Levels of Polychlorinated Dibenzo-p-dioxins (PCDDs), Polychlorinated Dibenzofurans (PCDFs), and Non-ortho-PCBs (NOPCBs), in Osprey plasma, collected from B.C. in 1992.

Project 92P91C23.

REPORT CRD-94-2.

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TABLE OF CONTENTS

PAGE

1. INTRODUCTION.....2

2. METHOD AND ANALYSIS.....2

a) Principle of method.....2

b) Analysis.....2

c) Quantitation.....3

d) Quality assurance protocol.....4

3. RESULTS.....4

4. REFERENCES.....5

5. APPENDICES: Figures and Tables

FLOW CHART. DIOXIN/FURAN/NOPCB extraction and cleanup.

TABLE 1. PCDDs/PCDFs in Osprey plasma, collected from B.C. in 1992. Project 92P91C23.

TABLE 2. NOPCBs in Osprey plasma, collected from B.C. in 1992. Project 92P91C23.

DATA SHEETS.

INTRODUCTION.

This report contains levels of PCDD and PCDF congeners (T4CDD/T4CDF to OCDD/OCDF), and NOPCB congeners (such as PCB-37, 77, 126, 169, and also PCB-81 and 189), detected in Osprey plasma, collected from B.C. in 1992. This work is identified under Project 92P91C23.

A total of ten Osprey plasma samples were received from the National Specimen Bank, NWRC in January 1994. Samples were collected in May 1991 and submitted for analysis by P.Whitehead (see DATA SHEETS attached).

Samples were analyzed for PCDDs, PCDFs and NOPCBs at NWRC-CRD in February and March 1994.

Table of results are attached.

METHOD AND ANALYSIS.

a) Principle of the "DIOXIN/FURAN/NOPCB" method.

Note that for plasma samples lipid extraction was altered from the neutral extraction used for other tissue types: Isotopically labelled internal standards (13-C-12-PCDDs/PCDFs/NOPCBs) were added to the plasma, and allowed to equilibrate for 30 min. Saturated aqueeous ammonium sulphate and absolute ethanol was added to the spiked plasma, and it was extracted 4 times with hexane. Hexane layers were combined, filtered through anhydrous sodium sulfate and volume was reduced to make it ready for GPC clean-up; removal of lipids and biogenic compounds by GPC **(1)** and alumina column cleanup; separation of PCDDs, PCDFs and NOPCBs from other contaminants by using carbon/fibre column **(2,3)**; further separation of PCDDs/PCDFs from the NOPCBs achieved by Florisil column chromatography; For details of the extraction and cleanup procedure, see Flow Chart attached. This new method was developed at the NWRC/CRD in 1993, by the combination and the modification of the two previously used methods, namely "Dioxins/Furans" **(2,3)** and "Non-ortho-PCBs" **(4)**. By using this new, combined method for sample preparation, and the highly sensitive new high resolution mass spectrometer for analysis, the total sample size required is reduced to 5 g from the 25-30 g (needed in the past) for egg, liver and fat tissues, and 10-15 g plasma. In case of these Osprey plasma samples, sample sizes were limited from 3.17 g to 4.2 g.

b) Analysis.

Quantative analysis for PCDDs/PCDFs and NOPCBs was performed with a VG Autospec double-focussing high resolution mass spectrometer linked to a HP 5890 Series II. high resolution gas chromatograph (equipped with a Carlo Erba CTC-A200S autosampler), computerized data system. The mass spectrometer was operated in the Selected Ion Monitoring (VOLTAGE SIR) mode.

MS conditions: EI; 70 EV; source temp = 280 °C; interface temp. = 280 °C.
PCDDs/PCDFs analysis - resolution used was 10000.
NOPCBs analysis - resolution used was 6000.

GC conditions: Temp 1 = 100 °C; Hold 3 min; Rate = 20 °C/min to 180 °C; Rate =5 °C/min to 325 °C; Injector temperature = 260 °C.
GC column was a 30 m DB-5 (J & W) 0.25 mm capillary column.

c) Quantitation.

Each sample was spiked with (13-C-12)-labelled PCDD and PCDF congeners (T4CDD/T4CDF to H7CDD/H7CDF and OCDD), and NOPCBs (PCB-77, -126 and -169) **internal standards** (Wellington Lab.), prior to lipid extraction, in order to be able to perform internal standard quantitation, and to calculate internal standard recoveries. Two other isotopically-labelled standards (1234-T4CDD and 123789-H6CDD) were added to the cleaned dioxin/furan extracts, and PCB-112 to the NOPCB fraction, just prior to analysis to serve as **recovery standards** (Wellington Lab.) for the quantification of internal standard recoveries.

