Spatial and Temporal Trends of Perfluoroalkyl Compounds in Fish Fillets Collected From Pool 2 of the Upper Mississippi River

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Key words:

ABSTRACT

In a 2011 study perfluoroalkyl compounds (PFCs) were analyzed in fish fillet samples taken from Pool 2 of the Upper Mississippi River, a 33 mile stretch of river inclusive of the Minneapolis/St. Paul, Minnesota metropolitan geographical area. Approximately 100 each of bluegill sunfish (BGS), freshwater drum (FWD), smallmouth bass (SMB) and white bass (WHB) were collected from 10 separate sampling reaches of approximately 3 miles in length. Fish fillet tissue were analyzed for perfluorinated carboxylic acids (PFCAs) (C4–C12), perfluorinated sulfonic acids (PFSAs) (C4, C6, and C8), and perfluorooctane sulfonamide (PFOSA). Perfluorooctane sulfonate (PFOS) was observed with the greatest frequency and at the greatest concentration in fish tissues ranging from 2.3 to 760 ng/g ww. Mean (geometric) PFOS concentrations in BGS, FWD, SMB and WHG were 20, 28, 29 and, 58 ng/g ww, respectively. Concentrations of perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHS), PFOSA and the nine C4-C12 PFCAs had species-specific geometric mean concentrations that were less than 5 ng/g ww. Comparison of the ~~current~~ fish data from this study to data collected in 2009 showed significantly lower ~~reductions of~~ perfluorooctane sulfonate (PFOS) fish tissue concentrations throughout Pool 2. The reduction in mean (geometric) PFOS concentrations from 2009 to 2011 for BGS, FWD, SMB and WHB were 60%, 60%, 43%, and 30% respectively. The measured declines in fish population PFOS levels for the Mississippi River Pool 2 region are consistent with the >10-year cessation of manufacturing of products based on perfluorooctanyl chemistry and with ongoing efforts in Minnesota to effectively control sources of PFCs to the Mississippi River.

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INTRODUCTION

The study of perfluorinated compounds (PFCs) in the environment has received considerable attention since the initial reports of global distribution in human populations and wildlife (1,2). Recent reviews of PFC monitoring in biota and humans have been reported (3,4). PFCs have been measured in freshwater and seawater environments (water and animals) at parts per trillion levels (3, 5-9).

Potential sources of exposure of PFCs to humans include inhalation of household dust, contact with consumer products, drinking water and consumption of food (10-12). Of these sources, food consumption has been estimated to contribute over 90% of the total lifetime exposure to non-occupationally exposed humans (13). Generally measureable concentrations of PFCs have been observed in freshwater fish and seafood and studies have shown that both can account for > 50% of the exposure in human populations in Canada, Spain, Poland, China, and Sweden (12, 14-17). However, fish and seafood have also been shown to be a minor source of PFC exposure in populations from Norway and the UK indicating that regional differences are important when evaluating human exposures (18-20). Overall, these data support the need to evaluate human exposure based on regional or site-specific data that includes not only fish PFC concentrations but also region-specific population differences and corresponding dietary habits.

Several studies have shown that fish collected from navigational Pool 2 in the Upper Mississippi River in the Minneapolis/St. Paul metropolitan area have measureable environmental concentrations of PFCs with PFOS having the greatest contribution to the total body burden of these fish (21-23). The Minnesota Pollution Control Agency (MPCA) released a 2009 study entitled, "Mississippi River Pool 2 Intensive Study of Perfluorochemicals in Fish and Water

2009" which reported PFOS concentrations in fillets of five species of fish (24). Prior to the 2009 study, only limited sampling was conducted of fish for the presence of PFOS in Pool 2 (23, 25). Fish from Pool 2 were first tested in 2004, and sporadic sampling had occurred in various locations in and/or immediately downstream of the pool in 2004, 2005, 2006 and 2008; however, relatively low numbers of fish were collected, and the fish species sampled varied between years. In 2009, MPCA conducted the first comprehensive evaluation of PFCs in fish throughout Pool 2, which involved five fish species sampled in four geographic sections spanning the entirety of the pool. Data from the MPCA study indicated that the freshwater drum had the highest arithmetic mean PFOS concentration at 229 ng g^{-1} (geometric mean of 70 ng g^{-1}). The study also concluded that no discernible trends were seen in PFOS levels in fish tissue over time for species that had been sampled since 2004. Using data from the 2009 MPCA study, the Minnesota Department of Health (MDH) issued a fish consumption advisory of one meal per month for the freshwater drum based on the ~~average~~ arithmetic mean concentration of PFOS exceeding 200 ng g^{-1} (26). Accordingly, Pool 2 was listed as impaired for PFOS. It should be noted that fish consumption advisories and the impairment determination were already in place based on other constituents.

