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# Exposure to persistent organic pollutants and sperm sex chromosome ratio in men from the Faroe Islands

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## Abstract

People in the Arctic as well as fishermen on the polluted Swedish east coast are highly exposed to persistent organic pollutants (POPs). These compounds have been shown to affect the sperm Y:X chromosome ratio. In present study, the aim was to investigate whether polychlorinated biphenyl (PCB) congeners and 1,1,-dichloro-2,2,-bis(*p*-chlorophenyl)ethane (*p,p′*-DDE) influence sperm sex chromosome ratio in Faroese men, and whether these men differ regarding Y:X ratio compared to Greenland Inuit and Swedish fishermen.

The study population (n = 449) consisted of young men from the general population (n = 276) as well as proven fertile men (n = 173). The Y:X ratio was assessed by fluorescent in situ hybridization. Serum concentrations of POPs were measured using gas chromatography. Associations between POP concentrations and Y:X ratio were calculated using linear and non-linear regression models as well as trend analysis and pairwise comparison of exposure data categorized into quartiles.

The selected POPs were associated with Y:X ratio in fertile Faroese men, but not in the total population; *p,p′*-DDE (95% CI for B = −0.005 to −0.001, p = 0.005) and ΣPCB (95% CI for B = −0.005 to −0.001, p = 0.012). Since *p, p′*-DDE and ΣPCB correlated significantly (r = 0.927, p < 0.001), the results involving the exposure variables can be regarded as a single finding. The Y:X ratio for the total Faroese population was 0.500 ± 0.018, which was statistically significantly lower than in both Inuit and Swedish fishermen (0.512 for both).



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In conclusion, Faroese men presented with lower Y:X ratio than Greenland Inuit and Swedish fishermen. Although no direct health effects are expected due to the lower Faroese Y:X ratio, it could be indicative of adverse effects on the reproductive system.

**Keywords**

PCB; Persistent organic pollutantsm; *p*,*p*′-DDE; Y:X ratio

## 1. Introduction

Globally, the secondary sex ratio is 0.515 at birth, or 106 males being born for every 100 females (Pyeritz, 1998). Over the last five decades, several studies have shown that the birth sex ratio in many industrialized countries is decreasing. A Danish study showed a statistically significant decrease in the birth sex ratios for Sweden, Norway, Denmark and Finland from 1945 to 1998 (Moller, 1998). Another study found a statistically significant decrease in male/female birth ratio between 1950 and 1995 in the Netherlands, Canada and the United States (Davis et al., 1998), while a study from 2003 showed a male/female birth ratio decline in Europe and North America over the second half of the 20th century (Grech et al., 2003).

The underlying reason for the drop in the secondary sex ratio is unclear, but one theory states that environmental or occupational agents from anthropogenic sources affecting the male reproductive system in a negative manner could be part of the explanation (Toppari et al., 1996). An example of this is, for instance, the accident in Seveso, Italy in 1976, leading to the release of large amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and a subsequent decrease in the birth sex ratio in the offspring of men exposed prior to adolescence (Mocarelli et al., 1996, 2000). Also, following the Yucheng poisoning in Taiwan in 1979, exposure to polychlorinated biphenyls (PCBs) and dibenzofurans, impaired sperm quality and an increased proportion of girls being born was observed (Chen et al., 1985). Studies carried out in the Great Lakes area in the USA suggested that maternal exposure to PCB would lead to a decrease in the birth sex ratio (Weisskopf et al., 2003), while paternal exposure leads to increased birth sex ratio (Karmaus et al., 2002).

Theoretically, a skewed birth sex ratio could be due to a deviation in sex chromosome ratio in sperm cells. Previously, a positive association between serum concentrations of CB-153, the most abundant PCB congener, and 1,1,-dichloro-2,2,-bis(*p*-chlorophenyl)ethane (*p*,*p*′-DDE) and the proportion of Y-chromosome bearing sperm cells was found in a cohort of Swedish fishermen, whose exposure originated from consumption of fish from the polluted Baltic sea (Tiido et al., 2005). Also, a negative correlation between CB-153 and the Y-chromosome bearing fraction of sperm cells was found in a Polish cohort that served as a European background population with respect to pollutant exposure. However, no associations at all between certain persistent organic pollutants (POPs) and Y:X chromosome ratio were observed in Inuit men from Greenland, who are among the highest to be exposed in the world (Tiido et al., 2006). These contradictory results were explained as inter-country differences in total exposure profiles, but might to some degree be due to genetic or epigenetic differences as well.

