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Levels of Polychlorodibenzo-*p*-dioxins and Dibenzofurans in Crab Tissues from the Newark/Raritan Bay System

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Hepatopancreas and muscle tissues of crabs collected from the Newark/Raritan Bay system, New Jersey, were analyzed for polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) by using capillary gas chromatography/high-resolution mass spectrometry (GC/HRMS) in the selected ion-monitoring, mass-profile mode. All hepatopancreas tissue samples were found to be contaminated with PCDD/Fs. Samples collected proximate to a former chemical manufacturing plant located on the Passaic River have the highest levels. The concentrations of PCDD/Fs decrease in animals taken at increasingly more remote sites from the alleged point source. The levels (up to 1 ppb) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in hepatopancreas tissues of the crabs taken from Newark Bay are elevated by a factor of 5–10 times those of samples from Raritan Bay. Even the muscle samples from animals taken from Newark Bay have detectable levels of 2,3,7,8-TCDD, whereas those from Raritan Bay have "no detectable" levels of 2,3,7,8-TCDD (detection limit 0.5–1.0 ppt). The levels of 2,3,7,8-substituted pentachloro- and hexachlorodibenzofurans are approximate 10 times lower. No other PCDD/Fs were detected at a detection limit range of 5–45 ppt.

Introduction

Contamination by polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) has been reported in waters, sediments, and several species of fish in the Newark Bay system, New Jersey (*1–4*). Results from the determination of selected PCDD/Fs in sediment cores and suspended particulate matter taken from the area strongly suggest that the major source of these compounds is a former chemical plant located on the Passaic River, approximately 3 km upstream of Newark Bay (see Figure 1) (*1–3*). The plant produced sodium 2,4,5-trichlorophenate that was used to synthesize 2,4,5-trichlorophenoxy acetic acid (2,4,5-T).

These PCDD/Fs can remain in aquatic ecosystems for long periods of time and have been detected in the tissues of aquatic species and sediments near other industrial locations in North America, Europe, and Japan (*4–9*). The low water solubility of PCDD/Fs and their high adsorption to sediment do not rule out movement in the environment. Normal sediment movement, dredging, and channel widening as well as uptake by mobile animals can cause dispersion of the toxic materials in the environment (*10, 11*). The New Jersey Department of Environmental Protection and Energy (NJDEPE) examined the levels of PCDD/Fs in the sediments and biota of Newark Bay and Arthur Kill (*3, 4*). The highest levels of 2,3,7,8-TCDD were found in the mid-gut glands (hepatopancreas) of blue crabs taken near the mouth of Passaic River just above the entry to Newark Bay. Only two crabs, however, were collected and analyzed. No data have been reported for the PCDD/Fs contamination in crabs from Raritan Bay, which is still open for commercial and recreational fishing.

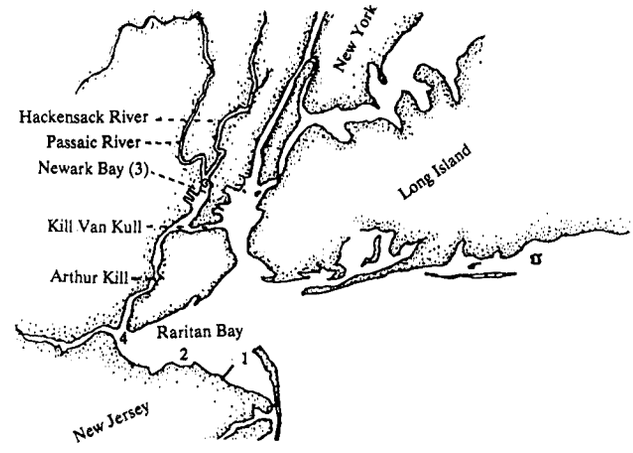

Figure 1. Map of the Newark/Raritan Bay system.

This paper describes the determination of various 2,3,7,8-substituted PCDD/Fs in muscle and hepatopancreas tissues of crabs collected from both Newark Bay and Raritan Bay. The levels and isomer distribution patterns of PCDD/Fs in crabs from different stations in the systems were determined to gain a better understanding of the fate and biomagnification of PCDD/Fs in the environment and to provide further evidence for the source(s) of PCDD/Fs contamination in this aquatic system.

