Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet

Grandjean, Philippe; Weihe, Pál; Nielsen, Flemming; Heinzow, Birger; Debes, Frodi; Budtz-Jørgensen, Esben

Published in:
Neurotoxicology and Teratology

Publication date:
2012

Document version:
Submitted manuscript

Citation for pulished version (APA):
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. *Neurotoxicology and Teratology*, *34*(4), 466-72.

Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet

Philippe Grandjean[a,b*], Pal Weihe[c], Flemming Nielsen [a], Birger Heinzow[d,e], Frodi Debes[c], Esben Budtz-Jørgensen[f]

[a] Institute of Public Health, University of Southern Denmark, Odense, Denmark [b] Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

[c] Faroese Hospital System, FR-100 Tórshavn, Faroe Islands

[d] State Agency for Social Services of Schleswig-Holstein, Kiel, Germany

[e] University of Notre Dame, School of Medicine, Sydney, Australia

[f] Department of Biostatistics, University of Copenhagen, Copenhagen N, Denmark

*Corresponding author at: Philippe Grandjean, Institute of Public Health, University of Southern Denmark, Winslowparken 17, 5000 Odense, Denmark. Phone: +45-6550.3769. Fax: +45-6591.1458. Email: pgrand@health.sdu.dk

Abstract

To determine the possible neurotoxic impact of prenatal exposure to polychlorinated biphenyls (PCBs), we analyzed banked cord blood from a Faroese birth cohort for PCBs. The subjects were born in 1986-1987, and 917 cohort members had completed a series of neuropsychological tests at age 7 years. Major PCB congeners (118, 138, 153, and 180), the calculated total PCB concentration, and the PCB exposure estimated in a structural equation model showed weak associations with test deficits, with statistically significant negative associations only with the Boston Naming test. Likewise, neither hexachlorobenzene nor *p,p'*-dichlorodiphenyldichloroethylene showed clear links to neurobehavioral deficits. Thus, these associations were much weaker than those associated with the cord-blood mercury concentration, and adjustment for mercury substantially attenuated the regression coefficients for PCB exposure. When the outcomes were joined into motor and verbally mediated functions in a structural equation model, the PCB effects remained weak and virtually disappeared after adjustment for methylmercury exposure, while mercury remained statistically significant. Thus, in the presence of elevated methylmercury exposure, PCB neurotoxicity may be difficult to detect, and PCB exposure does not explain the methylmercury neurotoxicity previously reported in this cohort.

Keywords: Methylmercury compounds Neuropsychological tests Polychlorinated biphenyls Prenatal exposure delayed effects Preschool child

1. Introduction

Environmental exposures are likely to involve more than a single neurotoxicant at a time. Because a large number of chemicals are suspected of being toxic to the developing brain (Grandjean and Landrigan 2006), assessment of complex exposures is a prerequisite for neuroepidemiological research. We have conducted extensive studies of birth cohorts in the Faroe Islands, where we mainly focused on methylmercury (Grandjean et al., 1997; Murata et al., 1999). In the Faroes, increased exposure to this neurotoxicant originates from the traditional habit of eating pilot whales (Vestergaard and Zachariassen 1987), the meat of which contains high mercury concentrations (Julshamn et al., 1987). In addition, lipophilic pollutants, such as polychlorinated biphenyls (PCBs), accumulate in the whale blubber (Borrell and Aguilar 1993) and are thought to cause developmental neurotoxicity (European Food Safety Authority 2005). We have previously found little evidence that PCB exposure in the Faroes contributes to neurobehavioral deficits, which seem to be mainly due to methylmercury (Budtz-Jorgensen et al., 2010; Grandjean et al., 2001; Steuerwald et al., 2000), but these assessments relied on incomplete estimates of the total PCB concentration in umbilical cord tissue obtained from half of the birth cohort members. We have now completed measurements of major PCB congeners and related pollutants in banked blood sampled from the umbilical cord from almost all births within the cohort, and are therefore able to update the initial findings.