*p*,*p*′-DDE is a metabolite of the pesticide *p*,*p*′-DDT while PCBs were produced for use in, among other things, carbon less paper, capacitors and transformers (Longnecker et al., 1997). Production of these chemicals has been banned in most countries. However, production of *p*,*p*′-DDT still takes place in certain areas, mainly in Africa, where it is used to fight malaria (Rogan and Chen, 2005). As these compounds are lipophilic and highly persistent to degradation, they easily bioaccumulate in adipose tissue and in the food chain (Jensen, 1987). Due to their semi-volatile nature they are able to migrate through cycles of evaporation and atmospheric cycling and thus be transported to locations great distances from where they were released (Ritter, 1995). Many of these compounds therefore cause contamination of Arctic food chains. Hence, populations like the ones in Greenland and the Faroe Islands whose diets traditionally consist of sea mammals, in which these chemicals bioaccumulate (Dam and Bloch, 2000; Fromberg et al., 1999; Weihe and Joensen, 2012) are heavily exposed (Grandjean et al., 2001; Jonsson et al., 2005; Petersen et al., 2007). Also, a diet heavy in fish from the contaminated Baltic sea has generated a population of POP exposed Swedish fishermen, in whom the exposure levels of CB-153 are comparable to that measured in Greenland Inuit (Jonsson et al., 2005).

Studies that measured serum POP exposure levels on the Faroe Islands have shown overall markedly higher concentrations than the average European population (Jonsson et al., 2005). For example, data on POPs exposure show that average serum concentrations of CB-153 on the Faroe Islands may be over ten-fold higher than in a general European population (Jonsson et al., 2005; Longnecker et al., 2003; Petersen and others, 2008) and the Faroese men are more highly exposed to *p*,*p*′-DDE (Petersen et al., 2007; Weihe and Joensen, 2012). In a previous study on Y:X chromosome ratio in sperm samples from Inuit on Greenland and Swedish fishermen, their Y:X ratios were both 0.512 (Tiido et al., 2006).

Based on this backdrop, we wished to investigate the impact of persistent organic pollutants on sperm sex chromosome ratio in Faroese men and secondly to compare the Faroese sperm sex chromosome ratio to the Swedish east coast fishermen and to the Inuit men.

## 2. Materials and methods

### 2.1. Subjects

Semen samples from Faroese men were obtained from three different sources: the UM, K1S and K5P cohorts. The UM cohort consists of young volunteers from the general population, born between 1981 and 1984. Of the 1023 men that were invited, 241 delivered a semen sample (participation rate 24%; see Supplemental material, Fig. S1) (Halling et al., 2013). The K1S cohort was generated from consecutive births at the three Faroese hospitals between 1986 and 1987 consisting of 510 boys and 512 girls. From this cohort 421 males were asked to participate in a semen study and the final sample population consisted of 240 of these men (participation rate 57%; see Supplemental material, Fig. S2) (Grandjean et al., 1992). The K5P cohort consists of men whose pregnant partner participated in a study focusing on fertility and environmental factors. Of the 376 males who were approached to participate in a semen study, 282 men consented, out of which 16 were excluded for various reasons, leaving a total of 266 proven fertile men in the study population (participation rate 71%; see Supplemental material, Fig. S3).

Blood and semen samples were collected at the same time for the UM and K5P cohorts, while semen samples from the K1S cohort were collected a year after the blood samples.

For this study, 212 semen samples from the UM cohort, 102 semen samples from the K1S cohort and 185 samples from the K5P cohort were available.

Of the total 499 semen samples available, 449 (90%) were included in the current study (181 from UM, 95 from K1S and 173 from K5P). Of the 50 remaining samples, 40 samples did not have enough cells for FISH and for 4 samples the FISH method failed. In addition, six samples were excluded from the analysis due to lack of exposure data.

In the comparison of all included and excluded samples, those men included were slightly older (mean = 28.7 years and 26.2 years, respectively; Table 1).

The men that were included also had higher sperm concentration (mean = 63.3 mill/mL; 21.7 mill/mL) than those who were excluded. There were no differences in POP serum concentrations between included and excluded men.

