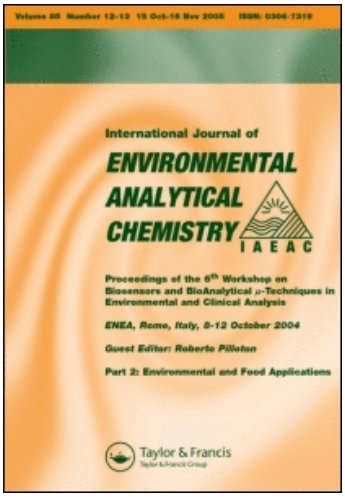

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Anna-Lea Rantalainen[a]; Norman F. Crewe[b]; Michael G. Ikonomou[b]
[a] Department of Chemistry, University of Jyväskylä, Jyväskylä, Finland [b] Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, B.C., Canada

To cite this Article Rantalainen, Anna-Lea , Crewe, Norman F. and Ikonomou, Michael G.(2000) 'Comparison of Three Techniques for Lipid Removal from Seal Blubber: Gel Permeation, Acid Treatment, and Dialysis with Semipermeable Membrane', International Journal of Environmental Analytical Chemistry, 76: 1, 31 — 47
To link to this Article: DOI: 10.1080/03067310008034116
URL: http://dx.doi.org/10.1080/03067310008034116

Intern. J. Environ. Anal. Chem., Vol. 76(1), pp. 31-47
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COMPARISON OF THREE TECHNIQUES FOR LIPID REMOVAL FROM SEAL BLUBBER: GEL PERMEATION, ACID TREATMENT, AND DIALYSIS WITH SEMIPERMEABLE MEMBRANE

ANNA-LEA RANTALAINEN[a*], NORMAN F. CREWE[b] and MICHAEL G. IKONOMOU[b]

[a]*University of Jyväskylä, Department of Chemistry, PO Box 35, 40351 Jyväskylä, Finland and* [b]*Fisheries and Oceans Canada, Institute of Ocean Sciences, 9860 W. Saanich Road, Sidney, B.C., V8L 4B2, Canada*

(Received 23 March 1999; In final form 18 August 1999)

Harbor seal blubber samples were analyzed for PCDD/Fs and PCBs by splitting the initial extract into three aliquots and applying three different techniques for lipid removal (the first step of sample cleanup methodology for GC-HRMS analysis): gel permeation chromatography (GPC), sulfuric acid treatment, and dialysis through semipermeable membrane. Correlation coefficients of analyte concentrations obtained from three sets of replicate samples ranged from 0.965 to 0.994. In addition, a number of seal blubber samples were processed without pre-extraction using only the dialysis technique. The analyte concentrations in these samples correlated well with the analyte concentrations obtained from dialyzed blubber extracts (correlation higher than 0.998). For all analyses (PCDD/Fs, NO- and MO-PCBs) the average surrogate standard recoveries for the GPC and dialysis techniques varied from 68 to 111 %. The recoveries for PCDD/Fs and MO-PCBs standards ranged from 61 to 89 % and 36 to 43% for the NO-PCBs when the acid treatment technique was used. Dialysis was proven to be an efficient technique for lipid removal of biological samples in comparison with the GPC and acid treatment techniques.

Keywords: Seal blubber; lipid removal; dialysis; semipermeable membrane

INTRODUCTION

Lipophilic organic pollutants, such as the polychlorinated-dibenzo-*p*-dioxins (PCDDs), -dibenzofurans (PCDFs), and -biphenyls (PCBs), bioaccumulate in the fatty tissues of fish and other marine organisms and in the blubber of marine mammals. Ultratrace chemical analyses of tissue samples taken from such organ-

* Corresponding author. Fax: +358–14–2602 501. E-mail: alranta@cc.jyu.fi

isms are often laborious and require many cleanup steps after original extraction in order for the final extracts to be compatible with GC-HRMS analysis [1, 2]. The first step in the sample cleanup process is the lipid removal from the extract. The most common techniques used in this process are gel permeation chromatography (GPC) and treatment with concentrated sulfuric acid. The aim of the acid treatment is to break down other co-extracted material since many organic compounds will hydrolyze (decompose) in concentrated acid. It is a more rigorous cleanup technique than size exclusion since it is not restricted by the size of the undesired interfering molecule in the specific matrix. However, depending on the analysis of interest acid treatment could react undiscriminatively with the target analytes and thus compromise the analytical result e.g. chlorinated cymenenes [3], chlorophenols and some of their methyl ethers are not persistent against the acid treatment.

Dialysis through semipermeable low density polyethylene membranes has been used for isolating lipophilic contaminants from large amounts of bulk lipid. Huckins et al. [4] were first to demonstrate polymeric film dialysis for organic contaminants spiked in carp lipid. Their results showed good analyte recoveries (88 and 101 %) for 9 persistent organic contaminants and the lipid carry over into the dialytic solvent was 7 % or less. In another study Meadows et al. [5] examined process variables associated with dialytic media. The effects of dialytic solvent, lipid type, temperature and dialysis time on analyte recovery and amount of co-dialyzed lipid on a per sample basis were studied. Strandberg et al. [6] have used dialysis technique for clean up of sediment, chicken egg and seal tissue extracts. They found that amount of lipid inside the membrane effects on lipid carry over and osmosis volume.