An **external standard mixture** (containing native **quantitation standards**, **internal standards** and **recovery standards**) was analyzed along with the samples. Relative response factors (RRF) for each pair of native analyte and (13-C-12)-labelled compound were calculated daily. Linearity of the instrument was tested previously by performing a 5 point calibration.

Two characteristic ions (the most abundant ions in the molecular cluster) were monitored for each native and (13-C-12)-labelled compounds, within established retention time windows.

Residue levels for PCDDs/PCDFs and NOPCBs were determined by using **internal standard quantitation** method. Internal standard quantitation was based on the integrated areas (sum of the two ions) measured for native congeners, compared directly to the integrated areas measured for the (13-C-12)-labelled internal standards in the sample, and using the isomer specific RRF values (determined on the same day). It is an assumption that native homologs behave in the same way during the cleanup procedures than (13-C-12)-labelled surrogates.

Quantitation of residues are achieved by using two automated computer programs "TRACES" and "DIOXIN", originally designed for processing PCDDs/PCDFs analyses, (developed by V.G. Analytical), and based on the sophisticated integrated spreadsheet modelling program "20/20" (developed by Access Technology). The first program "TRACES" detects and integrates peaks (for selected ions within selected retention windows); provides hard copy print-outs of the integrated chromatograms; and creates a list of the detected peaks (containing accurate masses, retention times, peak heights, integrated areas). This is a batch program capable of

processing up to 6 samples sequentially, using data collected from a multiple sample autoinjector analysis. The second program "DIOXIN" is used to generate calibration tables (containing isomer specific RRF values for target compounds); calculates % Recoveries for labelled internal standards; calculates residue data and Minimum Detection Limits for target compounds. Hard copies of the integrated chromatograms, residue levels for compounds detected, % recovery data for internal standards, along with the instrument-tuning data are kept in file for every sample.

d) Quality assurance protocol.

Recoveries for (13-C-12)-PCDDs/PCDFs and -NOPCBs were calculated by comparing the integrated areas of the labelled **internal standards** and areas of **the recovery standards** in samples, to the areas of these compounds measured in the **external standard mixture** analyzed along with the samples. Analysis of samples are usually accepted when recoveries for (13-C-12)-PCDDs/PCDFs are between 70% and 120%. In case of these Osprey plasma samples the **internal standard recoveries** for PCDDs/PCDFs were <70%, (see TABLE 1.) due to the small sample sizes, and losses during lipid extraction. Recoveries for NOPCBs were also <70% for the same reason (see TABLE 2.). Based on our previous results, it is an assumption that losses occurring for the labelled PCDD/PCDF/NOPCB internal standards are applicable to native PCDD/PCDF/NOPCB congeners. Considering the fact that calculations were based on internal standard quantitation, all reported levels (in TABLE 1., TABLE 2.) should be in an error of less than 15 %.

Identity of peaks was confirmed by monitoring **accurate masses** (4 decimal places) running the mass spectrometer in high resolution mode (10000 resolution for PCDDs/PCDFs, and 6000 resolution for NOPCBs); the proper **chlorine isotope ratio** (<20 % deviation from the correct ratio) for the two strongest ions in the M+ cluster; and the proper **retention time** (<5 sec deviation from the retention time obtained for authentic standard) on the DB-5 column.

Minimum Detection Limits (MDL, signal/noise = 3) were routinely calculated and reported for every congener detected.

Method blanks (distilled water spiked with 13-C-12-PCDDs/PCDFs/NOPCBs), were cleaned-up and analyzed along with the plasma samples .

RESULTS AND COMMENTS.

Levels for PCDDs and PCDFs, detected in Osprey plasma, collected form Kamloops in 1992, are reported in TABLE 1. The higher chlorinated dioxins, OCDD (from 2.84 ng/kg to 8.21 ng/kg), and 1234678-H7CDD were dominant in these samples. In Osprey eggs collected from the same location in 1992, OCDD was the major PCDD contaminant as well (see REPORT CRD-93-2). No 2378-T4CDD was

detected in these plasma samples, maybe because of the relatively high Minimum Detection Limit (MDL = 0.55 ng/kg - 1.19 ng/kg) caused by the small sample sizes (3.17-4.2 g) available. In the future we would like to receive 10-15 ml plasma, so the MDL would be 3-4 times lower, and it would be possible to detect <0.5 ng/kg 2378-T4CDD.