Regarding the subcohorts of the study group, the men in the UM cohort had significantly higher serum concentrations of *p,p′*-DDE than the men in the K1S cohort ($p < 0.001$). Also, the men in the K5P cohort, compared to the men in the K1S and the UM cohorts, were significantly older ($p < 0001$ for both) and had significantly higher serum concentrations of *p,p′*-DDE ($p < 0.001$ for both) and ΣPCB ($p < 0.001$ for both), respectively.

The project protocol was approved by the Faroese ethical review committee and the IRB at Harvard School of Public Health. Each subject provided written informed consent in accordance with the approved protocols.

For comparison, previously analyzed and reported FISH data of 161 Greenland Inuit and 155 Swedish fishermen (Tiido et al., 2006) were included in the current study to establish the possible differences in the Y: X chromosome ratio between the Faroese, Greenland Inuit and Swedish fishermen. These populations have been previously described (Tiido et al., 2006). In short, the Inuit were between 18.5 and 51.3 (mean 31) years old, proven fertile and had somewhat higher serum concentrations of *p,p′*-DDE than the Faroese cohort. The Swedish fishermen were between 23.8 and 67.5 (mean 47) years old and, in regard to exposure, had somewhat lower serum concentrations of *p,p′*-DDE than the Faroese. The birth sex ratios were similar in the Inuit, Swedish fishermen and the Faroese population: being 0.512, 0.515 and 0.517, respectively [CIA.gov].

### 2.2. Fluorescent in situ hybridization

Protein-nucleic acid (PNA) probes (DakoCytomation, Glostrup, Denmark) targeted against the centromere of the X chromosome (FITC-labeled) and the q-arm of the Y-chromosome (Rhodamine-labeled), respectively, were used for the FISH assay.

Semen samples were thawed at room temperature and 6 μL/sample was smeared onto positively-charged microscope slides (Superfrost Plus, Menzel Gläser, Braunschweig, Germany). The slides were left to air dry at room temperature for 24 h and subsequently

nuclear DNA of the samples was denatured by immersion in 0.5 M NaOH for 10 min. The slides were then dehydrated by immersion in a series of ethanol-baths (70%, 85% and 99% in sequence) for 1 min each, after which they were air dried for 15 min.

Five microliters/sample of the Y-probe (20 nM) and 7 μL/sample of the X-probe (20 nM) were mixed in a tube (Eppendorf, Hamburg, Germany) and the probe mixture denatured at 72 °C for 6 min. Twelve microliters of the mixture was added to each slide and subsequently covered with a 22 × 22 mm hybridization cover glass (Sigma-Aldrich, St. Louis, USA) and incubated in darkness for 75 min at room temperature in a humidified hybridization chamber.

Following hybridization, the cover glasses were removed by washing the slides in phosphate buffered saline (PBS) (Gibco, Life technologies Inc., Carlsbad, USA) with 0.1% Tween-20 (Scharlau Inc., Barcelona, Spain) for 2 min at room temperature. The slides were then moved to PBS with 0.1% Tween-20 at 58 °C for an additional 20 min after which the slides were rinsed in 2× saline sodium citrate (SSC) with 0.1% Tween-20 for 15 min at room temperature. The slides were mounted in 20 μL of Vectashield (Vector laboratories Inc., Burlingame, USA) containing 0.3 μg/mL DAPI and covered with 24 × 50 mm cover glasses (Waldemar Knittel Glasbearbeitungs, Braunschweig, Germany).

The slides were stored in darkness at 4 °C until analysis by fluorescent microscopy (Olympus America Inc., Center Valley, USA). DAPI-stained nuclei were counted at 400× magnification, whereas FISH-labeled cells were visualized using FITC and Rhodamine filters. Only cells with a clearly defined single nucleus were included. Each sample contained at least 500 countable cells and had a hybridization efficiency of = 95% (Tiido et al., 2006).

The Y:X chromosome ratio was calculated by dividing the number of Rhodamine-positive cells by the total number of probe-labeled cells. Hybridization efficiency was calculated by dividing the total number of labeled cells by the total number of DAPI-stained cells. All samples from the Faroese population were analyzed with the same method, in the same laboratory and during the same time period.

Since the samples from Inuit and Swedish men were analyzed earlier using a slightly different technique (Tiido et al., 2005, 2006), in order to validate that the two methods resulted in similar Y:X ratios, thirty-three of the Inuit samples were randomly selected and re-analyzed with the FISH technique used in this study.

The FISH technique used in the present study gave a hybridization efficiency of 99.3%.

