interlake or intersite differences and temporal trends in PHCZ contamination.

**Data Analysis.** Concentrations of PHCZs were adjusted with the recoveries of FBDE-160 or PCB-204 for ECNI or EI analysis, respectively, and reported on a lipid weight basis (lw) unless otherwise noted. Analytes with instrumental responses lower than IDLs were considered nondetectable (nd). A value was assigned to an individual measurement below IDL for statistical analyses using a regression plotting method,<sup>54</sup> whereas a half MLOQ was assigned if a measurement was

below MLOQ. All data were logarithmically transformed prior to the analysis of variance (ANOVA) with Tukey's posthoc test. The ANOVA and the principal component analysis (PCA) were conducted using OriginPro 9.0 (OriginLab Corporation). The nonparametric Kendall's tau test and Kendall–Theil robust line were employed for trends analysis using OriginPro 9.0 and KTRLLine software (U.S. Geological Survey), respectively.<sup>55</sup> A significance level of  $\alpha = 0.05$  was applied. The Kendall–Theil trend percentage was calculated using the equation:

$$\text{Kendall–Theil Trend \%} = \text{slope/average} \quad (1)$$

where slope represents the generated Kendall–Theil slope and average is the mean value of concentration data used for the estimation of Kendall–Theil slope.

The toxic equivalent (TEQ) of PHCZs was determined using eq 2:<sup>11</sup>

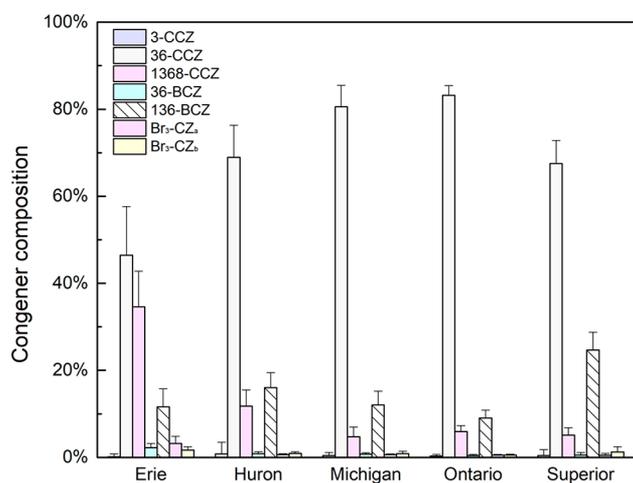
$$\text{TEQ}^{\text{PHCZs}} = \sum (C_i \times \text{REP}_i) \quad (2)$$

where  $C_i$  is the concentration of an individual PHCZ congener and  $\text{REP}_i$  represents its relative effect potency (REP) compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).<sup>21</sup> Riddell et al. reported the similarity between PHCZs and other dioxin-like compounds (e.g., coplanar PCBs and PCDDs) in the structure-dependent induction of cytochrome P450 CYP1A1 and CYP1B1 gene expression in aryl hydrocarbon-responsive MDA-MB-468 breast cancer cells.<sup>21</sup> The induction of CYP1A1 and CYP1A1-dependent activities is considered one of the most sensitive indicators of TCDD-like activity.<sup>56</sup> The presence of a structure-dependent correlation between the potencies of individual congeners has been used to estimate REPs for a group of halogenated substances with dioxin-like activities by comparison with TCDD, which was assigned an REP of 1.0. In the present study, we used the REP values reported by Riddell et al. to estimate the TEQ of PHCZs and employed eq 2 to determine the total dioxin toxicity of PHCZs relative to TCDD.<sup>11,21</sup>

## RESULTS AND DISCUSSION

**Bioaccumulation and Congener Profiles of PHCZs in Great Lakes Fish.** PHCZs were detected in fish mega-composites from all lakes and years, demonstrating broad exposure and substantial bioaccumulation potency (Table S2). Concentrations of  $\sum$ PHCZs (including 11 target PHCZ congeners and two detectable UNCs, Br<sub>3</sub>-CZ<sub>a</sub> and Br<sub>3</sub>-CZ<sub>b</sub>) ranged from 33.4 to 376 ng/g lw (or 0.8–72.2 ng/g wet weight or ww) in all mega-composites, with a median concentration of 101 ng/g lw (or 20.4 ng/g ww). Prior to the present work, there was only one study quantitatively determining PHCZs in biota, which reported a median  $\sum$ PHCZ concentration of 69.4 ng/g lw in various sport fish from the San Francisco Bay (U.S.).<sup>11</sup> In the same study, PHCZs were also detected in harbor seals (*Phoca vitulina*; 53.3–1058 ng/g lw; median 165 ng/g lw) and cormorant eggs (*Phalacrocorax auritus*; 29.6–584 ng/g lw; median 155 ng/g lw).<sup>11</sup> Biomagnification was determined from fish to harbor seal, with an estimated biomagnification factor (BMF) of 2.7–6.1.<sup>11</sup> Both studies had a 100% detection frequency for PHCZs regardless of species. Thus, despite very limited biota studies, data from the Great Lakes and San Francisco Bay convincingly demonstrate that PHCZs are bioavailable in both freshwater and marine ecosystems.