Experimental Section

Sample Collection. In September 1991 and June 1992, 30–40 adult, legal-size (~10-cm carapace width) blue crabs (*Callinectes sapidus*) were collected, by using an otter trawl or a crab rake, from each of four stations in the Newark/Raritan Bay: station 1, Sandy Hook Bay; station 2, East Beach of Raritan Bay; station 3, Newark Bay; and station 4, Wards Point (see Figure 1). The molting stage of the crabs was intermolt (C-4 stage of their molt cycle).

As soon as the crabs were removed from the net, they were placed in coolers containing crushed ice and stored overnight. On the next day, the crabs were sorted by size and sex, placed in labeled plastic bags while still alive, and frozen in a freezer at −80 °C. A total of 10–14 crabs from

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each station was chosen for the PCDD/Fs analysis. The remaining animals were stored in the freezer.

Before dissection, the crabs were measured, weighed, and staged for their position in the molt cycle. The muscle and hepatopancreas of 5–7 similar-sized animals of the same sex were each dissected while the animal was still frozen. The tissues were placed separately in glass scintillation vials with foil-lined caps that had been rinsed with methanol, acetone, and methylene choride. During the dissection, the vials were kept in an ice bath. The weight of the tissue in each vial was determined, and the vials were stored in the freezer at −80 °C.

Two composite samples of the muscle and two of the hepatopancreas (each from five to seven animals) were made for each station. The samples were homogenized and stored in a freezer at below −20 °C until the chemical analyses were performed.

PCDD/Fs Standards. The native PCDD/Fs standard compounds and the ^{13}C-labeled compounds that were used as internal standards:

2,3,7,8-TCDD	$[^{13}C_{12}]$-2,3,7,8-TCDD
1,2,3,7,8-P_5CDD	$[^{13}C_{12}]$-1,2,3,7,8-P_5CDD
1,2,3,4,7,8-H_6CDD	
1,2,3,6,7,8-H_6CDD	$[^{13}C_{12}]$-1,2,3,6,7,8-H_6CDD
1,2,3,7,8,9-H_6CDD	
1,2,3,4,6,7,8-H_7CDD	$[^{13}C_{12}]$-1,2,3,4,6,7,8-H_7CDD
1,2,3,4,6,7,8,9-OCDD	$[^{13}C_{12}]$OCDD
2,3,7,8-TCDF	$[^{13}C_{12}]$-2,3,7,8-TCDF
1,2,3,7,8-P_5CDF	$[^{13}C_{12}]$-1,2,3,7,8-P_5CDF
2,3,4,7,8-P_5CDF	$[^{13}C_{12}]$-2,3,4,7,8-P_5CDF
1,2,3,4,7,8-H_6CDF	$[^{13}C_{12}]$-1,2,3,4,7,8-H_6CDF
1,2,3,6,7,8-H_6CDF	
1,2,3,7,8,9-H_6CDF	
2,3,4,6,7,8-H_6CDF	
1,2,3,4,6,7,8-H_7CDF	$[^{13}C_{12}]$-1,2,3,4,6,7,8-H_7CDF
1,2,3,4,7,8,9-H_7CDF	
1,2,3,4,6,7,8,9-OCDF	

All standard compounds were purchased from Cambridge Isotope Laboratories, Woburn, MA, and Willington Environmental Consultants Inc., Guelph, Ontario, Canada. $[^{13}C_{12}]$-1,2,3,6,7,8-H_7CDD, $[^{13}C_{12}]$-1,2,3,7,8-P_5CDF, and $[^{13}C_{12}]$-1,2,3,4,6,7,8-H_7CDF were gifts from Twin City Testing Co, Minneapolis, MN. All organic solvents used in this study were at least HPLC grade and were purchased from Fisher Scientific, Pittsburgh, PA. Other chemicals were of analytical grade or better.

A 500-g catfish tissue was obtained commercially at a supermarket in Lincoln, NE, diced into small pieces, and carefully mixed. The catfish tissue was analyzed three times and was proven to contain no detectable PCDD/Fs. This tissue was used as both a method blank and a fortified matrix blank.

Calibration and Relative Response Factors. Calibration was done by using seven data points ranging from 10 to 1000 pg for 2,3,7,8-TCDD/F and three points for the other 2,3,7,8-substituted PCDD/Fs (i.e., 25, 50, and 75 pg for P_5CDD/F, H_6CDD/F and H_7CDD/F and 125, 250, and 500 pg for OCDD/F). The amounts of the internal standards were constant for all calibration runs: 50 pg for each labeled compound except $[^{13}C_{12}]$OCDD (150 pg). Relative response factors (RRF), the ratio of the intensity of a native PCDD/F signal to that of the corresponding internal standard, were obtained from the determination of the calibration standards. The RRF for each analyte was constant (less than 10% coefficient of variation) in the calibration ranges.

