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Isomer-Specific Analysis and Toxic Evaluation of Polychlorinated Naphthalenes in Soil, Sediment, and Biota Collected near the Site of a Former Chlor-Alkali Plant

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Concentrations and composition of polychlorinated naphthalene (PCN) congeners were determined in soil, sediments, blue crab, striped mullet, and boat-tailed grackle collected near a chlor-alkali plant to determine their congener profile, bioaccumulation properties, and toxic potential. Concentrations of total PCNs as high as 23 μg/g, dry wt, were found in sediments collected at the marsh contaminated by disposal of wastes from the chlor-alkali process. The spatial distribution of sediment–PCN concentrations was not related with those observed for polychlorinated biphenyls (PCBs). The PCN congener profile did not resemble those of any technical mixtures. Hepta- and octa-chloronaphthalenes were the most abundant congeners accounting for greater than 50% of the total PCN concentrations in soil and sediments. A characteristic profile of PCNs in samples collected at the chlor-alkali site suggests the formation of chloronaphthalene congeners during chlor-alkali process, as has been suggested for polychlorinated dibenzofurans (PCDFs). Concentrations of total PCNs in biota were 3–5 orders of magnitude less than in sediments. The profile of PCN congeners in biota was predominated by tetra- or penta-chloronaphthalenes, while hepta- and octa-chloronaphthalenes were not detected. Affinity of more chlorinated naphthalene congeners to sediment organic carbon and steric factors that affect membrane permeability have contributed to less bioavailability. The 2,3,7,8-TCDD equivalents (TEQs) estimated for PCNs in sediments and biota were greater than those reported for PCBs, PCDDs, or PCDFs. Our results suggest that the chlor-alkali process has been an important source of PCNs due to their formation in the process. The contribution of PCNs to dioxin-like toxicity in environmental media near the chlor-alkali process may overwhelm those due to PCBs, PCDDs, or PCDFs.

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Introduction

Polychlorinated naphthalenes (PCNs) are primarily industrial chemicals consisting of two fused aromatic rings substituted with one to eight chlorine atoms. The physical and chemical properties of PCNs are similar to polychlorinated biphenyls (PCBs) with high thermal stability and inertness, which favored their application in the electrical industry as dielectric fluids in transformers and capacitors and as cable insulators. PCNs were also used as engine oil additives, wood preservatives, electroplating masking compounds, and feedstocks for dye production (*1*). Besides their production as technical mixtures for use in various applications, chlorinated naphthalenes are also formed during combustion processes such as municipal solid waste incineration (MSWI) (*2–5*) and in metallurgical processes such as copper roasting (*6, 7*). Formation of chloronaphthalenes (CN) from pyrolysis of chlorinated solvents such as tetrachloroethylene and polyvinylidene chloride has also been reported (*8, 9*). Elevated concentrations of PCNs in environmental media collected near a chlorine manufacturing facility (*10*) and a magnesium refinery (*11*) were suggestive of their inputs from these industries. In addition, PCN congeners are also released from the use of PCBs due to their presence as co-contaminants in technical PCB mixtures (*10, 12*).

Technical PCN mixtures with chlorine contents ranging from 22 to 70 wt %, known as Halowaxes, have been produced by Koppers Company in the United States since the 1920s. PCNs have also been manufactured in other countries as Nibren waxes (Bayer, Germany), Seekay waxes (ICI, U.K.), Clonacire waxes (Prodelec, France), and Cerifal materials (Caffaro, Italy). The exact amount of production of technical PCN mixtures is not known, but it has been estimated to be approximately 150 000 tons, which is 10% of the total global production of PCBs (*13, 14*). Although the industrial production of technical PCN formulations has declined in the United States since the early 1980s, products containing these formulations are still present in the environment (*15*). Furthermore, the secondary formation of chlorinated naphthalenes in thermal processes such as MSWI suggests sustaining environmental sources of PCNs.

Similar to PCBs, several chlorinated naphthalene congeners are persistent, lipophilic (log K_{ow} 3.9–8.4), and bioaccumulate in the food chain (*16–21*). Chlorinated naphthalene congeners have been measured in air (*22, 23*), water (*15, 24*), sediment (*10, 25*), and biota (*10, 18–20, 26*) including human tissues (*27–29*) from various locations. Several of the 75 possible PCN congeners exhibit toxic effects similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These include induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD), chloracne, and liver damage (*30–34*). The relative toxicities of planar halogenated aromatic hydrocarbons (HAHs) are calculated by expressing their toxicity in relation to TCDD, the most potent compound in this class of chemicals. Toxic equivalency factors (TEFs) are fractional potencies that relate a compound's potency to that of TCDD. The TCDD equivalents (TEQs; estimated by multiplying concentrations with TEFs) of two hexachloronaphthalene congeners PCN-66 (1,2,3,4,6,7-HxCN) and PCN-67 (1,2,3,5,6,7-HxCN) in fish from Swedish lakes were 0.3–13% of the total TCDD-like toxicities from HAHs (*17*). Despite their bioaccumulation and toxic potential, studies describing congener-specific distribution, behavior, and fate of PCNs in the environment are scarce.

Distribution, bioaccumulation, and fate of PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated

dibenzofurans (PCDFs) in various environmental media near a chlor-alkali plant have been described earlier (*35*–*38*). In this study, the isomer-specific profile of PCNs was examined in soil, sediments, and biota including a benthic invertebrate (blue crab), fish (striped mullet), and bird (boat-tailed grackle) collected near this chlor-alkali plant in southeastern coastal Georgia to elucidate their sources, distribution pattern, and bioaccumulation properties. In addition, TEQs were estimated for toxic PCN congeners and compared with those reported for PCBs, PCDDs, and PCDFs in the same media to evaluate the relative significance of these four compound classes at this site.

