# Mass-Profile Monitoring in Trace Analysis: Identification of Polychlorodibenzothiophenes in Crab Tissues Collected from the Newark/ Raritan Bay system

**Zongwei Cai, Daryl E. Giblin, V. M. Sadagopa Ramanujam, and Michael L. Gross***

Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588

**Angela Cristini**

Biology Department, Ramapo College of New Jersey, Mahwah, New Jersey 07430

Tetra- and pentachlorodibenzothiophenes (TCDT and $P_5CDT$) and the sulfur analogues of tetra- and pentachlorodibenzofurans (TCDF and $P_5CDF$) were identified along with polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in tissues of crabs collected from the Newark/ Raritan Bay system. The use of mass-profile monitoring of selected ions in capillary gas chromatography/high-resolution mass spectrometry (GC/HRMS) resulted in a preliminary identification of an isomer of TCDT and of $P_5CDT$. Confirmation for this identification was accomplished by high-resolution peak matching and by acquiring a full spectrum by GC/MS. The accurate masses of three molecular ions, the full electron ionization (EI) mass spectrum, and the chromatographic retention time are all consistent with those of a 2,4,6,8-TCDT standard. Levels of 2,4,6,8-TCDT in the crab tissues are more than 5–10 times those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD); the highest level (12 ppb) occurring in the hepatopancreas tissue of crabs taken from Newark Bay. A good correlation was found between the levels of 2,3,7,8-TCDD and 2,4,6,8-TCDT ($r^2$ = 0.98) in the samples collected from four stations in the area, suggesting common sources of 2,4,6,8-TCDT and 2,3,7,8-TCDD.

## *Introduction*

Polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/ Fs) were found in muscle and hepatopancreas tissues of crabs collected from the Newark/Raritan Bay system, New Jersey, in September 1991 and June 1992 (*1*). More than 150 crabs were collected, composited, and analyzed for PCDD/Fs by using capillary gas chromatography/high-resolution mass spectrometry in the selected ion-monitoring (GC/HRMS-SIM) mode.

During the analysis of the crab hepatopancreas samples for PCDD/Fs, an unknown major component (approximately 10 times the level of 2,3,7,8-TCDD) was detected in a composited hepatopancreas tissue sample. The compound was present in the sample extract for PCDD/ Fs analysis despite using extensive cleanup procedures, indicating that it has similar chemical and physical properties as those of PCDD/Fs. Because it responded at the *m/z* values of ions used to monitor TCDD and, subsequently, appeared on the mass chromatograms for TCDD when the mass spectrometer was operated at a resolving power of 8000–10 000, the molecular ion of the compound has an accurate mass that deviates less than 50–60 ppm from that of TCDD.

It is very difficult to determine whether this major component is an isomer of TCDD or an interference by using the conventional peak-top monitoring even with GC/ HRMS at a mass resolving power of 10 000. In fact, it is not unreasonable that the compound could be assigned as 1,2,8,9-TCDD in peak-top monitoring because the unknown compound and this TCDD isomer have nearly identical retention times under the GC conditions that were used here and reported elsewhere (*2*).

This paper describes the identification of this material unknown to us and found in the crab tissues. The identification was accomplished by using the mass-profile monitoring technique that was applied in a GC/HRMS-SIM analysis (*3*). The mass-profile method provides higher specificity than the conventional peak-top monitoring method that is commonly used for PCDD/Fs determination. The mass-profile technique gives accurate masses for selected ions, in addition to the chromatographic retention times and signal intensities. By analyzing the mass-profile spectra, the unknown compound was not only immediately shown to be an interference but also identified by accurate mass information as an isomer of tetrachlorodibenzothiophene (TCDT) (**1**). A pentachlorodibenzothiophene ($P_5CDT$) isomer (**2**) was similarly identified. We also test for a correlation between the levels of the TCDT and those of 2,3,7,8-TCDD in crab tissues.

After the elemental composition was determined and a structure was proposed, a literature search was conducted. We learned that polychlorodibenzothiophenes (PCDTs) were previously found in environmental samples including soil (*4*), tissue from crabs in the area above the entry to Newark Bay (*2*), incineration samples (*2, 5–7*), and bleached pulp mill effluents (*8*).

