Supporting Information

Blood plasma sample preparation method for the assessment of thyroid hormone-disrupting potency in Effect-Directed Analysis

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Additional information on cow and polar bear plasma samples, chemical analysis to determine recoveries of spiked compounds, SPE cartridge selection and Tables (2)

Additional information on cow and polar bear plasma samples

Cow blood used for method validation was obtained from a slaughterhouse (Amsterdam, The Netherlands). Approximately 1 L of cow blood was heparinized with 10 mL of heparine lithium anti-coagulant (Sigma-Aldrich, Zwijndrecht, The Netherlands) dissolved in water, resulting in a final concentration of 3,000 units/L blood. Samples were carefully handled (e.g. no shaking) to avoid hemolysis (i.e. lysis of red blood cells) and the release of hemoglobin into the plasma. Whole blood was divided into 15 mL subsamples, that were centrifugated at 4,000 rpm for 10-15 minutes. The isolated plasma was stored in 15 mL aliquots at -18 ˚C until use.

The polar bear blood samples were collected from two adult (8 and 9 year old) female bears (estimated age based on tooth analysis as described by Christensen-Dalsgaard[1]) at Svalbard (Norway) in April 2008, between latitude=77.74N and 77.55N, and between longitude=18.44E and 22.12E. Capture and handling procedures followed standard protocols[2,3] and were approved by the National Animal Research Authority (NARA, Oslo, Norway) and the governor of Svalbard. The bioassay analyses of the current samples were performed within a much larger study (BearHealth). The percentage of plasma lipids (PL %) and the concentration of several OH-PCBs in the polar bear plasma samples were determined at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science (Oslo, Norway). The PL% was determined and the plasma sample was extracted and cleaned up according to Brevik *et al.*[4] with modifications as described by Bernhoft *et al.*[5] and Andersen *et al.*[6] Chemical analysis of OH-PCBs after derivatization was conducted as described by Løken *et al.*[7] and Berg *et*

al.[8] These samples were selected based on their medium TT_4 level (8 and 20 nM, respectively) and high PL% (1.5% for both). Two mL polar bear plasma was extracted and tested in the radioligand T_4*-TTR binding assay.

Chemical analysis to determine recoveries of spiked compounds

In the plasma extracts, the recoveries of OH-PBDEs were measured by gas chromatography-mass spectrometry (GC-MS) operated in electron capture negative ionization (ECNI) mode on an Agilent 6890 Network GC system with an Agilent 5975 inert XL mass selective detector and an Agilent7683 auto sampler (Agilent Technologies, Amstelveen, The Netherlands). A volume of 1 µL extract was injected at 275℃ in pulsed splitless mode at 380 kPa. For separation a CP-Sil8 CB column (48 m length, 0.25 mm I.D. and 0.25 µm film thickness) was used with helium as the carrier gas and methane as the reagent gas. The oven temperature program was: 1) 3 min at 90℃; 2) increase with 30℃ /min to 210℃; 3) 20 min at 210℃; 4) increase with 5℃/min to 290℃; 17 min at 290℃. The source temperature was held at 200℃, the quadrupole temperature at 106℃. The ionization energy was 50 eV.

The OHPs were analyzed by GC-MS operated in the selected ion (SIM) and full scan mode (scanning mass range: 50-400 m/z). GC-MS measurements were performed on a HP 6890 GC with a HP 5973 mass selective detector (Agilent Technologies, Amstelveen, The Netherlands), equipped with a 25 m SGE BPX5 column (0.22 mm I.D., 0.25 µm film thickness), and helium as the carrier gas. The oven temperature program was: 1 min at 60 ℃; increase with 5 ℃ /min to 210 ℃; increase with 10 ℃ /min to 300 ℃; 15 min at 300 ℃. Total run time was 55 min.

