

XENOHORMONE TRANSACTIVITIES IN SERUM ACROSS INUIT POPULATIONS

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ABSTRACT

Background: The persistent organic pollutants (POPs) are highly lipophilic and resistant to biodegradation. POPs are found in fish and sea birds, seals, whales and polar bears. Owing to the high intake of marine food Greenlandic Inuits display high body burden of POPs that varies according to local conditions and dietary preference.

The aims were 1) to determine the integrated xenohormone bioactivities as an integrated exposure marker of total lipophilic serum POPs comparing the effect on estrogen (ER) and androgen receptor (AR) transactivity between Greenlandic districts and 2) to evaluate associations of xenohormone transactivity to serum POP concentrations (14 PCBs and 10 pesticides) and lifestyle characteristics.

Methods: Serum samples from 121 men and 119 women from Nuuk, Sisimiut and Qaanaaq were extracted using SPE-HPLC fractionation to obtain the serum POP fraction free of endogenous hormones. These serum POP fractions were used for determination of xenohormone transactivity using ER and AR reporter gene assays.

Results: In overall, the xenohormone transactivities differed between districts as well as between genders. Mainly antagonistic ER and AR effects were found in the serum extracts from both genders. Heterogeneous correlations between xenohormone transactivities and POPs were observed being mainly negative for the xenoestrogenic and positive for the xenoandrogenic data. Lifestyle characteristics seemed to be predictors of the xenohormone transactivities.

Conclusions: We suggest that the observed xenohormone transactivities reflects the profile of the actual POP mixture found in serum, and that age, gender and food intake are predictors of the xenohormone transactivity.

1. INTRODUCTION

The increasing load of the environment by man-made pollutants is of concern for the human health. Due to long-range transport by atmospheric and oceanic currents [1, 2] the human exposure is not limited to individuals living close to the sources of the contaminants. The lipophilic persistent organic pollutants (POPs) includes polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs), polychlorinated biphenyls (PCBs) and certain organochlor pesticide residues e.g. 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT), its metabolite 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (*p,p'*-DDE), toxaphenes and chlordanes. Due to their high lipophilicity and resistance to biodegradation the POPs are biomagnified through the food chain and found in fatty tissues at high concentrations in predator fish and birds, seals, whales and polar bears [3]. Greenlandic Inuits display high body burden of POPs [4, 5] that significantly correlate with the level of n-3 polyunsaturated fatty acids in plasma, being strong indicators of the main source of POP contaminants in their traditional marine food [6-9].

In several Greenlandic districts the PCB body burden levels exceed Health Canada guidelines of concern (5 – 100 µL/L plasma) [10]. Also within Greenland strong regional differences are observed with the highest contaminant levels found in Inuits at the East coast [11], where also higher levels of PCBs and DDT are found in marine species and birds compared to the West coast [12, 13]. Some districts and settlements such as Qaanaaq (North West) still primarily rely on traditional foods, whereas the diet in the cities Nuuk and Sisimiut (South West) are more westernized [11, 14].

During the last two decades, a number of POPs have been identified and characterized as being estrogenic, anti-estrogenic and/or anti-androgenic [15-21], and to have a multitude of potential health effects on wildlife and humans, including immunotoxicity, carcinogenicity, and adverse

effects on reproductive, neurobehavioral, and endocrine functions [22-27]. Recently also endocrine-related human health effects of POPs on child development were reported at the individual or population level [28-30]. However, the toxicological assessment of humans is complicated. Not only is the analytical chemical approach for the detection of all xenobiotics practically impossible especially on large-scale surveys but additive enhancement of hormone actions has been reported *in vitro* for xenoestrogen mixtures [31, 32] and recently *in vivo* for antiandrogens [33]. Therefore, the assessment of the integrated biological effect of the actual chemical mixture in human blood is important and *ex vivo* cell systems have recently been introduced to enable the assessment of the integrated level of xenobiotic transactivity in human adipose tissue [23, 34, 35] or in human serum [36-40]. We have previously validated the SPE-HPLC extraction method and it was demonstrated that the ER- and AR-CALUX assays respond to a wide range of different compounds including PCBs and pesticides [15, 16] as well as to PCB-spiked serum extracts [38].

The aims of the present study were 1) to determine the integrated xenohormone transactivities as an integrated exposure marker of total lipophilic serum POPs comparing the effect on ER and AR transactivity between Greenlandic districts and 2) to evaluate the associations of xenohormone transactivity to serum POP concentrations and lifestyle characteristics.

2. METHODS

2.1 Study population and collection of blood samples

The subjects and sampling methods have been described in detail elsewhere [8]. However, because of the design this study included only a subset of the samples of the original study population [8]. All participants from Nuuk and Sisimiut in South West Greenland and Qaanaaq in North West Greenland were of Inuit decent, defined as having more than two grandparents born in Greenland. The data of the Sisimiut men was also a part of the EU project INUEDO (www.inuedo.dk). All participants completed a standard questionnaire including questions about demographic and lifestyle parameters. Venous blood samples were taken and prepared for determination of POPs and fatty acid profiles and stored at -80° C until analyzed as described [8].

2.2 Determination of POPs and fatty acids

Plasma samples were analyzed for POPs including *cis*-, *trans*- and oxy-chlordane, *p,p'*-DDE, *p,p'*-DDT, hexachlorobenzene (HCB), β -hexachlorocyclohexane (β -HCH), mirex, toxaphene 26, toxaphene 50 and 14 PCB congeners (CB28, CB52, CB99, CB101, CB105, CB118, CB128, CB138, CB153, CB156, CB170, CB180, CB183, CB187) by gas chromatography (GC) at the certified laboratory, Le Centre de Toxicologie, Sainte Foy, Quebec, Canada [8, 41]. All determined POPs were adjusted to the plasma lipid content analyzed from the corresponding samples [8, 41] and reported as $\mu\text{g} / \text{kg}$ lipid.

The fatty acid profiles were determined in plasma phospholipids at the Biology Department, University of Guelph, Canada [42]. The n-3 polyunsaturated fatty acids were reported on the sum of C18:3, n-3, C20:4, n-3, C20:5, n-3, C22:5, n-3 and C22:6, n-3, and the n-6 fatty acids was the sum of C18:2, n-6, C18:3, n-6, C20:2, n-6, C20:3, n-6 and C20:4, n-6.

