Behavioral Development Following Early Life Organochlorine Exposure

Research Year Report

Aske Hess Rosenquist

Health

Aarhus University

Department of Clinical Epidemiology, Aarhus University Hospital, Denmark

SUPERVISORS AND COLLABORATORS

Gunnar Toft, Associate Professor, DMSc, PhD (Main supervisor)
Department of Clinical Epidemiology
Aarhus University Hospital, Aarhus, Denmark

Birgit Bjerre Høyer, PostDoc, MHSc, PhD (Co-supervisor)
Department of Occupational and Environmental Medicine
Copenhagen University Hospital, Copenhagen, Denmark

Jordi Julvez, MSc, PhD, (Collaborator)
ISGlobal, Centre for Research in Environmental Epidemiology (CREAL)
Barcelona, Catalonia, Spain

Jordi Sunyer, Professor, MD, PhD (Collaborator)
ISGlobal, Centre for Research in Environmental Epidemiology (CREAL)
Barcelona, Catalonia, Spain

Henning Sloth Pedersen, MD (Collaborator)
Primary Health Care Clinic
Nuuk, Greenland

Virissa Lenters, MSc (Collaborator)
Division of Environmental Epidemiology, Institute for Risk Assessment Sciences
Utrecht University, Utrecht, The Netherlands

Bo A.G. Jönsson, Professor, MSc, PhD (Collaborator)
Division of Occupational and Environmental Medicine
Lund University, Lund, Sweden

Jens Peter Bonde, Professor, MD (Collaborator)
Department of Occupational and Environmental Medicine
Copenhagen University Hospital, Copenhagen, Denmark

PREFACE

This research year report is based on a study carried out during my research year at Department of Clinical Epidemiology, Aarhus University Hospital, from September 2015 to August 2016.

First of all, I would like to express my deepest gratitude to my supervisors Gunnar Toft and Birgit Bjerre Høyer, for being enthusiastic about the project and for guiding me throughout my research year. Despite busy schedules you have both had time for constructive feedback and countless meetings.

During my research year, I have had the pleasure of visiting Center for Research in Environmental Epidemiology (CREAL), Barcelona Institute for Global Health, Barcelona, Spain, for a month. I owe a special thanks to Jordi Sunyer and Jordi Julvez for having me there and for sparing their profound expertise on the area of epidemiologic research in relation to environment and human health.

I would also like to thank my other colleagues at the Department of Clinical Epidemiology for warm and intellectual surroundings. Finally, I owe a special thanks to the other research year students at Department of Clinical Epidemiology for an incredible year with sharing of knowledge as well as many great laughs.

Aske Hess Rosenquist, August 2016

FUNDING

The CLEAR and INUENDO studies were funded by European Commission's 7th and 5th Framework Programmes (FP7-ENV-2008-1-226217 and QLK4-CT-2001-00202). A. H. Rosenquist received grants from The Danish Council for Independent Research - Medical Sciences (5053-00064A); A.P. Møller Foundation (15-338); Oticon Foundation (15-3689); Spar Nord Foundation. The funding agencies had no role in study design, collection, analysis, or interpretation of the data

ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
CB-153	2,2′,4,4′,5,5′-hexachlorobiphenyl
CI	Confidence interval
CLEAR	Climate change, environmental contaminants and reproductive health
CPT	Continuous Performance Test
CRS-T	Conners' Rating Scale for Teachers
DDE	Dichlorodiphenyldichloroethylene
IQ	Intelligence quotient
INUENDO	Biopersistent organochlorines in diet and human fertility
LOD	Limits of detection
OR	Odds ratio
p,p'-DDE	1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene
PCBs	Polychlorinated biphenyls
SDQ	Strength and Difficulties Questionnaire

TABLE OF CONTENTS

ABSTRACT

Background: Studies have linked organochlorine exposure with adverse child development, but results are inconsistent and there is limited evidence on the aspect of social behavior.

Objective: To investigate the association between early life polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) exposure and adverse social behavior in children between 5 and 9 years of age.

Methods: In the INUENDO cohort, consisting of mother-child pairs from Greenland and Ukraine (n=1,018), maternal serum concentrations of biomarkers for PCB and DDE were examined. Postnatal cumulative exposure within the first 12 months of delivery was estimated using a pharmacokinetic model. Child behavior at follow-up was assessed using the Strength and Difficulties Questionnaire (SDQ).

Results: The pooled adjusted odds ratio (OR) [95% confidence interval (CI)] for abnormal behavior in relation to a doubling of prenatal exposure was 1.09 (0.86, 1.38) for PCB and 1.15 (0.90, 1.48) for DDE. For the subscale scores, a doubling of prenatal DDE exposure was associated with an increased OR (95% CI) for adverse conduct behavior in Greenland: OR 1.24 (1.00, 1.54), Ukraine: OR 1.58 (1.02, 2.44), and the pooled analysis: OR 1.25 (1.04, 1.51). A tendency towards increased hyperactivity was seen for both PCB and DDE. No association was found between postnatal exposures and any SDQ score.

Conclusion: Prenatal exposure to DDE and potentially PCB may increase the risk of conduct problems and to some extent hyperactivity in children from 5 to 9 years of age. This underlines the importance of minimal organochlorine exposure for women of childbearing age.

DANSK RESUMÉ

Baggrund: Studier har vist en sammenhæng mellem eksponering for organochloriner og den adfærdsmæssige udvikling af børn.

Formål: At undersøge sammenhængen mellem eksponering for organochlorinerne polychlorinated biphenyls (PCB) og dichlorodiphenyldichloroethylene (DDE) tidligt i livet og den adfærdsmæssige udvikling af børn mellem 5 og 9 år.

Metode: I en kohorte bestående af 1018 mor-barn par, blev biomarkører for PCB og DDE målt prænatalt i mødrenes serum. Postnatal eksponering i løbet af de først 12 måneder efter fødslen blev estimeret ud fra en farmakokinetisk model. Når børnene var mellem 5 og 9 år vurderede deres forældre deres adfærdsmæssige udvikling ud fra Strength and Difficulties Questionnaire (SDQ) spørgeskemaet.

Resultater: I den samlede kohorte var den justerede odds ratio (OR) [95% konfidensinterval (95% CI)] for en abnorm SDQ score i relation til en fordobling af den prænatale eksponering 1,09 (0,86, 1,38) for PCB og 1,15 (0,90, 1,48) for DDE. På subskalaerne var prænatal DDE associeret med en abnorm adfærdsmæssig score i både Greenland: OR 1,24 (1,00, 1,54), Ukraine: OR 1,58 (1,02, 2,44), og den samlede kohorte: OR 1,25 (1,04, 1,51). En tendens til øget hyperaktivitet blev fundet for både PCB og DDE. Ingen sammenhæng blev fundet mellem de postnatale eksponeringer og SDQ scoren hverken på den samlede skala eller på subskalaerne.

Konklusion: Prænatal eksponering for DDE og potentielt PCB kan muligvis øge risikoen for adfærdsmæssige problemer i børn mellem 5 og 9 år. Dette understreger vigtigheden af at minimere eksponeringen for organochloriner hos kvinder i den fødedygtige alder.

MANUSCRIPT

Introduction

Polychlorinated biphenyls (PCBs) have been widely used in e.g. hydraulic equipment, dyes, plasticizers, capacitors, transformers and flame retardants. Dichlorodiphenyltrichloroethane (DDT) has been used especially in pesticides and for vector control. Even though PCBs and DDT have been banned from use in most Western countries in the 1970s and 1980s, DDT is still used for disease vector control in some developing countries (1) and both are ubiquitous substances found in the environment all around the world (2).

The main exposure routes of organochlorine compounds like PCBs and DDT are through food consumption and breastfeeding, and given their lipophilic properties and long half-lives of 5 to 10 years, they bio-accumulate within human adipose tissue (3-5). The compounds can cross the placental barrier thus exposing the fetus even decades after the woman was exposed (4, 6). Environmental pollutants may act as developmental neurotoxins, causing brain injury during early life development at doses much lower than those affecting adults (7, 8). Therefore, *in utero* environmental pollutant exposure may pose a risk for the neurologic development of the fetus and perhaps potential long-term implications of the child.