A 2011 fish study was designed and conducted with the objectives of updating the previous 2009 MPCA Study, contributing to the knowledge of potential sources throughout the pool and investigating the temporal impact of the >10-year cessation of manufacturing of products based on perfluorooctanyl chemistry in the region and ongoing efforts in Minnesota to effectively control sources of PFCs to the Mississippi River.

MATERIALS AND METHODS

Study Site. The upper and lower extent of Mississippi River Pool 2 is defined by two dams and locks (Figure 1). The upper extent is bounded by the Ford Dam (Lock & Dam #1) located between Minneapolis and Saint Paul, just north of the confluence of the Mississippi and Minnesota Rivers, while the lower extent is defined by the Hastings Dam (Lock & Dam #2) located just upstream of Hastings, MN. A total of 10 sampling reaches of approximately 3 miles in length were identified in Pool 2 with 100-200 m "buffer zones" between each of the reaches. The 2011 Fish Study included a more highly geographically stratified sampling of Pool 2 than the 2009 MPCA Study. Instead of the four sections of varying lengths sampled previously by the MPCA in 2009, the 2011 survey more finely divided the various sections of the river into ten reaches of approximately equal lengths (compare Sections 1-4 versus Reaches 1-10; Figure 1). Based on this approach, the data density for a given species (fish sampled per river mile) also increased by 67%.

Fish Collection. The fish collection focused on species that may be harvested by licensed anglers for consumption or had been the focus of previous Mississippi River PFC assessment studies. The fish species collected included smallmouth bass (*Micropterus dolomieu*), white bass (*Morone chrysops*), freshwater drum (*Aplodinotus grunniens*), and bluegill (*Lepomis spp).* The sampling objective was to collect 10 fish per species in each of the ten sampling reaches. The primary fish collection technique was electrofishing (Smith-Root®, Vancouver, WA, USA), however limited hook and line sampling was also used when electrofishing was not effective. Due to precipitation events preceding the start of field efforts, water levels in Pool 2 were highly variable and elevated. As a result, electrofishing was hampered and three field collection rounds (Round 1 from May 31-June 9, 2011, Round 2 from August 1-11, 2011 and Round 3 from September 11-15, 2011) were necessary to complete the fish collection ~~from May 31 through September 15, 2011~~.

Upon collection, fish were transferred to clean coolers containing bagged ice and transported to a laboratory for processing. Fish were examined for general health and overt abnormalities, measured, weighed and photographed. Otoliths were removed for age determinations. Fish were filleted based on USEPA guidelines (27). The fillet type for all fish species was scaled and skin-on (major bones removed) which is consistent for data that is used for these species by the Minnesota Department of Health (MDH) to establish fish consumption advisories. Fillets were weighed and homogenized in a chemically-cleaned stainless food processor (Robo Coupe®, Jackson MS, USA) using a freeze-fracture method. Homogenized fillet tissue was then put into clean polyethylene bags and kept frozen until analysis. A total of 396 fish were collected for chemical analysis.

Water Collection. Three water samples were collected from each sampling reach during the middle round (August, ~~round~~ Round 2) of fish collection. Samples were taken from the water column approximately 12 to 24" below the surface and from the main river channel and/or locations where fish were collected. All samples were collected as grab samples using large polyethylene bottles and were distributed to 250 ml high density polyethylene (HDPE) sample bottles that contained internal standards (ISs) and surrogate recovery standards (SRSs).

