Persistent organic pollutant burden, experimental POP exposure and tissue properties affect metabolic profiles of blubber from grey seal pups

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Supporting Information

Title:

Persistent organic pollutant burden, experimental POP exposure and tissue properties affect metabolic profiles of blubber from grey seal pups.

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SI 1. Methodological details for POP detection.

The same analytical protocol was used in both laboratories at the University of Liege for the initial stages of POP extraction from blubber samples. Briefly, the lipid fraction of the blubber was extracted by Accelerated Solvent Extraction (ASE, Dionex 200, Thermo, USA) using a mixture of Hexane:Dichloromethane (90:10; V:V) at 125°C and 103.4 bar. The fat content was determined gravimetrically after solvent evaporation with a TurboVap LV concentration Evaporator workstation (Zymark TurboVap®LV, Charlotte, USA). The fat was then re-dissolved in 40ml of hexane and split into two equal fractions, one for PCB and PBDE determination and the other fraction for OCP determination.

NDL-PCBs, DL-PCBs and PBDEs analysed by GC-HRMS

In this fraction, a known amount of ^{13}C labelled homologues standard solution containing all the targeted PCBs and PBDEs was added to each sample as internal standard. The extract was then concentrated in 1 mL before being loaded for clean-up on a multilayer basic alumina and acid silica column. The column was eluted with hexane, followed by a solvent exchange to nonane in 100 μL as final volume. Recovery standards ($^{13}C_{12}$ PBDE 77, $^{13}C_{12}$ PBDE138 and $^{13}C_{12}$ PCB 80) were added into the vial prior to Gas Chromatography – High Resolution Mass Spectrometry (GC-HRMS) analysis. The analysis was performed with an Autospec Ultima High Res Mass Spectrometer (Waters, US) coupled to an Agilent 6890 GC (Agilent, US). The injection was carried out in splitless mode and the mass spectrometry via electron ionization EI (@40 eV) using a selected ion-monitoring (SIM) mode.

OCPs analysed by GC-ECD

For the OCPs, prior to extraction, 50 µL of a hexanic solution (100 pg μL^{-1}) of PCB congener 112 (Dr. Ehrenstorfer®, Augsburg, Germany) were added to samples as a surrogate internal standard in order to evaluate the efficiency of recovery. PCB 112 and mirex have never been detected in natural samples from these locations during pre-test analysis and can therefore act as standards. The clean-up of these extracts was conducted using H_2SO_4 98% followed be Florisil solid phase enrichment (Supelco, Envi-Florisil, Bellefonte, PA). Five µL of nonane were added as a keeper substance (resistant to evaporation) to the purified extract. Each extract was evaporated under nitrogen until only the nonane remained in the vial. The final extract was reconstituted with 45 µL of n-hexane and 500 µL of Mirex (100 pg µL-1 in hexane) as injection volume internal standard (Dr. Erhenstorfer® GmbH, Augsburg, Germany). Finally, these extracts were analysed by high-resolution gas chromatography (Thermo Quest Trace, 2000; Thermo Quest, Milan, Italy) equipped with a ^{63}Ni electron capture detector (ECD) and on-column injector. OCPs were analysed on a 60 m x 0.25 mm (0.25 mm film) DB5 ms capillary column (J&W Scientific, USA). Other analytical parameters are described in Debier et al., (2003). Quantification was performed using the internal standard. A calibration curve (1.5 - 250 pg μL^{-1}) was established for each compound of interest. The confirmation of the identity and concentrations of the compounds of interest were periodically performed by HRGC coupled to an ion trap MS (Trace GC Ultra and ITQ 1100 from ThermoQuest). The transfer line temperature was kept at 290 °C and the ion trap temperature was set at 250 °C. The electron ionization (EI) was performed at 70 eV and the ion trap was operating in MS/MS mode. The quality control (QC) was pork fat, free of the compounds of interest. The pork fat was spiked with 5 ng g^{-1} lipid weight nominal concentrations of a mixture of the seven OCPs forming the QC.