The calibration standards were analyzed after every third to fifth injection of sample. If a RRF value deviated by more than 20% of that determined every 3rd to 5th injection, a new calibration curve was obtained over the entire concentration range.

Sample Preparation. A 10-g portion (wet weight) of the composited crab tissue was accurately weighed and fortified with 5 ng each of the ^{13}C-labeled standards listed earlier, except that 10 ng of $[^{13}C_{12}]$OCDD was used. The sample preparation procedures were reported previously (*12, 13*). In short, the fortified tissue sample was digested with a 30% KOH solution in a mixture of water and ethanol (3:1) at room temperature, and the digest was extracted with hexane. The hexane extract was then submitted to a cleanup procedure that included sulfuric acid washing and column chromatography separations. The column chromatography made use of three separate solid phases: nonactivated silica, neutral alumina, and activated carbon/silica.

The samples were prepared and analyzed in batches. Each batch consisted of four or five tissue samples, a duplicate, a fortified matrix (catfish) blank, and a method blank. The final volume of sample extract was adjusted to be within the range of 10–50 μL. This insured that the amount of the analyte was within the linear range of the calibration curve.

Gas Chromatography/High-Resolution Mass Spectrometry System. Gas chromatography/high-resolution mass spectrometry (GC/HRMS) analysis was performed on a Carlo-Erba gas chromatograph/Kratos MS-50 double-focusing mass spectrometer/Kratos-MACH3 data system. The mass-profile technique (*14*) was applied in the selected ion monitoring (SIM) mode of GC/HRMS.

A fused silica capillary column (DB-5, 60 m × 0.32 mm i.d., 0.25-μm film thickness) from J & W Scientific, Folsom, CA, was used for sample analysis. The GC conditions were as follows: on-column injection (1 or 2 μL); helium carrier gas at a head pressure of 150 kPa; column temperature initially held at 80 °C for 1 min, then programmed to 300 °C at a rate of 15 °C/min.

A second DB-5 column (60 m × 0.25 mm i.d., 0.25-μm, J & W Scientific) was used, if necessary, to get better GC separation, especially for 1,2,3,6,7,8-H_6CDD and 1,2,3,7,8,9-H_6CDD as well as for 1,2,3,7,8,9-H_6CDF and 2,3,4,6,7,8-H_6CDF. The following temperature program was applied: column temperature initially held at 80 °C for 2 min, programmed to 230 °C at a rate of 35 °C/min and held for 5 min, then heated from 230 to 300 °C at 5 °C/min.

The GC column was directly introduced into the ion source via an interface operated at 250 °C. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV, source temperature 250 °C. Typical conditions for the selected ion monitoring were scan rate 0.1 s/mass window, amplifier bandwidth 1 kHz, and sweep width 300 ppm at 8000 mass resolving power (10% valley definition). No lock mass was used during the analysis; instead an ion of standard mass (from PFK) was used to check for drift just prior to analyte elution.

Selected ion monitoring of five groups of ion signals was used for the determination. Two ions were selected for each analyte, one as the confirmation ion and the other, more abundant one for quantification. An internal standard ion was also selected for each ^{13}C-labeled compound. Mass chromatograms and mass profiles for

the selected ions of the target compounds were obtained during GC elution over appropriate retention time windows and used for the determinations. The windows were defined by using PCDD/F mixtures as recommended in ref 17.

The SIM retention time windows were set for different ion masses: (1) TCDD/F, (2) P_5CDD/F, (3) H_6CDD/F, (4) H_7CDD/F, and (5) OCDD/F. The retention time range of each window was sufficiently long to cover the GC retention time of all analytes in the group.

More details of GC/HRMS conditions, identification criteria, and internal standard ratio quantification were described elsewhere (*13*). The methodologies used in this laboratory were validated in a variety of investigations (*15*, *16*).

Gas Chromatography/Low-Resolution Mass Spectrometry. To confirm further the presence of 2,3,7,8-TCDD/F, full-scan EI mass spectra (over the range m/z 35–500) were obtained for eluents from extracts of some hepatopancreas tissues. The spectra were compared with those obtained from authentic standards of 2,3,7,8-TCDD/F. The GC/MS analysis was performed by the same instrument described above, but the resolving power of the spectrometer was reduced to 2000, and the scan rate was 3 s/decade.

