Dioxin-like activity in the blood of Greenlandic Inuit and Danish women: a pilot study

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ABSTRACT

Objectives. (1) To determine whether plasma dioxin-like (DL) bioactivity differs between Inuit living in different Greenlandic districts, (2) to compare the DL activity of the Inuit having high burdens of POPs with a low-burden study group (Danish volunteers), and (3) to evaluate DL activity associations to POP exposure biomarkers and/or lifestyle factors.

Study design. The study was a cross-sectional survey, including randomized inhabitants (70) from 6 different Greenlandic districts and young Danish volunteers (22).

Methods. POPs and fatty acids profiles were analysed. Aryl hydrocarbon receptor (AhR) mediated DL-activity was determined by exposure of Hepa1.12cR AhR-CALUX reporter cell line to hexane: ethanol plasma extracts.

Results. The sum PCBs/POPs level of Inuit was more than10 times higher than the levels found in Danish volunteers, and for both study groups the level was positively associated with age. The TCDD equivalent of the determined DL-activity, AhR-TEQ, differed between the Greenlandic districts. The AhR-TEQ data of the combined Inuit was significantly higher compared with the Danish women. AhR-TEQ of Inuit were positively associated with plasma POPs after adjustment for age and/or the ratio of n-3 to n-6 fatty acids, whereas no correlations were found for the Danish samples.

Conclusions. AhR-TEQ differs between Inuit and Danish plasma samples. Plasma POP levels alone cannot be used as a biomarker for DL-activity. We suggest that the profile and level of plasma POPs, geographical location and diet have the greatest impact on plasma dioxin activity. Further studies are needed to elucidate the differences in geographical determinants of blood DL-activity.

Keywords: AhR-TEQ, n-3 and n-6 fatty acids, persistent organic pollutants (POPs)
INTRODUCTION

Human exposure to environmental contaminants such as persistent organic pollutants (POPs) is ubiquitous, and exposure can affect subjects living far from the sources of contaminants. POPs include polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and certain pesticide residues. The burden of POPs in the Arctic is recognized as one of the highest levels ever determined. That burden significantly correlates to the level found in plasma polyunsaturated fatty acids, an indicator of the main source of these contaminants, including seals, whales, fish and for some places polar bears as well (1,2).

Exposure to POPs elicit a number of species- and tissue-specific toxic responses, including carcinogenesis, reproductive- and hepatoxicity, thymic atrophy, alteration in vitamin A and thyroid hormone metabolism, impairment of immune responses, dermal lesions and weight loss (3–6). Exposure during foetal and early life is especially critical and can cause developmental effects (7). 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 has strengthened the need for further research to address the uncertainty and concern. In fact, several epidemiological studies indicate subtle changes in neurobehavioural and endocrine parameters in infants after exposure to POPs. This can occur in the Arctic as well as in the industrialized Western world (3,8–11).

There are a number of factors that complicate the toxicological assessment. First, no individual is exposed to a single contaminant. Rather, they are exposed to a complex mixture of contaminants throughout life, beginning during critical developmental phases. Moreover, a compound may have multiple sites of action mediated via different mechanisms, and metabolites of the substances (e.g., hydroxylated PCBs) can have different biological activities than the parent compound (12,13).

Toxicity scales have been developed for compounds that share a common mechanism of action. This concept was applied to mixtures of dioxin-like compounds (DLCs) that bind to the aryl hydrocarbon receptor (AhR), an intracellular ligand-dependent transcription factor expressed in most tissues of mammals. Dioxins and furans, as well as non- or mono-ortho chloro-substituted PCBs are ligands to the AhR, mediating the expression of proteins such as P450 Phase I and Phase II enzymes, which are involved in the metabolism and detoxification of many POPs (14).

