

Serum dioxin-like activity of Inuits across Greenlandic districts

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Abstract

Background: Human exposure to environmental contaminants is ubiquitous and all individuals carry a burden of the lipophilic persistent organic pollutants (POPs) including polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs), polychlorinated biphenyls (PCBs) and organochlorine pesticide residues. Exposure to POPs elicits a number of species- and tissue-specific toxic responses including effects on the reproductive-, immune- and thyroid system, many of which involve the aryl hydrocarbon receptor (AhR). A number of factors complicate the toxicological assessment e.g. no individual is exposed to a single contaminant alone but to a complex mixture of contaminants being life-long beginning during critical developmental windows. The aim of this study was to compare the actual level of integrated dioxin-like activity (DL-activity) in the lipophilic serum fraction containing the actual POP mixture among Inuits from different districts in Greenland, and to evaluate whether the DL-activity is correlated to the bio-accumulated POPs and /or lifestyle.

Methods: The study included 357 serum samples from the districts: Nuuk and Sissimiut (South West Coast), Quaanaaq (North Coast) and Tasiilaq (East Coast). The bio-accumulated serum POPs was obtained by ethanol: hexane extractions. Effects of the serum extract on the AhR transactivity was determined using the stable transfected Hepa 1.12cR mouse hepatoma cell line carrying an AhR-luciferase reporter gene (AhR-CALUX), and the data was evaluated for possible association to the serum levels of 14 PCB congeners, 10 organochlorine pesticide residues and life style factors.

Results: In total 85% of the Inuit samples elicited agonistic DL-activity in a district dependent pattern. The AhR-TCDD equivalent (AhR-TEQ) data of the genders was similar in the different districts. For the combined data the order of the AhR-TEQ was Tasiilaq > Nuuk ≥ Sissimiut > Qaanaaq possibly being related to the different composition of POP. In overall,

the DL-activity was inversely correlated to the levels of determined POPs, age and /or intake of marine food.

Conclusions: We suggest: i) the proportion of dioxin like compounds (DLCs) in the POPs mixture may be a dominating factor affecting the level of serum DL-activity; ii) the inverse association between serum DL-activity and POPs can be explained by the higher level of compounds antagonizing the AhR function probably due to selective POP bioaccumulation in the food chain.

1. Background

Contamination of the global environment with a complex mixture of persistent organic pollutants (POPs) has resulted from discharges, applications, and inadvertent formation of byproducts of incomplete combustion or industrial processes. Being resistant to both biotic and abiotic degradation, most POPs bioaccumulate and magnify in animals and humans [1, 2]. The vast number of compounds can be detected in tissue samples from organisms inhabiting even remote parts of the world [3]. The polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs), polychlorinated biphenyls (PCBs) and organochlorine pesticides are dominating among the POPs. Although the use of several organochlorine compounds, such as 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and PCBs, was restricted or banned in most countries in the 1970s, these compounds are still released into the environment because of ongoing use in developing countries and improper storage or disposal in developed countries. POPs, including PCBs and persistent organochlorine pesticides, can be transported to the Arctic regions by atmospheric and oceanic currents and have been found to bioaccumulate and biomagnify in the Arctic marine food web [4, 5]. The traditional diet of the indigenous people in the Arctic largely depends on fish, seabirds and marine mammals being associated with extraordinary high POP exposure [6-11], which possess a severe health risk [12]. Since 1994, organochlorines have been measured in fat and plasma samples taken from Inuits of Greenland indicating that the contaminant level in Greenland is very high [7, 9-11, 13]. It has been documented that exposure to POPs such as dioxins (e.g. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) and dioxin-like compounds (DLCs) such as *non-ortho* and *mono-ortho* PCBs may cause a series of negative effects both in animals and humans including carcinogenicity [14], immunotoxicity and adverse effects on reproductive, neurobehavioral [15]. The toxicity of dioxins and DLCs is mediated mainly through activation of the aryl hydrocarbon receptor (AhR), an intracellular ligand-dependent

transcriptional factor expressed in most tissues of mammals [16]. Upon receptor-ligand binding and translocation into the nucleus, the complex with the AhR nuclear translocator (ARNT) binds to the DNA dioxin-responsive elements (DREs), causing induction of gene transcription, for instance, encoding for metabolic enzymes [17].

Since dioxins and DLCs exist as complex mixtures of various congeners throughout the environment, the concept of TEQ (TCDD toxic equivalent) has been introduced to simplify risk assessment and regulatory control [1]. The classical TEQs are calculated by multiplying the concentration of individual PCDDs/PCDFs/PCBs by their respective Toxic Equivalency Factors (TEFs), which correspond to the relative potency of the congener to generate AhR-mediated effects compared to TCDD, the most potent AhR ligand. Studies have emphasized that assessment of the toxicological potential of a chemical mixture is much more complex than can be deduced by a calculated TEQ value [18-20]. There are several drawbacks using the TEF concept for risk assessment of mixtures of POPs such as expensive and time consuming gas chromatography mass spectrometry (GC-MS) determinations, small concentrations of individual congeners, presence of compounds not routinely measured or unknown substances with AhR affinity, the lack of TEF values for several POPs, and possible antagonistic or synergistic interactions between POPs [2, 20-22]. There is a need for an integrated risk assessment of dioxins and DLCs. The *in vitro* AhR mediated chemical activated luciferase gene expression (CALUX) bioassay has proven to be a quick and sensitive assay to detect the AhR mediated potential of pure chemicals [2, 21-23], extracts of environmental and biological matrices eliciting the integrated TEQ value (CALUX-TEQ) of complex mixtures as found in sediment, pore water [24], bovine and human milk [20, 25], human serum [26] and follicular fluid [27]. Compared to the calculated chemical-derived TEQ, the CALUX based AhR-TEQ might be more biological relevant for the risk assessment of dioxins and DLCs [28].

As a part of the human health program of the “Arctic Monitoring and Assessment Program (AMAP)”[29], the aim of the present study was i) to compare the AhR mediated dioxin-like activity (DL-activity) of the lipophilic serum fraction of subjects from different districts in Greenland and ii) to evaluate whether the DL-activity is associated to the level of POPs and /or lifestyle factors.

2. Methods

2.1. Subjects, sampling and POPs determination:

The subjects and sampling methods have been described in [11]. Briefly, the participants were of Inuit descent from Nuuk, Sisimiut (South West Greenland), Qaanaq (North West Greenland) and Tasiilaq (East Greenland). The men data of Sisimiut was also a part of the EU project INUENDO (www.inuendo.dk). Venous blood samples and questionnaire about demographic and lifestyle factors were collected. The serum was prepared as described [11] and then stored at -80 °C until analyzed.

As described [11] plasma was analyzed for POPs including cis-chlordane, trans-chlordane, oxychlordane, *p,p'*-DDE, *p,p'*-DDT, hexachlorobenzene (HCB), beta-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) and reported as $\mu\text{g} / \text{kg}$ lipid after adjusted to the plasma lipid content [11, 30]. Because of high POP intercorrelation (Bonefeld-Jorgensen and Long, submitted), the group-variables were used for evaluation of the data, namely, the sum of 14 PCB congeners ($\sum\text{PCB}_{14}$), the sum of pesticides (\sum pesticide) and finally the sum of all the determined POPs (\sum POP).

The fatty acid profiles were determined in plasma phospholipids at the Biology Department, University of Guelph, Canada [31]. 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, n6, C20:2, n-6, C20:3, n-6 and C20:4, n-6 [11]. The ratio between n-3 and n-6 is known to be a strong indicator of marine food intake and thus a good indicator of the relative consumption of traditional versus imported food [10, 32].

2.2. AhR-CALUX bioassay

The serum was extracted with a mixture of ammonium sulphate/ethanol/hexane (1:1:3) and the lipid extract was concentrated and cleaned up on Florisil columns to get actual mixture of POPs [26, 30]. The detailed descriptions of dissolving the extract samples and detecting DL-activity has been described in detail elsewhere [26, 30]. Briefly, the stably transfected mouse hepatoma cell line Hepa1.12cR cells carrying the AhR-luciferase reporter gene (kindly provided by MS Denison (University of California, Davis, CA, USA)) were exposed for 4 hours to the serum extract alone (termed AhRag) or in the presence of half maximum effective concentration of TCDD (termed AhRcomp) [26]. The luciferase activity and the cell protein were then determined and the luciferase activity of samples was presented as relative light units per microgram protein (RLU/ μg protein) [33]. The average intra and inter coefficient of variation (CV) of the solvent control was 9 % and 17 %, respectively and the intra CV of the test samples was 10 %.

No cell toxicity on Hepa1.12cR cells was observed after exposure to the serum extract samples determined as described [33].

2.3. Calculation and statistical analysis

In the independent assays the activity differences between the triple serum extract determinations and their respective solvent controls (% agonistic, % antagonistic and % additive/synergistic, Table 3) were evaluated using the Student t-test (Microsoft Excel).

The CALUX-based AhR-TEQs values were obtained by interpolation of the AhRag values onto the TCDD dose-response sigmoid Hill curve as described in [26].

Natural logarithmic transformation of AhR-TEQ / AhRcomp and POP data improved the normality (checked by Q-Q plots) and homogeneity of variance, and the statistical analysis was performed on the ln-transformed data. The comparisons of different variables (POPs, AhR-TEQ and AhRcomp) among the districts were performed with One-way ANOVA test. When ANOVA showed statistical significant differences complementary multiple

comparison *ad hoc* tests was performed. Test for equal variances was performed using Levene's test. The least-significant difference (LSD) test was used if the variables showed equal variance; otherwise Dunnett T3 test was used. The comparison of variables between the genders was performed by independent student t-test.

Bivariate correlations were evaluated by Pearson correlation analyses.

The overall associations between the AhR-mediated activities and POPs across the study groups and/or across genders were assessed by comparing the regression lines for each study group/gender by using multiple regression analysis.