Materials and Methods

Site and Samples. The chlor-alkali plant is located in southeastern coastal Georgia near the city of Brunswick, GA. The plant was established in 1955 adjacent to an intertidal, coastal estuarine marsh and was operated until 1994, when it was designated as a Federal Superfund site. Disposal of process wastes into large holding pits near the top of the marsh and directly into the marsh and tidal creek has resulted in extensive contamination of soils on-site and also of sediments in the marsh (for map, see ref *35*). Details regarding the site and PCBs and PCDDs/PCDFs contamination patterns have been described earlier (*35*–*38*).

Soil samples were taken from waste holding pits near the top of the marsh (excavated soil) in February 1996. Wastes from chlor-alkali processes have been landfilled in these holding pits for several years. Surficial sediments (0–5 cm) were collected from two intertidal locations during low tide-one in the contaminated marsh (marsh sediment), about 200 m down from the holding pits, and the other at Purvis Creek (creek sediment), about 800 m down from the holding pits. Soil and sediments collected from several quadrates were pooled. Samples were placed in I-Chem jars and stored at −20 °C prior to analysis. Sediments were freeze-dried and passed through a 500-μm sieve prior to chemical analysis. Subsamples of soil and sediments were homogenized and analyzed for total organic carbon. Crabs and fish were collected in March 1997, and birds were collected in August 1995 in Purvis Creek, 1–2 km away from the chlor-alkali plant. This creek has been receiving drains from the contaminated marsh. Striped mullet (*Mugil cephalus*) were collected by gillnet (mean length: 25 cm, weight: 344 g), and blue crabs (*Callinectes sapidus*) were captured in traps (mean carapace width: 15.7 cm). Two boat-tailed grackle (*Quiscalus major*) were collected by shooting with steel shot (mean weight: 177 g). Muscle tissue from individual fish and grackle and hepatopancreas from crabs were taken. Individuals from same species were pooled for analysis.

Chemical Analysis. Chlorinated naphthalene congeners were analyzed following the method described earlier (*3*, *39*) with slight modifications. Five grams of soil or sediment was homogenized with anhydrous sodium sulfate and extracted with toluene in a Soxhlet apparatus for 20 h. The toluene extract was concentrated and cleaned up on a acidic silica/silica gel (Wakogel C-200) column. Two grams of acidic silica (25% concentrated sulfuric acid, w/w) was transferred over a layer of 2 g of silica gel packed in a glass column (30 cm × 10 mm i.d.). Chlorinated naphthalene congeners were eluted with *n*-hexane (100 mL).

Biological samples (5–10 g) were homogenized with sodium sulfate and extracted with 400 mL of methylene chloride and hexane (3:1) in a Soxhlet apparatus for 16 h. The extract was rotary evaporated at 40 °C, and an aliquot was used for the determination of fat content gravimetrically. The remaining extract was cleaned up on a acidic silica/silica gel column similar to that described for soil samples except 5 g of acidic silica was used instead of 2 g. Chlorinated naphthalene congeners were eluted with *n*-hexane (100 mL). The eluate was concentrated to 1 mL and transferred to a carbon column packed with 1 g of activated carbon-impregnated silica gel and 1.5 g of anhydrous sodium sulfate. PCNs were eluted with toluene (200 mL) after an initial rinsing with 150 mL of *n*-hexane.

A gas chromatograph (Hitachi M-80B GC) coupled with a mass spectrometric detector (GC/MSD) was used for the determination of PCN congeners. Injections were made splitless and solvent cut. The solvent cut mode is a special provision that allows injection of a relatively large volume of sample extracts (5–10 μL) onto a glass insert. Nitrogen is passed to evaporate the solvent on the glass insert, which is then released onto the column and purged with carrier gas. Fused silica capillary columns coated with DB-1701 (30 m × 0.25 mm i.d.) and DB-5 (30 m × 0.25 mm i.d.) at 0.25 μm film thickness were used for the separation and identification of individual congeners. The column oven temperature was programmed from 160 to 280 °C at a rate of 4 °C/min. Injector and detector temperatures were 280 and 180 °C, respectively. Helium was used as the carrier gas. The MSD was operated at an electron impact (EI) energy of 70 eV. PCN homologues were determined by selective ion monitoring (SIM) at *m/z* 230 and 232, 264 and 266, 300 and 302, 334 and 336, 368 and 370, and 402 and 404 for tri-, tetra-, penta-, hexa-, hepta-, and octa-chloronaphthalenes, respectively. Tri- through hexa-chloronaphthalenes were quantified against corresponding isomers present in Halowax 1014 while hepta- and octa-chloronaphthalenes were quantified using Halowax 1051 as the standard. The elution pattern and composition of PCN homologues in Halowaxes 1014 and 1051 have been determined earlier (*5*). In addition, the elution order of PCN isomers in capillary GC columns (OV-1701 and DB-5) was determined and reported as relative retention indices (RRI) (Table 1). Capillary columns, TC-1701, DB-1701, and OV-1701, have similar properties, and PCN isomers elute in the same order in these columns except 1,3,5,8-tetra-CN, which coelutes with 1,3,6,8-tetra-CN in DB-1701. Several PCN congeners were synthesized (*3*, *5*, *39*) for the identification of individual chloronaphthalenes in Halowaxes, which were used as standards. The RRI were assigned for individual PCN isomers based on their retention relative to *n*-alkanes, viz., $C_{20}H_{42}$ to be 2000 and $C_{30}H_{62}$ to be 3000. Identification of individual CN peaks to their GC elution order was based on RRI and an appropriate isotopic ratio. Peak area was used to determine concentrations of each homologue and isomer. Extracts were injected in duplicate or triplicate to confirm the identity of peaks and results. Instrumental calibration was done by using external standards, Halowaxes 1014 or 1051, or individual CN congeners. A procedural blank was analyzed within each sample series to check for interfering peaks. The detection limit of individual congeners varied depending on the sample mass, response factor, and interferences. The detection limit of CNs in sediments was 1 ng/g, dry wt, whereas those in biota varied from 2 to 60 pg/g, wet wt. PCN congeners are referred by their IUPAC numbers throughout the manuscript (*4*). Structures and IUPAC numbers of tri- through hepta-CN congeners are presented in Table 1. Recoveries of tri-, tetra-, penta-, hexa-, and hepta-CN congeners spiked in sediments or tissue extracts at the 3.3-μg level and passed through the analytical steps used for biological matrices were 66, 84, 91, 91, and 98%, respectively; whereas, the method used for soil and sediments had a overall recovery of 94–107%. Reported concentrations were not corrected for the recovery.

