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Environmental Contamination and Toxicology

Investigation of American Lobster, *Homarus americanus*, for the Presence of Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans

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The polychlorinated dibenzo-p-dioxins (PCDD) are a group of 75 compounds of current environmental concern. Most attention has been focussed on the 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8,-TCDD) because of high toxicity exhibited in laboratory animal studies (Schwetz et al. 1973). Polychlorinated dibenzofurans (PCDF) comprise 135 compounds similar to the PCDD in structure and toxicity. The 2,3,7,8- tetrachlorodibenzofuran (2,3,7,8-TCDF) is the most toxic of this group (McKinney and McConnell 1983). Two principal sources of PCDD and PCDF in the environment are incineration (Brocco et al. 1984) and as by-products in the manufacture and use of pentachlorophenols (Ahling et al. 1977).

American lobsters (Homarus americanus) have not been previously investigated for the presence of PCDD and PCDF. Many other animals have been analyzed, including herring gulls (Gilbertson, 1983), cattle (Firestone et al. 1979), poultry (Firestone et al. 1971), and freshwater fish (Tosine et al. 1983). Lobsters were examined in this study because of their proximity to possible sources of PCDD and PCDF and an ability to concentrate hydrophobic organics such as polycyclic aromatic hydrocarbons (PAH) in the digestive gland (Uthe and Musial 1986).

MATERIALS AND METHODS

Sample site 1 (Fig. 1, Table 1) is within the Miramichi River estuary which receives input from a wood-preservation plant located a few km up the Miramichi river (Kieley et al. 1986). Site 2 is within Chaleur Bay approximately 13 km from a lead smelter. Sites 3 and 4 are in the South Arm and mouth of Sydney Harbour. The South Arm of Sydney Harbour receives the effluent from a coal-coking plant and steel mill and has resulted in substantial contamination of lobsters by polycyclic aromatic hydrocarbons (Uthe and Musial 1986). Site 5 is distant from known sources of PCDD and PCDF and is considered as the control sample site.

Market-sized lobsters (about 1 pound) were collected by trapping in wooden lobster pots, sites 1 and 2 on May 8 and May 15, 1983,

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Table 1. Lobster sample sites and parameters.

Sample Site	Date of Collection	Location	Replicate	Number of Lobster in Sample	Wet weight of tissue extracted	Description of area sampled
1	May 8, 1983	Miramichi Bay, New Brunswick	A B	8 8	40 40	Area suspected of pent-chlorophenol contamination.
2	May 15, 1983	Limestone Point, New Brunswick	A B	4 4	41 42	Area approximately 13 km from lead smelter and 20 km from Bathurst, New Brunswick
3	May 21-22, 1984	South Arm, Sydney Harbour, Nova Scotia	A B	5 5	60 59	Area known to be receiving effluent from coal-coking and steel plants.
4	May 21-22, 1984	Mouth, Sydney Harbour	A B	4 4	41 44	Approximately 7 km fom South South Arm, Sydney Harbour, site 3.
5	May 21-22, 1984	Port Morien Nova Scotia	A	5	75	In 'clean' area approximately 25 Km from Sydney Harbour.