Up to date few studies on DL- activity in human serum have been reported [26, 34-37], and thus the knowledge is limited about which dietary or other life-style determinants might affect serum DL- activity. Our hypothesis is that potential determinants of POP bioaccumulation might also be potential determinants for serum DL- activity. Guided by the literature [38], age and seafood intake are known determinants affecting the POP serum level, and intake of seabird might as well influence the POP level. N-3 fatty acids being determinants of seafood intake [9-11], and life style factors such as smoking and BMI might also influence the serum POP level [10]. Using the multiple linear regression model, assessing the relation between the POPs and AhR activities, the impact of potential confounders were evaluated by entering variables together with $\sum\text{PCB}_{14}$, \sum pesticide and \sum POP as follows: in the first step, age and then seafood intake (represented by n-3/n-6 ratio) were included in the model, then additionally smoking years and BMI were included in the model. Finally, the bird intake was additionally included in the model. The potential confounder was defined if the regression coefficient (β) changes more than 10 % and when a significant association ($p \leq 0.05$) was obtained after adjustment.

The inter-district variations in serum levels of POPs, AhR-TEQ and AhRcomp were also assessed by linear regression models and age was in these models considered as a potential confounder of these variables.

The statistical analysis was performed on SPSS 13.0 (SPSS Inc. Chicago, IL, USA). The statistical significant level was set to $p \leq 0.05$.

3. Results

3.1. The demographical and life style factors of study groups

As reported [11], the age of the participants was from 18-77 with the median age of 35 years old. The Nuuk men were older than the other participants, whereas the age range of the remaining male and female participants of all four districts were similar (Table 1). No significant difference was observed for the BMI of the participants from all districts. For the smoking years, Nuuk men and women had the highest median, whereas the other three districts were similar for both sexes (Table 1). The Nuuk and Sisimiut men had the highest and the lowest median n3/n6 ratio, respectively, whereas for women higher n3/n6 ratio was observed for Qaanaq and Tasiilaq. The median seabird intake of the Nuuk men was the highest whilst for the rest of subjects similar median seabird intake was observed (Table 1). Nuuk and Sisimiut participants had higher median consumption of dairy food than that of Qaanaq and in overall male participants had lower dairy food intake than women (Table 1).

3.2. The plasma level of POPs

For the men, similar median levels of \sum PCB₁₄, \sum pesticide and \sum POP were observed among Nuuk, Qaanaq and Tasiilaq and all were significantly higher than that of Sisimiut with the order of Nuuk \geq Qaanaq \geq Tasiilaq $>$ Sisimiut. Tasiilaq and Qaanaq women had significantly higher POPs levels than that of Nuuk and Sisimiut women (Table 2) as reported by Deutch et al [11]. In general, male participants had higher median POP levels than women in all districts. For the combined sex data, Tasiilaq and Qaanaq had similar POPs level which were significantly higher than that of Nuuk and Sisimiut participants (Tasiilaq \geq Qaanaq $>$ Nuuk $>$ Sisimiut). After adjustment of age, the pattern of regional difference did not change (data not shown).

The dioxin-like PCBs (DL-PCBs) determined in this study were CB105, CB118 and CB156. The Nuuk men had higher median percentage of \sum DL-PCB in \sum PCB₁₄ than men from

other districts (Nuuk > Tasiilaq \geq Qaanaq \geq Sisimiut). No significant difference of Σ DL-PCB percentage between women from different districts was observed but a lower trend for that of Qaanaq women (Table 2). Women had significantly higher percentage of DL-PCBs than men. In overall, the median percentage of Σ DL-PCB for the combined data was in the order of Nuuk \geq Tasiilaq \geq Sisimiut > Qaanaq. (Table 2).

3.3. The serum level of AhR-TEQ and AhRcomp activity

91% of all male and 79 % of all female serum samples showed significantly agonistic AhR activity. The levels of AhR-TEQ differed significantly among the districts for the separate gender (Table 3). The order of median level of AhR-TEQ was Tasiilaq \geq Sisimiut \geq Nuuk > Qaanaq for men (Fig. 2 A), and Nuuk \geq Tasiilaq \geq Sisimiut > Qaanaq for women (Fig. 2B). For both genders the Qaanaq had a lower AhR-TEQ level than the participants from other districts (Table 3).

AhRcomp activity was assessed by co-exposure with serum extract and 60 pM TCDD (EC₅₀ (TCDD)). The median AhRcomp activity of men was in the order of Qaanaq \geq Sisimiut \geq Tasiilaq > Nuuk, reflecting the higher percentage of samples elicited antagonistic effect on the TCDD induced AhR activity in the serum samples of Nuuk and Tasiilaq (Table 3, Fig. 2C). Higher percentage of serum samples of men from Qaanaq and Sisimiut elicited a further increase of the TCDD induced AhR activity (Table 3, Fig. 2C). The serum samples of women from the four districts had similar AhRcomp level, mainly eliciting a further increase of the TCDD induced AhR activity (Table 3, Fig. 2D).

No significant difference of the AhR-TEQ level was found between the genders in each district (Table 3). The Nuuk men had a lower level of AhRcomp than Nuuk women, whereas similar AhRcomp levels were found for the two genders for Sisimiut, Qaanaq and Tasiilaq participants (Table 3).

Adjustment for age did not change the regional and gender difference of AhR-TEQ and AhRcomp (data not shown).

3.4. The association between AhR-TEQ, AhRcomp and POPs

3.4.1. The correlations between the single POPs.

Pearson correlation analyses showed that most PCB congeners were significantly and mutually intercorrelated ($r > 0.82$; $p < 0.001$), whereas CB 28, CB52, CB128 and CB101 had a relatively lower correlation coefficients to other PCB congeners ($r = 0.29-0.82$; $p < 0.05$). Also the determined pesticides were intercorrelated ($r > 0.75$ up to $r = 0.99$). The grouping variables (\sum PCB_14 and \sum pesticide) intercorrelated with $r = 0.97$ and the sum of determined POPs as \sum POP was therefore acceptable.

3.4.2. The correlations between AhR-TEQ, AhRcomp and POPs

3.4.2.1. Multiple regressions of AhR-TEQ and AhRcomp on POPs across the study groups and genders

Multiple regression analysis of the combined districts and genders data was performed to analyze for homogeneity / heterogeneity of the associations between POPs and AhR-TEQ / AhRcomp (supplementary Table). The analyses indicated that the associations of POPs and AhR-TEQ were allowed to be evaluated in each district for combined gender, whereas the correlation of AhRcomp activity and POPs must be evaluated in each single district for men data and as combined districts for women.

However, in order to obtain the overall trend of the studied groups and a better statistical power, the data based on the combined genders of the single districts for POPs and AhRcomp and the combined genders across the districts were also evaluated for the correlation of POPs and AhR-TEQ / AhRcomp.

3.4.2.2. Correlations between AhR-TEQ, AhRcomp and POPs

Few significant correlations between AhR-TEQ and POPs were found for the single districts for the two sexes. Significantly inverse correlations between AhR-TEQ and POP variables were observed for both the Qaannaq women and the combined Qaannaq participants (Table 4A, Fig. 3A). Moreover, a negative correlation between AhR-TEQ and POPs was observed for the combined men data and the total combined data of districts and sexes (Table 4A, Fig. 3B). After adjustment of age and n-3/n-6 ratio, the significant associations of AhR-TEQ and different POPs in Qaannaq women disappeared.

As shown in Table 4 B, few significant correlations between AhRcomp and POPs were observed for the two sexes in the single districts but Tasiilaq women showing an inverse correlation. In overall, negative association between AhRcomp and POP data was found but Sisimiut men where AhRcomp activity was positively related to the determined $\sum\text{PCB}_{14}$. For the combined Nuuk data, negative correlations of AhRcomp and POPs were observed, which disappeared upon adjustment for both age and n-3/n-6 ratio (Table 4B, Fig. 3 C). The combined Tasiilaq data and the total combined data showed an inverse relationship between AhRcomp and POPs before and after adjustment for age and n-3/n-6 (Table 4B, Fig. 3C). The significantly inverse correlations of AhRcomp and POPs observed for all men data disappeared upon adjustment for both age and n-3/n-6 ratio (Table 4B, Fig. 3D).

Further adjustment for BMI, smoking year and bird intake did not change the pattern of associations of POPs and AhR-TEQ, AhRcomp (data not shown).

Scattered and few significant correlations of AhR-TEQ / AhRcomp and the single PCB and pesticides and /or $\sum\text{DL-PCBs}$ were observed (data not shown). Since the congeners of PCBs were highly intercorrelated and the CALUX-TEQ represents the integrated activity of

mixture, it is hard to assess which of the compounds having the highest impact on the AhR-TEQ / AhRcomp level.

3.5. The correlations of life style factors and AhR –CALUX activity

3.5.1. AhR-TEQ

As shown in Table 5, scattered and few correlations between AhR activities and life style factors were found in the single districts and the separate genders, most of which involved the age, and /or the n-3/n-6 ratio. A significantly negative correlation between age and AhR-TEQ was observed for Sisimiut men and the combined men data. For Sisimiut women, a positive correlation between BMI and AhR-TEQ and for Qaanaq women a negative correlation of the n-3/n-6 ratio and AhR-TEQ were observed. Significantly negative correlation between age and AhR-TEQ was also found for the combined Qaanaq data and the total combined data (Table 5). For the combined Nuuk, Sisimiut and Qaanaq data a significantly, positive correlation between AhR-TEQ and consumption of dairy food was found (Table 5), suggesting that dairy products may be one source of DLCs exposure for the Inuits living in West Greenland.

3.5.2. AhRcomp activity

The AhRcomp data was also in overall inversely related to age and /or the n3 /n6 ratio, but also the seabird intake seemed to be a determinant. A negative correlation for AhRcomp to seabird intake was observed for the Qaanaq men and all men data (Table 5). Moreover, the AhRcomp of all men was found to be inversely correlated to age, the n-3/n-6 ratio and smoking years. Negative correlations between AhRcomp and the n-3/n-6 ratio and age and smoking years were found for the women of Nuuk and Qaanaq, respectively. For the combined sex data of Nuuk subjects, negative correlations between AhRcomp and age, the n-3/n-6 ratio and seabird intake were found (Table 5). When combined all data (districts and

sexes), AhRcomp was found to be negatively correlated to the age, the n3/n6 ratio (Table 5). Besides, seabird intake was found to be negatively correlated to AhRcomp for all men data, the total combined data of Nuuk, Sisimiut and Qaanaq groups (Table 5).