The compositional profiles of PHCZs based on age normalized (Figure 2) or non-normalized data (Figure S5)

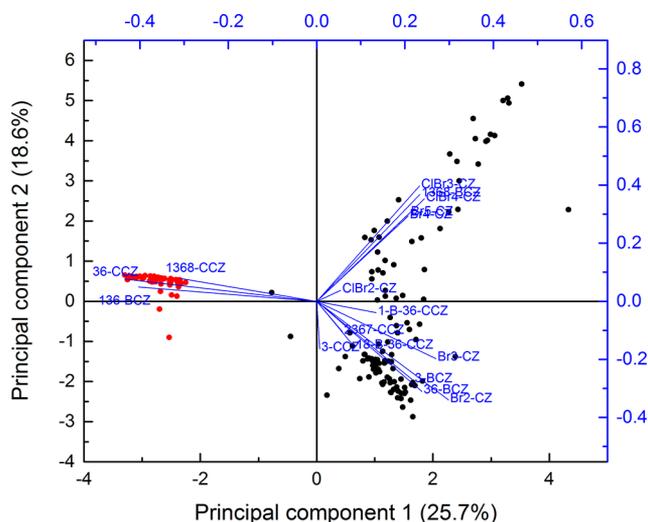


**Figure 2.** Age-normalized congener compositions of PHCZs in Great Lakes fish. Error bars represent standard deviations.

resemble each other. Congener compositions in Great Lakes fish were dominated by 36-CCZ, followed by 136-BCZ or 1368-CCZ (Figure 2). These three congeners, combined together, contributed 88.1 to 100% of the total PHCZs. Other frequently detected congeners included 3-CCZ, 36-BCZ, and two tribromocarbazoles (i.e., Br<sub>3</sub>-CZ<sub>a</sub> and Br<sub>3</sub>-CZ<sub>b</sub>). The dominance of PHCZs with halogen substitution at the *para*- and/or *ortho*- positions relative to nitrogen at the carbazole agree with the results from laboratory-based chemical or enzymatic synthesis of PHCZs,<sup>26,57</sup> reflecting a favored electrophilic aromatic substitution pattern.<sup>58</sup> The frequently detected PHCZs have an estimated log *K*<sub>ow</sub> (octanol–water partitioning coefficient) of 4.58–6.85, falling into the range of log *K*<sub>ow</sub> (i.e., 4.5–8) predicted for a chemical that can bioaccumulate and biomagnify in aquatic food webs.<sup>59</sup>

The PHCZ congener profiles in Great Lakes fish were similar to that reported in San Francisco Bay fish, where 36-CCZ and 136-BCZ were the most abundant congeners. However, distinctly different composition profiles were observed between Great Lakes fish and sediment; the latter was dominated by brominated carbazoles substituted with 2–5 bromine atoms (Figure 3).<sup>9</sup> The discrepancy between fish and sediment clearly suggests congener-specific bioaccumulation potency or resistance to metabolism. Sediment data for PCA analysis were cited from Guo et al., who obtained surface sediment in 2010–2012 from a number of sites in LM, LS and LH.<sup>9</sup> Lake- or site-specific contamination sources and/or sedimentation made a considerable impact on the composition,<sup>9</sup> leading to substantial variation of compositions as reflected by the PCA biplot (Figure 3). By contrast, fish samples were from relatively limited sites (i.e., two from each lake). The bioaccumulation or metabolic potency of individual PHCZ congeners may be relatively consistent in the same species. This likely resulted in a more consistent composition profile in fish (Figure 3). Although fish and sediment samples used for PCA analysis differed in sites and years, we believe these differences would not confound the PCA results, given the distinctly different composition patterns observed between fish and sediment.

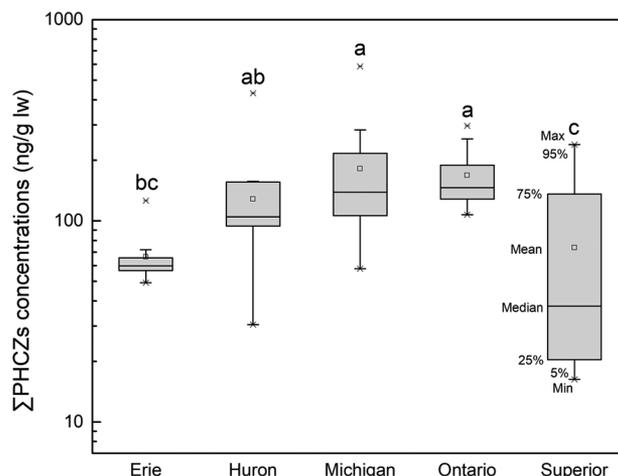
**Spatial Distribution Patterns.** Age normalized data were used for spatial analysis to minimize the impact by interlake



**Figure 3.** Biplot from the principal component analysis of PHCZ congener compositions in Great Lakes fish (red dots) and sediment (black dots). Sediment data were from Guo et al.<sup>9</sup>

variances in trophic structure or fish age (Tables S2 and S3). As statistical analyses did not reveal significant differences in  $\sum$ PHCZ concentrations between industrial and nonindustrial sites from each lake, the two sites were combined for lake comparison. The two LE species (i.e., lake trout and walleye) were also combined for statistical analysis, given that they did not exhibit significant concentration differences ( $p = 0.58$ ). The mix of hatchery and wild lake trout also did not bring systematic bias to the analysis. For example, although greater than 50% of the LO lake trout collected from years 2007, 2009, 2011, 2013, and 2015 had hatchery coded wire tags (Figure S1), neither  $\sum$ PHCZ concentrations nor compositions differed significantly from even and odd collection years.

Significant spatial differences in fish  $\sum$ PHCZ concentrations were found among five lakes (ANOVA,  $p < 0.01$ ; Figure 4). Fish concentrations of  $\sum$ PHCZs were the highest from LM (mean  $\pm$  standard deviation:  $181 \pm 141$  ng/g lw after age normalization) and LO ( $168 \pm 55.1$  ng/g lw), followed by LH ( $128 \pm 102$  ng/g lw), whereas LE ( $66.1 \pm 20.3$  ng/g lw) and

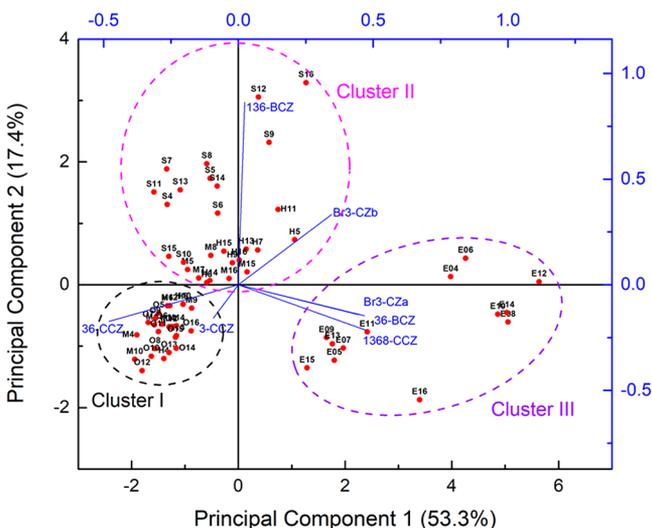


**Figure 4.** Comparison of the total concentrations of PHCZs ( $\sum$ PHCZs, age normalized) in fish mega-composites from five lakes. Boxes without a common letter indicate a statistically significant difference in concentrations.

