Screening of Shellfish Samples for Planar Chlorobiphenyls, Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and the planar chlorobiphenyls (non-orthochlorinated ones and their mono- and di-ortho chlorinated analogs) are the topics of intense analytical discussion. These compounds are extremely toxic requiring their measurement at $pg\,g^{-1}$ wet mass concentrations in foodstuffs. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is considered to be the most toxic of these compounds. Planar chlorobiphenyls and PCDFs are similar in structure and toxicity to the PCDDs. The 2,3,7,8-tetrachlorodibenzofuran is the most toxic of the 135 PCDF congeners and IUPAC No. 126 is the most toxic of the 209 chlorinated biphenyls (CBs). These compounds are lipophilic contaminants and are found in the digestive gland of American lobster (*Homarus americanus*).

Present methods for measuring these contaminants in shellfish involve selective, complicated and multiple sample cleanup procedures,[1] generally requiring clean room facilities and expensive instrumentation, namely, capillary gas chromatography combined with high resolution mass spectroscopy. We have developed two separate methods for measuring these compounds in shellfish[2,3]. Combining the two methods, we were able to develop one procedure to determine them all in a single digestive gland extract.

2. Material and Methods

Market-sized lobsters (usually 10) were captured with commercial gear in: Halifax (large industrial Harbour), Sydney (site of a massive coal tar dump), and Petit-de-Grat (site of a large fish plant) Harbours, Nova Scotia; at the dredge spoil dump at Saint John Harbour (New Brunswick); and in St. Margarets Bay, Nova Scotia (Control) (Figure 1). The digestive glands were removed and two pools per site prepared and stored frozen. The method used saponification followed by gel-permeation chromatography, sulphuric acid treatment of the concentrated extracts, and separation on a Florisil:carbon column. The Florisil:carbon procedure was according to King et al.,[3] with the following exceptions: a 60 ml filter funnel (with medium porosity) replaced the 15 ml funnel, the filter paper was cut to fit 60 ml funnel (43 mm), 400 mg of Florisil:carbon (240:1 w/w) topped with 5.0 g of anhydrous sodium sulphate replaced 210 mg of Florisil:carbon (15:1 w/w) topped with 1.5 g sodium sulphate, apply 5.0 ml sample to the new column bed and two 5.0 ml rinsing of sample tube instead of 1.0 ml sample and rinsing. Add 40 ml 1:1 v\v dichloromethane:cyclohexane (omnisolve, BDH Canada) and complete collection as Fraction 1 (CBs). Add 500 ml of toluene (omnisolv, BDH Canada) and elute Fraction 2 (non-orthochlorinated: IUPAC No. 37, 77, 126 and

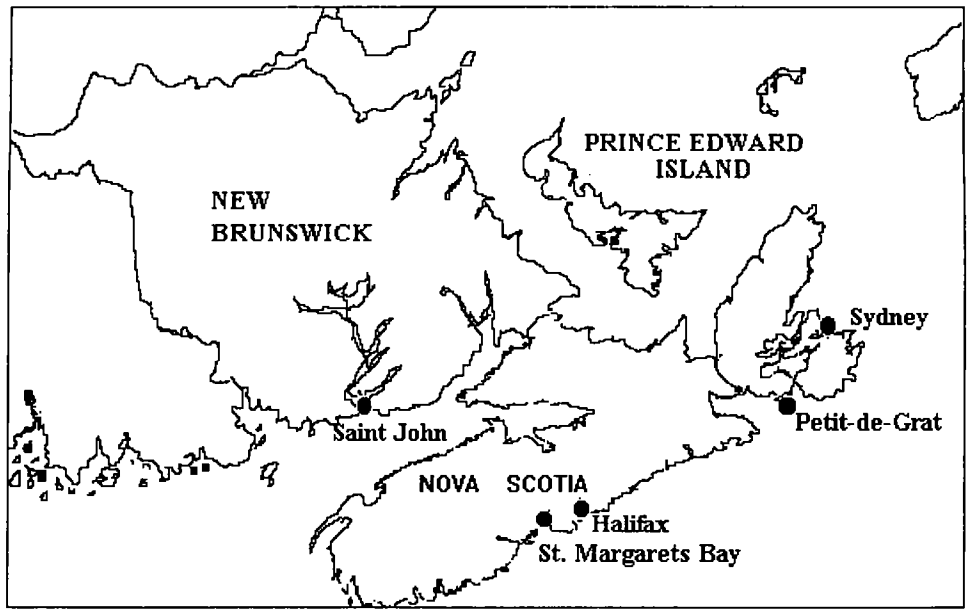

Figure 1. Location Map.