A common practice in assessing the risk of mixtures comprising DLCs is to calculate the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxic equivalents (TEQs) by multiplying the concentration of each DLC by its toxic equivalency factor (TEF). The TEF corresponds to the relative AhR potency of a particular compound compared with that of TCDD, the most potent dioxin. Thus the classical TEQ/TEF risk assessment accounts for potential dioxin-like (DL) properties mediated via AhR. Non-ortho and mono-ortho chloro PCBs are DL-PCBs. The latter group is made up of weak AhR ligands with very low or no TEF values, whereas di-ortho PCBs are non-DL-PCBs (15). The 3 most highly bioaccumulated di-ortho PCBs (138,
153, 180) make up to 55% of the total PCBs in human matrices (16,17) and they are found in Inuit breast milk and the blood of north shore fishermen in Arctic Quebec at concentrations between 10 and 30 times higher compared with Caucasian controls, respectively (18). The higher chlorinated PCBs have the potential to inhibit the AhR function (19,20). These results emphasize that assessment of the toxicological potential of a chemical mixture is much more complex than can be deduced by a given TEQ value.

There are several drawbacks to using the chemical-derived TEQ for the risk assessment of mixtures of POPs, such as expensive and time consuming gas chromatography-mass spectrometry determinations, small concentrations of individual congeners, the presence of compounds not routinely determined or unknown AhR-agonists, the lack of TEF value for several POPs and the possible additive, antagonistic or synergistic interactions (5,21,22). The AhR-chemical-activated-luciferase-expression (AhR-CALUX) bioassay can measure the activity of pure compounds (21,23) as well as the integrated activity of total DLCs, and thus the net AhR-TEQ of complex mixtures as found in bovine and human milk (24,25), and human serum and follicular fluid (17,26–29).

The aim of the present survey study was (1) to compare the level of DL-activity in plasma among Inuit living in 6 different districts of Greenland, (2) to compare the DL-activity in individuals with high and low POP burdens and (3) to further evaluate correlations of AhR-CALUX and AhR-TEQ to POP exposure biomarkers and lifestyle factors.

**MATERIAL AND METHODS**

**Subjects and sampling**

*Inuit:* The blood samples were collected under the Human Health Programme of AMAP (Arctic Monitoring and Assessment Programme) in a geographical survey including 6 Greenlandic districts regarding assessment of lifestyle versus burden from POPs. The subjects and sampling methods have been described (30). The samples were collected during the period of September 1997 to April 1998 from Scoresbysund (central east), Tassilaq (south-east), Nanortalik (south-west), Nuuk (south-west), Ilulissat (central west) and Upernavik (central west). Blood samples and questionnaires about lifestyle and demographics were included in the survey. The blood samples were analysed for POPs and fatty acid profiles (30). The project was accepted by the Ethical Commission for Scientific Investigations in Greenland.

*Danish women:* The blood samples were selected from individuals participating in a project with the aim to evaluate whether menstrual discomfort could be reduced by dietary supplements such as fatty acids (31). The young women suffering from dysmenorrhea were recruited through Danish newspapers and on university bulletin boards. Out of 70 who completed the experimental period, we randomly selected 22 blood samples taken before any form of diet supplement (controls). The project was approved by the Science Ethical Committee of the city of Aarhus.

**Ethanol-hexane extraction of POPs from the plasma/serum samples**

The extraction of plasma (Inuit) and serum (Danish women) samples to obtain the fraction containing lipophilic POPs for AhR-CALUX
activity measurements was performed at a certified laboratory, Le Centre de Toxicologie, Sainte-Foy, Quebec, Canada. We compared the DL-activity of serum and plasma samples from the same individuals, and a similar activity level was observed (not shown). Samples (2 ml) were ethanol: hexane extracted and cleaned up on 2 Florisil columns as described (32). The recovery of congeners using parallel plasma samples spiked with $^{13}$C- or $^{14}$C-PCB standards was calculated to 98–100%. The 500 µl hexane extracts for AhR-CALUX determination were stored at -80°C until analyses; then evaporated to almost dryness under a nitrogen stream and resuspended in dimethyl sulfoxide (DMSO) (BDH Laboratory Supplies, pool, UK) to a concentration where 1 µl equals 50 µl of whole plasma/serum.