The analysis of PCBs, PBDEs and OH-PCBs was performed on an Agilent 7890A GC-system with micro electron capture detection (μECD) and with an Agilent 7683 autosampler. The system was equipped with a 50 m CP-Sil8 CB column (0.25 mm I.D., 0.33 μm film thickness) and a 50 m CP-Sil19 CB column (0.2 mm I.D., 0.33 μm film thickness), and helium as the carrier gas and nitrogen as the make-up gas. The oven temperature program was: 1) 3 min at 50°C; 2) increase with 5°C /min to 300°C; 3) 10 min at 300°C. The samples were injected in split mode (1:50). Hydroxylated compounds were derivatized with diazomethane prior to GC measurement to improve the quality of the chromatography and the detection power. Ethereal diazomethane was generated from N-methyl-N-nitroso-ureum and 50% w/v potassium hydroxide in diethyl ether according to Vogel[9].

Perfluorinated compounds were analyzed by triple quadrupole liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS; G6410A, Agilent). A Waters Symmetry C18 column (particle size 5μm, i.d. 2.1mm, length 50mm) was used in combination with a Waters Symmetry C18 guard column (particle size 5 μm, i.d. 3.9mm, length 20mm). Another Waters Symmetry C18 column (5 μm, 2.1x 50mm) was placed between the degasser and the LC pump to retain PFCs originating from the LC system, mainly from the tubing and the degasser. The injection volume used was 20 μL. The mobile phase consisted of methanol (solvent A) and 2 mM ammonium acetate in water (solvent B), and had a flow rate of 0.3 $mLmin^{-1}$. The column temperature was set at 25 ºC. The gradient elution program was 90% solvent B during the first 0.5 min, a linear gradient from 90% to 0% solvent B over the next 49.5 min and 100% solvent A for another 0.5 min. The column was reconditioned for 14.5 min. The operating conditions

for the ESI source were as follows: capillary voltage 1000 V, source temperature 325 ºC, gas flow rate 6 L/min and nebulizer gas pressure 25 psi. The quantifier and qualifier ions coming from two selected transitions used for each target PFC, and the internal standards together with their corresponding quantifier ions are published elsewhere[10].

Target compounds were quantified in all cases by external standard calibration using the different spiking mixtures. The detection limits of the analytical chemical methods are: 3-5 ng/mL for OH-PBDEs, 1-5 ng/mL for OHPs, 1 ng/mL for PCBs and PBDEs, 0.1-1 ng/mL for OH-PCBs and 0.1-0.5 ng/mL for PFASs.

SPE cartridge selection

In a preliminary experiment, different types of cartridges were tested to find the most appropriate SPE material for our purpose, i.e. Oasis® hydrophilic-liphophilic balance (HLB) and ion-exchange (MCX, WCX, MAX and WAX) cartridges from Waters (Milford MA, USA) and reversed phase Strata-X cartridges from Phenomenex® (Torrance CA, USA). The extraction procedures are outlined in Table S1. The plasma samples spiked with OHPs, OH-PCBs and the PCB/PBDE mixtures were extracted in triplicate on seven different SPE cartridge types. Different protocols were followed for conditioning, equilibration, washing and elution of the cartridges (Table S1).

TABLE S1: Applied extraction and solvent protocols of the tested SPE-cartridges

Step	Process	Mixed-mode cation exchange sorbent **Oasis® MCX**	Weak anion exchange sorbent **Oasis® WAX**	Mixed-mode cation exchange sorbent for gas chromatography **Oasis® MCX-GC**	Weak cation exchange sorbent **Oasis® WCX**	Mixed-mode anion exchange sorbent **Oasis® MAX**	Hydrophilic-liphophilic balance sorbent **Oasis® HLB**	Polymeric reversed phase sorbent from Phenomenex **Strata-X**
1	**Condition**	**3 mL MeOH**		3 mL DCM / MeOH (1:1,v/v)	3 mL MeOH		3 mL DCM / MeOH (1:1,v/v)	3 mL MeOH
2	**Equilibrate**	**3 mL water**		3 mL water	3 mL water		3 mL water	3 mL water
3	**Load the sample**							
4	**Wash**	**3 mL 1.8% formic acid in water**		3 mL 5% MeOH in water	3 mL 5% NH_4OH in water		3 mL 5% MeOH in water	3 mL 5% MeOH in water
5	**Drying the sorbent**							
6	**Elution 1 (E1)**	**4*0.75 mL 100 % MeOH**		4*0.75 mL 5% MeOH in DCM	4*0.75 mL 100 % MeOH		4*0.75 mL DCM	4*0.75 mL MeOH:ACN (1:1)
7	**Elution 2 (E2)**	**4*0.75 mL 5 % NH_4OH in MeOH**		-	4*0.75 mL 2 % formic acid in MeOH		4*0.75 mL 10 % MeOH in DCM	-