LS fish ( $73.5 \pm 71.0$  ng/g lw) contained the lowest concentrations. A large variation in  $\sum$ PHCZ concentrations was observed in LS fish, which may be partially attributed to the concentration difference between two LS sites prior to 2011 (Table S2). A similar pattern has been observed for some other PBT chemicals (e.g., PCBs and Hg) in LS fish.<sup>46,60</sup>

The spatial pattern of PHCZs was similar to that of PBDEs in Great Lakes fish and herring gulls (*Larus argentatus*). Some of the highest  $\sum$ PBDE concentrations were found in fish or gull egg composites from LM and LO sites in the period of 2004–2006 during which the peak PBDE contamination was reported in Great Lakes ecosystems.<sup>6,61</sup> By contrast, some of the lowest fish PBDE concentrations were reported in LE and LS.<sup>6</sup> Lake Michigan appeared to be the most contaminated lake with respect to PBDEs and some other POPs, mainly due to high human population densities and intense industrial activities along its shorelines.<sup>2</sup> Hydrological factors (e.g., water retention time or water depth) may also affect spatial distribution.<sup>6,46</sup> Lakes Erie has a relatively shorter water residence time (i.e., 2.6 yr) than the other lakes (i.e., 6–191 yr).<sup>6,62</sup> Hydraulic retention time may affect the turnover and bioaccumulation rates of chemicals in aquatic systems.<sup>6,63</sup> Thus, the interlake variation in PHCZ contamination may be partially, if not all, from a combination of anthropogenic and hydrological influences.

Congener compositions of PHCZs also exhibited spatial differences in fish among five lakes. Although 36-CCZ was overall the most abundant congener (Figure 2), the PCA analysis of congener compositions revealed three separate clusters (Figure 5). Cluster I contained mainly LM and LO



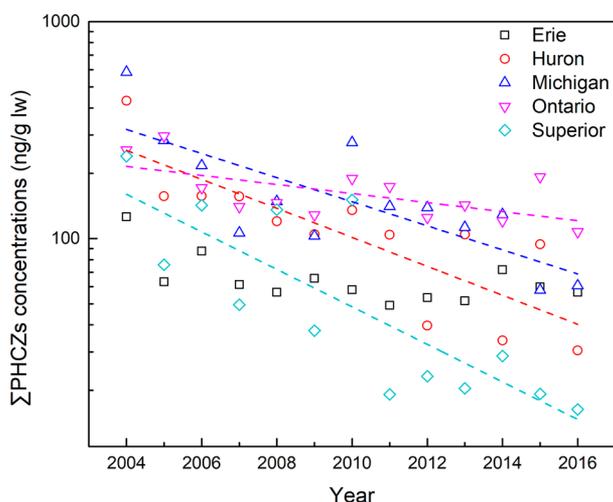
**Figure 5.** Biplot from the principal component analysis of PHCZ congener compositions (age normalized) in fish mega-composites from five lakes. The E, H, M, O, and S represent fish mega-composites collected from Lakes Erie, Huron, Michigan, Ontario, and Superior, respectively. The digit following a letter indicates the sampling year.

samples as well as some LH samples, which was characterized by the predominance of 36-CCZ (average compositions >75%). Cluster II contained all LS and some LM and LH samples, characterized by relatively elevated compositions of 136-BCZ compared to other lakes. Lake Erie samples were all included in cluster III, which was characterized by elevated contributions from 1368-CCZ and 36-BCZ, as well as Br<sub>3</sub>-CZ<sub>a</sub>. The average ( $\pm$ standard deviation) percentage of 1368-

CCZ ( $32.2 \pm 4.4\%$ ) in LE fish was much greater than that in other lakes (ranging from  $3.8 \pm 0.6\%$  to  $10.9 \pm 3.5\%$ ). The difference between LE and other lakes in congener profiles was not due to a combination of walleye and lake trout from LE for analysis, given that neither congener compositions nor concentrations of PHCZs exhibited significant differences between the two species (two-way ANOVA,  $p > 0.05$ ). Instead, it may suggest additional input of selected PHCZs to LE from unrecognized sources.

Overall, spatial differences in congener compositions and/or concentrations may suggest interlake differences in the sources and input of selected PHCZs, which can be complicated by other factors such as hydrological characteristics and trophic status. However, an in-depth interpretation of spatial distributions is limited by our understanding of the dominant sources of PHCZs to the lakes. Previous studies suggested a complexity of natural and anthropogenic sources for PHCZs.<sup>9,18</sup> Deposition of 36-BCZ and 136-BCZ with the Great Lakes sediment cores has increased in the second half of the 20th century but started to decline from the late 1990s, which suggests anthropogenic input of these congeners. Halogenated carbazoles have been involved in the production of various industrial products, including dyes, pharmaceuticals, and organic semiconductors.<sup>9,23,64</sup> A full array of chlorinated carbazoles has been detected at sites near a chlorine production facility.<sup>65</sup> All of these point to possible anthropogenic sources. However, natural sources may exist for some halogenated carbazoles. The 36-BCZ, 36-CCZ, and some other halogenated carbazoles were reported in marine cyanobacteria<sup>66,67</sup> and have been produced from carbazole via enzymatic synthesis,<sup>26,39</sup> supporting a natural source. Therefore, the complexity of potential sources complicates the interpretation of spatial distributions of PHCZs, thus confounding our understanding of interlake or intersite contamination differences. Additional approaches, such as the molecular-level <sup>14</sup>C analysis, may better elucidate if a PHCZ congener originates from natural or industrial sources<sup>68</sup> but are beyond the scope of this work.