2.3 SPE-HPLC fractionation of the serum samples

To obtain the serum fraction containing the actual mixture of bio-accumulated lipophilic POPs a solid phase extraction (SPE) and high performance liquid chromatography (HPLC) fractionation was performed on 3.6 ml serum [38]. The first fraction (F1: 0.00 – 5.30 min.) was defined to include most POPs and free for endogenous hormones [38]. This SPE-HPLC F1 extract was evaporated and stored at -80° C for later ER or AR mediated CALUX assay.

A set of serum control samples were prepared by combining batches of male and female serum (blood bank of Aarhus Sygehus, DK), respectively, and distributed into 3.6 ml portions and stored at -80° C. One serum sample from each sex was, on a weekly basis, processed by the SPE-HPLC method in parallel with the study samples serving as serum control for the cleanup procedure.

On the day of analysis the SPE-HPLC F1 extracts (samples and controls) were thawed and processed as previously described [36, 39]. The samples were analyzed randomly attempting to analyze samples from different districts in each independent assay.

2.4 Receptor chemically activated luciferase gene expression (CALUX) assays

SPE-HPLC serum extracts were analyzed in order to assess the ability of the lipophilic POPs to affect the ER or AR transactivity (referred to as XER and XAR, respectively). Moreover, we determined the effect of the serum extracts in the presence of the respective high affinity ligand of the receptors (the ER agonist 17 β -estradiol (E2); the synthetic AR agonist methyltrienolone (R1881)) to mimic the physiological process assessing the ability of the lipophilic POPs to compete with the endogenous hormones / ligands (called XERcomp and XARcomp).

The ER transactivation response (ER-CALUX) were determined in the stable transfected MVLN cells, a derivative of the ER positive MCF-7 cell line, carrying the ERE-luciferase reporter vector as described previously [36, 43]. AR-CALUX transactivity was determined in the Chinese Hamster

Ovary cells (CHO-K1) by transient co-transfection with the mouse mammary tumor virus-luciferase (MMTV-LUC) reporter vector (kindly provided by Dr. Ronald M. Evans, Howard Hughes Medical Institute, San Diego, CA, USA) and the AR expression plasmid pSVAR0 (kindly provided by Dr. A.O. Brinkmann, Erasmus University, Rotterdam, The Netherlands) [39]. The determined luciferase activity per well was corrected for cell density using protein measurements and expressed in relative luciferase units per microgram protein (RLU / μg protein) equal to a response of 0.328 ml whole serum. The controls and the SPE-HPLC F1 serum extracts were determined at least in triplicate. If one of the triplicate values deviated more than 30% from the other two values, the mean was calculated from two wells only. The mean of the samples were related to the respective solvent controls.

In each independent assay a concentration-response control for the receptor ligand (E2 or R1881) was analyzed in parallel. The solvent controls \pm E2 or R1881 were handled and analyzed in the same way as the SPE-HPLC F1 extracts [36, 39]. The average ER-CALUX intra-coefficient of variation (CV) of the serum extracts and inter-CV of solvent controls were below 5%, and for AR-CALUX the intra-CV of the serum extracts and inter-CV of solvent controls were 11% and 14%, respectively. No visual cell toxicity was observed upon exposure of MVLN or CHO-K1 cells to the serum extracts.

2.5 Statistical analysis

In each independent CALUX-assay transactivity differences between the triple serum extract determinations and their respective solvent controls (% agonistic, % decreased, % further increased and % antagonistic) were tested by the Student's T-test in Microsoft Excel ($p \leq 0.05$). For the XER data eliciting significant agonistic transactivity, the E2 equivalents (XER-EEQ) were calculated by interpolation to the E2 concentration-response curves using Sigma plot. The equivalence factor for

AR could not be calculated since the weak agonistic responses did not reach the linear range of the R1881 dose-response curve.

To improve normal distribution the serum ER and AR transactivity and the POP data were natural logarithmic transformed for the statistical analysis. The analyses were performed on continuous data. One-way ANOVA was used to compare levels of xenohormone transactivities and POPs among the districts. If differences were observed, multiple comparison *ad hoc* test were performed using the least significant difference (LSD) pair wise multiple comparison test for the variables with equal variance ($p > 0.05$) and Dunnett's T3 test for the variables with an unequal variance ($p \leq 0.05$). The homogeneity of variance was tested by Levene's test. The comparisons of means between xenohormone serum transactivity in men and women were performed by Student's t-test. Pearson's correlation analyses (two-tailed) were used to evaluate the interrelationship between the 14 PCB congeners and the 10 pesticides as well as associations between xenohormone transactivity and lifestyle characteristics.

Associations between the xenohormone transactivity and the POPs were analyzed in a linear regression model. To our knowledge no experience about which dietary and/or other life-style characteristics that might influence the xenohormone transactivity is reported. We hypothesized that a potential predictor of POP bioaccumulation might also be a potential predictor for serum xenohormone transactivity. As known from literature age and seafood affect the serum POP levels [44, 45]. Also body mass index (BMI), smoking years and bird intake might influence the serum POP levels [8]. Using the multivariate linear regression model, assessing the relation between the xenohormone transactivities and the POPs the impact of the lifestyle characteristics were evaluated by entering variables together with the POPs. Due to the small sample sizes this was not done for the separate sexes in each district.

The statistical analysis was performed in SPSS 13.0 (SPSS Inc, Chicago, IL). The term statistically significant level is used to denote a p-value ≤ 0.05 .

3. RESULTS

3.1 Lifestyle characteristics of the study groups

Demographic and lifestyle factors that potentially influence the ER and AR mediated transactivities of the study groups are shown in Table 1. The age of the participants was 18-72 years. The Nuuk men were older ($p < 0.001$) than the rest of the study groups, having similar age range. BMI did not differ between the participants. Nuuk men had the highest smoking years value ($p < 0.05$), the highest n-3/n-6 fatty acid ratio ($p < 0.01$) and the highest level of seabird intake ($p < 0.03$) compared to the rest of the subjects. The consumption of dairy food was highest in Nuuk and Sisimiut women ($p < 0.05$) and generally male participants had lower dairy food intake than women ($p < 0.05$). Age and n-3/n-6 ratio was significantly correlated ($p < 0.05$) except for Nuuk women.