## *Experimental Section*

The blue crabs (*Callinectes sapidus*) were collected from station 3 in Newark Bay and station 1 (Sandy Hook), station 2 (East Branch), and station 4 (Wards Point) in Raritan Bay in September 1991 and June 1992 (see Figure 1). The procedures for collection, extraction, and cleanup as well as for the GC/HRMS-SIM analysis are described in the preceding paper (*1*). A 60 m × 0.32 mm DB-5 column with on-column injection and a temperature program from 80 °C (hold 1 min) to 300 °C at a rate of 15 °C/min was used. The same sample extracts that were previously analyzed for PCDD/Fs were also analyzed for PCDTs. The GC/HRMS analysis was performed on a Carlo-Erba gas chromatograph/Kratos MS-50 double-focusing mass

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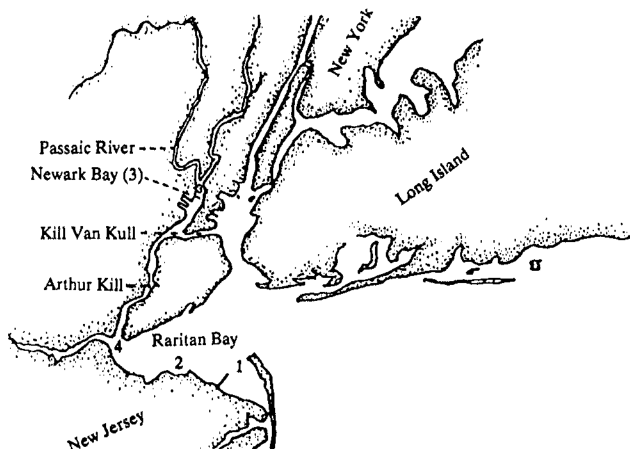


**Figure 1.** Map of the sample collection area. Station 1, Sandy Hook Bay; Station 2, East Beach of Raritan Bay; Station 3, Newark Bay; Station 4, Wards Point. Stations 1, 2, and 4 are located in Raritan Bay.

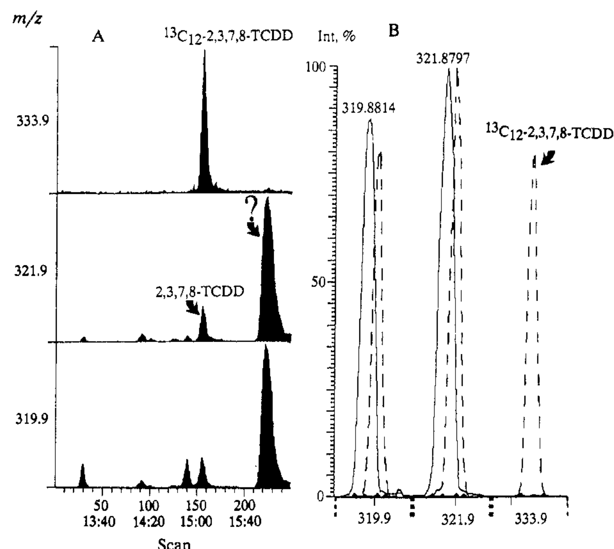


**Figure 2.** Mass chromatograms (A) and mass-profile spectra (B) for the substance ultimately identified as 2,4,6,8-TCDT (solid line) and for 2,3,7,8-TCDD (dashed line) from a hepatopancreas tissue sample that was composited from six male crabs collected from station 3 in September 1991. Three ions were selected in the SIM program: M (*m/z* 319.9) and M + 2 (*m/z* 321.9) of native TCDD and M + 2 (*m/z* 333.9) of [$^{13}C_{12}$]TCDD. The retention times of 2,3,7,8-TCDD and 2,4,6,8-TCDT were 15:06 and 15:58 min, respectively. Coelution of 2,4,6,8-TCDT and 1,2,8,9-TCDD would have been seen as a doublet in the mass profile. The small, unlabeled peaks in the chromatograms were not assigned.

spectrometer/Kratos-MACH3 data system. The mass-profile monitoring technique was applied in the SIM mode (*4*).

The isotopically labeled compounds [$^{13}C_{12}$]-2,3,7,8-TCDD and [$^{13}C_{12}$]-1,2,3,7,8-$P_5$CDD were used as internal standards for the quantification of the PCDTs. The quantification was made by assuming that the mass spectrometric responses for the PCDTs are equal to those for the corresponding PCDDs. This assumption was made because the concentration of a 2,4,6,8-TCDT standard solution that was made available to us was not known.

High-resolution peak matching conducted in real time with the gas chromatography separation was used for more accurate measurement of the masses and isotope distribution patterns of the molecular ion clusters of the PCDTs. The peak matching experiments were performed by switching from two perfluorokerosene (PFK) reference ions to the theoretical masses of three TCDT molecular ions during the elution time window of the unknown compound. The mass peaks of the unknown were within 3 ppm of the theoretical values.