Prenatal exposure to both PCBs and DDT has been linked to behaviors associated with attention deficit hyperactivity disorder (ADHD) among children at 8 years of age (9, 10). In addition, previous studies have shown a link between PCBs and a lowered intelligence quotient (IQ) in children around 8-9 years of age (11, 12), and DDT has been shown to be inversely associated with verbal and memory skills in children at 4 years of age (13). Regarding adverse social behavior, DDT has been positively but insignificantly linked in children at 7-8 years of age (14). However, studies are not entirely consistent and some show no association between PCB and DDT exposure and cognitive developmental changes (15-17).

In this prospective cohort study, we investigate the association between early life exposure to the organochlorine compounds PCBs and DDT and behavioral development of children at 5 to 9 years of age. To our knowledge, this is the first study examining both prenatal and postnatal exposure to PCB and DDT in relation to child behavior measured as Strength and Difficulties Questionnaire (SDQ) scores. To assess the behavioral development, we used SDQ both as a total score and subdivided into five subscales.

Methods

Study population

The study population consists of mother-child pairs from the INUENDO (Biopersistent organochlorines in diet and human fertility) birth cohort. Pregnant women attending antenatal care visits were enrolled between May 2002 and February 2004 at 3 hospitals and 8 antenatal clinics in Kharkiv (Ukraine), and 19 local hospitals in municipalities and settlements in Greenland. Furthermore, women from Warsaw in Poland were included in the cohort, but given a low participation rate at follow-up (n = 92) these women were not included in this study. With few exceptions the antenatal health programs covered all pregnant women in these locations. To be eligible for the study, the woman had to be pregnant, at least 18 years of age, and born in the country of study.

At baseline, 1238 women were included in the study with a participation rate of 90% in Greenland and 26% in Ukraine. The enrolled women were interviewed and had a venous blood sample drawn. Further details on the recruitment procedure and baseline characteristics can be found in Toft et al. (18).

A follow-up was conducted between January 2010 and May 2012 when the children were 5 to 9 years of age (19). Parents or guardians responded to retrospective questions concerning behavioral development and other characteristics in a face-to-face interview or by filling in a questionnaire themselves.

In total, 1018 mother-child pairs had blood samples available and were followed up. Only singleton births were included. The study population was evenly distributed between Greenland (52%) and Ukraine (48%).

Ethics

The data collection for the study was approved by local ethical committees: the Ethical Committee for Human Research in Greenland (approval no. 2010-13), and the Commission on Ethics and Bioethics Kharkiv National Medical University in Ukraine (protocol number 7, October 7 2009). The storage and use of data is registered at the Danish Data Protection Agency. All participants signed an informed consent prior to study enrolment.

Prenatal exposure

At baseline, a venous blood sample was drawn from the pregnant women. In Greenland, the median (10th-90th percentile) gestational week for the blood drawing was 25 (13-37) and in Ukraine it was 23 (9-40). Cubital vein blood samples were drawn into 10 ml vacuum tubes for serum collection without additives (Becton Dickinson, Maylan, France). Sera were stored at -20 ºC until shipment. After arrival at the Department of Occupational and Environmental Medicine in Lund, Sweden, sera were stored at -80 ºC until analyzed.

Maternal serum concentrations of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (*p,p'*-DDE), biomarkers for PCBs and DDT, respectively, were analyzed using gas chromatography-mass spectrometry following solid phase extraction. CB-153 was chosen as a proxy for PCB exposure since it is strongly correlated with other PCB congeners and has a long half-life in humans (20, 21). Similarly, DDE is a proxy for DDT since it is a direct breakdown product with a long biological half-life. The levels of CB-153 and *p,p'*-DDE were adjusted for lipids based on serum concentrations of cholesterol and triglycerides determined by enzymatic methods (22).

The limits of detection (LOD) were 0.05 ng/mL for CB-153 and 0.1 ng/mL for *p,p'*-DDE. All sample analyses were performed twice on different days and the mean concentration of these two was used. If the difference between the two samples was above 30%, a third and, if necessary, a fourth re-analysis was performed. However, at sample concentrations below 0.2 ng/mL a deviation of 0.1 ng/mL between the duplicate samples was accepted. For CB-153, 24 samples were below the LOD and for *p,p'*-DDE, 9 samples were below the LOD. When concentrations were below the LOD, they were set to half of the LOD based on fresh weight concentrations for all subsequent analyses. Further details on the coefficients of variation and the analysis of the serum samples can be found in Jönsson et al. (23). For external quality control the laboratory participates in an international Round Robin intercomparison program.

Postnatal exposure

To estimate the cumulative postnatal exposure to PCB and DDT for the first 12 months after delivery, a pharmacokinetic model developed by Verner et al. was used (24). The pharmacokinetic model has previously been validated in a Slovakian birth cohort and a Canadian Inuit birth cohort (24, 25). The model inputs were age of the mother at delivery, maternal pre-pregnancy weight, duration of the exclusive breastfeeding, duration of partial breastfeeding, gestational age at birth,

the sex of the child, child's birth weight, child's weight at follow-up, and age at measurement, up to two previously recorded weight measurements, maternal serum levels of CB-153 and DDE during pregnancy, gestational age at blood sampling, and half-life of the compounds (24). Since the duration of exclusive breastfeeding had the largest influence on the model estimates, only mother-child pairs with available breastfeeding data during the first 12 months and measured maternal levels of PCB-153 and *p,p'*-DDT (n=977) were included in the postnatal models.

Based on the above, we estimated the cumulative exposure of PCB-153 and *p,p'*-DDE during the first 12 months based on the area under the curve for the estimates at each individual month. The model has previously been used by Høyer et al. in the same birth cohort (26). The pharmacokinetic modeling was performed using acslX software (Aegis Technologies Group, Inc., Huntsville, AL, USA).

Outcome assessment

We assessed behavioral development using country specific versions in the respective language of the Strength and Difficulties Questionnaire (SDQ). SDQ is a standardized screening tool comprising 25 questions, including both strengths and difficulties, on five different scales (emotional symptoms, conduct problems, hyperactivity, peer relationship problems, and prosocial behavior) (27). SDQ can be applied internationally and has been thoroughly validated (28, 29). The questionnaire is used to identify the mental health in children 2 to 17 years of age. Each item is marked "not true", "somewhat true", or "certainly true" and summed-up each scale is ranked 0-10 points. A high score on the prosocial scale reflects strengths whereas higher score on the other four scales reflects difficulties. The subscales can be used individually or as a combined measure of behavior using the sum of the first four scales (emotional, conduct, hyperactivity, and peer problems). The total SDQ score is ranged 0-40 points. In a cohort of 9,998 British 5-15 years internal consistency was tested, showing a Cronbach α of 0.82 for the parent reported SDQ score.

In the present study, parents completed the SDQ at follow-up when the children were between 5 and 9 years of age, reflecting their child's behavior during the preceding 6 months. As outcomes we used the total SDQ score and the SDQ subscale scores according to the guidelines (27, 29). The SDQ scores were categorized into normal, borderline and abnormal scores based on cut-off values (Total SDQ Score: normal 0-13, borderline 14-16, abnormal 17-40; Emotional Symptoms Score: normal 0-3, borderline 4, abnormal 5-10; Conduct Problems Score: normal 0-2, borderline 3, abnormal 4-10; Hyperactivity Score: normal 0-5, borderline 6, abnormal 7-10; Peer Problems

Score: normal 0-2, borderline 3, abnormal 4-10; Prosocial Behavior Score: normal 6-10, borderline 5, abnormal 0-4) (27).