**Temporal Trends.** During the study period (2004–2016), significant changes in  $\sum$ PHCZ concentrations ( $p < 0.05$ ) were observed in fish from all lakes except for LE, where only a marginal trend was observed (Kendall's tau =  $-0.39$ ,  $p = 0.07$ ; Figure 6). The trend slopes ranged from  $-10.2\%$  per year in LS to  $-3.9\%$  in LO (Table S5). The declining trends were mainly driven by the changes of two major congeners, 36-CCZ and 136-BCZ, which had concentrations declining significantly at most study sites with trend slopes (i.e.,  $-10.4$  to  $-3.8\%$  and  $-13.5$  to  $-6.2\%$ , respectively) steeper than other congeners. These results agreed with the findings from the Great Lakes sediment core study. The 36-CCZ and 3-CCZ had concentrations continuously declining from the 1600s in Great Lakes sediment cores, although the concentrations fluctuated in the 1900s.<sup>9</sup> Concentrations of 136-BCZ, 36-BCZ, and several other brominated PHCZs with unknown halogen substitution patterns had similar trends in sediment cores as that of 36-CCZ before 1900, but began to increase around 1950, followed by a decrease or fluctuation in recent decades.<sup>9</sup> The lack of a significant trend in LE may be due to a relatively constant input of PHCZs to the lake or the lake-specific physical features, i.e., a relatively shallow water depth and a fast turnover rate of water in LE compared with the other four lakes.<sup>62,63</sup> Bhavsar et al. also suggested that among-lake differences in the change of food web structure could lead to lake-specific temporal trends of contaminants by altering the



**Figure 6.** Temporal trends of age normalized PHCZ concentrations in Great Lakes fish mega-composites. Dashed lines represent statistically significant correlations between PHCZ concentration and sampling year in fish from Lake Huron (Kendall's tau =  $-0.82$ ,  $p < 0.001$ ), Michigan (Kendall's tau =  $-0.62$ ,  $p < 0.01$ ), Ontario (Kendall's tau =  $-0.44$ ,  $p = 0.04$ ), and Superior (Kendall's tau =  $-0.64$ ,  $p < 0.01$ ), respectively. No significant trend was observed in fish from Lake Erie (Kendall's tau =  $-0.39$ ,  $p = 0.07$ ; line not shown on plot).

way contaminants are accumulated in predatory fish.<sup>69</sup> The lake-specific trends were also reported for legacy chemicals such as PCBs. Although PCBs concentrations exhibited significant decreases during the period of 1976–2006 in lake trout and walleye from LS, LH, and LO, the levels did show slight increases in LE fish.<sup>69</sup> However, the scarcity of knowledge on lake-specific PHCZ sources and various aspects of PHCZs' environmental behavior (e.g., sediment or air–water partition, resistance to degradation or metabolism, and food-web transfer) prevents us from in-depth interpretation of the lake-specific temporal trends.

Compositions of major PHCZ congeners did not exhibit significant temporal changes at most sites except in LO and LS where the percentage of 1368-CCZ increased significantly ( $p = 0.01$  and  $0.04$ , respectively), while that of 136-BCZ had a significant decrease in LO ( $p = 0.01$ ) and LE fish ( $p = 0.04$ ; Table S5). The increasing compositions of 1368-CCZ observed in LO and LS are mainly due to the fact that its concentrations at these sites did not change significantly over time or decreased less rapidly than other main congeners such as 36-CCZ and 136-BCZ, leading to an increase of the relative abundance of 1368-CCZ to the total PHCZ concentrations. The same reason could explain the marginal increase of the composition of a minor congener  $\text{Br}_3\text{-CZ}_b$  in some lakes (Table S5).

Overall, these temporal trends pointed to a decreasing input of PHCZs to the Great Lakes in past decades. It is expected that the concentrations of PHCZs in Great Lakes ecosystems may continue to decrease without significant anthropogenic input in the future. However, the trends may be confounded by factors such as climate change and eutrophication. Resuspension of sediments in shallow water may be increased due to increasing storms and less ice cover caused by a warmer climate.<sup>46</sup> Olsson et al. suggested the important influence of sediment resuspension on sediment contamination by DDT and PCBs in the Baltic Sea.<sup>70</sup> Thus, in shallow lakes or sites, resuspension of old sediment particles which usually represent a

former more polluted time period may increase pollutant concentrations in surface sediment.<sup>70</sup> Eutrophication and invasive species may cause lake-specific changes in food webs and trophic structures, subsequently altering the bioaccumulation and biomagnification rates of PHCZs in predatory fish. Previous studies suggested that eutrophication could increase mercury methylation rates by changing sediment microbial metabolism or increasing the organic carbon loading in sediment<sup>71</sup> and increase the association of PCBs with dissolved organic matter in marine microcosms.<sup>72</sup> Thus, these factors, combined together, may influence lake-specific changes of PHCZ concentrations and subsequent exposure to aquatic organisms.

We would like to point out that the age normalization approach could bring potential bias to temporal trend analyses. The trends of PBT chemicals chosen for age normalization could potentially affect the trend analyses for PHCZs. For comparison, Table S4 summarizes the results of temporal analyses using non-normalized data. Similar temporal trends were observed when using data with or without age transformation in LO or LE where fish age did not exhibit a significant temporal trend. The data without age normalization also suggested declining trends in  $\sum$ PHCZ concentrations in fish mega-composites collected from LH and LS during even years, whereas no significant trends were found in odd year samples from these two lakes. Additionally, no significant trends were found in LM (even or odd year collection) based on non-normalized data. Nevertheless, given significant increases of fish age in LH, LS, and LM (even years only), age factor could substantially bias the spatiotemporal analyses. The potential biases can be essentially minimized by age normalization to homogenize PHCZ data based on chemical exposure times (age). We have shown that age adjustment for legacy contaminants is important to account for variability in age/length relationships resulting from the recent perturbations in the Great Lakes food webs.<sup>45,46,73</sup> Additionally, as we mentioned earlier, the use of mega-composites may limit trend analyses due to the lack of a variability assessment. Given the wide distribution and bioaccumulation of PHCZs in Great Lakes fish, future analysis will investigate the occurrence of these compounds in more detail, including the variability of concentrations and compositions among individual fish and its influence on spatiotemporal analyses.