Results and Discussion

Soil and Sediments. Concentrations of tri- through octa-CN congeners (total PCNs) in excavated soil, marsh, and creek sediments were 17.9, 19.6, and 23.4 μg/g, dry wt, respectively (Table 2). The spatial distribution of PCN

TABLE 1. Relative Retention Indices of Tri- through Hepta-Chloronaphthalene Isomers in DB-5 and TC-1701

isomer	DB-5[b]	TC-1701	isomer	DB-5	TC-1701
tri-CN					
1,3,6- (20)[a]	1759	1847	1,2,6,8- (40)	2052	2182
1,3,5- (19)	1761	1838	1,4,5,8- (46)	2086	2212
1,3,7- (21)	1769	1860	1,2,3,8- (31)	2101	2237
1,4,6- (24)	1772	1856	1,2,7,8- (41)	2114	2268
1,2,4- (14)	1776	1856	penta-CN		
1,2,5- (15)	1796	1884	1,2,3,5,7- (52)	2145	2226
1,2,6- (16)	1802	1905	1,2,4,6,7- (60)	2145	2226
1,2,7- (17)	1812	1919	1,2,4,5,7- (58)	2168	2253
1,6,7- (25)	1812	1916	1,2,4,6,8- (61)	2178	2268
2,3,6- (26)	1819	1936	1,2,3,4,6- (50)	2186	2280
1,2,3- (13)	1827	1929	1,2,3,5,6- (51)	2190	2288
1,3,8- (22)	1842	1956	1,2,3,6,7- (54)	2217	2345
1,4,5- (23)	1852	1956	1,2,4,5,6- (57)	2227	2336
1,2,8- (18)	1896	2030	1,2,4,7,8- (62)	2235	2351
tetra-CN			1,2,3,5,8- (53)	2243	2358
1,3,5,7- (42)	1911	1972	1,2,3,6,8- (55)	2243	2383
1,2,4,6- (33)	1950	2025	1,2,4,5,8- (59)	2261	2384
1,2,4,7- (34)	1950	2025	1,2,3,4,5- (49)	2275	2399
1,2,5,7- (37)	1950	2029	1,2,3,7,8- (56)	2309	2475
1,3,6,7- (44)	1970	2069	hexa-CN		
1,4,6,7- (47)	1974	2059	1,2,3,4,6,7- (66)	2378	2479
1,2,5,6- (36)	1993	2092	1,2,3,5,6,7- (67)	2378	2479
1,3,6,8- (45)	1993	2095	1,2,3,4,5,7- (64)	2405	2514
1,2,3,5- (28)	2000	2092	1,2,3,5,6,8- (68)	2405	2514
1,3,5,8- (43)	2000	2092	1,2,3,5,7,8- (69)	2415	2531
1,2,3,6- (29)	2006	2115	1,2,4,5,6,8- (71)	2425	2544
1,2,3,7- (30)	2017	2128	1,2,4,5,7,8- (72)	2425	2544
1,2,3,4- (27)	2018	2110	1,2,3,4,5,6- (63)	2472	2603
1,2,6,7- (39)	2018	2136	1,2,3,4,5,8- (65)	2493	2635
1,2,4,5- (32)	2029	2128	1,2,3,6,7,8- (70)	2505	2670
2,3,6,7- (48)	2034	2175	hepta-CN		
1,2,4,8- (35)	2038	2141	1,2,3,4,5,6,7- (73)	2694	2772
1,2,5,8- (38)	2052	2165	1,2,3,4,5,6,8- (74)	2694	2784

[a] Numbers in parentheses correspond to the numbering of CNs as proposed by Wiedmann and Ballschmiter (*4*) and the corresponding structures according to the IUPAC rules. [b] DB-5 was 60 m long, and carrier gas was used at 140 kPa; whereas, TC-1701 was 30 m long, and carrier gas used at 70 kPa.

concentrations at this marsh site was different from those observed for PCBs, PCDDs, and PCDFs, which decreased by 40–60-fold along the contamination gradient from excavated soil to creek sediment (*35, 36*). The organic carbon-normalized concentrations of total PCNs decreased in the following order: excavated soil > creek sediments > marsh sediments. The concentrations of total PCNs in creek sediments were 2.4 times greater than those reported for total PCBs.

