Rapid HPLC/PDA analysis of marine fish and invertebrates for dioxin-like and other chlorobiphenyl congeners

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1. Introduction

Analytical costs can be substantially decreased by first using rapid techniques to estimate concentrations of contaminants [1)] and then confirming these concentrations in priority samples by detailed methods, e.g., gas chromatography/mass spectrometry (GC/MS). We have recently developed a rapid method, using high-performance liquid chromatography with photodiode array detection (HPLC/PDA), for determining selected chlorinated hydrocarbons (CHs) in tissues of marine biota. [2)] Among the analytes that can be measured are certain chlorobiphenyl congeners (CBs), including the "dioxin-like" CBs, the DDTs and hexachlorobenzene (HCB). The quantitative accuracy of HPLC/PDA was established through analysis of standard reference materials (SRMs) and by confirming analytical results using GC/MS. In this paper, we demonstrate the utility of this rapid method in determining concentrations of dioxin-like and other CB congeners in 222 tissue samples of three marine species from seven sites in the Northeastern (U.S.A.) coastal area.

2. Experimental[1]

During 1993-1994, three commercially and recreationally important marine species were collected from recreationally popular waters of the coastal region of the Northeastern United States (Figure 1). Winter flounder (*Pleuronectes americanus*) were captured by otter trawl at several sites. When possible, Northern lobster (*Homarus americanus*) and blue mussel (*Mytilus edulis*) were collected as bycatch during the trawls. The edible

[1]Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

tissues (lobster body muscle and hepatopancreas, flounder muscle and whole body of blue mussel) were excised (10 samples per site, when possible) and stored at -20°C until analysis.

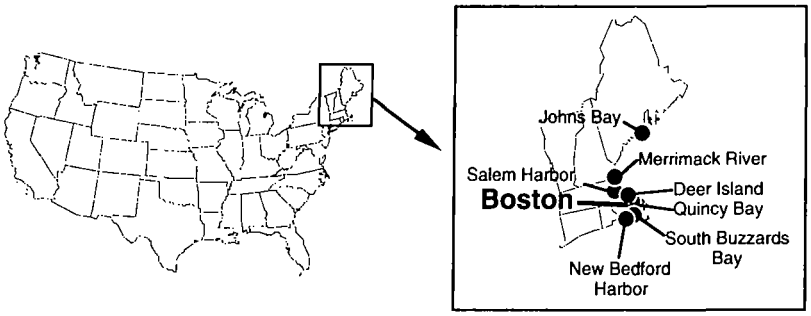

Figure 1. Area of the Northeastern U.S. coast from which samples were collected.

The method of Krahn *et al.* [2] was used to analyze for CHs in the tissues. Briefly, analytes were extracted from tissue matrices with 1:1 hexane/pentane (v/v) and interfering compounds were separated from the CBs on a gravity-flow column packed with acidic, basic and neutral silica gel eluted with 1:1 hexane/methylene chloride (v/v). Subsequently, the planar CB congeners were resolved from the DDTs and other CBs by HPLC on Cosmosil PYE analytical columns cooled to 9°C and were measured by an ultraviolet (UV) photodiode array (PDA) detector. Each set of analyses included the following quality assurance samples: a method blank, a sample duplicate and a control material or SRM. In this paper, individual CBs are identified by their IUPAC numbers. [3] Total CB concentrations were calculated as follows: Total CBs = ∑CB concentrations + ∑ unidentified peak concentrations (based on response for CB 101). In addition, the dioxin-like toxicity of these samples was calculated by the method of Ahlborg *et al.* [4] in terms of toxic equivalents (TEQs) relative to the 2,3,7,8-tetrachlorodibenzo[*p*]dioxin (dioxin) standard.