The pesticide concentrations in each sample and in the QC were corrected for initial fat weight, and the recovery percentage of the surrogate PCB 112. Recovery rates in QCs ranged between 96% ± 10.3 % and 113 % ± 12.5 % according to the OCP and the recovery rates of the PCB 112 surrogate internal standard was aways between 70 and 110 %, in good agreement with requirements of SANCO (SANCO, 2014). The limit of detection (LOD) was 0.02 ng g^{-1} lipid weight and the measured limit of quantification (LOQ) determined with PCB spiked pork fat was measured at 0.07 ng g^{-1} lipid weight.

References

1. Debier, Cathy, Paddy P. Pomeroy, Cédric Dupont, Claude Joiris, Vinciane Comblin, Eric Le Boulengé, Yvan Larondelle, and Jean-Pierre Thomé. "Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals Halichoerus grypus." *Marine Ecology Progress Series* 247 (2003): 237-248.
2. SANCO, 2014. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANCO/12571/2013 Supersedes SANCO/12495/2011. Off. J. Eur. Communities,2002, pp. 8-36 48.

SI Table 1. Candidate GAMM models exploring different summed POP types, potential interactions and the top models in the selection process.

Response variable	Predictor variables	Interactions	dAIC	R_2	Akaike's weight
Glucose uptake (µmoles / hour/ 100 mg)	∑DL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑DL-PCB + nutritional state ∑DL-PCB + tissue depth Nutritional state + tissue depth	3.6	0.36	0.14
	∑DL-PCB, nutritional state, tissue depth, experimental conditions	Nutritional state + tissue depth	0	0.36	0.82
	∑NDL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑NDL-PCB + nutritional state ∑NDL-PCB + tissue depth Nutritional state + tissue depth	14.5	0.34	0
	∑NDL-PCB, nutritional state, tissue depth, experimental conditions	Nutritional state + tissue depth	8.7	0.35	0.01
	∑PBDE, nutritional state, tissue depth, experimental conditions, sex, mass	∑PBDE + nutritional state ∑PBDE + tissue depth	12.6	0.34	0

		Nutritional state + tissue depth			
	∑PBDE, nutritional state, tissue depth, experimental conditions	Nutritional state + tissue depth	6.9	0.35	0.03
	∑DDX, nutritional state, tissue depth, experimental conditions, sex, mass	∑DDX + nutritional state ∑DDX + tissue depth Nutritional state + tissue depth	13.7	0.34	0
	∑DDX, nutritional state, tissue depth, experimental conditions	Nutritional state + tissue depth	14.6	0.34	0
Lactate generation (µmoles / hour/ 100 mg)	∑DL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑DL-PCB + nutritional state ∑DL-PCB + tissue depth Nutritional state + tissue depth	0.3	0.3	0.46
	∑NDL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑NDL-PCB + nutritional state ∑NDL-PCB + tissue depth Nutritional state + tissue depth	11.3	0.28	0
	Nutritional state, tissue depth, sex	Nutritional state + tissue depth			

	∑PBDE, nutritional state, tissue depth, experimental conditions, sex, mass	∑PBDE + nutritional state ∑PBDE + tissue depth Nutritional state + tissue depth	14	0.27	0
	∑DDX, nutritional state, tissue depth, experimental conditions, sex, mass	∑DDX + nutritional state ∑DDX + tissue depth Nutritional state + tissue depth	11.4	0.27	0
	∑DL-PCB, nutritional state, tissue depth, sex	∑DL-PCB + nutritional state Nutritional state + tissue depth	0	0.3	0.54
Glycerol generation (µmoles / hour/ 100 mg)	∑DL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑DL-PCB + nutritional state ∑DL-PCB + tissue depth Nutritional state + tissue depth	12.5	0.75	0
	∑NDL-PCB, nutritional state, tissue depth, experimental conditions, sex, mass	∑NDL-PCB + nutritional state ∑NDL-PCB + tissue depth Nutritional state + tissue depth	7.2	0.75	0.02
	∑PBDE, nutritional state, tissue depth,	∑PBDE + nutritional state	16	0.74	0

	experimental conditions, sex, mass	∑PBDE + tissue depth Nutritional state + tissue depth			
	∑DDX, nutritional state, tissue depth, experimental conditions, sex, mass	∑DDX + nutritional state ∑DDX + tissue depth Nutritional state + tissue depth	0	0.75	0.61
	∑DDX, nutritional state, tissue depth	∑DDX + nutritional state Nutritional state + tissue depth	1	0.75	0.38

SI Table 2. Model output from all final GAMMs analysing metabolic characteristics in explant experiments with their standard errors, estimates and p values. If predictor variables have parentheses, this indicates the tested group.