The concentrations of PCNs in soil and sediments were 2–3 orders of magnitude greater than those reported for sediments collected from Gdańsk Basin in the Baltic Sea (*18*) and Lake Vänern in Sweden (*10*). The sediment PCN concentrations were 8-fold greater than the mean concentrations reported for sediments from the Trenton Channel of the Detroit River, MI, although the maximum concentration reported for the Trenton Channel sediments was 3-fold greater than that observed in this study (*25*).

Elevated concentrations of PCNs in soil and sediments analyzed in this study suggest that chlor-alkali plants have been a potential source of PCNs in the environment. Technical mixtures of PCNs such as Halowaxes 1013 and 1014 have been used as impregnants for graphite electrodes used in the chlor-alkali process (*1*). While our previous studies have reported the use of Aroclor 1268, a greatly chlorinated PCB mixture, as a lubricant on graphite electrodes used at this chlor-alkali facility (*35*), the present study provides evidence for the use or formation of PCNs in this process. PCN contamination arising from the use of Aroclor 1268 is less likely due to the differences in the spatial contamination gradients between these two contaminants at this site and greater concentrations of PCNs than PCBs in creek sediments. In fact, the relative proportions of PCNs in Aroclors 1254 and 1260 were 0.00035% and 0.00027%, respectively (*12*), which is 3–4 orders of magnitude less than those estimated for soil and sediments analyzed in this study.

Hexa- and hepta-CN congeners contributed 70% of the total PCN concentrations in soil and sediments (Figure 1). This distribution of PCN homologues was different from those found in Halowaxes 1051 or 1014. Particularly, the proportions of hepta-CN in soil and sediments were 3- and 8-fold greater than those found in Halowaxes 1051 and 1014, respectively. On the other hand, the proportion of octa-CN in soil and sediments was less than that in Halowax 1051 but greater than that in Halowax 1014. Despite the reports on the use of Halowaxes 1013 and 1014 as impregnants for carbon electrodes used in chlor-alkali process (*1*), a different profile of PCN homologues in soil and sediments suggests their formation in chlor-alkali process. Physicochemical and biological processes can alter PCN congener profile, but no information is available in this regard. Lower chlorinated naphthalenes have greater water solubility and vapor pressure; therefore, their relative proportion in sediments may be expected to be minimal. Evaporation and/or microbial dechlorination of greatly chlorinated PCN congeners are unlikely. Indeed, a similar profile of PCN congeners in soil and sediments suggests that anaerobic microbial dechlorination in sediments was limited. Enrichment in hepta- and octa-CN congeners relative to those in Halowaxes 1013 and 1014 suggests that these congeners may have been formed in the chlor-alkali process, similar to that discussed for the formation of hepta- and octa-chlorodibenzofurans in the chlor-alkali process (*36*). Graphite electrodes used in the chlor-alkali industry are made up of wood-pitch, a material providing aromatic precursors from which PCNs may have been formed during the electrolysis of brine for the produc-

TABLE 2. Concentrations and Relative Composition (in Parentheses; % wt) of PCN Homologues in Soil (μg/g, dry wt), Sediments (μg/g, dry wt), and Biota (ng/g, wet wt)

CN congener	excavated soil	marsh sediment	creek sediment	blue crab	striped mullet	boat-tailed grackle
organic carbon (%)	2	10	5.6	NA[a]	NA	NA
lipid content (%)	NA	NA	NA	18	1.2	4.0
tri-CN	0.09 (<1)	0.1 (<1)	0.05 (<1)	2.36 (18)	0.52 (9)	0.24 (22)
tetra-CN	0.64 (4)	0.65 (3)	0.53 (2)	7.18 (54)	2.28 (38)	0.23 (22)
penta-CN	2.23 (12)	2.44 (13)	2.58 (11)	2.93 (22)	2.15 (36)	0.36 (34)
hexa-CN	5.81 (32)	6.47 (33)	7.28 (31)	0.78 (6)	1.11 (18)	0.23 (22)
hepta-CN	7.14 (40)	7.69 (39)	9.64 (41)	<0.04 (<1)	<0.02 (<1)	<0.06 (<6)
octa-CN	2.03 (11)	2.26 (11)	3.35 (14)	<0.06 (<1)	<0.03 (<1)	<0.04 (<4)
total	17.9	19.6	23.4	13.26	6.05	1.06

[a] NA, not analyzed.

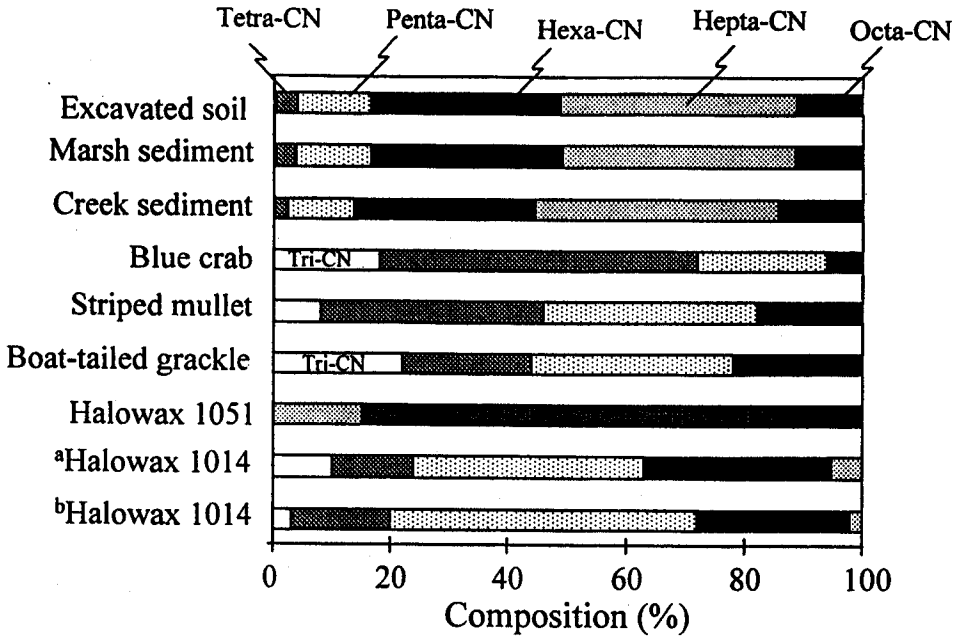

FIGURE 1. Composition (%) of PCN congeners in soil, sediment, biota, and Halowax mixtures. Fnt a: Ref 23. Fnt b: Ref 5.