4. Discussion

In the present study we compared the *ex vivo* DL- activity in the lipophilic fraction of serum from Greenlandic Inuits among different Greenlandic districts and evaluated whether the DL- activity was related to the bioaccumulated POPs and / or life style factors. The serum levels of POPs, AhR-TEQ and AhRcomp activity differed among the two sexes living in different Greenlandic districts (Northwest- Qaanaq, South West- Nuuk and Sisimiut and East- Tasiilaq). Also the association of the DL-activity to the determined POPs differed between the districts and genders. The marked observations were an inverse association between AhR-TEQ, AhRcomp and POPs where the Qaanaq women had the main influence on the combined AhR-TEQ data and Nuuk and Tasiilaq on the combined AhRcomp data.

As reported [11], the POPs levels of Tasiilaq and Qaanaq participants (including men and women) were in general higher than that of Nuuk and Sisimiut subjects (Tasiilaq \geq Qaanaq $>$ Nuuk $>$ Sisimiut), which is consistent with previous reports that inhabitants of East Greenland had higher POP levels than those in the West Greenland [6, 10, 39] and in accordance with the levels of POPs in the marine biota of North West Greenland and East Greenland being higher than that of Central West [39]. A greater prevalence of western lifestyle habits (the consumption of imported food) was reported for Nuuk and Sisimiut subjects [11, 13], which can explain the lower POP burden in Sisimiut and Nuuk subjects compared to Qaanaq and Tasiilaq. However, in the present study, Nuuk men elicited higher POP levels than men from other districts, which may be due to their higher age and high intake of seafood and seabird [11, 40].

The pattern of regional differences of serum AhR-TEQ levels did not follow the district-distribution of POPs levels. The AhR-TEQ level of Nuuk men having the highest POP level was lower than that of Sisimiut men with the lowest POPs level, whereas Qaanaq women with higher POPs level elicited the lowest AhR-TEQ level. However, the regional differences

of AhR-TEQ seemed to mimic the district variation of the ratio of the Σ DL-PCB to the Σ PCB₁₄. For both sexes, the DL-PCB proportion of Qaanaq subjects was in general lower than that of Nuuk, Tasiilaq and Sisimiut (men: Nuuk > Tasiilaq \geq Qaanaq \geq Sisimiut; women: Sisimiut \geq Nuuk \geq Tasiilaq \geq Qaanaq) and consistently lower AhR-TEQ level was observed for Qaanaq men and women (men: Tasiilaq \geq Sisimiut \geq Nuuk > Qaanaq; women: Nuuk \geq Tasiilaq \geq Sisimiut > Qaanaq). Similarly, male participants in all districts had higher POP burden but lower DL-PCB proportion and accordingly a tendency of lower AhR-TEQ level compared to female was observed in the present study. Our study further support the previous report that DL-PCBs were major contributors of the total TEQ concentration in the plasma of Arctic Inuits [41]. Therefore, the total POPs level may not be a suitable proxy of the DLCs levels and thus the composition of the POPs in the body must be taken into account for the assessment of DL- activity of biological samples [26].

Ayotte and coworkers reported that the average plasma AhR-TEQ level of Inuit adults living in Arctic Quebec was 184 pg/g lipid [41], being compatible with the serum AhR-TEQ level of Greenlandic Inuits determined in the present study (161 pg /g lipid). Previously, we reported a lower AhR-TEQ level for Greenlandic Inuit males (197 pg /g lipid) than for European men from Sweden, Warsaw and Kharkiv with the average serum AhR-TEQ level of 317 pg/g lipid [26]. The present study further supports that Greenlandic Inuits have lower DLCs level than European individuals.

In the present study, the competitive AhR mediated activities (AhRcomp) differed between districts and between sexes. Compared to Qaanaq and Sisimiut men, Nuuk and Tasiilaq men had the lowest AhRcomp level and being in accordance with the higher frequency of samples eliciting antagonistic effect on the TCDD induced AhR activity, suggesting the presence of compounds having the potency to competing with TCDD for the AhR activation. The higher incidence of additive/synergistic effect on the TCDD induced effect of Sisimiut and Qaanaq

men as well as women from all districts suggest the presence of compounds further enhancing the TCDD effects. Therefore these subjects might be in risk of health problem when toxicants such as TCDD and related compounds simultaneously exist in the body. The differences in the incidence of additive/synergistic and antagonistic effects might be caused by the different POP composition of Inuits from different districts and for genders also difference in diet intake might have an impact on the AhR function [10, 11].

Humans are exposed to a combination of several xenobiotics. It is likely that the interaction between these contaminants, additivity and /or synergy and /or antagonism, will be reflected in the final toxic effect. The *in vivo* and *in vitro* studies have reported that PCB mixtures and individual PCB congeners antagonized TCDD effects [42, 43], and Aroclor 1254 was reported to inhibit TCDD induced 7-ethoxyresorufin O-deethylase (EROD) activity [44]. A mixture of PCBs, based on the congeners identified in human milk, significantly decreased TCDD induced rat liver EROD activity [42]. For the POPs determined in the serum extract in this study, only 3 DL-PCBs (CB105, CB-118 and CB156) and HCB were previously shown to have agonistic AhR potentials [1, 45, 46], while other PCBs such as PCB153, PCB 52, PCB128 have the potential to antagonize the AhR pathway [47]. Chlordane was reported to decrease the basal and TCDD induced CYP 1A1 promoter activity in MCF-7 cells [48]. Furthermore, most of the determined pesticides and PCBs in this study were reported as xenoestrogens (p,p'-DDT, p,p'-DDE, toxaphene, chlordane) [49-52] or antiestrogens (CB138, CB153, CB180) [53, 54]. Previous studies showed that o,p'-DDT and other xenoestrogenic pesticides such as endosulfan and dieldrin significantly decreased the basal and TCDD induced AhR mediated gene expression [55, 56]. Besides, other less studied groups of halogenated organic chemicals having physical and chemical properties in common with the well-studied POPs can be expected to accumulate in the Arctic environment in a similar manner. Some polybrominated diphenylethers (PBDEs) are lipophilic and persistent,

and consequently they bioaccumulate [57]. Reports have documented the existence of PBDEs in both the Arctic abiotic and biotic samples [29], and PBDEs were found in the body of marine animals in Greenland [58]. Similar to other polyhalogenated aromatic hydrocarbons (PHAHs) such as PCBs, several PBDEs competitively binds to the AhR [59], and decreased the TCDD induced effect in rodent hepatoma cell lines, human hepatoma and breast carcinoma cell lines [60-63]. BDE77 was shown to inhibit the TCDD induced AhR-mediated gene expression both in mouse and rat hepatoma cells, suggesting its antagonizing AhR property [61]. The clean-up procedure used in this study might include the PBDEs in the serum extract. As humans are exposed to a complex mixture and many quantitatively important PBDEs and PCBs can act as AhR antagonists most likely in concert when present together, this could actually influence the overall effect of DLCs by decreasing the net serum DL- activity. Since the serum DL-activity determined in the present study is the integrated net effect of the AhR agonists and antagonists of the constituents in the mixture, the negative correlation of AhR-TEQ, AhRcomp and POPs / PCBs / pesticides observed might be due to a higher concentration of compounds having antagonistic activity on AhR transactivity.

PCBs generally biomagnify within the aquatic food-chains. However, the biomagnification of PCBs in the aquatic food-chain is congener-specific evidenced by that the concentration of CB77, CB126 and CB169 showed no obvious biomagnification although the concentration of CB138 increased from plankton to piscivores to herring gulls [64]. The lack of biomagnification of CB77 was attributed to its rapid elimination by aquatic species [65] and the CB105 and CB118 are more easily metabolized congeners than non DL-PCBs such as CB153 [66]. Owing to this selective biotransformation effect during the bioaccumulation in the food chain, the concentration of some DL-PCBs might be reduced, whereas non DL-PCBs accumulate causing the variation of the composition of PCBs in the body of Inuits.

We observed that the serum AhR-TEQ was negatively correlated to age, being in accordance to the frequency of marine food intake and POPs bioaccumulation over time. We also observed that for Qaanaq women the serum AhR-TEQ inversely correlated to seafood intake as indicated by the n-3/n-6 ratio, suggesting that the DL- activity might follow the intake of n6 fatty acids possibly with the origin from non-marine diet being supported by our previous report (Bonefeld-Jorgensen and Long, submitted). Moreover, it was reported that the important source of DLCs for the population of the western world is dairy food intake [67-69]. In the present study, we observed that the consumption of dairy food was higher for the Nuuk and Sisimiut participants than that of Qaanaq and a positive correlation between dairy food intake and serum AhR-TEQ level was observed for the combined Nuuk and Sisimiut data and the combined Nuuk, Sisimiut and Qaanaq data, respectively. Being more westernized, the import of western food and intake of milk products was reported to be higher for the subjects living in Nuuk and Sisimiut, while the intake of local Greenlandic products was relatively higher in Qaanaq [11]. Hence, the dairy food might be an important source of DLCs for the Inuits living in bigger cities such as Nuuk and Sisimiut, relating to the higher AhR-TEQ level observed for Nuuk and Sisimiut compared to Qaanaq.

5. Conclusions

The serum DL-activity differed among Greenlandic districts, suggesting that the difference of serum DL- activity depends on the composition of bioaccumulated POPs. The observed inverse correlation of POPs and AhR-TEQ or AhRcomp data suggests the presence of compounds with high antagonistic impact on the AhR signalling pathway, probably due to the selective POP bioaccumulation in the food chain. Further study is needed to elucidate this preliminary conclusion.

Abbreviations

AhR aryl hydrocarbon receptor

AhRag agonistic AhR activity

AhRcomp competitive AhR activity

ARNT AhR nuclear translocator

CALUX Chemical activated luciferase gene expression

DDT 2, 2-bis (p-chlorophenyl)-1, 1, 1-trichloroethane

DLCs dioxin-like compounds

DL-activity dioxin-like activity

DL-PCBs dioxin-like PCBs

DREs dioxin-responsive elements

EROD 7-ethoxyresorufin O-deethylase

GC-MS gas chromatography mass spectrometry

HCB hexachlorobenzene

β-HCH Hexachlorocyclohexane

PCDDs/PCDFs polychlorinated dibenzo-p-dioxins/furans

PCBs polychlorinated biphenyls

PBDEs polybrominated diphenylethers

PHAHs polyhalogenated aromatic hydrocarbons

POPs persistent organic pollutants

TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin

TEFs Toxic Equivalency Factors

TEQs TCDD toxic equivalents


Competing interests

The authors declare that they have no competing interest

Author's contributions

ML and ECB-J were responsible for design of the DL-activity study and preparation of the manuscript; ML performed the mechanistic work and data evaluation. BD has provided the epidemiological data concerning POPs, questionnaires and fatty acids.