**Environmental Implications.** To provide a broader picture of the importance of PHCZ contamination, PHCZs were compared with some legacy PBTs in Great Lakes fish.<sup>5–7,47</sup> The median concentration ratios of  $\sum$ PHCZs to  $\sum$ PBDEs,  $\sum$ PCBs,  $\sum$ DDTs (dichlorodiphenyltrichloroethanes), or  $\sum$ Chlordanes were 0.34, 0.02, 0.10, or 0.63, respectively (Figure S6). Recent annual loading of  $\sum$ PHCZs in LH, LM, and LS sediment was estimated to be 811–1325 kg/yr, greater than those of BDE-209 (109–491 kg/yr) and  $\sum$ PCBs (54.2–558 kg/yr), which are two prominent groups of halogenated chemicals in the environment.<sup>9</sup> Potential biomagnification as demonstrated in the San Francisco Bay study may lead to elevated concentrations in predatory species such as piscivorous birds in the Great Lakes region.<sup>11</sup>

Environmental concerns of PHCZs also arise from their potential toxicity, particularly dioxin-like effects. PHCZ congeners have a coplanar structure resembling that of polychlorinated dibenzo-*p*-dioxins or dibenzofurans (PCDDs or PCDFs). Exposure of zebrafish (*Danio rerio*) to 27-BCZ and 2367-CCZ at nanomolar levels resulted in phenotypes similar

to dioxin-induced cardiotoxicity, including swollen yolk sac, pericardial sac edema, elongated and unlooped heart and lower jaw shortening, and induced CYP4501A expression primarily in the heart area.<sup>22</sup> In the evaluation of structure-dependent induction of CYP1A1 and CYP1B1 gene expression in breast cancer cells, Riddell et al. reported the induction responses for the most active PHCZs (e.g., 2367-CCZ) to be at a magnitude similar to that for TCDD. The estimated REPs of mono- to tetra-PHCZs compared to TCDD range from 0.000013 to 0.00066.<sup>21</sup> In the present study, we estimated the median TEQ<sup>PHCZs</sup> ranging from 8.7 pg of TEQ/g lw in LS fish to 25.7 pg of TEQ/g lw in LH fish (Table S2). Great Lakes fish contained TEQ<sup>PHCZs</sup> significantly greater than those of San Francisco Bay fish (2.5–34.8 pg TEQ/g lw;  $p = 0.003$ ) but less than harbor seal blubber (7.6–174 pg TEQ/g lw;  $p = 0.002$ ) from the same area.<sup>11</sup> These two studies represent the only available work investigating TEQ<sup>PHCZs</sup> in biota.

The present study provides the first insight into the bioaccumulation and spatiotemporal distributions of PHCZs in Great Lakes ecosystems. Our findings strongly demonstrate the bioavailability of this group of CECs in aquatic ecosystems. Available data, although limited, suggest that PHCZs are PBT chemicals and potentially a class of POPs with a global distribution. However, there exist a number of missing pieces in the puzzle of this group of contaminants, including their origin or sources, rates and kinetics of degradation or metabolism, and the occurrences and fate in nonaquatic systems. Additionally, little is known about potential human exposure to PHCZs, including exposure pathways, intake rates, and possible health effects. A better elucidation of the environmental and human health risks of PHCZs requires additional field and laboratory studies.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b00427.

