Estrogen-like activities in blood cleared for endogenous steroid hormones across European and Inuit populations.

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Introduction

Human exposure to environmental contaminants is ubiquitous and can affect individuals living close to as well as remote from the sources of contaminants. All individuals carry a burden of the lipophilic persistent organic pollutants (POPs) and heavy metals in their body. POPs includes polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and certain pesticide residues e.g. dichloro-diphenyl-trichloroethane/dichloro-diphenyl-dichloroethylene (DDT/DDE), toxaphenes, β-hexachlorocyclohexane (β-HCH), chlordanes, hexachlorobenzene, and Mirex (1).

Exposure to POPs elicits a number of species- and tissue-specific toxic responses including effects on the reproductive-, immune- and thyroid system. Study on wildlife populations have documented adverse effects, including reproductive end developmental effects that correlate with exposure to one or more of these endocrine modulating chemicals. Especially the exposure during foetal and early life is critical. Although, no clear cut evidence for adverse endocrine-related human health effects has been obtained, the reasonable suspicion based on wildlife, animal and laboratory studies strengthened the need for further research to address the uncertainty and concern (1, 2).

There are a number of factors that complicate the toxicological assessment. Firstly, it is important to remember that no individual is exposed to a single contaminant but to a complex mixture of contaminants, which is life-long beginning during critical developmental windows.

Aim **T**he aim of the present study was to compare the actual level of estrogen-like activity in serum fractions containing the lipophilic POPs but free of endogenous hormones between different European and Inuit populations for finally to evaluate whether the xeno-estrogenic activity is correlated to bio-accumulated POPs and/or lifestyle.

Methods

Study population: The study is a part of the EU project INUENDO with the specific objective to elucidate the fertility in European and Inuit populations with high respectively low intake of POC. The main study includes questionnaires to the women concerning time to pregnancy and semen sampling from the men. The blood samples for xeno-hormone activity analysis were taken from the spouses / partners of pregnant women chosen randomly from the different districts: 75 of 461 from Greenland; 100 out of 195 from Sweden, 100 out of 267 from Poland, and 88 out of 302 from Ukraine.

SPE-HPLC extraction: To obtain the serum fraction containing the actual mixture of bio-accumulated POPs a SPE-HPLC extraction was performed. Similar to the described methods (3, 4) POPs were extracted from the serum samples by solid phase extraction (SPE) using Oasis HLB cartridges from Waters. The crude serum extract was then further processed using high performance liquid chromatography (HPLC) in order to separate the POPs from the endogenous hormones to avoid a false response in the ER-CALUX assay.

ER-transactivation (ER-CALUX): The effect of the serum extract on the function of the estrogen receptor (ER) trans-activity was determined using the stable transfected MVLN human breast cancer cell line carrying an ER-luciferase reporter gene (5) measuring the relative luciferase unit per microgram protein (RLU/µg protein) (6).

Results

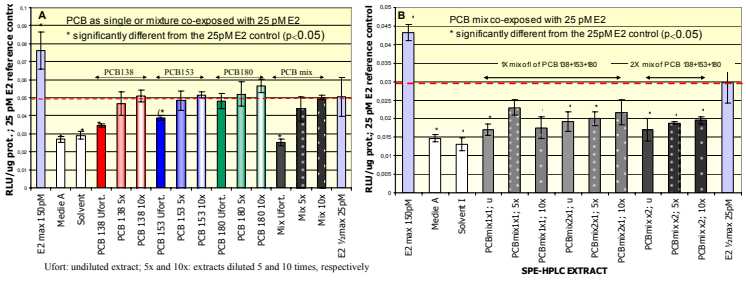
Table 1. Recovery of selected serum POPs after SPE-HPLC determined by GC/MS

Compound	Male volunteers average (%)	Blood bank male (%)	Blood bank female (%)	Blood bank average (%)
Arochlor 1260	38	35	33	34
PCB 118	79	64	76	70
PCB 138	39	38	35	37
PCB 153	38	33	31	32
PCB 170	28	25	N/A	25
PCB 180	25	22	21	22
p,p'-DDE	45	40	45	43
HCB	21	38	50	44

Eight selected POPs were determined in serum samples from 7 young Danish male volunteers and from serum controls donated by the local blood bank, both with low levels of POPs close to the detection limit. Recovery was determined by comparing POP-levels in untreated serum with POP-levels in SPE-HPLC processed samples (table 1).