PCDDs, PCDFs, non-*ortho*-chlorinated PCBs (NO-PCBs), and mono-*ortho*-chlorinated PCBs (MO-PCBs) has been determined in blubber of harbor seals *(Phoca vitulina)* from the Strait of Georgia and Quatsino Sound, British Columbia, Canada. That study focused on spatial trends of these particular contaminants in seals from Canada's West Coast. All the samples analyzed were cleaned using GPC. The results have been published earlier by Addison et al.[7]. Sixteen out of those 29 seal blubber samples had been divided in three aliquots after initial extraction and two additional lipid removal techniques were applied to aliquots of the sixteen samples: sulfuric acid treatment and dialysis through semipermeable membranes. The objectives of the present study were: a) to compare the cleanup efficiency of conventional methods such as GPC and acid treatment versus a novel approach, i.e. dialysis through semipermeable membranes; and b) to explore the applicability of the latter to different sample matrices without pre-extraction (i.e. fish tissue and blubber) and its effectiveness in handling high amounts of blubber (2 to 10 g neat blubber).

MATERIALS AND METHODS

Semipermeable membranes used in this study were 3.1 cm wide, low density polyethylene (PE), layflat tubing (Cope Plastics, Inc., Fargo, ND, USA). The wall thickness of the PE tubing was 104.4 μm, and the weight per unit length was 0.037 g/cm. All solvents used in this study were pesticide grade (BDH Ltd., Vancouver, B.C., Canada).

The blubber used was taken from harbor seals *(Phoca vitulina)* from Vancouver Island, B.C., Canada. Three samples of female seals (codes 216F, 217F and 220F) and seven male seals (codes 218F, 221F – 226F) were obtained from Quatsino Sound and four samples of female seals (codes 228F, 230F – 233F) and one male seal (code 229F) from Strait of Georgia. Map of sampling sites and more detailed description of seal habitat, sampling, and other measurements (age, blubber thickness, lipid content of blubber) have been published before [7].

Analysis

A 6 g sub sample of seal blubber was spiked with a suite of ^{13}C-labeled PCDDs, PCDFs and PCBs surrogate standards (Table III). The sample was ground with 200 g of activated sodium sulfate (coarse grain) via mortar / pestle until the mixture was completely homogenized. It was transferred to a freestanding column and 250 mL of DCM was allowed to percolate through at a rate of about 5 mL per minute [8]. The extract was concentrated and transferred to a centrifuge tube. It was then mixed thoroughly and divided quantitatively into three aliquots. Each aliquot was subjected to a different technique for lipid removal during the sample cleanup process.

The first aliquot was processed via an automated GPC system (70 g BioBeads S-X3 [7]) equipped with a single GPC column and a sample concentrator. The second aliquot was dissolved in hexane (1:1, v/v) and the sample was transferred into a 35 cm piece of hexane-rinsed semipermeable membrane, which was then placed in a 600 mL beaker and dialyzed with 400 mL of hexane for 24 hours. The dialysate was removed and replaced with another 400 mL of hexane for an additional 24 hour dialysis. The combined dialysates were concentrated by rotary evaporation (this technique will be referred to as DIAL in subsequent discussions). The third aliquot was dissolved in 4 mL of hexane and the sample was shaken for 2 to 3 minutes with 7 mL of concentrated sulfuric acid in a centrifuge tube (this technique will be referred to as ACID). The acid layer was extracted three times with 3 mL of hexane. The acid treated sample and the three portions

of the hexane extracts were combined in a large centrifuge tube that was rinsed with toluene washed distilled water (15 mL) to remove any traces of acid. The water used for rinsing was also extracted with 5 mL of hexane, which was combined in turn with the other hexane fractions. All extracts were concentrated to 1 mL under nitrogen evaporation and further processed via alumina column cleanup.

In addition to processing the blubber samples through three different lipid removal techniques, some blubber samples were also processed by completely bypassing the sample extraction step (see above). Seal blubber samples (2, 5 or 10 g) were weighed, surrogate standards were added to them and the samples were mixed with small amounts of hexane (1:1, v/v) in a beaker. Most of the blubber dissolved completely in hexane. When solid parts (interconnecting tissue) were left undissolved, they were cut up with scissors and scalpel. The mixture was placed into a piece of PE tubing and dialyzed as described before. Rest of the analytical procedure was the same as it was for other samples.