3. **Results and Discussion**

The preliminary results we report in this paper are a portion (222 samples) of a larger effort to document concentrations of selected CHs in fish and invertebrate species from U.S. coastal waters [5] and to provide these data for use in risk evaluation and risk management. Rigorous validation and quality assurance measures were used to assure that these data are comparable to those measured using other analytical methods. For

example, analyses of SRMs (e.g., NIST cod liver oil SRM 1588 and NIST whale blubber SRM 1945), as well as control materials (NIST whale blubber control material and a fish tissue control material), have demonstrated excellent agreement with certified, advisory or consensus values. [2,6,7] Moreover, analyses (n = 99) of the NIST whale blubber control material over a two-year period have shown both excellent accuracy and precision. For example, the overall mean of relative standard deviations based on 99 analyses of 15 analytes with measurable concentrations in the control material was 17 ± 5%.

Mean concentrations of individual CB congeners that can be measured by HPLC/PDA are shown for marine species from seven sites (Figure 2). In addition, total CBs and total TEQs were calculated for the samples (Figure 2; see Experimental for calculation methods). Concentrations of the DDTs and the pesticide HCB were also determined using this method [2,5] (data are not shown). The highest concentrations of CBs were found in lobster hepatopancreas samples from New Bedford Harbor and from two Boston Harbor sites (Quincy Bay and Deer Island). The TEQs of dioxin-like CBs were highest in lobster hepatopancreas samples from New Bedford Harbor, Quincy Bay, Deer Island and South Buzzards Bay. From these types of data, statistical comparisons can be made to indicate site, species or tissue differences based on individual or summed contaminant concentrations. For example, statistically significant differences were found in ∑CB congener concentrations among the sites surveyed for each of the tissues analyzed (see Figure 2).

In conclusion, the HPLC/PDA method has allowed us to rapidly and cost-effectively measure several CHs (including the dioxin-like congeners) in a relatively large number of tissue samples from marine species. This large database will provide a better tool for assessing dioxin-like toxicity in various tissues than has been possible in previous studies that have relied on expensive analyses (e.g., GC/high resolution MS) for dioxin-like CB congeners in relatively few samples.

4. Acknowledgements

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5. References

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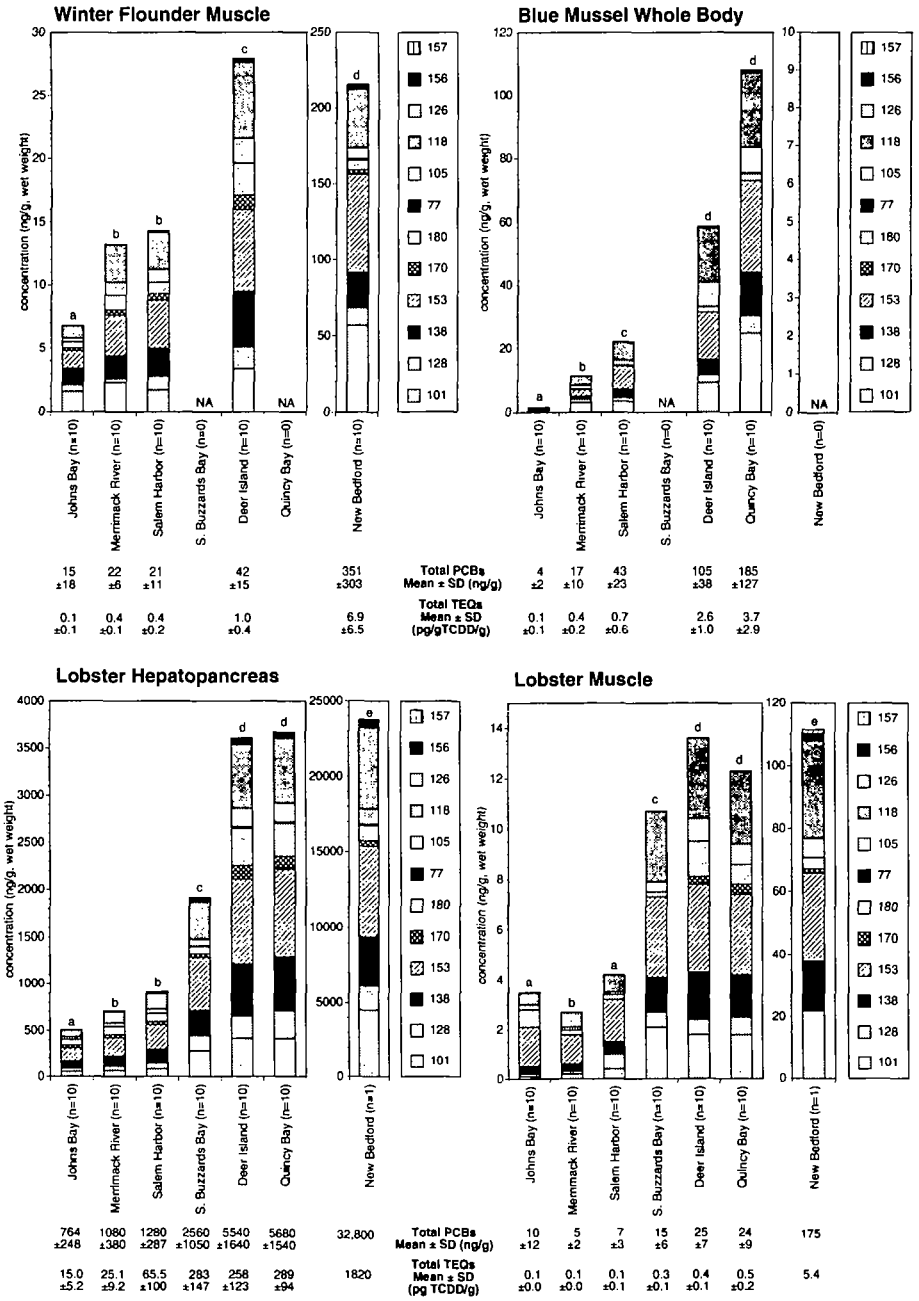

