



ENVIRONMENTAL HEALTH PERSPECTIVES

<http://www.ehponline.org>

Sperm Aneuploidy in Faroese Men with Lifetime Exposure to Dichlorodiphenyldichloroethylene (DDE) and Polychlorinated Biphenyl (PCB) Pollutants

Melissa J. Perry, Heather A. Young, Philippe Grandjean,
Jónrit Halling, Maria Skaalum Petersen, Sheena E. Martenies,
Parisa Karimi, and Pál Weihe

<http://dx.doi.org/10.1289/ehp.1509779>

Received: 2 February 2015

Accepted: 9 October 2015

Advance Publication: 4 November 2015

Note to readers with disabilities: *EHP* will provide a [508-conformant](#) version of this article upon final publication. If you require a 508-conformant version before then, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.



Sperm Aneuploidy in Faroese Men with Lifetime Exposure to Dichlorodiphenyldichloroethylene (DDE) and Polychlorinated Biphenyl (PCB) Pollutants

Melissa J. Perry,¹ Heather A. Young,² Philippe Grandjean,³ Jónrit Halling,⁴ Maria Skaalum Petersen,⁴ Sheena E. Martenies,¹ Parisa Karimi,¹ and Pál Weihe⁴

¹Department of Environmental and Occupational Health, Milken Institute School of Public Health, The George Washington University, Washington, DC, USA; ²Department of Epidemiology and Biostatistics, Milken Institute School of Public Health, The George Washington University, Washington, DC, USA; ³Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark; and Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; ⁴Department of Occupational Medicine and Public Health, the Faroese Hospital System, Tórshavn, Faroe Islands

Address correspondence to Melissa J. Perry, Department of Environmental and Occupational Health, Milken Institute School of Public Health, The George Washington University, 950 New Hampshire Ave NW Suite 400, Washington, DC 20051 USA. Telephone: 202-994-1734. E-mail: mperry@gwu.edu

Running title: Organochlorines and sperm aneuploidy

Acknowledgments: This study was supported by the National Institute of Environmental Health Sciences (ES009718, ES017457, ES000002, ES014460), the European Commission through its

Sixth Framework Programme for RTD (contract no. FOOD-CT-2006–016253, PHIME), and the Faroese Research Council.

The authors thank Niels Jørgensen, MD, University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark, who provided instrumental support and guidance with the semen quality analysis.

Competing financial interests: The authors of this paper declare they have no actual or potential competing financial interest and that their freedom to design, conduct, interpret, and publish research is not compromised by any controlling sponsor as a condition of review or publication.

Abstract

Background: While it is known that sperm aneuploidy contributes to early pregnancy losses and congenital abnormalities, causes are unknown and environmental contaminants are suspected.

Objectives: Our goal was to evaluate associations between lifetime exposure to organochlorines, specifically dichlorodiphenyldicholorethylene (*p,p'*-DDE) and polychlorinated biphenyls (PCBs) and sperm aneuploidy in men from the general population of the Faroe Islands, a population with a known history of organochlorine exposures.

Methods: Serum and semen samples from men (n=90) ages 22-44 participating in Faroe Islands health studies were analyzed for *p,p'*-DDE and PCB (118, 138, 153, and 180) and adjusted for total lipids. Cord blood and age 14 serum were available for a subgroup (n=40) and also analyzed for *p,p'*-DDE and PCBs. Sperm fluorescence *in situ* hybridization (FISH) for chromosome X, Y, and 18 was used to determine rates of XX18, XY18, YY18 and total disomy. Multivariable adjusted Poisson models were used to estimate the relation between organochlorine exposure and sperm disomy outcomes.

Results: Adult *p,p'*-DDE and total PCB serum concentrations were each associated with significantly increased rates of XX18, XY18 and total disomy. Age 14 *p,p'*-DDE and PCB concentrations were each associated with significantly increased rates of XX, XY and total disomy at adult age. Associations between cord blood concentrations of *p,p'*-DDE and PCBs and sperm disomy at adult age were not consistently significant.

Conclusions: Organochlorine exposures measured at age 14 and in adulthood were associated with sperm disomy in this sample of high exposure men, suggesting the impacts of persistent pollutants on testicular maturation and function need deeper investigation.

Introduction

Environmental endocrine disrupting chemicals have been associated with impaired male reproductive function (Bergman et al. 2013; Woodruff et al. 2008) and may impact sperm development (Schiffer et al. 2014). Aneuploidy, i.e., an abnormal chromosome complement in the fetus, is thought to contribute to up to 50% of early pregnancy losses (Hassold et al. 2007). The most common aneuploidies in humans at birth involve an abnormal number of X or Y chromosomes (Hassold and Hunt 2001) and at least 50% of XXY trisomies originate from the father (Hassold et al. 2007). European data from consecutive birth studies show that the incidence of Klinefelter syndrome (XXY) appears to be increasing, but no increases have been observed in the incidence of XXX or XYY trisomies (Morris et al. 2008). Because XXY trisomies frequently arise from nondisjunction of the XY (paternal) bivalent during meiosis I, and increases in XXX trisomy (predominately maternally-derived) have not been observed, underlying environmental causes affecting non-disjunction during spermatogenesis are suspected.