Statistical analyses

Spearman's correlation coefficients were calculated to assess the correlations between both prenatal and postnatal levels of PCB and DDE. The association between pregnancy and postnatal exposure to CB-153 and p'p'-DDE and behavioral changes was investigated using logistic regression models. The exposures were log2 transformed allowing us to examine the odds ratio (OR) for abnormal SDQ scores associated with a doubling of the exposures. The SDQ scores of the children were dichotomized into normal and borderline versus abnormal according to the approach by Goodman et al. (27, 28).

Potential confounders were selected *a priori* based on existing literature and only variables indicated to confound the association in previous studies were included. The possible confounders were obtained from the blood samples, baseline interviews, and follow-up interviews. We did a stringent prioritization of the strongest confounders and chose to adjust for: maternal age at birth (continuous, years), maternal smoking during pregnancy (serum cotinine level during pregnancy ≤ 10 / >10 ng/mL), sex of the child, and age of the child at follow-up (continuous, years) (30-33). To evaluate the less strong confounders not chosen for the primary analysis, secondary analyses were performed to further adjust for parity (1st, 2nd or above), educational level of the mother (left school at or before the age of 15 years, left school at age 16-17 years, left school at age 18 or older), breastfeeding duration (0, <6, 6-12, >12 months), gestational age at blood drawing (continuous, weeks), and gestational age at birth (continuous, weeks). Furthermore, we assessed effect modification of the association between the exposure and outcome by country as well as sex of the child using stratified analyses and by including interaction terms in the model. In a sensitivity analysis, we used the top 10 percentile of the SDQ scores as the abnormal group instead of the cut-off points given by Godman (27).

All analyses were stratified by country as well as pooled, adjusted for country, and both crude and adjusted ORs were calculated.

All statistical analyses were performed using the Stata software version 14.1 (Stata Corporation, College Station, TX, USA). A p-value less than 0.05 was considered statistically significant.

Results

Characteristics of the 1018 mother-child pairs included in the study are shown in Table 1. The mean SDQ score was 8 in Greenland and 9 in Ukraine, whereas the percentages of children with abnormal SDQ scores were 6% and 5%, respectively.

In Greenland, 56% of the women smoked during pregnancy and 11% had alcohol consumption above 7 units per week, whereas in Ukraine, only 15% smoked during pregnancy and none reported alcohol consumption above 7 units per week. In general, the women from Greenland were more often multiparous and were slightly older than women from Ukraine who were mostly primiparous. Also, the women from Greenland were younger when leaving school and 45% breastfed their children for more than 12 months compared to 22% in Ukraine.

Among Greenlandic women, the median (10th-90th percentile) pregnancy serum concentration was 107 ng/g lipids (30-369) for PCB and 229 ng/g lipids (75-954) for DDE. Conversely, among Ukrainian women, the median (10th-90th percentile) pregnancy serum concentrations were 27 ng/g lipids (11-54) for PCB and 639 ng/g lipids (329-1303) for DDE. The same pattern of considerably higher amounts of PCB in Greenlandic women and DDE in Ukrainian women was seen in the estimated postnatal exposures.

The Spearman's correlation coefficient between PCB and DDE was 0.92 in Greenland and 0.46 in Ukraine for the prenatal exposures and 0.93 in Greenland and 0.76 in Ukraine for the postnatal exposures. In Greenland, the Spearman's correlation coefficient between the prenatal exposures and postnatal exposures was 0.81 for PCB and 0.82 for DDE. In Ukraine, the equivalent coefficient was 0.56 for PCB and 0.49 for DDE.

The results of crude and adjusted ORs for abnormal total SDQ score in relation to a doubling of prenatal and postnatal PCB and DDE are presented in Table 2. Overall, no clear association was observed between log2-transformed continuous exposure variables of neither prenatal nor postnatal exposure to PCB or DDE in relation to total SDQ scores. The pooled crude OR (95% CI) for a doubling of prenatal exposure was 1.00 (0.81, 1.24) for PCB and 1.07 (0.86, 1.34) for DDE; similarly, the pooled adjusted OR (95% CI) was 1.09 (0.86, 1.38) for prenatal PCB and 1.15 (0.90, 1.48) for prenatal DDE. The same pattern was observed when stratified by country for both Greenland and Ukraine; the adjusted OR (95%) for prenatal DDE exposure was 1.09 (0.82, 1.45) in Greenland and 1.46 (0.87, 2.44) in Ukraine.

The pooled adjusted OR (95% CI) for a doubling of postnatal exposure was 0.89 (0.74, 1.06) for PCB and 0.91 (0.76, 1.09) for DDE. The pattern of an OR slightly below 1 for the postnatal exposures was consistent in both the crude and adjusted analyses stratified by country and pooled; no clear association was seen. To test if these results were due to a beneficial component of breastfeeding in general, the breastfeeding duration was included in the models. The ORs were then slightly above 1, however, the result was still not significant (data not shown).

The OR for abnormal SDQ subscale scores associated with a doubling of prenatal PCB and DDE is presented in Table 3. For PCB, the adjusted OR (95% CI) of abnormal conduct score was 1.19 (0.99, 1.42) in the pooled analysis, while no clear association was found on the hyperactivity scale. For DDE, the adjusted OR (95% CI) of having an abnormal score on the conduct problems scale was increased in both Greenland: OR 1.24 (1.00, 1.54), Ukraine: OR 1.58 (1.02, 2.44), and the pooled analysis: OR 1.25 (1.04, 1.51). The pattern was consistent in both the crude and adjusted analysis. The OR of having an abnormal score on the hyperactivity subscale in relation the prenatal DDE was increased in both countries, and in the pooled analysis the OR (95% CI) was 1.43 (1.06, 1.92). No associations were found on the emotional problems, peer problems, or prosocial score scale, for neither prenatal PCB nor DDE.

The OR for abnormal SDQ subscale scores in relation to the postnatal exposures to PCB and DDE showed no clear association on any of the subscales (see Supplemental Material, Table S1).

Subanalyses were performed adjusting for parity, maternal educational level, breastfeeding duration, gestational age at blood drawing, and gestational age at birth, and none of these changed the estimates significantly (data not shown). Additionally, the test for effect modification by country and sex of the child showed no interaction (see Supplemental Material, Table S2). In the sensitivity analysis using the top 10 percentile of the SDQ scores as the abnormal group, the overall pattern did not change (see Supplemental Material, Table S3).

Discussion

We found a consistent association between a doubling of prenatal exposure to DDE and increased crude and adjusted OR for conduct problems in Greenland, Ukraine, and in the pooled analysis. Additionally, we found a tendency towards associations between prenatal PCB and DDE and hyperactivity problems, but the associations were less consistent. The association with total SDQ scores was not significant. Likewise, postnatal exposure was not associated with any adverse changes of neither the total SDQ score nor the subscale scores. To our knowledge, this is the first study to evaluate the impact of prenatal as well as postnatal exposure to PCB and DDE on adverse social behavior measured as SDQ.

Our findings suggest that prenatal exposure to DDE and potentially PCB may increase the risk of conduct problems and to some extent hyperactivity in children from 5 to 9 years of age. It adds to the growing evidence of adverse developmental effects of *in utero* exposure to neurotoxins such as PCBs and DDE (34). In a cohort of 607 mother-child pairs from Massachusetts, Sagiv et al. reported an association between umbilical cord blood levels of PCB and DDE, and ADHD like symptoms in children 7 to 11 years of age measured with the Conners' Rating Scale for Teachers (CRS-T) (9). These results of changes in social behavior are consistent with ours, even though our association of hyperactivity was less strong. Likewise, Verner et al. found that cord blood levels of PCB were associated with ADHD related behavior, on the CRS-T, in a cohort of 441 children at 8 years of age (35). In the same study weaker associations were found between adverse CRS-T scores and estimated postnatal exposure for the first 12 months after delivery calculated using a pharmacokinetic model (35). In our study, we used the same pharmacokinetic model to calculate postnatal exposure to PCB and DDE, resulting in similar attenuated exposure-outcome associations. Another study showed maternal blood levels of PCB to be associated with lowered attention function in a German birth cohort, but the number of participants was low (n=117) (36).

