ELECTRONIC SUPPLEMENTARY INFORMATION FOR EM-ART-06-2010-000310.R1

PBDEs AND PCBs IN THE LIVER OF THE ST. LAWRENCE ESTUARY BELUGA (*DELPHINAPTERUS LEUCAS*): A COMPARISON OF LEVELS AND TEMPORAL TRENDS WITH THE BLUBBER

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Samples

Since 1982, a carcass monitoring program has been carried out in order to assess causes of mortality of the beluga whale from the SLE [1, 2]. Beached carcasses were examined and, if in a reasonably good state of preservation, were transported to the faculty of veterinary medicine at Ste-Hyacinthe (QC), where a complete necropsy was performed. Carcasses that were not transported were documented, biological parameters were determined and teeth were removed for age determination. Blubber was sampled and whenever possible, liver, brain, muscle, kidney, blood and other tissues were sampled in order to evaluate and assess trends and dynamic of POPs [1- 6].

Carcass state was coded according to Geraci and Lounsbury classification [7]. For example, code of 1 refers to a good carcass condition while code of 4 means that the carcass is in fair condition. In such a case, a necropsy is not required, and only the blubber and a few teeth are removed for age determination. Biological parameters including total length and sex were recorded for each whale. Total length was measured from the rostrum to the notch of tail fluke. The age estimation is based on counting the number of growth layer groups (GLGs) on longitudinal sectioned teeth and considering the deposition of one GLG yearly [8].

Table 1 Data of individual belugas stranded between 1993 and 2007 on the SLE shores

Sample ID	Stranding date	Stranding location	Gender	Length (m)	Age (GLG)	Carcass Code	Lipid (%)	Water (%)	Total protein (%)
DL-02-93	May-93	Ruisseau castor	M	3.97	50	3.0	3.3	74.8	17.6
DL-01-94	May-94	Tadoussac	M	4.56	55	2.5	1.4	73.7	23.2
DL-11-95	October-95	Les Escoumins	M	4.19	52	2.5	3.4	67.7	24.1
DL-12-95	October-95	Grandes-Bergeronnes	M	4.00	22	3.5	1.7	n.d[a]	n.d
DL-01-96	May-96	Les Escoumins	M	4.27	42	2.5	1.3	n.d	n.d
DL-02-98	May-98	Ste. Flavie	M	4.01	24	3.0	1.1	74.4	22.7
DL-07-98	August-98	Petit Matane	M	4.06	35	2.0	3.2	71.5	22.4
DL-08-98	September-98	Port Cartier	M	3.96	44	3.5	3.9	73.3	20.7
DL-09-98	December-98	Ste. Flavie	M	4.06	36	3.0	1.9	75.3	22.9
DL-01-99	April-99	Ste. Flavie	M	4.02	31	3.0	2.9	67.0	21.3
DL-02-99	April-99	Métis sur Mer	M	4.16	35	3.5	2.4	74.6	20.3
DL-04-99	June-99	Trois Pistoles	M	3.98	34	2.5	2.9	72.0	21.4
DL-03-00	July-00	Les Escoumins	M	4.11	35	3.5	3.1	69.0	29
DL-01-01	April-01	Pointe au père	M	4.40	48	3.0	2.0	71.0	26.1
DL-04-01	July-01	Ile au Caribou	M	4.10	54	3.5	3.7	72.6	25.8
DL-07-01	November-01	Baie des Ha! Ha!	M	3.85	55	3.5	2.5	74.9	24.9
DL-03-02	June-02	Matane	M	4.33	30	3.0	2.5	72.0	29.0
DL-08-02	November-02	Matane	M	4.25	24	3.0	2.9	76.9	19.9
DL-09-02	November-02	Baie des sables	M	4.20	40	3.0	1.5	71.0	27.5
DL-102-02	May-02	Rivière Ouelle	M	4.20	74	4.0	5.6	66.1	28.4
DL-01-2003	May-03	Ste. Flavie	M	4.13	33	4.0	3.2	76.0	19.8
DL-08-2003	November-03	Tadoussac	M	3.74	33	3.0	2.7	69.5	29.9
DL-10-2004	December-04	Rivière à Claude	M	4.23	29	3.5	2.1	71.0	28.5
DL-101-2004	April-04	Île Verte	M	3.90	44	4.0	1.2	74.9	24.1
DL-105-2004	July-04	Ste. Anne des Monts	M	4.18	39	4.0	10.7	72.0	18.8
DL-108-2004	September-04	Baie des mille vaches	M	4.51	30	4.0	1.6	76.8	20.1