Due to their lipophilic nature and persistence, organochlorine contaminants biomagnify in the food chain (Agency for Toxic Substance and Disease Registry 1989) and occur at measurable levels in a large proportion of the general population (Centers for Disease Control and Prevention 2009). They transcend the blood-testis barrier and can alter endocrine homeostasis essential for testicular function (Schiffer et al. 2014; Woodruff et al. 2008). In epidemiologic studies, organochlorine exposures have been associated with decreased semen quality (reviewed in Meeker and Hauser 2010) and miscarriage (Eskenazi et al. 2009; Toft 2014; Venners et al. 2005).

Environmental chemicals including non-persistent pesticides (reviewed in Perry 2008) and benzene (Xing et al. 2010) have been associated with sperm aneuploidy in epidemiologic studies. Because it is the most frequent type of sperm aneuploidy (Hassold and Hunt, 2001), most environmental exposure studies have focused on sex chromosome disomy, which involves an extra X or Y chromosome. To our knowledge, only one previously published study has investigated the relationship between organochlorine compounds and human sperm sex-chromosome disomy (McAuliffe et al. 2012). Among men recruited from a hospital based fertility clinic, significant positive associations with dichlorodiphenyldicholorethylene (*p,p'*-DDE) and polychlorinated biphenyls (PCBs) and sex chromosome disomy were found. There was a significant inverse association between PCBs and XX disomy. The aim of the present study was to investigate whether environmental exposure to *p,p'*-DDE and PCBs (118, 138, 153, and 180) were associated with sperm sex-chromosome disomy in a group of adult men who had elevated lifetime exposures to the pollutants. Due to environmental contamination particularly from a seafood rich diet including pilot whale, the Faroes population is exposed to above-average levels of persistent organic pollutants than other populations (Longnecker et al. 2003; Weihe and Joensen 2012). This population provides a unique context for cross-sectional and prospective investigations of pollutant exposures and male reproductive functioning among men recruited from communities rather than clinical settings.

Methods

Study Subjects

Study population and recruitment

The study population consisted of adult men from the general Faroese population. Study population recruitment practices and inclusion criteria have been described previously (Halling et al. 2013; Petersen et al. 2015). Briefly, the parent study population consisted of 747 Faroese men recruited from three study groups - a birth cohort, a group of fertile men, and a group of randomly selected men from the population register. The birth cohort was established in the Faroes in 1986 -1987. Biological samples and information on physical health and environmental exposures were obtained at birth and during follow-up examinations for cohort members at ages 14 and 22. Two hundred and forty men participating in the 22-year follow-up from the original birth cohort agreed to participate in a study of semen quality and were examined in 2009-2010 (Halling et al. 2013).

The second group consisted of 266 proven fathers to children in a new Faroese birth cohort established in 2007-2009. Men were excluded if the pregnancy was achieved using fertility treatment (Petersen et al. 2015).

The third study group was recruited from men consecutively listed in the population register born between January 1981 and December 1984. Two hundred and forty one men from this group agreed to participate in a cross-sectional semen quality study and were examined between 2007 and 2009 (Halling et al., 2013).

The parent studies were approved by the local Science Ethical Committee for the Faroe Islands and the Institutional Review Board of the Harvard School of Public Health. All subjects signed an informed consent form prior to participation. The study reported here focused on a subsample of men identified from semen samples available from the parent studies. The number who did not provide a semen sample was 3 for the birth cohort, 3 for the fathers cohort, and 21 for the population register. To be included from the birth cohort, men also needed to have had exposure data from cord blood and age 14 samples. Ninety samples were randomly selected from the available samples without respect to demographic or semen parameter characteristics and without regard to the parent study. Semen samples were analyzed using sperm fluorescence *in-situ* hybridization (FISH) analysis (birth cohort men n=40; fathers cohort n=12; population register men n=38). For men recruited from the birth cohort, data were also available on *in utero* exposures via cord blood samples for n=40 subjects and exposures at age 14 for n=33 subjects.

Semen samples and analysis

Semen analysis methods have been described previously (Halling et al. 2013). Briefly, at the time of study recruitment, the men produced a semen sample via masturbation in a private room at the laboratory. Abstinence time was recorded at the time of sample collection. Three technicians trained in quality control measures at the National University Hospital (Rigshospitalet) in Denmark conducted all of the semen analysis for evaluation by World Health Organization (WHO) guidelines (WHO 1999). Semen values were dichotomized on the basis of reference values for three main semen quality parameters, i.e., sperm count (15 million sperm per milliliter of ejaculate), motility (40% total motile sperm), and morphology (<4% normal forms) (WHO 2010).

Sperm Disomy Analysis

To detect sex chromosome disomy (aneuploidy involving an extra X or Y chromosome), a single investigator blinded to exposure status performed fluorescence *in situ* hybridization (FISH) analysis. The procedures for this analysis have been described in detail previously (McAuliffe et al. 2012). Briefly, the FISH procedure was carried out for three chromosomes of interest: X, Y and 18 (autosomal control) with a series of non-overlapping field images taken for each prepared FISH slide using a fluorescence laser scanning wide field microscope. Sex chromosome disomy was the primary outcome of interest because of its reproductive health impacts: a) it is the most frequent form of sperm aneuploidy and occurs twice as frequently as disomy in the autosomes; b) sperm that are disomic for X or Y are capable of fertilization; and c) sex chromosome disomy results in viable offspring. The images were scored with custom MATLAB software designed to utilize scoring criteria for size and shape as reported by Baumgartner et al. (1999). A co-localization analysis allowed the software to identify sperm nuclei and the number of signals contained therein. The method has been shown to produce results quantitatively and qualitatively comparable to manual scoring (Perry et al. 2007; Perry et al. 2011).