**AhR-CALUX analyses**

The stably transfected mouse hepatoma cell line Hepa1.1 2cR cells carrying the AhR-luciferase reporter gene (33) provided by M. Denison were cultured and seeded into a 96-well white plate CulturPlate™ (Packard Instrument), and after 24 hours, they were exposed for 4 hours to an extract equal to 50 µl plasma/serum (reference solvent control DMSO, ≤0.1%) as described (27). Each test sample was analysed in triplicate at least 3 times. The luciferase activity was corrected to cell protein per well and expressed in relative light units per µg protein (RLUs/µg protein). The average intra coefficient of variation (CV) of samples and controls was 10% and 11%, and the inter CV of samples and controls was 25% and 20%, respectively.

In each independent experiment, a dose-response titration of TCDD (98%, Cambridge, UK) was performed at concentrations ranging from $2 \times 10^{-12}$ to $5 \times 10^{-9}$ M TCDD, diluted in DMSO and subsequently dissolved in the medium. The detection limit was defined as the lowest concentration of the sample which showed statistically significant elevated response compared with solvent control ($p \leq 0.05$). In our lab, through many independent analyses the lowest TCDD concentration eliciting significantly induced AhR transactivity in each assay was $2 \times 10^{-12}$ M (equal to 64 fg/well).

For calculation of AhR-TEQ, the AhR-CALUX data were fitted into a parallel TCDD dose-response curve using the SigmaPlot-8 software and reported as pg/g lipid after adjusting to the plasma lipid content. Cell cytotoxicity was tested in parallel as described (34); only non-toxic data were evaluated.

**Statistical analyses**

The statistical analysis was performed in SPSS 13.0 (SPSS Inc, Chicago, IL) with the significance level $p \leq 0.05$. Normal distribution was assessed by Q-Q plots. The natural logarithm transformed AhR-CALUX data as well as lipid adjusted POPs improved the normality and homogeneity of variance. The statistical analyses were performed on the ln-transformed data. The data were treated as continuous variables.

The statistical power depends on the endpoint and the prevalence of the exposure. The combined Inuit sample numbers (70) and Danish sample numbers (22) enrolled in the present survey study were enough for comparison of the POP levels and dioxin-like activity between combined Greenlandic Inuit and Danish women with a statistical power of 80–100% (i.e., risk of Type II error [$\beta$]<20%) at the significant level of 0.05 (i.e., risk of Type I error [$\alpha$]=5%).
Comparisons of means for AhR activity outcomes between the districts were performed by the One-way-ANOVA test. When ANOVA indicated significant district differences, complementary multiple comparison ad hoc tests were used. The test for homogeneity of variance was performed with Levene's test. The least significant difference (LSD) pairwise multiple comparison test was used for the variables with equal variance (p≥0.05) and Dunnett's T3 test was used for the variables with an unequal variance (p≤0.05).

The differences between sum of the Inuit and Danish data were assessed using the independent Student's t-test. Spearman's rank correlation was used to assess non-parametric correlations between the single POP markers.

A potential source of POP bioaccumulation might also be a potential predictor for xenobiotic serum activity. As the literature has shown (35,36), age and seafood intake are examples of exposure risk factors. N-3 fatty acids are known indicators of seafood intake (1), and we used the ratio of n-3/n-6 fatty acids as measures of seafood. Smoking was evaluated as a potential confounder of AhR-CALUX /AhR-TEQ levels for the combined data of Inuit and Danish women. Multivariate linear regression analyses were used to assess the relation between the POP biomarkers and AhR-CALUX outcomes. The impact of potential confounders were evaluated by entering single and blocks of variables together with sum DL-PCBs, sum non-DL-PCBs, sum PCBs and sum POPs. In the first step, age and then the ratio of n-3/n-6 (continuous variables) were included in the model, and in the second, smoking status (cigarettes per day) was also included as a continuous variable.

RESULTS

POPs and characteristics of Inuit and Danish women

Fourteen PCBs and 15 pesticide residues were determined in the plasma of 70 Inuit and 22 Danish volunteers (DK). The level of POPs in Inuit was between 7 and 40 times higher compared with the DK samples (Table I). The sum of mono-ortho DL-PCBs and of non-coplanar non-DL-PCBs were 218 and 3,310 μg/kg lipid for Inuit and 18 and 193 μg/kg lipid for Danish women, respectively. The plasma POPs level differed among Inuit from different districts (p=0.001) with the highest found in Scoresbysund and the lowest in Upernavik (p=0.001) (Fig. 1A). The plasma lipid (Table I) significantly differed between Inuit and the DK samples (p=0.009).