### 2.3. Analysis of POPs

Serum concentrations of *p*,*p*′-DDE were measured by gas chromatography with electron-capture detection as previously described (Petersen et al., 2006, 2007); this method was also used to measure serum concentrations of the different PCB congeners by solid phase extraction and gas chromatography (Heilmann et al., 2006; Petersen et al., 2006). The total PCB burden (∑PCB) in this study was defined as a mixture of the three major congeners

(CB-138, CB-153 and CB-180) multiplied by 2, since the sum of these three congeners represents close to 50% of the total PCB concentration (Grandjean et al., 1995).

The level of detection (LOD) was set at 0.004 μg/g lipid and the two samples that registered values below LOD were assumed to equal 0.002 μg/g.

Serum levels of ΣPCB correlated significantly with levels of *p,p′*-DDE ($p < 0.001$; $r = 0.927$), which is comparable with the correlation between CB-153 and *p,p′*-DDE found in a previous study (Tiido et al., 2005).

### 2.4. Statistical analysis

The Y:X chromosome ratio was summarized as the mean ± SD of Y: X ratio over all samples that fulfilled the inclusion criteria.

Exposure data was log transformed prior to analysis to gain a Gaussian distribution of residuals.

An exploratory spline analysis was performed in order to elucidate specific non-linear patterns in the relationship between serum concentrations and Y:X chromosome ratio. The linear relationship between the Y:X chromosome ratio and levels of exposure was investigated using a linear regression model in R (R Development Core Team 2010, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/).

In order to further investigate any possible non-linear relationships between exposure and Y:X chromosome ratio, the exposure data was categorized into quartiles, where the lowest quartile was pairwise compared to the three upper quartiles using ANOVA. Also, a trend analysis, using ordinal levels for each exposure quartile, was performed. These calculations were performed using PASW v20 statistical software (SPSS, Inc., Chicago, IL, USA).

A p-value of 0.05 was defined as the level of statistical significance. The results of the spline analysis are presented as p-value, adjusted $r^2$ and the results of the linear regression are presented as p-value and 95% CI over the regression slope. For the pairwise comparison of quartiles, the results are presented as p-value and 95% CI for p. For the trend analysis, the results are presented as B-value, p-value and 95% CI for B.

As the UM and K1S subcohorts were of comparable age and with an unknown fertility status because of young age, they were merged to enable the easier comparison with the older, proven fertile K5P subcohort (For background characteristics for the merged cohorts, see Supplemental material, Table SI).

Several covariates were considered for their separate effect on the Y: X ratio, but were later discarded (see Supplemental material, Table SII).

Differences in the Y:X chromosome ratio between the Faroese, the Inuit and the Swedish populations were tested using a univariate analysis of variance model. The results are presented as p-value and 95% CI of the difference.

The results of the two FISH assays used to analyze the Faroese and the Swedish/Inuit samples respectively, were compared by a paired samples *t*-test, which did not indicate any differences in the results gained from the two different techniques (p = 0.642; mean difference = 0.00136; 95% CI of the difference, −0.00456 to 0.00729).

Also, to more clearly elucidate possible sources of POP exposure, the relationship between frequency of ingestion of several different kinds of traditional Faroese sea food and serum exposure levels of ΣPCB and *p,p′*-DDE was investigated (see Supplemental material, Table SIII).

## 3. Results

### 3.1. Y:X chromosome ratio in Faroese men

The Y:X chromosome ratio was 0.500 ± 0.018 for the total Faroese cohort; 0.501 ± 0.020 for UM, 0.500 ± 0.018 for K1S and 0.500 ± 0.016 for K5P (Fig. 1).

### 3.2. Association between Y:X chromosome ratio and serum POP exposure levels

The explorative spline analysis did not show any significant nonlinear associations between the Y:X ratio and serum concentrations to either *p,p′*-DDE or ΣPCB in the total, merged UM + K1S cohort or in the UM or K1S subcohorts. In the K5P subcohort there were statistically significant associations between Y:X ratio and serum concentrations of both *p,p′*-DDE (p = 0.026; $r^2$ = 0.024) and ΣPCB (p = 0.030; $r^2$ = 0.022; see Supplemental material, Figs. S4 & S5).