Exposure analysis

A two-stage solid-phase extraction method, followed by gas chromatography analysis with electron capture detection was used to quantify the four most prevalent PCB congeners, CB-118, CB-138, CB-153, and CB-180 (Grandjean et al. 1995; Petersen et al. 2006) along with *p,p'*-DDE. The concurrent and age-14 results were adjusted for total serum lipid content and reported as μg per gram lipid, while the cord blood concentrations were expressed in terms of

volume of whole blood (Grandjean et al. 2012). As in a previous study (McAuliffe et al. 2012), PCB exposure was represented by the sum of serum concentrations of the four most prevalent PCB congeners 118, 138, 153, and 180 (Σ_4 PCBs). The median limit of detection was 0.03 $\mu\text{g/L}$ (LOD), which, at a mean lipid concentration of 7.45 g/l, corresponds to 0.004 $\mu\text{g/g}$ lipid. Non-detectable levels of PCB congeners and *p,p'*-DDE were assumed to equal 0.002 $\mu\text{g/g}$ lipid; this level corresponded to half the limit of detection.

Statistical Analysis

Descriptive statistics for demographic and semen parameters were summarized using frequency distributions or means and standard deviations, as appropriate. Descriptive statistics for exposures and disomy data were summarized as means and standard deviations, geometric means, medians, and relevant percentiles. Poisson regression (SAS GENMOD procedure) was used to model the association between each of the disomy measures and organochlorine exposures (*p,p'*-DDE and Σ_4 PCBs) in unadjusted and adjusted analyses.

The number of sperm scored and the number of disomic nuclei identified were summed for each subject and used as the unit of analysis. In Poisson regression, the offset variable allows for control of time/space variation in the denominator. In this study the source of variation was the number of nuclei scored per subject. The Poisson model was fitted using each disomy measure (XX18, YY18, XY18, or total sex chromosome disomy) as the outcome variable, the natural logarithm of the number of sperm counted as the offset variable, the organochlorine exposure of interest as the independent variable, with age, abstinence time, smoking status, log of sperm concentration, motility, and morphology as potential confounders in the adjusted analyses. Covariates were identified based on *a priori* considerations (Blackwell and Zaneveld;

Hassan and Killick 2003; Vine 1996) and retained in the final models based on potential associations with the disomy outcomes and the organochlorine exposures. Sperm concentration, motility, and morphology were adjusted for because they have been associated with disomy in some prior studies (e.g., Martiin et al. 2003; McAuliffe et al. 2012b; Vegetti, et al., 2000). They were each found to be significant independent predictors of disomy outcomes and were retained in the final models. Because semen concentrations were skewed and residuals were not normally distributed, they were natural log-transformed prior to inclusion in the models. Age, log sperm concentration, motility, and morphology were included as continuous variables, while smoking (ever - including current and former - versus never) and abstinence time (≤ 2 days, 3-4 days, ≥ 5 days) were included as categorical variables. Differences between rate ratios with cohort affiliation in the model compared to the model without cohort affiliation were 10% or less. There were no changes in p-values when cohort affiliation was included; it was not a significant variable and it was not retained in the final model. Serum organochlorine concentrations were categorized as tertiles and entered into the model. Concentrations were highly correlated at birth, at age 14 and in adults ($r=0.77$ to $r=0.93$). To avoid redundant variables and inflated standard errors (Holforda et al. 2000; Kleinbaum et al. 2013) organochlorine concentrations were not entered simultaneously. Incidence rate ratios (IRRs) and 95% confidence intervals were calculated for each model.

For a subset of men, Pearson correlations were examined for exposures at each of the three time points (adult, age 14 years, and prenatal). Poisson regression models as described above were constructed for the subset of men at the three time points for p,p' -DDE and Σ_4 PCBs. Due to the possible bias from standardizing exposure measures by serum lipids for the adult and

age 14 values (Schisterman et al. 2005), models were re-run with lipid concentration as a separate covariate. Because rate ratios calculated with lipid concentration as a separate covariate differed by less than 10%, and the significance of p-values did not change, results are presented here in the latter form. All models were run separately using Σ_3 PCBs (138, 153, 180). Because the rate ratios differed by less than 10% and p-values were unchanged in the models that included Σ_4 PCBs (118, 138, 153, 180) (data not shown), results are presented here for Σ_4 PCBs only. Complete case analysis was used for calculating means and observations with missing data were automatically dropped from models. This affected four observations, bringing the number of observations in the adjusted models to 86. A p-value of ≥ 0.05 was used to define statistical significance.

Results

Table 1 describes demographic characteristics and semen parameters for the total sample stratified by cohort affiliation (i.e., birth cohort, fathers cohort, and population register). The men had an average age of 25 years (range: 22-44.5) and a mean BMI of 25.3 kg/m² (range: 19.7-46.1). Slightly over half of the men (53%) had never smoked. Twelve percent (n=11) had sperm concentrations <15 million/mL, 2% (n=2) had <40% progressively motile sperm, and 75% (n=65) had <4% normally shaped sperm. Of the total 90 men evaluated, 12 were men with proven fertility (fathers cohort). In comparison to the other men in the study, more men with proven fertility were older (mean age 35 years vs 23 in the other men), more had <4% normally shaped sperm, and more were smokers.

The sperm disomy results summarized in Table 2 show a median of 7164 sperm nuclei were scored per subject. The observed median percentages of XX18, YY18, XY18, and total disomy were 0.2, 0.26, 0.54, and 1.17, respectively.