Experimental - Analytical. *Standards and Reagents.* All target analytes, ISs, SRSs, and mass transitions (MS/MS mode) are shown in Table S1, Supporting Information. Perfluoroheptanoic acid (PFHpA) was purchased from Sigma Aldrich (Milwaukee, WI, USA) while perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorododecanoic acid (PFDoA) were purchased from Oakwood Products (West Columbia, SC, USA). Predominately linear isomeric standards of perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHS), perfluorooctane sulfonate (PFOS) , and the following stable isotope labeled reference standards

were purchased from Wellington Laboratories (Guelph, ON Canada): [1,2,3,4-$^{13}C_4$]PFBA, [1,2,3,4,5-$^{13}C_5$]PFPeA, [1,2-$^{13}C_2$]PFHxA, [1,2,3,4,5-$^{13}C_5$]PFHpA, [1,2,3,4, 5, 6,7, 8-$^{13}C_8$]PFOA, [1,2,3,4,5,6,7,8,9-$^{13}C_9$]PFNA, [1,2,3,4,5-$^{13}C_6$]PFDA, [1,2,3,4,5,6,7-$^{13}C_7$]PFUnA, [1,2-$^{13}C_2$]PFDoA, [1,2,3-$^{13}C_3$]PFHS, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOS, and [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOSA. The stable isotope labeled standard [$^{18}O_2$] PFBS was acquired from RTI International (Research Triangle Park, NC) for use as an internal standard. The following isotopically labeled reference standards were purchased from Wellington Laboratories (Guelph, ON Canada) for use as surrogate recovery standards: [1,2,3,4-$^{13}C_4$]PFOA, [1,2,3,4-$^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA. Standards containing both branched and linear isomers of PFOS (potassium salt, Lot #217), PFOA (ammonium salt, Lot # 332), and FOSA were obtained from 3M Company (St. Paul, MN, USA). All chemicals and reagents used in extraction procedures were from Sigma-Aldrich or VWR Scientific (Bridgeport, NJ, USA) while HPLC grade methanol and acetonitrile were purchased from EM Science (Gibbstown, NJ, USA).

Fish Tissue Analysis. Analytical methods for fish tissues as published by Malinsky et al. (28) were followed in this study. Briefly, frozen fillet samples were homogenized frozen with dry ice to achieve a fine powder consistency. After homogenization, the tissue was transferred to a polyethylene bag which was stored unsealed at -20 °C to allow the residual dry ice to sublime. Approximately 0.5 grams of each sample homogenate was transferred to centrifuge tubes and each sample aliquot received a fixed quantity of stable isotope labeled ISs and SRS for use in quantitation, and for evaluation of analyte recovery, respectively. Extraction of each sample was performed by homogenization in 2.5 ml of acetonitrile, followed by centrifugation and analysis by high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS).

Samples were analyzed using an Agilent 1100 HPLC system with binary pump (Palo Alto, CA) HPLC system interfaced with an AB Sciex (Framingham, MA) API 5000 mass spectrometer equipped with a TurboIon ion™ electro-spray interface and operated in negative-ion mode. PFCs were separated from sample extracts chromatographically using an Oasis HLB online column (3 mm x 20 mm; 25µm particle size) at 30°C using a 2mM ammonium acetate in water and acetonitrile mobile phase system at a flow rate of 400 µL min^{-1} and an injection volume of 40 µL.

Water Analysis. The analytical methods described by Wolf et. al. (29) were followed in this study. Briefly, all samples, calibration standards, and associated quality control samples were extracted using either a pre-conditioned Waters tC18 solid phase extraction (SPE) cartridge (PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHS, PFOS and PFOSA) or a pre-conditioned Oasis HLB SPE cartridge (PFBA, PFPeA, PFHxA, PFHpA, and PFOA). Target analytes were extracted with methanol into polypropylene vials with a 25x concentration factor. Samples were then analyzed using an Agilent 1200 series (Palo Alto, CA) HPLC system interfaced with an AB Sciex (Framingham, MA) API 5500 mass spectrometer equipped with a TurboIon ion™ electro-spray interface and operated in negative-ion MS/MS mode. Analysis of the target analytes was performed using a Betasil™ C18 analytical column (2.1 mm x 100 mm; 5µ particle size) held at 30°C with 2 mM ammonium acetate in water and methanol mobile phase system. The flow rate was 300 µL min^{-1} and the injection volume was either 5 or 10 µL.