The median age of Inuit was 35, differing among districts, and also was significantly different from the median age of 24 for the Danish volunteers (Table II). The plasma n-3/n-6 ratio of Inuit was similar among the districts except for Nuuk, which was similar to the n-3/n-6 ratio of Danish samples (DK). However, the n-3/n-6 ratio of DK was significantly lower than that of the combined Inuit data. The Inuit showed a higher number with a smoking habit than the Danish (Table II).

AhR-CALUX outcomes

In total, 71% of the Inuit samples significantly induced the AhR-CALUX level up to approximately twofold relative to the solvent control DMSO (p<0.05). The level of AhR-CALUX activity differed significantly among the districts (Table II), with the highest and lowest activity level found in Nuuk and Upernavik (p=0.03), respectively (Table II). The lipid adjusted AhR-
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**Table I. Medians for lipid adjusted POPs of the study groups.**

| District, n descriptives | Beta-HCH | Chlor-dane | DDT sum | HCB | Mirex | Tox sum | Sum PCBs | Sum POPs | Lipid g/l |
|-------------------------|----------|------------|---------|-----|-------|---------|----------|----------|----------|
| Scoresbysund, 15m       | 100      | 1474       | 2147    | 296 | 149   | 146     | 9146     | 13458    | 6.1      |
| µg/kg lipid             | 24-290   | 149-9485   | 278-5394| 68-629| 3-328 | 22-339  | 1116-20400| 5-9      |
| min-max                 |          |           |         |     |       |         |          |          |
| Tasiilaq, 10 w          | 38       | 797       | 1132    | 199 | 44    | 214     | 2380     | 4804     | 7.1      |
| µg/kg lipid             | 6-82     | 172-2175   | 397-3091| 47-403| 8-149 | 75-437  | 657-5767 | 3-8      |
| min-max                 |          |           |         |     |       |         |          |          |
| Nanortalik, 5 m         | 37       | 1714      | 3241    | 231 | 95    | 326     | 3797     | 9441     | 6.5      |
| µg/kg lipid             | 15-46    | 568-2122   | 1260-4949| 75-371| 55-200| 92-569  | 1804-5764| 5-7      |
| min-max                 |          |           |         |     |       |         |          |          |
| Nuuk, 15 m              | 25       | 474       | 811     | 126 | 24    | 128     | 1031     | 2619     | 6.2      |
| µg/kg lipid             | 11-69    | 86-1240    | 270-1995| 50-531| 10-53 | 16-542  | 449-2792 | 4-10     |
| min-max                 |          |           |         |     |       |         |          |          |
| Ilulissat, 14 m         | 50       | 1327      | 1321    | 426 | 42    | 287     | 2373     | 5826     | 7.4      |
| µg/kg lipid             | 13-98    | 145-2784   | 360-3979| 69-1000| 13-102| 35-714  | 511-3754 | 4-10     |
| min-max                 |          |           |         |     |       |         |          |          |
| Upernavik, 11 m         | 31       | 423       | 647     | 136 | 19    | 106     | 845      | 2209     | 5.9      |
| µg/kg lipid             | 15-72    | 159-2586   | 329-2972| 39-397| 8-172 | 42-456  | 527-4637 | 5-8      |
| min-max                 |          |           |         |     |       |         |          |          |
| Total 6 districts, 70   | 39       | 802       | 1282    | 234 | 39    | 162     | 2051     | 4609     | 6.3      |
| µg/kg lipid             | 6-290    | 86-9486    | 269-5394| 39-1000| 3-328 | 16-714  | 449-20400| 3-10     |
| min-max                 |          |           |         |     |       |         |          |          |
| Danish women, 22        | 17       | 0.03      | 123     | 27  | -     | -       | 172      | 339      | 5.7      |
| µg/kg lipid             | 8-30     | 0-0.08    | 51-710  | 13-41 | -     | -       | 103-393  | 4-8      |
| min-max                 |          |           |         |     |       |         |          |          |

Note: The median and minimum–maximum values of POPs in plasma given as µg/kg lipid. By mistake the Tassilaq district includes women instead of men. However, the lipid corrected POP data did not differ significantly between men and women, and thus the 10 women were included in the district survey analyses.