Despite the evidence of adverse behavioral effects of prenatal exposure to PCBs and DDE, the literature is still inconsistent, with some studies showing a null result. In a cohort of 917 children at 7 years of age from the Faroe Islands, Grandjean et al. showed an association between cord blood levels of DDE and attention function measured by the Continuous Performance Test (CPT) (37). Furthermore, in the same study, four PCB-congeners were measured as well but showed no clear association (37). This is in contrast to our study, which shows an association between prenatal exposure to DDE, and potentially PCB, in relation to conduct problems and to some extent

hyperactivity. Still, it is difficult to compare the studies given different outcome ascertainment methods and behavioral areas (i.e. cognition vs. behavior), and exposure measured in maternal serum versus cord blood. In a Danish cohort of 876 pregnant women, PCB and DDE were measured in serum between 1988 and 1989 and the children were followed up through national registers until 2011 for diagnosis or prescription of medication for ADHD (38). The study showed no association between prenatal exposure to PCB and DDE, and ADHD (38). Similarly, these results of ADHD medication and diagnosis of children followed up beyond childhood are difficult to compare to our study where behavioral symptoms, including hyperactivity and conduct problem dimensions, were measured in children between 5 and 9 years of age by the parents. A follow-up on two Spanish birth cohorts (n=475) showed an association between hexachlorobenzene and adverse social competences and ADHD like behavior, but the investigators found no association between PCB and DDE and the two outcomes (39). On the basis of the existing literature it may be difficult to make a clear conclusion on the effects of the compounds since not all studies are comparable. Differences may be due to different rating scales, ages of the children, exposure levels, measured congeners, and whether the compounds are tested in cord blood, serum, or placental tissue.

The possible biological mechanism behind behavioral disturbances and early life exposure to PCBs and DDT is still unknown. However, studies have shown an association between the compounds and altered thyroid function (40, 41), thereby suggesting an altered development of the fetal brain (42). A review by Bell states that PCBs act as endocrine disrupters working at more than one pathway with quite diverse actions (43).

This study has some limitations. Participation rates at baseline were 90% in Greenland and 26% in Ukraine, therefore introduction of selection bias cannot be ruled out. A non-response analysis at baseline showed almost no difference in age or parity among participants and non-participants in neither Greenland nor Ukraine suggesting little indication of selection bias (18). Also, the decision to participate at both baseline and follow-up was made without knowledge of the prenatal or postnatal exposure levels.

Blood samples were collected throughout pregnancy, mainly in the second and third trimester. Since DDE and PCBs tend to decrease throughout pregnancy, exposure misclassification might occur. We addressed this in a sensitivity analysis adjusting for gestational age of blood sampling, which did not change the results (data not shown). For that reason, gestational age at blood sampling does not seem to be of major concern.

Also, we had no measurements of the postnatal exposure to CB-153 and *p,p'*-DDE and used a pharmacokinetic model to estimate the cumulative exposure the first 12 months after delivery. The model has been validated in similar settings (24, 25) and has previously been used in the same birth cohort (26). However, an exact measure of the postnatal exposure would have been preferred.

As the outcome measure, we used the thoroughly validated and internationally recognized SDQ (28, 29). SDQ is not a diagnostic tool, but can be used as a screening measure to assess the mental health of children as both a total score and on five different subscales. The literature gave no clear indication of which behavioral problems the compounds could cause. Therefore, we chose not only to look at the total SDQ score but also the subscale scores, since a possible effect on the subscales would not necessarily be of notice on the total SDQ score.

Since SDQ cut-off points were not validated in the Greenlandic and Ukrainian populations, we used the general cut-off points given by Goodman for the British population (27). This could lead to misclassification of the outcome estimate. We addressed this in a sensitivity analysis using the top 10 percentile as cut-off, which did not alter the results considerably. Furthermore, studies have shown comparability of the SDQ across different European countries and to some extent beyond Europe as well (44, 45).

The prevalence of participants in the abnormal group was only 6% in Greenland and 5% in Ukraine, which is in line with other similar studies (28, 46). Still, to ensure sufficient power, we chose to adjust our main analysis only for maternal age at birth, maternal smoking during pregnancy, sex of the child, and child age at follow-up. Other covariates such as parity, educational level, gestational age at blood sampling and gestational age at birth, were addressed in supplementary analyses and did not change the estimate essentially (data not shown).

The strengths of our study include the prospective follow-up where exposures were collected without knowledge of the outcome. Likewise, the outcome at follow-up was collected without knowledge of the exposure levels. The follow-up period of 5 to 9 years enabled us to assess the consequences of the exposure on children at school age. Also, the study population of mother-child pairs from Greenland and Ukraine enabled us to evaluate consistency of exposure-outcome associations across different regions, ethnicities, and exposure contrasts.

Conclusion

The results of this study indicate that prenatal exposure to DDE may increase the risk of conduct problems and to some extent hyperactivity in children from 5 to 9 years of age. For prenatal PCB exposure association was less consistent. No associations were observed for postnatal exposures. The study contributes to the growing evidence of adverse behavioral development following *in utero* exposure to organochlorines like PCBs and DDE, and underlines the importance of minimal organochlorine exposure for women of childbearing age.

SUPPLEMENTARY

The following section of the Research Year Report contains additional methodological considerations of the study design and statistics, and a discussion of strengths and limitations including especially bias and confounding. Furthermore, perspectives and future studies are presented.

Methodological considerations

Study design

The present study was a prospective cohort study of women from the INUENDO birth cohort consisting of mother-child pairs from Greenland and Ukraine. A prospective cohort study, is defined as a study where a group of subjects are assembled in the present and follow them into the future in relation to a specific outcome (47). Exposures and various characteristics can thereby be collected at baseline before the outcome has happened, in contrast to retrospective cohort studies where exposure information is identified from past records and followed up to the present time.

In this study exposure was measured prenatally as pregnancy serum concentrations of PCB and DDE. Also, a pharmacokinetic model was used to calculate the theoretical cumulative postnatal exposure to PCB and DDE during the first 12 months after delivery (24). The study population was pregnant women attending antenatal care visits between May 2002 and February 2004 at 3 hospitals and 8 antenatal clinics in Kharkiv (Ukraine), and 19 local hospitals in municipalities and settlements in Greenland. Follow-up was conducted between January 2010 and May 2012 when the children were 5 to 9 years of age. The outcome was collected retrospectively by the parents, who filled out the SDQ rating their child's behavior during the preceding 6 months.

One of the advantages with prospective cohort studies is that it can be collected in a standardized manner, thereby decreasing the chance of measurement bias (48). Also information on risk factors and confounders such as socioeconomic status, educational level, genetic inheritance, geography, and lifestyle can be collected in a prospective cohort study.

The aim of our study was to examine the effect of prenatal and postnatal exposure to PCB and DDE on child social behavior assessed both as a total SDQ score and as 5 subscale scores divided into emotional problems, conduct problems, hyperactivity, peer problems, and prosocial behavior.

Statistical analysis

The association between prenatal and postnatal exposure to PCB and DDE and SDQ score was investigated using logistic regression. Logistic regression is the most commonly used method for the analysis of a binary outcome (49). We used the total SDQ score as well as the subscale scores and divided them into two categories - normal or abnormal - according to the guidelines (27). We also considered linear regression and negative binomial regression, but SDQ did not fit the assumptions. Furthermore, we wanted a more clinical angle on the project and chose the normal/abnormal dichotomization.

Strengths and limitations

One of the main strengths is the prospective study design where the exposures are measured without knowledge of the outcome. Furthermore, the outcome was collected blinded, therefore neither parents nor investigators had any knowledge of the prenatal and postnatal exposure levels. The exposure and outcome was collected in a standardized manner, thereby minimizing the chance of measurement bias. Another strength is the long follow-up period of 5 to 9 years enabling us to assess the consequences of early life exposure to PCB and DDE on the mental health of school age children. Also, having both women-child pairs from Greenland and Ukraine enables us to assess the exposure-outcome association across regions, ethnicity, and exposure contrasts.

