Polychlorinated Naphthalenes and Polychlorinated Biphenyls in Benthic Organisms of a Great Lakes Food Chain

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Abstract. Invasion of zebra mussels, *Dreissena polymorpha*, and round gobies, *Neogobius melanostomus*, into the Great Lakes has altered the food web structure and thereby the pathways of toxic contaminants such as polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs). In this study, concentrations of PCNs and PCBs were measured in organisms of a Great Lakes benthic food chain encompassing zebra mussels. PCNs were found in all of the benthic organisms, including phytoplankton, algae, amphipods, zebra mussels, round goby, and smallmouth bass, *Micropterus dolomieui*. Concentrations of PCNs were greater in samples collected from the Raisin River than in samples from the St. Clair River. Biomagnification factors (BMF) for tetra- through octa-CN congeners in going from algae to zebra mussels from the St. Clair River ranged from 3 to 10. No major biomagnification of PCNs was found in round gobies, when concentrations were related to those in their prey species, zebra mussels. The biomagnification potential of PCNs appears to be similar to that of PCBs in the benthic food chain investigated in this study, despite the fact that PCNs may be metabolized by organisms higher in the food chain. Among several congeners, the BMFs of PCN congeners 35, 42, 43/45, 52/60, 58, and 66/67 were highest in round gobies. PCNs accounted for 1–22% of the total TEQs (toxic equivalents) of PCBs and PCNs in benthic organisms analyzed in this study. PCB congener 126 was the major contributor to TEQs, accounting for 72–99% of the PCB-TEQs in the food chain organisms analyzed.

Polychlorinated naphthalenes (PCNs), like polychlorinated biphenyls (PCBs), are widespread, persistent environmental pollutants. PCNs have been detected in samples of air, water, and

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biota collected from the remote oceans, including the Arctic Ocean (Harner *et al.* 1998; Helm *et al.* 2002; Corsolini *et al.* 2002). Although PCNs are ubiquitous pollutants, reports of their accumulation in the food chain are scarce, compared with PCBs. Biomagnification of PCNs in pelagic and benthic food chains of the Baltic Sea has been documented (Falandysz *et al.* 1996; Falandysz 1998; Lundgren *et al.* 2002). These studies have shown that, among 75 PCN congeners, only a few biomagnify in the food chain (Falandysz *et al.* 1997; Lundgren *et al.* 2002). Hexachloronaphthalene congeners 66/67, 69, and 71/72 were the most bioaccumulative in four-horned sculpins, *Myoxocephalus quadricornis*, when concentrations were compared with those in their prey species, amphipods (Lundgren *et al.* 2002). In sea eagles and cormorants, chloronaphthalene (CN) congeners 42, 52/60, 54, 58, 61, 64/68, 66/67, 69, and 71/72 were the most bioaccumulative ones (Falandysz *et al.* 1997). Moreover, patterns of PCN congeners retained in biota, particularly those at the lower trophic levels in the food chain, vary considerably, depending on the source of exposure (Falandysz *et al.* 1996; Järnberg *et al.* 1997; Kannan *et al.* 1998, 2000). Therefore, biomagnification of PCN congeners is expected to vary depending on location and species. Differences in CN congener composition in benthic organisms, coupled with congener-specific biomagnification, necessitate a site-specific approach to the evaluation of food chain transfer of PCNs. While earlier studies have examined trophic transfer of PCNs in the Baltic Sea (Falandysz *et al.* 1997; Lundgren *et al.* 2002), no information on the accumulation of PCNs in organisms in a Great Lakes food chain has been reported prior to this study. In this study, freshwater aquatic organisms were collected from the International Joint Commission (IJC) designated Areas of Concern (AOC) including the Raisin River (a tributary of Lake Erie), the Saginaw River (a tributary to Lake Huron), and the St. Clair River, Michigan, USA. The major objective of this study was to examine the concentrations and transfer of PCNs and PCBs in the zebra mussels, *Dreissena polymorpha*–round gobies, *Neogobius melanostomus*–smallmouth bass, *Micropterus dolomieui*, food chain. This study is of particular significance because the invasion

of the Great Lakes by zebra mussels in the late 1980s and round gobies in the early 1990s (Jude et al. 1992) represents a new mechanism for the mobilization of toxic organic contaminants, such as PCNs and PCBs, from benthic organisms to higher trophic levels. Zebra mussels are filter feeders and feed primarily on algae, plankton, and other suspended materials in the water column (Bruner et al. 1994). Large round gobies (> 50-mm length) feed primarily on zebra mussels less than 20 mm in length (Jude et al. 1995; French and Jude 2001). Prior to the invasion of round gobies into the Great Lakes, relatively few species there consumed zebra mussels (French 1993). Therefore, toxic substances accumulated in zebra mussels were released to decomposers only after the mussels died. Because zebra mussels are an important component of the round goby diet, the invasion and spread of round gobies created a mechanism for the transfer of toxic substances in zebra mussels to higher trophic levels. This is because round gobies are reported in the diets of game fishes such as walleye, smallmouth bass, and rock bass (Jude et al. 1995). The effects of the invasion of zebra mussels and of round gobies on the movement of toxic substances from benthic organisms to higher trophic levels are a subject of potential public and environmental health concern (Morrison et al. 1998).

In this study, samples were collected from the Raisin, Saginaw, and St. Clair Rivers, where both zebra mussels and round gobies have invaded. Phytoplankton, amphipods, and benthic algae were also collected from the Raisin and St. Clair Rivers for determination of the extent and profile of PCN accumulation in these lower trophic level organisms. PCBs, including coplanar congeners, were also analyzed in the samples for comparison of their trophic transfer with those of PCNs in zebra mussels and round gobies. 2,3,7,8-Tetrachloro-dibenzo-p-dioxin equivalents (TEQs) of PCNs (PCN-TEQs) and PCBs (PCB-TEQs) were calculated for evaluation of the relative importance of these two class of organochlorine pollutants in the benthic chain of the Great Lakes.