3.2 POP levels and intercorrelations

For PCB28, PCB52 and PCB128 more than 70% of the samples were at the detection limit (0.01 μg) whereas 43% and 32% of the samples were at the detection limit for PCB101 and PCB105, respectively.

High intercorrelations between the PCBs ($r > 0.82$) were observed except for PCBs of lower concentration: PCB28 ($0.24 < r < 0.64$), PCB52 ($0.24 < r < 0.64$), PCB101 ($0.66 < r < 0.82$) and PCB128 ($0.54 < r < 0.74$). The pesticides were highly intercorrelated ($r > 0.75$).

However, different POP correlations were observed for the study groups. Generally in Nuuk and Qaanaaq the correlations among PCBs and pesticides were higher than in Sisimiut, and higher correlations were observed for men compared to women.

Due to difference in chemical structure and toxicological properties we grouped the POPs as following: $\Sigma\text{PCB-12}$ (all PCBs except PCB28 and PCB52), $\Sigma\text{PCB-estrogenic}$ (PCB28 + PCB52 + PCB99 + PCB 101) [46], $\Sigma\text{PCB-antiestrogenic}$ (all PCBs except PCB28 + PCB52 + PCB99 + PCB 101), $\Sigma\text{DL-PCB}$ (dioxin like PCB105 + PCB118 + PCB156), ΣPCB ,

Σ chlordanes, Σ toxaphenes, DDT+DDE, Σ pesticide and total Σ POP. However, since the results regarding the associations between xenohormone transactivities and the defined POP groups were similar, we only show the results for Σ PCB, Σ pesticide and Σ POP.

In general, male participants had higher POP levels than women for all districts (Table 2). For the men, similar median levels of Σ PCB, Σ pesticide and Σ POP were observed in Nuuk and Qaanaaq, being significantly higher than in Sisimiut ($p < 0.001$). Qaanaaq women had significantly higher POP levels than that of Nuuk and Sisimiut women ($p < 0.001$) as reported by Deutch et al [8]. For the combined sex data, significant different POP levels were observed between the districts in the order Qaanaaq > Nuuk > Sisimiut ($p \leq 0.003$). The pattern of regional difference did not change after adjustment for age (data not shown).

3.3 Xenoestrogenic transactivities

The serum POP extracts of both sexes predominantly decreased/inhibited the XER and XERcomp transactivities (Table 3). The XER transactivities of Sisimiut subjects were significantly lower than Nuuk (male $p = 0.012$; female $p < 0.001$), and for females the Sisimiut XER transactivities were lower than Qaanaaq ($p = 0.012$).

For all districts except Nuuk women the median XERcomp value was below the reference level, and the XERcomp transactivities differed among the districts (Nuuk > Qaanaaq > Sisimiut; $p < 0.001$) for both men and women except between Sisimiut and Qaanaaq women. A higher percentage of men elicited antagonistic effect on XERcomp transactivity compared to women ($p < 0.001$).

3.3.1 Correlation between xenoestrogenic transactivities and lifestyle characteristics

XER transactivities were negatively correlated to age in Nuuk and Sisimiut (Table 4A), whereas positive correlations were observed for XER and n-3/n-6 fatty acid ratio and smoker years in Qaanaaq and for the combined men (Table 4A).

For XERcomp negative correlations were observed to n-3/n-6 fatty acid ratio in Nuuk and for all women, while positive correlations were found to age and smoker years for all men and the total combined data (Table 4A).

3.3.2 Association between xenoestrogenic transactivities and POPs

Few and scattered significant correlations were observed between the xenoestrogenic transactivities and the single PCBs and pesticides (data not shown).

The XER data of men from Nuuk (n = 32) and Sisimiut (n = 51) showed significant positive and negative associations, respectively, to Σ PCB (Nuuk: $\beta = 0.36$, $p = 0.04$; Sisimiut: $\beta = -0.40$, $p < 0.04$), Σ pesticide (Nuuk: $\beta = 0.34$, $p = 0.05$; Sisimiut: $\beta = -0.45$, $p = 0.001$) and Σ POP (Nuuk: $\beta = 0.36$, $p = 0.04$; Sisimiut: $\beta = -0.43$, $p = 0.001$).

Negative correlations were observed between XERcomp transactivities and POPs for the combined genders in Sisimiut (Figure 1A), which did not substantially change after adjustment for lifestyle characteristics (Table 5A). No correlations were observed for Nuuk and Qaanaaq samples. Also the combined women and the total combined data across the districts correlated negatively to the POPs upon adjustment for age. Positive correlations were only observed for the combined men across districts, which disappeared upon adjustment for lifestyle characteristics.

3.4 Xenoandrogenic transactivities

The XAR transactivities (Table 3) did not differ significantly between the districts neither for men nor women. The XARcomp transactivities (Table 3) for men differed between districts ($p < 0.001$) and the XARcomp transactivity for Nuuk women was higher ($p = 0.001$) than Sisimiut and Qaanaaq women, which did not mutually differ.

3.4.1 Correlation between xenoandrogenic transactivities and lifestyle characteristics

The XAR transactivities only correlated negatively to seabird intake in Sisimiut (Table 4B).

In Nuuk the XARcomp transactivities correlated positively to age, n-3/n-6 fatty acid ratio, BMI, and seabird intake. Age and smoker years were also positively correlated to the XARcomp in the total data. Only in Sisimiut a negative correlation to n-3/n-6 ratio were observed (Table 4B).

3.4.2 Association between xenoandrogenic transactivities and POPs

Few and scattered significant correlations were observed between the xenoandrogenic transactivities and the single PCBs and pesticides (data not shown). For the XAR transactivity no correlations were observed.