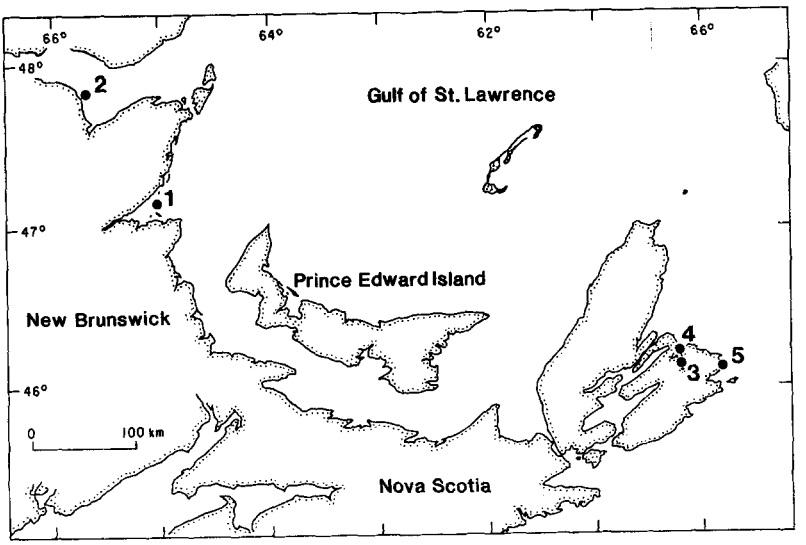

Figure 1. Location of lobster sampling sites in the Atlantic region of Canada

respectively. They were taken immediately by car to Halifax and held in aquaria at the Halifax Fisheries Research Laboratory until June 8, 1983, when they were killed. The site 1 replicates were composed of the digestive glands of two lobsters from each of four locations (total of 8 per replicate) within the Miramichi River estuary. The site 2 replicates were composed of whole digestive glands from four lobsters from Limestone Point, New Brunswick in Chaleur Bay. Samples from sites 3, 4, and 5 were collected on May 21-22, 1984, taken to Halifax and held in the same manner as those from sites 1 and 2 until May 29, 1984, when they were killed. The sites 3 and 4 replicates were composed of the left lobes of digestive glands from five lobsters while the site 5 replicates were composed of the left lobes of the digestive glands of four lobsters. In all cases the digestive glands were dissected immediately after death and frozen immediately after dissection. They were then shipped frozen, by air, from Halifax to Toronto for analysis. Each whole individual sample as received in Toronto was processed. Analysis was limited to the digestive gland because it contains the largest amount of lipid in the animal (Stewart et al. 1967) and is eaten by lobster consumers. Tissues high in fat are well known to accumulate higher levels of organochlorines compared to low-fat tissues (e.g. Ernst et al. 1976). A blank container was left open during the autopsy procedure and processed as a "blank" sample.

Table 2. Chlorinated dibenzo-p-dioxins and dibenzofurans in lobster digestive glands (duplicate determinations picograms/gram wet weight, corrected for recovery.[a])

Site	1		2		3		4		5	
dioxins										
tetra	ND(2)[b]	ND(2)	ND(2)	ND(2)	ND(2)	ND(2)	ND(5)	ND(4)	ND(3)	ND(3)
penta	ND(3)	ND(3)	ND(3)	ND(3)	ND(3)	ND(3)	ND(5)	ND(5)	ND(4)	ND(4)
hexa	7^{3}[c]	4^{3}	24^{3}	19^{3}	ND(4)	ND(4)	ND(10)	ND(8)	ND(6)	ND(6)
hepta	26^{2}	1^{2}	3^{2}	4^{2}	4^{1}	13^{2}	ND(25)	ND(10)	ND(10)	ND(10)
octa	11	1	8	2	4	ND(5)	ND(25)	ND(10)	ND(10)	ND(10)
TOTAL	44	6	35	25	8	13	ND	ND	ND	ND
furans										
tetra	190^{9}	270^{8}	160^{10}	170^{10}	300^{5}	590^{10}	30^{5}	66^{5}	37^{5}	85^{7}
penta	81^{5}	140^{8}	95^{9}	100^{8}	35^{3}	53^{3}	ND(5)	ND(5)	3^{3}	5^{3}
hexa	21^{7}	40^{5}	120^{5}	160^{5}	ND(4)	ND(4)	ND(10)	ND(8)	ND(6)	ND(6)
hepta	2^{2}	2^{2}	6^{2}	16^{2}	ND(5)	ND(5)	ND(25)	ND(10)	ND(10)	ND(10)
octa	ND(2)	ND(2)	ND(2)	ND(2)	ND(5)	ND(5)	ND(25)	ND(10)	ND(10)	ND(10)
TOTAL	300	450	380	450	340	640	30	66	40	90

[a] Average % recoveries (± sd) of internal standard: ^{13}C-2378-TCDD: 38±5, ^{13}C-OCDD: 32±12.
[b] ND = not detected, limits of detection in parenthesis.
[c] Superscripts are number of isomers observed.

All samples were spiked with isotopically labelled $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-octachlorodibenzo-p-dioxin (^{13}C-OCDD). After acid digestion overnight, with concentrated hydrochloric acid, samples were extracted with 3x100 mL portions of "distilled-in-glass" grade hexane. Extracts were cleaned-up by chromatography by passing through a dual-column system consisting of acidic silica, silica, basic silica, silver nitrate, and activated alumina (Lamparski et al. 1979).