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References

1. Van den Berg, M., L. Birnbaum, A.T. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X. van Leeuwen, A.K. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski, Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect*, 1998. 106(12): p. 775-92.
2. Safe, S.H., Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol*, 1994. 24(2): p. 87-149.
3. Muir, D.C., R. Wagemann, B.T. Hargrave, D.J. Thomas, D.B. Peakall, and R.J. Norstrom, Arctic marine ecosystem contamination. *Sci Total Environ*, 1992. 122(1-2): p. 75-134.
4. Vorkamp, K., F. Riget, M. Glasius, M. Pecseli, M. Lebeuf, and D. Muir, Chlorobenzenes, chlorinated pesticides, coplanar chlorobiphenyls and other organochlorine compounds in Greenland biota. *Sci Total Environ*, 2004. 331(1-3): p. 157-75.
5. Barrie, L.A., D. Gregor, B. Hargrave, R. Lake, D. Muir, R. Shearer, B. Tracey, and T. Bidleman, Arctic contaminants: sources, occurrence and pathways. *Sci Total Environ*, 1992. 122(1-2): p. 1-74.
6. Van Oostdam, J.C., E. Dewailly, A. Gilman, J.C. Hansen, J.O. Odland, V. Chashchin, J. Berner, J. Butler-Walker, B.J. Lagerkvist, K. Olafsdottir, L. Soininen, P. Bjerregard, V. Klopov, and J.P. Weber, Circumpolar maternal blood contaminant survey, 1994-1997 organochlorine compounds. *Sci Total Environ*, 2004. 330(1-3): p. 55-70.
7. Deutch, B. and J.C. Hansen, High human plasma levels of organochlorine compounds in Greenland. Regional differences and lifestyle effects. *Dan Med Bull*, 2000. 47(2): p. 132-7.
8. Bonefeld-Jorgensen, E.C. and P. Ayotte, *Toxicological Properties of POPs and Related Health Effects of Concern for the Arctic Populations (Chapter 6)*, in *AMAP assessment 2002: Human Health in the Arctic*. 2003, Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xiv + 137pp: Oslo, Norway. p. 57-74.
9. Deutch, B., J. Dyerberg, H.S. Pedersen, G. Asmund, P. Moller, and J.C. Hansen, Dietary composition and contaminants in north Greenland, in the 1970s and 2004. *Sci Total Environ.*, 2006a. 370: p. 372-381.
10. Deutch, B., H.S. Pedersen, and J.C. Hansen, Dietary composition in Greenland 2000, plasma fatty acids and persistent organic pollutants. *Sci Total Environ*, 2004. 331(1-3): p. 177-88.
11. Deutch, B., H.S. Pedersen, and J.C. Hansen, Contaminants, diet, plasma fatty acids and smoking in Greenland. *Science of Total Environment*, 2006b. in press.
12. Kinloch, D., H. Kuhnlein, and D.C. Muir, Inuit foods and diet: a preliminary assessment of benefits and risks. *Sci Total Environ*, 1992. 122(1-2): p. 247-78.
13. Dewailly, E., G. Mulvad, H.S. Pedersen, P. Ayotte, A. Demers, J.P. Weber, and J.C. Hansen, Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. *Environ Health Perspect*, 1999. 107(10): p. 823-8.
14. Steenland, K., P. Bertazzi, A. Baccarelli, and M. Kogevinas, Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen. *Environ Health Perspect*, 2004. 112(13): p. 1265-8.

15. Lindstrom, G., K. Hooper, M. Petreas, R. Stephens, and A. Gilman, Workshop on perinatal exposure to dioxin-like compounds. I. Summary. *Environ Health Perspect*, 1995. 103 Suppl 2: p. 135-42.
16. Rowlands, J.C. and J.A. Gustafsson, Aryl hydrocarbon receptor-mediated signal transduction. *Crit Rev Toxicol*, 1997. 27(2): p. 109-34.
17. Safe, S. and V. Krishnan, Cellular and molecular biology of aryl hydrocarbon (Ah) receptor-mediated gene expression. *Arch Toxicol Suppl*, 1995. 17: p. 99-115.
18. Bonefeld Jorgensen, E.C. and P. Ayotte, Toxicological properties of persistent organic pollutants and related health effects of concern for the arctic populations. *AMAP Assessment 2002: Human Health in the Arctic*, 2003. chapter 6: p. 57-74.
19. Van Overmeire, I., Clark, G.C., Brown, D.J., Chu, M.D., Cooke, W. M., Denison, M.S., Baeyens, W., Srebrnik S., Goeyens, L., Trace contamination with dioxin-like chemicals: evaluation of bioassay-based TEQ determination for hazard assessment and regulatory responses. *Environmental Science & Policy*, 2001. 4(6): p. 345-357.
20. Laier, P., Cederberg, T., Larsen, J.C., Vinggaard, A.M., Applicability of the CALUX bioassay for screening of dioxin levels in human milk samples. *Food Additives and Contaminants*, 2003: p. 1-13.
21. Aarts, J.M., M.S. Denison, M.A. Cox, M.A. Schalk, P.M. Garrison, K. Tullis, L.H. de Haan, and A. Brouwer, Species-specific antagonism of Ah receptor action by 2,2',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphenyl. *Eur J Pharmacol*, 1995. 293(4): p. 463-74.
22. Long, M., P. Laier, A.M. Vinggaard, H.R. Andersen, J. Lynggaard, and E.C. Bonefeld-Jorgensen, Effects of currently used pesticides in the AhR-CALUX assay: comparison between the human TV101L and the rat H4IIE cell line. *Toxicology*, 2003. 194(1-2): p. 77-93.
23. Garrison, P.M., K. Tullis, J.M. Aarts, A. Brouwer, J.P. Giesy, and M.S. Denison, Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin-like chemicals. *Fundam Appl Toxicol*, 1996. 30(2): p. 194-203.
24. Murk, A.J., J. Legler, M.S. Denison, J.P. Giesy, C. van de Guchte, and A. Brouwer, Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. *Fundam Appl Toxicol*, 1996. 33(1): p. 149-60.
25. Aarts, J.M.M.J.G., P.H. Cenjin, B.M.G. Blankvoort, A. Murk, A. Brouwer, T.F.H. Bovee, W.A. Traag, L.A.P. Hoogenboom, S. Patandin, N. Weisglas-Kuperus, P.J.J. Sauer, and M.S. Denison, Application of the chemical-activated luciferase expression (CALUX) bioassay for quantification of dioxin-like compound in small samples of human milk and blood plasma. *Organohal Compds*, 1996. 27: p. 285-290.
26. Long, M., B.S. Andersen, C.H. Lindh, L. Hagmar, A. Giwercman, G.C. Manicardi, D. Bizzaro, M. Spano, G. Toft, H.S. Pedersen, V. Zvyezday, J.P. Bonde, and E.C. Bonefeld-Jorgensen, Dioxin-like activities in blood across European and Inuit populations. *Environ Health*, 2006. 5(1): p. 14.
27. Windal, I., M.S. Denison, L.S. Birnbaum, N. Van Wouwe, W. Baeyens, and L. Goeyens, Chemically activated luciferase gene expression (CALUX) cell bioassay analysis for the estimation of dioxin-like activity: critical parameters of the CALUX procedure that impact assay results. *Environ Sci Technol*, 2005. 39(19): p. 7357-64.
28. Brouwer, A., U.G. Ahlborg, M. Van den Berg, L.S. Birnbaum, E.R. Boersma, B. Bosveld, M.S. Denison, L.E. Gray, L. Hagmar, E. Holene, and et al., Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. *Eur J Pharmacol*, 1995. 293(1): p. 1-40.

29. AMAP, Arctic Monitoring and Assessment Programme. <http://www.amap.no/>.
30. Butler Walker, J., L. Seddon, E. McMullen, J. Houseman, K. Tofflemire, A. Corriveau, J.P. Weber, C. Mills, S. Smith, and J. Van Oostdam, Organochlorine levels in maternal and umbilical cord blood plasma in Arctic Canada. *Sci Total Environ*, 2003. 302(1-3): p. 27-52.
31. Deutch, B., H.S. Pedersen, E.C. Jorgensen, and J.C. Hansen, Smoking as a determinant of high organochlorine levels in Greenland. *Arch Environ Health*, 2003. 58(1): p. 30-6.
32. Tjonneland, A., K. Overvad, E. Thorling, and M. Ewertz, Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. *Am J Clin Nutr*, 1993. 57(5): p. 629-33.
33. Bonefeld-Jorgensen, E.C., H.T. Grunfeld, and I.M. Gjermansen, Effect of pesticides on estrogen receptor transactivation in vitro: a comparison of stable transfected MVLN and transient transfected MCF-7 cells. *Mol Cell Endocrinol*, 2005. 244(1-2): p. 20-30.
34. Covaci, A., G. Koppen, R. Van Cleuvenbergen, P. Schepens, G. Winneke, N. van Larebeke, V. Nelen, R. Vlietinck, and G. Schoeters, Persistent organochlorine pollutants in human serum of 50-65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 2: Correlations among PCBs, PCDD/PCDFs and the use of predictive markers. *Chemosphere*, 2002. 48(8): p. 827-32.
35. Pauwels, A., P.H. Cenijn, P.J. Schepens, and A. Brouwer, Comparison of chemical-activated luciferase gene expression bioassay and gas chromatography for PCB determination in human serum and follicular fluid. *Environ Health Perspect*, 2000. 108(6): p. 553-7.
36. Warner, M., B. Eskenazi, D.G. Patterson, G. Clark, W.E. Turner, L. Bonsignore, P. Mocarelli, and P.M. Gerthoux, Dioxin-Like TEQ of women from the Seveso, Italy area by ID-HRGC/HRMS and CALUX. *J Expo Anal Environ Epidemiol*, 2004.
37. Ayotte, P., E. Dewailly, G.H. Lambert, S.L. Perkins, R. Poon, M. Feeley, C. Larochelle, and D. Pereg, Biomarker measurements in a coastal fish-eating population environmentally exposed to organochlorines. *Environ Health Perspect*, 2005. 113(10): p. 1318-24.
38. James, R.A., I. Hertz-Picciotto, E. Willman, J.A. Keller, and M.J. Charles, Determinants of serum polychlorinated biphenyls and organochlorine pesticides measured in women from the child health and development study cohort, 1963-1967. *Environ Health Perspect*, 2002. 110(7): p. 617-24.
39. Riget, F., R. Dietz, K. Vorkamp, P. Johansen, and D. Muir, Levels and spatial and temporal trends of contaminants in Greenland biota: an updated review. *Sci Total Environ*, 2004. 331(1-3): p. 29-52.
40. Johansen, P., D. Muir, G. Asmund, and F. Riget, Human exposure to contaminants in the traditional Greenland diet. *Sci Total Environ*, 2004. 331(1-3): p. 189-206.
41. Ayotte, P., E. Dewailly, J.J. Ryan, S. Bruneau, and G. Lebel, PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (Arctic Quebec). *Chemosphere*, 1997. 34(5-7): p. 1459-68.
42. Chu, I., P. Lecavalier, H. Hakansson, A. Yagminas, V.E. Valli, P. Poon, and M. Feeley, Mixture effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and polychlorinated biphenyl congeners in rats. *Chemosphere*, 2001. 43(4-7): p. 807-14.
43. Chen, G. and N.J. Bunce, Interaction between halogenated aromatic compounds in the Ah receptor signal transduction pathway. *Environ Toxicol*, 2004. 19(5): p. 480-9.