169), PCDDs, and PCDFs) under vacuum . Fractions 1 and 2 are analyzed by gas chromatography-mass spectrometry (GC-MS) in the selected ion mode (SIM) and the SIM methods are according to King et al.[2,3]. Operating the GC-MS in the SIM modes permits the class separation of these compounds. Separate class isomers are summed to yield a class total for PCDDs and PCDFs. The method is very useful for rapidly screening PCDDs, PCDFs and planar CBs in shellfish tissue.

3. Result and Discussion

Recoveries for CBs classes (Fraction 1 of Florisil:carbon column) are found in Table 1. Fraction 1 contains nonplanar CBs and the mono- and di-ortho analogs of the non-ortho chlorinated CBs. Fortification studies yielded recoveries that ranged from 71 to 96% for CBs (Fraction 1 of the Florisil:carbon column) with relative standard deviations (RSDs) (n=3 for each class and concentration) ranging from 1.7 to 11.6 for individual classes (Table 1). As expected, as the amount of spike decreased, recoveries became more erratic. Fortification studies yielded recoveries for the non-ortho chlorinated CBs comparable to literature values[3,4].

Recoveries ranged from 95 to 102 for PCDDs and 91 to 102 for PCDFs (Fraction 2 of Florisil:carbon column) with RSDs (n=3 for each concentration except octachlorodibenzo-*p*-dioxin and dibenzofuran) ranging 1.9 to 8.3 and 7.6 to 9.2 respectfully (Table 2). Figure 2 shows SIM-extracted ion (306) chromatograms: A. Halifax Harbour lobster digestive gland extract (Fraction 2 off Florisil: carbon) containing 2,3,7,8-tetrachlorodibenzofuran and B. 2,3,7,8-tetrachlorodibenzofuran standard. The procedure was used to measure non-ortho (their mono- and diortho analogs) chlorinated CBs, PCDDs, and PCDFs in the digestive gland of lobster from five sites (Tables 3 and 4).

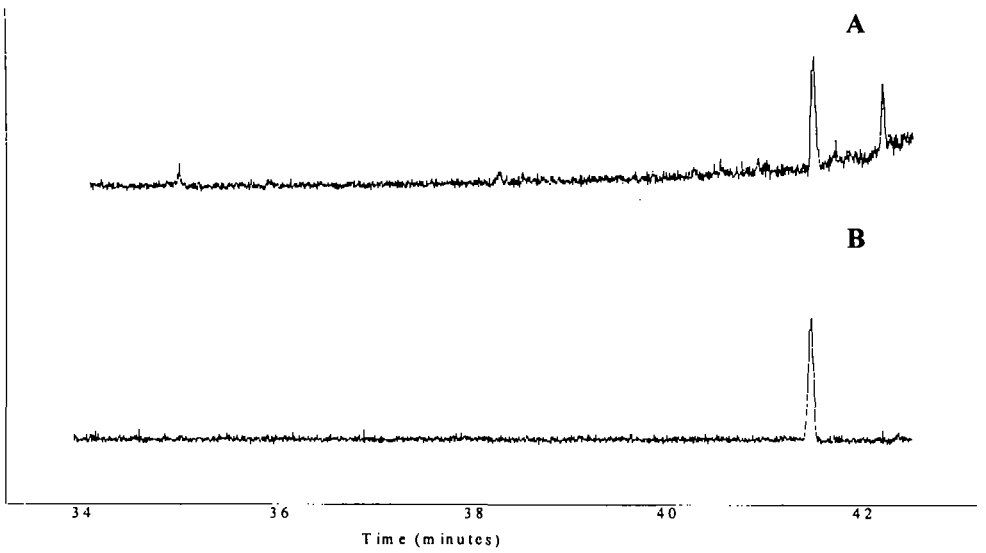

Figure 2. SIM-extracted ion (306) chromatograms: A. Halifax Harbour lobster digestive gland extract (Fraction 2 off Florisil:carbon) containing 2,3,7,8-tetrachlorodibenzofuran; B. 2,3,7,8-tetrachlorodibenzofuran standard.