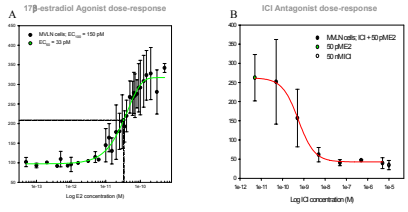
Figure 1. Effect of PCBs on ER function upon directly cell exposure (A) or after serum spiking followed by SPE-HPLC extraction (B)

Physiological GRL mean level used: CB138 (2.73 mg/l), CB153 (4.00 mg/l), CB180 (2.71 mg/l)



Ufort: undiluted extract; 5x and 10x: extracts diluted 5 and 10 times, respectively

Figure 2. Agonistic and antagonistic dose-response of the MVLN (ER-CALUX) cells



A. **T**he 17β-estradiol (E2) dose response of MVLN determined in the concentration range of 0.05 pM to 500 pM elicited a detection limit, EC_{25}, EC_{50}, EC_{75} and EC_{100} to 0.5 pM, 16 pM, 33 pM, 60 pM and 150 pM, respectively.

B. **T**he antagonistic ICI dose-response of the E2 induced luciferase activity in MVLN cells determined in the concentration range of 50 pM to 5 µM ICI. 50 nM ICI exerted a maximum antagonistic response to background level of the 50 pM E2 (EC_{70}) induced luciferase activity.

Table 2. Agonistic and antagonistic ER responses of hormone-free serum extracts

Country	N	Basal ER-activity %agonistic	Basal ER-activity %antagonistic	E2 induced ER-activity %add./syn.	E2 induced ER-activity %antagonistic	DDE G-mean (ng/ml)	PCB153 G-mean (ng/ml)
Greenland							
Sisimiut	50	0	36	2	76	3,1	1,1
Ammassalik	25	4	32	0	60	8,5	3,4
Sweden	100	12	12	3	19	1,3	1,0
Poland	100	21	5	13	7	4,0	0,1
Ukraine	88	14	17	1	30	4,8	0,2

Basal ER-activity: *Serum extract alone* induced ER-activity as measured by the relative luciferase activity (RLU) pr µg cell protein. % agonistic and % antagonistic indicates increased or decreased activity, respectively, compared to the solvent control, which is set to 100%.
E2 induced ER-activity: *Serum extract + 25 pM 17β-estradiol* (E2) induced ER activity measured by RLU/µg cell protein. % additive/synergistic and % antagonistic indicates an increased or decreased activity, respectively, compared to the 25 pM E2 control, which is set to 100%.
G-mean: *geometric mean* (ng/ml serum).

Summary

Table 1. We have established an SPE - HPLC serum extraction method to isolate the fraction containing the pool of lipophilic xenohormone compounds from human serum free of endogenous hormones. This hormone free serum fraction was applied to MVLN (ER CALUX) cells for determination of the integrated xeno-estrogenic activity of the actual POP mixture.

FIG. 1 Exposure of the MVLN cells either directly (A) or upon serum spiking and SPE-HPLC extraction (B) to the highly bioaccumulated PCB congeners 138, 153 and 180 showed that an effect of the PCBs on ER function at a concentration level found in human serum can be assessed by the established SPE-HPLC-ER-CALUX method.

Table 2. SPE-HPLC serum extracts from Greenland, Sweden, Poland and Ukraine were analysed by the SPE-HPLC-ER-CALUX system. Very few serum extracts from Greenland elicited agonistic effect whereas up to 36% and 76 % of the samples elicited an antagonistic effect on basal and E2 induced ER transactivation, respectively. In contrast 12%, 21% and 14% of serum extracts from Sweden, Poland and Ukraine, respectively, induced an ER agonistic activity and less than 20% of the samples exerted an E2-ER antagonistic activity but from Ukraine, where 30% of the samples antagonized the E2:ER transactivation.

Discussion

The geometric means of DDE showed the highest value in Ammassalik > Ukraine > Poland > Sisimiut > Sweden, whereas the geometric means of PCB153 showed the highest value at Ammassalik > Sisimiut > Sweden > Ukraine > Poland. DDE and PCB153 were shown *in vitro* to have estrogenic and anti-estrogenic potential, respectively. The relatively high level of both DDE and PCB153 in samples from Greenland might be responsible for the antagonistic effect of these serum extracts. Whether the relatively high DDE level but lower PCB153 level in Ukraine > Poland > Sweden serum extracts might be involved in the higher estrogenic activity of these extracts require further investigations.

Conclusion

In conclusion the study shows that the xeno-estrogenic activity in hormone free serum extracts differs between European populations and Inuits, which may be explained by differences in the pattern and levels of bioaccumulated POPs.

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Project coordinator: Professor Jens Peter Bonde, KH University Hospital of Aarhus, Århus, Denmark.

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