Tables S1–S7 and Figures S1–S8, as well as a detailed description of the age normalization method (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Liu, L. Y.; Salamova, A.; Venier, M.; Hites, R. A. Trends in the levels of halogenated flame retardants in the Great Lakes atmosphere over the period 2005–2013. *Environ. Int.* **2016**, *92–93*, 442–449.
- (2) Venier, M.; Salamova, A.; Hites, R. A. Halogenated flame retardants in the Great Lakes environment. *Acc. Chem. Res.* **2015**, *48*, 1853–1861.
- (3) Venier, M.; Dove, A.; Romanak, K.; Backus, S.; Hites, R. Flame retardants and legacy chemicals in Great Lakes' water. *Environ. Sci. Technol.* **2014**, *48*, 9563–9572.
- (4) Batterman, S.; Chernyak, S.; Gwynn, E.; Cantonwine, D.; Jia, C.; Begnoche, L.; Hickey, J. P. Trends of brominated diphenyl ethers in fresh and archived Great Lakes fish (1979–2005). *Chemosphere* **2007**, *69*, 444–457.
- (5) Carlson, D. L.; De Vault, D. S.; Swackhamer, D. L. On the rate of decline of persistent organic contaminants in lake trout (*Salvelinus namaycush*) from the Great Lakes, 1970–2003. *Environ. Sci. Technol.* **2010**, *44*, 2004–2010.
- (6) Crimmins, B. S.; Pagano, J. J.; Xia, X.; Hopke, P. K.; Milligan, M. S.; Holsen, T. M. Polybrominated diphenyl ethers (PBDEs): turning the corner in Great Lakes trout 1980–2009. *Environ. Sci. Technol.* **2012**, *46*, 9890–9897.
- (7) Salamova, A.; Pagano, J. J.; Holsen, T. M.; Hites, R. A. Post-1990 temporal trends of PCBs and organochlorine pesticides in the atmosphere and in fish from Lakes Erie, Michigan, and Superior. *Environ. Sci. Technol.* **2013**, *47*, 9109–9114.
- (8) Guo, J.; Venier, M.; Salamova, A.; Hites, R. A. Bioaccumulation of dechloranes, organophosphate esters, and other flame retardants in Great Lakes fish. *Sci. Total Environ.* **2017**, *583*, 1–9.
- (9) Guo, J.; Li, Z.; Ranasinghe, P.; Bonina, S.; Hosseini, S.; Corcoran, M. B.; Smalley, C.; Rockne, K. J.; Sturchio, N. C.; Giesy, J. P.; Li, A. Spatial and temporal trends of polyhalogenated carbazoles in sediments of upper Great Lakes: Insights into their origin. *Environ. Sci. Technol.* **2017**, *51*, 89–97.
- (10) Guo, J.; Chen, D.; Potter, D.; Rockne, K. J.; Sturchio, N. C.; Giesy, J. P.; Li, A. Polyhalogenated carbazoles in sediments of Lake Michigan: a new discovery. *Environ. Sci. Technol.* **2014**, *48*, 12807–12815.
- (11) Wu, Y.; Tan, H.; Sutton, R.; Chen, D. From sediment to top predators: Broad exposure of polyhalogenated carbazoles in San Francisco Bay (U.S.A.). *Environ. Sci. Technol.* **2017**, *51*, 2038–2046.
- (12) Wu, Y.; Moore, J.; Guo, J.; Li, A.; Grasman, K.; Choy, S.; Chen, D. Multi-residue determination of polyhalogenated carbazoles in aquatic sediments. *J. Chromatogr. A* **2016**, *1434*, 111–118.
- (13) Pena-Abaurrea, M.; Robson, M.; Chaudhuri, S.; Riddell, N.; McCrindle, R.; Chittim, B.; Parette, B.; Jin, U.-H.; Safe, S.; Poirier, D.; Ruffolo, R.; Dyer, R.; Fletcher, R.; Helm, P. A.; Reiner, E. J. Environmental levels and toxicological potencies of a novel mixed halogenated carbazole. *Emerg. Contam.* **2016**, *2*, 166–172.
- (14) Chen, W. L.; Xie, Z.; Wolschke, H.; Gandrass, J.; Kotke, D.; Winkelmann, M.; Ebinghaus, R. Quantitative determination of ultra-trace carbazoles in sediments in the coastal environment. *Chemosphere* **2016**, *150*, 586–595.
- (15) Heim, S.; Schwarzbauer, J.; Kronimus, A.; Littke, R.; Woda, C.; Mangini, A. Geochronology of anthropogenic pollutants in riparian wetland sediments of the Lippe River (Germany). *Org. Geochem.* **2004**, *35*, 1409–1425.
- (16) Kronimus, A.; Schwarzbauer, J.; Dsikowitzky, L.; Heim, S.; Littke, R. Anthropogenic organic contaminants in sediments of the Lippe river. *Water Res.* **2004**, *38*, 3473–3484.
- (17) Grigoriadou, A.; Schwarzbauer, J. Non-target screening of organic contaminants in sediments from the industrial coastal area of Kavala City (NE Greece). *Water, Air, Soil Pollut.* **2011**, *214*, 623–643.