Sample ID	Stranding date	Stranding location	Gender	Length (m)	Age (GLG)	Carcass Code	Lipid (%)	Water (%)	Total protein (%)
DL-01-2005	May-05	St.Ulric	M	4.20	39	3.0	3.5	70.0	24.5
DL-05-2005	September-05	Les Escoumins	M	4.37	28	3.5	3.2	71.0	25.7
DL-01-2006	July-06	Pointe au père	M	4.36	47	3.0	2.9	72.6	22.8
DL-101-2006	March-06	Cloridorme	M	4.57	43	4.0	14.9	59.9	21.0
DL-09-2007	September-07	Forestville	M	4.05	33	2.5	2.5	70.3	25.8
DL-10-2007	November-07	Baie des Sables	M	3.92	21	2.0	1.9	74.4	21.5
DL-107-2007	October-07	Les Méchins	M	4.17	37	4.0	2.2	69.7	26.3
DL-08-1993	October-93	Les Méchins	F	3.55	25	2.5	0.9	71.0	26.2
DL-04-1994	September-94	St. Joachim Tourelles	F	3.39	20	3.0	2.0	72.6	18.4
DL-05-1995	June-95	St. Ulric	F	3.70	52	3.0	1.3	70.7	26.6
DL-02-1997	May-97	Iles aux lièvres	F	3.38	57	2.0	2.7	71.7	22.4
DL-03-1997	June-97	Rivière Ouelle	F	3.63	42	2.0	1.0	70.4	22.4
DL-105-1997	September-97	Bic	F	3.91	60	4.0	2.4	71.9	27.7
DL-03-1998	May-98	Anse au persil	F	3.77	53	2.5	1.6	71.0	24.4
DL-04-1998	May-98	Ste. Flavie	F	3.56	41	2.5	3.1	71.9	22.8
DL-06-1998	July-98	Bic	F	3.88	22	3.5	4.5	72.6	22.5
DL-03-1999	May-99	Iles aux lièvres	F	3.66	54	4.0	2.8	71.5	n.d
DL-05-1999	July-99	St. Ulric	F	3.57	22	2.5	3.1	72.0	21
DL-07-1999	August-99	Trois Pistoles	F	3.38	20	3.0	2.2	70.0	24.5
DL-107-1999	November-99	Baie des Rochers	F	3.64	30	4.0	0.7	78.0	17.6
DL-05-2000	October-00	Bic	F	4.01	34	3.0	6.2	67.0	22.6
DL-02-2001	May-01	Ste. Thérèse de cap Colombier	F	3.75	40	3.0	4.5	68.0	31.2
DL-01-2002	April-02	St. Irénée	F	3.76	28	2.0	3.5	72.0	22
DL-05-2002	July-02	Île Verte	F	3.71	40	2.0	1.7	73.0	24.4
DL-02-2003	June-03	St. Ulric	F	3.56	46	2.0	6.9	69.4	20.7
DL-06-2003	October-03	Longue Rive	F	3.83	45	2.5	26.0	55.0	16
DL-07-2003	October-03	Rimouski	F	3.10	33	2.5	2.1	73.0	23.4