The study also contains limitations affecting the validity of the study. Validity of an epidemiological study can be divided into internal validity and external validity (48). Internal validity of a study is the degree to which the results of the study are correct for the study population. External validity is the degree to which the results can be generalized to other populations. The internal validity of a study is dependent on the study design, data collection, and data analysis which relates to the random error and systematic error. Random error occurs by chance and cannot be explained, but decreases with increasing study size (47). Systematic error includes information bias, selection bias, and confounding, and remains unaffected by study size

Selection bias

Selection bias is a systematic error that stems from the procedures used to select participants and factors that alters the participation in a given study (47). If the association between exposure and outcome differs between the participants and non-participants, this could bias the results and interpretation of the association.

Our study included a sample of women attending antenatal care visits between 2002 and 2004 at 3 hospitals and 8 antenatal clinics in Kharkiv in Ukraine, and 19 local hospitals in municipalities and settlements in Greenland. Therefore, our study population does not include women from all over the countries and this must be considered when interpreting the results. If women who choose to participate are less exposed than women who refuse to participate this may introduce selection bias. Also, at baseline participation rates were 90 % in Greenland but only 25 % in Ukraine. This was addressed in a non-responsive analysis at baseline by Toft et al. (18), which showed almost no

difference in age or parity among participants and non-participants in neither Greenland nor Ukraine. Still, the participation rates of our study can introduce bias.

Information bias

Information bias is when information collected about or from the study participants is wrong (47). Information bias can lead to misclassification if a study subject is being placed in a wrong exposure group based on flawed information (e.g. if a “normal” child ends up in the “abnormal” group or a non-smoker ends up in the smoker group). The misclassification can either be non-differential or differential. Non-differential misclassification occurs when the misclassification is not related to exposure or outcome, meaning that the misclassification is the same in all the groups that are compared. Differential misclassification occurs when the misclassification is related to exposure or outcome, e.g. if cases under-report their smoking status during pregnancy whilst controls do not. A non-differential misclassification will normally only lead to an underestimation of the results, meaning that a given association will be closer to null than the actual effect. Differential misclassification can both underestimate and overestimate the results depending on the study design.

In our study the decision to participate at both baseline and follow-up was made without knowledge of prenatal or postnatal exposure levels. Likewise, the people analyzing the blood samples and collecting the data were unaware of the exposure. Therefore, we believe that potential misclassification will only be non-differential.

Confounding

In statistics, confounding is a confusion of effects where the exposure effects are mixed with other variables leading to bias or misinterpretation of the result (47). If you e.g. study the associations between educational level and cardiovascular events. If people with a high education has a healthier lifestyle (e.g. less smoking, healthier diet, more physical exercise, less obesity, etc.) then the educational level may seem to have an effect on cardiovascular events. The direct reason for less cardiovascular events is not the educational level itself but the lifestyle factors that follows it. Confounding is a systematic error and can either increase or decrease an observed association between exposure and outcome.

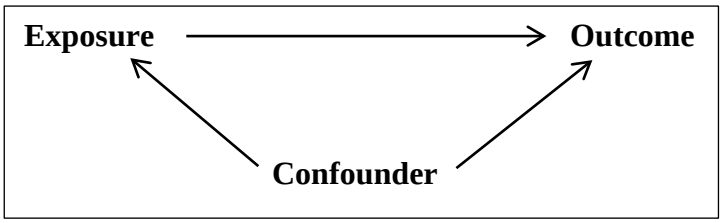
For a variable to be a confounder it must meet three criteria (see Supplemental Figure 1) (48):

1. The confounder must be associated with the exposure.

2. The confounder must be associated with the outcome

3. The confounder must not be an intermediate in the causal chain.

Supplemental Figure 1. Correlation between exposure, outcome and confounder.



Confounding can be prevented by three different methods (47). In the first method, randomization, subjects are randomly assigned to the different exposure groups (e.g. to a treatment A, treatment B, placebo group etc.). Randomization balances potential confounding variables, but it is not always possible to design a study like that. In the second method, restriction, the study subjects are chosen within a certain range of characteristics thereby minimizing potential confounding of these characteristics. The downside of restriction is that it decreases generalizability to other populations than the very specific population used for the study. In the third method, matching, exposed and unexposed subjects are matched in pairs based on a certain range of characteristics. If the confounding factors could not be handled in the study design, potential cofounders can be adjusted for in the given analysis.

There are different approaches to identify potential condounders. One approach, is through the literature (48). Another is to screen the different variables for the effect on the regression estimate, some studies only include confounders that change the estimate by 10% or more.

In our study, we did a stringent prioritizing of the variables based on the existing literature and adjusted for maternal age at birth (continuous, years), maternal smoking during pregnancy (serum cotinine level during pregnancy ≤ 10 / >10 ng/mL), sex of the child, and age of the child at follow-up (continuous, years). To ensure sufficient power we chose only the strongest confounders and evaluated the less strong in secondary analyses. We choose to control for confounding by multivariable adjustment in the logistic regression models. Results are both presented crude and adjusted, so that the difference can be interpreted by the reader. Information on the potential confounders was obtained from blood samples, baseline interviews and follow-up interviews. Despite our attempt to adjust for all potential confounders, residual confounding may still alter our results.

Effect measure modification

Effect measure modification is whether the presence or absence of a specific variable changes the effect of the exposure on the outcome (48). We tested effect modification of country and sex on the association between exposure and outcome, which showed no interaction. Therefore we included age in the adjusted models and country in the pooled adjusted models.

Confidence intervals

A confidence interval indicates the level of precision of a point estimate (47). The interval ranges from below to above the point estimate; a wide interval implies less precision whereas a narrow interval implies a high precision. Our study included a relatively large cohort, still the precision of the point estimates varies and depends on the number of missing values of the covariates and whether or not we stratified by country or used the pooled number including both Greenland and Ukraine. E.g. the CI was wider when stratified by country and narrower when pooled.

Clinical perspectives and future studies

PCB and DDE are ubiquitous environmental contaminants found in our everyday life. They accumulate within human adipose tissue. During pregnancy these contaminants can cross the placental barrier thereby exposing the fetus (4, 6). Altered neurological development is of utmost importance for the general public health. Our study showed that a doubling of DDE is associated with increased odds for conduct problems in Greenland and Ukraine – individually and pooled. We also observed a tendency towards increased hyperactivity for both PCB and DDE, but the results were less consistent. We found no association with total SDQ score. Likewise, postnatal exposure was not associated with neither total SDQ score nor the subscale scores.

Our study adds to the growing evidence of the harm of organochlorine exposure in relation to behavioral development. It is challenging to compare the different studies in the literature given different cohorts, outcome ascertainment methods etc. Still, this study implies that prenatal exposure to organochlorines should be taken seriously. Since PCB and DDE accumulates in the human body for decades, not only women of child bearing age should avoid exposure, children and adolescents should as well. We found no association of any of the compounds and total SDQ score, this might be because the total score is not specific enough. Others might argue that only five questions on the subscale is to unprecise. This said, it is important to notice that SDQ is not a clinically diagnostic tool, but should be seen as a screening tool to assess the mental health of children.

Future studies should look into PCB and DDE in similar cohorts but with longer follow-up time. Thereby it would be possible to examine e.g. whether a change in the childhood persists into adolescence. We are also looking into the effect of maternal phthalates levels during pregnancy on the children's behavior measured as SDQ in the same cohort (INUENDO). Phthalates have the same ability of crossing the placental barrier during pregnancy (50, 51) and studies have shown a link between phthalates and autism spectrum disorders (ASD) (52), and attention deficit hyperactivity disorder (ADHD) like symptoms (53). Furthermore, SDQ is widely used in the Scandinavian countries and further country specific validation of the questionnaire could be useful in future studies. Also, we are lacking studies directly comparing results from the SDQ with results from other questionnaires.