Response variable	Predictor variables	Estimate	Standard Error	P value
Glucose uptake (µmoles / hour/ 100 mg)	∑DL-PCB	-0.66	0.18	**< 0.001**
	Tissue Depth (Outer)	-0.33	0.07	**< 0.001**
	Nutritional State (Feeding)	-0.07	0.09	0.44
	Experiment (+PCB)	0.22	0.05	**< 0.001**
	Interaction: Tissue depth and Nutritional State (Outer)	0.22	0.10	**0.04**
	Intercept	-2.48	0.26	**< 0.001**
Lactate generation (µmoles / hour/ 100 mg)	∑DL-PCB	-0.01	0.005	**0.03**
	Tissue Depth (Outer)	-0.006	0.002	**0.001**
	Nutritional State (Feeding)	-0.02	0.008	**0.003**
	Sex (male)	0.006	0.003	**0.05**
	Interaction: Tissue depth and Nutritional State (Outer)	-0.005	0.003	0.06
	Intercept	0.03	0.007	**< 0.001**
Glycerol generation (µmoles / hour/ 100 mg)	∑DDX	0.01	0.02	0.5
	Tissue Depth (Outer)	-0.05	0.03	0.1
	Nutritional State (Feeding)	0.15	0.03	**< 0.001**
	Experiment (+PCB)	0.001	0.002	0.7
	Sex (male)	-0.005	0.005	0.3
	Mass	-0.001	0.0004	0.1
	Interaction: Tissue depth and nutritional state	0.01	0.003	**< 0.001**
	Interaction: ∑DDX and Nutritional state (feeding)	-0.05	0.01	**< 0.001**
	Interaction: ∑DDX and Tissue	0.02	0.01	0.2

	Depth (Outer)			
	Intercept	0.08	0.06	0.08

SI Table 3. Doses of each PCB type given in the explant PCB culture conditions, with the estimated percentage change for each PCB type measured in blubber tissue.

∑DL-PCB within total dose: 1.6ng. ∑NDL-PCB within total dose: 202.6ng

PCB	total dose (ng)	% increase (feeding)	% increase (fasting)
1	0.581		
2	0.031		
3	0.163		
4	3.764		
5	0.153		
6	1.734		
7	0.316		
8	8.476		
9	0.663		
10	0.235		
11	0		
12	0.071		
13	0.255		
14	0		
15	2.560		
16	3.978		
17	4.080		
18	11.036		
19	1.030		
20	0.908		
21	0		
22	3.601		
23	0.010		
24	0.163		
25	0.734		
26	1.621		
27	0.51		
28	**8.792**	**1588-26291**	**178-28010**
29	0.102		
30	0		
31	9.496		
32	2.428		
33	6.355		
34	0.031		
35	0.061		
36	0		
37	1.030		
38	0		
39	0		