Table 3 summarizes the distribution of lipid-adjusted *p,p'*-DDE and selected PCB concentrations. The medians for *p,p'*-DDE and Σ_4 PCBs were 0.28 $\mu\text{g/g}$ and 0.39 $\mu\text{g/g}$, respectively. The serum organochlorine levels were higher in the men of proven fertility as compared to the other men (median *p,p'*-DDE 0.79 vs. 0.25 in the other men; median Σ_4 PCBs 0.94 vs 0.36 in the other men), consistent with their older age, but their semen parameters and disomy rates did not differ significantly (data not shown). Table 3 also summarizes the distribution of *p,p'*-DDE and Σ_4 PCB exposures for the subgroups of men who had prenatal and age 14 exposure measurements. Median values were lowest in the adult sample.

Correlations between the prenatal and adult, and the prenatal and age 14 log transformed *p,p'*-DDE values (Table 4) were weak (Pearson's $r=0.20$; Pearson's $r=0.22$, respectively), while the correlation between age 14 and adult was moderate (Pearson's $r=0.52$). Correlations between the prenatal and adult, and the prenatal and age 14 log transformed Σ_4 PCBs values were weak (Pearson's $r=0.25$; Pearson's $r=0.15$, respectively), while the correlation between age 14 and adult again was moderate (Pearson's $r=0.75$).

Tertile cutpoints were different among the three age groups for *p,p'*-DDE, whereas tertile cutpoints among the three age groups for PCBs were similar (Table 5). Poisson regression models using exposure tertiles showed that for the adults, *p,p'*-DDE was associated with increased rates of XX18 (IRR=1.52; 95% CI: 1.35, 1.72) and XY18 (IRR=1.40; 95% CI: 1.30,

1.51) and total disomy (IRR=1.32; 95% CI: 1.25, 1.35) comparing the highest to the lowest tertile. For YY and Σ_4 PCBs, the incidence rate ratio increased for tertile 2 (IRR=1.16; 95% CI: 1.03, 1.32), and decreased for tertile 3 (IRR=0.85; 95% CI: 0.74, 0.96). Unadjusted results (not shown) were similar to the adjusted results detailed in Table 5. Age 14 exposures showed similar associations between *p,p'*-DDE and sperm disomy as seen in adults. Age 14 exposures between PCBs and sperm disomy were consistently further from the null. Disomy associations with *p,p'*-DDE in cord blood in tertile 2 were consistently negative in contrast with corresponding associations in adults that were more likely to be null. Disomy associations with *p,p'*-DDE in cord blood in tertile 3 were generally null in contrast with corresponding associations in adults that were generally positive.

Because age differences between the fathers cohort and the other men could have introduced systematic differences, the 12 men from the fathers cohort were removed and the same analyses represented in Table 5 were repeated. Results when these men were omitted showed patterns of statistical significance that were consistent with the results when the men were included (data not shown).

Discussion

Within this sample of young men from the Faroe Islands there was an increase in the rate of an extra X chromosome and total sex-chromosome disomy by elevated exposures measured in adulthood to *p,p'*-DDE and PCBs, and these results persisted after adjustment for potential confounders. There was evidence of a negative association between *p,p'*-DDE and YY18, whereas results for PCBs and YY18 were inconsistent, with a significant positive association in the second tertile and significant negative association in the third tertile.

Aneuploidy occurs during the disruption of meiosis during gametogenesis. It is not known how hormone-disrupting pollutants like *p,p'*-DDE and PCBs interfere with the meiotic phase, but infertile men often have an impaired chromosome synapsis and an increased frequency of chromosomes that are missing a recombination site (Martin 2006; Sun et al. 2008). These errors make the cells susceptible to meiotic arrest and production of aneuploid gametes. Altered recombination impacts nondisjunction; non-recombinant chromosomes are susceptible to nondisjunction due to reduced connections among homologous chromosome pairs (Ferguson et al. 2007). Chemicals known to disrupt hormone signaling have been shown to affect mammalian recombination and germ cell aneuploidy (reviewed in Pacchierotti and Eichenlaub-Ritter, 2011). Changes in the endocrinologic environment of the testis affect the rate of meiotic segregation errors in mice (Oppedissano et al. 2002), and *p,p'*-DDE has been shown to impact calcium ion channels (CatSper) to affect Ca²⁺ increases which impact sperm capacitation, chemotaxis, hyperactivation, and acrosomal exocytosis (Schiffer et al. 2014). Links between organochlorine exposures and early fetal loss (reviewed in Eskenazi et al. 2009) add additional human evidence to the potential links between organochlorines and gametogenesis, and reinforce that connections between organochlorine action and chromosome disomy need further *in vivo* and epidemiologic study.

The Faroes birth cohort allowed for a subgroup evaluation of organochlorine exposures at three developmental time points and positive associations were seen particularly between *p,p'*-DDE and PCBs measured at age 14 and adult sperm disomy rates. Because prenatal exposure was determined from the concentrations only in whole blood from the umbilical cord, imprecision in this exposure measurement and the small number of observations may have

affected association of cord blood levels with subsequent sperm disomy measured in adulthood. There is evidence that prenatal exposures can predispose adult men to impaired semen quality and testis cancer (McLachlan et al. 1998) and infertility (Juul et al. 2014), suggesting that early development of testicular cells is particularly vulnerable to adverse exposures. To our knowledge, this is the first study evaluating associations between prenatal and early developmental exposure to these compounds and associations with subsequent sperm chromosomal abnormalities.