As statistical analysis of several analytes in regard to their possible effects on several neurobehavioral outcomes would be difficult to interpret from large numbers of regression analyses, we have used structural equation models to identify the relative

and joint effect of these potential neurotoxicants on neurodevelopment, while adjusting for methylmercury exposure. In addition, as experimental evidence has suggested a possible interaction between methylmercury and PCBs (Coccini et al., 2006; Fischer et al., 2008), we have also explored this possibility.

2. Materials and methods

2.1 Cohort establishment and exposure assessment

A birth cohort was generated in 1986-1987 at the three hospitals in the Faroe Islands (Grandjean et al., 1992). A questionnaire completed by the midwife contained summary information on maternal diet during pregnancy, while other relevant information was retrieved from the medical records.

In connection with each singleton birth, both cord blood and cord tissue were collected and frozen for subsequent analysis. Because of the limited volume of cord blood available and difficulties in analyzing these samples for PCBs at the time, cord specimens were initially used for PCB analyses. For validation purposes, 50 whole-blood samples from the cord were subsequently analyzed for PCBs by a method that required 4 mL of blood (Grandjean et al. 2001).

Using a more advanced analytical method, we have now measured the major PCB congeners and related substances in 0.5 mL whole blood available from almost all cohort members. Our analytical method is based on solid phase extraction (SPE) and gas chromatography routinely used for serum or plasma (Petersen et al., 2007); it was modified to allow analysis of whole blood. Thawed samples were vortex mixed for 15 seconds before transferring 500 µL whole blood to a conical test tube containing 300 µL

concentrated formic acid and 2300 µL Milli-Q water. The mixture was vortex mixed for 10 seconds and ultrasonicated for 5 minutes to precipitate proteins before centrifugation. The liquid was added onto an SPE cartridge (AccuBond II C18,Agilent Technologies, Palo Alto, CA) that had been preconditioned with 2.5 mL isooctane, 2.5 mL methanol and 2.5 mL Milli-Q water. After washing with Milli-Q water, elution was carried out with 2 x 2.5 mL isooctane into a tarred conical test tube. The solvent was evaporated at 30°C under a gentle stream of nitrogen until approximately 100 µL remained. The tube was weighed and the residue adjusted to a volume of 250 µL with isooctane. An aliquot of 4 µL was injected in splitless mode into an Agilent 6890 Plus Gas Chromatographic system (Agilent Technologies, Palo Alto, CA). The sample was then split between two separate narrow bore capillary columns each connected with two electron capture detectors. Analytical standards were from Dr. Ehrenstorfer GmbH, Germany. Isooctane and methanol were of HPLC grade and the formic acid was of analytical grade (Sigma-Aldrich, St. Louis, MO).The recovery of the PCBs and DDE averaged 87% (range 84–97 %); results were not adjusted for incomplete recovery. Spiked quality control samples were included in each series of samples. The laboratory participates regularly and successfully in the German Quality Assessment Programme (Q-EQUAS) for serum-PCB analyses coordinated by the University Erlangen-Nüremberg, Germany.

Results are reported in ng per mL whole blood. Lipid concentrations in cord serum are low and less variable than those in non-fasting adult serum. As lipid determination was not feasible for the material available, an average lipid concentration of 3 mg/mL may be used for converting the volume-based results into µg/g lipid. The

median limit of detection ranged from 0.08 to 0.16 ng/mL for the analytes, i.e., approximately 0.016-0.032 µg/g lipid. The repeatability (intra-day precision) and the reproducibility (inter-day variation assessed over four days) were between 6% and 16%.

The sum of the three major congeners (CB-138, CB-153, and CB-180) represents about 50% of the total PCB concentration in milk from the cohort mothers (Grandjean et al., 1995), and in maternal serum from a subsequent Faroese cohort (Steuerwald et al. 2000). The sum of these three congeners multiplied by 2.0 was therefore used as a surrogate for the total concentration of PCBs. CB-118, hexachlorobenzene (HCB) and *p,p'*-dichlorodiphenyldichloroethylene (DDE) were detectable in virtually all samples and were therefore included in the data analysis.