This present study contributes to the field of environmental contaminants in relation to child behavioral development. To our knowledge it is the first study to evaluate prenatal as well as postnatal exposure to PCB and DDE on adverse social behavior measured as SDQ. It is an important topic where only limited information is available.

REFERENCES

1. van den Berg H. Global status of DDT and its alternatives for use in vector control to prevent disease. Environ Health Perspect. 2009;117(11):1656-63.

2. Ibarluzea J, Alvarez-Pedrerol M, Guxens M, Marina LS, Basterrechea M, Lertxundi A, et al. Sociodemographic, reproductive and dietary predictors of organochlorine compounds levels in pregnant women in Spain. Chemosphere. 2011;82(1):114-20.

3. Malarvannan G, Dirinck E, Dirtu AC, Pereira-Fernandes A, Neels H, Jorens PG, et al. Distribution of persistent organic pollutants in two different fat compartments from obese individuals. Environ Int. 2013;55:33-42.

4. Glynn A, Aune M, Darnerud PO, Cnattingius S, Bjerselius R, Becker W, et al. Determinants of serum concentrations of organochlorine compounds in Swedish pregnant women: a cross-sectional study. Environ Health. 2007;6:2.

5. Ribas-Fitó N, Grimalt JO, Marco E, Sala M, Mazón C, Sunyer J. Breastfeeding and concentrations of HCB and p,p′-DDE at the age of 1 year. Environmental Research. 2005;98(1):8-13.

6. Vizcaino E, Grimalt JO, Fernandez-Somoano A, Tardon A. Transport of persistent organic pollutants across the human placenta. Environ Int. 2014;65:107-15.

7. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet (London, England). 2006;368(9553):2167-78.

8. Rosas LG, Eskenazi B. Pesticides and child neurodevelopment. Current opinion in pediatrics. 2008;20(2):191-7.

9. Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. Am J Epidemiol. 2010;171(5):593-601.

10. Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. Environ Health Perspect. 2010;118(12):1654-67.

11. Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. Environ Health Perspect. 2012;120(6):904-9.

12. Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal PCB exposure and intelligence (IQ) in 9-year-old children. Environ Health Perspect. 2008;116(10):1416-22.

13. Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, et al. In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers. Am J Epidemiol. 2006;164(10):955-62.

14. Sioen I, Den Hond E, Nelen V, Van de Mieroop E, Croes K, Van Larebeke N, et al. Prenatal exposure to environmental contaminants and behavioural problems at age 7-8years. Environ Int. 2013;59:225-31.

15. Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. Epidemiology. 2012;23(5):689-98.

16. Gray KA, Klebanoff MA, Brock JW, Zhou H, Darden R, Needham L, et al. In utero exposure to background levels of polychlorinated biphenyls and cognitive functioning among school-age children. Am J Epidemiol. 2005;162(1):17-26.

17. Orenstein ST, Thurston SW, Bellinger DC, Schwartz JD, Amarasiriwardena CJ, Altshul LM, et al. Prenatal organochlorine and methylmercury exposure and memory and learning in school-age children in communities near the New Bedford Harbor Superfund site, Massachusetts. Environ Health Perspect. 2014;122(11):1253-9.

18. Toft G, Axmon A, Giwercman A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, et al. Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross-sectional study. Environ Health. 2005;4:26.

19. Høyer BB, Ramlau-Hansen CH, Vrijheid M, Valvi D, Pedersen HS, Zviezdai V, et al. Anthropometry in 5- to 9-Year-Old Greenlandic and Ukrainian Children in Relation to Prenatal Exposure to Perfluorinated Alkyl Substances. Environ Health Perspect. 2015;123(8):841-6.

20. DeVoto E, Fiore BJ, Millikan R, Anderson HA, Sheldon L, Sonzogni WC, et al. Correlations among human blood levels of specific PCB congeners and implications for epidemiologic studies. American journal of industrial medicine. 1997;32(6):606-13.

21. Ritter R, Scheringer M, MacLeod M, Moeckel C, Jones KC, Hungerbuhler K. Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. Environ Health Perspect. 2011;119(2):225-31.

22. Rylander L, Nilsson-Ehle P, Hagmar L. A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. Chemosphere. 2006;62(3):333-6.

23. Jonsson BA, Rylander L, Lindh C, Rignell-Hydbom A, Giwercman A, Toft G, et al. Inter-population variations in concentrations, determinants of and correlations between 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE): a cross-sectional study of 3161 men and women from Inuit and European populations. Environ Health. 2005;4:27.

24. Verner MA, Sonneborn D, Lancz K, Muckle G, Ayotte P, Dewailly E, et al. Toxicokinetic modeling of persistent organic pollutant levels in blood from birth to 45 months of age in longitudinal birth cohort studies. Environ Health Perspect. 2013;121(1):131-7.

25. Verner MA, Ayotte P, Muckle G, Charbonneau M, Haddad S. A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. Environ Health Perspect. 2009;117(3):481-7.

26. Høyer BB, Ramlau-Hansen CH, Henriksen TB, Pedersen HS, Goralczyk K, Zviezdai V, et al. Body mass index in young school-age children in relation to organochlorine compounds in early life: a prospective study. Int J Obes (Lond). 2014;38(7):919-25.

27. Goodman R. The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry. 1997;38(5):581-6.

28. Goodman R, Ford T, Simmons H, Gatward R, Meltzer H. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. The British journal of psychiatry : the journal of mental science. 2000;177:534-9.

29. Goodman R. Psychometric properties of the strengths and difficulties questionnaire. Journal of the American Academy of Child and Adolescent Psychiatry. 2001;40(11):1337-45.

30. Fergusson DM, Woodward LJ. Maternal age and educational and psychosocial outcomes in early adulthood. J Child Psychol Psychiatry. 1999;40(3):479-89.

31. Ellis LC, Berg-Nielsen TS, Lydersen S, Wichstrom L. Smoking during pregnancy and psychiatric disorders in preschoolers. Eur Child Adolesc Psychiatry. 2012;21(11):635-44.

32. Pauly JR, Slotkin TA. Maternal tobacco smoking, nicotine replacement and neurobehavioural development. Acta Paediatr. 2008;97(10):1331-7.

33. Zahn-Waxler C, Shirtcliff EA, Marceau K. Disorders of childhood and adolescence: gender and psychopathology. Annu Rev Clin Psychol. 2008;4:275-303.

34. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. The Lancet Neurology. 2014;13(3):330-8.

35. Verner MA, Hart JE, Sagiv SK, Bellinger DC, Altshul LM, Korrick SA. Measured Prenatal and Estimated Postnatal Levels of Polychlorinated Biphenyls (PCBs) and ADHD-Related Behaviors in 8-Year-Old Children. Environ Health Perspect. 2015;123(9):888-94.

36. Neugebauer J, Wittsiepe J, Kasper-Sonnenberg M, Schoneck N, Scholmerich A, Wilhelm M. The influence of low level pre- and perinatal exposure to PCDD/Fs, PCBs, and lead on attention performance and attention-related behavior among German school-aged children: results from the Duisburg Birth Cohort Study. Int J Hyg Environ Health. 2015;218(1):153-62.

37. Grandjean P, Weihe P, Nielsen F, Heinzow B, Debes F, Budtz-Jorgensen E. Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet. Neurotoxicol Teratol. 2012;34(4):466-72.

38. Strom M, Hansen S, Olsen SF, Haug LS, Rantakokko P, Kiviranta H, et al. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up. Environ Int. 2014;68:41-8.

39. Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. Environ Health Perspect. 2007;115(3):447-50.

40. Julvez J, Debes F, Weihe P, Choi AL, Grandjean P. Thyroid dysfunction as a mediator of organochlorine neurotoxicity in preschool children. Environ Health Perspect. 2011;119(10):1429-35.

41. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. Environ Health Perspect. 2008;116(10):1376-82.

42. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. European journal of endocrinology / European Federation of Endocrine Societies. 2004;151 Suppl 3:U25-37.

43. Bell MR. Endocrine-disrupting actions of PCBs on brain development and social and reproductive behaviors. Curr Opin Pharmacol. 2014;19:134-44.