Abbreviations: m=men; w=women; Beta-HCH=(β-hexachlorocyclohexane); Chlordane=(sum of α-, γ-, cis-, nona-, trans-, and oxychlordanes); DDT sum=(DDT+DDE, dichloro-diphenyl-trichloroethane and dichloro-diphenyl-dichloroethylene); HCB=(Hexachlorobenzene); Mirex=(a chlorinated hydrocarbon that was commercialized as an insecticide); sum PCBs=(polychlorinated biphenyls, sum of all 14 PCB congeners: mono-ortho DL-PCBs (#105, #118, #156), non-coplanar non-DL-PCBs (#28, #52, #99, #101, #128, #138, #153, #170, #180, #183, #187); Tox sum=(toxaphene parlar 26 and 50; parlar 62 and 69 were generally below detection limit); Sum POPs=sum PCBs plus all the organochlorines listed.

TEQ showed that the Ilulissat data were significantly lower than the Nuuk and Scoresbysund samples eliciting the highest AhR-TEQ level (Table II, Fig. 1B).

Similar to the Inuit samples, 64% of the DK samples elicited significant induced AhR activity relative to the solvent control (p<0.05). The range of AhR-CALUX and thus the AhR-TEQ data were much broader for DK than Inuit samples. Moreover, the median of both AhR-CALUX and AhR-TEQ for DK samples differed significantly from the combined Inuit data, being higher and lower, respectively (Table II, Figs. 2A and 2B).

**Figure 1A.** POP levels of Inuit in different Greenlandic districts.
Table II. Characteristics and plasma AhR-CALUX and AhR-TEQ values of the study groups.

| District       | Scoresbysund | Tasilaq | Nanortalik | Nuuk | Itulissat | Upernavik | Total | ANOVA\(^3\) | Danish\(^4\) | t-Test\(^5\) |
|----------------|--------------|---------|------------|------|-----------|-----------|-------|--------------|-------------|-------------|
| Age (n, s)     |              |         |            |      |           |           |       |              |             |             |
| (15, m)        | (10, w)      | (5, m)  | (15, m)    | (14, m) | (11, m)   | (70)      | (21, w) |              |             |             |
| Median         | 36           | 37      | 36         | 31   | 43        | 33        | 35    | 0.004**      | <0.001***   |             |
| Min–max        | 19-60        | 23-45   | 23-52      | 19-36| 23-59     | 25-38     | 19-60 | 16-36        |             |             |
| n-3/n-6        | (15)         | (10)    | (5)        | (15) | (14)      | (11)      | (70)  |              |             |             |
| Mean           | 0.46         | 0.44    | 0.66       | 0.21 | 0.35      | 0.34      | 0.3751 |              |             |             |
| Median         | 0.40         | 0.42    | 0.38       | 0.16 | 0.31      | 0.33      | 0.313 |              |             |             |
| Min–max        | 0.14-1.11    | 0.15-0.74| 0.17-1.89 | 0.05-0.46 | 0.13-0.66 | 0.10-0.72 | 0.05-1.89 | 0.09-0.23 | <0.001***   |             |
| smoking\(^6\)  | (15)         | (10)    | (5)        | (15) | (14)      | (11)      | (70)  |              |             |             |
| Mean           | 10           | 7.8     | 2.4        | 8.8  | 8.5       | 9.2       | 8.5   |              |             |             |
| Median         | 10           | 6.0     | 2.0        | 6.0  | 8.0       | 10.0      | 8.0   | 0.426        | 0.0          | 0.050**     |
| AhR-CALUX\(^4\)(n) | (14) | (9)      | (5)        | (10) | (13)      | (11)      | (62)  | (22)         |             |             |
| Mean           | 1.27         | 1.09    | 1.21       | 1.34 | 1.12      | 1.08      | 1.19  |              |             |             |
| Median         | 1.24         | 1.11    | 1.17       | 1.34 | 1.12      | 1.08      | 1.16  | 1.89         | <0.001***   |             |
| Min–max        | 1.05-1.70    | 0.96-1.26 | 1.11-1.39 | 0.96-1.88 | 0.83-1.37 | 0.83-1.53 | 0.83-1.88 | 0.76-8.42 |             |             |
| % agonist      | 86           | 56      | 100        | 80   | 69        | 55        | 71    |              |             |             |
| AhR-TEQ, pg/g (n) | (14) | (9)      | (5)        | (10) | (13)      | (11)      | (62)  | (22)         |             |             |
| Mean           | 241          | 202     | 216        | 232  | 182       | 200       | 213   |              |             |             |
| Median         | 238          | 171     | 205        | 236  | 167       | 186       | 207   | 0.096        | 152         | <0.001***   |
| Min–max        | 150-414      | 133-391 | 175-292    | 145-342 | 118-284  | 124-329  | 118-414 | 55-693      |             |             |