GC/full-scan mass spectral analyses were performed in the EI mode under the following mass spectrometric conditions: resolving power 2000, scanning rate 3 s/decade, and source temperature 250 °C. The GC separation conditions were the same as those used for the preliminary SIM experiment, the peak matching, and the full mass spectrum scan.

### *Results and Discussions*

**Preliminary Detection of PCDTs.** An unknown chlorinated material was detected initially in the mass-profile mode that was set up to quantify various TCDDs. It was immediately realized that the exact masses of the ions of the unknown were not those of a TCDD isomer—the mass peak profiles were shifted by approximately 50 ppm from those of TCDD. Example data for the analysis of a hepatopancreas tissue sample that was composited from six male crabs collected at station 3 in September 1991 is illustrated in Figure 2.

The mass chromatograms (Figure 2A) acquired by integrating all signals in the mass-profile windows centered at *m/z* 319.8965, *m/z* 321.8936, and *m/z* 333.9339, and extending over ±150 ppm in mass, show that the retention time of this major component (15:58 min) is very close to that of 1,2,8,9-TCDD (~10 s difference under the GC conditions used here). The material would be falsely identified in the conventional peak-top monitoring mode even at a mass resolving power of 10 000. The mass-profile data, however, show clearly that the mass peaks obtained from the unknown component are shifted slightly to lower masses than those of 2,3,7,8-TCDD (see Figure 2B). Centroid analysis of the mass-profile spectra indicates that the accurate masses of the two selected ions are approximately 50 ppm less than those expected for TCDD. Thus, the component cannot be a TCDD isomer because the maximum error for the accurate mass measurement in this analysis is less than 5 ppm.

The observed accurate masses (*m/z* 319.8814 and *m/z* 321.8797) obtained by estimating the shift in peak center agree closely with the masses of M and M + 2 ions of a tetrachlorodibenzothiophene (TCDT) isomer (*m/z* 319.8788 and *m/z* 321.8759) rather than those of TCDD (*m/z* 319.8965 and *m/z* 321.8936). The accurate masses in conjunction with the isotope distribution patterns indicate that the two ions have atomic compositions of $C_{12}H_4{}^{35}Cl_4S$ and $C_{12}H_4{}^{35}Cl_3{}^{37}ClS$, which are the compositions of the M and M + 2 ions of TCDT, respectively.

The TCDT isomer was also detected in other crab tissue samples during their analysis for TCDD. The accurate masses of the ions selected for TCDT were measured by locating the peak center in the mass window and interpolating on the basis of the mass width of the window. The mass differences ($\Delta M$) between the detected mass of the M + 2 ion for the TCDT isomer and the theoretical value for TCDD range from −40 to −60 ppm, which agree well with the theoretical mass difference (−55 ppm).

Similarly, an isomer of $P_5$CDT was seen in the mass window used for $P_5$CDD determination during the analysis of the hepatopancreas tissue samples collected from station

3 in September 1991 and June 1992. Its retention time relative to that of 1,2,3,7,8-$P_5CDD$ is 1.073. A average $\Delta M$ of −43 ppm for the M + 2 ion of the putative $P_5CDT$ isomer compared to $P_5CDD$ was obtained for the tissue samples containing the unknown, whereas the theoretical mass difference for $P_5CDD$ and $P_5CDT$ is −50 ppm.

The mass difference between the molecular ions of PCDDs and PCDTs is 0.0177 mass unit (the mass difference between $O_2$ and S). This small difference in mass does not permit complete separation of ion signals with a mass resolving power of 10 000. In fact, a resolving power of greater than 18 000 is required to separate the PCDTs from the corresponding PCDDs when conventional peak-top monitoring is used for the analysis. With mass-profile monitoring, however, one can separate an interference having a mass difference as small as 0.0032–0.0046 unit from that of an analyte because a variation of 10 ppm in mass can be easily established. A mass resolving power of 100 000 is needed to remove completely the interference when using the conventional peak-top mode.

**Confirmation and Identification of 2,4,6,8-TCDT.** *High-Resolution Peak Matching Analysis.* High-resolution peak matching provides more accurate mass measurement for selected ions of an analyte because assumptions of width and linearity of the mass range across a small mass window are not made. In our experiment, accurate masses for the three most abundant ions of the TCDT molecular ion cluster (i.e., M, M + 2, and M + 4) were determined as the compound eluted from the gas chromatograph. The results show that the errors between experimental mass and theoretical mass are less than 3 ppm for the detection of TCDT in the composited hepatopancreas sample taken from station 3. The experimental isotopic patterns of the ions [0.80 for M/(M + 2) and 0.43 for (M + 4)/(M + 2)] are also in close agreement with the theoretical values [0.74 for M/(M + 2) and 0.52 for (M + 4)/(M + 2)].