Materials and Methods

Sampling

Smallmouth bass, largemouth bass, round gobies, and zebra mussels were collected from various locations within 1 km of the Raisin River mouth to Lake Erie (Figure 1). Smallmouth bass were collected near the mouth of the Raisin River and below the highway 24 bridge dam. Amphipods, phytoplankton, and benthic algae were collected at one site within 1 km of the river mouth. Smallmouth bass, round gobies, zebra mussels, phytoplankton, and benthic algae were collected from the St. Clair River at its confluence with the Belle River, at Marine City, and at Algonac (Figure 1). Round gobies and zebra mussels were collected from the Saginaw River. All of the samples were collected during July–November 1998 and 1999 (Table 1). Several hundreds of zebra mussels were collected from their rocky substrates at each location by snorkeling or SCUBA diving. Zebra mussels ranged in size from 3 to 24 mm. Round gobies generally eat zebra mussels ranging in size from 3 to 12 mm (Jude et al. 1995). Fish were collected by seining or by electroshocking. Amphipods were collected by turning over rocks and using forceps to collect individual organisms. Phytoplankton was collected using a 10-μm plankton net. The phytoplankton samples collected using the 10-μm net may contain zooplankton and suspended particles. Nevertheless, zooplankton levels are gener-
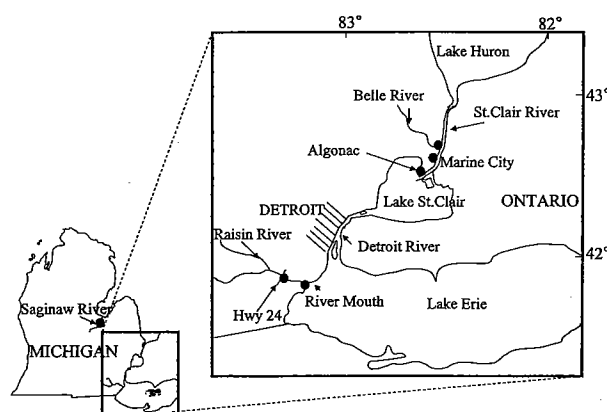

Fig. 1. Map of the lower peninsula of Michigan, showing sampling locations

ally low in these rivers. Benthic algae were collected by scraping hard surfaces with a toothbrush (for nonfilamentous forms), or by hand-picking tufts of filamentous algae. Skinless fillets were analyzed for fish except round gobies, for which the whole body was analyzed. Soft tissues were analyzed for zebra mussels. Tissues from several individuals per species were pooled to obtain adequate mass for extraction.

Chemical Analysis

Chloronaphthalene (CN) and chlorobiphenyl (CB) congeners were analyzed following the method described elsewhere, with some modifications (Kannan et al. 2000). Samples (approximately 20 g for fish and zebra mussels, 1–4 g for phytoplankton, algae, and amphipods) were homogenized with anhydrous sodium sulfate and extracted in a Soxhlet apparatus for 16 h using dichloromethane and hexane (400 mL, 3:1). An aliquot of the concentrated extract was used for the determination of fat content by gravimetry. Extracts were then treated with sulfuric acid in a separatory funnel. The solution was concentrated to 2 ml and passed through silica gel packed glass columns. Aliquots of extracts were injected into a gas chromatograph (Perkin Elmer series 600) equipped with a ^{63}Ni electron capture detector (GC-ECD) for the determination of total PCB concentrations.

Identification and quantification of di-, mono-, and non-ortho PCBs and individual PCN congeners were accomplished with a Hewlett Packard 6890 series high-resolution gas chromatograph (HRGC) coupled to a JEOL JMS-700D high-resolution mass spectrometer (HRMS). Di-, mono-, and non-ortho coplanar PCBs and PCN congeners were separated from other ortho-substituted PCBs and pesticides by passing the extracts through a porous Hypercarb graphite carbon column (100 × 4.6 mm, 7-μm particle size; Thermo Hypersil-Keystone, Bellefonte, PA). Details of the fractionation procedure have been described elsewhere (Horii et al. 2001). A Shimadzu liquid chromatograph pump (LC-10AD) was employed to deliver solvents. The first fraction, eluted with 50% dichloromethane in hexane at a rate of 2.5 ml/min (20 ml), contained di- and mono-ortho substituted PCBs. The graphite column was then reversed and eluted with toluene (50 ml) to collect non-ortho coplanar PCBs and PCNs. The toluene fraction was further fractionated by passing the extracts through a Cosmosil 5-PYE column (pyrenyl ethyl group; 250 × 4.6 mm, 5-μm particle size; Nacalai Co., Osaka, Japan). The first fraction, eluted with 10 ml 10% dichloromethane in hexane, contained non-ortho coplanar congeners 77, 81, 126, and 169, and lower CNs. The second and third fractions, eluted with the same solvent mixture, contained more highly chlorinated naphthalenes. The fourth fraction contained the remaining

Table 1. Concentrations of total PCBs (ng/g, wet wt) and total PCNs (pg/g, wet wt) in aquatic organisms from the Raisin, Saginaw, and St. Clair Rivers, Michigan, USA