Recoveries determined by GC-ECNI-MS and GC-µECD measurement are summarized in Table S2. Best recoveries were obtained for the OHPs (107±8.1%) and OH-PCBs (93.8±15.5%) with the Oasis® MCX and Oasis® HLB (83±13.1% and 80±18.5%, respectively) cartridges applying the normal procedure with MeOH elution. For PCBs and PBDEs none of the tested cartridges gave recoveries >30 %. Extracts eluted from StrataX cartridges have not been injected onto the GC because they contained particles and several solvent layers. Generally, extracts from Oasis® MAX and Oasis® WCX cartridges had very low recoveries (<10%) for most of the compounds (these results are not listed in Table S2). Considering the recoveries obtained after SPE with different cartridges the Oasis® MCX cartridge was chosen for further method evaluation.

TABLE S2: Comparison of different cartridges - recoveries (%±SD) of the spiking compounds measured in fraction $E_{1, hex}$ from extracts of spiked cow plasma after SPE

GC-ECNI-MS/GC-µECD	Oasis® MCX	Oasis® MCX-GC	Oasis® WAX	Oasis® HLB
2,4,5-Trichlorophenol	**113 ± 9.8**	85 ± 33.7	84 ± 4.0	92 ± 0.6
2,4,6-Tribromophenol	**110 ± 5.4**	86 ± 37.6	14 ± 0.8	95 ± 0.0
Pentachlorophenol	**95 ± 3.8**	107 ± 49.3	1.0 0.1	67 ± 1.6
Triclosan	**110 ± 9.2**	17 ± 0.0	39 ± 5.5	77 ± 1.8
AVERAGE	**107 ± 8.1**	74 ± 39.2	35 ± 36.6	83 ± 13.1
OH-PCB 118	**97 ± 2.1**	54 ± 1.5	7.0 ± 0.2	88 ± 0.6
OH-PCB 107	**105 ± 11.3**	51 ± 0.1	9.0 ± 0.0	86 ± 1.2
OH-PCB 138	**102 ± 8.7**	41 ± 0.2	31 ± 0.7	52 ± 3.0
OH-PCB 187	**71 ± 1.5**	50 ± 0.3	14 ± 0.8	92 ± 5.2
AVERAGE	**93.8 ± 15.5**	49 ± 5.6	15 ± 10.9	80 ± 18.5
BDE 28	**32 ± 7**	35 ± 2.7	20 ± 1.5	47 ± 7.2
PCB 153	**29 ± 7**	23 ± 1.1	13 ± 0.9	31 ± 6.0
PCB 138	**30 ± 7**	23 ± 0.6	13 ± 1.0	32 ± 5.4
BDE 49	**30 ± 7**	25 ± 0.8	16 ± 0.9	38 ± 4.2
BDE 47	**30 ± 7**	15 ± 0.6	7.0 ± 0.7	22 ± 3.8
PCB 180	**29 ± 7**	19 ± 0.2	10 ± 0.1	29 ± 3.8
PCB 170	**30 ± 7**	17 ± 1.0	9.0 ± 0.7	24 ± 3.6
BDE 100	**26 ± 6**	8.0 ± 0.3	6.0 ± 1.1	16 ± 4.0
AVERAGE	**30 ± 1.7**	21 ± 8	12 ± 4.7	30 ± 9.7

References to Supporting Information

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