In Nuuk significant positive correlations were found between XARcomp and all three Σ POPs (Σ PCB, Σ pesticide and Σ POP) (Table 5B and Figure 1B) not changing upon adjustment for lifestyle characteristics. The combined male data across the districts showed negative correlations between XARcomp and the POPs upon adjustment for age and n-3/n-6 ratio. For the total combined data only the non-adjusted XARcomp positively correlated to Σ PCB and Σ POP.

3.5 The ratio of xenoandrogenic to xenoestrogenic serum transactivity

Generally women showed lower XAR/XER ratio and significantly lower XARcomp/XERcomp ratio ($p < 0.02$) compare to men (Table 3). Qaanaaq men elicited lower XARcomp/XERcomp ratio ($p < 0.001$) compare to Nuuk and Sisimiut men, whereas for women the XARcomp/XERcomp ratios were similar for all three districts.

4. DISCUSSION

In the present study we determined the actual xenohormone serum transactivity as a biomarker of POP exposure and evaluated whether the transactivity was associated to POP levels and the lifestyle characteristics across the Greenlandic districts Nuuk, Sisimiut and Qaanaaq.

In general, serum POP extracts, free of endogenous hormones, elicited low level of agonistic ER and AR transactivity (XER, XAR) for both genders. The effect of the serum extract on ligand induced receptor transactivity (XERcomp, XARcomp) was performed as a mimic of *in vivo* physiological hormone processes. Predominantly, antagonistic XERcomp and XARcomp transactivities were found except for serum extracts of Nuuk and Sisimiut men eliciting further increased XARcomp transactivities.

In accordance with previous studies, Qaanaaq inhabitants, who intake more marine and traditional diet, had in general significantly higher levels of POPs compared to those in Nuuk and Sisimiut, eating more westernized diet. However, in the present study the serum samples of men in Nuuk contained a higher level of POPs due to their higher average age and intake of seafood and seabird [8]. In all three districts the serum POP levels of men were significantly higher than for women, reflecting the higher intake of traditional food by men. Moreover, despite a high intercorrelation between POPs, the correlations between districts and genders differed as a result of different exposure pattern.

In Arctic populations determinants of serum POP concentration are age, n-3/n-6 fatty acid ratio as a marker of the intake of marine food [9, 47], smoking, male gender and district [8]. In this study we observed associations between the xenohormone transactivities and age, n-3/n-6, BMI, smoker years and seabird intake suggesting, that these lifestyle characteristics are predictors of the xenohormone transactivities. However, heterogeneous associations were observed for the study groups suggesting, that also sex and district influence the

xenohormone transactivity. Thus the determinants of the serum POP concentration seem also to be predictors of the xenohormone transactivities supporting the use of xenohormone transactivities as an integrated biomarker of POP exposure. In Nuuk and Sisimiut XER and XERcomp correlated negatively to age or n-3/n-6 whereas XARcomp correlated positively to age and n-3/n-6, suggesting that increased serum POP level might cause an antagonized ER but increased AR function, respectively. However, for Qaanaaq subjects no correlations between xenohormone transactivity and age or n-3/n-6 ratio was found. For the total study population and the combined male data positive correlations between xenohormone transactivities and lifestyle characteristics were observed. We suggest that the differences in food intake and life styles between sexes and districts affect the profile and level of serum POPs and consequently the serum POP related xenohormone transactivity.

The XERcomp transactivities for the total study group correlated inversely to the age-adjusted POP levels, supporting the antiestrogenic actions by the POPs in Greenland [36]. Additionally, XER and XERcomp transactivities for Sisimiut correlated negatively to all three Σ POPs. However, no consistent correlations were found across the districts, which could reflect the different composition of the POPs in the body as well as the statistical power.

In support to the present report we found in a previous study [36], evaluating the xenoestrogenic serum transactivity of male study groups from Europe and Greenland, a negative correlation between the transactivity and the two POP proxy markers PCB153 and *p,p'*-DDE for Inuit's. Although using another approach it was recently reported that high levels of PCBs in Slovakia male serum samples were associated with a decreased ER mediated activity and increased AhR mediated activity [46]. Data on the ER-mediated activity in human blood samples are still scarce, but few similar studies using the E-screen MCF-7 cell proliferation as the end point of xenoestrogenic transactivities have been reported [23, 34, 35, 37]. However, in contrast to our studies no correlation between POPs and

xenoestrogenicity were found. We believe that the high impact of POP burden in the Inuits is responsible for the inhibited ER function in line with our earlier *in vitro* study [16].

Humans are exposed to a complex mixture of compounds that may act in concert and influence the overall effect of the serum transactivity. Therefore, it remains to be demonstrated mechanistically how environmental chemicals characterized either as weak estrogens or antiestrogens [16, 37, 46, 48-56] or antiandrogens [15, 16, 18, 21, 57-61] will respond in a concerted action and it must be taken into account that different POP composition can cause different xenobiotic transactivities. The future use of the integrated estrogenicity of tissue fluid for health risk assessment was supported by a recent report showing association between xenoestrogenic transactivity of adipose extracts and breast cancer risk of leaner women [23].

In the present study we found for Nuuk and Sisimiut men a high frequency of samples further increasing the XARcomp transactivity, whereas antagonized XARcomp transactivities were observed for Qaanaaq men and for women from all districts. Numerous POPs have been shown to be anti-androgenic *in vivo* and *in vitro* [15, 16, 18, 21, 57-61]. In contrast, androgenic effects have been found in the environment. Craft mill effluent from a river in Florida contained a chemical mixture that induced AR-dependent gene expression *in vitro* [62] and the endocrine basis for masculinised female fish detected in waters near craft mill effluents was confirmed [62].

For the combined genders of Nuuk positive correlations were observed between XARcomp transactivities and age, n-3/n-6, BMI and seabird intake. Furthermore, positive correlations between XARcomp and the POPs were found. We speculate whether the relatively broad range of age in combination with differences in food intake causes these associations between XARcomp and POP levels for Nuuk participants although it can not be assessed whether POPs or lifestyle characteristics exert the main impact on the XARcomp transactivity.

For the combined male data negative correlations were observed between XARcomp transactivities and the POPs after adjustment for age. We previously observed negative correlations between XARcomp and the POP proxy marker *p,p'*-DDE for combined European study groups [39]. Thus, for men a higher POP level might cause an antagonized AR function.