A few plasma contained traces of PCDFs (see TABLE 1.).

Unlike in the Osprey egg samples (REPORT CRD-93-2.), in which PCB-126 was the major NOPCB, in these plasma samples the major NOPCB was PCB-37 > PCB-77 > PCB-126 (see TABLE 2.).

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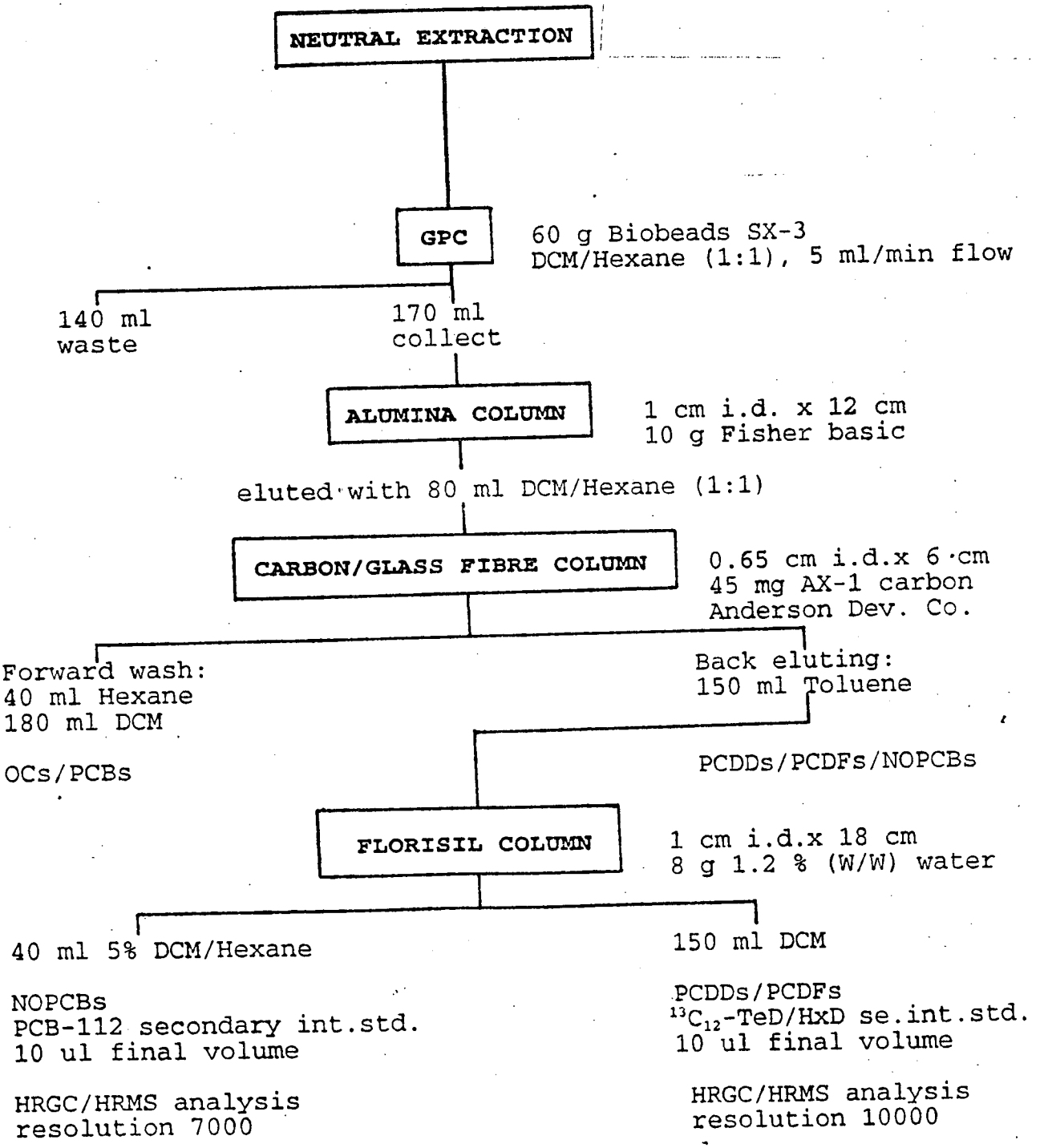
N.B. Sample preparation and technical help was provided by A.M. Idrissi (NWRC).