Additional clean-up was performed with a dual-HPLC column system using reversed-phase C_{18} five micron spherical stationary phase and isocratic methanol mobile phase. (Tosine et al. 1983). The resulting solutions were concentrated to 10µl and analyzed by gas chromatography-mass spectrometry (GC-MS) operated in the selected ion monitoring (SIM) mode. A 30 metre J&W DB-1 fused silica capillary column was employed with the following conditions; injection temperature 250°C; initial temp. 80°C for 2.0 minutes; program rate, 15°C/min. to 250°C, then 5°C/min. to 300°C for 10 minutes. The GC was operated in spitless injection mode. Instrumentation used was a Finnigan 4500 GC-MS with INCOS data system. During the GC run, selected ions characteristic of the PCDD and PCDF congeners (tetra-to octachlorinated) were monitored. Two or three characteristic ions from the molecular ion region of each congener group were employed. Three conditions were required for a peak to be identified as a PCDD or PCDF congener: 1 coincident response at three characteristic ions; 2 isotope ratios were within ± 15% of theoretical values and 3 peaks eluted within pre-determined retention time windows for each specific congener group.

Quantification was by comparison of area responses of sample peaks with external standards representative of each congener group and concentrations are expressed in picograms per gram (ppt) on a wet weight basis. Data were corrected for recovery of ^{13}C-2,3,7,8-TCDD and ^{13}C-OCDD internal standards. For additional confirmation two samples were analyzed by a VG-ZAB double-focussing mass spectrometer at mass resolution 12,000.

RESULTS AND DISCUSSION

Data from the GC-MS analysis of 10 extracts of lobster digestive glands are shown in Table 2. No TCDD congeners were detected in any of the samples, while TCDF was the only congener found in all samples. Generally, the higher chlorinated congeners (hepta, octa) predominated for PCDD, while the lower chlorinated congeners (tetra, penta) were the most abundant PCDF. Composite samples taken near sources of known pollution (sites 1,2,3) exhibited greater PCDD and PCDF concentrations than samples from remote locations (site 5). Average digestive gland PCDD concentrations were 22 ppt in lobsters captured near contamination sources. No PCDDs were detected in the "clean" area sample. Average PCDF concentrations were 400 ppt in contaminated samples and 60 ppt in clean area samples. While known sources of contamination existed

near locations 1,2, and 3, the limited data in Table 2 do not necessarily show a direct relationship between PCDD and PCDF in lobsters and any specific source. The low levels observed on the sample from site 4 suggest that movement of TCDF out of the South Arm of Sydney Harbour is limited. However, the relatively high TCDF levels in lobsters from site 2 are contradictory but may result from an unidentified source in the immediate area.

Because of the natural variation in biological systems and the trace levels detected, it was not expected that replicate samples would produce identical results, however, the replicate data shown in Table 2 are generally close and seem to reflect a real difference between "clean" and "contaminated" locations. Almost all replicate data agree within a factor of two.

Although extensive sample clean-up and selective GC-MS detection were employed, some interfering peaks were observed in the low resolution GC-MS (LRMS) determination. These interferences were eliminated by analysis of the samples by high resolution GC-MS (HRMS; MS resolution 12,000). A Varian 6000 gas chromatograph, equipped with a J&W on-column injector and a 30 metre J&W DB-1 fused silica column was used to introudce the samples into a VG ZAB MS2F mass spectometer operated in the electron impact mode. Gas chromatographic conditions were the same as those used for LRMS. Two representative samples were chosen for HRMS analysis, sample 1-B and sample 3-B. Peaks detected in the LRMS analysis for hepta- and octachlorinated dioxin, and for the tetra- and pentachlorinated furans were also detected by HRMS, and had the same isomer patterns and relative retention times.

Some congeners were initially not detected in the LRMS determination, because interferences obscured one of the confirmation ions. The HRMS data were used to identify isomer patterns for the hexachlorinated dioxins and hexa- and heptachlorinated furans, which were then quantified using the LRMS data. For these congeners, identification was by correspondence of LRMS and HRMS isomer patterns and relative retention times, in addition to one or two characteristic ions in the LRMS data which were free of interferences.

Isomer patterns are important because they may give clues to the specific sources of pollutants. For hexachlorinated and heptachlorinated PCDD and PCDF, within-congener isomer patterns were the same in all samples where these compounds were detected. Patterns for TCDF were also similar in all samples, except for the relative abundance of one peak which eluted at the correct rentention time for 2,3,7,8-TCDF. This peak varied from 5% to 47% of total TCDF. The percent of total TCDF for this peak from each site is 28% (1), 7% (2,3), 13% (4) and 35% (5). Average variations between replicates are much smaller than variations between locations. Some differences were also observed in patterns of pentachlorinated furans. Samples 4-A, 4-B, 3-A, 3-B, and 2-A all showed identical patterns, while 2-B, 1-A, and 1-B had different isomer patterns.

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