In contrast to our method, cell-based reporter assays for determination of total androgenic activity in whole human serum including endogenous hormones have previously been described [63-65]. Paris et al. [63] reported that the total androgenic activity in pubertal girls was approximately 15% of that in boys. In our study the determined xenoandrogenic transactivity of the serum POP fraction free of endogenous hormones in women was approximately 90% of that in men supporting the removal of endogenous hormones but reflecting the higher POP levels in men.

Interestingly, we found higher androgenicity for the male Inuits compared to the daily serum controls of Danish men (see section 2.3) as well as male European study groups [39]. At this point it is not possible to assess whether this change in xenohormone transactivity can have any physiological role for the hormone homeostasis, but it was proposed, that exposure to a mixture of chemicals with anti-androgenic or estrogenic properties may affect the androgen-estrogen ratio and thereby influence the risk of cryptorchidism [66].

In another parallel study we determined serum dioxin-like transactivity mediated via the aryl hydrocarbon receptor (AhR) [67]. Dioxins are known to exert anti-estrogenic actions [68, 69]. Interestingly, xenoestrogenic transactivities correlated negatively to the AhR transactivity supporting the presence of antiestrogenic POPs in the serum. The highest frequency of decreased XER and antagonized XERcomp transactivity was observed for Sisimiut and Qaanaaq men, that also showed the highest frequency of further increased

AhRcomp transactivity [67] suggesting an antiestrogenic action of serum POPs mediated via AhR for Sisimiut and Qaanaaq men.

Some AhR agonists elicit anti-androgenic effects although the exact mechanisms are not clear [70-72]. In support, the lowest and highest AhR transactivity [67] and opposite the highest and lowest XARcomp values (this study) were observed in Nuuk and Qaanaaq men, respectively. XARcomp correlated negatively to the AhR transactivity suggesting the presence of AhR agonistic substances in the serum. Whether the observed changes in xenohormone and dioxin-like transactivity have any effect on human health can at this point only be speculative. We did however previously observed negative correlations for xenoestrogenic transactivity and dioxin-like transactivity to sperm chromatin integrity and DNA damage for Inuits and positive correlations between xenoandrogenic and dioxin-like transactivity and sperm chromatin integrity and DNA damage for Europeans [73, 74]. Further studies are needed to elucidate the possible association between POP related xenohormone transactivity and human health risk biomarkers.

5. CONCLUSIONS

Whether our *ex vivo* data mimic the cellular and molecular effects *in vivo* awaits further studies. It must be taken into consideration that the presented data gives the final effect on the given receptor alone and that other factors such as receptor and/or co-factor interactions might precede the final data. We suggest that the xenoestrogenic and xenoandrogenic actions observed in the present study reflect the ability of serum POPs to interfere with the ER / AR functions. We conclude that the integrated serum POP xenohormone transactivity reflects the POP profile of the actual mixture found in human blood, and that age, gender and/or food intake are predictors of the xenohormone transactivity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

TK, MG and ECB-J drafted the manuscript and were responsible for data evaluation and statistical analyses. TK carried out the AR-CALUX analyses. MG carried out the ER-CALUX analyses. PSH carried out the SPE-HPLC fractionation of the serum samples and commented on the manuscript. BD evaluated the POP data and questionnaires concerning lifestyle characteristics and established the database. ECB-J was responsible for the design of the study. All authors read and approved the final manuscript.

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FIGURE LEGEND

Figure 1. Illustration of selected associations to Σ POP

A: The significant correlation for the XERcomp in Sisimiut. **B:** The significant correlation for XARcomp in Nuuk. **C:** The non-significant correlation for XARcomp in Qaanaaq. Similar associations were observed for Σ PCB and Σ pesticide.

Table 1. Lifestyle characteristics

		<i>Men</i>				<i>Women</i>				<i>Men + Women</i>			
		Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All
Age (years)	n	32	51	38	121	45	42	32	119	77	93	70	240
	median	55	30	34	36	38	32	34	35	42	31	34	36
	mean	54	31	33	38	36	33	32	34	44	31	33	36
	min	38	18	19	18	19	18	18	18	19	18	18	18
	max	72	46	45	72	45	44	44	45	72	46	45	72
BMI (kg/m ²)	n	30	51	38	119	43	42	32	117	73	93	70	236
	median	28	26	27	27	26	26	24	25	26	26	25	26
	mean	27	27	27	27	26	27	25	26	26	27	26	26
	min	22	19	20	19	18	19	18	18	18	19	18	18
	max	35	36	41	41	32	47	34	47	35	47	41	47
Smoking (years)	n	29	51	37	117	42	42	30	114	71	93	67	231
	median	35	11	18	18	20	12	16	15	22	12	17	16
	mean	31	12	17	18	16	12	15	14	22	12	16	16
	min	0	0	0	0	0	0	0	0	0	0	0	0
	max	59	26	30	59	33	27	29	33	59	27	30	59
n-3/n-6	n	32	37	38	107	45	38	32	115	77	75	70	222
	median	0.55	0.22	0.39	0.35	0.25	0.29	0.37	0.29	0.31	0.25	0.38	0.31
	mean	0.69	0.29	0.50	0.48	0.29	0.32	0.43	0.34	0.45	0.30	0.47	0.41
	min	0.20	0.10	0.09	0.09	0.12	0.14	0.15	0.12	0.12	0.10	0.09	0.09
	max	1.65	0.73	1.45	1.65	0.72	0.82	1.26	1.26	1.65	0.82	1.45	1.65
Seabird intake (per month)	n	30	48	36	114	44	41	31	116	74	89	67	230
	median	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	mean	4.6	2.7	2.4	3.1	1.4	1.3	3.5	1.9	2.7	2.0	2.9	2.5
	min	0	0	0	0	0	0	1	0	0	0	0	0
	max	20	28	20	28	8	2	28	28	20	28	28	28
Dairy food consumption (per month)	n	31	50	38	119	45	42	32	119	76	92	70	238
	median	36	51	32	40	56	57	37	50	48	51	36	44
	mean	37	49	31	40	53	49	35	47	47	49	33	43
	min	4	8	3	3	2	0	0	0	2	0	0	0
	max	76	84	58	84	84	84	84	84	84	84	84	84