1 n=number, s=sex, m=men, w=women.
2 Ratio of plasma n-3/n-6 fatty acids; n-3 is a marker for the level of seafood intake.
3 Cigarettes per day.
4 AhR-CALUX activities in plasma/serum extract measured as relative luciferase units (RLU) per μg cell protein (RLU/μg).
5 The reference control was solvent control (<0.1% DMSO). % agonist: the frequency of samples showing significantly increased RLU/μg compared with the solvent control (p<0.05); AhR-TEQ was calculated from the agonistic AhR-CALUX data and given as pg/g plasma lipid.
6 One-way ANOVA (p-value) evaluation of the total data of Inuit, ln transformed data were used.
7 Danish women.
8 Independent t-test between total Inuit data and Danish data, ln transformed data used but age (normal distributed), n-3/n-6 and smoking.
9 The p value reflects that Nuuk only was different from all the other districts. ▲=p<0.05 vs. Scoresbysund and Nuuk; *p≤0.05; **p≤0.01; ***p≤0.001.

Figure 1B. AhR-TEQ level of Inuit in different Greenlandic districts.
Association between AhR activity data and plasma POPs

Most of the determined POPs were highly intercorrelated at the significance level \( p<0.001 \) with an overall Spearman correlation coefficient \( r_s = 0.50-0.99 \), whereas the PCBs 101, 28, 52 and 128 showed a lower intercorrelation range \( r_s = 0.26-0.34 \). Multivariate regression analyses of the age and/or n-3/n-6 adjusted combined Inuit AhR-CALUX and AhR-TEQ data elicited significant association to the total of sum PCBs and sum POPs (Table III). The age and n-3/n-6 adjusted data did not change upon adjustment for smoking. A similar trend was observed when sum DL-PCBs and sum non-DL-PCBs were analysed separately in the multivariate regression model (not shown). None of the determined single POPs was linearly associated to the AhR activity outcomes. Evaluation of the single districts alone showed few and scattered significant associations between AhR activity data and POP levels (not shown). However, taking into account the high intercorrelation between the congeners and the relatively low sample number, and thus the low power for the single districts, the observed significances might be chance findings.

Multivariate regression analyses of the DK samples showed no association between POPs and AhR-
Associations between possible determinants and AhR-CALUX outcomes

For Inuit, the POPs were positively and highly correlated to age (β=0.56; p≤0.001) (Table IV), and more than 50% might be explained by lifelong accumulation of the POPs. For the DK samples, an association between age and sum PCBs (β=0.52; p=0.016) was also observed, but no association between age and sum POPs was observed, possibly because of missing data or data below detection limit of organochlorines for approximately 40% of the individuals (not shown).

For the combined Inuit data, the AhR-TEQ (not AhR-CALUX) was negatively correlated with age (β=-0.33; p=0.009), whereas the n-3/n-6 ratio tended to be negatively associated with AhR-CALUX (β=-0.236; p=0.065) (Table IV). For the DK samples, neither age nor the n-3/n-6 ratio was linearly correlated with the 2 AhR-CALUX outcomes. No correlation was found between smoking and plasma AhR-activities (not shown).

Note: The analyses were performed on ln transformed POPs and CALUX data. The raw data were adjusted for age and the n-6/n-3 fatty acid ratio (ln-transformed). Smoking did not influence the data for either the Inuit or Danish samples.