Species	Location	Length range (mm)	Weight range (g)	Date of collection	Lipid (%)	Total PCBs (ng/g)	Total PCNs (pg/g)
Raisin River							
Smallmouth bass	Highway 24	340–410	620–920	29 Sep 98	0.67	43.5 (6,490)[a]	28.2 (4,210)
Smallmouth bass	Highway 24	260–310	240–510	29 Sep 98	0.59	45.2 (7,660)	15.4 (2,610)
Smallmouth bass	Highway 24	170–210	67–135	29 Sep 98	0.5	60.7 (12,100)	11.30 (2,260)
Largemouth bass	River mouth to Lake Erie	345–366	755–990	22 Sep 99	0.98	1,750 (178,000)	223 (22,700)
Round goby	River mouth	70–96	4.6–13	22 Sep 99	2.61	4,710 (181,000)	1,140 (43,600)
Round goby	Port of Monroe	77–87	7.5–10.2	15 Oct 98	2.48	2,090 (84,300)	336 (13,600)
Round goby	Port of Monroe	95–109	14.6–21	15 Oct 98	2.18	1,990 (91,100)	264 (12,100)
Zebra mussel	Port of Monroe	8–20	NA	15 Oct 98	2.41	814 (33,800)	1,120 (46,400)
Zebra mussel	River mouth	10–19	NA	6 Aug 99	3.19	2,920 (91,400)	1,750 (54,800)
Phytoplankton	River mouth	NA	NA	13 Oct 99	0.99	10,200 (1,030,000)	12,200 (1,230,000)
Amphipod	River mouth	NA	NA	1 Sep 99	1.28	4,760 (372,000)	1,100 (85,900)
Benthic algae	River mouth	NA	NA	1 Sep 99	0.25	194 (77,600)	781 (313,000)
Saginaw River							
Round goby	Independence Bridge	92–105	12.2–18.5	17 Oct 99	2.6	372 (14,300)	189 (7,280)
Round goby	Independence Bridge	133–144	44–50	17 Oct 99	3.24	488 (15,100)	322 (9,950)
Zebra mussel	Independence Bridge	4–15	NA	17 Oct 99	1.22	356 (29,200)	362 (29,700)
St. Clair River							
Smallmouth bass	Near Belle River mouth	174–203	94–132	6 Aug 99	0.76	55.7 (7,330)	32.4 (4,270)
Round goby	Near Belle River mouth	112–120	78.3–25	30 Sep 98	1.55	181 (11,700)	42.7 (2,750)
Round goby	Marine City	128–133	34–39	29 Jul 99	2.71	98.7 (3,640)	130 (4,810)
Round goby	Marine City	77–82	7–8.1	22 Sep 99	1.35	81.3 (6,020)	28.7 (2,130)
Round goby	Near Belle River mouth	88–102	9.6–15.3	30 Sep 98	2.01	146 (7,260)	49.9 (2,480)
Zebra mussel	Marine City	12–24	NA	28 Nov 98	0.73	31 (4,250)	53 (7,260)
Zebra mussel	Marine City	13–21	NA	6 Aug 99	0.74	31 (4,190)	22.2 (3,000)
Phytoplankton	Algonac	NA	NA	2 Sep 99	0.29	768 (265,000)	1,370 (471,000)
Benthic algae	Marine City	NA	NA	29 Jul 99	0.18	7.3 (4,060)	1.96 (1,090)

[a] Values in parentheses are lipid-normalized concentrations.

PCN congeners. This column fractionation enabled the separation of several PCN congeners, that would otherwise coelute in GC analysis. Each fraction was concentrated and injected into a HRGC interfaced with an HRMS. A Hewlett Packard 6890 series II GC coupled to a JEOL JMS-700D HRMS was used to determine non-*ortho* PCBs and PCNs. A capillary column coated with DB-17 at a 0.25-μm film thickness (30 m × 0.25-mm i.d.; J&W Scientific, Folsom, CA) was used for the separation of individual isomers. The column oven temperature was programmed from 70°C (1 min) to 180°C at a rate of 15°C/min, then to 270°C at 2°C/min, with a final hold time of 10 min for PCNs, and 70°C (1 min) to 200°C at a rate of 15°C/min, then to 270°C at 4°C/min, with a final hold time of 15 min for PCBs. Both of the MS were operated in an electron impact (38-eV energy and 500-μA ion current), selected ion monitoring mode, at a resolution R >10,000 (10% valley). Further details of the instrumental analysis are presented elsewhere (Yamashita et al. 2000a). PCB and PCN congeners are represented by their IUPAC numbers (Wiedmann and Ballschmiter 1993). Total PCBs and PCNs represent the sum of all of the identified tri- to deca-CB and di- to octa-CN congeners, respectively. Data are presented on a wet- and lipid-weight basis, to allow for comparisons, to examine food chain transfer, and for use in risk assessments.

The laboratory has participated in National Institute of Standards and Technology's intercomparison exercises for organochlorines including PCNs. Our results for PCBs and PCNs were within 20% of the mean value reported for the control material or standards. PCB congener 30 was spiked as a surrogate standard (during Soxhlet extraction) for total PCB analysis. Recoveries of PCB 30 ranged from 78 to 128%. ^{13}C-Labeled PCDD/DF congeners were spiked as internal standards for PCN congener analysis. Recoveries of ^{13}C-labeled con-
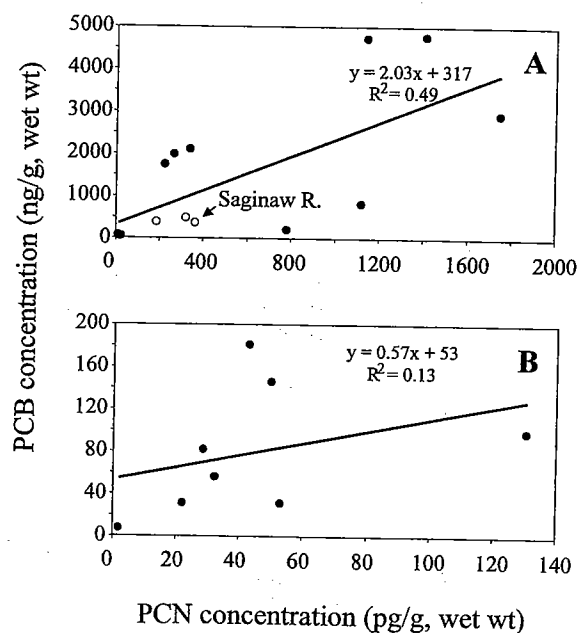

PCN concentration (pg/g, wet wt)