Data Analysis. Statistical analyses were performed with SAS (SAS, Ver. 9.3; Cary, NC, USA). Kolmogorov-Smirnov test and probability plots were used to evaluate normality and if necessary, data were log-transformed to approximate normality. Variance homogeneity was evaluated with Levene's test. Species and location PFC concentration differences were evaluated

with mixed models (PROC MIXED) and least square means to account for unequal sample sizes. Tukey's test with the Kramer approximation was used to evaluate differences between treatments (species and/or location). T-tests with the Satterthwaite approximation were used to evaluate differences between groups while correlations were evaluated with the Spearman's rank test. Unless noted, all PFC concentrations in surface water and fish fillet tissues were expressed as a geometric mean and its standard error. Throughout this paper differences with $p < 0.05$ were considered to be significant.

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Quality Assurance. *Water Samples.* A total of three sample bottles were collected from each location (sample, sample duplicate, and 25 ng L^{-1} field matrix spike (FMS)). Mean FMS recoveries (with percent relative standard deviations) in all the river samples of C4–C12 PFCAs, C4, C6, and C8 PFSAs, PFOSA, and [1,2,3,4- $^{13}C_4$]PFOA, [1,2,3,4- $^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA SRSs are 87.5-124% (6.8-22%). Detailed quality control results for the water samples are presented in Table S2, Supporting Information.

Quality Assurance. *Fish Samples.* All fish samples were analyzed in duplicate. In addition, every tenth sample included a third and fourth sample aliquot that were fortified with perfluorinated carboxylic acids (PFCAs) (C4–C12), perfluorinated sulfonic acids (PFSAs) (C4, C6, and C8), and perfluorooctane sulfonamide (PFOSA) as low and high laboratory matrix spike samples (LMSs). All samples, sample duplicates, and LMSs were fortified with the three SRSs [1,2,3,4- $^{13}C_4$]PFOA, [1,2,3,4- $^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA. Mean recoveries and percent relative standard deviations in all study samples (samples, sample duplicates, and LMSs) (n > 970) of the three SRSs [1,2,3,4- $^{13}C_4$]PFOA, [1,2,3,4- $^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA are 107% ± 13% (n=974), 103% ± 10% (n=974) and 106% ± 11% (n=971), respectively. Mean

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LMS recoveries and percent relative standard deviations (n=47) of all the analytes and the three SRSs are 91.7-113% and 8-20%, respectively.

Mean laboratory control sample (LCS) recoveries and %RSDs (n > 224) in control bluegill fillet fortified at three levels of 1.00, 10.0 and 100 ng/g, and each level in triplicate (n > 224) with C4–C12 PFCAs, C4, C6, and C8 PFSAs, PFOSA, and the three SRSs [1,2,3,4-$^{13}C_4$]PFOA, [1,2,3,4-$^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA are 113% ± 21% for PFBA, 106% ± 24% for PFPeA, 102% ± 13% for PFHxA, 93.6% ± 10% for PFHpA, 96.1% ± 15% for PFOA, 99.2% ± 11% for PFNA, 102% ± 11% for PFDA, 96.7% ± 9.7% for PFUnA, 98.9% ± 9.8% for PFDoA, 98.8% ± 13% for PFBS, 97.0% ± 9.8% for PFHS and 95.8% ± 6.9% for PFOSA. The average SRS recovery and RSD for linear LCSs were as follows: 109% ± 12% for $^{13}C_4$-PFOA, 105% ± 8.6% for $^{13}C_2$-PFUnA and 105% ± 8.5% for $^{13}C_4$-PFOS. The LCS results demonstrate excellent method accuracy and precision for all target analytes and SRSs (Tables S3, S4, S5 in Supporting Information).

Quality Assurance. Interlaboratory. The PFC analyses of all 396 fish and all water study samples were conducted by the primary analytical testing laboratory (3M Environmental Laboratory). For quality assurance, confirmatory analysis of 41 fish representing 10% of the total number of fish and all 30 water samples collected from the 10 reaches in sampling Round 2 (August) were also analyzed by a second analytical testing laboratory (AXYS Analytical Services Ltd.).