CWS/1993.
DIOXIN/FURAN/NOPCB
EXTRACTION AND CLEAN-UP



PCDDs/PCDFs in Osprey plasma, collected from B.C. in 1992.
Project no: 92P91C23.

TABLE 1.

4DIFUR2

Levels are in ng/kg (wet wt.)

USOX # L93-	57356		57357		57358		57359		57360		57361		57362		57363		57364		57365		Blank		Blank		Blank	
SPECIMEN #	TT2A		TT4A		TC1A		TC4A		TC5A		TT2B		TT4B		TC1B		TC4B		TC5B							
ANAL. #	B-175		B-176		B-177		B-178		B-179		B-180		B-181		B-182		B-183		B-184		B-174		B-185		B-186	
No. in pool	1		1		1		1		1		1		1		1		1		1							
Location	W. Kamloops		W. Kamloops		E. Kamloops		E. Kamloops		E. Kamloops		W. Kamloops		W. Kamloops		E. Kamloops		E. Kamloops		E. Kamloops							
wt.* g	3.37		4.20		3.25		3.17		3.97		3.75		3.57		3.88		3.52		3.21							
PCDDs	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL
2378-T4D	ND	0.55	ND	0.60	ND	0.91	ND	1.14	ND	0.68	ND	0.75	ND	1.19	ND	0.99	ND	0.94	ND	1.11	ND	0.72	ND	0.89	ND	0.99
TotalT4D	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
12378-P5D	ND	0.52	ND	0.55	ND	0.62	ND	1.23	ND	0.39	ND	0.36	ND	0.76	ND	0.97	ND	0.57	ND	0.61	ND	0.45	ND	0.52	ND	0.42
Total P5D	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
123679-H6D	ND	0.49	ND	0.29	ND	0.43	ND	0.50	ND	0.34	ND	0.29	ND	0.38	ND	0.39	ND	0.45	ND	0.56	ND	0.20	ND	0.37	ND	0.39
123478-H6D	ND	0.49	ND	0.29	ND	0.43	ND	0.50	ND	0.34	ND	0.29	ND	0.38	ND	0.39	ND	0.45	ND	0.56	ND	0.20	ND	0.37	ND	0.39
123678-H6D	ND	0.49	ND	0.29	ND	0.43	ND	0.50	ND	0.34	ND	0.29	ND	0.38	ND	0.39	ND	0.45	ND	0.56	ND	0.20	ND	0.37	ND	0.39
123789-H6D	ND	0.49	ND	0.29	ND	0.43	ND	0.50	ND	0.34	ND	0.29	ND	0.38	ND	0.39	ND	0.45	ND	0.56	ND	0.20	ND	0.37	ND	0.39
Total H6D	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
1234679-H7D	ND	0.21	ND	0.26	ND	0.62	ND	0.32	ND	0.38	ND	0.43	ND	0.52	ND	0.64	ND	1.70	ND	0.83	ND	0.15	ND	0.54	ND	0.25
1234678-H7D	1.25	0.26	1.48	0.26	1.38	0.62	2.35	0.32	ND	0.38	1.33	0.43	1.51	0.52	ND	0.64	1.87	1.70	1.37	0.83	ND	0.15	ND	0.54	ND	0.25
Total H7D	1.25		1.48		1.38		2.35		ND		1.33		1.51		ND		1.87		1.37		ND		ND		ND	
12346789-OD	5.73	0.25	4.44	0.33	4.31	0.23	5.01	0.41	3.31	0.19	4.77	0.32	8.21	0.24	2.84	0.71	4.69	0.51	5.62	0.81	0.27	0.14	ND	0.33	ND	0.10
PCDFs	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL	Levels	MDL
2378-T4F	ND	0.83	ND	0.62	ND	0.77	ND	0.93	0.68	0.69	1.49	0.75	0.91	0.70	ND	0.88	ND	1.07	ND	1.03	ND	0.36	ND	0.75	ND	0.46