Safety. The 2,3,7,8-substituted PCDD/Fs and PCDTs are highly toxic and suspected carcinogens. Therefore, sample extraction and cleanup were conducted in a high hazard laboratory that is solely dedicated to dioxin-related determinations. The handling of standard solutions and the sample extracts was done by experienced chemists under well-defined and controlled conditions. The safety guidelines for dioxin determination were described in the U.S. EPA method 1613 (*17*).

Results and Discussion

Identification of PCDD/Fs in Crab Tissue Samples. The compounds 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-P_5CDF, 2,3,4,7,8-P_5CDF, and 1,2,3,4,7,8-H_6CDF were identified in the composited crab tissues, although other 2,3,7,8-substituted PCDD/Fs were not found at detection limits of 5–20 ppt for P_5CDD/Fs, H_6CDD/Fs, and H_7CDD/Fs and of up to 45 ppt for OCDD/F (see Table 4).

Strict criteria are required for the GC/HRMS analysis for positive identification of PCDD/Fs in an environmental or biological sample. Our criteria include acceptable recoveries of ^{13}C-labeled internal standards (within the range of 40–120%), correct accurate mass for all selected ions, correct retention time, and proper isotope and signal-to-noise ratios (to within ±0.1 and greater than 3:1, respectively).

A unique feature of the method applied in this study is the use of mass-profile data, which provide much higher certainty than that achieved by conventional high-resolution MS techniques commonly used for PCDD/F determination (*14*). The GC/HRMS-SIM operated in the mass-profile mode gives accurate mass measurements, in addition to the chromatographic retention time and signal intensities, which are also obtained from conventional peak-top monitoring mode, but its use is accompanied with a small loss of sensitivity (*14*). Figure 2 shows the mass chromatograms and mass-profile data for the analysis of TCDD in a crab hepatopancreas sample collected from station 3. The mass-profile spectra (right side of the figure)

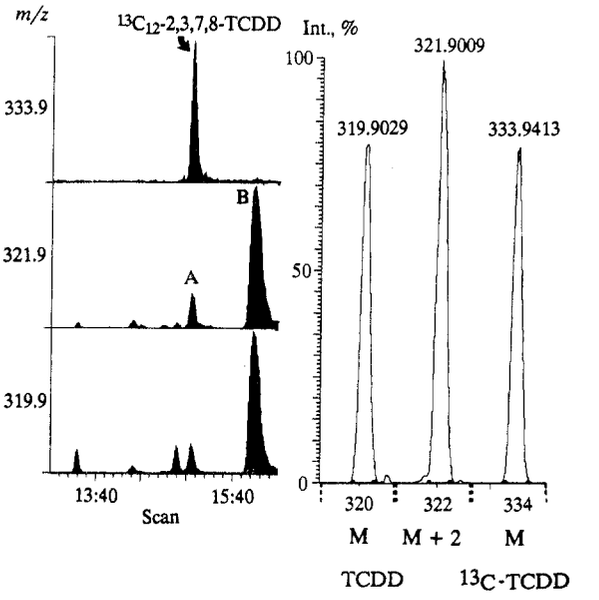

Figure 2. Mass chromatograms (left) and mass profiles (right) for analysis of TCDD in a composited crab hepatopancreas sample collected from station 3. Peak A, 2,3,7,8-TCDD; peak B, TCDT isomer (*28*).

Table 1. Errors Found in Mass Measurements of Ion Used for Quantification of PCDD/Fs

analyte	ΔM^a (ppm)	av ΔM^b (ppm)	no.[c]
2,3,7,8-TCDD	−8.0 to 6.2	3.1	15
2,3,7,8-TCDF	−7.6 to 4.9	3.8	14
1,2,3,7,8-P_5CDF	−4.1 to 0	2.6	8
2,3,4,7,8-P_5CDF	−2.9 to 5.9	3.6	8
1,2,3,4,7,8-H_6CDF	−7.0 to 0.2	2.7	5

[a] Ranges of mass errors for the detected compound in crab tissue samples. [b] Average mass error is obtained by considering each mass error as absolute value of mass deviation. [c] Number of the tissue samples containing the analyte.

were acquired for 2,3,7,8-TCDD (A) by a narrow mass scan over the ion of interest followed by a switch to the adjoining mass ion. The isotope abundance ratio obtained from the abundance of the two selected ions (M and M + 2) for TCDD, the accurate masses of the selected ions, and the retention time of the GC peak are used together for the identification of 2,3,7,8-TCDD.