Fig. 2. Relationship between total PCN and total PCB concentrations in benthic organisms collected from the Raisin and Saginaw (A) and St. Clair (B) rivers. Data for phytoplankton from the Raisin River were excluded
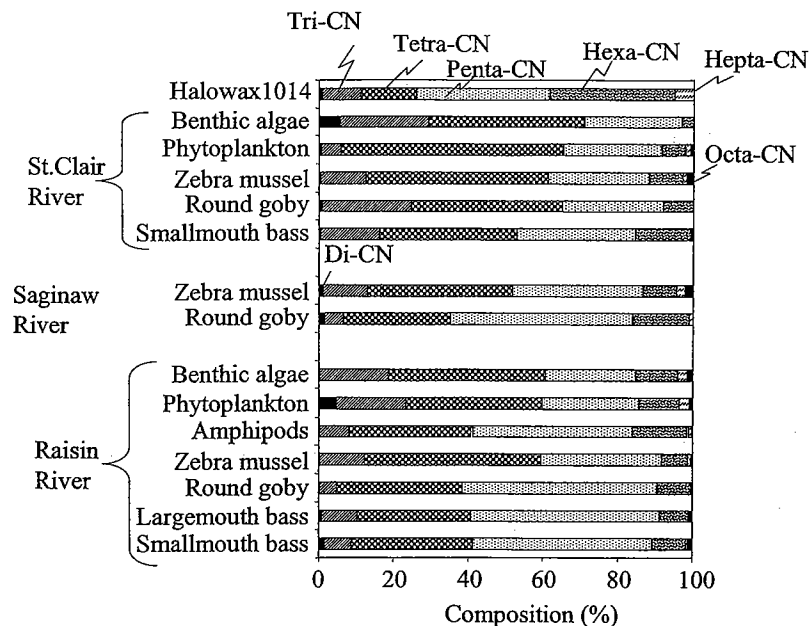
Fig. 3. Mean composition (%) of PCN homologues in aquatic organisms collected from Michigan waters compared to the PCN homologue composition of Halowax 1014

geners ranged from 70 to 135%. Reported concentrations were not corrected for the recoveries. Procedural blanks were analyzed for every set of five samples to check for interferences. Calculated concentrations were reported as less than the limit of detection, if either the observed isotope ratio was not within ± 20% of the theoretical ratio or the peak area was not greater than the specified threshold (three times the noise). The detection limits of individual coplanar PCBs and PCN congeners varied from 0.1 to 3 pg/g, wet wt.

Results and Discussion

Concentrations

Concentrations of total PCNs in freshwater aquatic organisms analyzed in this study varied from 1.96 to 12,200 pg/g, wet wt (Table 1). The lowest concentration was found in benthic algae collected from the St. Clair River near Marine City and the highest concentration was found in phytoplankton collected from the Raisin River near its mouth to Lake Erie. Concentrations of PCBs also varied widely, from 7.3 to 10,200 ng/g, wet wt. Concentrations of total PCNs in fish were 3 to 4 orders of magnitude lower than total PCB concentrations. High concentrations of PCBs and PCNs found in phytoplankton collected near the Raisin River mouth to Lake Erie suggest the presence of local sources of contamination. Despite their lower trophic status in the food chain, amphipods and benthic algae, also collected at this location, contained anomalously high concentrations of PCBs and PCNs.

Overall, samples collected from the Raisin River contained the greatest concentrations of PCNs and PCBs, followed in decreasing order by samples from the Saginaw and St. Clair Rivers. Concentrations of PCNs in zebra mussels and fish collected from the Saginaw and St. Clair Rivers were lower than those from the Raisin River. For example, lipid-normalized concentrations of PCNs in round gobies collected from the
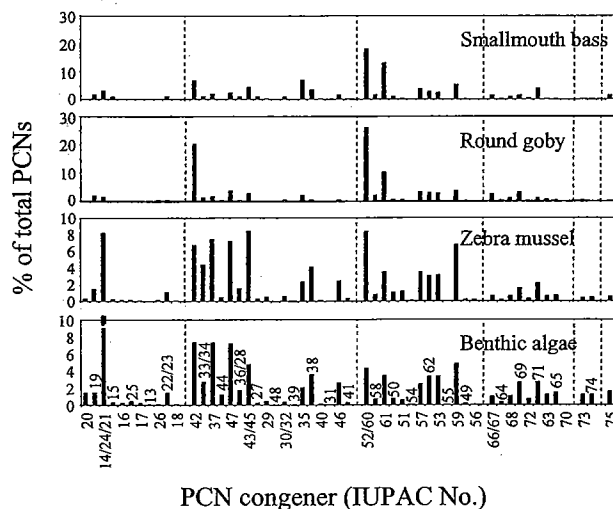

Fig. 4. Profiles of PCN congeners in benthic organisms from the Raisin River, Michigan. Concentrations of individual congeners are reported as the percentage total PCN concentration