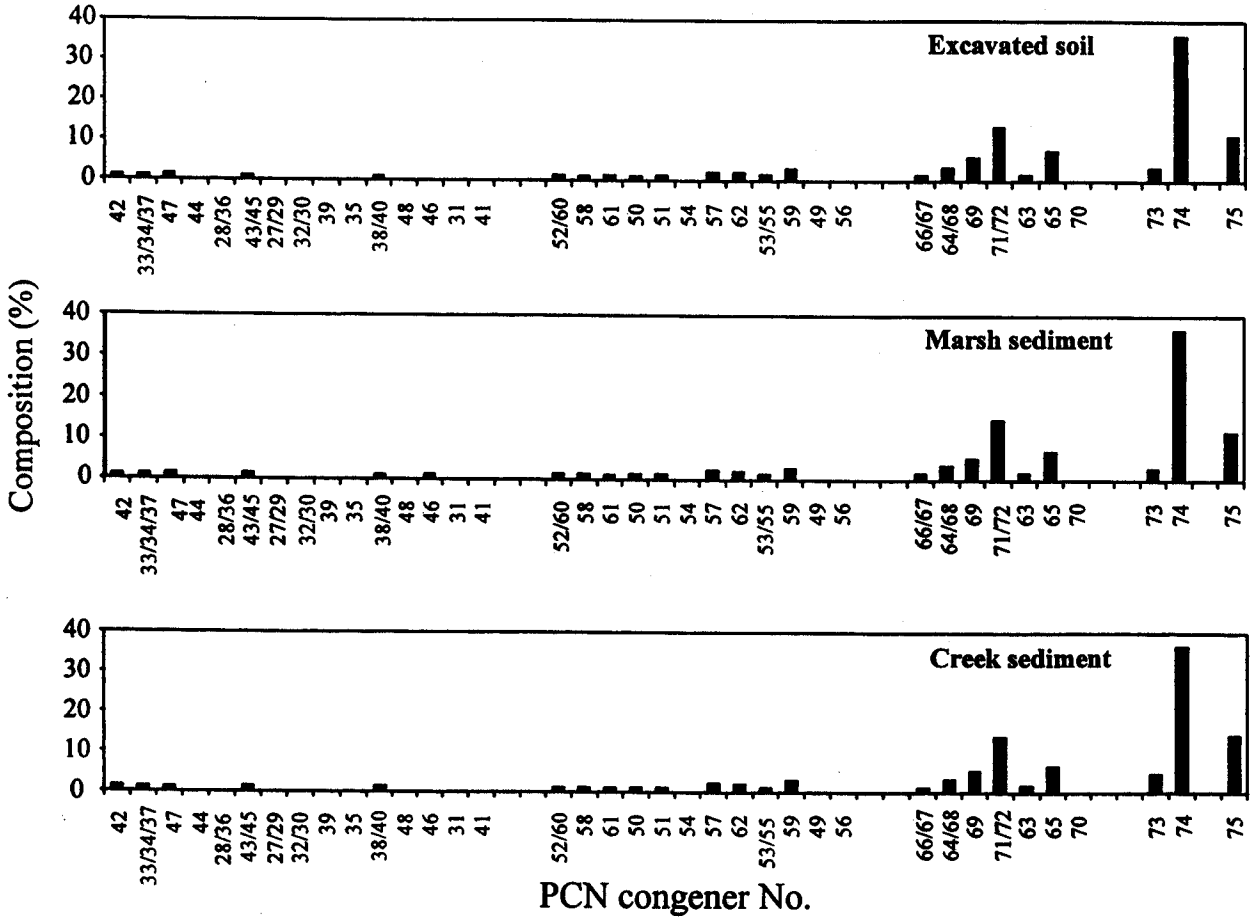

FIGURE 2. Relative composition (%) of PCN isomers and congeners in soil and sediments collected near a chlor-alkali plant.

tion of chlorine. Thus, multiple pathways for the sources of PCNs from the chlor-alkali industry such as the use of Halowaxes and the formation of CN congeners are considered.