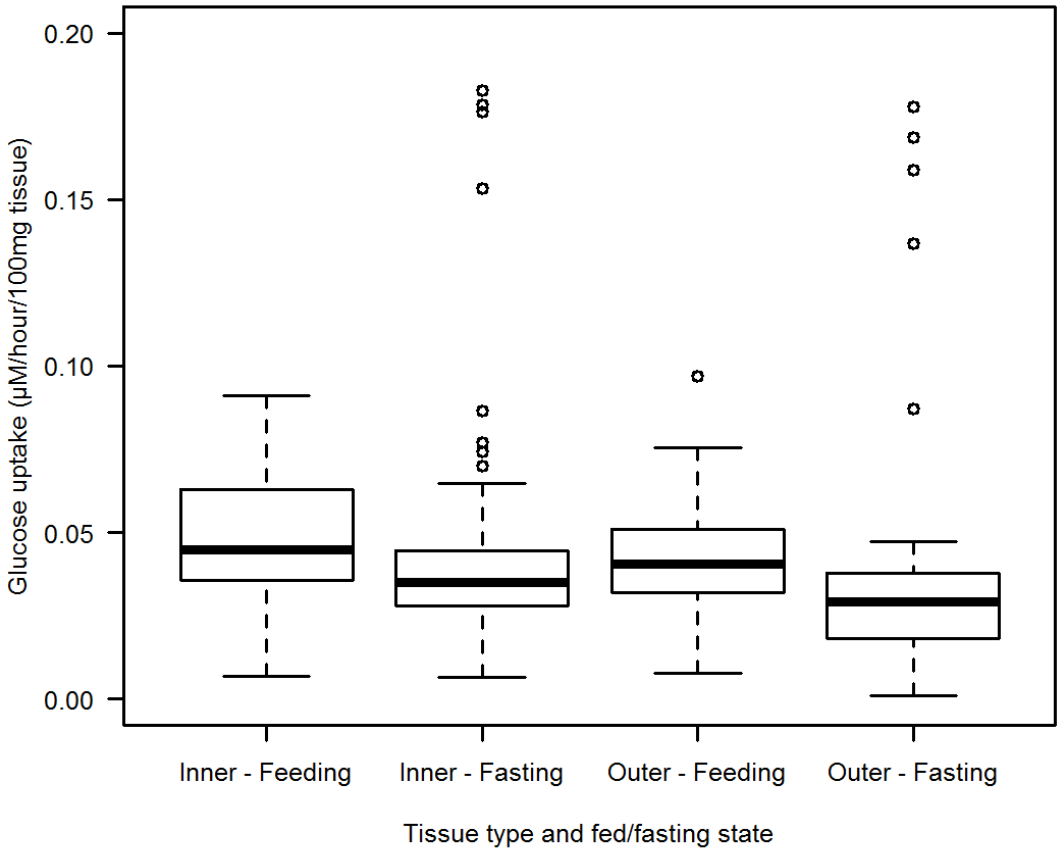
40	0.592		
41	0.775		
42	1.622		
43	0.255		
44	4.610		
45	1.244		
46	0.490		
47	1.265		
48	1.622		
49	3.488		
50	0.010		
51	0.326		
52	**4.957**	**157-3371**	**30.04 -1601**
53	0.959		
54	0.020		
55	0		
56	0.082		
57	0.010		
58	0		
59	0.388		
60	0.071		
61	0		
62	0		
63	0.051		
64	1.887		
65	0		
66	0.400		
67	0.061		
68	0		
69	0		
70	0.612		
71	1.193		
72	0		
73	0		
74	0.388		
75	0.061		
76	0		
77	0		
78	0		
79	0		
80	0		
81	0		
82	0		
83	0		
84	0.173		
85	0		

86	0		
87	0.428		
88	0		
89	0		
90	0		
91	0.061		
92	0.326		
93	0		
94	0		
95	2.897		
96	0.041		
97	0.133		
98	0		
99	0.051		
100	0		
101	**3.274**	**59-374**	**21-191**
102	0.041		
103	0		
104	0		
105	**0.214**	**30-311**	**7-336**
106	0		
107	0		
108	0		
109	0		
110	1.387		
111	0		
112	0		
113	0		
114	**0**	**0**	**0**
115	0		
116	0		
117	0		
118	**0.510**	**25-256**	**7-208**
119	0		
120	0		
121	0		
122	0		
123	**0**	**0**	**0**
124	0		
125	0		
126	0		
127	0		
128	0.561		
129	0.143		
130	0.224		
131	0.071		