Sample ID	Stranding date	Stranding location	Gender	Length (m)	Age (GLG)	Carcass Code	Lipid (%)	Water (%)	Total protein (%)
DL-101-2003	May-03	Baie des Rochers	F	3.46	50	4.0	1.8	72.0	23
DL-102-2003	May-03	New Richmond	F	3.54	28	4.0	0.8	81.0	17
DL-02-2004	June-04	Port au Persil	F	3.47	45	2.5	3.2	73.0	22.3
DL-03-2004	June-04	Rimouski	F	3.30	61	2.5	1.5	72.4	23
DL-107-2004	August-04	Grosse roche	F	3.88	39	4.0	19.2	65.3	n.d
DL-02-2005	May-05	Ste. Luce	F	3.77	33	3.5	3.9	69.0	24.8
DL-03-2005	August-05	Pointe au père	F	3.62	32	3.0	7.0	70.6	n.d
DL-06-2005	September-05	Baie des Ha! Ha! Saguenay	F	3.73	37	3.5	3.3	69.0	28.4
DL-102-2006	May-06	Notre Dame du Portage	F	3.45	21	4.0	1.6	79.4	17.5
DL-103-2006	July-06	Rivière à Claude	F	3.69	44	4.0	3.2	80.5	15
DL-03-2007	July-07	Île Verte	F	3.74	40	3.0	1.2	72.7	24.4
DL-04-2007	July-07	Anse Pleureuse	F	3.80	25	3.5	6.1	73.2	22.7

[a]: not determined

Liver characterization

Water, lipid and protein contents were determined in beluga liver.

Water content

1.25g of liver wet weight was dried at 70˚C over night. Water percentage was calculated using the following formula:

% water = [1- (dried weight sample / wet weight sample) *100].

Lipid content

This parameter was determined gravimetrically by measuring the total non volatile solvent (Dichloromethane/Hexanes 50/50 v/v) extractable material on subsamples taken from the original extracts.

% lipid = [(lipid extract weight *100) / liver wet weight]

Protein content

The total protein was determined following the Kjeldahl method [9]. Briefly, proteinic nitrogen is converted into ammonia (NH_3) at the time of hot digestion with sulphuric acid concentrate, peroxide and a K/Cu/Ti catalyst.

Ammonia is fixed by acid excess in the form of sulphate ammonium. In alkaline solution, this salt releases ammonia, which is collected by drive with the vapour in some boric acid. The nitrogen concentration is determined by titrating with hydrochloric acid.

The percentage of proteins is obtained by multiplying by a factor predetermined according to the nature of the product to analyze. In fish, this factor is 6.25 (the Quebec Department of Agriculture, Fisheries and Food, MAPAQ). This value was used for the liver.

Chemicals

All chromatography grade solvents used for chemical analyses were provided by EM Science (Gibbstown, NJ, USA). Anhydrous sodium sulphate (BDH, Toronto, ON, Canada), alumina oxide (Bio-Rad Laboratories, Hercules, CA, USA), silica gel (EM Science) were extracted three times with dichloromethane (DCM) followed by n-hexane prior to their use. Bio-Beads SX-3 for the gel permeation chromatography (GPC) was obtained from Bio-Rad laboratories. Standard solutions were supplied by AccuStandards Inc. (New Haven, CT, USA) or Wellington Laboratories (Guelph, ON, Canada) while ^{13}C labelled compounds were obtained from Cambridge Isotope Laboratories (Andover, MA, USA) or Wellington Laboratories.

Organohalogen residue analyses

PBDEs and PCBs were determined in liver samples according to analytical methods described previously for marine mammal blubber with slight modifications [10, 11]. Briefly, homogenized liver (12g wet weight) were chemically dried with anhydrous sodium sulphate before being transferred into a glass column. Lipids and lipophilic compounds were extracted from the sample and 40% was used for chemical analysis. Labelled surrogates of five PBDEs and eight PCBs were added in all extracts. Lipids were removed from the extract by Gel Permeation Chromatography (GPC). The cleaning-up of each extract was performed by elution through a two-layer column packed with silica, hydrated alumina and anhydrous sodium sulphate. Final extracts were reduced in volume and spiked with an instrument performance solution containing two additional labelled $^{13}C_{12}$ PCBs (PCB-111 and -189) for a final volume of 100µL.