Isomer-specific analysis of tetra- through octa-CN congeners in soil and sediments revealed the presence of 35 congener pairs comprising 47 of 49 isomers theoretically possible (Table 3). A few tetra-, penta-, and hexa-CN congeners coeluted in pairs or triplicate and could not be isolated using DB-1701 or DB-5 columns. Tetra-CN isomers, 1,2,3,8- (PCN 31) and 1,2,7,8- (PCN 41), were undetected. These two isomers have been reported in fly ash from MSWI (*3*, *5*). The congeners 1,2,3,4,5,6,8-hepta-CN (PCN 74), 1,2,4,5,6,8-/1,2,4,5,7,8-hexa-CN (PCNs 71/72), 1,2,3,4,5,6,7,8-

TABLE 3. Concentrations of PCN Isomers in Soil, Sediment (ng/g, dry wt), and Biota (pg/g, wet wt) Collected near a Chlor-Alkali Facility

congener	IUPAC No.	excavated soil	marsh sediment	creek sediment	blue crab	striped mullet	boat-tailed grackle
			Tetra-CN				
1,3,5,7-	42	120	118	97	1850	989	25
1,2,4,6-/1,2,4,7-/1,2,5,7-	33/34/37	180	163	135	3060	468	149
1,4,6,7-	47	51	54	53	361	250	<3
1,3,6,7-	44	15	11	14	162	37	<3
1,2,3,5-/1,2,5,6-	28/36	15	26	19	500	44	<3
1,3,5,8-/1,3,6,8-	43/45	85	93	67	346	355	<3
1,2,3,4-/1,2,3,6-	27/29	3.7	2.4	3.8	45	32	<3
1,2,4,5-/1,2,3,7-	32/30	4.1	5.9	3.7	39	6.3	4.8
1,2,6,7-	39	4.3	4.9	2.7	291	5.2	<3
1,2,4,8-	35	7.0	6.9	5.7	78	23	17
1,2,5,8-/1,2,6,8-	38/40	127	135	108	291	71	35
2,3,6,7-	48	<1	1.1	<1	148	<2	<3
1,4,5,8-	46	27	30	22	16	<2	<3
1,2,3,8-	31	<1	<1	<1	<5	<2	<3
1,2,7,8-	41	<1	<1	<1	<5	<2	<3
			Penta-CN				
1,2,3,5,7-/1,2,4,6,7-	52/60	242	280	276	1430	1010	246
1,2,4,5,7-	58	75	82	83	132	337	25
1,2,4,6,8-	61	205	255	242	170	405	27
1,2,3,4,6-	50	45	54	45	113	12	7.8
1,2,3,5,6-	51	33	41	34	440	18	4.6
1,2,3,6,7-	54	<1	<1	<1	14	2.9	<3
1,2,4,5,6-	57	413	457	474	269	100	20
1,2,4,7,8-	62	371	394	445	238	68	13
1,2,3,5,8-/1,2,3,6,8-	53/55	282	263	273	64	139	10.3
1,2,4,5,8-	59	539	579	686	67	65	6.5
1,2,3,4,5-	49	21	28	22	<5	<2	<3
1,2,3,7,8-	56	3.6	7.4	<1	<5	<2	<3
			Hexa-CN				
1,2,3,4,6,7-/1,2,3,5,6,7-	66/67	158	171	202	334	135	60
1,2,3,4,5,7-/1,2,3,5,6,8-	64/68	598	710	749	69	252	44
1,2,3,5,7,8-	69	1040	1040	1200	107	216	49
1,2,4,5,6,8-/1,2,4,5,7,8-	71/72	2400	2860	3220	183	362	46
1,2,3,4,5,6-	63	298	340	371	52	30	10
1,2,3,4,5,8-	65	1330	1350	1530	36	115	22
1,2,3,6,7,8-	70	<1	<1	<1	<5	<2	<3
			Hepta-CN				
1,2,3,4,5,6,7-	73	639	586	1080	<20	<10	<30
1,2,3,4,5,6,8-	74	6500	7110	8560	<20	<10	<30
			Octa-CN				
	75	2030	2260	3350	<60	<30	<40

octa-CN (PCN 75), 1,2,3,4,5,8-hexa-CN (PCN 65), and 1,2,3,5,7,8-hexa-CN (PCN 69) were the major components in soil and sediments accounting for 37, 14, 12, 7, and 5%, respectively, of the total PCN concentrations (Figure 2). Predominance of 1,2,3,4,5,6,7-hepta-CN (PCN 73), comprising 40–50% of the total PCN concentrations in sediment and graphite sludge from a chlor-alkali plant in Sweden has been reported (*10*). Nevertheless, soil and sediments analyzed in this study contained about 3–4% of this congener in total PCNs. These results suggest the differences in the profiles of CN congeners in samples collected from different chlor-alkali plants.

Biota. Among the biological samples analyzed, blue crab hepatopancreas contained the greatest concentrations (13.3 ng/g, wet wt) followed by striped mullet (6.05 ng/g) and grackle (1.06 ng/g) muscle tissues (Table 2). The lipid-normalized concentrations of total PCNs in blue crab, mullet, and grackle were 74, 500, and 27 ng/g, respectively. Concentrations of PCNs in striped mullet were greater than those reported for various species of fish from the Baltic Sea (*40*) and lakes and rivers in Sweden (*10*). On the other hand, the concentration of total PCNs in grackle was less than those reported for predatory birds such as white-tailed sea eagles from the Baltic Sea (*10, 19*).

The profile of PCN homologues in biota was different from those observed in soil or sediments. Tetra-CN congeners accounted for the greatest proportion in blue crab and striped mullet whereas penta-CNs predominated in grackle (Table 2). Hepta- and octa-CNs were not detected in biota. The profile of PCN congeners in biota did not resemble those found in greatly chlorinated Halowax mixtures 1014 and 1051. Although, the pattern of PCNs in blue crab hepatopancreas matched with those of Halowax 1013, this does not necessarily mean exposure to this technical mixture because neither sediments nor other biological samples contained similar PCN distribution. A greater water solubility of lower chlorinated naphthalenes may suggest that these congeners are more bioavailable than higher chlorinated naphthalenes. Species-specific accumulation or metabolism of PCN congeners may also influence their distribution in biota. The observed profiles of PCN homologues in biota were similar to those reported from the Baltic Sea, with great proportions of tetra- or penta-CNs in crabs and fish and penta-CNs in birds (*10, 18, 19, 40*).