In our previous cross-sectional study, we investigated the association of *p,p'*-DDE and the same PCB congener concentrations in serum with sperm sex-chromosome disomy in 192 men from subfertile couples at the Massachusetts General Hospital in Boston (McAuliffe et al. 2012). A significant positive trend was found for XX, XY, and total sex chromosome disomy by increasing quartiles of serum *p,p'*-DDE, and there was evidence of a negative association with YY that was not significant. These positive associations for *p,p'*-DDE with XX, XY, and total disomy and evidence of a negative association with YY were similar to the patterns seen here among men recruited from community rather than fertility clinic settings. As in our prior study, we saw less consistent findings for PCBs and XX and YY disomy. In our prior study we saw a significant negative association for PCBs and XX, whereas there was positive association in the present study. For YY, there was a positive association in our prior study, whereas in the present study there was a positive association in the second exposure tertile and a negative association in the third tertile. This may in part be attributable to accuracy of frequency estimates because XX and YY have lower frequencies than XY and total disomy.

It is not possible to compare to other studies of sperm disomy beyond the previous cross

sectional study, and studies are needed to prospectively evaluate maternal and paternal organochlorine exposures and risk for Turner or Klinefelter syndromes. The ratio of Y to X chromosome bearing sperm in relation to organochlorine exposures has been measured in sperm and observed from live births (secondary sex ratio). High PCB exposures have been associated with a higher Y:X ratio in sperm among men from Sweden and Greenland (Tiido et al. 2006). Associations between organochlorine exposures and altered secondary sex ratio among live births as been observed to be biphasic, with an increased ratio associated with acute exposure and a decreased ratio associated with persistent and chronic exposures (Karmaus et al., 2002; Hertz-Picciotto et al., 2008).

The Faroes population is exposed to higher levels of persistent organic pollutants compared to other populations (Longnecker et al. 2003; Weihe and Joensen 2012), and the serum concentrations of *p,p'*-DDE and PCBs (138,153, and 180) in this study were much higher than those reported from the US general population in 2003-04 in the most recent 2009 NHANES report (Centers for Disease Control and Prevention 2009), and as seen in our previous study (McAuliffe et al., 2012). For example, the median for *p,p'*-DDE was 200 ng/g lipid in men in the 2003-04 NHANES, as compared to 510 ng/g lipid in the Faroese men. PCB 180 was 18.5 ng/g lipid in the 2003-04 NHANES men and 150 ng/g lipid in the Faroes.

The cord blood results were not lipid adjusted and subject to exposure misclassification. The small sample size did not limit detection of significant cross sectional associations in adulthood and separate prospective associations involving exposures measured at age 14. PCBs were highly correlated with *p,p'*-DDE at age 14 and in adults and it was not possible to separate out which time point - age 14 or adulthood - contributed most strongly to this association. Tertile

cutpoints for *p,p'*-DDE were different among the three age groups, limiting dose response comparisons across time points. There is the potential for bias in the results due to uncontrolled confounding.

Whereas prior studies investigating environmental exposures and sperm disomy have focused on men seeking evaluation of, or treatment for infertility, men in this study were recruited from the general Faroese population. The population's wide range of organochlorine exposures and homogeneity in demographic and lifestyle characteristics is an advantage for conserving power and reducing confounding. Taken together, the results reported here further demonstrate links between organochlorine exposures and sperm abnormalities and illustrate that the impacts of persistent pollutants on testicular maturation and function need deeper investigation.

References

- ATSDR (Agency for Toxic Substances and Disease Registry). 2008. Toxicological Profile for DDT. US Department of Health and Human Services. CAS#: DDT 50-29-3; DDE 72-55-9; DDD 72-54-8. Available: http://www.atsdr.cdc.gov/toxprofiles/ddt_addendum.pdf [accessed 30 December 2014].
- Baumgartner A, Van Hummelen P, Lowe XR, Adler ID, Wyrobek AJ. 1999. Numerical and structural chromosomal abnormalities detected in human sperm with a combination of multicolor FISH assays. *Environ Mol Mutagen* 33:49–58.
- Bergman A, Heindel JJ, Kasten T, Kidd KA, Jobling S, Neira M, et al. 2013. The impact of endocrine disruption: a consensus statement on the state of the science. *Environ Health Perspect* 121:A104–A106.
- Blackwell JM, Zaneveld LJD. 1992. Effect of abstinence on sperm acrosin, hypoosmotic swelling, and other semen variables. *Fertil Steril* 58(4):798-802.
- CDC (Centers for Disease Control and Prevention). 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Available: <http://www.cdc.gov/ExposureReport/pdf/FourthReport.pdf> [Accessed 30 December 2014].
- Eskenazi B, Chevrier J, Rosas LG, Anderson HA, Bornman MS, Bouwman H, et al. 2009. The Pine River statement: human health consequences of DDT use. *Environ Health Perspect* 117:1359-1367.
- Ferguson KA, Wong EC, Chow V, Nigro M, Ma S. 2007. Abnormal meiotic recombination in infertile men and its association with sperm aneuploidy. *Hum Mol Genet* 16:2870-2879.
- Giwercman A, Rylander L, Rignell-Hydbom A, Jönsson BA, Pedersen HS, Ludwicki JK, et al. 2007. Androgen receptor gene CAG repeat length as a modifier of the association between persistent organohalogen pollutant exposure markers and semen characteristics. *Pharmacogenet Genomics* 17:391-401.
- Grandjean P, Brown SS, Reavey P, Young DS. 1995. Biomarkers in environmental toxicology: state of the art. *Clin Chem* 41:1902-1904.
- Grandjean P, Weihe P, Nielsen F, Heinzow B, Debes F, Budtz-Jørgensen E. 2012. Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet. *Neurotoxicol Teratol* 34:466-472.