Each extract was injected in gas chromatography coupled to an ion trap mass detector operated in MS-MS mode (GC/MS/MS). The injection of each sample was performed in a separate run for PBDEs and PCBs. The quantification was then achieved by comparing retention times and integrated area in the sample chromatogram to chromatograms of standard PBDEs and PCBs solutions analysed on the GC/MS/MS. Concentrations were calculated using relative response factor (RRF). The final concentration of the extracted compounds was corrected for the recovery of the labelled surrogates added during analysis, which characterize the losses of the target compounds during the extraction and purification process.

Forty individual PBDEs and 45 individual PCBs for which authentic standards were available were measured in all liver samples. The following PBDE congeners were analysed: mono (-1, -2, -3), di-tri (-7, -8, -10, -11, -12, -13, -15, -17, -25, -28, -30, -32, -33, -35, -37), tetra (-47, -49, -66, -71, -75, -77), penta (-85, -99, -100, -116, -118, -119, -126), hexa (-138, -140, -153, -154, -155, -166) and hepta (-181, -183, -190) bromo-substituted PBDE congeners. Only seven PBDE congeners were systematically detected in all samples. PBDE concentrations were reported as $\sum_7$PBDEs and as tetra (-47, -49), penta (-99, -100) and hexa (-153, -154 and -155) PBDE homolog groups.

Analysed PCB congeners were di-tri (-8, -15, -18, -28, -31, -33, -37), tetra (-40, -44, -49, -52, -66+70, -74, -77), penta (-87, -95, -99, -101, -105, -110, -118, -123, -126), hexa (-128, -138, -149, -151, -153, -156,-169), hepta (-170, -171, -177, -180, -183, -187, -191), octa (-194, -195, -199, -205), nona (-206, -208) and deca (-209) chloro-substituted PCB congeners. In all samples, 32 PCB congeners were systematically detected. PCB

concentrations were also reported as $\sum_{32}$ PCBs and as homolog groups which combine the following congeners for tetra (-44, -49, -52, -66+70 and -74), penta (-87, -95, -99, -101, -105, -110 and -118), hexa (-128, -138, -149, -151, -153 and -156), hepta (-170, -171, -177, -180, -183, -187 and -191) and octa-deca (-194, -195, -199, -205, -206, -208 and -209).

1 **Patterns, concentrations and time trends of PBDEs and PCBs in SLE beluga liver**

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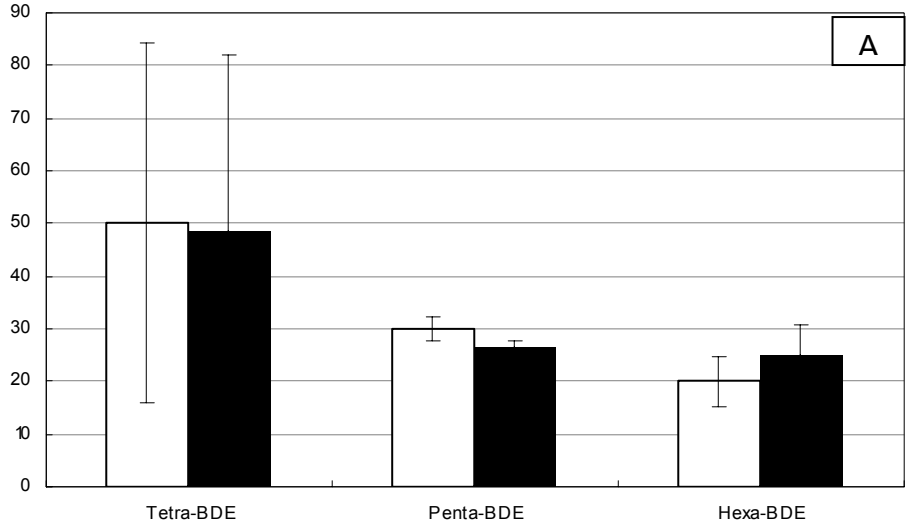