After the lipid removal the final cleanup and fractionation was performed on neutral alumina and carbon fiber columns [8]. Recovery standards $^{13}C_{12}$-1,2,3,4-tetrachlorodibenzo-*p*-dioxin and $^{13}C_{12}$-1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin were added to the dioxin fraction and $^{13}C_{12}$-2,2′,4,5,5′-pentachlorobiphenyl was added to the PCB-fractions. PCDDs, PCDFs, NO-PCBs, and MO-PCBs were analyzed by HRGC-HRMS.

RESULTS AND DISCUSSION

Comparison of the three lipid removal techniques

Blubber sample aliquots from sixteen different animals were processed in parallel using three different lipid cleanup techniques (GPC, ACID, and DIAL). The results from all these analyses are summarized in Table I. In addition, a number of samples were analyzed using dialysis without pre-extraction technique in replicate. These results are presented in Table II. Although all the 2378-chloro substituted PCDD/Fs were monitored only those listed in the tables were detected with significant concentrations. PCB-78 and PCB-159 were monitored, but their concentrations were below detection limits of 0.1 pg/g for 10 g sample and thus are not included in the tables. Data of sample 222F were excluded in Table I, because acid treated sample could not be analyzed. Instead, results from dialyzed aliquot of sample 222F are reported in Table II.

TABLE I PCDD/Fs, NO-PCBs [pg/g] and MO-PCBs [ng/g] in seal blubber by using GPC, DIAL, and ACID lipid removal techniques. ND = not detected

SAMPLE	*216F*			*217F*			*218F*			*220F*			*221F*		
	GPC	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*
2378-TCDD	1.0	0.9	0.8	1.0	1.2	1.7	1.4	1.3	1.5	0.9	0.8	1.5	0.6	0.5	0.5
12378-PeCDD	1.7	1.9	2.1	1.8	1.5	2.0	1.4	1.8	2.0	1.1	1.2	3.9	0.9	1.1	1.2
123678-HxCDD	7.4	7.3	6.5	4.1	3.8	4.0	2.8	2.9	3.0	4.5	4.1	6.8	5.8	6.1	6.4
123789-HxCDD	0.5	ND	0.3	0.4	ND	ND	0.4	ND	0.4	0.8	0.3	3.3	0.5	0.3	ND
1234678-HpCDD	1.1	1.0	1.0	0.9	0.9	2.8	0.5	ND	0.5	1.0	0.9	6.3	0.9	1.0	1.4
OCDD	1.9	1.3	1.5	1.5	3.0	ND	1.3	1.2	1.7	1.6	1.0	11	1.5	1.1	2.2
2378-TCDF	8.4	9.1	8.3	9.9	9.0	9.9	11	11	11	3.7	4.1	4.0	2.7	2.9	3.2
12378-PeCDF	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND	ND	1.6	ND	ND	0.3
23478-PeCDF	0.5	0.5	0.5	0.5	0.4	ND	0.5	0.8	ND	0.4	0.3	1.6	0.3	ND	0.4
123678-HxCDF	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND	2.7	ND	ND	ND
1234678-HpCDF	ND	ND	0.2	ND	ND	ND	ND	ND	ND	ND	ND	3.8	ND	ND	0.4
OCDF	1.1	0.4	ND	0.7	0.4	ND	0.5	ND	0.6	1.0	0.3	10	0.5	ND	ND
PCB-80	19	16	18	24	30	22	17	35	24	7.9	9.2	10	1 1	1 1	9.8
PCB-79	1.7	2.4	1.7	1.3	1.4	1.6	1.7	2.0	1.9	0.6	0.5	0.7	1.2	1.1	1.3
PCB-81	6.5	6.7	6.6	6.8	7.9	8.8	9.0	11	8.7	1.6	1.6	1.6	3.2	3.6	8.2
PCB-77	37	36	36	38	37	38	61	61	51	11	11	14	13	13	94
PCB-126	81	84	65	79	83	65	80	87	67	23	25	21	47	46	43
PCB-169	5.5	5.9	5.8	5.2	5.1	7.0	5.5	5.7	5.5	2.5	1.7	3.9	4.5	3.2	3.4
PCB-60	1.6	1.9	1.1	1.4	1.2	0.7	1.3	1.3	1.7	0.5	0.5	0.7	0.9	0.7	1.2
PCB-118	49	56	47	39	31	39	25	31	34	13	13	14	31	25	30