In the linear regression model, no statistically significant associations were found for the total cohort. However, a positive, although statistically non-significant, trend for associations between the Y:X ratio and serum concentrations of both *p,p′*-DDE or ΣPCB was found in the merged UM + K1S cohort and in the UM or K1S subcohorts, while statistically significant negative associations were found between the Y:X ratio and both *p,p′*-DDE (p = 0.026; $r^2$ = 0.029) and ΣPCB (p = 0.030; $r^2$ = 0.027) in the K5P subcohort (Table 2; Fig. 2A–B).

In the pairwise comparison of quartiles of categorized serum concentrations, the only statistically significant differences in the Y:X ratio were found in the K5P cohort, between the lowest and the fourth quartile for *p,p′*-DDE (p = 0.015; 95% CI for p = 0.0017 to 0.0153) and the lowest and both the third and the fourth quartile for ΣPCB (p = 0.016; 95% CI for p = 0.0016 to 0.0153 and p = 0.028; 95% CI for p = 0.0009 to 0.0146, respectively).

A trend analysis found no significant associations between either *p,p′*-DDE or ΣPCB and the Y:X chromosome ratio in the total, merged UM + K1S cohort or in the UM or K1S subcohorts. There were, however, significant negative associations between the Y:X ratio and categorized serum concentrations of both *p,p′*-DDE (B = −0.003; p = 0.005; 95% CI = −0.005 to −0.001) and ΣPCB (B = −0.003; p = 0.012; 95% CI = −0.005 to −0.001; Table 3) in the K5P cohort.

### 3.3. Inter-population comparison of Y:X chromosome ratio

Comparison of Y:X chromosome ratio between the Faroese population, the Greenland Inuit and the Swedish fishermen showed that the Faroese men had significantly lower mean sperm sex chromosome ratio than both the Inuit (−0.012; $p < 0.001$; 95% CI −0.015 to −0.009) and Swedish fishermen (−0.012; $p < 0.001$; 95% CI −0.016 to 0.009).

## 4. Discussion

Our main finding was that the sperm Y:X chromosome ratio in fertile Faroese men was negatively associated with serum concentrations of *p,p′*-DDE and ΣPCB. This was, however, not observed among men from the general Faroese population. One explanation for this could be that the POP concentration needed to affect the Y:X ratio was not reached in the general population, which consisted of younger men than the cohort of fertile men.

Inconsistent patterns like these have also been seen in other epidemiological studies evaluating the effect of POPs on Y:X- and sex ratio (Michalek et al., 1998; Mocarelli et al., 1996; Tiido et al., 2006). A plausible explanation, in this case, is that the mean 11 year difference in age may be linked to a time-related change in the environmental exposure profile, both regarding exposure load and the composition of compounds, i.e. that the fertile men have heavier exposure due to the longer period of time they have been exposed to various factors, or that the effect is due to something else than environmental pollutants. For example, the dietary habits of the Faroe Islands as in many other locations have changed towards a so called western diet during the last 10–20 years as have many other life-style factors.

As a secondary finding, it was noted that the Y:X chromosome ratio in men from the Faroe Islands was lower than that in Swedish fisher-men and Greenland Inuit as well as compared to other European populations (Tiido et al., 2005, 2006). Despite the proximate location of Greenland and the Faroese Islands, inter-population differences in exposure profiles may be present. It has for instance been shown that the people of the Faroe Islands are highly exposed to methyl mercury (Weihe and Joensen, 2012) at a level neither seen in Greenland nor in Sweden (Bjornberg et al., 2005; Johansen et al., 2007; Lindberg et al., 2004). However, whether this exposure affects the Y:X chromosome ratio is currently not known.

The young Faroese men as well as the Swedish fishermen were regarded as representative of the general population regarding fertility and selection bias was therefore not presumed to be of concern as the age-distributions as well as the number of children was similar among the participants and the non-participants (Tiido et al., 2005). The selection of the Inuit men and the fertile Faroese men on the other hand, was performed on the criteria of fatherhood (Toft et al., 2005). Nevertheless, it seems unlikely that such selection has any impact on the association between serum concentrations of POPs and the Y:X chromosome ratio.

In the present study, PCB exposure was estimated by adding concentrations of three different congeners, while in older studies, PCB exposure was assessed by means of CB-153. However, CB-153 is considered the major PCB congener and, in a previous study performed using the K1S cohort, the concentration of this compound was found to be highly

correlated to the concentrations of CB-138 and CB-180 (Barr et al., 2006); all three considered as having non-dioxin like, anti-estrogenic properties (Bonefeld-Jorgensen et al., 2001; Pliskova et al., 2005), and could therefore be assumed to have similar biological effects.