Table 2. Lipid adjusted serum levels of the POPs

Σ PCB: sum of 14 PCB congeners. Σ pesticide: sum of the 10 pesticide residues. Σ POP: sum of all 24 POPs

		Men				Women				Men + Women			
		Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All
Σ PCB (μ g/kg lipid)	n	32	51	36	119	44	42	30	116	76	93	66	235
	median	2680	540	1830	1350	300	350	1190	450	760	500	1380	750
	mean	3040	750	2630	1940	490	460	1250	680	1560	620	2000	1310
	min	490	130	620	130	97	67	99	67	97	67	99	67
	max	11100	2370	7100	11100	2430	1380	3490	3490	11100	2370	7100	11100
Σ pesticide (μ g/kg lipid)	n	32	51	36	119	44	42	30	116	76	93	66	235
	median	3500	740	2590	1590	480	500	1670	640	990	680	2150	940
	mean	4070	1010	3740	2660	680	700	1890	1000	2110	870	2900	1840
	min	410	140	620	140	84	76	78	76	84	76	78	76
	max	19100	3540	14400	19100	2880	3010	7530	7530	19100	3540	14400	19100
Σ POP (μ g/kg lipid)	n	32	51	36	119	44	42	30	116	76	93	66	235
	median	6420	1360	4390	3020	760	830	2850	1110	1900	1250	3410	1880
	mean	7250	1770	6380	4640	1190	1170	3140	1690	3750	1500	4910	3180
	min	910	290	1240	290	180	150	190	150	180	150	190	150
	max	30800	5910	19900	30800	5370	4350	10800	10800	30800	5910	19800	30800

Table 3. Xenoestrogenic and xenoandrogenic transactivities of the serum POP fraction

		Male serum				Female serum				Male + female serum			
		Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All	Nuuk	Sisimiut	Qaanaaq	All
XER RLU/ μ g protein	<i>n</i>	32	51	36	119	45	42	32	118	77	92	68	237
	median	1.00	0.95	0.97	0.97	1.05	0.90	1.01	0.99	1.01	0.92	0.99	0.97
	mean \pm SD	1.00 \pm 0.08	0.94 \pm 0.08	0.98 \pm 0.09	0.97 \pm 0.09	1.03 \pm 0.08	0.94 \pm 0.23	1.00 \pm 0.10	0.99 \pm 0.16	1.02 \pm 0.08	0.94 \pm 0.17	0.99 \pm 0.09	0.98 \pm 0.13
	% agonistic	6	2	14	7	4	5	6	5	5	3	10	6
	% decreased	9	33	25	24	9	20	28	18	9	27	27	21
EEQ pg/g lipid	<i>n</i>	2	0	4	6	3	2	5	10	5	2	9	16
	median	22	-	135	88	14	43	37	33	20	43	88	35
	mean \pm SD	22 \pm 2.8	-	119 \pm 85	114 \pm 104	18 \pm 9.3	43 \pm 18	67 \pm 49	48 \pm 41	20 \pm 7.2	43 \pm 18	90 \pm 68	75 \pm 78
XERcomp RLU/ μ g protein	<i>n</i>	32	51	38	121	45	42	32	118	77	92	70	239
	median	0.97	0.82	0.93	0.89	1.03	0.96	0.91	0.96	1.00	0.85	0.92	0.93
	mean \pm SD	0.99 \pm 0.09	0.84 \pm 0.09	0.91 \pm 0.12	0.90 \pm 0.12	1.02 \pm 0.11	0.95 \pm 0.13	0.90 \pm 0.12	0.96 \pm 0.13	1.01 \pm 0.11	0.89 \pm 0.12	0.91 \pm 0.12	0.93 \pm 0.13
	% further increased	6	2	0	3	34	10	6	14	17	5	3	8
	% antagonistic	16	75	37	47	34	0	50	23	21	41	44	35
XAR RLU/ μ g protein	<i>n</i>	34	33	38	110	41	28	33	102	75	61	71	212
	median	1.21	1.12	1.13	1.16	1.14	0.99	1.01	1.06	1.17	1.09	1.06	1.12
	mean \pm SD	1.22 \pm 0.27	1.20 \pm 0.29	1.16 \pm 0.27	1.20 \pm 0.27	1.17 \pm 0.29	1.01 \pm 0.18	1.09 \pm 0.27	1.10 \pm 0.26	1.19 \pm 0.28	1.11 \pm 0.26	1.13 \pm 0.27	1.15 \pm 0.27
	% agonistic	18	22	11	17	15	0	12	10	16	11	11	13
	% decreased	6	3	0	3	2	0	0	1	4	2	0	2
XARcomp RLU/ μ g protein	<i>n</i>	34	33	38	110	41	28	33	102	75	61	71	212
	median	1.38	1.17	0.84	1.17	0.94	0.80	0.84	0.87	1.16	0.99	0.84	0.99
	mean \pm SD	1.40 \pm 0.27	1.24 \pm 0.37	0.90 \pm 0.22	1.18 \pm 0.36	0.99 \pm 0.22	0.81 \pm 0.13	0.86 \pm 0.21	0.90 \pm 0.21	1.18 \pm 0.32	1.05 \pm 0.36	0.88 \pm 0.22	1.05 \pm 0.99
	% further increased	50	17	5	23	5	0	6	4	25	10	6	14
	% antagonistic	0	6	24	11	12	25	21	19	7	11	23	13
XAR/XER RLU/ μ g protein	<i>n</i>	28	33	33	99	41	28	29	98	69	61	62	197
	median	1.13	1.27	1.14	1.22	1.06	1.10	1.01	1.07	1.09	1.23	1.08	1.17
	mean \pm SD	1.19 \pm 0.27	1.29 \pm 0.34	1.19 \pm 0.28	1.23 \pm 0.30	1.14 \pm 0.27	1.09 \pm 0.24	1.08 \pm 0.25	1.11 \pm 0.25	1.16 \pm 0.27	1.20 \pm 0.32	1.14 \pm 0.27	1.12 \pm 0.28
XARcomp/ XERcomp RLU/ μ g protein	<i>n</i>	28	33	34	100	41	28	29	98	69	61	63	198
	median	1.41	1.38	0.99	1.32	0.94	0.84	0.92	0.92	1.11	1.14	0.95	1.10
	mean \pm SD	1.41 \pm 0.29	1.50 \pm 0.47	1.03 \pm 0.31	1.33 \pm 0.43	0.98 \pm 0.22	0.89 \pm 0.20	0.96 \pm 0.26	0.95 \pm 0.23	1.16 \pm 0.33	1.22 \pm 0.48	1.00 \pm 0.28	1.14 \pm 0.39