Raisin, Saginaw, and St. Clair Rivers were 23,100, 8,610, and 3,050 pg/g, respectively, whereas those in zebra mussels were 50,600, 29,700, and 5,130 pg/g, respectively. The lower River Raisin has been identified by the IJC as an AOC due to contamination by PCBs and heavy metals. Several industrial operations have been suggested as potential sources of PCBs (Collier and Cieniawski 2002). A 1997 sediment-dredging project resulted in the removal of approximately 27,000 cubic yards of PCB-contaminated sediments (maximum concentration 49,000 µg/g, dry wt) from the Raisin River near Monroe. Despite this, PCB concentrations in sediments in the remediated area (>200 µg/g) and downstream depositional zones were significantly higher than were upstream concentrations
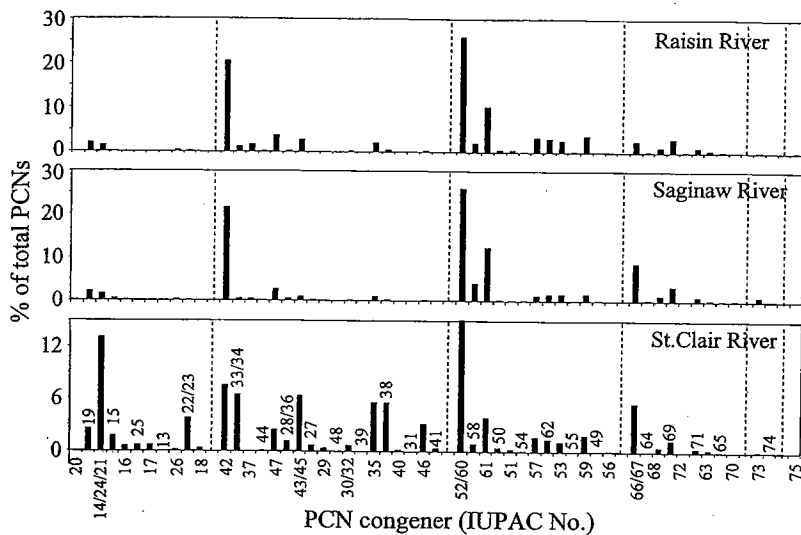
Fig. 5. Contributions (%) of individual PCN congeners to total PCN concentrations in round gobies collected from the Raisin, Saginaw, and St. Clair Rivers. IUPAC numbers are listed in their gas chromatographic elution order
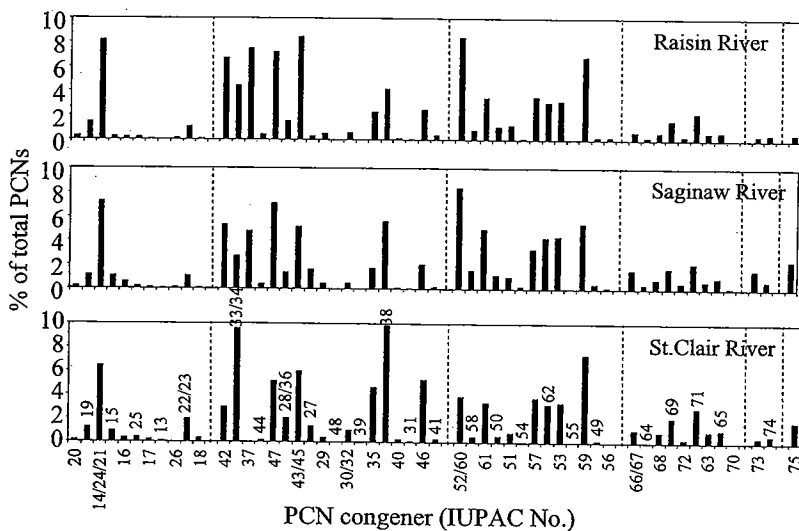

Fig. 6. Contributions (%) of individual PCN congeners to total PCN concentrations in zebra mussels collected from the Raisin, Saginaw, and St. Clair Rivers. IUPAC numbers are listed in their gas chromatographic elution order

(Collier and Cieniawski 2002). Moreover, bioaccumulation of PCBs by oligochaete worms (*Lumbriculus variegates*) was ~100 times higher in the remediated area than at an upstream reference site (Collier and Cieniawski 2002). Because of the exposure to point sources of pollution in the Raisin River, PCB and/or PCN concentrations in phytoplankton, amphipods, and benthic algae collected at this site were not included in the evaluation of their food chain transfers.

When data for phytoplankton were removed, concentrations of PCNs were significantly correlated with those of total PCBs, in organisms collected from the Raisin River (Figure 2). Nevertheless, PCN concentrations were positively, but weakly, correlated with those of total PCBs in St. Clair River organisms (Figure 2). High concentrations of PCBs in Raisin River organisms imply that these organisms are simultaneously exposed to PCNs present as impurities in technical PCB mixtures (Yamashita *et al.* 2000b). The mean concentration of PCNs in technical Aroclor mixtures is 39 μg/g, which corresponds to 0.0039% of the total PCB content (Yamashita *et al.* 2000b). Concentrations of PCNs in aquatic organisms analyzed in this

study ranged from 0.01 to 0.4% (mean: 0.08%) of the total PCB concentrations. A greater proportion of PCNs in these aquatic organisms than in technical PCB mixtures suggests that, in addition to technical PCB mixtures, other sources have contributed to the organisms' PCN exposures. Technical PCN mixtures such as Halowaxes, as well as incineration-related activities, could contribute to PCN contamination in the environment.

Concentrations of total PCNs in zebra mussels (46,400–54,800 pg/g, lipid wt) and round gobies (12,100–43,600 pg/g, lipid wt) from the Raisin River were in the higher range of the values reported in the literature (Falandysz *et al.* 1996, 1997; Järnberg *et al.* 1993, 1997; Corsolini *et al.* 2002). Concentrations of PCNs found in round gobies were comparable to those (710–33,000 pg/g, lipid wt) reported for fishes from Michigan waters of the Great Lakes (Kannan *et al.* 2000). Total PCN concentrations ranging between 540 and 1500 pg/g, lipid wt, were reported in four-horned sculpins from the Gulf of Bothnia (Lundgren *et al.* 2002). Concentrations of PCNs in muscle tissue of burbot and cod from the Baltic Sea ranged from 2900
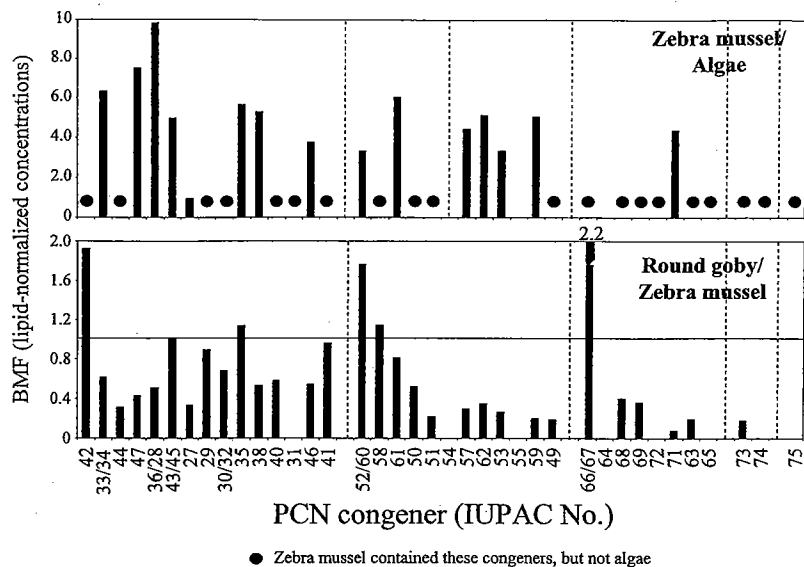
Fig. 7. Biomagnification factors (BMFs: ratio of lipid-normalized concentrations; predator/prey) for PCN congeners between algae and zebra mussels and between zebra mussels and round gobies