Despite the predominance of hepta- and octa-CNs in soil and sediments, these congeners were not accumulated in the biota analyzed. Although biodegradation may reduce the relative abundance of these congeners, information

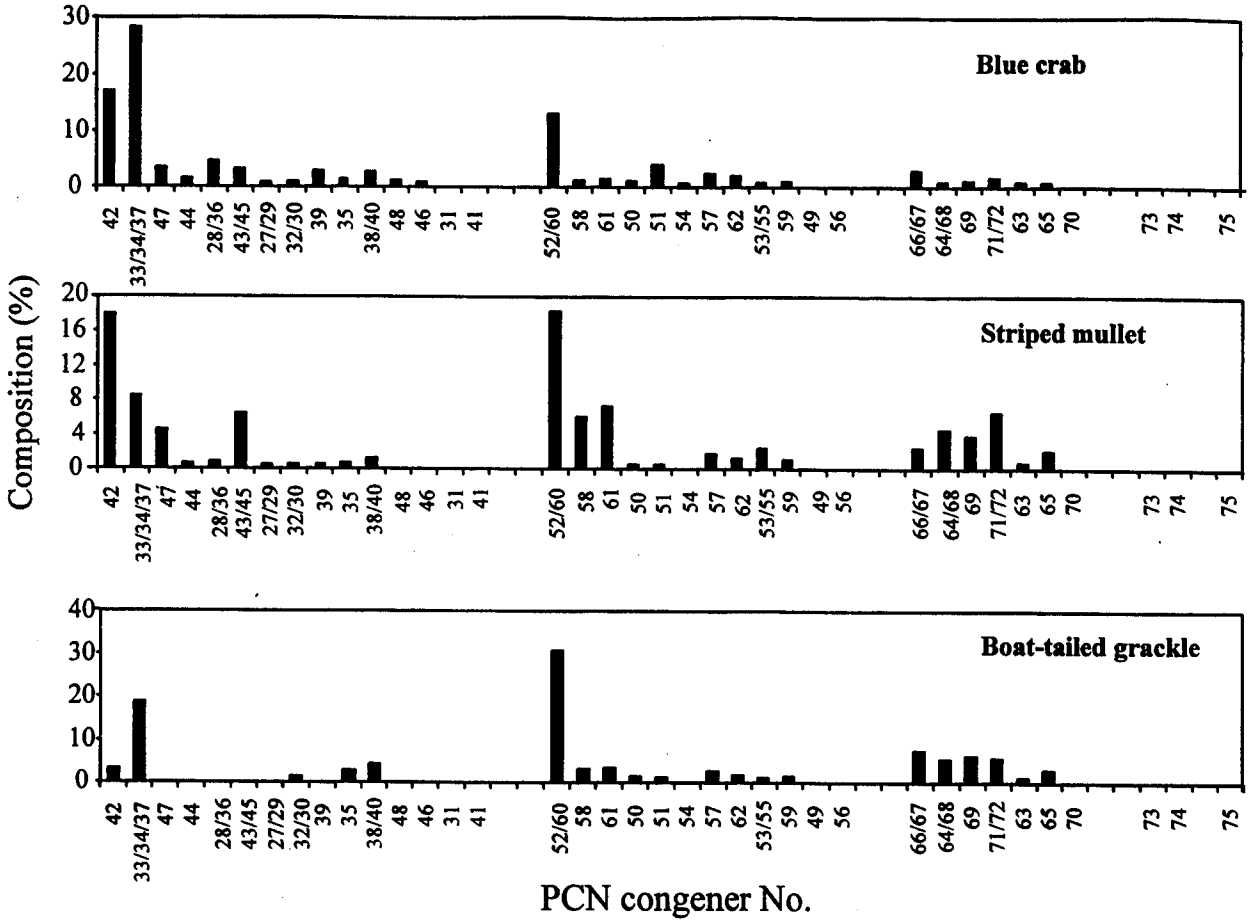

FIGURE 3. Relative composition (%) of PCN isomers and congeners in blue crab, striped mullet, and boat-tailed grackle collected near a chlor-alkali plant.

TABLE 4. Concentrations of Dioxin-Like PCN Congeners and Their Toxic Equivalents (TEQs; in Parentheses) in Soil, Sediment (pg/g, dry wt), and Biota (pg/g, wet wt)

congener	IUPAC No.	TEF[a]	excavated soil	marsh sediment	creek sediment	blue crab	striped mullet	boat-tailed grackle
1,2,6,8-	40[b]	1.65E-05	127000	135000	108000	291	71	35
1,2,3,6,7-	54	1.7E-04	ND[c]	ND	ND	14 (0.002)	2.9 (0.0005)	ND
1,2,3,4,5,6-	63	2E-03	298000 (596)	340000 (680)	371000 (742)	52 (0.1)	30 (0.06)	10 (0.02)
1,2,3,4,6,7-/1,2,3,5,6,7-	66/67[d]	2.27E-03	158000 (358)	171000 (387)	202000 (458)	334 (0.76)	135 (0.31)	60 (0.14)
1,2,3,5,6,8-	68[e]	1.5E-04	598000	710000	749000	69	252	44
1,2,3,5,7,8-	69	2E-03	1040000 (2080)	1040000 (2080)	1200000 (2400)	107 (0.21)	216 (0.43)	49 (0.10)
1,2,3,6,7,8-	70	5.9E-04	ND	ND	ND	ND	ND	ND
1,2,3,4,5,6,7-	73	3.45E-03	639000 (2210)	586000 (2020)	1080000 (3730)	ND	ND	ND