- (18) Wu, Y.; Qiu, Y.; Tan, H.; Chen, D. Polyhalogenated carbazoles in sediments from Lake Tai (China): Distribution, congener composition, and toxic equivalent evaluation. *Environ. Pollut.* **2017**, *220*, 142–149.
- (19) Mumbo, J.; Pandelova, M.; Mertes, F.; Henkelmann, B.; Bussian, B. M.; Schramm, K. W. The fingerprints of dioxin-like bromocarbazoles and chlorocarbazoles in selected forest soils in Germany. *Chemosphere* **2016**, *162*, 64–72.
- (20) Mumbo, J.; Henkelmann, B.; Abdelaziz, A.; Pfister, G.; Nguyen, N.; Schroll, R.; Munch, J. C.; Schramm, K. W. Persistence and dioxin-like toxicity of carbazole and chlorocarbazoles in soil. *Environ. Sci. Pollut. Res.* **2015**, *22*, 1344–1356.
- (21) Riddell, N.; Jin, U. H.; Safe, S.; Cheng, Y.; Chittim, B.; Konstantinov, A.; Parette, R.; Pena-Abaurrea, M.; Reiner, E. J.; Poirier, D.; Stefanac, T.; McAlees, A. J.; McCrindle, R. Characterization and biological potency of mono- to tetra-halogenated carbazoles. *Environ. Sci. Technol.* **2015**, *49*, 10658–10666.
- (22) Fang, M.; Guo, J.; Chen, D.; Li, A.; Hinton, D. E.; Dong, W. Halogenated carbazoles induce cardiotoxicity in developing zebrafish (*Danio rerio*) embryos. *Environ. Toxicol. Chem.* **2016**, *35*, 2523–2529.
- (23) Parette, R.; McCrindle, R.; McMahan, K. S.; Pena-Abaurrea, M.; Reiner, E.; Chittim, B.; Riddell, N.; Voss, G.; Dorman, F. L.; Pearson, W. N. Halogenated indigo dyes: A likely source of 1,3,6,8-tetrabromocarbazole and some other halogenated carbazoles in the environment. *Chemosphere* **2015**, *127*, 18–26.
- (24) Pevery, A. A.; Hites, R. A. Comment on "Halogenated indigo dyes: A likely source of 1,3,6,8-tetrabromocarbazole and some other halogenated carbazoles in the environment". *Chemosphere* **2016**, *144*, 273–274.
- (25) Parette, R.; McCrindle, R.; McMahan, K. S.; Pena-Abaurrea, M.; Reiner, E.; Chittim, B.; Riddell, N.; Voss, G.; Dorman, F. L.; Pearson, W. N.; Robson, M. Response to the comment on "Halogenated indigo dyes: A likely source of 1,3,6,8-tetrabromocarbazole and some other halogenated carbazoles in the environment". *Chemosphere* **2016**, *150*, 414–415.
- (26) Mumbo, J.; Lenoir, D.; Henkelmann, B.; Schramm, K. W. Enzymatic synthesis of bromo- and chlorocarbazoles and elucidation of their structures by molecular modeling. *Environ. Sci. Pollut. Res.* **2013**, *20*, 8996–9005.
- (27) Zhu, L. Y.; Hites, R. A. Temporal trends and spatial distributions of brominated flame retardants in archived fishes from the Great Lakes. *Environ. Sci. Technol.* **2004**, *38*, 2779–2784.
- (28) Xia, X.; Crimmins, B. S.; Hopke, P. K.; Pagano, J. J.; Milligan, M. S.; Holsen, T. M. Toxaphene analysis in Great Lakes fish: a comparison of GC-EI/MS/MS and GC-ECNI-MS, individual congener standard and technical mixture for quantification of toxaphene. *Anal. Bioanal. Chem.* **2009**, *395*, 457–463.
- (29) Xia, X.; Hopke, P. K.; Crimmins, B. S.; Pagano, J. J.; Milligan, M. S.; Holsen, T. M. Toxaphene trends in the Great Lakes fish. *J. Great Lakes Res.* **2012**, *38*, 31–38.
- (30) Znananski, T. J.; Holsen, T. M.; Hopke, P. K.; Crimmins, B. S. Mercury temporal trends in top predator fish of the Laurentian Great Lakes. *Ecotoxicology* **2011**, *20*, 1568–1576.
- (31) Great Lakes fish stocking database. Available at: <http://www.glf.org/fishstocking/exactsearch.htm> (accessed October 3, 2017).
- (32) Schmalz, P. J.; Hansen, M. J.; Holey, M. E.; McKee, P. C.; Toney, M. L. Lake trout movements in northwestern Lake Michigan. *N. Am. J. Fish. Manag.* **2002**, *22*, 737–749.
- (33) Kapuscinski, K. L.; Hansen, M. J.; Schram, S. T. Movements of lake trout in U.S. waters of Lake Superior, 1973–2001. *N. Am. J. Fish. Manag.* **2005**, *25*, 696–708.
- (34) U.S. EPA. Great Lakes fish monitoring and surveillance program: Quality assurance project plan for sample collection activities. Available at: [https://www.epa.gov/sites/production/files/2016-02/documents/glfmsp\\_qapp\\_version\\_2\\_111312\\_merged\\_508\\_0.pdf](https://www.epa.gov/sites/production/files/2016-02/documents/glfmsp_qapp_version_2_111312_merged_508_0.pdf) (accessed October 11, 2017).
- (35) Swackhamer, D. L.; Hites, R. A. Occurrence and bioaccumulation of organochlorine compounds in fishes from Siskiwit Lake, Isle Royale, Lake Superior. *Environ. Sci. Technol.* **1988**, *22*, 543–548.
- (36) Sharma, C. M.; Rosseland, B. O.; Almvik, M.; Eklo, O. M. Bioaccumulation of organochlorine pollutants in the fish community in Lake Arungen. *Environ. Pollut.* **2009**, *157*, 2452–2458.
- (37) Gewurtz, S. B.; Bhavsar, S. P.; Fletcher, R. Influence of fish size and sex on mercury/PCB concentration: importance for fish consumption advisories. *Environ. Int.* **2011**, *37*, 425–434.
- (38) Wellenkamp, W.; He, J. X.; Vercnocke, D. Using maxillae to estimate ages of lake trout. *N. Am. J. Fish. Manag.* **2015**, *35*, 296–301.
- (39) Chen, Y.; Lin, K.; Chen, D.; Wang, K.; Zhou, W.; Wu, Y.; Huang, X. Formation of environmentally relevant polyhalogenated carbazoles from chloroperoxidase-catalyzed halogenation of carbazole. *Environ. Pollut.* **2018**, *232*, 264–273.
- (40) Vives, I.; Grimalt, J. O.; Lacorte, S.; Guillamón, M.; Barceló, D.; Rosseland, B. O. Polybromodiphenyl ether flame retardants in fish from lakes in European high mountains and Greenland. *Environ. Sci. Technol.* **2004**, *38*, 2338–2344.
- (41) McIntyre, J. K.; Beauchamp, D. A. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. *Sci. Total Environ.* **2007**, *372*, 571–584.
- (42) Volta, P.; Tremolada, P.; Neri, M. C.; Giussani, G.; Galassi, S. Age-dependent bioaccumulation of organochlorine compounds in fish and their selective biotransformation in top predators from Lake Maggiore (Italy). *Water, Air, Soil Pollut.* **2009**, *197*, 193–209.
- (43) Gui, D.; Yu, R.; He, X.; Tu, Q.; Chen, L.; Wu, Y. Bioaccumulation and biomagnification of persistent organic pollutants in Indo-Pacific humpback dolphins (*Sousa chinensis*) from the Pearl River Estuary, China. *Chemosphere* **2014**, *114*, 106–113.
- (44) El-Shaarawi, A. H.; Backus, S.; Zhu, R.; Chen, Y. Modelling temporal and spatial changes of PCBs in fish tissue from Lake Huron. *Environ. Monit. Assess.* **2011**, *173*, 611–623.
- (45) Paterson, G.; Ryder, M.; Drouillard, K. G.; Haffner, G. D. Contrasting PCB bioaccumulation patterns among Lake Huron lake trout reflect basin-specific ecology. *Environ. Toxicol. Chem.* **2016**, *35*, 65–73.
- (46) Zhou, C.; Cohen, M. D.; Crimmins, B. A.; Zhou, H.; Johnson, T. A.; Hopke, P. K.; Holsen, T. M. Mercury temporal trends in top predator fish of the Laurentian Great Lakes from 2004 to 2015: Are concentrations still decreasing? *Environ. Sci. Technol.* **2017**, *51*, 7386–7394.
- (47) Chang, F.; Pagano, J. J.; Crimmins, B. S.; Milligan, M. S.; Xia, X.; Hopke, P. K.; Holsen, T. M. Temporal trends of polychlorinated biphenyls and organochlorine pesticides in Great Lakes fish, 1999–2009. *Sci. Total Environ.* **2012**, *439*, 284–290.
- (48) Crane, D. P.; Einhouse, D. W. Changes in growth and diet of smallmouth bass following invasion of Lake Erie by the round goby. *J. Great Lakes Res.* **2016**, *42*, 405–412.
- (49) Warner, D. M.; Lesht, B. M. Relative importance of phosphorus, invasive mussels and climate for patterns in chlorophyll a and primary production in Lakes Michigan and Huron. *Freshwater Biol.* **2015**, *60*, 1029–1043.
- (50) Lepak, R. F.; Krabbenhoft, D. P.; Ogorek, J. M.; Tate, M. T.; Bootsma, H. A.; Hurley, J. P. Influence of cladophora-quagga mussel assemblages on nearshore methylmercury production in Lake Michigan. *Environ. Sci. Technol.* **2015**, *49*, 7606–7613.
- (51) Cornwell, E. R.; Goyette, J.-O.; Sorichetti, R. J.; Allan, D. J.; Kashian, D. R.; Sibley, P. K.; Taylor, W. D.; Trick, C. G. Biological and chemical contaminants as drivers of change in the Great Lakes–St. Lawrence river basin. *J. Great Lakes Res.* **2015**, *41*, 119–130.
- (52) Swackhamer, D. L.; Pearson, R. F.; Schottler, S. P. Toxaphene in the Great Lakes. *Chemosphere* **1998**, *37*, 2545–2561.
- (53) He, J. X.; Bence, J. R. Modeling annual growth variation using a hierarchical Bayesian approach and the von Bertalanffy growth function, with application to lake trout in southern Lake Huron. *Trans. Am. Fish. Soc.* **2007**, *136*, 318–330.
- (54) Newman, M. C. *Quantitative Methods in Aquatic Ecotoxicology*; Lewis Publishers, Inc.: Boca Raton, FL, 1995; pp 21–40.
- (55) Granato, G. E. Kendall-Theil Robust Line (KTRLine version 1.0): a visual basic program for calculating and graphing robust nonparametric estimates of linear regression coefficients between two