With the exception of one split sample analysis, the data for fish tissue samples was in good agreement between the laboratories with the average relative percent differences (RPD) for PFASs and PFCAs generally 25% or less. PFBS was the lone exception with an average RPD of approximately 90%; however, the significance of this difference is difficult to evaluate given that

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it was based on only 2 samples approximating analytical method limits of quantitation. The split fish sample results between the 3M Environmental and AXYS laboratories indicate very good agreement. The PFOS results for AXYS were typically higher than for the corresponding 3M split sample. The average relative percent difference was 16% for the 2011 data set. Interlaboratory splits between the same two labs were also analyzed for the 2009 fish samples, with an average relative percent difference of about 14% (MPCA 2009). In both studies, these differences are within acceptable interlaboratory variation (30, 31).

An additional post-study QC interlaboratory comparison for PFOS in fish tissue was completed at both the 3M Environmental and AXYS laboratories with the analysis of blinded duplicate standard reference material fish tissues (National Institute of Standards and Technology standard reference material (NIST SRM)) for lake trout tissue from Lake Superior (SRM 1946) and Lake Michigan (SRM 1947) and splits of previously analyzed archived Pool 2 fish tissue study samples. Results from this QC study showed good agreement between PFOS concentration measured in original analysis of the 2011 fish tissue samples as conducted by both laboratories. In addition, PFOS concentrations measured by both laboratories for the SRM samples fell within the acceptable range reported by NIST. Based on average PFOS concentrations, the relative percent difference between the reported NIST SRM concentration and those measured by 3M and AXYS labs was less than 10%, thus indicating excellent accuracy by both laboratories.

The interlaboratory comparison based on water PFC concentrations was limited due to the large number of non-detects reported by both laboratories. Using data from matched samples with measured concentrations by both laboratories, the RPD ranged from 1.6 to 135% with an overall arithmetic average and median of 36% and 33%, respectively. For PFOS, the RPD ranged from 17 to 56% with an arithmetic average of 35%.

RESULTS AND DISCUSSION

PFC Concentrations in Water. A complete set of water samples for all ten reaches was collected during the second round of fish collection (August). Results for these samples are presented in Table 1. ~~Of water samples collected for all 10 reaches in Round 2 (August),~~ eConcentrations of PFDA, PFUnA, PFDoA and PFOSA in all thirty samples were less than their LOQ ~~(Table 1)~~. Similarly, with the exception of one sample from Reach 10, all results for PFHS, PFHpA and PFNA were also less than their LOQ. Conversely, PFOA was detected in all ten study reaches and PFBA in nine of the ten reaches. In Reaches 1-4, the only PFCs with measureable levels were PFOA and PFBA with concentrations ranging from <2 – 3.16 and <10 – 12.8 ng/L, respectively. For PFOS, water concentrations ranged from the LOQ up to 136 ng L^{-1} with approximately 70% of the samples being less the LOQ of 2 ng L^{-1}. The greatest PFC concentrations were measured in Reach 10 followed by concentrations reported in Reach 5. The PFC concentrations observed at both of these locations may have been influenced by point source mixing zones and as such, may not be representative of ~~a~~ the river cross section ~~of the river~~ at these locations within Pool 2. Downstream concentrations decreased with distance from these point sources and PFC concentrations in samples collected from locations on the ~~far~~ opposite bank ~~from these outfalls~~ were less than the LOQ. ~~The effect of water level on PFC concentrations appeared to be negligible based on samples collected from the same locations in both rounds (Supplemental materials). Overall difference between sampling rounds for all measurable PFC concentrations was less than 2-fold.~~

Comparison of water sample analyses for PFOS between 2009 and 2011 indicate a similar pattern of non-detectable results in most parts of the river, with higher detectable levels in the most downriver area of Pool 2, (Section 4 in 2009, Reach 10 in 2011). The high frequency of

non-detectable water samples in both years limits the ability to assess temporal changes. The detection limit for PFOS in 2011 (approximately 2 ng L^{-1}) is lower than the limit in 2009 (approximately 5 ng L^{-1}).