to 10,000 pg/g, lipid wt (Järnberg et al. 1997). Concentrations of total PCNs in muscle tissue of tuna and swordfish from the Italian coast of the Mediterranean Sea ranged from 170 to 430 pg/g, lipid wt (Kannan et al. 2002). Comparison with these results suggests extensive contamination by PCNs in fish collected from the Raisin River. However, PCN concentrations here were lower than the 504,000 pg/g, lipid wt, found in striped mullet collected near a chlor-alkali plant along the Georgia coast, USA (Kannan et al. 1998).

Homologue Profiles

Tetra- and penta-CNs were the predominant homologues, accounting for 62–86% of the total PCN concentrations in the biological samples analyzed in this study (Figure 3). Tetra- and penta-CNs are the major homologues formed during the incineration of solid wastes. The predominance of these homologues has been reported in fly ash originating from solid waste incinerators (Nakano et al. 1993; Takasuga et al. 1994). In Aroclor 1242, a technical PCB mixture, tetra-CNs are the major homologues (Yamashita et al. 2000b). Halowax 1014, a technical PCN mixture, contains penta- and hexa-CNs as the predominant homologues. Some PCN isomers that are prevalent in Halowax 1014 were also the predominant ones found in biological samples analyzed in this study. For instance, PCN congeners 35 (1,2,4,8-), 36 (1,2,3,5-), 43 (1,3,5,8-), and 46 (1,4,5,8-) are prevalent in Halowaxes but not in fly ash. The predominance of tetra-CNs in benthic organisms and the presence of PCN isomers 35, 36, 43, and 46 suggest exposure to multiple sources.

In general, the composition of tetra-CNs decreased, while penta-CNs increased, with an increase in trophic level (Figure 4). For instance, the composition of tetra- and of penta-CNs in zebra mussels from the Raisin River was 47 and 32%, respectively, whereas that in smallmouth bass was 32 and 48%, respectively. Similarly, the respective compositions of tetra- and penta-CNs were 49 and 37% in zebra

mussels and 27 and 32% in smallmouth bass from the St.Clair River. The relative increase in more highly chlorinated homologues with an increase in trophic level may be due to elimination of less highly chlorinated congeners by metabolism and/or due to selective enrichment of more highly chlorinated congeners. Similar to the pattern in this study, tetra- and penta-CN homologues were the predominant congeners in sediments and benthic organisms from the Baltic Sea (Falandysz et al. 1996; Lundgren et al. 2002; Järnberg et al. 1997). Higher trophic organisms such as birds collected from the Great Lakes contained high proportions of hexa-CNs (Kannan et al. 2001a). These results suggest that the less highly chlorinated naphthalene congeners are metabolized in the higher trophic level organisms.

Differences in sources, species-specific accumulation, and metabolism/elimination can contribute to the observed differences in PCN homologue patterns. The profiles of PCN congeners in organisms collected from the St. Clair River were different from the profiles in organisms from the Raisin and Saginaw Rivers. For example, round gobies collected from the St. Clair River contained higher proportions of tri- and tetra-CNs than did those collected from the Raisin River (Figure 5). Tri-CN congeners 14/24/21 and 22/23 and tetra-CN congeners 33/34/37, 35, 38, 43/45, and 46 were prominent in round gobies collected from the St. Clair River. However, these congeners were either absent or present only in trace amounts in round gobies collected from the Raisin and Saginaw Rivers. PCN congeners 23, 24, 33/34/37, 35, 43, and 46 are more abundant in Halowaxes (Falandysz et al. 2000) than in fly ash (Schneider et al. 1998). Congeners 25, 26, 30/32, and 39 are less abundant in Halowaxes (Falandysz et al. 2000) but are present in fly ash. These latter congeners were either absent or prsent only at trace levels in St. Clair River organisms. Similarly, zebra mussels collected from the St. Clair River contained those congeners that were abundant in Halowaxes (Figure 6). These results suggest that Halowaxes are the major sources of PCNs in samples collected from the St. Clair River.

Table 2. Concentrations of PCN-TEQs and PCB-TEQs (pg/g, wet wt) in aquatic organisms collected from the Raisin, Saginaw, and St. Clair Rivers, Michigan, USA[a]

	Raisin River						
	SMB	LMB	RG	ZM	Amphipod	Phytoplankton	B. algae
PCN-TEQs	0.002 (2.2)[b]	0.027 (0.76)	0.085 (1.7)	0.097 (4.06)	0.25 (1.89)	1.5 (6.8)	0.092 (14.8)
PCB-TEQs	0.089 (97.8)	3.51 (99.2)	4.79 (98.3)	2.29 (95.9)	13 (98.1)	20.6 (93.2)	0.531 (85.2)
Total TEQs	0.091	3.54	4.88	2.39	13.3	22.1	0.62

Note. SMB—smallmouth bass; LMB—largemouth bass; RG—round goby; ZM—zebra mussel; B. algae—benthic algae. Values have been rounded to three significant digits.
[a] TEF for PCNs from Blankenship *et al.* 2000; Villeneuve *et al.* 2000; Griesy *et al.* 1997.
[b] Values in parentheses are % of the total TEQ contribution.