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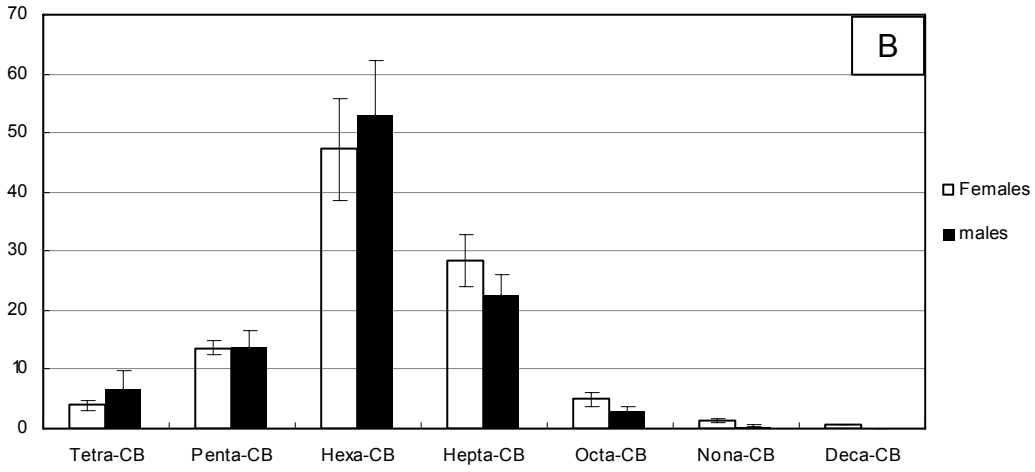

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5 **Fig. 1** Relative contributions (%; mean±SD) of homolog groups to the $\sum_7$PBDE (A) and
6 $\sum_{32}$PCBs (B)
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REFERENCES

1) DFO, 2007. *DFO Canadian Science Advisory Secrandariat*, Proceeding Series 2007/005.
2) L. Measures, *Le Naturaliste Canadien*, 2008, 132, 75.
3) D. Martineau, P. Béland, C. Desjardins, and A. Lagacé, *Arch. Environ. Contam. Toxicol.*, 1987, 16, 137.
4) P. Béland, S. DeGuise, C. Girard, A. Lagace, D. Martineau, R. Michaud, D. C. G. Muir, R. J. Norstrom,; É. Pelletier, E. Ray and L. R. Shugart, *Journal of Great Lakes Research*, 1993, 19, 766.
5) D. C. G. Muir, K. Koczanski, B. Rosenberg, and P. Béland, *Environ. Pollut.*, 1996b, 93, 235.
6) J. M. Gauthier, É. Pelletier, C. Brochu, S. Moore, C. D. Metcalfe, and P. Béland, *Mar. Pollut. Bull.*, 1998, 36, 102.
7) J. R. Geraci, and V. J. Lounsbury, ed. Baltimore, MD, National Aquarium in Baltimore, 2nd edn., 2005, 372 p.
8) R. E. A. Stewart, S. E. Campana, C. M. Jones, and B. E. Stewart, *Canadian Journal of Zoology*, 2006, 84, 1840.
9) R. B. Bradstreet, New York: Academic Press, 1965, 239 p.
10) M. Lebeuf, B. Gouteux, L. Measures, and S. Trottier, *Environ. Sci. Technol.*, 2004, 38, 2971.
11) M. Lebeuf, M. Noël, S. Trottier, and L. Measures, *Sci. Total Environ.*, 2007, 383, 216.