- Halling J, Petersen MS, Jørgensen N, Jensen TK, Grandjean P, Weihe P. 2013. Semen quality and reproductive hormones in Faroese men: a cross-sectional population-based study of 481 men. *BMJ Open* 1:3(3).
- Hassan MAM, Killick SR. 2003. Effect of male age on fertility: Evidence for the decline in male fertility with increasing age. *Fertil and Steril* 79:1520-1527.
- Hassold T, Hunt P. 2001. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2:280-291.
- Hassold T, Hall H, Hunt P. 2007. The origin of human aneuploidy: where we have been, where we are going. *Hum Mol Genet* 15;16 Spec No. 2:R203-8.
- Hertz-Picciotto I, Jusko TA, Willman EJ, Baker R, Teplin S, Charles M. 2008. A cohort study of in utero polychlorinated biphenyl (PCB) exposures in relation to secondary sex ratio. *Environ Health*:7:37.
- Holforda TR, Zhenga T, Maynea ST, Zahm SH, Tessaric JD, Boyled P. 2000. Joint effects of nine polychlorinated biphenyl (PCB) congeners on breast cancer risk. *Int J Epidemiol* 29 (6): 975-982.
- Juul A, Almstrup K, Andersson AM, Jensen TK, Jørgensen N, Main KM, et al. 2014. Possible fetal determinants of male infertility. *Nat Rev Endocrinol* 10:553-562.
- Karmaus W, Huang SY, Cameron L. 2002. Parental concentration of dichlorodiphenyl dichloroethene and polychlorinated biphenyls in Michigan fish eaters and sex ratio in offspring. *J Occup Environ Med*. 44(1):8-13.
- Kleinbaum, D., Kupper, L., Nizam, A., & Rosenberg, E. 2013. Applied regression analysis and other multivariable methods. Cengage Learning.
- Longnecker MP, Wolff MS, Gladen BC, Brock JW, Grandjean P, et al. 2003. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. *Environ Health Perspect* 111:65-70.
- Martin RH, Rademaker AW, Greene C, Ko E, Hoang T, Barclay L, Chernos J. 2003. A comparison of the frequency of sperm chromosome abnormalities in men with mild, moderate, and severe oligozoospermia. *Biol Reprod* 69: 535-539.
- Martin RH. 2006. Meiotic chromosome abnormalities in human spermatogenesis. *Reprod Toxicol* 22:142-147.

- McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ. 2012a. Environmental Exposure to Polychlorinated Biphenyls and *p,p'*-DDE and Sperm Sex-Chromosome Disomy. *Environ Health Perspect* 120:535-540.
- McAuliffe ME, Williams PL, Korrick SA, Dadd R, Perry MJ. 2012b. The association between sperm sex chromosome disomy and semen concentration, motility, and morphology. *Hum Reprod* 27:2918-2926.
- McLachlan JA, Newbold RR, Li S, Negishi M. 1998. Are estrogens carcinogenic during development of the testes? *APMIS* 106:240-242.
- Meeker JD, Hauser R. 2010 Exposure to polychlorinated biphenyls (PCBs) and male reproduction. *Syst Biol Reprod Med* 56:122-131.
- Morris JK, Alberman E, Scott C, Jacobs P. 2008. Is the prevalence of Klinefelter syndrome increasing? *Eur J Hum Genet* 16:163-170.
- Oppedisano L, Haines G, Hrabchak C, Fimia G, Elliott R, Sassone-Corsi P, et al. 2002. The rate of aneuploidy is altered in spermatids from infertile mice. *Hum Reprod* 17:710-717.
- Pacchierotti F, Eichenlaub-Ritter U. Environmental hazard in the aetiology of somatic and germ cell aneuploidy. *Cytogenet Genome Res.* 2011;133(2-4):254-68.
- Perry MJ, Chen X, Lu X. 2007. Automated scoring of multiprobe FISH in human spermatozoa. *Cytometry A* 71:968-972.
- Perry MJ. 2008. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. *Hum Reprod Update* 14:233-242.
- Perry MJ, Chen X, McAuliffe ME, Maity A, Deloid GM. 2011. Semi-automated scoring of triple-probe FISH in human sperm: methods and further validation. *Cytometry A* 79:661-666.
- Petersen MS, Halling J, Damkier P, Nielsen F, Grandjean P, Weihe P. 2006. Caffeine N3-demethylation (CYP1A2) in a population with an increased exposure to polychlorinated biphenyls. *Eur J Clin Pharmacol* 62:1041-1048.
- Petersen MS, Halling J, Weihe P, Jensen TK, Grandjean P, Nielsen F, Jørgensen N. Spermatogenic capacity in fertile men with elevated exposure to polychlorinated biphenyls. *Environ Res.* 2015 Apr;138:345-51.