SAMPLE	216F			217F			218F			220F			221F		
	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID
PCB-114	2.0	2.2	1.9	1.0	0.9	0.9	0.7	0.9	1.0	0.4	0.4	0.5	1.4	1.3	1.4
PCB-105	20	25	21	15	12	17	10	13	14	5.7	5.8	6.4	14	12	16
PCB-167	1.1	1.3	1.1	1.2	1.0	1.6	0.8	1.0	1.2	0.4	0.4	0.5	0.9	0.7	1.0
PCB-156	14	17	16	5.6	4.6	7.6	3.7	4.8	5.5	3.3	3.3	4.1	14	13	21
PCB-157	4.0	4.4	4.8	1.5	1.3	2.2	1.1	1.3	1.6	0.9	0.9	1.1	4.3	3.7	6.7
PCB-189	0.7	0.9	1.3	0.4	0.4	0.7	0.2	0.3	0.4	0.2	0.3	0.4	0.6	0.8	1.2
SAMPLE	223F			224F			225F			226F			228F		
	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID
2378-TCDD	1.5	1.9	1.7	1.8	2.0	2.1	1.1	ND	0.8	0.9	ND	1.0	5.6	5.5	5.7
12378-PeCDD	4.0	4.2	4.6	3.5	3.5	3.8	1.6	1.2	1.3	1.7	1.2	1.3	11	12	12
123678-HxCDD	23	24	21	20	21	20	5.8	5.3	5.0	8.8	8.9	8.4	176	186	171
123789-HxCDD	1.3	1.1	1.6	0.9	ND	1.5	0.7	ND	0.3	0.5	ND	0.4	4.1	4.6	4.8
1234678-HpCDD	3.1	3.1	3.4	2.9	3.3	3.4	1.1	1.0	0.8	1.3	1.3	1.5	1.8	1.8	1.9
OCDD	2.7	2.0	3.3	2.7	2.0	3.0	2.4	ND	1.3	1.9	2.7	2.3	4.1	3.8	4.6
2378-TCDF	7.4	7.9	7.9	7.2	7.5	7.6	6.5	7.1	6.4	7.1	7.7	7.4	47	51	49
12378-PeCDF	0.5	ND	ND	0.3	ND	0.6	0.5	ND	ND	0.2	ND	ND	0.2	ND	ND
23478-PeCDF	0.9	ND	0.7	0.8	0.8	0.9	0.7	ND	0.4	0.4	ND	ND	0.9	ND	0.9
123678-HxCDF	0.4	ND	ND	0.3	ND	0.5	0.4	ND	ND	0.2	ND	0.2	ND	ND	0.2
1234678-HpCDF	1.2	1.2	1.0	ND	1.2	1.4	ND	ND	0.4	ND	ND	ND	ND	ND	0.3
OCDF	1.5	ND	ND	0.8	ND	1.5	1.3	ND	ND	0.9	ND	ND	1.2	ND	ND
PCB-80	11	12	6	11	13	12	16	21	24	21	21	39	26	26	14

SAMPLE	223F			224F			225F			226F			228F		
	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID
PCB-79	0.9	1.3	1.0	0.7	0.7	0.7	1.6	2.3	1.9	1.7	2.0	1.4	1.3	0.7	0.6
PCB-81	2.6	2.9	2.8	2.3	2.3	2.1	6.3	7.1	6.8	6.1	7.9	6.8	3.3	3.0	2.6
PCB-77	11	10	11	7.0	7.1	16	54	43	39	38	39	33	3.7	3.2	4.5
PCB-126	46	46	39	38	38	39	68	61	56	69	69	52	145	149	140
PCB-169	2.4	2.2	2.1	2.1	2.3	7.4	12	4.7	5.5	5.4	4.9	4.9	5.0	5.1	5.5
PCB-60	1.6	1.3	1.0	1.1	0.8	1.0	1.1	1.0	1.3	1.6	1.3	1.6	3.6	4.3	2.4
PCB-118	51	39	45	36	31	32	30	30	33	37	32	33	150	160	150
PCB-114	2.0	1.4	1.5	1.5	1.3	1.3	1.1	1.2	1.3	1.3	1.2	1.3	5.0	5.4	5.0
PCB-105	25	18	31	15	13	13	13	15	15	16	14	14	66	75	81
PCB-167	1.3	1.0	1.7	0.8	0.8	0.9	0.7	0.9	1.0	0.9	0.9	1.0	3.2	3.9	4.3
PCB-156	11	8.3	17	13	11	13	7.9	11	12	7.8	7.2	7.9	33	46	51
PCB-157	3.4	2.5	5.4	3.9	3.6	4.0	2.0	2.9	3.2	2.3	2.0	2.2	9.2	12	14
PCB-189	0.5	0.4	1.2	0.7	0.6	0.7	0.4	0.7	1.0	0.4	0.4	0.6	1.8	2.4	3.5
SAMPLE	229F			230F			231F			232F			233F		
	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID	GPC	DIAL	ACID
2378-TCDD	7.3	5.3	4.6	2.6	2.8	2.8	3.4	4.2	3.6	3.4	4.0	3.4	3.4	3.9	3.5
12378-PeCDD	10	8.1	7.1	7.0	6.9	7.4	12	11	12	9.1	8.3	9.7	11	8.7	10
123678-HxCDD	451	329	309	116	112	116	198	215	195	79	74	78	77	78	75
123789-HxCDD	9.4	9.6	7.0	4.0	4.3	4.4	2.7	4.4	3.5	1.9	1.9	2.0	1.7	2.0	2.7
1234678-HpCDD	4.8	2.8	2.7	1.7	1.5	1.4	1.6	1.7	1.6	1.6	1.6	1.4	1.4	1.2	1.6
OCDD	3.9	3.0	2.7	2.3	1.8	1.9	1.5	1.8	1.2	2.6	6.7	1.3	1.9	1.6	2.2