2011 PFC Concentrations in Fish.

Measurable PFC concentrations were reported in all fish species collected from Pool 2 (Table 2). However, ~~R~~reported concentrations of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS and PFHS in fish fillets collected from Pool 2 were dominated by non-detects where the total frequency of detection for these PFCs were only 16, 12, 15, 14, 25, 5 and 32%, respectively. As the chain length of PFCs increased, the frequency of detection of the PFC in fish also increased. Using the carboxylic acids as an example, the frequency of detection went from 25% for PFOA ~~with~~to 55% for PFNA and 100%~~being detected in 55% if the samples while~~ for PFDA, PFUnA, PFDoA, PFOS and PFOSA ~~it was 100%~~. With the exception of PFOS, tissue PFC concentrations generally did not exceed 5 ng g^{-1} ww in fish collected in this study. The highest mean PFC concentrations were consistently observed in the WHB while the lowest mean concentrations for all but PFNA were seen in the BGS~~,~~ (SMB had the lowest PFNA levels). For PFOS, the pool wide geometric mean concentration for all fish analyzed from Pool 2 was 31 ng g^{-1} ww while geometric mean concentrations for BGS, FWD, SMB and WHB were 20, 28, 29 and 58 ng g^{-1} ww, respectively (Table 2). A statistically significant differences in PFOS concentration ($P<0.05$) was only observed between bluegill and white bass. Evaluation of analyte concentration patterns in fish tissue showed that approximately 97% of the total PFC tissue levels were accounted for by PFOS (86%), PFDA (4.8%), PFUnA (2.7%), PFDoA (2.4%) and PFOSA (0.8%). An examination of individual PFC spatial trends indicated that, with the exception of PFOS, no clear concentration gradient was observed across the sampling reaches (Table 3). For

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BGS A
FWD B
SMB B
WHB C

is A vs B or B vs C stat. significantly diffrent?

Comment [u7]: Discuss possibility of moving this table to SI and replacing it in the manuscript with a figure illustrating PFOS levels, by reach and species. See accompanying draft figure. Also note errors in PFOS levels for SMB, reaches 3-10 (same as FWD).

PFOS, there was a clear directional shift in the cumulative distribution in PFOS concentrations for all the fish species with the greatest concentrations being observed in sampling reaches 5, 6, 9, and 10. These represent areas with known nearby point sources of PFCs to Pool 2.

Temporal Trends in Fish PFOS Concentration: To date, only the 2009 MPCA study provides sufficient data to evaluate the temporal changes on PFOS in fish tissues for the entirety of Pool 2. The 2011 results indicate overall lower PFOS levels in fish tissue for the four species sampled in Pool 2, compared to the 2009 study where geometric mean concentrations in bluegill, freshwater drum, smallmouth bass and white bass were 51 ± 7.4, 89 ± 12, 51 ± 6.6, and 83 ± 4.9, respectively . Furthermore, there was a statistically significant ($p < 0.0001$) reduction of approximately 60%, 60%, 43%, and 30% in the pool-wide geometric mean PFOS fish concentrations in 2011 when compared to 2009 in bluegill, freshwater drum, smallmouth bass and white bass, respectively. The overall geographic pattern of fish tissue concentrations in 2011 was similar to 2009, in that the highest concentrations for each species occur in the southern-most section of Pool 2 followed by a middle section identified as Reaches 5 and 6 in the 2011 study. To allow for direct statistical comparisons, fish samples from 2011 from the 10 reaches were assigned to the appropriate section (1–4) from 2009 based on collection location coordinates in 2011. Scatterbox presentations of these data sets are provided in (Figure 2). Notably lower concentrations are observed for the 2011 dataset for all species and all areas in the river. However, the magnitude of the reductions in PFOS concentrations from 2009 to 2011 followed a similar geographic pattern observed the fish tissue concentration with the least reduction occurring in Sections 1 and 2 while the greatest reduction were observed in the most downriver areas, Sections 3 and 4. The greatest change in PFOS concentrations was observed in Section 4 where statistically significant ($p < 0.05$) reductions of approximately 88%, 68% and

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70% were observed in freshwater drum, bluegill and smallmouth bass, respectively. In contrast, the reduction of PFOS concentrations in white bass was much more consistent throughout Pool 2 averaging 26% in Sections 1 to 3 and 36% in Section 4.