Total T4F	ND		ND		ND		ND		0.68		1.49		0.91		ND		ND		ND		ND		ND		ND	
12478-P5F	ND	0.42	ND	0.52	ND	0.62	ND	1.18	ND	0.42	ND	0.63	ND	0.91	ND	0.97	ND	0.74	ND	0.83	ND	0.48	ND	0.24	ND	0.61
12378-P5F	ND	0.42	ND	0.52	ND	0.62	ND	1.18	ND	0.42	ND	0.63	ND	0.91	ND	0.97	ND	0.74	ND	0.83	ND	0.48	ND	0.24	ND	0.61
23478-P5F	ND	0.42	ND	0.52	ND	0.62	ND	1.17	ND	0.42	ND	0.63	ND	0.90	ND	0.99	ND	0.75	ND	0.84	ND	0.48	ND	0.24	ND	0.61
23457-P5F	ND	0.42	ND	0.52	ND	0.62	ND	1.17	ND	0.42	ND	0.63	ND	0.90	ND	0.99	ND	0.75	ND	0.84	ND	0.48	ND	0.24	ND	0.61
Total P5F	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
123468-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
124678-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
124689-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
123478-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
123678-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
123789-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
234678-H6F	ND	0.19	ND	0.36	ND	0.43	ND	0.54	ND	0.28	ND	0.28	ND	0.32	ND	0.43	ND	0.45	ND	0.56	ND	0.17	ND	0.40	ND	0.15
Total H6F	ND		ND		ND		ND		ND		ND		ND	0.00	ND		ND		ND		ND		ND		ND	
1234678-H7F	0.37	0.21	0.38	0.25	0.55	0.20	ND	0.54	ND	0.42	ND	0.29	ND	0.22	ND	0.44	ND	0.74	ND	0.51	ND	0.15	ND	0.30	ND	0.15
1234689-H7F	ND	0.21	ND	0.25	0.00	0.20	ND	0.54	ND	0.42	ND	0.29	ND	0.22	ND	0.44	ND	0.74	ND	0.51	ND	0.15	ND	0.30	ND	0.00
1234789-H7F	ND	0.26	ND	0.25	0.00	0.25	ND	0.54	ND	0.42	ND	0.29	ND	0.29	ND	0.44	ND	0.74	ND	0.51	ND	0.19	ND	0.30	ND	0.15
Total H7F	0.37		0.38	0.00	0.55		ND		ND		ND		ND	0.00	ND		ND		ND		ND		ND		ND	
12346789-OF	ND	0.30	ND	0.32	0.00	0.31	ND	0.25	ND	0.18	ND	0.28	ND	0.15	ND	0.64	ND	0.51	ND	0.73	ND	0.10	ND	0.42	ND	0.13
RECOVERIES	% Recoveries for 13C12-Ds/Fs																									
2378-T4D	85		67		90		54		96		75		76		78		88		85		93		89		86	
12378-P5D	74		61		81		48		88		66		68		63		83		75		89		95		86	
123678-H6D	94		70		87		53		78		72		68		77		88		88		89		87		93	
1234678-H7D	75		55		75		49		69		60		59		54		70		64		91		90		75	
12346789-OD	46		39		50		37		54		37		37		36		53		43		75		81		71	
2378-T4F	85		66		88		49		93		70		75		75		86		82		89		88		82	
12378-P5F	75		61		81		49		88		69		73		67		84		75		86		96		82	
123478-H6F	97		73		99		51		84		72		77		79		96		87		86		87		94	
1234678-H7F	76		55		76		48		74		63		63		58		69		69		80		76		73	