44. Obel C, Heiervang E, Rodriguez A, Heyerdahl S, Smedje H, Sourander A, et al. The Strengths and Difficulties Questionnaire in the Nordic countries. Eur Child Adolesc Psychiatry. 2004;13 Suppl 2:II32-9.

45. Woerner W, Fleitlich-Bilyk B, Martinussen R, Fletcher J, Cucchiaro G, Dalgalarrondo P, et al. The Strengths and Difficulties Questionnaire overseas: evaluations and applications of the SDQ beyond Europe. Eur Child Adolesc Psychiatry. 2004;13 Suppl 2:II47-54.

46. Elberling H, Linneberg A, Olsen EM, Goodman R, Skovgaard AM. The prevalence of SDQ-measured mental health problems at age 5-7 years and identification of predictors from birth to preschool age in a Danish birth cohort: the Copenhagen Child Cohort 2000. Eur Child Adolesc Psychiatry. 2010;19(9):725-35.

47. Rothman KJ. Epidemiology: An Introduction. 2nd ed: Oxford University Press; 2012.

48. Fletcher RH, Fletcher SW, Fletcher GS. Clinical Epidemiology: The Essentials. 5th ed: Lippincott Wiliams & Wilkins; 2014.

49. Kirkwood BR, Sterne JAC. Essential Medical Statistics 2nd ed: Blackwell Science; 2003.

50. Mose T, Mortensen GK, Hedegaard M, Knudsen LE. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod Toxicol. 2007;23(1):83-91.

51. Ejaredar M, Nyanza EC, Ten Eycke K, Dewey D. Phthalate exposure and childrens neurodevelopment: A systematic review. Environ Res. 2015;142:51-60.

52. Testa C, Nuti F, Hayek J, De Felice C, Chelli M, Rovero P, et al. Di-(2-ethylhexyl) phthalate and autism spectrum disorders. ASN Neuro. 2012;4(4):223-9.

53. Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, Kim JW, et al. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry. 2009;66(10):958-63.

TABLES

Table 1. Characteristics of mothers and their children.

Characteristics	Greenland (n = 525)	Ukraine (n = 493)	Pooled (n = 1018)
Exposure [median (10th-90th percentile)]			
Prenatal CB-153 (ng/g lipids)	107 (30-369)	27 (11-54)	45 (15-253)
Prenatal *p,p'*-DDE (ng/g lipids)	299 (75-954)	639 (329-1303)	465 (124-1158)
Estimated postnatal CB-153 (ng/g lipids)	2647 (558-9618)	516 (104-1419)	1001 (173-6368)
Estimated postnatal *p,p'*-DDE (ng/g lipids)	7075 (1282-23,133)	12,459 (2914-34,724)	9642 (1836-28,807)
Outcome [mean score (% of children with abnormal scores)]			
Total difficulties (score 0 to 40)	8 (6%)	9 (5%)	8 (6%)
Emotional problems (score 0 to 10)	2 (8%)	1 (5%)	2 (7%)
Conduct problems (score 0 to 10)	1 (11%)	2 (8%)	1 (10%)
Hyperactivity (score 0 to 10)	2 (4%)	3 (5%)	3 (5%)
Peer problems (score 0 to 10)	2 (10%)	2 (5%)	2 (7%)
Prosocial behavior (score 0 to 10)	6 (13%)	6 (10%)	6 (11%)
Maternal characteristics			
Maternal age at delivery, median (10th-90th percentile)	26 (20-36)	24 (19-32)	25 (20-35)
Parity, n (%)			
1st	166 (33)	402 (82)	568 (57)
2nd or above	335 (67)	91 (18)	426 (43)
Smoking during pregnancy, n (%)			
Yes (serum cotinine > 10 ng/mL)	295 (56)	75 (15)	370 (37)
No (serum cotinine ≤ 10 ng/mL)	230 (44)	413 (84)	643 (63)
Alcohol consumption when trying to conceive, n (%)			
≤ 7 units per week	465 (89)	493 (100)	958 (94)
> 7 units per week	60 (11)	0 (0)	60 (6)
Educational level, left school at age (years), n (%)			
≤ 15	44 (10)	25 (6)	69 (8)
16-17	169 (36)	116 (27)	285 (32)
≥ 18	251 (54)	293 (67)	544 (60)

Table 1. Characteristics of mothers and their children *(continued)*.

Child characteristics			
Sex, n (%)			
Male	284 (54)	261 (53)	545 (54)
Female	239 (46)	229 (47)	468 (46)
Age at follow-up, median (10th-90th percentile)	8 (7-9)	7 (7-8)	7 (7-9)
Total breastfeeding duration (months), n (%)			
0	18 (4)	42 (9)	60 (6)
<6	120 (25)	165 (34)	285 (30)
6-12	124 (26)	175 (36)	299 (31)
>12	213 (45)	108 (22)	321 (33)
Gestational age at blood sample (weeks), median (10th-90th percentile)	25 (13-37)	23 (9-40)	24 (10-39)
Gestational age at birth (weeks), n (%)			
≥ 37 weeks	498 (95)	481 (98)	979 (97)
< 37 weeks	25 (5)	9 (2)	34 (3)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene.

Table 2. Odds ratios (95% CI) for abnormal total SDQ scores associated with a doubling of prenatal and postnatal PCB and DDE.

	Greenland				**Ukraine**				**Pooled**[a]			
	n	Crude OR	n	Adjusted OR[b]	n	Crude OR	n	Adjusted OR[b]	n	Crude OR	n	Adjusted OR[b]
Prenatal CB-153	516	0.93 (0.73, 1.19)	452	1.02 (0.77, 1.36)	492	1.22 (0.80, 1.84)	456	1.24 (0.77, 1.99)	1008	1.00 (0.81, 1.24)	908	1.09 (0.86, 1.38)
Prenatal *p,p'*-DDE	516	1.00 (0.79, 1.27)	452	1.09 (0.82, 1.45)	492	1.41 (0.88, 2.25)	456	1.46 (0.87, 2.44)	1008	1.07 (0.86, 1.34)	908	1.15 (0.90, 1.48)
Estimated postnatal CB-153	482	0.77 (0.62, 0.96)	426	0.81 (0.63, 1.05)	485	0.97 (0.75, 1.26)	450	0.94 (0.70, 1.25)	967	0.85 (0.72, 1.00)	876	0.89 (0.74, 1.06)
Estimated postnatal *p,p'*-DDE	482	0.84 (0.68, 1.02)	426	0.87 (0.69, 1.11)	485	1.00 (0.76, 1.33)	450	0.98 (0.72, 1.32)	967	0.89 (0.76, 1.05)	876	0.91 (0.76, 1.09)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; OR, odds ratio; PCB, polychlorinated biphenyls; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene; SDQ, Strength and Difficulties Questionnaire.

[a] Crude and adjusted OR for the pooled cohort is adjusted for country.

[b] Adjusted for maternal age, maternal smoking during pregnancy, sex of the child, and age of the child at follow-up.

Table 3. Odds ratios (95% CI) for abnormal SDQ subscale scores associated with a doubling of prenatal PCB and DDE.