The high correlation between CB-153 and *p,p′*-DDE found in this study indicates that, in this specific case, the findings associated with one of the exposure variables can be interpreted as belonging to both.

No methodological bias is expected as the method used to analyze Y:X ratio in the Faroese men compares well with older FISH-techniques. Also, the very high hybridization efficiency almost completely eliminates any potential bias due to uneven hybridization failure between the sex chromosomes.

We did not adjust our calculations for the effect of age, since age was clearly associated with the level of exposure and including the former in the regression model would imply a risk of "over adjustment" thereby creating a false loss of association between POP concentrations and Y:X ratio. Also, a previous study investigating exposure to environmental pollutants and the Y:X chromosome ratio found that neither smoking, age nor hormonal parameters were associated with the fraction of Y-chromosome bearing sperm cells in the Swedish fishermen used for comparison in this study (Tiido et al., 2005).

### 4.1. Conclusions

In conclusion, negative associations between the investigated POPs and the Y:X chromosome ratio were observed in older, but not in younger men from the Faroe Islands. Furthermore, the Faroese sperm sex chromosome ratio was significantly lower than that of previously analyzed populations of Greenland Inuit and Swedish fishermen. Clearly, there is a need for further studies to properly establish the effect of POPs on the sperm sex chromosome ratio.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.09.001.

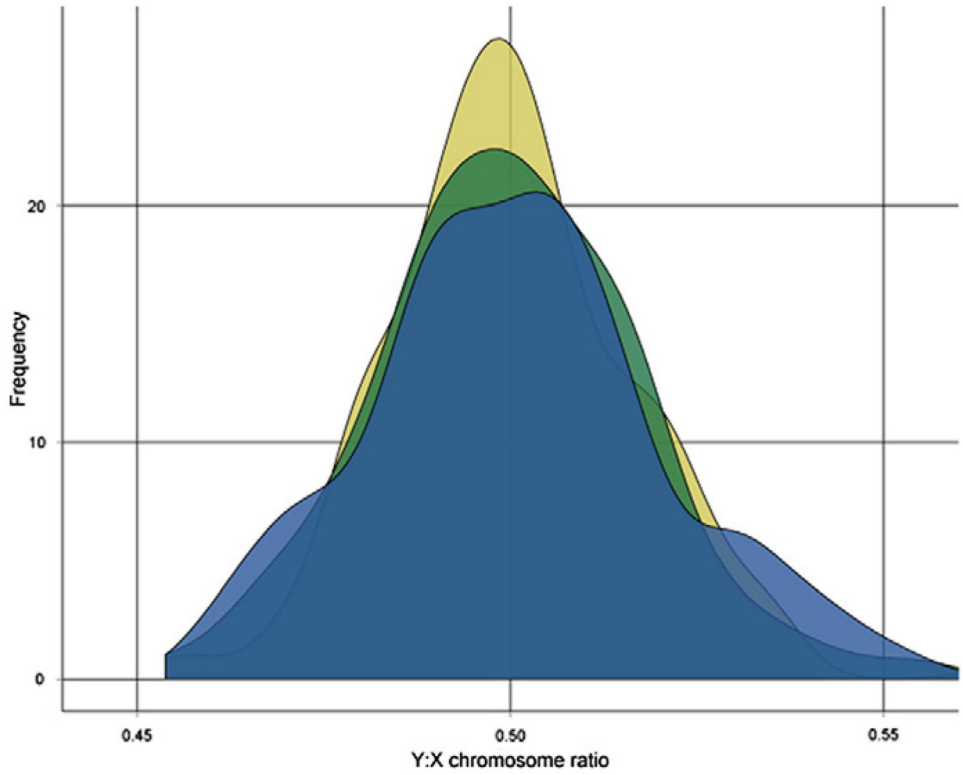


**Fig. 1.**

Distribution curves of the Y:X chromosome ratio as observed for the UM (blue, foremost curve), K1S (green, middle curve) and K5P (yellow, back curve) cohorts.