Blank - method blank spiked with 13C12-Ds/Fs.
MDL - Minimum Detection Limit (signal/noise = 3); ng/kg (wet wt.) .
ND - Not detected (signal/noise <3).
Analyses were performed by using the new AUTOSPEC GC/MC, at 10000 resolution.

NOPCBs in Osprey plasma, collected from B.C. in 1992. **TABLE 2.**
Project no.: 92P91C23.

Levels are in ng/kg (wet wt.) 4NOPCB2.

USOX # LS?	Specimen #	Anal. #	No. in pool	Geographical Location	wt.* g	Residue levels												% Int.Std.Recov. 13C-		
						PCB-37		PCB-81		PCB-77		PCB-126		PCB-169		PCB-189				
						Level	MDL	Level	MDL	Level	MDL	Level	MDL	Level	MDL	Level	MDL	77	126	169
57356	TT2A	B-175	1	W. Kamloops	3.37	27.13	0.82	ND	0.66	6.63	0.66	0.93	0.56	ND	0.36	ND	0.36	60	61	57
57357	TT4A	B-176	1	W. Kamloops	4.20	13.98	0.69	ND	0.67	4.75	0.67	1.31	0.54	ND	0.48	ND	0.48	70	76	70
57358	TC1A	B-177	1	E. Kamloops	3.25	25.27	1.37	ND	0.98	10.52	0.98	1.74	1.03	ND	1.78	ND	1.09	49	48	45
57359	TC4A	B-178	1	E. Kamloops	3.17	20.93	0.82	ND	1.39	10.42	1.39	3.47	1.47	ND	2.73	ND	1.14	50	52	47
57360	TC5A	B-179	1	E. Kamloops	3.97	15.22	1.13	ND	0.97	8.17	0.97	1.75	1.31	ND	1.91	ND	1.06	28	28	25
57361	TT2B	B-180	1	W. Kamloops	3.75	31.83	1.29	ND	1.16	7.41	1.16	1.13	0.68	ND	1.80	ND	0.64	61	57	54
57362	TT4B	B-181	1	W. Kamloops	3.57	30.30	2.10	ND	1.68	7.66	1.68	ND	2.08	ND	2.20	ND	1.57	24	22	21
57363	TC1B	B-182	1	E. Kamloops	3.88	22.39	0.89	ND	0.84	8.91	0.84	2.17	0.98	ND	2.10	ND	0.86	61	58	53
57364	TC4B	B-183	1	E. Kamloops	3.52	15.19	1.08	ND	0.80	7.91	0.80	1.99	0.96	ND	1.07	ND	0.94	70	73	65
57365	TC5B	B-184	1	E. Kamloops	3.21	29.09	0.79	0.79	0.78	11.39	0.78	2.49	0.94	ND	1.11	ND	1.06	69	70	62
Blank		B-174				1.65	0.49	ND	0.60	ND	0.60	ND	0.57	ND	0.35	ND	0.35	47	46	44
Blank		B-185				1.35	0.67	ND	0.51	ND	0.51	ND	1.09	ND	1.06	ND	0.50	59	61	57
Blank		B-186				ND	0.53	ND	0.52	ND	0.52	ND	0.62	ND	0.62	ND	0.43	65	72	69

Blank - method blank spiked with 13C12-NOPCBs.
MDL - Minimum Detection Limit (signal/noise = 3); ng/kg (wet wt.).
ND - Not detected (signal/noise <3).
Analyses were performed by using the new AUTOSPEC GC/MS, at resolution of 7000.
wt.* - the sample size (g, in wet wt.) used for analysis.

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NATIONAL REGISTRY OF TOXIC CHEMICAL RESIDUES
BUREAU NATIONAL D'INSCRIPTION DES RÉSIDUS CHIMIQUES TOXIQUES

NE RIEN ECRIRE DANS LA CASE C

PROJECT / PROJET: 92 PYLC23 — Sent

COLLECTION FORM NO. / FORMULE DE PRELEVEMENT N°

COLLECTOR / ECHANTILLONNEUR: P. Whitehead

SUBMITTOR / EMETTEUR: P. Whitehead

COLLECTING ORGANIZATION / ORGANISATION CHARGEE DU PRELEVEMENT: CWS

USOX	Laboratory Number / Laboratoire n°	Specimen Number / Echantillon n°	Units in Specimen / Unités dans l'échantillon	Tissue or Part / Tissu or partie	Species / Espèce	Age Class / Catégorie d'âge	Sex / Sexe	Collection Date / Date du prélèvement	Collection Site - Emplacement du prélèvement: Lat. Deg.	Lat. Min.	Long. Deg.	Long. Min.	Location / Endroit	PROVINCE	Collecting Technique / Technique de prélèvement	Condition when Collected / Etat lors du prélèvement	Storage location
92-57256		TT2A'	1	plasma	OSPR				50	47	121	10	W. Kamloops	BC	syringe	frozen	
92-57257		TT4A'	1	"	"				50	43	121	18	↓		filter 0.5	"	
92-57258		TC1A'	1						50	46	119	47	E. Kamloops		centrifuge		
92-57259		TC4A'	1						50	40	120	09	↓				
92-57260		TC5A'	1						50	40	120	09	↓				
92-57261		TT2B'	1						50	47	121	10	W. Kamloops				
92-57262		TT4B'	1						50	43	121	18	↓				
92-57263		TC1B(r)2							50	46	119	47	E Kamloops				
92-57264		TC4B(r)2							50	40	120	09	↓				
92-57265		TC5B(r)2		↓	↓	--			50	40	120	09	↓		✓	✓	

STORAGE TECHNIQUE / TECHNIQUE D'ENTREPOSAGE: frozen

STORAGE CONTAINER / RECIPIENT D'ENTREPOSAGE: glass vial

LABORATORY STORAGE TECHNIQUE / TECHNIQUE D'ENTREPOSAGE POUR LE LABORATOIRE

LABORATORY STORAGE CONTAINER / RECIPIENT D'ENTREPOSAGE POUR LE LABORATOIRE