*GC/Low-Resolution MS (EI) Full-Scan Analysis.* Full low-resolution EI mass spectra were acquired during the elution of those samples containing sufficient TCDT (~1 ng). The EI mass spectrum of the TCDT (not shown) from the composited hepatopancreas sample gives major ion fragments at $m/z$ 285 for [M − Cl], $m/z$ 287 for [M + 2 − Cl], $m/z$ 250 for [M − 2Cl], and $m/z$ 252 for [M + 2 − 2Cl] ions and is consistent with the TCDT assignment.

The positions of chlorines in the detected TCDT, however, cannot be specified on the basis of these measurements. An authentic sample of 2,4,6,8-TCDT was obtained from the Environmental Research Laboratory, U.S. EPA, Narragansett, RI. The GC retention time and the full-scan mass spectrum of the analyte in samples were compared with those obtained from the analysis of the authentic standard sample under the same GC and MS conditions. The data of the GC retention times indicate that the major TCDT isomer detected in the crab tissues is likely 2,4,6,8-TCDT. The EI mass spectrum of 2,4,6,8-TCDT obtained from the tissue samples is nearly identical to that obtained from the standard. The isomer of the $P_5CDT$ detected in the hepatopancreas tissue samples cannot be specified owing to lack of standards for the $P_5CDTs$.

**Levels of PCDTs in Crab Tissues.** Internal standards [$^{13}C_{12}$]-2,3,7,8-TCDD and [$^{13}C_{12}$]-1,2,3,7,8-$P_5CDD$ were used to quantify the PCDTs. The quantification was carried out by assuming that the mass spectrometric responses for TCDT and $P_5CDT$ isomers are equal to those for TCDD and $P_5CDD$, respectively. Therefore, the levels of PCDTs should be viewed as estimates (see Table 1 for results) not only because the assumption regarding relative response has not been tested but also because the recovery for PCDTs may be different than those of corresponding PCDDs.

**Table 1. Levels (ppt) of 2,3,7,8-TCDD[3] and 2,4,6,8-TCDT in the Crab Tissue Samples**

| | | | | Sep 1991[b,e] | | June 1992[b,e] | |
|---|---|---|---|---|---|---|---|
| sample ID | station | sex | tissue[c] | 2,3,7,8-TCDD | 2,4,6,8-TCDT | 2,3,7,8-TCDD | 2,4,6,8-TCDT |
| NJCL8E, 11D | 3 | male | mu | 50 | 610 | 30 | 260 |
| NJCL8D, 11F | 3 | female | mu | 40 | 480 | 20 | 270 |
| NJCL9A, 10A | 1 | male | hp | 50 | 300 | 80 | 510 |
| NJCL8A | 1 | female | hp | 40 | 290 | | |
| NJCL7C, 10B | 2 | male | hp | 90 | 490 | 70 | 510 |
| NJCL7D, 10G | 2 | female | hp | 95 | 1500 | 45 | NA[d] |
| NJCL8C, 10C | 3 | male | hp | 940 | 1200 | 425 | 4600 |
| NJCL8B, 10E | 3 | female | hp | 690 | 10000 | 480 | 4300 |
| NJCL9B, 10D | 4 | male | hp | 210 | 1400 | 80 | 310 |
| NJCL9C, 10F | 4 | female | hp | 60 | 430 | 60 | 440 |

[a] Levels of 2,3,7,8-TCDD have been reported in ref 1. [b] Time of sample collection. [c] mu = muscle; hp = hepatopancrease. [d] NA = not analyzed. [e] Detection limits are 1 and 3 ppt for 2,3,7,8-TCDD and 2,4,6,8-TCDT, respectively.

