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Application of an automated cleanup system for the determination of polychlorinated dibenzo-p-dioxins, dibenzofurans and coplanar polychlorinated biphenyls in fish from various watersheds in Maine, USA.

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Introduction

Concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) were determined in fish from Maine watersheds applying an automated cleanup apparatus and HRGC/HRMS. Small mouth bass and white suckers were collected at several locations on the Penobscot River. In general, concentrations in small mouth bass were below quantification levels with the exception of 2,3,7,8-Tetra-CDF. In contrast, detectable levels of PCDDs/PCDFs were found in all of the white suckers ranging from 26 - 77 pg/g on a fat basis. The PCDFs were consistently greater than the PCDDs with the Tetra-CDD/CDFs, and the Hexa- and Hepta-CDDs being the major homologues found. This paper describes the use and evaluation of an automated cleanup apparatus, the PowerPrep™, for the analysis of PCDDs/PCDFs in fish tissue. Although over 300 fish samples were cleaned up using this system, here we report the results from a subset of the Pensobscot River samples.

Methods and Materials

Sample Collection: Small mouth bass (*Micropeterus dolomieui)* and white suckers (*Catostomus commersoni*) were collected by either hook and line or gill nets in the Penobscot River, Maine by Maine Department of Environmental Protection (MDEP) and others in 2001-2002. Collected fish were aluminum foil wrapped and shipped on ice for storage at MDEP. Frozen fish were received by chain of custody to the University laboratory for analysis. Thawed frozen fish were ground whole either as composites or individuals (white suckers), or filleted (small mouth bass), and the homogenate was stored in pre-cleaned jars at -20°C until analysis.

Sample Preparation: US EPA Methods 1613[1] and 8290[2] employing HRGC-HRMS and isotope dilution techniques were followed with minor modifications as per Ryel et al.[3] Twenty five grams wet weight tissue was blended with baked anhydrous sodium sulfate and extracted with a mixture of methylene chloride:hexane 50:50 (v/v) by Soxhlet apparatus for 18-24 h. Samples were spiked with $^{13}C_{12}$ PCDD/PCDF labeled surrogates prior to extraction. Extracts were concentrated using a Kuderna-Danish concentrator, and then taken to dryness for the determination of lipid content gravimetrically. Lipid extracts were re-constituted, filtered with a 0.45 μm filter, brought up to 12 mL in hexane, and then spiked with $^{37}Cl_4$ labeled 2,3,7,8-TCDD cleanup standard prior to cleanup.

Automated Cleanup: An automated sample preparation system Power Prep ™ manufactured by Fluid Management Systems (FMS,Waltham, MA) was employed for sample clean up in place of conventional manual column procedures. The computer operated system uses six-port valves that deliver solvents onto three or four different disposable pre-packed Teflon columns. Our procedure was designed to purify and collect PCDDs/PCDFs in one fraction, and

coplanar PCBs in two other separate fractions, a non-ortho fraction and a mono-ortho fraction. Here we report the results of the PCDDs/PCDFs analysis only.

Samples were cleaned up with a series of four columns purchased from FMS: including jumbo multilayer acid/base/neutral (ABN) silica, regular multilayer ABN silica, basic Alumina, and carbon/celite. Cleaned up fractions were transferred to automated concentrator Turbo-Vap II (Zymark) and reduced to approximately 0.4 to 0.5 mL. The sample was then transferred with methylene chloride to a 1.1 mL silanized vial containing the $^{13}C_{12}$ labeled internal standards in tridecane. The sample was concentrated to a final volume of 10 uL by nitrogen evaporation.

Instrumental Analysis and Data Processing: Identification and quantification of PCDDs/PCDFs was performed on a 6890 Agilent gas chromatograph interfaced to VG Autospec Ultima high resolution mass spectrometer operating in electron impact (EI) and selected ion monitoring mode (SIM) with resolution > 10,000 (10% valley). Splitless injections of 2 µL were made on an Agilent 6890 gas chromatograph with separation on 0.25 mm i.d. x 60 m DB-5MS (Agilent) fused silica column. The M/M+2 ions were used in the acquisition for Penta-CDD in place of M+2/M+4 as specified in US EPA Method 1613. This modification was made due to an interfering ion with the same mass as the M+4. Ion ratio criteria were also modified from 1.55 to 0.62. HRMS data was processed using Opus Quan data system and quantification was achieved using a six point calibration curve. Reporting limits were congener dependent (0.1 pg/g for Tetra-CDD/CDF; 0.5 pg/g for Penta-, Hexa- and Hepta-CDDs/CDFs; and 1.0 pg/g for Octa-CDD/CDF).

Results and Discussion

General Problems with Fish Cleanup: If the samples are left to sit for any period of time, a stringy solid may form. This material does not filter but will dissolve with the addition of methylene chloride. Since the sample must be in 100% hexane for cleanup by Power Prep,™ exchanging the solvent from a methylene chloride/hexane mixture back into hexane alone results in the reformation of the precipitate. Sonicating and shaking the sample generally help by either bringing the precipitate back into solution or breaking it up enough so that it is able then pass through the Power Prep.™

The lipid content in the small mouth bass and white sucker samples ranged from 0.1 to 2.0 g per sample. Although the jumbo multilayer ABN silica column should alone remove up to 3 g of lipid, we did not find this to be the case with fish fat. The jumbo acid silica column had better capacity to cleanup fish samples. Therefore, based on our cleanup of over 300 fish samples, we the first column in the column cleanup sequence. Other notable observations were made involving fish lipid and species. We noted that, regardless of species, fish samples that contain the same

Table 1. Percent recoveries of surrogates and cleanup standard for fish analysis.