44. Bannister, R., D. Davis, T. Zacharewski, I. Tizard, and S. Safe, Aroclor 1254 as a 2,3,7,8-tetrachlorodibenzo-p-dioxin antagonist: effects on enzyme induction and immunotoxicity. *Toxicology*, 1987. 46(1): p. 29-42.
45. van den Berg, M., R.E. Peterson, and D. Schrenk, Human risk assessment and TEFs. *Food Addit Contam*, 2000. 17(4): p. 347-58.
46. Hahn, M.E., J.A. Goldstein, P. Linko, and T.A. Gasiewicz, Interaction of hexachlorobenzene with the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in vitro and in vivo. Evidence that hexachlorobenzene is a weak Ah receptor agonist. *Arch Biochem Biophys*, 1989. 270(1): p. 344-55.
47. Suh, J., J.S. Kang, K.H. Yang, and N.E. Kaminski, Antagonism of aryl hydrocarbon receptor-dependent induction of CYP1A1 and inhibition of IgM expression by di-ortho-substituted polychlorinated biphenyls. *Toxicol Appl Pharmacol*, 2003. 187(1): p. 11-21.
48. Coumoul, X., M. Diry, C. Robillot, and R. Barouki, Differential regulation of cytochrome P450 1A1 and 1B1 by a combination of dioxin and pesticides in the breast tumor cell line MCF-7. *Cancer Res*, 2001. 61(10): p. 3942-8.
49. Bonefeld Jorgensen, E.C., H. Autrup, and J.C. Hansen, Effect of toxaphene on estrogen receptor functions in human breast cancer cells. *Carcinogenesis*, 1997. 18(8): p. 1651-4.
50. Bonefeld-Jorgensen, E.C., The Human Health Effect Programme in Greenland, a review. *Sci Total Environ*, 2004. 331(1-3): p. 215-31.
51. Welch, R.M., W. Levin, and A.H. Conney, Estrogenic action of DDT and its analogs. *Toxicol Appl Pharmacol*, 1969. 14(2): p. 358-67.
52. Cassidy, R.A., C.V. Vorhees, D.J. Minnema, and L. Hastings, The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol Appl Pharmacol*, 1994. 126(2): p. 326-37.
53. Bonefeld-Jorgensen, E.C., H.R. Andersen, T.H. Rasmussen, and A.M. Vinggaard, Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. *Toxicology*, 2001. 158(3): p. 141-53.
54. Rasmussen, T.H., F. Nielsen, H.R. Andersen, J.B. Nielsen, P. Weihe, and P. Grandjean, Assessment of xenoestrogenic exposure by a biomarker approach: application of the E-Screen bioassay to determine estrogenic response of serum extracts. *Environ Health*, 2003. 2(1): p. 12.
55. Coumoul, X., M. Diry, C. Robillot, and R. Barouki, Differential regulation of cytochrome P450 1A1 and 1B1 by a combination of dioxin and pesticides in the breast tumor cell line MCF-7. *Cancer Res*, 2001. 61(10): p. 3942-8.
56. Jeong, H.G. and J.Y. Kim, Effects of o,p'-DDT on the 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible CYP1A1 expression in murine Hepa-1c1c7 cells. *Food Chem Toxicol*, 2002. 40(11): p. 1685-92.
57. Rahman, F., K.H. Langford, M.D. Scrimshaw, and J.N. Lester, Polybrominated diphenyl ether (PBDE) flame retardants. *Sci Total Environ*, 2001. 275(1-3): p. 1-17.
58. Christensen, J.H., M. Glasius, M. Pecseli, J. Platz, and G. Pritzl, Polybrominated diphenyl ethers (PBDEs) in marine fish and blue mussels from southern Greenland. *Chemosphere*, 2002. 47(6): p. 631-8.
59. Meironyte, D., K. Noren, and A. Bergman, Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. *J Toxicol Environ Health A*, 1999. 58(6): p. 329-41.
60. Chen, G. and N.J. Bunce, Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. *Toxicol Sci*, 2003. 76(2): p. 310-20.

61. Peters, A.K., K. van Londen, A. Bergman, J. Bohonowych, M.S. Denison, M. van den Berg, and J.T. Sanderson, Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4IIE cells. *Toxicol Sci*, 2004. 82(2): p. 488-96.
62. Peters, A.K., S. Nijmeijer, K. Gradin, M. Backlund, A. Bergman, L. Poellinger, M.S. Denison, and M. Van den Berg, Interactions of polybrominated diphenyl ethers with the aryl hydrocarbon receptor pathway. *Toxicol Sci*, 2006. 92(1): p. 133-42.
63. Peters, A.K., J.T. Sanderson, A. Bergman, and M. van den Berg, Antagonism of TCDD-induced ethoxyresorufin-O-deethylation activity by polybrominated diphenyl ethers (PBDEs) in primary cynomolgus monkey (*Macaca fascicularis*) hepatocytes. *Toxicol Lett*, 2006. 164(2): p. 123-32.
64. IPCS, Concise International Chemical Assessment Document 55: Polychlorinated biphenyls: human health aspects. 2003.
65. Koslowski, S.E., C.D. Metcalfe, R. Lazar, and G.D. Haffner, The distribution of 42 PCBs, including three coplanar congeners, in the food web of the western basin of Lake Erie. *Journal of Great Lakes Research*, 1994. 20: p. 260-270.
66. Demers, A., P. Ayotte, J. Brisson, S. Dodin, J. Robert, and E. Dewailly, Plasma concentrations of polychlorinated biphenyls and the risk of breast cancer: a congener-specific analysis. *Am J Epidemiol*, 2002. 155(7): p. 629-35.
67. Baars, A.J., M.I. Bakker, R.A. Baumann, P.E. Boon, J.I. Freijer, L.A. Hoogenboom, R. Hoogerbrugge, J.D. van Klaveren, A.K. Liem, W.A. Traag, and J. de Vries, Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands. *Toxicol Lett*, 2004. 151(1): p. 51-61.
68. Llobet, J.M., J.L. Domingo, A. Bocio, C. Casas, A. Teixido, and L. Muller, Human exposure to dioxins through the diet in Catalonia, Spain: carcinogenic and non-carcinogenic risk. *Chemosphere*, 2003. 50(9): p. 1193-200.
69. Taioli, E., R. Marabelli, G. Scortichini, G. Migliorati, P. Pedotti, A. Cigliano, and V. Caporale, Human exposure to dioxins through diet in Italy. *Chemosphere*, 2005. 61(11): p. 1672-6.

Figure legends

Fig. 1. Geographical localisation of Nuuk, Sisimiut, Qaanaq and Tasiilaq

Fig.2. The DL-activity of the different groups and sexes. A) The AhR-TEQ in different districts; B) The AhR-TEQ in different sexes; C) The AhRcomp activity in different districts and D) The AhRcomp activity in different sexes. The reference line of the control is given as dash line.

Fig. 3. The correlation between \sum POP and DL- activity in the serum of Greenlandic Inuits.

A) \sum POP versus AhR-TEQ among different districts; B) \sum POP versus AhR-TEQ between sexes; C) \sum POP versus AhRcomp among different districts. D) \sum POP versus AhRcomp between genders. The regression coefficients see Table 4 A & B

Table 1. Characteristics of study groups

		Men					Women					Men+Women				
		Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All
Age (years)	n	50	52	43	41	186	45	42	36	48	171	95	94	79	89	357
	median	54	30	34	34	37	38	31	34	29	34	44	31	34	32	35
	mean	54	31	34	34	39	36	33	33	30	33	46	31	33	32	36
	Min	35	18	19	19	18	19	18	18	19	18	19	18	18	19	18
	Max	77	46	45	45	77	45	44	44	45	45	77	46	45	45	77
BMI (kg / m ²)	n	46	52	43	41	182	43	42	36	48	169	89	94	79	89	351
	median	28	26	27	26	26	26	26	24	25	25	26	26	26	25	26
	mean	28	27	27	26	27	26	27	26	26	26	27	27	27	26	26
	Min	22	19	20	21	19	18	19	18	17	17	18	19	18	17	17
	max	35	36	41	33	41	32	47	34	40	47	35	47	41	40	47
Smoking (years)	n	45	52	41	40	178	42	42	34	48	166	87	94	75	88	344
	median	34	12	18	15	18	20	12	16	12	14	23	12	17	13	16
	mean	31	12	17	15	19	16	13	16	13	14	23	12	16	14	16
	Min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	max	59	26	31	31	59	33	27	29	31	33	59	27	31	31	59
n-3/n-6	n	50	37	43	41	171	45	38	36	48	167	95	75	79	89	338
	median	0.61	0.22	0.40	0.40	0.40	0.25	0.29	0.40	0.41	0.33	0.36	0.25	0.40	0.40	0.36
	mean	0.74	0.29	0.48	0.45	0.50	0.29	0.32	0.48	0.46	0.38	0.51	0.30	0.48	0.45	0.44
	Min	0.20	0.10	0.09	0.12	0.09	0.12	0.14	0.15	0.18	0.12	0.12	0.10	0.09	0.12	0.09
	Max	1.93	0.73	1.45	1.03	1.93	0.72	0.82	1.53	1.0	1.53	1.93	0.82	1.53	1.03	1.93
seabird intake (per month)	n	47	49	41	na	137	44	41	35	na	120	91	90	76	na	257
	median	2.0	1.0	1.0	na	2.0	1.0	1.0	1.0	na	1.0	2.0	1.0	1.0	na	1.0
	mean	5.5	2.6	2.6	na	3.6	1.4	1.3	3.4	na	1.9	3.5	2.0	3.0	na	2.8
	Min	0	0	0	na	0	0	0	1.0	na	0	0	0	0	na	0
	Max	20	28	20	na	28	8.0	2.0	28	na	28	20	28	28	na	28
Dairy food consumption (per month)	n	48	51	43	na	142	45	42	35	na	122	93	93	78	na	264
	median	41	51	32	na	41	56	57	37	na	49	48	51	36	na	43
	mean	39	49	30	na	40	53	49	36	na	47	46	49	33	na	43
	Min	2	8	3	na	2	2	0	0	na	0	2	0	0	na	0
	Max	84	84	58	na	84	84	84	84	na	84	84	84	84	na	84