Prenatal exposure to methylmercury was determined through analysis of the total mercury concentration in cord blood by cold-vapor atomic absorption (Grandjean et al. 1992). Twenty-three subjects did not have a blood-mercury analysis. Sufficient volumes of banked cord blood from these subjects were available for analysis on a Direct Mercury Analyzer DMA-80 (Milestone, Sorisole, Italy), and complete methylmercury exposure data for the subjects examined at age 7 can now be presented.

Concurrent exposure to PCBs was assessed from serum obtained at the time of the examination, although this was only possible for the 101 cohort subjects with sufficient serum for the PCB analysis (Barr et al., 2006). Our previous report (Grandjean et al. 2001) took into account the methylmercury exposure at age 7 based on analysis of hair (Grandjean et al. 1997), but we now include also the results of the blood-mercury analyses for 694 cohort members at age 7 (Grandjean et al., 1999).

2.2 Neuropsychological tests

At age 7 years, 923 (90%) of the cohort members participated in a thorough clinical examination with a focus on nervous system function (Grandjean et al. 1997). The individual tests have been previously described (Grandjean et al. 2001) and are briefly outlined below.

In the Neurobehavioral Evaluation System (NES2) Finger Tapping Test (Dahl et al., 1996; Letz and Baker 1988), the scores were the maximum number of taps with the preferred hand, the non-preferred hand, and both hands. In the NES2 Hand-Eye Coordination Test (Dahl et al. 1996; Letz and Baker 1988), the score was the average deviation from the stimulus in the best two trials. The third computer-assisted test, the NES2 Continuous Performance Test (CPT) in a 4-minute attention test using a series of animal silhouettes flashed on the computer screen (Dahl et al. 1996; Letz and Baker 1988). The scores were the total number of missed responses and the average reaction time during the last three minutes. Only the supervised CPT data from the first year were used. These outcomes were considered motor tests.

The Bender Visual Motor Gestalt Test (Schlange et al., 1977) is a visuospatial test, where we scored the errors in the copying condition using the Göttingen system. In the recall condition, we summed the number of recognizable figures.

Among the verbally mediated tests, we used three Wechsler Intelligence Scale for Children-Revised (WISC-R) subtests (Wechsler 1974), the Digit Spans where the score was total number of correct trials in the forward condition; the Similarities, where we used the WISC-R raw scores; and the Block Designs, again using WISC-R criteria combining a basic score for producing a correct design with bonus points for quick

performance.

The California Verbal Learning Test (Children)(Delis et al., 1994) was translated into Faroese. We scored the total number of correct responses during the five learning trials, the spontaneous recall after an interference list (short recall), and the spontaneous recall of the initial twenty minutes later (long delay) and the number correctly recognized. In the Boston Naming Test (Kaplan et al., 1983), we scored the number of line drawings of objects correctly named, both spontaneously and after semantic and phonemic cueing.

2.3 Statistical analysis

Statistical analysis was carried out as previously described (Grandjean et al. 1997; Grandjean et al. 2001), but now with total blood-PCB concentration as an independent exposure variable after logarithmic transformation. We also examined individual PCB congeners 118, 138, 153, and 180, along with HCB and DDE as independent exposure indicators, one by one. After excluding seven children with neurological disease of other origin, multiple regression analyses were conducted with the neuropsychological test results as dependent variables and standard sets of covariates as independent predictors (Grandjean et al. 1997; Grandjean et al. 2001). In addition to age and sex as obligatory covariates for all outcomes, all neuropsychological tests were also adjusted for the maternal score on Raven's Progressive Matrices (Raven 1958), medical risk for neurobehavioral deficit, maternal and paternal education level, paternal employment, and day care, as previously described (Grandjean et al. 1997). Since the original analyses, a Raven score had been obtained from 30 additional mothers, so that this

parameter was missing only from 39 subjects. The computer-assisted tests were further adjusted for the child's acquaintance with computers and computer games. Because the Similarities test was administered by two different examiners, adjustment for examiner was included. Two-sided *p*-values were calculated throughout.