Analyses of the long-term temporal trends of fish PFOS concentrations within the entirety of Pool 2 is complicated by differences in the species that have been collected since 2004 as well as low sample sizes (23-25). However, in all studies conducted prior to 2009, most fish collected from Pool 2 were from locations within Section 4 (Figure 3). In Section 4, PFOS concentrations in bluegill, smallmouth bass and white bass follow the same basic trend with concentrations being elevated in 2005 and then decreasing with time with the greatest decreases occurring between 2009 and 2011. Thus, even with the limitations that are inherent ~~this~~ in such small datasets, the general trends observed in Pool 2 are consistent with that observed in other systems that have observed decreased PFOS concentrations since 2000 that includes lake trout ~~form~~ from Lake Ontario (32) and herring gull eggs from the Laurentian Great Lakes (33) that appear to be associated with decreased inputs of PFOS to those environments. The measured declines in fish population PFOS levels in the current study for the Mississippi River Pool 2 region are also consistent with the >10-year cessation of manufacturing of products based on perfluorooctanyl chemistry and with ongoing efforts in Minnesota to effectively control sources of PFCs to the Mississippi River. However, to more quantitatively define the rate and magnitude of these changes additional fish data are needed.

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Acknowledgements

We wish to thank individuals from the Minnesota Department of Natural Resources for the information they shared concerning fish habitat, populations, movements, etc that proved extremely valuable in terms of enabling the achievement of our sampling objectives. The

authors declare the following competing financial interest(s): Mr. Hohenstein, Ms. Wolf, Mr. Ellefson and Drs. Lange and Reagen are employees of the 3M Company that has manufactured PFCs. In addition, this study was funded by 3M.

ASSOCIATED CONTENT

Supporting Information

Additional details are provided including statistical calculations, QC recovery results, and study findings. This material is available free of charge via the Internet at http://pubs.acs.org.

Tables

Table 1. Geometric mean concentrations of perfluorinated compounds in fish (ng/g ww) from Pool 2 of the Mississippi River in 2011.

Table 2. Summary statistics of PFOS concentrations measured in fish tissue from Pool 2.

Table 3. Spatial distribution of perfluorinated compounds (PFCs) in fish collected from the Pool 2 of the Upper Mississippi River.

Figures

Figure 1. Areas of fish and water sample collection in 2009 and 2011.

Figure 2. Comparison of PFOS fish tissue concentrations in 2009 and 2011 by species and river section (24).

Comment [u11]: I thought we were going to get rid of letter designations.

Comment [u12]: Note errors in SMB PFOS data.

Comment [u13]: Point source descriptions should be "Metropolitan Council Wastewater Treatment Facility" and "3M Cottage Grove Site"

Comment [u14]: Remove references to MDH advisories.

Figure 3. Temporal trends of PFOS concentrations (geometric mean ± standard error) in fish collected from the tail waters of Pool 2 of the Mississippi River.

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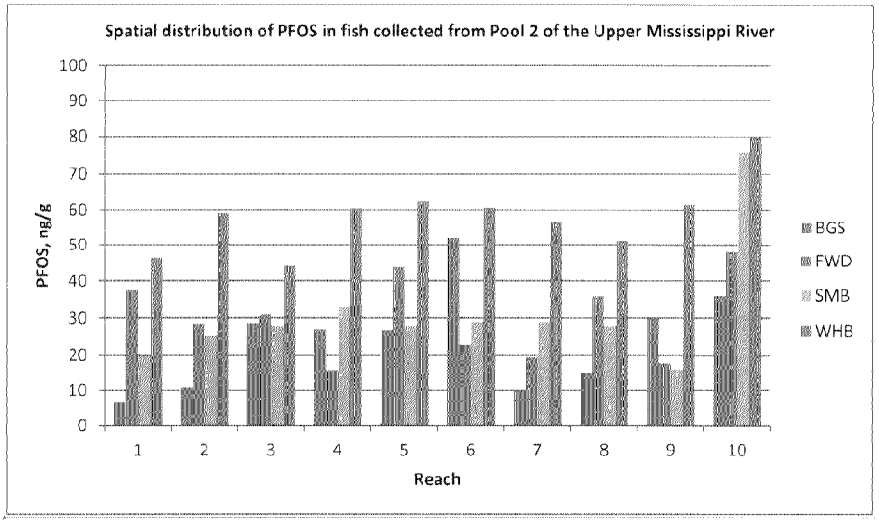
Possible alternative to Table 3:



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