Table 4. Pearson 2-tailed correlation between xenohormone transactivities and lifestyle characteristics

4A. Xenoestrogenic transactivities

		Nuuk			Sisimiut			Qaanaaq			Combined men			Combined women			Combined data		
		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
<i>XER</i>	age	77	-.24	.04	93	-.22	.04	68	.19	.12	119	.14	.14	119	-.01	.93	238	.04	.50
	n-3/n-6	77	.04	.76	75	-.18	.12	68	.04	.78	105	.20	.04	115	-.05	.57	220	.04	.52
	BMI	73	-.11	.34	93	.02	.82	68	-.17	.17	117	-.07	.47	117	-.04	.70	234	-.06	.39
	smokeysrs	71	-.10	.40	93	-.05	.65	65	.31	.01	115	.25	.01	114	.04	.69	229	.11	.09
	seabird	67	-.22	.07	86	.09	.41	64	-.04	.76	109	.06	.54	108	.05	.63	217	.04	.58
	dairy food	76	.11	.36	91	-.05	.62	67	-.11	.40	117	-.07	.44	117	-.06	.51	234	-.05	.41
<i>XERcomp</i>	age	77	-.09	.43	93	.01	.91	70	.04	.75	121	.36	<.001	119	.07	.46	240	.19	<.01
	n-3/n-6	77	-.28	.01	75	-.06	.62	70	-.23	.06	107	.09	.37	115	-.27	<.01	222	-.12	.09
	BMI	73	-.12	.31	93	-.05	.62	70	-.15	.18	119	-.09	.31	117	-.05	.61	235	-.10	.14
	smokeysrs	71	-.01	.97	93	.14	.19	67	.11	.36	117	.39	<.001	114	.08	.40	231	.19	<.01
	seabird	67	-.09	.50	86	-.08	.46	66	-.05	.69	111	.12	.21	108	-.04	.67	219	-.00	.99
	dairy food	76	-.03	.79	91	-.01	.95	69	-.05	.70	119	-.14	.13	117	.05	.59	236	-.01	.93

4B. Xenoandrogenic transactivities

		Nuuk			Sisimiut			Qaanaaq			Combined men			Combined women			Combined data		
		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
<i>XAR</i>	age	75	-.02	.87	61	-.04	.75	71	-.06	.60	105	.02	.82	102	-.02	.83	207	.03	.71
	n-3/n-6	75	.17	.15	53	-.15	.27	71	-.08	.51	99	.001	.99	100	.03	.80	199	.04	.57
	BMI	71	-.09	.48	61	-.20	.13	71	-.04	.76	103	-.10	.30	100	-.16	.12	203	-.10	.15
	smokeysrs	69	-.17	.18	61	.14	.28	67	.04	.78	100	-.01	.96	97	-.03	.76	197	.01	.85
	seabird	67	.13	.30	59	-.28	.03	68	-.01	.97	99	.02	.84	95	-.14	.19	194	.01	.91
	dairy food	74	.11	.33	59	.16	.22	69	-.10	.43	103	.09	.37	99	.05	.63	202	.05	.46
<i>XARcomp</i>	age	75	.40	<.001	61	-.13	.31	71	.03	.82	105	.33	.001	102	.03	.80	207	.26	<.001
	n-3/n-6	75	.45	<.001	53	-.28	.04	71	.03	.78	99	.03	.78	100	.002	.98	199	.09	.22
	BMI	71	.29	.02	61	-.02	.86	71	.07	.59	103	.01	.92	100	.10	.31	203	.10	.15
	smokeysrs	69	.21	.08	61	-.07	.62	67	.09	.49	100	.16	.12	97	.05	.63	197	.18	.01
	seabird	67	.35	<.01	59	-.02	.89	68	-.04	.75	99	.13	.19	95	-.14	.18	194	.13	.07
	dairy food	74	-.13	.27	59	.02	.89	69	.20	.11	103	.17	.09	99	.14	.18	202	.10	.17

Table 5. Linear regression analyses before and upon adjustment for lifestyle characteristics

A. XERcomp transactivities

		Nuuk			Sisimiut			Qaanaaq			Combined men			Combined women			Combined data		
		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
ΣPCB	Non-adjusted	76	-.12	.30	93	-.22	.04	66	-.09	.48	119	.22	.02	116	-.14	.14	235	-.08	.23
	+age	76	-.12	.52	93	-.26	.02	66	-.15	.30	119	-.00	.99	116	-.18	.07	235	-.39	<.01
	+n-3/n-6	76	.21	.20	75	-.24	.06	66	.10	.52	105	.27	.06	112	.05	.62	217	-.02	.82
	+age+n-3/n-6	76	.21	.34	75	-.26	.05	66	.03	.86	105	.10	.47	112	.01	.92	217	-.13	.15
$\Sigma pesticide$	Non-adjusted	76	-.17	.14	93	-.21	.04	66	-.15	.23	119	.17	.07	116	-.20	.03	235	-.12	.08
	+age	76	-.21	.21	93	-.26	.02	66	-.26	.09	119	-.07	.50	116	-.24	.01	235	-.27	<.001
	+n-3/n-6	76	.09	.58	75	-.26	.04	66	.03	.87	105	.13	.39	112	-.02	.84	217	-.10	.28
	+age+n-3/n-6	76	.03	.88	75	-.28	.04	66	-.08	.65	105	-.03	.86	112	-.06	.58	217	-.20	.03
ΣPOP	Non-adjusted	76	-.15	.19	93	-.21	.04	66	-.13	.29	119	.20	.03	116	-.18	.06	235	-.10	.13
	+age	76	-.18	.30	93	-.26	.02	66	-.23	.13	119	-.04	.71	116	-.22	.03	235	-.26	.001
	+n-3/n-6	76	.14	.39	75	-.26	.05	66	.05	.76	105	.21	.16	112	.01	.94	217	-.06	.48
	+age+n-3/n-6	76	.09	.65	75	-.27	.04	66	-.05	.79	105	.04	.80	112	-.04	.76	217	-.17	.06