continuous variables. *Techniques and Methods of the U.S. Geological Survey*; USGS: Reston, VA, 2006; Book 4, Chapter A7, p 31.

(56) Safe, S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* **1990**, *21*, 51–88.

(57) Xu, X.; Wang, D.; Li, C.; Feng, H.; Wang, Z. Characterization of the reactivity and chlorinated products of carbazole during aqueous chlorination. *Environ. Pollut.* **2017**, *225*, 412–418.

(58) Bonesi, S. M.; Erra-Balsells, R. On the synthesis and isolation of chlorocarbazoles obtained by chlorination of carbazoles. *J. Heterocycl. Chem.* **1997**, *34*, 877–889.

(59) Kelly, B. C.; Ikononou, M. G.; Blair, J. D.; Morin, A. E.; Gobas, F. A. Food web-specific biomagnification of persistent organic pollutants. *Science* **2007**, *317*, 236–239.

(60) Hickey, J. P.; Batterman, S. A.; Chernyak, S. M. Trends of chlorinated organic contaminants in great lakes trout and walleye from 1970 to 1998. *Arch. Environ. Contam. Toxicol.* **2006**, *50*, 97–110.

(61) Gauthier, L. T.; Hebert, C. E.; Weseloh, D. V. C.; Letcher, R. J. Dramatic changes in the temporal trends of polybrominated diphenyl ethers (PBDEs) in herring gull eggs from the Laurentian Great Lakes: 1982–2006. *Environ. Sci. Technol.* **2008**, *42*, 1524–1530.

(62) U.S. EPA. Physical features of the Great Lakes. Available at: <https://www.epa.gov/greatlakes/physical-features-great-lakes> (accessed April 10, 2017).

(63) Luross, J. M.; Alae, M.; Sergeant, D. B.; Cannon, C. M.; Whittle, D. M.; Solomon, K. R.; Muir, D. C. G. Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. *Chemosphere* **2002**, *46*, 665–672.

(64) Kuehl, D. W.; Durhan, E.; Butterworth, B. C.; Linn, D. Tetrachloro-9H-carbazole, a previously unrecognized contaminant in sediments of the Buffalo River. *J. Great Lakes Res.* **1984**, *10*, 210–214.

(65) Takasuga, T.; Takemori, H.; Yamamoto, T.; Higashino, K.; Sasaki, Y.; Weber, R. The fingerprint of chlorinated aromatic compounds in contaminated sites from chloralkali process and a historic chlorine production using GC-HR-TOF-MS screening. *Organohalogen Compd.* **2009**, *71*, 2224–2229.

(66) Lee, S. C.; Williams, G. A.; Brown, G. D. Maculalactone L and three halogenated carbazole alkaloids from *Kyrtuthrix maculans*. *Phytochemistry* **1999**, *52*, 537–540.

(67) Dembitsky, V. M. Bromo- and iodo-containing alkaloids from marine microorganisms and sponges. *Russ. J. Bioorg. Chem.* **2002**, *28*, 170–182.

(68) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307*, 917–920.

(69) Bhavsar, S. P.; Jackson, D. A.; Hayton, A.; Reiner, E. J.; Chen, T.; Bodnar, J. Are PCB levels in fish from the Canadian Great Lakes still declining? *J. Great Lakes Res.* **2007**, *33*, 592–605.

(70) Olsson, M.; Bignert, A.; Eckhell, J.; Jonsson, P. Comparison of temporal trends (1940s–1990s) of DDT and PCB in Baltic sediment and biota in relation to eutrophication. *Ambio* **2000**, *29*, 195–201.

(71) Gray, J. E.; Hines, M. E. Biogeochemical mercury methylation influenced by reservoir eutrophication, Salmon Falls Creek Reservoir, Idaho, USA. *Chem. Geol.* **2009**, *258*, 157–167.

(72) Gunnarsson, J. S.; Rosenberg, R. Eutrophication increases the association of PCB to dissolved organic matter in marine microcosms. *Mar. Pollut. Bull.* **1996**, *33*, 100–111.

(73) Huestis, S. Y.; Servos, M. R.; Whittle, D. M.; van den Heuvel, M.; Dixon, D. G. Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in lake Ontario lake trout, 1977 to 1993. *Environ. Toxicol. Chem.* **1997**, *16*, 154–164.