Table 1. Example table of mean recoveries and RSDs (n=3) of added chlorinated biphenyl to lobster digestive gland tissue.

Class	Fortification/ $pg \cdot g^{-1}$ wet wt.	Mean recovery (%)	RSD (%)
mono	50-2000	71.0	9.9
di	50-2000	82.9	11.6
tri	50-2000	87.0	6.1
tetra	50-2000	90.9	1.7
penta	50-2000	91.1	8.7
hexa	50-5000	94.0	6.7
hepta	50-5000	96.1	9.9
octa	50-5000	93.7	4.6
nano	50-5000	92.4	5.0

Table 2. Example table of mean percentage recoveries and RSDs (n=3) of added polychlorinated dibenzo-*p*-dioxins and dibenzofurans to Florisil:carbon column.

Fortification/ $pg \cdot g^{-1}$ wet wt.	PCDDs Class	Mean Rec (%)	RSD (%)	PCDFs Class	Mean Rec (%)	RSD (%)
20-250	T_4CDD	99.0	8.3	T_4CDF	90.9	8.5
20-250	P_5CDD	94.7	7.5	P_5CDF	96.7	7.6
50-250	H_6CDD	100.5	7.2	H_7CDF	95.6	9.2
50-250	H_7CDD	102.1	1.9	H_7CDF	95.1	8.3
50-250	O_8CDD	100.7	4.1	O_8CDF	101.5	8.6

Table 3. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans concentration ($pg \cdot g^{-1}$ wet wt.) in the digestive glands of lobster (the number in parentheses are the number of peaks observed by GC-LRMS).

	Halifax Harbour	Sydney Harbour	Petit-de-Grat Harbour	Saint John Harbour	Control
PCDDs					
T_3CDD	1100(1)	nd	nd	nd	nd
T_4CDD	70(2)	nd	nd	nd	nd
P_5CDD	nd	nd	nd	nd	nd
H_6CDD	nd	nd	nd	nd	nd
H_7CDD	nd	nd	nd	nd	nd
O_8CDD	nd	nd	nd	nd	nd
PCDFs					
T_4CDF	61(2)	nd	67(2)	nd	nd
P_5CDF	nd	nd	nd	nd	nd
H_6CDF	nd	nd	nd	nd	nd
H_7CDF	nd	nd	nd	nd	nd
O_8CDF	nd	nd	nd	nd	nd
∑PCDD and PCDF	1231		67		

Table 4. Planar Chlorobiphenyl Concentrations in Lobster Digestive Gland ($ng\,g^{-1}$ wet wt.; concentrations $\geq 0.05\ ng\,g^{-1}$ wet wt. are shown).

IUPAC No.	Halifax Harbour	Saint John Dump Site	Petit-de-Grat	Sydney Harbour	Control
Non-ortho					
37	3.4	0.1	1.4	0.4	0.2
77	7.0	0.4	2.7	0.6	0.3
126	1.1	0.2	0.7	0.2	
169			0.1		
Mono-ortho					
105	150	10	80	14	6.5
118	330	25	240	40	20.9
156	40	2.6	19	4.1	2.1
189	1.7	0.30	0.9	2.9	0.2
Di-ortho					
138/158	420	22	320	340	34
170	41	8.7	18	64	3.8
180	160	31	89	320	14
194	14	2.2	7.3	34	2.4

4. Conclusion

The method reduces time and cost of analytical reagents. The introduction of a vacuum in the Florisil:carbon column step reduces separation time from hours to minutes. The minimum amount of carbon required to achieve separation on the larger column (60 ml) was 1.6 mg. Good analytical practices are essential in order to maintain good blank control. An operational blank is performed with each batch of five samples. In the procedure minimum exposure to the laboratory air precluded the need for special filtration of the incoming air. Most operations are performed in enclosed systems, thus reducing background levels considerably.

The method allows estimation of non-ortho chlorinated CBs, PCDDs and PCDFs concentration in shellfish tissue in a conventional laboratory setting without multiple sample purification steps, expensive instrumentation and a clean room facility. Because results can vary 20 to 30 % using external standardization, the present procedure is advocated for screening only. Although the procedure was developed for shellfish, it should be readily applicable to other tissues.

5. References

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