1 Non-adjusted data.
2 n-3/n-6: ratio of n-3 to n-6 fatty acids in plasma. For definition of AhR-CALUX and AhR-TEQ, see Table II. The Inuit data did not change upon analyses of the women and men separately. *p≤0.05; **p≤0.01; ***p≤0.001.

For sum PCBs and sum POPs, see Abbreviations at Table I.
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DISCUSSION

The geographical differences in POP bioaccumulation between Greenlandic districts and its positive associations to age and n-3/n-6 ratio, respectively, have been reported several times over the years (1, 2). However, the present data of plasma DL-activity differences among these districts has been reported here for the very first time.

In accordance with the study aims, we measured the dioxin-like activity in plasma extracts of Inuit and Danish women. Both AhR-CALUX activity and the calculated lipid adjusted AhR-TEQ differed between the various Greenlandic districts. Moreover, the combined Inuit data were significantly different from the data of Danish samples, where the Inuit median level for AhR-CALUX was lower but the lipid adjusted AhR-TEQ level was higher than that of Danish women. The significant plasma lipid differences between the 2 study groups may explain the AhR outcome differences, stressing the utmost necessity in data adjustment to plasma lipid for comparisons of data between different study groups and studies.

As reported earlier (30), we found significant POP differences among the Greenlandic districts, with the highest POPs level in Scoresbysund and the lowest level in Upernavik and Nuuk. The district profile of AhR-TEQ did not follow the district profile of POP levels. The sum POP levels of Nuuk were 5 times below the levels of Scoresbysund. However, Nuuk samples had the highest median AhR-activity and an AhR-TEQ level similar to Scoresbysund. This indicates that the actual exposure to compounds eliciting DL-activity might not necessarily follow the sum of PCBs and organochlorine pesticides. Because of the low level of samples from each district and thus low statistical power, the present data can only be regarded as preliminary for the single districts. However, our new published data for Nuuk and other Greenlandic districts support our above conclusion (37).

Table IV. Linear regression analyses of AhR activity outcomes and possible determinants.

| sum POPs | sum PCBs | AhR-CALUX | AhR-TEQ | Age |
|---------|----------|-----------|---------|-----|
| Inuit   | n=70     | n=70      | n=62    | n=62| n=70|
| Age     | p<0.001*** | p<0.001*** | p=0.120 | p=0.009** | - |
| [95% CI; [95% CI; [95% CI; [95% CI; [95% CI; |
| 0.556   | 0.565    | -0.199    | -0.331  | .036-.075 | .008-.001 | .018-.003 |
| 0.110-.234 | 0.008-.001 | 6.651-11.93 | 1.285-2.670 | 1.288-.004 | .128-.004 | .200-.031 | 5.42-.21.28 |

1 Ratio of plasma n-3/n-6 fatty acids; (*) borderline significant; *p≤0.05; **p≤0.01; ***p≤0.001.

For sum PCBs and sum POPs, see Abbreviations at Table I.
Previously, it has been reported that the high plasma POP levels in Greenlandic Inuit is highly correlated to plasma n-3 levels that follows the intake of traditional food, including marine mammals (seal and whales) and polar bears. In larger towns such as Nuuk and Sisimiut, a more Westernized diet is consumed and body POPs were found inversely correlated with the intake of Westernized diet (1). The type of diet therefore influences plasma POP levels.

The combined Inuit AhR-CALUX and AhR-TEQ data were negatively associated to age, and the ratio of plasma n-3/n-6 fatty acids suggest that the DL-activity mainly follows the intake of n-6 fatty acids possibly from non-marine fat/animals, such as domestic animals. Our hypothesis is supported by the similar lower ratio of n-3/n-6 found in the younger Nuuk volunteers (this might be due to their increased consumption of Westernized food [1]) and the young Danish women, both groups being significantly different from the other Inuit study groups with respect to their n-3/n-6 ratio. The older volunteer’s diet includes more marine food with high levels of POPs and n-3 fatty acids (1,2). In a recent study including older men from Nuuk, relatively higher plasma POP levels and higher n-3/n-6 ratio were found (1), and in accordance with the present study, a relatively lower plasma AhR-TEQ was determined for that group (37). The negative association between the n-3/n-6 ratio to AhR-CALUX and AhR-TEQ, as well as the negative relation between age and the AhR activity outcomes for Inuit support the anti-AhR effect of several POPs (19,20). Previous studies have shown that meat such as beef, poultry and pork is a major source of DLCs for humans (38,39).