SAMPLE	*229F*			*230F*			*231F*			*232F*			*233F*		
	GPC	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*	*GPC*	*DIAL*	*ACID*
2378-TCDF	56	44	40	12	11	13	25	24	27	60	57	64	66	63	71
12378-PeCDF	0.5	ND	0.3	ND	ND	ND	0.4	ND	0.3	0.5	ND	0.4	0.5	0.5	0.6
23478-PeCDF	2.0	ND	1.4	1.8	1.6	ND	1.4	1.3	1.3	2.9	2.4	2.2	3.3	2.8	2.7
123678-HxCDF	0.6	ND	0.2	ND	ND	0.2	0.2	ND	0.1	0.4	ND	0.2	0.3	ND	0.4
1234678-HpCDF	ND	ND	ND	ND	ND	ND	ND	ND	0.3	ND	ND	0.3	ND	ND	ND
OCDF	1.1	ND	ND	1.2	ND	ND	1.7	ND	ND	1.4	ND	0.3	0.8	ND	ND
PCB-80	13	27	21	16	17	7.7	27	28	23	30	43	20	22	41	15
PCB-79	1.8	7.9	2.3	0.3	ND	0.5	ND	2.1	2.0	1.7	2.5	4.1	1.8	3.0	2.1
PCB-81	2.5	3.4	2.4	1.3	1.5	1.7	12	13	12	5.0	5.1	5.4	4.1	4.5	5.2
PCB-77	8.7	9.1	12	4.9	3.9	9.0	28	30	29	17	17	17	16	16	29
PCB-126	169	169	152	60	61	59	150	153	143	157	156	136	146	152	122
PCB-169	15	16	17	3.0	3.4	3.3	6.1	6.4	6.6	7.5	6.8	7.8	8.1	8.0	8.8
PCB-60	3.7	2.7	2.7	1.3	1.2	0.9	3.8	3.6	2.7	3.9	3.7	2.7	3.5	4.1	NA
PCB-118	290	160	160	51	44	47	140	130	120	140	130	120	130	130	NA
PCB-114	13	8.0	7.9	1.5	1.3	1.4	5.9	5.3	5.0	6.7	5.7	5.7	5.8	5.6	NA
PCB-105	140	81	77	23	19	24	69	64	59	73	64	74	67	62	NA
PCB-167	ND	6.8	5.9	1.2	1.0	1.4	3.2	2.4	2.6	ND	3.6	4.0	4.1	4.5	NA
PCB-156	200	120	110	9.2	7.1	11	45	39	42	68	62	85	61	53	NA
PCB-157	50	32	31	2.6	2.0	3.6	13	11	12	19	17	24	18	16	NA
PCB-189	14	6.3	7.6	0.3	0.3	0.6	2.1	1.8	2.4	2.6	2.8	4.8	3.1	2.0	NA

TABLE II PCDD/Fs, NO-PCBs [pg/g] and MO-PCBs [ng/g] in seal blubber dialyzed without pre-extraction and in the representative samples dialyzed after extraction (DIAL)

SAMPLE	*220F*	*220F*	*220F*	*220F*	*222F*	*222F*	*224F*	*224F*	*229F*	*229F I*	*229F II*	*232F*	*232F*	*232F*
	DIAL	*2 g*	*5 g*	*10 g*	*DIAL*	*2 g*	*DIAL*	*2 g*	*DIAL*	*2 g*	*2 g*	*DIAL*	*2 g*	*5 g*
2378-TCDD	0.8	1.0	0.8	0.6	1.4	1.7	2.0	2.0	5.3	5.2	5.0	4.0	3.8	3.6
12378-PeCDD	1.2	1.0	1.1	0.9	6.9	7.2	3.5	3.3	8.1	7.3	6.9	8.3	10	11
123678-HxCDD	4.1	2.4	3.2	2.3	81	70	21	19	329	303	302	74	79	86
123789-HxCDD	0.3	ND	ND	0.2	1.5	2.1	ND	ND	9.6	7.6	8.6	1.9	22	2.3
1234678-HpCDD	0.9	0.8	0.8	0.5	1.7	1.6	3.3	2.9	2.8	3.4	3.0	1.6	1.6	1.5
OCDD	1.0	ND	0.4	0.3	1.1	ND	2.0	2.4	3.0	3.7	2.7	6.7	1.4	0.8
2378-TCDF	4.1	4.6	4.2	4.3	26	22	7.5	7.6	44	46	40	57	68	65
12378-PeCDF	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7	ND	ND	0.4	0.5
23478-PeCDF	0.3	ND	ND	0.2	1.1	1.0	0.8	ND	ND	1.9	1.4	2.4	3.0	2.6
123678-HxCDF	ND	ND	ND	ND	0.2	ND	ND	ND	ND	0.5	ND	ND	ND	ND
1234678-HpCDF	ND	ND	ND	ND	0.4	ND	1.2	1.1	ND	ND	ND	ND	ND	ND
OCDF	0.3	ND	ND	ND	0.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB-80	9.2	10	9.2	4.6	20	49	13	19	27	36	29	43	31	41
PCB-79	0.5	0.3	0.3	0.2	ND	1.1	0.7	1.2	7.9	2.3	2.5	2.5	3.8	2.9
PCB-81	1.6	1.6	1.5	0.4	2.2	7.6	2.3	2.5	3.4	4.4	22	5.1	2.8	5.4
PCB-77	11	10	10	1.7	8.8	50	7.1	7.1	9.1	15	8.7	17	7.8	16
PCB-126	25	21	22	9.1	49	96	38	34	169	143	153	156	156	153
PCB-169	1.7	1.6	1.5	1.3	4.4	4.9	2.3	2.4	16	12	15	6.8	12	7.5