Mass-profile data provide the accurate masses of the selected ions, which can be compared with the theoretical values. Errors or differences between the true and measured mass (ΔM) of the quantification ions for the detected PCDD/Fs are listed in Table 1. The data obtained from analysis of all tissue samples show that mass errors range from −8 to 6 ppm for the targeted compounds. The average mass error obtained for all unknown samples is less than 4 ppm (see Table 1).

For confirmation of 2,3,7,8-TCDD/F in the tissue samples, low-resolution mass spectra were obtained for GC eluants of those samples containing sufficient 2,3,7,8-TCDD/F. The full-scan mass spectra of the analytes in the samples (not shown) match well those spectra obtained from the analysis of authentic standards.

Quantification of PCDD/Fs. The internal standard method was employed to quantify the PCDD/Fs in the samples. The ratios of areas of mass-profile peaks of the selected quantification ions and the internal standard ions were used. The ratios were related to actual concentrations

Table 2. Wet Weight Concentration (ppt) of 2,3,7,8-TCDD/F in Crab Tissue Samples Collected in September 1991

sample ID	station	sex[a]	tissue[b]	2,3,7,8-TCDD	rec[c] (%)	2,3,7,8-TCDF	rec[c] (%)
NJCL9E	1	M	mu	ND (6)[d]	80	ND (8)	85
NJCL9D	1	F	mu	ND (5)	70	ND (7)	70
NJCL7A	2	M	mu	ND (5)	75	ND (14)	85
NJCL7B	2	F	mu	ND (10)	80	ND (15)	80
NJCL8E	3	M	mu	45	60	15	70
NJCL8D	3	F	mu	40	65	ND (8)	70
NJCL8F	4	M	mu	ND (12)	70	ND (10)	70
NJCL9F	4	F	mu	ND (7)	75	ND (5)	75
duplicate 1[e]	*4*	*F*	*mu*	*ND (8)*	*60*	*ND (7)*	*65*
NJCL9A	1	M	hp	50	70	100	80
NJCL8A	1	F	hp	40	45	75	60
duplicate 2	*1*	*F*	*hp*	*35*	*50*	*90*	*55*
NJCL7C	2	M	hp	90	90	150	90
NJCL7D	2	F	hp	90	85	140	90
NJCL8C	3	M	hp	940	60	200	80
NJCL8B	3	F	hp	690	65	160	70
duplicate 3	*3*	*F*	*hp*	*600*	*55*	*185*	*50*
NJCL9B	4	M	hp	210	80	220	85
NJCL9C	4	F	hp	60	80	125	85
duplicate 4	*4*	*F*	*hp*	*65*	*60*	*115*	*65*
method blank 1[e]				*ND (3)*	*65*	*ND (3)*	*75*
method blank 2				*ND (5)*	*75*	*ND (3)*	*70*
method blank 3				*ND (5)*	*60*	*ND (4)*	*80*
fortified matrix blank 1[f]				*475*	*50*	*470*	*50*
fortified matrix blank 2				*495*	*55*	*465*	*65*
fortified matrix blank 3				*490*	*70*	*495*	*60*

[a] M = male crab; F = female crab. Each male or female composite was from 5 to 7 crabs. [b] mu = muscle; hp = hepatopancreas. [c] Rec = recovery of ^{13}C-labeled internal standard added at 500 ppt. [d] ND = not detected (the detection limit is in parentheses). Better detection limits of 1 ppt for 2,3,7,8-TCDD were obtained after the method was modified (see text for details). [e] Results for duplicate and control samples are given in italics. [f] Fortified level of 2,3,7,8-TCDD/F is 500 ppt.