Trophic Transfer

For the determination of food-chain transfer of PCNs, lipid-normalized concentrations of samples collected from a given location at a given time were used. Lipid normalization was necessary because of the lipophilic nature of the compounds and because of differences in the tissues analyzed for mussels (whole-body soft tissue) and fish (edible muscle tissues for smallmouth bass and whole-body for round gobies). Samples from the Raisin River were not all collected from the same location. Lower-trophic level organisms—phytoplankton, amphipods, and benthic algae—were collected near a point source and, therefore, contained anomalously high concentrations of PCNs and PCBs. Therefore, these lower-trophic level organisms from the Raisin River were excluded from this trophic transfer analysis. However, benthic algae, zebra mussels and round gobies were all collected in 1999 from the St. Clair River near Marine City. This provided an opportunity for us to evaluate the food chain transfer of PCNs and PCBs from algae to round gobies in the St. Clair River. Lipid-normalized concentrations of PCNs in benthic algae, zebra mussel, and round gobies collected in 1999 near Marine City were 1090, 3000, and 3470 pg/g, respectively, suggesting a biomagnification factor (BMF: lipid-normalized concentration in predator/prey) of ~3 between benthic algae and zebra mussels and round gobies. There was no significant biomagnification of PCNs between zebra mussels and round gobies from the St. Clair River. In fact, concentrations of PCNs in round gobies (mean: 8615 pg/g) were ~3-fold less than those in zebra mussels (29,700 pg/g, lipid wt) collected from the Saginaw River. Similarly, concentrations of PCNs in round gobies (12,900 pg/g) from the Raisin River near Monroe were ~3-fold less than those in zebra mussels (46,400 pg/g) collected from the same site. This may be due to metabolism and elimination of certain less chlorinated PCN congeners and/or enrichment of highly chlorinated congeners in round gobies. However, concentrations of PCNs in smallmouth bass collected in the St. Clair River near the Belle River confluence were 2-fold greater than those in round gobies collected from the same location. Variations in the biomagnification potential of PCNs at different locations may be attributed to differences in the dietary compositions of the species analyzed. In addition to zebra mussels, round gobies feed on aquatic insects and on eggs of other fish species (French and Jude 2001). Therefore, evaluation of biomagnification potential should take into consideration of the dietary composition of the predator species in

addition to geographic and biological (e.g., sex, lipid, season) variables. The BMFs of PCNs in zebra mussels are similar to what was reported for cormorants (1.1 to 3.5) from the Baltic Sea (Falandysz *et al.* 1997). BMFs between PCNs in amphipods (prey species) and the PCN levels in four-horned sculpins ranged from 0.4 to 0.65 (Lundgren *et al.* 2002).

The biomagnification potential of each PCN congener varied considerably (Figure 7). On the basis of mean lipid-normalized concentrations of various isomers in benthic algae and zebra mussels from the St. Clair River, BMFs of di-CNs in zebra mussels were less than one, whereas those for hexa-CNs were ~10. The overall BMF between ΣPCNs in algae and ΣPCNs in zebra mussels was ~4.9. The BMFs between PCN congener levels in zebra mussels and the levels in round gobies were less than 1. In general, BMFs increased with an increase in the chlorination level of PCN congeners.

Lipid-normalized concentrations of tetra- to octa-CN congeners in benthic algae, zebra mussels, and round gobies collected from the St. Clair River near Marine City were used to calculate congener-specific BMFs (lipid-normalized concentrations related as zebra mussel to algae and round goby to zebra mussel). Several PCN congeners that were found in zebra mussels were not found in their diet, algae, at a detection limit of 0.1 pg/g, wet wt (or 56 pg/g lipid wt) (Figure 7). Although these congeners had been expected to have biomagnified, BMFs were not calculated for these congeners. For those congeners that were found both in zebra mussels and algae, the BMFs varied from 3 to 10 (mean: 4.9). Most of the congeners had high bioaccumulation rates in zebra mussels relative to algae. Zebra mussels, due to their relatively high lipid content, may also concentrate PCNs directly from the water column. Therefore, the calculated BMF for PCNs in zebra mussels is accumulation from both water column and algae/diet. The BMFs of PCN congeners between zebra mussels and round goby were low, varying from < 0.1 to 2.2. BMFs of PCN congeners 35, 42, 43/45, 52/60, 58, and 66/67 were greater than 1. PCN congeners with chlorine substitution at the 2,3,6,7 positions have been shown to biomagnify considerably (Lundgren *et al.* 2002). Hexa-CN congeners 66/67 are both substituted at the 2,3,6,7 positions, and these two congeners are the most highly biomagnified ones found in the food chain thus far. Similarly, CN congeners with no vicinal (adjacent) unsubstituted carbon atoms biomagnify considerably (Falandysz *et al.* 1997). For example, CN congeners 42, 52/60, 58, and 66/67, prevalent in zebra mussels, have no vicinal unsubstituted carbons. Although congeners 35 and 43/45 have two vicinal

Table 2. Continued

Saginaw River		St. Clair River				
RG	ZM	SMB	RG	ZM	Phytoplankton	B. algae
0.084 (13.4)	0.043 (14.4)	0.009 (2.7)	0.011 (2.5)	0.004 (22.2)	0.106 (4.9)	<0.001
0.541 (86.6)	0.255 (85.6)	0.319 (97.3)	0.424 (97.5)	0.014 (77.8)	2.05 (95.1)	<0.001
0.63	0.298	0.33	0.44	0.018	2.16	<0.001

Table 3. Mean concentrations (pg/g wet wt) of dioxin-like PCBs in samples collected from the Raisin, Saginaw, and St. Clair Rivers, Michigan, USA