SAMPLE	*220F*	*220F*	*220F*	*220F*	*222F*	*222F*	*224F*	*224F*	*229F*	*229F I*	*229F II*	*232F*	*232F*	*232F*
	DIAL	*2 g*	*5 g*	*10 g*	*DIAL*	*2 g*	*DIAL*	*2 g*	*DIAL*	*2 g*	*2 g*	*DIAL*	*2 g*	*5 g*
PCB-60	0.5	0.5	0.7	0.6	1.2	1.7	0.8	0.9	2.7	3.4	3.2	3.7	2.9	3.3
PCB-118	13	12	13	12	36	40	31	30	160	180	180	130	100	110
PCB-114	0.4	0.4	0.5	0.4	1.5	1.6	1.3	1.4	8.0	8.5	8.6	5.7	5.1	5.6
PCB-105	5.8	5.6	6.0	5.7	18	19	13	13	81	85	89	64	53	56
PCB-167	0.4	0.2	0.3	0.2	1.1	1.1	0.8	1.0	6.8	8.3	7.0	3.6	1.6	3.2
PCB-156	3.3	2.2	3.1	1.9	12	11	11	12	120	99	110	62	45	46
PCB-157	0.9	0.6	0.8	0.5	3.8	3.5	3.6	3.7	32	29	30	17	13	13
PCB-189	0.3	0.1	0.2	0.1	0.6	0.4	0.6	0.8	6.3	5.8	6.2	2.8	2.5	2.1

In most cases all three techniques applied provided good results. The overall inter method-accuracy and precision in determining the analytes with concentrations higher than the detection limits was satisfactory. There were some exceptions however, i.e. the high PCDD/Fs concentrations detected in the acid treated sample of the 220F aliquot. The significantly higher concentrations observed were attributed to laboratory sample cross-contamination especially when the corresponding concentrations detected in the parallel samples treated with GPC or dialysis were below detection limits. Altogether the parallel sample analyses gave similar results, comparable to what is normally obtained from replicate analysis. For example sample 233F is a replicate of 232F and the results obtained show as much variation between replicate samples as they do among the three techniques. Even hepta- and octachlorinated PCDD/F concentrations were similar to each other, which is opposite to Bergqvist et al. [9], observing much higher concentrations of HpCDD/F, OCDF and OCDD in samples, when lipid was removed by semipermeable membrane dialyses than those treated with traditional lipid removal method.

The correlation coefficients between samples treated with the three different techniques were 0.994 ± 0.006 for GPC/DIAL, 0.966 ± 0.045 for GPC/ACID, and 0.965 ± 0.045 for DIAL/ACID. Thus, in comparison the ACID results differed from the GPC and DIAL results more than what was observed between the GPC and DIAL data.

Dialysis of blubber samples without pre-extraction

The extraction-dialysis technique works as a continuous extraction process. The sample (ground if necessary) is mixed with a few mL of organic solvent and transferred into a semipermeable membrane and dialyzed. The analytes are continuously extracted from the sample matrix with the solvent inside the membrane. Dialysis of the compounds through the membrane to the larger solvent volume (125 mL or more) takes place until their concentrations in the dialytic solvent and the solvent inside the membrane reach equilibrium. The extraction (i.e. the equilibrium concentration) is achieved in one or two days and by that time most of the compounds of interest have migrated into the dialysate [6]. The dialysate is further processed and the final extract is analyzed by GC-HRMS.