Table 3. Wet Weight Concentration (ppt) of 2,3,7,8-TCDD/F in Crab Tissue Samples Collected in June 1992

sample ID	station	sex[a]	tissue[b]	2,3,7,8-TCDD	rec[c] (%)	2,3,7,8-TCDF	rec[c] (%)
NJCL11A	1	M	mu	ND (5)[d]	80	ND (5)	80
NJCL11B	2	M	mu	ND (10)	70	ND (5)	80
NJCL11C	2	F	mu	ND (10)	70	ND (5)	75
NJCL11D	3	M	mu	30	75	ND (6)	80
duplicate 1[e]	*3*	*M*	*mu*	*40*	*60*	*ND (10)*	*55*
NJCL11F	3	F	mu	20	75	ND (6)	80
NJCL11G	4	M	mu	ND (10)	65	ND (5)	80
NJCL11H	4	F	mu	ND (10)	65	ND (15)	65
NJCL10A	1	M	hp	80	75	100	80
NJCL10B	2	M	hp	70	70	110	75
NJCL10G	2	F	hp	45	70	70	70
duplicate 2	*2*	*F*	*hp*	*65*	*50*	*90*	*60*
NJCL10C	3	M	hp	475	80	130	75
duplicate 3	*3*	*M*	*hp*	*425*	*60*	*140*	*50*
NJCL10E	3	F	hp	580	85	150	60
duplicate 4	*3*	*F*	*hp*	*480*	*70*	*150*	*65*
NJCL10D	4	M	hp	80	55	115	50
NJCL10F	4	F	hp	60	75	110	85
method blank 1[e]				*ND (5)*	*60*	*ND (5)*	*70*
method blank 2				*ND (5)*	*55*	*ND (3)*	*50*
method blank 3				*ND (3)*	*60*	*ND (3)*	*55*
fortified matrix blank 1[f]				*495*	*45*	*465*	*45*
fortified matrix blank 2				*525*	*65*	*550*	*55*

[a] M = male crab; F = female crab. Each male or female composite was from 5 to 7 crabs. [b] mu = muscle; hp = hepatopancreas. [c] Rec = recovery of ^{13}C-labled internal standard added at 500 ppt. [d] ND = not detected (the detection limit is in parentheses). Better detection limits of 1 ppt for 2,3,7,8-TCDD were obtained after the method was modified (see text for details). [e] Results for duplicate and control samples are given in italics. [f] Fortified level of 2,3,7,8-TCDD/F is 500 ppt.

by using the relative response factors obtained from calibration plots.

Tables 2 and 3 give the analytical results including wet weight concentration levels, detection limits, and ^{13}C-labeled internal standard recoveries for 2,3,7,8-TCDD/F in the crab tissue samples collected in September 1991 and June 1992, respectively. The tables also include the results for duplicate samples, method blanks, and fortified matrix blanks.

The recovery of the internal standard that was fortified in the sample calculated by comparing the areas for peaks centered at the exact m/z of the internal standard in a sample run and those in a standard run. The recoveries (40–110%) satisfy the requirement in EPA method 1613 (*17*). The detection limit (DL) is defined as the concentration of a PCDD/F required to produce a signal with an intensity of at least three times that of the baseline noise. Detection limits for 2,3,7,8-TCDD/F in the first round of

Table 4. Concentrations (ppt) of 2,3,7,8-Substituted PCDD/Fs Detected in Crab Pancreas Samples Collected from Newark/Raritan Bays

compound	station 3		station 4	
	composite 1	composite 2	composite 1	composite 2[a]
	Crabs Collected in September 1991			
2,3,7,8-TCDD	940	690	210	60
2,3,7,8-TCDF	200	160	220	125
1,2,3,7,8-P_5CDF	70	25	45	20
2,3,4,7,8-P_5CDF	90	15	50	25
1,2,3,4,7,8-H_6CDF	95	45	20	ND (8)[b]
	Crabs Collected in June 1992			
2,3,7,8-TCDD	450	530	80	60
2,3,7,8-TCDF	135	150	115	110
1,2,3,7,8-P_5CDF	45	40	25	ND (10)
2,3,4,7,8-P_5CDF	70	50	ND (15)	ND (10)
1,2,3,4,7,8-H_6CDF	50	75	ND (15)	ND (15)

[a] Composite 1 is for male crab, and composite 2 is for female crab. Each composite was made from 5 to 7 animals. [b] ND = not detected (the detection limit is in parentheses).

analyses were 3–15 ppt, as shown in the tables. Better detection limits of 0.5–1.0 ppt for 2,3,7,8-TCDD, however, were obtained by decreasing the resolving power of the mass spectrometer to 3000; by selecting only three ions (M and M + 2 of native TCDD and M + 2 of ^{13}C-labeled TCDD) for the SIM program instead of the 30 ions used for five groups in the PCDD/Fs analysis; and by injecting a larger fraction of sample extract (30% instead of 10%).