		Greenland		Ukraine		Pooled[a]	
		Crude OR	Adjusted OR[b]	Crude OR	Adjusted OR[b]	Crude OR	Adjusted OR[b]
Prenatal CB-153	Emotional	0.89 (0.72, 1.09)	0.88 (0.70, 1.11)	1.13 (0.74, 1.72)	1.16 (0.71, 1.90)	0.93 (0.77, 1.12)	0.94 (0.76, 1.15)
	Conduct	1.16 (0.96, 1.40)	1.18 (0.96, 1.46)	1.32 (0.93, 1.87)	1.32 (0.89, 1.96)	1.19 (1.01, 1.41)	1.19 (0.99, 1.42)
	Hyperactivity	1.18 (0.88, 1.58)	1.35 (0.96, 1.89)	0.90 (0.59, 1.37)	0.96 (0.60, 1.53)	1.08 (0.85, 1.38)	1.24 (0.94, 1.62)
	Peer	1.00 (0.87, 1.15)	1.04 (0.88, 1.22)	1.14 (0.87, 1.50)	1.19 (0.88, 1.61)	1.03 (0.91, 1.17)	1.05 (0.92, 1.21)
	Prosocial	1.09 (0.91, 1.30)	1.17 (0.94, 1.45)	0.88 (0.65, 1.18)	0.83 (0.59, 1.16)	1.03 (0.89, 1.20)	1.06 (0.89, 1.26)
Prenatal *p,p'*-DDE	Emotional	0.95 (0.78, 1.16)	0.94 (0.76, 1.17)	1.25 (0.77, 2.02)	1.24 (0.71, 2.15)	0.99 (0.82, 1.19)	0.97 (0.79, 1.19)
	Conduct	1.22 (1.00, 1.48)	1.24 (1.00, 1.54)	1.58 (1.07, 2.33)	1.58 (1.02, 2.44)	1.29 (1.08, 1.54)	1.25 (1.04, 1.51)
	Hyperactivity	1.22 (0.91, 1.65)	1.36 (0.95, 1.94)	1.40 (0.86, 2.29)	1.42 (0.84, 2.40)	1.27 (0.98, 1.65)	1.43 (1.06, 1.92)
	Peer	1.07 (0.93, 1.24)	1.11 (0.94, 1.30)	1.30 (0.96, 1.78)	1.36 (0.96, 1.91)	1.11 (0.98, 1.26)	1.12 (0.97, 1.29)
	Prosocial	1.16 (0.97, 1.39)	1.24 (1.00, 1.54)	0.96 (0.67, 1.38)	0.99 (0.66, 1.47)	1.12 (0.95, 1.31)	1.16 (0.97, 1.39)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; OR, odds ratio; PCB, polychlorinated biphenyls; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene; SDQ, Strength and Difficulties Questionnaire.

[a] Crude and adjusted OR for the pooled cohort is adjusted for country.

[b] Adjusted for maternal age, maternal smoking during pregnancy, sex of the child, and age of the child at follow-up.

SUPPLEMENTAL MATERIAL

Table S1. Odds ratios (95% CI) for abnormal SDQ subscale scores associated with a doubling of postnatal PCB and DDE.

		Greenland		Ukraine		Pooled[a]	
		Crude OR	Adjusted OR[b]	Crude OR	Adjusted OR[b]	Crude OR	Adjusted OR[b]
Postnatal CB-153	Emotional	0.85 (0.71, 1.02)	0.87 (0.71, 1.07)	0.94 (0.73, 1.23)	0.95 (0.71, 1.29)	0.88 (0.76, 1.02)	0.91 (0.77, 1.07)
	Conduct	0.95 (0.81, 1.13)	1.00 (0.84, 1.20)	1.00 (0.81, 1.25)	0.94 (0.74, 1.19)	0.97 (0.85, 1.11)	0.98 (0.85, 1.13)
	Hyperactivity	0.85 (0.66, 1.09)	0.85 (0.64, 1.13)	0.76 (0.59, 0.99)	0.75 (0.57, 0.99)	0.81 (0.68, 0.97)	0.81 (0.67, 0.99)
	Peer	0.96 (0.84, 1.09)	1.01 (0.88, 1.17)	1.14 (0.95, 1.36)	1.15 (0.95, 1.40)	1.01 (0.91, 1.12)	1.05 (0.94, 1.18)
	Prosocial	1.10 (0.93, 1.30)	1.19 (0.98, 1.44)	0.92 (0.76, 1.12)	0.91 (0.74, 1.12)	1.02 (0.90, 1.16)	1.05 (0.92, 1.21)
Postnatal *p,p'*-DDE	Emotional	0.91 (0.76, 1.08)	0.93 (0.77, 1.13)	0.96 (0.73, 1.28)	0.97 (0.71, 1.33)	0.92 (0.80, 1.07)	0.94 (0.80, 1.10)
	Conduct	1.00 (0.85, 1.17)	1.04 (0.87, 1.23)	1.04 (0.83, 1.32)	0.97 (0.75, 1.24)	1.01 (0.88, 1.16)	1.00 (0.87, 1.16)
	Hyperactivity	0.89 (0.70, 1.13)	0.87 (0.67, 1.14)	0.85 (0.64, 1.12)	0.82 (0.61, 1.10)	0.87 (0.73, 1.04)	0.86 (0.71, 1.05)
	Peer	1.01 (0.89, 1.14)	1.06 (0.92, 1.22)	1.19 (0.98, 1.44)	1.20 (0.98, 1.47)	1.06 (0.96, 1.18)	1.09 (0.97, 1.22)
	Prosocial	1.17 (0.98, 1.38)	1.25 (1.03, 1.51)	0.95 (0.78, 1.17)	0.96 (0.77, 1.20)	1.08 (0.95, 1.23)	1.12 (0.97, 1.29)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; OR, odds ratio; PCB, polychlorinated biphenyls; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene; SDQ, Strength and Difficulties Questionnaire.

[a] Crude and adjusted OR for the pooled cohort is adjusted for country.

[b] Adjusted for maternal age, maternal smoking during pregnancy, sex of the child, and age of the child at follow-up.

Table S2. Test for effect modification by country and sex of the child.

	Difference in slope (β)	
	Boys versus girls[a]	Greenland versus Ukraine[b]
Prenatal CB-153	-0.07 (-0.43, 0.30)	0.27 (-0.22, 0.75)
Prenatal *p,p'*-DDE	-0.20 (-0.69, 0.29)	0.34 (-0.18, 0.87)
Estimated postnatal CB-153	0.02 (-0.29, 0.34)	0.23 (-0.11, 0.57)
Estimated postnatal *p,p'*-DDE	-0.04 (-0.40, 0.33)	0.18 (-0.16, 0.53)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene.

[a] Using girls as reference

[b] Using Greenland as reference

Table S3. Odds ratios (95% CI) for top 10 percentile cut-off of total SDQ scores associated with a doubling of prenatal and postnatal PCB and DDE

	Greenland				**Ukraine**				**Pooled**[a]			
	n	Crude OR	n	Adjusted OR[b]	n	Crude OR	n	Adjusted OR[b]	n	Crude OR	n	Adjusted OR[b]
Prenatal CB-153	516	1.04 (0.86 ; 1.27)	442	1.12 (0.90 ; 1.41)	492	0.96 (0.70 ; 1.31)	456	1.02 (0.72 ; 1.43)	1008	1.02 (0.86 ; 1.20)	898	1.10 (0.91 ; 1.32)
Prenatal *p,p'*-DDE	516	1.14 (0.93 ; 1.40)	442	1.20 (0.95 ; 1.52)	492	1.04 (0.72 ; 1.51)	456	1.06 (0.70 ; 1.58)	1008	1.12 (0.94 ; 1.34)	898	1.16 (0.95 ; 1.40)
Estimated postnatal CB-153	482	0.87 (0.73 ; 1.03)	417	0.93 (0.77 ; 1.14)	485	0.93 (0.76 ; 1.13)	450	0.94 (0.76 ; 1.16)	967	0.89 (0.78 ; 1.02)	867	0.94 (0.82 ; 1.09)
Estimated postnatal *p,p'*-DDE	482	0.94 (0.80 ; 1.11)	417	0.99 (0.81 ; 1.20)	485	0.96 (0.77 ; 1.18)	450	0.94 (0.76 ; 1.18)	967	0.95 (0.83 ; 1.08)	867	0.97 (0.84 ; 1.12)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; OR, odds ratio; PCB, polychlorinated biphenyls; *p,p'*-DDE, 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene; SDQ, Strength and Difficulties Questionnaire.

[a] Crude and adjusted OR for the pooled cohort is adjusted for country.

[b] Adjusted for maternal age, maternal smoking during pregnancy, sex of the child, and age of the child at follow-up.