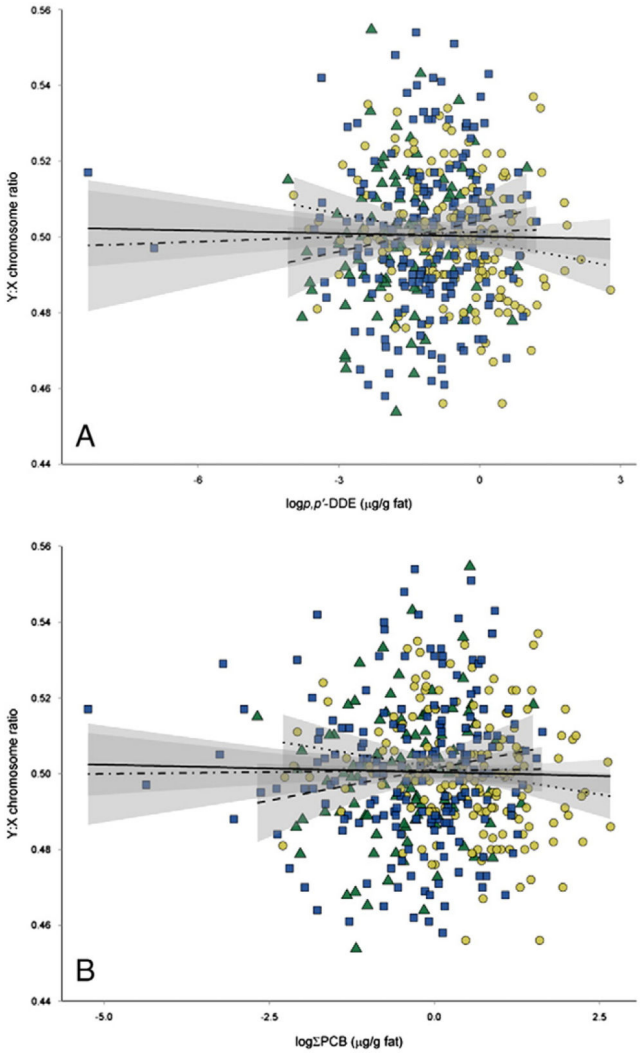


**Fig. 2.**

A. Scatterplot of Y:X chromosome ratio compared to log-transformed serum levels of *p,p′*-DDE. The solid regression line marks the total Faroese cohort, blue squares and dot–dash regression line mark the UM cohort, green triangles and dashed regression line mark the K1S cohort and yellow circles and dotted regression line mark the K5P cohort. The shaded areas show 95% CI. B. Scatterplot of Y:X chromosome ratio compared to log-transformed serum levels of ΣPCB. The solid regression line marks the total Faroese cohort, blue squares and dot–dash regression line mark the UM cohort, green triangles and dashed regression line mark the K1S cohort and yellow circles and dotted regression line mark the K5P cohort. The shaded areas show 95% CI.

**Table 1**

Characteristics of the Faroese study populations.

| Characteristics | Included | | Excluded | | p-Value[a] |
|---|---|---|---|---|---|
| | | n | | n | |
| *Total* | | | | | |
| Age[b] (years) | 28.7 ± 6.6 | 448 | 26.2 ± 3.9 | 46 | <0.001 |
| Abstinence time[b] (days) | 3.9 ± 2.3 | 434 | 3.9 ± 2.2 | 44 | 0.929 |
| Semen volume[b] (mL) | 4.30 ± 1.90 | 446 | 4.22 ± 1.91 | 46 | 0.797 |
| Sperm concentration[b] (mill/mL) | 63.3 ± 57.6 | 449 | 21.7 ± 68.1 | 46 | <0.001 |
| *p,p′*-DDE[c] (μg/g fat) | 0.359 (0.687) | 449 | 0.312 (0.736) | 42 | 0.332 |
| ΣPCB[c,d] (μg/g fat) | 1.014 (1.496) | 449 | 0.809 (1.405) | 42 | 0.333 |
| *UM* | | | | | |
| Age (years) | 25.3 ± 0.7 | 181 | 25.4 ± 0.8 | 31 | 0.525 |
| Abstinence time (days) | 3.6 ± 3.0 | 167 | 3.6 ± 1.7 | 29 | 0.966 |
| Semen volume (mL) | 4.31 ± 1.70 | 180 | 4.14 ± 1.90 | 31 | 0.636 |
| Sperm concentration (mill/mL) | 60.0 ± 49.1 | 181 | 26.5 ± 82.0 | 31 | 0.034 |
| *p,p′*-DDE (μg/g fat) | 0.341 (0.506) | 181 | 0.272 (0.675) | 31 | 0.919 |
| ΣPCB (μg/g fat) | 0.929 (1.165) | 181 | 0.651 (1.222) | 31 | 0.905 |
| *K1S* | | | | | |
| Age (years) | 22.7 ± 0.5 | 95 | 22.9 ± 0.7 | 7 | 0.693 |
| Abstinence time (days) | 4.0 ± 2.4 | 94 | 5.4 ± 4.0 | 7 | 0.416 |
| Semen volume (mL) | 4.09 ± 1.71 | 95 | 4.59 ± 1.95 | 7 | 0.530 |
| Sperm concentration (mill/mL) | 67.5 ± 60.4 | 95 | 7.7 ± 11.9 | 7 | <0.001 |
| *p,p′*-DDE (μg/g fat) | 0.182 (0.214) | 95 | 0.112 (1.449) | 5 | 0.798 |
| ΣPCB (μg/g fat) | 0.654 (0.727) | 95 | 0.927 (3.295) | 5 | 0.913 |
| *K5P* | | | | | |
| Age (years) | 35.7 ± 5.8 | 172 | 32.6 ± 5.8 | 8 | 0.190 |
| Abstinence time (days) | 4.0 ± 1.2 | 173 | 3.5 ± 1.7 | 8 | 0.440 |
| Semen volume (ml) | 4.40 ± 2.16 | 171 | 4.20 ± 2.18 | 8 | 0.815 |
| Sperm concentration (mill/mL) | 64.4 ± 64.2 | 173 | 15.4 ± 22.3 | 8 | <0.001 |