The hepatopancreas tissues of the crabs taken from all four stations contain measurable levels of 2,3,7,8-TCDD/F (see Tables 2 and 3). The hepatopancreas samples from station 3 have the highest concentration level, followed by those from station 4, station 2, and station 1. The 2,3,7,8-TCDD/Fs, however, were detected in the muscle samples only from station 3, and the levels are less than 50 ppt.

Levels of the 2,3,7,8-substituted PCDD/Fs found in the hepatopancreas tissue samples collected from stations 3 and 4 are listed in Table 4. Other 2,3,7,8-substituted PCDD/Fs were not seen at detection limits of 5–20 ppt for P_5-, H_6-, and H_7CDD/Fs and of 45 ppt for OCDD/F, whereas recoveries of the corresponding ^{13}C-labeled internal standards were >40%.

Because PCDD/Fs are highly lipophilic, the lipid contents of the crab muscle and hepatopancreas tissues were measured by using a method reported in the literature (*18*, *19*). The lipid contents of the hepatopancreas range from 6% to 9%, whereas the muscle tissues have less than 1% lipid.

The content of PCDD/Fs is higher in the hepatopancreas than in the muscle tissues of the crabs collected from all four stations. In those animals (station 3) where 2,3,7,8-TCDD could be detected in both tissues, the levels in the hepatopancreas are 10–20 times higher than those in the muscle (Tables 2 and 3). The muscle tissue of crabs collected at stations 1, 2, and 4, located in Raritan Bay, were found not to be contaminated with the 2,3,7,8-substituted PCDD/Fs. Cristini and Cooper (*20*) examined the uptake into the blue crab of tritium-2,3,7,8-TCDD in food fortified with the radiolabeled standard. The data indicate that the hepatopancreas rapidly accumulate the highest levels of 2,3,7,8-TCDD and that the buildup continues throughout the 96-h study. Although the gills, stomach, and muscle also accumulated 2,3,7,8-TCDD throughout the period, and levels were much lower.

PCDD/F Levels at Different Collection Sites. The data in Tables 2–4 show the distribution of PCDD/Fs levels as a function of distance from the putative source of PCDD/Fs as identified in previous work (*1–3*); that is, a chemical manufacturing plant located along Passaic River. The crabs from station 3, located at a site in Newark Bay that is closest to the plant, have the highest concentration levels of 2,3,7,8-TCDD/F. The animals from station 3 also contain other 2,3,7,8-sustituted PCDD/Fs (see Table 4). The second highest levels are in tissue samples from animals at station 4, located on the west site of Raritan Bay and near the mouth of Arthur Kill. The lowest levels of 2,3,7,8-TCDD/F were found in the crabs taken from station 1, a site that is the most remote from the alleged point source.

The results of this study, therefore, point to the Passaic or Hackensack Rivers as possible sources of the contamination. Because most of the PCDD is 2,3,7,8-TCDD and the former chemical plant is a major 2,3,7,8-TCDD contamination site, this site is implicated as the origin of the 2,3,7,8-TCDD in the crab tissues (*1–3*).

Our results also show that most, but not all, male crabs contain higher levels of PCDD/Fs than female crabs. The data are limited, and thus, the conclusion should be regarded as tentative.

PCDD/F Levels in Crabs Collected in Summer and Winter. Crabs exhibit distinct seasonal cycles that include periods of growth and reproduction accompanied by extensive feeding activity from late spring through mid-fall. The lower water temperature in winter causes a resting stage characterized by reduced feeding. Our results show that the animals collected in September 1991 (i.e., after the extensive summer feeding state) have higher dioxin contamination than those collected in June 1992. To achieve a better understanding of the levels as a function of seasons, we intend to analyze crab samples collected during the winter.

Route of Bioaccumulation in Crab. Food chains and food organisms may be more important than sediments as immediate sources of the PCDD/Fs found in the tissues of crabs from this system. A previous study demonstrated that juvenile *Callinectes sapidus* can accumulate 2,3,7,8-TCDD present in foods (*20*). The crab is known to be a voracious consumer of many benthic invertebrates including the bivalve mollusc *Mya arenaria*. Some clams of this species from Newark Bay and Authur Kill contain an average of 137 ppt of PCDD (*21*). It is hypothesized that in the field, the clams accumulate the PCDD/Fs when

they ingest food particles as they filter feed. This is supported by Brown et al. (22), who showed that *Mya arenaria* concentrate tritium-labeled 2,3,7,8-TCDD in their food.