- Schiffer C, Müller A, Egeberg DL, Alvarez L, Brenker C, Rehfeld A, et al. 2014. Direct action of endocrine disrupting chemicals on human sperm. *EMBO Rep* 15:758-765.
- Schisterman EF, Whitcomb BW, Louis GMB, Louis TA. 2005. Lipid Adjustment in the Analysis of Environmental Contaminants and Human Health Risks. *Environ Health Perspect* 113(7):853-857.
- Sun F, Mikhaail-Philips M, Oliver-Bronet M, Ko E, Rademaker A, Turek P, et al. 2008. Reduced meiotic recombination on the XY bivalent is correlated with an increased incidence of sex chromosome aneuploidy in men with non-obstructive azoospermia. *Mol Hum Reprod* 14:399-404.
- Tiido T, Rignell-Hydbom A, Jonsson BA, Giwercman YL, Pedersen HS, Wojtyniak B, et al. 2006. Impact of PCB and p,p'-DDE contaminants on human sperm Y:X chromosome ratio: studies in three European populations and the Inuit population in Greenland. *Environ Health Perspect* 114:718-724.
- Toft G. 2014. Persistent organochlorine pollutants and human reproductive health. *Dan Med J* 61:B4967.
- Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MM, Bonduelle M, Liebaers I, Van Steirteghem A. 2000. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. *Hum Reprod* 15: 351-365.
- Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A, et al. 2005. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. *Am J Epidemiol* 162:709-716.
- Vine MF. 1996. Smoking and Male Reproduction: A Review. *Int J Androl* 19(6):323-337.
- Weihe P, Joensen HD. 2012. Dietary recommendations regarding pilot whale meat and blubber in the Faroe Islands. *Int J Circumpolar Health* 71:18594; doi: 10.3402/ijch.v71i0.18594.
- WHO (World Health Organization). 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction, 4th Edition. Cambridge: Cambridge University Press.

WHO (World Health Organization). 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th Edition. Available:

http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf [accessed 30 December 2014].

Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. 2008. Proceedings of the Summit on Environmental Challenges to Reproductive Health and Fertility: Executive Summary. *Fertil Steril* 89:281-300.

Xing C, Marchetti F, Li G, Weldon RH, Kurtovich E, Young S, et al. 2010. Benzene exposure near the U.S. permissible limit is associated with sperm aneuploidy. *Environ Health Perspect* 118:833-839.

Table 1. Characteristics of adult Faroese men for the total sample and by cohort [n (%) or mean \pm SD]

Characteristic	Total Sample (n=90)	Birth Cohort (n=40)	Fathers Cohort (n=12)	Population Register (n=38)
Age (years)	25.0 \pm 4.8	22.6 \pm 0.49	35.0 \pm 5.6	24.1 \pm 0.39
BMI (kg/m ²)	25.3 \pm 4.4	24.8 \pm 4.1	26.0 \pm 3.8	25.6 \pm 4.6
Semen Concentration				
<15 million/mL	11 (12.2)	5 (12.5)	1 (8.3)	5 (13.2)
Motility				
<40% motile	2 (2.2)	1 (2.5)	0 (0)	1 (2.6)
Morphology (n=87) ^a				
< 4% normal	65 (74.7)	27 (71.1)	10 (83.3)	28 (75.7)
Abstinence time				
\leq 2 days	31 (34.4)	14 (35.0)	2 (16.7)	15 (39.5)
3-4 days	44 (48.9)	16 (40.0)	8 (66.7)	20 (52.6)
\geq 5 days	15 (16.7)	10 (25.0)	2 (16.7)	3 (7.9)
Smoking (n=89) ^b				
Yes (current/former)	42 (47.2)	17 (42.5)	7 (58.3)	18 (48.6)
No	47 (52.8)	23 (57.5)	5 (41.7)	19 (51.4)

^aMorphology data missing for 2 birth cohort participants and 1 population register participant

^bSmoking data missing for 1 population register participant

Table 2. Number of nuclei scored and % disomy for Faroese men (n=90)

Variable	Mean \pmSD	Median	25th Percentile	75th Percentile
Nuclei (n)	7164 \pm 4011	7098	4442	10,093
% X18	41.51 \pm 6.40	43.09	38.90	46.08
% Y18	39.50 \pm 5.28	40.74	36.80	43.45
% XX18	0.30 \pm 0.26	0.20	0.12	0.37
% YY18	0.29 \pm 0.17	0.26	0.15	0.43
% XY18	0.81 \pm 0.54	0.54	0.43	1.03
% Total Disomy	1.39 \pm 0.79	1.17	0.85	1.69

Table 3. Distribution of *p,p'*-DDE and PCBs for adult Faroese men (n=90)

Exposure	<LOD N (%)	Geometric Mean	Mean \pm SD	Median	Interquartile range (Q25-Q75)
Adult (n=90)					
<i>p,p'</i> -DDE	0 (0)	0.28	0.51 \pm 0.64	0.28	0.13-0.69
Σ_4 PCBs	0 (0)	0.37	0.53 \pm 0.48	0.39	0.19-0.65
PCB 118	10 (11.1)	0.02	0.04 \pm 0.04	0.02	0.01-0.04
PCB 138	11 (12.2)	0.04	0.12 \pm 0.18	0.04	0.01-0.13
PCB 153	0 (0)	0.16	0.22 \pm 0.19	0.17	0.09-0.31
PCB 180	0(0)	0.10	0.15 \pm 0.13	0.12	0.05-0.21
Age 14 (n=33)					
<i>p,p'</i> -DDE	0 (0)	0.79	1.14 \pm 1.15	0.72	0.47-1.17
Σ_4 PCBs	0 (0)	0.51	0.66 \pm 0.52	0.54	0.32-0.84
Cord blood (n=40)					
<i>p,p'</i> -DDE	0 (0)	0.45	0.60 \pm 0.49	0.43	0.27-0.86
Σ_4 PCBs	0 (0)	0.48	0.59 \pm 0.42	0.46	0.35-0.69

Adult and age 14 concentrations $\mu\text{g/g}$ lipid; cord blood concentrations $\mu\text{g/mL}$; Σ_4 PCBs =Sum of PCB 118, 138, 153, and 180. <LOD values for PCB 118 and 138 were imputed before measures of central tendency were estimated.