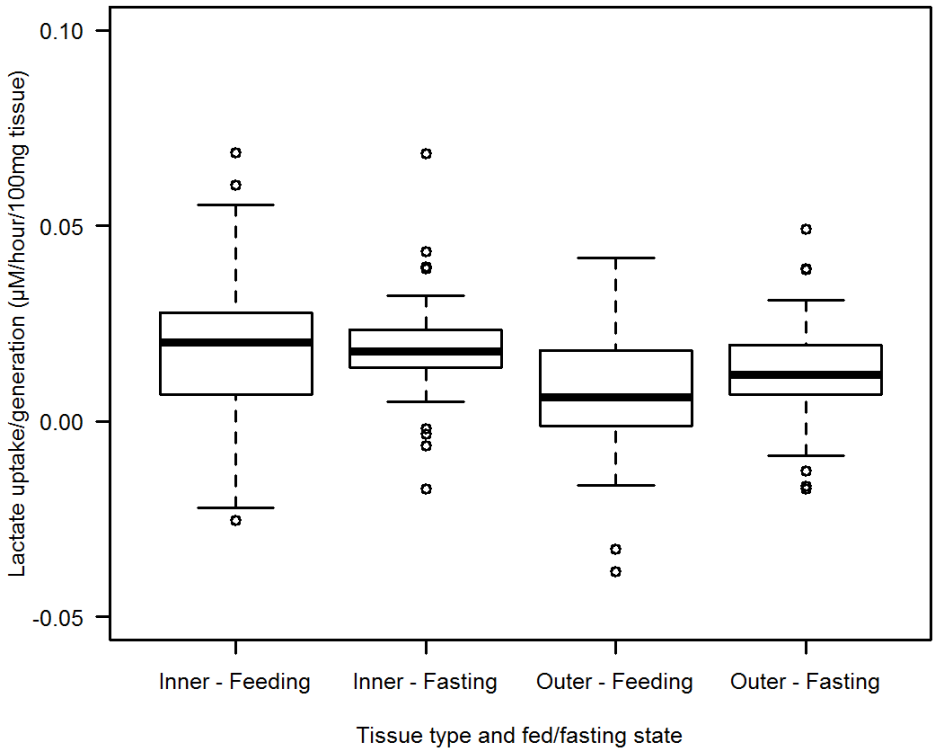
132	3.019		
133	0.061		
134	0.367		
135	1.112		
136	1.479		
137	0.020		
138	**6.538**	**42-137**	**38-82**
139	0		
140	0		
141	2.734		
142	0		
143	0		
144	0.622		
145	0		
146	1.183		
147	0		
148	0		
149	8.956		
150	0		
151	3.101		
152	0		
153	**9.353**	**35-115**	**30-70**
154	0		
155	0		
156	**0.551**	**102-574**	**75-373**
157	**0.020**	**20-84**	**17-46**
158	0.592		
159	0		
160	0		
161	0		
162	0		
163	2.479		
164	0.714		
165	0		
166	0		
167	**0.204**	**553-25185**	**111-5074**
168	0		
169	0		
170	4.090		
171	1.102		
172	0.714		
173	0.092		
174	5.059		
175	0.184		
176	0.592		
177	2.570		

178	0.857		
179	2.081		
180	**11.424**	**136-872**	**128-897**
181	0		
182	0		
183	2.397		
184	0		
185	0.571		
186	0		
187	5.477		
188	0		
189	**0.112**	**124-1167**	**104-662**
190	0.816		
191	0.173		
192	0		
193	0.500		
194	2.101		
195	0.826		
196	1.051		
197	0.071		
198	0.112		
199	1.897		
200	0.265		
201	0.255		
202	0.367		
203	1.479		
204	0		
205	0.102		
206	0.622		
207	0.061		
208	0.163		
209	0		

SI Figure 1. Glucose uptake in inner and outer blubber tissue from feeding and fasting grey seal pups (n = 27 feeding and n = 23 fasting pups) with median, upper and lower quartiles, 1.5 x interquartile range and outliers shown. Significant differences between groups are detailed in the main text, as not all significant parameters and interactions influencing glucose uptake are plotted. Individual was included as a random effect in the model.



SI Figure 2. Lactate production in inner and outer blubber tissue from feeding and fasting grey seal pups (n = 27 feeding and n = 23 fasting pups) with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences between groups are detailed in the main text, as not all significant parameters and interactions influencing lactate generation/uptake are plotted. Individual was included as a random effect in the model.



SI Figure 3. Glycerol generation in inner and outer blubber tissue from feeding and fasting grey seal pups (n = 27 feeding and n = 23 fasting pups) with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences between groups are detailed in the main text, as not all significant parameters and interactions influencing glycerol generation are plotted. Individual was included as a random effect in the model.