The uptake of PCDD/Fs in the crabs is unlikely to be via water. Cantelmo (23) showed that crabs reduce the water permeability of their gills and guts as they acclimatize to the low salinities found in estuaries such as those investigated here. Furthermore, PCDD/Fs are not highly soluble in water.

The more likely route of exposure of PCDD/Fs to the crabs is via ingestion of foods. Moreover, results from the study on the relative importance of water, sediment, and food chain on the bioaccumulation of 2,3,7,8-TCDD in lake trout indicate that the food chain is the primary route, followed by contact with contaminated sediment (24).

Comparison of Levels of 2,3,7,8-TCDD and OCDD in Crabs and in Sediments. A poor correlation exists between the levels of 2,3,7,8-TCDD and OCDD in samples of sediment cores and crab tissues. Although levels of OCDD are 10–300 times higher than those of 2,3,7,8-TCDD in the sediment core and suspended matter samples from Newark Bay and Arthur Kill (1, 2), OCDD was not detected in the crab tissue samples analyzed here. Recoveries of [$^{13}C_{12}$]OCDD, however, are acceptable at values ranging from 50 to 85%. A previously published study (4) showed that levels of 2,3,7,8-TCDD in the hepatopancreas tissue of two crabs collected from Newark Bay are between 73 and 88 times higher than those of OCDD. Similar relations apply to the levels of 2,3,7,8-PCDD/Fs in carp and sediment samples collected from Petenwell Reservior on the Wisconsin River (25). The authors of that latter study observed that the level of OCDD in sediment is approximately 800 times higher than that in composited fish tissue, although both sediment and fish tissue contain similar levels of 2,3,7,8-TCDD.

There are several explanations for the preferential accumulation of 2,3,7,8-TCDD. There may be differences in the bioavailability of the compounds for crabs or for organisms that crabs feed upon. Kuehl et al. (26) found that the bioavailability of PCDD/Fs for carp from sediment decreases dramatically with increasing chlorine substitution. de Voogt et al. (25) reported that the bioaccumulation factor (defined as the concentration of a chemical in fish to the concentration in food at equilibrium) of 2,3,7,8-TCDD is more than 10 times that of OCDD for rainbow trout. A study of uptake and depuration of six ^{14}C-labeled PCDDs by rainbow trout fry and fathead minnows shows that OCDD has low rates of uptake but relatively high rates of elimination of radioactivity with a rapid initial depuration followed by a slower clearance phase (27). The biological degradation of 2,3,7,8-TCDD and OCDD may also differ. There are, however, no reported data concerning metabolism and the elimination half-lifes for TCDD and OCDD in crabs.

Preliminary Identification of a TCDT Isomer in Hepatopancreas Tissue. A major component (peak B in Figure 2) that appeared in the GC retention window for TCDD was identified as an isomer of tetrachlorodibenzothiophene (TCDT), the sulfur analogue of TCDF. The levels of the TCDT are more than 5–10 times those of 2,3,7,8-TCDD in hepatopancreas tissue, assuming that th mass spectrometric response for TCDT is equal to tha for TCDD. The details of the identification and the correlation with 2,3,7,8-TCDD levels given in this paper are reported in the companion paper (28).

Conclusion

Analysis of 2,3,7,8-substituted PCDD/Fs in tissues of crabs collected from four stations in the Newark/Raritan Bay system shows that 2,3,7,8-TCDD contamination exists in both bay systems. An accumulation of those compounds may lead to significant human exposure because the blue crab is taken from this bay system by both commerical and recreational fisherman.

The results of this study of the PCDD/Fs levels in crab tissues support the conclusion obtained from the analysis of sediment cores regarding the environmental impact of a specific point source. The levels of PCDD/Fs decrease monotonically in animals taken at increasingly remote sites from a highly contaminated site. Comparison of the PCDD/F patterns of the aquatic species to those found in the sediment cores provides information regarding the sources of PCDD/Fs and the environmental fate of 2,3,7,8-TCDD and OCDD. Because there is a poor correlation between the levels of 2,3,7,8-TCDD and OCDD in crab and sediment samples collected from Newark Bay, there must be differences in environmental and biological degradation of these dioxin congeners.

Our findings suggest that organisms on which crab feed in the area and sediments taken from the Raritan Bay may also be contaminated with PCDD/Fs.

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