| Characteristics | Included | | Excluded | | p-Value[a] |
|---|---|---|---|---|---|
| | | n | | n | |
| *p,p′*-DDE (μg/g fat) | 0.623 (0.993) | 173 | 0.858 (0.425) | 6 | 0.065 |
| ΣPCB (μg/g fat) | 1.807 (2.208) | 173 | 1.660 (1.704) | 6 | 0.516 |

[a] p-Value represents the difference between included and excluded subjects.

[b] Values are mean ± standard deviation.

[c] Values are median (interquartile range).

[d] The sum of PCB-congeners 138, 153 and 180, multiplied by 2.

## Table 2

Linear regression analysis of association between Y:X chromosome ratio and continuous log-transformed concentration levels of *p,p′*-DDE and ΣPCB.[a]

| Cohort | *p,p′*-DDE | ΣPCB[b] |
|---|---|---|
| Total | 0.740 (−0.0016 to 0.0011) | 0.653 (−0.0019 to 0.0012) |
| UM | 0.723 (−0.0020 to 0.0028) | 0.892 (−0.0025 to 0.0028) |
| K1S | 0.137 (−0.0009 to 0.0062) | 0.132 (−0.0010 to 0.0078) |
| K5P | 0.026 (−0.0044 to −0.0003) | 0.030 (−0.0054 to −0.0003) |
| UM + K1S | 0.273 (−0.0009 to 0.0030) | 0.413 (−0.0013 to 0.0032) |

[a] Values are p-value and values within parenthesis show 95% CI over the regression slope.

[b] The sum of PCB-congeners 138, 153 and 180, multiplied by 2.

**Table 3**

Trend analysis of Y:X chromosome ratio and the association with categorized exposure levels of *p,p′*-DDE and ΣPCB.[a]

| Cohort | *p,p′*-DDE | | | ΣPCB[b] | | |
|---|---|---|---|---|---|---|
| | B | p- Value | 95% CI for B | B | p- Value | 95% CI for B |
| Total | −0.001 | 0.928 | −0.002 to 0.001 | 0.000 | 0.519 | −0.002 to 0.001 |
| UM | 0.002 | 0.234 | −0.001 to 0.004 | 0.001 | 0.265 | −0.001 to 0.004 |
| K1S | 0.002 | 0.186 | −0.001 to 0.005 | 0.003 | 0.090 | 0.000 to 0.006 |
| K5P | −0.003 | 0.005 | −0.005 to −0.001 | −0.003 | 0.012 | −0.005 to −0.001 |
| UM + K1S | 0.001 | 0.173 | −0.001 to 0.003 | 0.002 | 0.095 | 0.000–0.004 |

[a] Values are B, p-value and 95% CI for UM + K1S.

[b] The sum of PCB-congeners 138, 153 and 180, multiplied by 2.