na: not available

Table 2. The plasma POP levels in the subjects of the different district

	Men					Women					Men+Women					
	Nuuk	Sisimiut	Qaannaq	Tasiiliaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	
Σ PCB ₁₄ ($\mu\text{g}/\text{kg}$ lipid)	n	38	51	41	37	167	44	42	34	47	167	82	93	75	84	334
	median	2682	540.9	1854	1890	1526	295.7	346	1188	1660	635.5	912.4	495.3	1379	1806	1068
	mean	3094	752.1	2699	2079	2057	492.4	459.7	1406	1809	1041	1698	620.0	2113	1928	1549
	Min	490	132	617	318	132	97.5	67.2	99	112	67.2	97.5	67.2	99	112	67.2
	Max	11113	2366	7352	4527	11113	2428	1384	5980	6701	6701	11113	2366	7352	6701	11113
Σ pesticide ($\mu\text{g}/\text{kg}$ lipid)	n	38	51	41	41	171	44	42	34	48	168	82	93	75	89	339
	median	3401	737.8	2630	2242	2126	481.6	496.3	1671	2054	924.6	1336	685.3	2156	2218	1478
	mean	4655	1195	4457	2674	3024	739.7	765.7	2290	2577	1585	2521	949.5	3474	2560	2311
	Min	405	141	617	194	155	83.6	75.6	77.9	153	85.5	83.6	75.6	77.9	153	85.5
	Max	19118	3542	14366	7224	21394	2881	3014	7529	6995	8425	19118	3542	14366	7224	21394
Σ POP ($\mu\text{g}/\text{kg}$ lipid)	n	38	51	41	37	167	44	42	34	47	167	82	93	75	84	334
	median	6423	1359	4419	4276	3600	764.1	831.7	2854	3672	1471	2371	1253	3445	4096	2437
	mean	7266	1772	6532	4525	4801	1194	1167	3432	4158	2477	4008	1499	5127	4320	3639
	Min	908	294	1241	510	294	183	151	186	264	151	183	151	186	264	151
	Max	30782	5915	19816	11306	30783	5370	4345	12441	12482	12842	30783	5915	19816	12842	30783
$\Sigma\text{DL-PCB} / \Sigma\text{PCB}_{14}$ (%)	n	38	51	41	37	167	44	42	34	47	167	82	93	75	84	334
	median	8.42	7.07	7.53	7.51	7.70	9.01	9.26	8.45	8.96	9.00	8.79	7.92	7.88	8.40	8.30
	mean	8.36	7.41	7.44	7.73	7.71	9.02	9.40	8.78	9.02	9.10	8.71	8.31	8.05	8.45	8.39
	Min	5.0	4.0	4.0	6.0	4.0	4.0	6.0	6.0	6.0	4.0	4.0	4.0	4.0	6.0	4.0
	Max	11.0	11.0	11.0	11.0	11.0	13.0	15.0	13.0	12.0	15.0	13.0	15.0	13.0	12.0	15.0

ΣPCB_{14} : sum of 14 PCB congeners; Σ pesticide: sum of 10 pesticides; ΣPOP : $\Sigma\text{PCB}_{14} + \Sigma$ pesticide; $\Sigma\text{DL-PCBs}$: sum of dioxin-like PCBs (PCB105, PCB118, PCB156).

Table 3. The serum levels of AhR-CALUX activity of habitants

		Men					Women					Men+Women				
		Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All	Nuuk	Sisimiut	Qaannaq	Tasiilaq	All
AhR-TEQ ¹ (pg/g lipid)	n	21	42	38	38	139	12	32	32	44	120	33	74	70	82	259
	median	166	189	110	240	153	235	188	122	228	167	188	189	115	239	161
	mean	206	247	118	296	219	326	221	131	332	248	250	236	124	315	232
	Min	95	26	58	98	26	115	93	63	100	63	94	26	58	98	26
	Max	1019	1202	271	1452	1452	1055	1032	259	1987	1987	1055	1202	271	1987	1987
	% agonist	79	88	100	97	91	64	64	100	91	79	71	76	100	94	85
AhRcomp ² (RLU/μg protein)	n	38	51	43	38	170	45	42	36	44	167	83	93	79	82	337
	median	0.78	1.21	1.35	1.04	1.15	1.41	1.33	1.31	1.28	1.32	1.08	1.26	1.33	1.18	1.23
	mean	0.82	1.24	1.38	1.12	1.15	1.50	1.38	1.33	1.92	1.54	1.19	1.30	1.36	1.55	1.35
	Min	0.38	0.59	0.77	0.31	0.31	0.9	0.94	0.74	0.25	0.25	0.38	0.59	0.74	0.25	0.25
	Max	1.18	2.46	2.17	3.24	3.24	4.54	3.88	1.87	15	15	4.54	3.88	2.17	15	15
	% add/syn	0	44	54	29	33	49	64	58	46	54	27	53	56	38	43
% antagonist	32	4	2	32	16	0	0	11	21	8	15	2	6	26	12	

¹AhR-TEQ (TCDD equivalents): The AhR-TEQ of samples eliciting significantly agonistic activity was calculated by interpolation to the TCDD dose-response curve using the Sigmaplot program, given as pg/g serum lipid. The % agonistic indicates the % of samples eliciting a significant increase in AhR activity compared to the solvent control. ²AhRcomp: AhR competitive activity of serum extract + 60 pM TCDD (EC₅₀) given as RLU/ μg protein. EC₅₀ solvent control = 1 RLU/ μg protein; % add/syn and %antagonistic indicates the % of samples responding with a further increase or decrease of the EC₅₀ (TCDD) induced activity, respectively.

Table 4A. Multivariate linear regression analysis of AhR-TEQ and POPs

		Nuuk			Sisimiut			Qaanaq			Tasiilaq			All		
Men ^a		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
Σ PCB ₁₄	raw ¹	21	-.01	.97	41	-.24	.13	37	-.14	.41	33	-.06	.72	135	-.21	.01
	+age	21	.05	.86	41	-.09	.64	37	.05	.80	33	.03	.87	135	-.16	.11
	+n-3/n-6 ²	21	-.01	.98	29	-.25	.25	37	-.30	.23	33	-.11	.54	123	-.22	.07
	+age+n-3/n-6	21	.07	.87	29	-.06	.81	37	-.14	.60	33	.01	.98	123	-.16	.21
Σ pesticide	raw	21	.08	.72	41	-.27	.087	37	-.20	.23	37	-.13	.46	139	-.25	<.01
	+age	21	.15	.57	41	-.14	.44	37	-.01	.98	37	-.05	.78	139	-.21	.04
	+n-3/n-6 ²	21	.16	.61	29	-.34	.13	37	-.49	.06	37	-.20	.23	127	-.37	<.01
	+age+n-3/n-6	21	.22	.53	29	-.20	.39	37	-.30	.32	37	-.09	.60	127	-.31	.01
Σ POP	raw ¹	21	.04	.86	41	-.26	.10	37	-.19	.24	33	-.11	.56	135	-.16	.01
	+age	21	.11	.69	41	-.12	.50	37	-.01	.96	33	-.02	.93	135	-.20	.06
	+n-3/n-6 ²	21	.09	.78	29	-.30	.17	37	-.48	.07	33	-.15	.40	123	-.29	.02
	+age+n-3/n-6	21	.16	.66	29	-.15	.54	37	-.29	.31	33	-.04	.82	123	-.23	.06
Women ^b																
Σ PCB ₁₄	raw ¹	12	.06	.85	32	.23	.20	32	-.46	.01	43	-.09	.55	119	-.04	.70
	+age	12	-.01	.99	32	.25	.24	32	-.40	.05	43	-.21	.35	119	-.002	.99
	+n-3/n-6 ²	12	.58	.19	28	.17	.43	32	-.32	.18	43	-.13	.50	115	.06	.62
	+age+n-3/n-6	12	.53	.31	28	.20	.38	32	-.25	.36	43	-.23	.35	115	.08	.53
Σ pesticide	raw ¹	12	.11	.74	32	.25	.16	32	-.53	<.01	44	-.11	.46	120	-.06	.52
	+age	12	.06	.89	32	.26	.19	32	-.50	.01	44	-.28	.20	120	-.03	.74
	+n-3/n-6 ²	12	.69	.12	28	.19	.39	32	-.51	.04	44	-.17	.35	116	.01	.97
	+age+n-3/n-6	12	.65	.20	28	.20	.37	32	-.47	.09	44	-.31	.19	116	.02	.89
Σ POP	raw ¹	12	.09	.79	32	.24	.18	32	-.51	<.01	43	-.11	.49	119	-.05	.57
	+age	12	.03	.95	32	.25	.22	32	-.48	.02	43	-.24	.28	119	-.02	.83
	+n-3/n-6 ²	12	.66	.14	28	.17	.42	32	-.47	.07	43	-.15	.43	115	.04	.77
	+age+n-3/n-6	12	.62	.23	28	.20	.39	32	-.42	.14	43	-.27	.27	115	.05	.67
Men + Women ^c																
Σ PCB ₁₄	raw ¹	33	-.19	.29	74	-.05	.65	70	-.32	.01	77	-.10	.40	254	-.15	.02
	+age	33	-.09	.79	74	.03	.83	70	-.22	.01	77	-.07	.65	254	-.10	.18
	+n-3/n-6 ²	33	.12	.72	58	-.15	.30	70	-.34	.02	77	-.15	.24	238	-.16	.06
	+age+n-3/n-6	33	.24	.60	58	-.09	.53	70	-.26	.09	77	-.11	.49	238	-.12	.17
Σ pesticide	raw ¹	33	-.13	.47	74	-.07	.58	70	-.38	<.01	82	-.12	.28	254	-.18	<.01
	+age	33	.06	.84	74	.01	.94	70	-.30	.03	82	-.12	.38	254	-.13	.07
	+n-3/n-6 ²	33	.29	.37	58	-.16	.29	70	-.47	<.01	82	-.21	.09	238	-.24	<.01
	+age+n-3/n-6	33	.41	.29	58	-.12	.44	70	-.40	.02	82	-.17	.22	238	-.21	.02
Σ POP	raw ¹	33	-.16	.38	74	-.06	.59	70	-.37	<.01	77	-.12	.30	254	-.17	.01
	+age	33	-.001	.997	74	.01	.92	70	-.29	.04	77	-.10	.49	254	-.12	.09
	+n-3/n-6 ²	33	.23	.50	58	.16	.28	70	-.45	<.01	77	-.18	.17	238	-.20	.02
	+age+n-3/n-6	33	.36	.395	58	-.11	.46	70	-.31	.02	77	-.14	.36	238	-.16	.07