All hepatopancreas tissues collected from the four stations in the Newark/Raritan Bay contain 2,4,6,8-TCDT. The results reported here are wet weight concentrations. The TCDT isomer was also detected in muscle tissue from crabs taken at station 3, which is located in Newark Bay, and the levels are ~500 ppt. The results in Table 1 show that the levels of 2,4,6,8-TCDT are more than 5–10 times those of 2,3,7,8-TCDD in the crab tissues. The hepatopancreas tissue samples from station 3 contain the highest level of 2,4,6,8-TCDT, followed by those from station 4, station 2, and station 1. The levels of 2,4,6,8-TCDT decrease monotonically in animals taken at increasingly remote locations from the putative source of 2,3,7,8-TCDD contamination (see the next section for details). A $P_5CDT$ isomer is in the hepatopancreas tissue samples collected from station 3; its concentration is approximately 20 times less than that of 2,4,6,8-TCDT. No $P_5CDTs$, however, were detected in all other crab tissue samples at a detection limit of 10 ppt. The detection limit for 2,4,6,8-TCDT is 3 ppt.

**Possible Source of PCDTs Contamination.** The source of the contamination of 2,3,7,8-TCDD in the Newark Bay area was discussed by Bopp et al. (*9*) after analyzing sediment cores that were dated with radionuclide tracers. The authors suggested that the 2,3,7,8-TCDD contamination originates from the production of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in a former chemical manufacturing facility located on the Passaic River (approximately 3 km upstream of Newark Bay). This proposal is also supported by the analytical results of PCDD/Fs in crab tissues collected from the Newark/Raritan Bay system (*1*). The distribution of PCDD/Fs levels was found to decrease with increasing distance from the putative source.

The concentration levels of 2,3,7,8-TCDD, reported in ref 1, and 2,4,6,8-TCDT in the hepatopancreas tissue samples collected in both September 1991 and June 1992 are well-correlated ($r^2$ = 0.98) (see Figure 3), suggesting that the source of 2,4,6,8-TCDT is the same as that of 2,3,7,8-TCDD. The major TCDD and TCDF isomers in

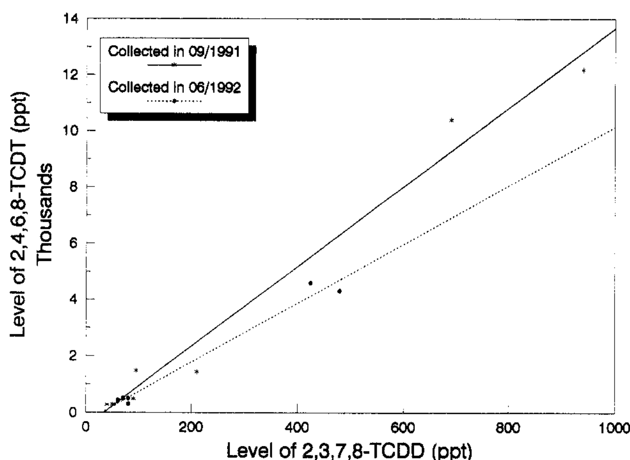


**Figure 3.** Correlation of the levels of 2,3,7,8-TCDD and 2,4,6,8-TCDT in hepatopancreas tissue of crabs collected from four stations in the Newark/Raritan Bay. The coefficient of variation ($r^2$) for both lines is better than 0.98.

the crab tissues are 2,3,7,8-TCDD/F, whereas the major TCDT isomer is 2,4,6,8-TCDT. The 2,3,7,8-TCDD isomer is clearly a byproduct from the production of 2,4,5-T (*9*). Although it is unlikely that 2,4,6,8-TCDT is formed via an analogous route as that of 2,3,7,8-TCDD, its route of formation remains unknown. Possibilities are a byproduct in chemical synthesis at the chemical plant or a product from environmental processing of materials discharged from the plant.

A wide GC retention time window was established to detect tetrachlorothioanthrene (TCTA), the sulfur analogue of TCDD. No TCTA isomers were detected in the crab tissue samples. The 2,3,7,8-TCTA isomer, however, has been detected in the environment and is also of concern (*10*).

## Conclusion

The application of mass-profile monitoring for the trace analysis of PCDD/Fs in tissues of crabs collected from the Newark/Raritan Bay system not only prevented false identification of major trace constituents as PCDD isomers but also permitted their identification as TCDT and $P_5$CDT. GC/low-resolution full-scan mass spectra, GC/high-resolution peak matching, and comparisons with authentic compounds support the identification of 2,4,6,8-TCDT in crab hepatopancreas tissue samples. The levels of 2,4,6,8-TCDT correlate well with those of 2,3,7,8-TCDD in samples collected from four stations in the area, indicating that the source of 2,4,6,8-TCDT may be the same as that of the 2,3,7,8-TCDD. Because 2,4,6,8-TCDT may be toxic (*4, 11*) and because its levels in tissue of crabs, some of which are consumed by humans, are more than 5–10 times that of 2,3,7,8-TCDD, its environmental and biological origins and fate require investigation.

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