Table 4. Pearson correlations (p-values) for natural log transformed organochlorine exposures in cord blood, age 14, and adult samples

	Age 14 Σ_4PCB	Adult Σ_4PCB	Cord Blood <i>p,p'</i>-DDE	Age 14 <i>p,p'</i>-DDE	Adult <i>p,p'</i>-DDE
Cord Blood Σ_4PCB	0.15 (0.41)	0.25 (0.12)	0.88 (<0.0001)	0.10 (0.58)	0.14 (0.40)
Age 14 Σ_4PCB		0.75 (<0.001)	0.09 (0.62)	0.77 (<0.001)	0.59 (0.003)
Adult Σ_4PCB			0.26 (0.10)	0.54 (0.001)	0.93 (<0.0001)
Cord Blood <i>p,p'</i>-DDE				0.22 (0.23)	0.20 (0.21)
Age 14 <i>p,p'</i>-DDE					0.52 (0.002)
Adult <i>p,p'</i>-DDE					

Adult and age 14 concentrations $\mu\text{g/g}$ lipid; cord blood concentrations $\mu\text{g/mL}$; Σ_4 PCBs =Sum of PCB 118, 138, 153, and 180

Table 5. Adjusted^a IRRs (95% CI) for XX, YY, XY, and total sex-chromosome disomy, by *p,p'*-DDE and Σ_4 PCBs tertiles at different time points

Exposure	Tertile 2	Tertile 3
Cord Blood <i>p,p'</i>-DDE (n=40)	0.34-0.54 $\mu\text{g}/\text{mL}$	>0.54 $\mu\text{g}/\text{mL}$
XX18	0.58 (0.48, 0.71)	1.07 (0.87, 1.32)
YY18	0.80 (0.68, 0.94)	0.84 (0.69, 1.02)
XY18	0.67 (0.60, 0.75)	0.97 (0.86, 1.09)
Total Disomy	0.68 (0.63, 0.74)	0.94 (0.86, 1.03)
Age 14 <i>p,p'</i>-DDE (n=33)	0.54-1.08 $\mu\text{g}/\text{g}$ lipid	>1.08 $\mu\text{g}/\text{g}$ lipid
XX18	1.02 (0.77, 1.35)	1.77 (1.39, 2.26)
YY18	0.57 (0.46, 0.71)	0.39 (0.30, 0.50)
XY18	0.85 (0.73, 0.99)	1.45 (1.26, 1.65)
Total Disomy	0.79 (0.71, 0.89)	1.19 (1.08, 1.32)
Adult <i>p,p'</i>-DDE (n = 86)	0.18-0.39 $\mu\text{g}/\text{g}$ lipid	>0.39 $\mu\text{g}/\text{g}$ lipid
XX18	0.90 (0.79, 1.03)	1.52 (1.35, 1.72)
YY18	0.88 (0.78, 0.99)	0.93 (0.82, 1.05)
XY18	1.03 (0.95, 1.12)	1.40 (1.30, 1.51)
Total Disomy	0.98 (0.92, 1.04)	1.32 (1.25, 1.35)
Cord Blood Σ_4PCBs (n=40)	0.40-0.59 $\mu\text{g}/\text{mL}$	>0.59 $\mu\text{g}/\text{mL}$
XX18	1.06 (0.86, 1.31)	1.07 (0.87, 1.31)
YY18	1.22 (1.03, 1.45)	0.94 (0.78, 1.14)
XY18	1.08 (0.96, 1.22)	1.16 (1.03, 1.30)
Total Disomy	1.09 (0.99, 1.18)	1.09 (0.99, 1.19)
Age 14 Σ_4PCBs (n=33)	0.40-0.59 $\mu\text{g}/\text{g}$ lipid	>0.59 $\mu\text{g}/\text{g}$ lipid
XX18	1.21 (0.82, 1.80)	1.93 (1.42, 2.62)
YY18	1.58 (1.12, 2.21)	0.63 (0.46, 0.86)
XY18	1.66 (1.32, 2.08)	2.23 (1.88, 2.65)
Total Disomy	1.64 (1.37, 1.92)	1.81 (1.58, 2.06)
Adult Σ_4PCBs (n=86)	0.28-0.56 $\mu\text{g}/\text{g}$ lipid	>0.56 $\mu\text{g}/\text{g}$ lipid
XX18	0.76 (0.66, 0.87)	1.22 (1.09, 1.38)
YY18	1.16 (1.03, 1.32)	0.85 (0.74, 0.96)
XY18	0.90 (0.83, 0.98)	1.13 (1.05, 1.22)
Total Disomy	0.94 (0.88, 1.00)	1.10 (1.04, 1.16)

^aAdjusted for age, abstinence time, smoking, log sperm concentration, morphology, and motility

Adult and age 14 concentrations $\mu\text{g}/\text{g}$ lipid; cord blood concentrations $\mu\text{g}/\text{mL}$; Σ_4 PCBs =Sum of PCB 118, 138, 153, and 180