Further investigations are needed to identify the main source of dioxin plasma activity of Greenlandic Inuit.

For both Inuit and Danish subjects, a correlation between sum PCBs and age was observed. Multiple linear regression analyses of the combined Inuit or Danish data found no association of AhR-CALUX or AhR-TEQ raw data to the single POPs, sum DL-PCBs, sum non-DL-PCBs, sum PCBs or sum POPs, respectively. However, upon adjustment for n-3/n-6 and/or age, the Inuit DL-activity data became highly significant associated to the sum DL-PCBs, sum non-DL-PCBs, sum PCBs and sum POPs, whereas these adjustments did not change the Danish data. The explanation might be that differences in age and diet intake for Inuit in our study groups weakens the association between POPs and AhR-activity, and the significant association between POPs and AhR-activity appear upon adjustment and thus equalize age and n-3/n-6 differences.

The relative concentrations of non-DL-PCBs and DL-PCBs can differ between regions depending on exposure sources. In Canadian Inuit, the serum ratio of non-DL-PCBs (e.g., PCB-153) to the DL-PCB levels was higher than for Canadian Caucasians from the Arctic area (18,40). In the present study, among the 14 PCB congeners the plasma levels of 11 non-DL-PCBs and 3 mono-ortho-DL-PCBs (#105, #118, #156) were determined. The ratio of the lipid adjusted sum non-DL-PCBs to sum mono-ortho-DL-PCBs was 151 and 11 for Inuit and Danish women, respectively (not shown). Thus the present study supports the Canadian data, showing the same trend of a higher level of non-DL-PCBs to DL-PCBs.
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for Inuit as opposed to Caucasians. The ratio of non-DL-PCBs to DL-PCBs might also differ between the different Greenlandic districts and affect the AhR-TEQ level since some PCBs can inhibit induced AhR function (19,20) (unpublished data from our laboratory).

We suggest that the ratio of non-DL-PCBs to DL-PCBs, as well as the level and composition of plasma POPs are the main factors that influence the net plasma AhR activity and AhR-TEQ.

Currently, we are studying the relation between dioxin congeners and total DL-activity in the breast milk of Danish women. In general, we have found similar results using WHO-TEQ and AhR-TEQ. However, the results clearly showed that the inter-ratio of dioxins and levels of mono-ortho and non-ortho PCBs in the mixture influence the AhR-CALUX activity. Thus, the different proportion of PCDD/Fs, organochlorine pesticides and PCBs influence the identity of WHO-TEQ and AHR-TEQ (unpublished).

Summary and conclusion

The sum PCB/sum POP levels in Inuit were more than 10 times the level of Danish volunteers, and for both study groups the level was associated with age. For Inuit, the plasma AhR-CALUX and AhR-TEQ was positively associated to bioaccumulated POPs upon adjustment for age and/or n-3/n-6. No interactive pattern for AhR-activity-POP-fatty acids was found for the Danish samples. No single compounds were linearly related to the determined AhR-CALUX and AhR-TEQ data. We did not determine the plasma concentration of dioxins and other DL compounds except those for 3 DL-PCBs.

Therefore, taking into consideration the very low concentrations of PCBs found in the Danish serum samples, the similar (although slightly higher AhR-TEQ for Inuit) AhR-CALUX levels found for the Inuit and Danish women suggests that for Danish women, exposure to dioxins and other DL compounds might contribute more to the net AhR activity than the bioaccumulated PCBs. Although the observed differences between Inuit and Danish volunteers might be influenced by gender, we believe that geographical location (different exposures) and diet are the main impact factors for the AhR activity outcomes. We suggest that age, differences in dietary intake, along with composition and levels of plasma POPs are important factors which must be taken into consideration in future epidemiological-mechanistic study designs of DL-activity related to POPs.

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Conflict of interest statement

The authors declare that they have no competing interest.

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