The analyses were performed on ln transformed data with AhR-TEQ as dependent and POPs as independent variables before and upon adjusted for the potential confounders.

¹: non-adjusted data.

²: ratio of n-3 to n-6 fatty acids in serum.

^a: Combining districts is not allowed according to the multiple regression analyses.

^b: Combining districts is not allowed according to the multiple regression analyses.

^c: According to the multiple regression analyses, combining sex for each district is allowed while combining sexes for all districts is not allowed.

For Σ PCB₁₄, Σ pesticide and Σ POP, AhR-TEQ, see the legend of Table 2 and Table 3.

Table 4B. Multivariate linear regression analysis of AhRcomp and POPs

		Nuuk			Sisimiut			Qaannaq			Tasiilaq			All		
		n	β	p	n	β	p	n	β	p	n	β	p	n	β	p
Men^a																
Σ PCB ₁₄	raw ¹	38	-.11	.52	51	-.13	.36	41	-.07	.68	34	-.18	.31	164	-.24	<.01
	+age	38	-.16	.47	51	-.03	.87	41	-.001	1.00	34	-.29	.15	164	-.05	.56
	+n-3/n-6 ²	38	-.24	.37	37	.29	.12	41	.08	.68	34	-.20	.27	150	-.01	.95
	+age+n-3/n-6	38	-.30	.34	37	.42	.04	41	.11	.61	34	-.29	.15	150	.10	.35
Σ pesticide	raw ¹	38	-.09	.59	51	-.20	.17	41	-.12	.46	38	-.27	.11	168	-.24	<.01
	+age	38	-.10	.59	51	-.12	.45	41	-.07	.75	38	-.36	.05	168	-.06	.53
	+n-3/n-6 ²	38	-.16	.52	37	.22	.27	41	.01	.97	38	-.29	.10	154	-.04	.73
	+age+n-3/n-6	38	-.16	.52	37	.29	.17	41	.04	.87	38	-.36	.06	154	.07	.49
Σ POP	raw ¹	38	-.10	.55	51	-.18	.22	41	-.10	.53	34	-.19	.28	164	-.24	<.01
	+age	38	-.13	.53	51	-.09	.57	41	-.04	.84	34	-.30	.13	164	-.06	.53
	+n-3/n-6 ²	38	-.20	.43	37	.25	.20	41	.04	.86	34	-.21	.25	150	-.02	.87
	+age+n-3/n-6	38	-.22	.43	37	.34	.10	41	.07	.76	34	-.30	.13	150	.09	.46
Women^b																
Σ PCB ₁₄	raw ¹	44	.08	.60	42	.17	.28	34	-.31	.07	43	-.18	.24	163	-.09	.24
	+age	44	.16	.37	42	.09	.59	34	-.15	.46	43	-.51	.02	163	-.11	.17
	+n-3/n-6 ²	44	.18	.25	38	.09	.62	34	-.49	.06	43	-.27	.14	159	-.11	.25
	+age+n-3/n-6	44	.25	.14	38	.07	.71	34	-.33	.25	43	-.56	.02	159	-.13	.21
Σ pesticide	raw ¹	44	.07	.67	42	.09	.57	34	-.25	.15	44	-.21	.18	164	-.11	.18
	+age	44	.11	.49	42	-.001	.995	34	-.07	.72	44	-.55	.01	164	-.13	.13
	+n-3/n-6 ²	44	.18	.26	38	-.03	.85	34	-.37	.17	44	-.30	.10	160	-.14	.18
	+age+n-3/n-6	44	.22	.19	38	-.06	.76	34	-.19	.52	44	-.59	.01	160	-.15	.15
Σ POP	raw ¹	44	.08	.62	42	.12	.45	34	-.27	.12	43	-.195	.21	163	-.10	.21
	+age	44	.14	.42	42	.03	.85	34	-.10	.64	43	-.54	.01	163	-.12	.15
	+n-3/n-6 ²	44	.19	.24	38	.01	.97	34	-.42	.12	43	-.29	.12	159	-.13	.21
	+age+n-3/n-6	44	.24	.15	38	-.02	.94	34	-.24	.42	43	-.59	.01	159	-.14	.17
Men + Women^c																
Σ PCB ₁₄	raw ¹	82	-.55	<.01	93	-.05	.64	75	-.14	.22	77	-.22	.06	327	-.24	<.01
	+age	82	-.35	.02	93	-.07	.58	75	-.04	.76	77	-.39	.01	327	-.19	<.01
	+n-3/n-6 ²	82	-.39	.01	75	-.06	.66	75	-.07	.62	77	-.27	.03	309	-.21	<.01
	+age+n-3/n-6	82	-.21	.25	75	-.08	.55	75	-.001	1.00	77	-.41	.01	309	-.18	.02
Σ pesticide	raw ¹	82	-.53	<.01	93	-.10	.34	75	-.15	.20	82	-.23	.04	332	-.24	<.01
	+age	82	-.30	.03	93	-.13	.27	75	-.04	.80	82	-.38	.01	332	-.18	<.01
	+n-3/n-6 ²	82	-.34	.02	75	-.12	.35	75	-.08	.61	82	-.28	.02	314	-.21	.01
	+age+n-3/n-6	82	-.17	.28	75	-.14	.29	75	.01	.93	82	-.40	<.01	314	-.18	.02
Σ POP	raw ¹	82	-.54	<.01	93	-.09	.42	75	-.15	.21	77	-.22	.05	327	-.24	<.01
	+age	82	-.32	.03	93	-.11	.35	75	-.04	.80	77	-.40	.02	327	-.19	<.01
	+n-3/n-6 ²	82	-.37	.01	75	-.10	.43	75	-.07	.62	77	-.27	.03	309	-.21	<.01
	+age+n-3/n-6	82	-.19	.28	75	-.13	.35	75	.011	.95	77	-.41	.01	309	-.18	.02

The analyses were performed on ln transformed data with AhRcomp as dependent and POPs as independent variables before and upon adjusted for the potential confounders.

¹: non-adjusted data.

²: ratio of n-3 to n-6 fatty acids in serum.

^a: Combining districts is not allowed according to the multiple regression analyses.

^b: Combining districts is allowed according to the multiple regression analyses.

^c: Combining districts and sex is not allowed according to the multiple regression analyses.

For Σ PCB₁₄, Σ pesticide and Σ POP and AhRcomp, see the legend of Table 2 and Table 3.

Table 5. The Pearson correlation coefficients of serum AhR-TEQ / AhRcomp and life style factors