B. XARcomp transactivities

		Nuuk			Sisimiut			Qaanaaq			Combined men			Combined women			Combined data		
		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
ΣPCB	Non-adjusted	74	.53	<.001	61	.09	.49	68	.17	.16	104	-.05	.62	99	-.06	.56	203	.15	.03
	+age	74	.59	.001	61	.17	.24	68	.21	.15	104	-.39	.001	99	-.07	.50	203	.03	.73
	+n-3/n-6	74	.45	<.01	53	.21	.14	68	.23	.14	98	-.22	.14	97	-.04	.75	195	.19	.06
	+age+n-3/n-6	74	.52	.01	53	.22	.13	68	.25	.13	98	-.42	<.01	97	-.06	.68	195	.10	.29
$\Sigma pesticide$	Non-adjusted	74	.53	<.001	61	.06	.65	68	.12	.35	104	-.04	.68	99	-.06	.55	203	.13	.06
	+age	74	.54	.001	61	.13	.36	68	.14	.36	104	-.36	<.01	99	-.07	.50	203	.01	.95
	+n-3/n-6	74	.45	<.01	53	.19	.18	68	.15	.35	98	-.20	.19	97	-.05	.74	195	.15	.12
	+age+n-3/n-6	74	.47	.01	53	.20	.18	68	.17	.35	98	-.36	.01	97	-.06	.67	195	.08	.43
ΣPOP	Non-adjusted	74	.53	<.001	61	.08	.56	68	.14	.26	104	-.04	.67	99	-.06	.57	203	.15	.04
	+age	74	.58	.001	61	.15	.29	68	.17	.25	104	-.38	.001	99	-.07	.52	203	.02	.83
	+n-3/n-6	74	.47	<.01	53	.21	.15	68	.19	.24	98	-.21	.17	97	-.04	.77	195	.18	.07
	+age+n-3/n-6	74	.51	.01	53	.22	.14	68	.21	.23	98	-.40	<.01	97	-.06	.70	195	.10	.33

Adjustment for BMI, smoker years and bird intake did not further change the data.

Figure 1A

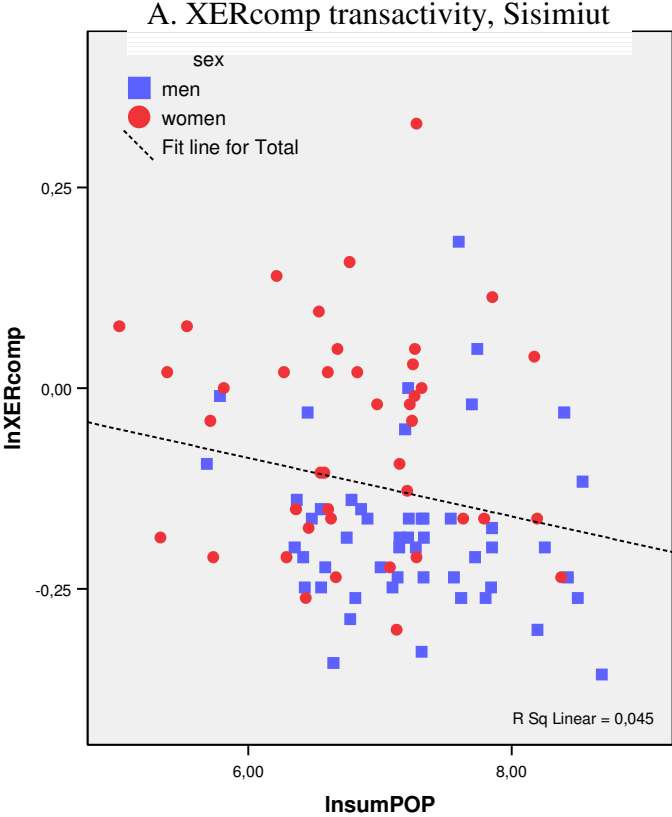


Figure 1B

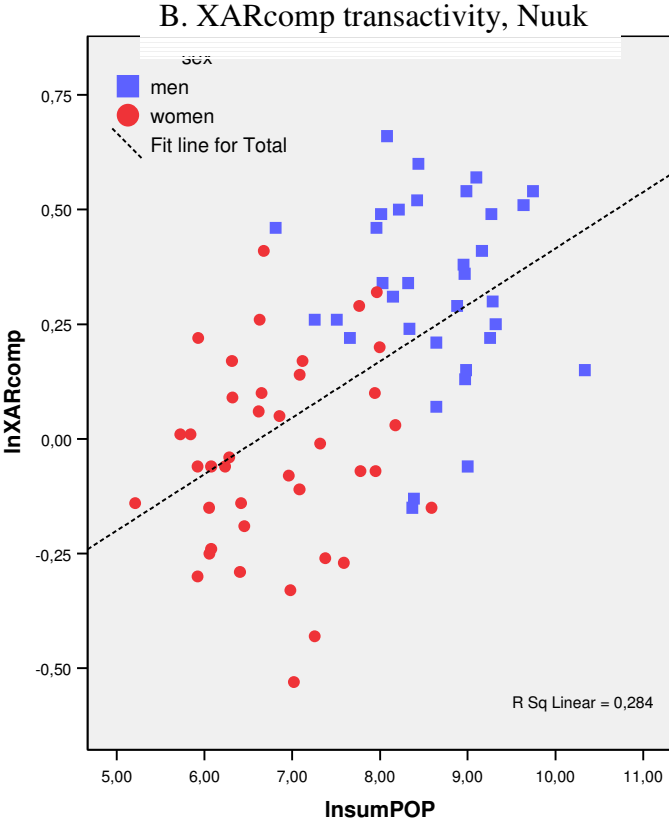


Figure 1C