The seal blubber samples of various sizes (2, 5, and 10 g) were dialyzed without extraction or other processing using semipermeable membranes. With this approach extraction and lipid removal are taking place in one sample workup step. To our knowledge this is the first study where the dialysis technique was applied for the analysis of tissue samples without pre-extraction. The PCDD/F and PCB concentrations detected for all samples processed with this technique

are summarized in Table II. For comparison also parallel samples dialyzed after pre-extraction are presented in the table. Good replication was obtained from the analysis of variable amounts of blubber from the same sample, the correlation coefficients of analyte concentrations in replicate samples were always greater than 0.998. There were some discrepancies with the NO-PCB determination of samples 220F (10 g) and 222F (2 g). Although the PCDD/F and MO-PCB determinations were consistent among the other five 220F replicates analyzed, the concentrations of all NO-PCBs in the 10 g sample were considerably lower than those measured in the other dialyzed 220F samples. Sample 222F had higher PCB-80, PCB-77 and PCB-126 concentrations than the pre-extracted 222F sample aliquots. At this point we do not have explanations for this observation. The present experiment has shown that the direct dialysis technique, without pre-extraction, can be used to extract contaminants from high lipid content tissues very conveniently. It performs well even with high amounts of lipid (tested with 10 g blubber) which is a challenge with conventional techniques such as GPC. This dialysis technique is at its best in processing large series of high lipid samples.

TABLE III Surrogate method recoveries (%) of the internal standard for gel permeation (*GPC*), dialysis (*DIAL*), acid treatment (*ACID*), and dialysis of tissue without pre-extraction (*tissue*)

SAMPLE	*GPC*	*DIAL*	*ACID*	*tissue*	*tissue*	*tissue*
Amount of tissue dialyzed	–	–	–	**2g**	**5g**	**10g**
Number of samples	**16**	**16**	**16**	**6**	**2**	**1**
$^{13}C_{12}$-2378-TCDD	86	91	65	97	89	82
$^{13}C_{12}$-12378-PeCDD	111	108	78	110	96	92
$^{13}C_{12}$-123678-HxCDD	82	81	69	83	75	69
$^{13}C_{12}$-1234678-HpCDD	103	96	81	89	84	80
$^{13}C_{12}$-OCDD	108	96	89	87	80	73
$^{13}C_{12}$-2378-TCDF	84	91	61	97	89	82
$^{13}C_{12}$-12378-PeCDF	94	98	65	94	92	88
$^{13}C_{12}$-123478-HxCDF	86	89	68	91	96	89
$^{13}C_{12}$-1234678-HxCDF	92	92	67	95	92	85
$^{13}C_{12}$-PCB-77	77	87	36	66	76	104
$^{13}C_{12}$-PCB126	68	76	43	81	77	99
$^{13}C_{12}$-PCB169	81	96	39	97	81	74
$^{13}C_{12}$-PCB-118	87	98	71	97	82	66

During lipid dialysis in low density polyethylene membrane a small amount of lipid escapes to the dialytic solvent. Meadows and his co-workers [5] showed that the amount of lipid that migrated into the dialytic solvent depended on temperature and dialysis time. In our experiment the temperature was 20 °C and the dialysis time was 48 hours. Under these conditions we found the lipid crossover into the dialytic solvent to be approximately 3 %. This is consistent with Strandberg et al. observing 2.5 % of seal blubber lipid to migrate into the cyclohexane dialytic solvent [6]. Recovered amount of lipid remained did not interfere since these levels of lipid can be easily removed during sample cleanup (see experimental). In case where the lipid crossover becomes a noticeable interference in the final extracts DCM can be used as dialytic solvent, because minimum lipid crossover is then expected [5].

Spike recoveries of three techniques

In our laboratory GPC has been used to process hundreds of tissue samples and the surrogate internal standard recoveries obtained from the overall analytical method were between 65 and 115 % for PCDD/Fs and in general were better than 60 % for the PCBs [8, 10]. Similarly in this study the surrogate internal standard recoveries for the 16 seal blubber samples processed with the GPC technique ranged between 68 and 111 % (Table III). By comparison the PCDD/F and PCB surrogate recoveries obtained from the two techniques (GPC and DIAL) were very similar and within the acceptable limits which range from 30 % to 120 %.

The samples treated with concentrated sulfuric acid had significantly lower recoveries for all surrogate internal standards in comparison to GPC and DIAL. The NO-PCBs analyses resulted in the lowest recoveries as they ranged between 36 and 43 %. This is borderline performance as most analytical protocols in environmental analyses call for better than 30% surrogate internal standard recoveries. Recoveries of the PCDD/F and MO-PCB standards were above 60 % but consistently lower when compared against the results obtained from the GPC and the DIAL techniques. The low recoveries obtained can only be attributed to either decomposition of the compounds during acid treatment or sample losses during liquid-liquid extraction. However, we can not conclusively differentiate between the two since our experiment was not designed to probe such details.

In Table III are also included the % surrogate internal standard recovery data obtained when different size aliquots of the same blubber sample (2, 5, and 10 g) were processed via the DIAL technique without pre-extraction. Statistically similar results were obtained from all analyses. Surrogate recoveries for samples of all sizes processed via DIAL technique with and without pre-extraction were consistent and well within the acceptable limits.