Surrogate/Cleanup Standard	*Ave %Rec*	*% RSD*	*Surrogate/Cleanup Std.*	*Ave % Rec*	*% RSD*
^{13}C-2,3,7,8-TCDF	98.1	20.4	^{13}C-1,2,3,4,7,8,9-HpCDF	100	16.7
^{13}C-1,2,3,7,8-PeCDF	89.9	17.1	^{13}C-2,3,7,8-TCDD	96.5	19.3
^{13}C-2,3,4,7,8-PeCDF	92.9	16.6	^{13}C-1,2,3,7,8-PeCDD	95.1	16.5
^{13}C-1,2,3,4,7,8-HxCDF	101	14.5	^{13}C-1,2,3,4,7,8-HxCDD	105	17.1
^{13}C-1,2,3,6,7,8-HxCDF	98.6	14.8	^{13}C-1,2,3,6,7,8-HxCDD	90.0	16.0
^{13}C-2,3,4,6,7,8-HxCDF	98.8	14.0	^{13}C-1,2,3,4,6,7,8-HpCDD	92.2	15.8
^{13}C-1,2,3,7,8,9-HxCDF	109	13.9	^{13}C-OCDD	73.1	23.4
^{13}C-1,2,3,4,6,7,8-HpCDF	91.6	14.4	^{37}Cl-2,3,7,8-TCDD	83.4	16.8

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Table 2. Concentration (pg/g Fat Wt) of PCDDs and PCDFs in Penobscot River White Suckers

Fish Id	2874	2875	2876	2877	2878	2879	2880	2881	2882	**Mean**	**Range**
Lipid (%)	13.3	13.0	12.8	13.7	12.0	13.6	11.1	15.0	7.2	12.0	
2,3,7,8-TCDF	34	41	35	66	55	44	50	23	35	43	23 - 66
1,2,3,7,8-PeCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
2,3,4,7,8-PeCDF	bql	4.6	bql	5.2	6.2	4.7	5.1	bql	bql	5.2	bql - 6.2
1,2,3,4,7,8-HxCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
1,2,3,6,7,8-HxCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
2,3,4,6,7,8-HxCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
1,2,3,7,8,9-HxCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
1,2,3,4,6,7,8-HpCDF	bql	bql	bql	bql	bql	bql	bql	3.3	bql		bql – 3.3
1,2,3,4,7,8,9-HpCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		
OCDF	bql	bql	bql	bql	bql	bql	bql	bql	bql		bql - 12
ΣPCDFs	34	46	35	71	61	49	55	26	35	48	26 - 71
2,3,7,8-TCDD	1.8	2.2	1.9	3.4	2.5	2.6	2.2	1.2	1.5	2.2	1.5 - 3.4
1,2,3,7,8-PeCDD	bql	bql	bql	4.1	4.8	bql	bql	bql	bql	4.5	bql - 4.8
1,2,3,4,7,8-HxCDD	bql	bql	bql	bql	bql	bql	bql	bql	bql		
1,2,3,6,7,8-HxCDD	5.2	6.4	4.7	6.1	8.5	7.4	8.8	7.8	9.4	7.1	4.7 – 9.4
1,2,3,7,8,9-HxCDD	bql	bql	bql	bql	bql	bql	bql	bql	bql		
1,2,3,4,6,7,8-HpCDD	7.9	9.7	7.8	9.9	9.9	11	11	11	13	10	7.8 - 13
OCDD*											
Σ PCDDs	15	18	14	23	26	21	22	20	24	20	14 - 26
ΣPCDFs/PCDDS	49	64	49	94	87	70	77	47	59	66	47 - 87
I-TEQ**	1.3	1.7	1.3	2.6	2.2	1.8	1.6	1.2	1.0	1.6	1.0 - 2.6

bql = below quantification limit; OCDD*= found in blanks; I-TEQ** = based on wet wt. with non-detects entered as 1/2 of detection limit.

amount of lipid, can have different effects on column loading. In contrast, we also found that the type of species of fish can affect column performance. For example, small mouth bass contained less fat than the white suckers, but these samples were more likely to overload both the jumbo and regular multilayer ABN columns.

Advantages of the Power Prep™ for fish cleanup: The greatest advantage of using the Power Prep™ is its speed in sample cleanup. Each unit can hold up to 6 modules which may be operated simultaneously. The cleanup from start to finish only takes 3 h per sample, whereas manual cleanup can take days. The automation of the series of cleanup columns allows the operator to multitask and reduces his/her exposure to solvents and acids. Another advantage is the reduction in sample transfers which results in good surrogate and cleanup standard recoveries (Table 1). All recoveries were well within the acceptable range of US EPA Method 1613.

Analysis of the Penobscot fish for PCDDs/PCDFs showed that small mouth bass had little or no reportable levels, while the white suckers had ΣPCDFs ranging from 26 - 71 pg/g fat, and ΣPCDDs ranging from 14 - 26 pg/g, fat (Tabel 2). Average lipid content of the two species varied considerably with the small mouth bass at 0.8% and white suckers at 12%. The fish lipid content may be the primary factor in explaining the differences in the levels of the PCDD/PCDFs observed. Other factors may include habitat as white suckers are bottom dwellers and would be exposed to more contamination from sediment than small mouth bass. I-TEQs were calculated on a wet weight bases using I-TEF[4] values for PCDDs/PCDFs (Table 2). Non-detected analytes were entered as of ½ of the detection limit. I-TEQs were all above 1.0 ppt with a mean of 1.6 ppt (1.0 – 2.6 ppt).

Overall, the automated cleanup apparatus is a convenient time saving technique for sample cleanup over traditional gravity column methods for the determination of PCDDs/PCDFs, and has been successfully applied for the analysis of fish.

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