	Johns Bay (n=10)	Merrimack River (n=10)	Salem Harbor (n=10)	S. Buzzards Bay (n=0)	Deer Island (n=10)	Quincy Bay (n=10)	New Bedford (n=10)
Winter Flounder Muscle — Total PCBs Mean ± SD (ng/g)	15 ±18	22 ±6	21 ±11		42 ±15		351 ±303
Winter Flounder Muscle — Total TEQs Mean ± SD (pg/gTCDD/g)	0.1 ±0.1	0.4 ±0.1	0.4 ±0.2		1.0 ±0.4		6.9 ±6.5
Blue Mussel Whole Body — Total PCBs Mean ± SD (ng/g)	4 ±2	17 ±10	43 ±23		105 ±38	185 ±127	
Blue Mussel Whole Body — Total TEQs Mean ± SD (pg/gTCDD/g)	0.1 ±0.1	0.4 ±0.2	0.7 ±0.6		2.6 ±1.0	3.7 ±2.9	
Lobster Hepatopancreas — Total PCBs Mean ± SD (ng/g)	764 ±248	1080 ±380	1280 ±287	2560 ±1050	5540 ±1640	5680 ±1540	32,800
Lobster Hepatopancreas — Total TEQs Mean ± SD (pg TCDD/g)	15.0 ±5.2	25.1 ±9.2	65.5 ±100	283 ±147	258 ±123	289 ±94	1820
Lobster Muscle — Total PCBs Mean ± SD (ng/g)	10 ±12	5 ±2	7 ±3	15 ±6	25 ±7	24 ±9	175
Lobster Muscle — Total TEQs Mean ± SD (pg TCDD/g)	0.1 ±0.0	0.1 ±0.0	0.1 ±0.1	0.3 ±0.1	0.4 ±0.1	0.5 ±0.2	5.4

Figure 2. Concentrations of CB congeners in various tissues of 3 marine species captured in Northeastern U.S. coastal waters. Bars with unlike letters differ significantly ($p < 0.05$) using Analysis of Variance and Fisher's Least Significant Difference test. Also, the site mean ± SD of total PCBs and total TEQs are given (see Experimental).