<i>Nuuk</i>	Men						Women						Men + Women					
	AhR-TEQ			AhRcomp			AhR-TEQ			AhRcomp			AhR-TEQ			AhRcomp		
	n	r	p	n	β	p	n	r	p	n	β	p	n	r	p	n	β	p
age	20	-.08	.74	37	-.02	.91	11	.12	.72	44	-.13	.41	32	-.19	.28	82	-.53	<.01
n-3/n-6	20	-.01	.97	37	-.02	.91	11	-.32	.32	44	-.30	.05	32	-.26	.15	82	-.50	<.01
BMI	19	.40	.08	35	.24	.15	11	.07	.84	42	-.19	.22	31	.13	.48	78	-.17	.14
smoking	19	.04	.86	32	.12	.52	8	.56	.15	34	.22	.21	27	.13	.53	66	-.22	.08
seabird	19	.001	1.0	34	.01	.94	8	-.47	.20	37	-.09	.56	28	-.19	.32	72	-.43	<.01
diary food	20	-.26	.25	36	-.12	.48	11	.34	.29	44	.014	.93	32	.03	.85	81	.19	.09
<i>Sisimiut</i>																		
age	41	-.32	.04	50	-.21	.14	31	.08	.65	41	.21	.18	73	-.18	.12	92	.01	.91
n-3/n-6	29	-.06	.75	36	-.31	.06	27	.21	.28	37	.29	.07	57	-.10	.48	74	.08	.55
BMI	41	-.05	.76	50	-.16	.28	31	.52	<.01	41	-.18	.27	73	.19	.10	92	-.16	.14
smoking	35	-.36	.03	43	-.12	.44	29	.11	.56	33	.01	.94	64	-.15	.22	76	-.03	.78
seabird	38	-.01	.97	46	-.12	.43	28	-.17	.38	38	-.05	.74	67	-.05	.72	85	-.12	.28
diary food	40	.15	.37	49	.06	.69	30	-.003	.99	40	.23	.15	71	.09	.44	90	.14	.18
<i>Qaanaq</i>																		
age	37	-.28	.08	42	-.11	.48	31	-.33	.07	35	-.34	.05	69	-.30	.01	78	-.21	.66
n-3/n-6	37	-.002	.99	42	-.16	.30	31	-.42	.02	35	-.06	.74	69	-.17	.15	78	-.11	.31
BMI	37	-.02	.49	42	-.08	.60	31	-.11	.54	35	.04	.83	69	-.13	.28	78	-.02	.87
smoking	33	-.12	.49	37	-.12	.49	29	-.23	.22	33	-.35	.05	62	-.20	.12	70	-.21	.08
seabird	35	-.19	.27	39	-.33	.04	30	-.05	.81	34	-.23	.19	66	-.01	.37	74	-.28	.17
diary food	37	-.02	.91	42	.15	.34	29	-.01	.96	35	.14	.42	67	.001	.99	76	.14	.22
<i>Tasiilaq</i>																		
age	37	-.19	.25	37	.05	.76	43	.04	.81	43	.10	.53	81	-.07	.54	81	.03	.76
n-3/n-6	37	.26	.12	37	.01	.95	43	.12	.90	43	.02	.91	81	.12	.28	81	.007	.95
BMI	37	-.002	.91	37	-.002	.99	43	-.27	.08	43	-.08	.60	81	-.19	.10	81	-.07	.52
smoking	33	.01	.97	33	-.003	.99	41	.06	.72	41	.05	.75	74	.03	.79	74	.02	.90
seabird	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
diary food	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
<i>All</i>																		
age	138	-.19	.03	169	-.35	<.01	119	-.09	.34	166	.03	.66	258	-.16	.01	336	-.20	<.01
n-3/n-6	126	.10	.27	155	-.20	.01	115	-.12	.19	162	-.04	.59	242	-.02	.81	318	-.16	.01
BMI	137	-.02	.86	167	-.01	.77	119	.01	.87	164	-.02	.23	257	-.01	.93	332	-.09	.10
smoking	120	-.17	.07	145	-.23	.01	107	-.01	.96	141	.04	.68	227	-.11	.11	286	-.11	.06
seabird*	94	-.07	.47	121	-.35	<.01	68	-.20	.10	111	-.13	.16	163	-.12	.12	233	-.33	<.01
diary food*	99	.13	.18	129	.02	.79	72	.21	.08	119	.13	.15	172	.17	.03	249	.11	.08

na: not available

*: For the combined data of Nuuk, Sisimiut and Qaanaq.



Fig. 1.

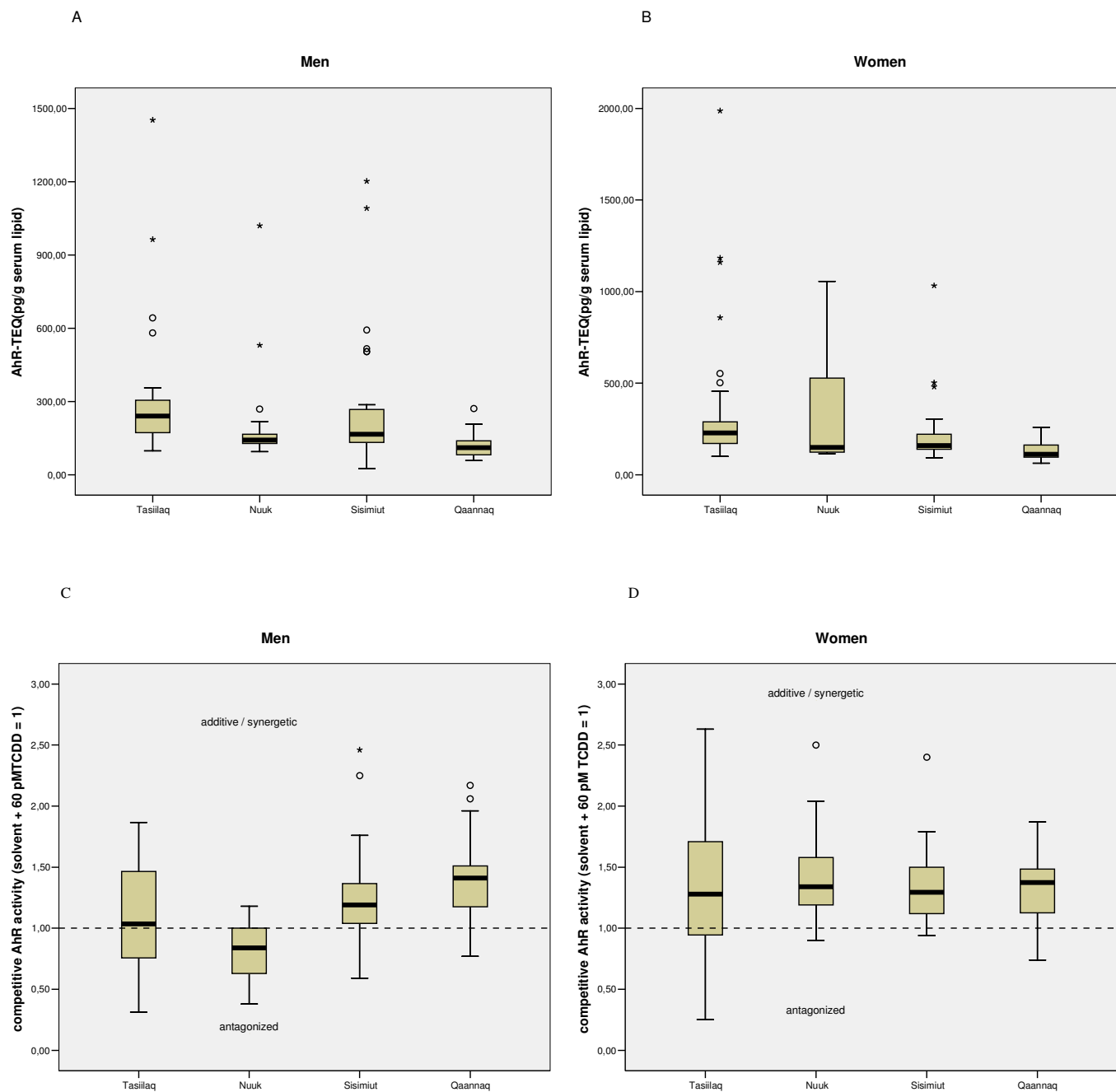


Fig. 2

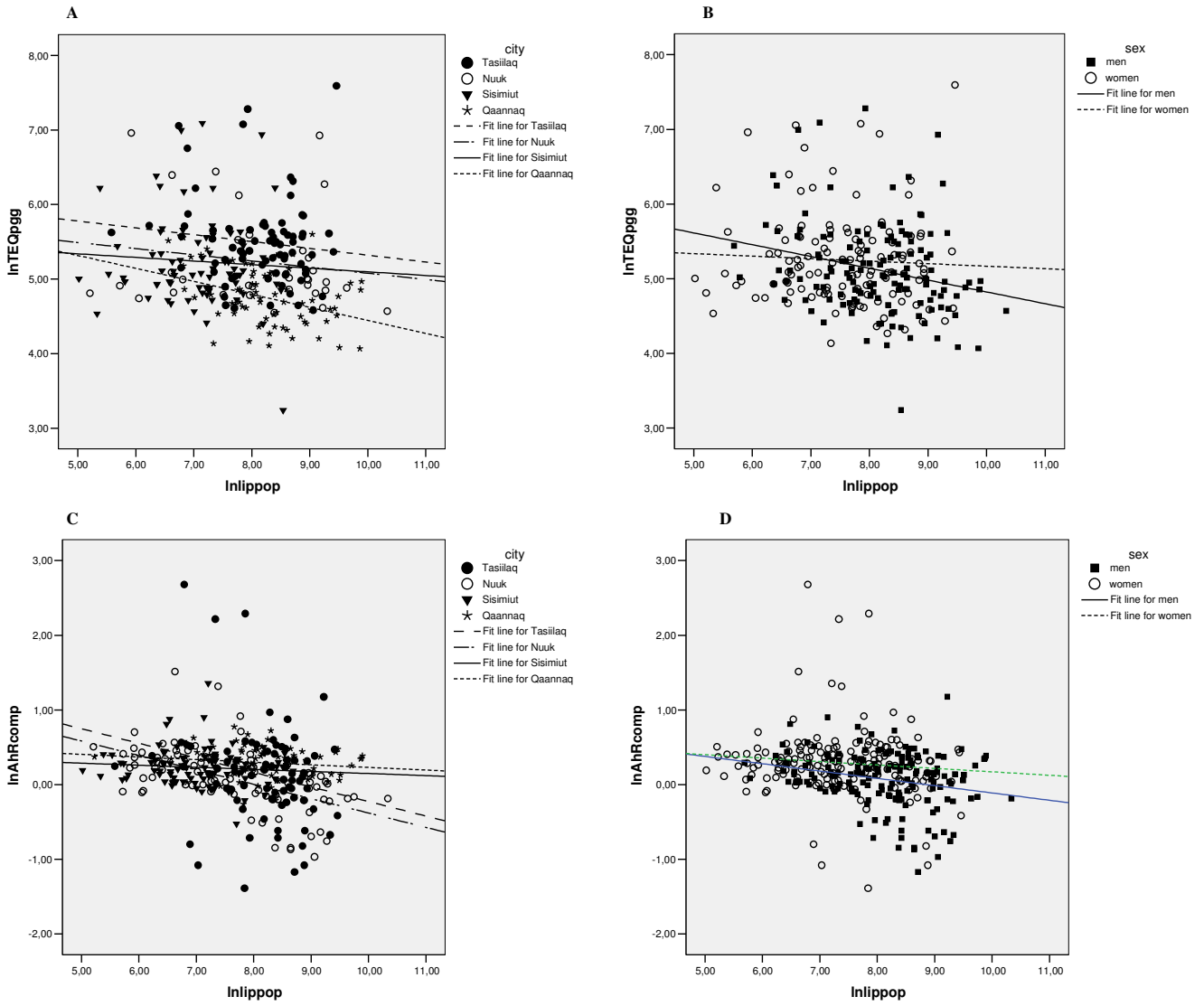


Fig. 3.

Additional files provided with this submission:

Additional file 1 : suppl-table.doc : 79Kb

<http://www.ehjournal.net/imedia/1726956898125426/sup1.DOC>