TABLE IV Expenses per sample and feasibility of the three lipid removal techniques. The time required is estimated for a batch of ten samples and that is divided by ten. Note that GPC is often needed 2–3 times per sample. Estimated time required and costs include lipid removal using the technique and concentration of the sample

Technique	*Solvent required*	*Active/total time required*	*Estimated costs (normalized)*	*Amount of lipid removed*	*Cross contamination*	*Advantages (+) and disadvantages (-)*
GPC Automated	850 mL 1:1 DCM:hex	20 min./ 1.5 h	100 %	1 g	Yes	+ Gentle technique – Expensive – Cross contamination
ACID	20 mL hex	1.5 h/2 h	6 %	5 g	No	+ Effective also as a cleanup technique – Only for persistent molecules
DIAL	270 mL hex	40 min./5 h	37 %	10 g	No	+ Large sample size + Gentle technique – Discriminates large molecules

A comparison of the techniques in terms of overall analytical costs

The GPC apparatus used in this study was an automated system and equipped with a single column and a sample concentrator unit [8]. Up to 23 samples could be loaded and processed automatically. The time that is required to process a 2 g blubber sample was about 2 1/2 hours since a maximum of 1 g of lipid could be processed via the GPC column with each loading (Table IV). The total solvent volume used per 1 g sample was 850 mL dichloromethane: hexane (v/v 1:1); the first 150 mL fraction that eluted after sample loading was not collected, the next 350 mL was collected (it contained the analytes of interest) and subsequently the column was washed with an additional 350 mL of the binary solvent. In a non automated GPC approach where several gravity fed columns are used in parallel to process simultaneously the corresponding number of samples the total amount of solvent used on a per sample basis is less since there is no need for the solvent blanks between samples. There is some time saving with the automated system but higher risk of sample cross contamination exists.

Acid treatment is very effective in destroying the entire lipid and other interfering macromolecules in tissue samples. In addition, the overall analytical procedure for samples treated with acid is shorter than the corresponding GPC and DIAL since some of the sample cleanup steps may become unnecessary. These features make this technique the most economical both in terms of total amount of solvent used and sample processing time. One of the drawbacks of the acid treatment approach, however, is the fact that the acid might undiscriminatively attack target analytes like chlorophenols and their methyl ethers. This is a major limitation and thus the ACID technique can not be applied universally in environmental sample work-up analytical methodologies.

Large volumes of hexane (800 mL) were used for dialysis of the blubber extracts. However, later experiments showed that 250 mL of solvent was sufficient for dialyzing extracts of up to 2 g of blubber sample. Dialysis required 48 hours of passive time, but many samples could be processed simultaneously. The DIAL technique requires significantly less solvent than GPC and can handle high lipid content samples and large sample sizes, i.e. up to 10 g blubber, as the GPC is limited to 1 g per loading. The ACID technique uses less solvent than DIAL but it compromises the recovery of analytes. The DIAL technique may be discriminatory against high molecular weight compounds if the porous size of the membrane used is not of compatible with the size of the target analytes. The membrane to be used should be chosen carefully and the technique tested on spike recovery experiments using model test compounds before used for the processing of real samples. DIAL is not prone to sample cross contamination, as is the case with automated GPC.

Dialysis of seal blubber without pre-extraction provided major savings in solvents and processing time, because there was one step of procedure less than with other techniques. Dialysis is very low cost technique, because it does not require any expensive equipment or glassware. The present experiment showed that DIAL is the method of choice for processing high lipid content samples.

CONCLUSIONS

This study was undertaken to compare three different techniques for removing lipid from environmental tissue samples subjected to ultra trace GC-HRMS analysis for persistent organohalogen contaminants such as PCDD/Fs and PCBs. Gel permeation chromatography, concentrated sulfuric acid treatment, and dialyses through semipermeable membranes were used to isolate or destroy the lipid from seal blubber extracts. The analytical variables examined were the inter method efficiency and the intra method precision for detecting the target analytes. All three techniques produced statistically similar concentrations for PCDD/Fs, NO-PCBs, and MO-PCBs in the samples examined. Therefore any of the techniques is feasible in analyses of persistant organic pollutants in environmental samples. When contaminants other than these analyzed in this study are subject of interest the lipid removal technique to be applied should be carefully selected and tested, because any of these techniques may exclude certain analytes.

Dialysis with semipermeable membrane as a lipid removal technique was shown to be competitive with GPC and acid treatment techniques. For example internal standard recoveries were very good and comparable to those of GPC technique. The dialysis technique without pre-extraction was also successfully applied to large size samples (up to 10 g seal blubber). The dialysis technique, especially without pre-extraction, is a very convenient and economical way of processing high lipid samples. This method (without pre-extraction) should be tested also for different sample matrices to find more applications for this innovative dialysis technique.

Acknowledgements

We thank Cathy Hung (University of British Columbia co-op student) for helping with the sample processing. The Finnish Academy of Sciences is acknowledged by Rantalainen for funding the study, Dr. Hal Rogers for providing the opportunity for this work to be undertaken at the Institute of Ocean Sciences and professor emeritus Jaakko Paasivirta for revising the manuscript.

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