Congener No.	Raisin River							Saginaw River		St. Clair River				
	SMB	LMB	RG	ZM	Amphipod	Phyto-plankton	B. algae	RG	ZM	SMB	RG	ZM	Phyto-plankton	B. algae
						Non-*ortho* PCBs								
81	1.5	122	153	149	716	806	19.6	10.5	11.8	2.2	2.2	<1	51.8	<1
77	25.1	2,530	5,040	6,040	20,300	29,900	1670	215	341	37.5	33.1	4.9	1,430	2.5
126	3.4	123	176	75.3	472	765	18.9	20.3	8.75	12.7	16.6	<1	79.4	<1
169	6.3	2.2	3.89	0.88	<1	<1	0.5	1.1	<1	4.4	4.2	<1	<1	<1
						Mono-*ortho* PCBs								
123	31.8	3,900	5,360	5,270	6020	10,400	368	410	347	88.9	201	<3	816	<3
118	557	92,400	80,400	40,100	126,000	172,000	5,980	8,770	5,240	1,830	3,400	52	18,700	6.8
114	16.1	2,650	2,165	1,080	4,890	4,670	172	284	160	64.8	107	<3	490	<3
105	210	32,800	34,500	11,400	62,300	92,000	2,500	3,000	1,740	994	1,650	24.5	7,590	<3
167	73.3	1,450	1,390	525	2,250	5,310	123	300	173	209	265	5.8	753	<3
156	93.6	2,940	2,660	1,100	4,860	12,100	327	603	210	367	546	5.3	1,550	<3
157	23.8	685	570	233	1,080	2,710	74.1	139	65.3	90.3	123	<3	349	<3
189	20.5	295	270	84	422	<3	35.3	50	23.3	51	70.4	<3	61.4	<3
						Di-*ortho* PCBs								
180	1200	26,200	25,200	7,190	26,400	63,600	1,880	4,140	2,250	3,400	4,890	43	6,650	3.3
170	359	7,630	6,400	2,250	8,620	21,700	782	1,250	608	1,100	1,510	15	2,040	<3

Note. SMB—smallmouth bass; LMB—largemouth bass; RG—round goby; ZM—zebra mussel; B. algae—benthic algae.

unsubstituted carbon atoms, they biomagnified considerably between zebra mussels and round gobies.

For the comparison of BMFs of PCNs with those of PCBs, lipid-normalized concentrations of total PCBs were compared among benthic algae, zebra mussels, and round goby. Lipid-normalized concentrations of total PCBs in benthic algae, zebra mussels, and round gobies collected in 1999 from the St. Clair River near Marine City were 4060, 4190, and 4830 ng/g, respectively. BMFs for PCBs between algae and zebra mussels and round gobies were ~1. No notable magnification of PCBs was observed in round gobies relative to zebra mussels. An earlier study has reported BMFs for PCBs between plankton and zebra mussel vary from 1 to 3 (Ravera 2001; Willman et al. 1999). This is similar to the pattern observed for PCNs. However, round gobies collected from the Raisin River contained PCB concentrations 2- to 3-fold greater than those in zebra mussels. As noted earlier, BMFs appear to vary depending on location, and may be influenced by the dietary composition of predatory species and local sources of contamination. Furthermore, lipid-normalization of concentrations of lipophilic pollutants is suggested to be inappropriate in organisms with low lipid content and in organisms with varying classes of lipids (Bergen et al. 2001).

Toxic Potential

PCN congeners that elicit dioxin-like activity were found in most of the biota analyzed. These included congeners 52/60 (1,2,3,5,7-/1,2,4,6,7-), 54 (1,2,3,6,7-), 57 (1,2,4,5,6-), 56 (1,2,3,7,8-), 66/67 (1,2,3,4,6,7-/1,2,3,5,6,7-), 68 (1,2,3,5,6,8-), 69 (1,2,3,5,7,8-), 63 (1,2,3,4,5,6-), 70 (1,2,3,6,7,8-), and 73 (1,2,3,4,5,6,7-). TEQs were calculated based on relative potencies derived from H4IIE bioassays (Blankenship et al. 2000; Villeneuve et al. 2000). Concentrations of PCN-TEQs in biota analyzed in this study varied from <0.001 to 1.5 pg/g, wet wt (Table 2). Phytoplankton collected from the Raisin River contained the highest PCN-TEQs. For comparison, concentrations of dioxin-like PCBs and their TEQs were also measured and calculated using H4IIE-TEFs (Giesy et al. 1997) (Table 3). TEQs contributed by dioxin-like PCBs ranged from <0.001 to 20.6 pg/g, wet wt. PCBs accounted for 78 to 99% of the ΣTEQs, whereas PCNs accounted for 1 to 22% of the TEQs. The highest contribution by PCNs to TEQs was found in zebra mussels collected from the St. Clair River. The range of TEQs contributed by PCNs in benthic organisms analyzed in this study was similar to ranges reported previously (Järnberg et al.

1993, 1997; Kannan *et al.* 2000; Corsolini *et al.* 2002). However, in point source areas, such as the Detroit River and in the vicinity of a chlor-alkali plant in Georgia, PCNs contributed much higher TEQs than did PCBs or PCDDs/Fs (Kannan *et al.* 1998, 2000, 2001b).

Among dioxin-like PCB congeners analyzed, PCB 126 was the major contributor to TEQs, accounting for 72–99% of the PCB-TEQs (Table 3). The contribution of each PCN congener to TEQs varied, depending on the species. In general, PCN congeners 66 and 69 were the major contributors to PCN-TEQs, accounting for 61–96% of the PCN-TEQs.

These results suggest that zebra mussels and round gobies can act as conduits for the transfer of PCBs and PCNs from lower- to higher-trophic level organisms. The biomagnification factor for PCNs between algae and zebra mussels was comparable to those for PCBs. In general, PCNs biomagnified to some extent (~3 fold) between algae and zebra mussels, although no remarkable biomagnification occurred between zebra mussels and round gobies. PCNs contributed up to 22% of the total TEQs in zebra mussels collected the St. Clair River.

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