[a] From ref *34*; for congeners 63 and 69 from ref 31. [b] Colelutes with congener 38. [c] ND, not detected. [d] PCN congeners 66 and 67 coelute, and therefore, an average TEF was used. [e] Coelutes with congener 64.

regarding biotransformation of these greatly chlorinated PCN congeners is scarce, but biodegradation seems unlikely since no metabolism of penta- through octa-CN congeners was observed in rabbits (*41*). Steric hindrance in the uptake of large molecules through biological membranes has been suggested. Molecules with an effective cross section (ECS) of >9.5 Å were not taken up through the gills (*42*). The ECS of hepta- and octa- CNs were 9.7 and 9.8 Å, respectively. This suggests that steric hindrance may also have also contributed to reduced uptake in biota as has been reported for nona- and deca-chlorobiphenyls (*37, 38*). However, a recent study showed that the dietary uptake efficiencies of hepta- and octa-CNs in pike (*Esox lucius*) were 68 and 35%, respectively, (*43*). Occurrence of hepta-CN congeners has been reported in biota, although at relatively lesser concentrations (*18, 20*). Lack of bioavailability of planar congeners, particularly highly chlorinated PCNs, due to their tendency to bind to sediment organic carbon has been considered as an important factor that contributed lesser accumulation in biota. Biota–sediment accumulation factors (BSAF: concentration in tissue normalized to lipid content divided by concentration in sediment normalized to organic carbon) were estimated for total PCN concentrations in biota relative to the concentrations measured in creek sediment. The BSAFs for PCNs were in the range of 0.0012–0.00007. These values were compared with those measured for PCBs at the same location. The lipid-normalized concentrations of PCBs in blue crab, striped

mullet, and boat-tailed grackle were 197, 283, and 83 μg/g, respectively (*37*), while the organic carbon-normalized concentration of PCBs in creek sediments was 171 μg/g (*35*). The BSAFs for PCBs were in the range of 0.48−1.7, which were 3−4 orders of magnitude greater than those estimated for PCNs. These results suggest that PCNs tend to bind strongly to sediment organic carbon, and therefore, their bioavailability may be limited in organic-rich sediments.

Isomer-specific profiles of PCNs in biota revealed different patterns among species analyzed (Table 3, Figure 3). Blue crab and fish contained greater proportions of tetra-CN congeners 42, 33/34/37, 47, and 43/45 and penta-CN congeners 52/60, 58, and 61; collectively accounting for 68−69% of the total concentrations. In addition to these congeners, hexa-CN congeners 66/67, 64/68, 69, and 71/72 were abundant in mullet, constituting 20% of the total PCN concentrations. The distribution of CNs in grackle muscle was characterized by the presence of a few congeners, of which PCNs 33/34/37 and 52/60 accounted for 50% of the total concentrations. Compared to those in fish and crab, few tetra-CN congeners were found in grackle, which suggests efficient metabolism and elimination of less chlorinated congeners in birds (Figure 3).

Toxic Potential. PCN congeners are planar, and some of them exhibit Ah-receptor mediated cytochrome P450 induction, analogous to TCDD. The TEFs have been reported for chloronaphthalene congeners 63, 64/68, 66/67, 69, 71/72, and 73 based on EROD induction in in vitro bioassays using H4IIE rat hepatoma cells (*31*). Hexa-CN congeners 63, 66/67, and 69 were the most potent among the congeners examined, and each was assigned a TEF of 0.002. Development of TEFs for other PCN congeners has been hindered by the lack of sufficient quantities of pure, well-characterized individual congeners. Recently, to develop TEFs, 25 individual PCN congeners were tested for their potency as Ah-receptor agonists in H4IIE bioassays (*34*). In addition to the congeners mentioned above, PCN congeners of IUPAC Nos. 5, 40, 54, 68, 70, and 73 were shown to exhibit dioxin-like activity.

The 2,3,7,8-TCDD equivalents (TEQs) were estimated for the active PCN congeners in soil, sediment, and biota collected at the chlor-alkali plant (Table 4). Although CN congeners 40 and 68 were active in bioassays and present at measurable concentrations in soil, sediment, and biota, they coelute in pairs with other CN congeners for which no TEFs were available. Therefore, TEQs were not estimated for these congeners. The TEQs estimated for PCNs in sediments were greater than 5 ng/g, dry wt, which was greater than those observed for PCDDs, PCDFs, and PCBs (*35*−*37*). The contribution of different planar halogenated aromatic hydrocarbons to TEQs in soil and sediments were in the following order:

excavated soil: PCBs > PCNs > PCDFs > PCDDs

marsh and creek sediments: PCNs > PCBs > PCDFs > PCDDs

Hexa-CN congener 69 and hepta-CN congener 73 contributed to greater than 80% of the TEQs in soil and sediments.

The TEQs for PCN congeners 66/67, 54, 63, and 69 in biota were 0.25−1.1 pg/g, wet wt. These values were greater than those of the TEQs reported for non-ortho-PCBs, PCDDs, and PCDFs in blue crab, striped mullet, and boat-tailed grackle from the same location (*37*). Nevertheless, this location has been contaminated by a unique mixture of technical PCBs, Arolcor 1268, which has relatively less abundant non-ortho congeners that would explain less contribution of PCBs to total TEQs, despite the total PCB concentrations as great as 480 μg/g, dry wt, in marsh sediments (*35*) and 48 μg/g, wet wt, in blue crab hepatopancreas were found (*37*). This study suggests that PCNs are important toxic contaminants formed in chlor-alkali process.

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