Two neuropsychological outcomes were transformed for the residuals to approach a Gaussian distribution, i.e., the logarithmic transformation of the number of missed responses on the CPT, and the square root of the WISC Block Designs score (Grandjean et al. 1997).

Because of the correlation between PCB and mercury exposure biomarkers, the possible confounding or effect modification by mercury exposure was investigated in regression analyses where a PCB effect was suggested. In addition to the confounders, these analyses included both the mercury and PCB exposure variables, along with a product term between the two exposure biomarkers (Budtz-Jorgensen et al., 1999).

We also used structural equation models (Bollen 1989; Skrondal and Rabe-Hesketh 2004) to determine the joint effects of the major PCBs on two broad neurobehavioral functions. In this factor analysis approach, the independent exposure variables are considered as contributors to the latent variable representing the true PCB exposure. A separate model was attempted, where both HCB and DDE were added as exposure indicators along with the PCB congeners. In the structural part of the model, the latent exposure variable is assumed to affect the latent motor or verbally-mediated functions, which are reflected in the individual test results. We have previously used this methodology (Budtz-Jorgensen et al., 2002; Budtz-Jorgensen et al. 2010; Debes et al., 2006), because information from multiple exposures and multiple outcomes can be

included simultaneously, thereby affording a greater statistical power, while avoiding the need for adjustment for multiple comparisons (Budtz-Jorgensen et al. 2002). The goodness of fit was determined by the Root Mean Square Error of Approximation (RMSEA) and a χ^2 test comparing the expected and the observed covariance (Kline 2011). Models were considered to have an excellent fit at *p* values above 0.05 for the χ^2 test and at upper confidence limits for the RMSEA below 0.05.

3. Results

The overall characteristics for the cohort are shown in Table 1. The log transformed concentrations of the more highly chlorinated PCB congeners: CB-118, CB-138, CB-153, and CB-180 showed close correlations (r above 0.70 for log transformed values). Less chlorinated congeners (CB-28, CB-52, and CB-101) showed many results below the detection limit; they were less clearly associated with the other PCBs (r below 0.26 for log transformed values) and were therefore not considered any further. Paired values for the total PCB concentration in cord whole blood and the wet-weight-based PCB concentration in cord tissue are shown in Figure 1. The whole-blood PCB concentrations covered a 300-fold range from a minimum of 0.05 ng/L to a maximum of 16.8 ng/L, with an interquartile range of 1.11-3.29 ng/L. Thus, the relative ranges were similar for mercury and PCB, and log transformed cord-blood concentrations of PCB and mercury correlated well, though not closely (r = 0.41). Concurrent exposures to PCB and methylmercury also correlated well with one another (r = 0.34), as well as with the respective prenatal levels (r = 0.45 and 0.38 for PCB and mercury).

Correlations of the total PCB concentration with covariates thought to be

associated with traditional diets were in agreement with expectations, e.g., for the number of whale blubber meals during pregnancy (r = 0.23). In regard to potentially important genetic, environmental and social variables (Dietrich and Bellinger 1994; Grandjean et al. 2001), few associations were identified. Thus, children of Faroese-born mothers (who are more likely to eat blubber) had a higher PCB exposure than those with non-Faroese (Danish) mothers. Maternal age and parity were also positively associated with PCB exposure. A lower PCB exposure was associated with longer education and a higher Raven score. As these findings were in accordance with previous results, adjustment of neuropsychological test results was carried out as already described (Grandjean et al. 1997; Grandjean et al. 2001).

Table 2 shows the regression coefficients for the logarithmic transformation of PCB concentrations, and Table 3 for chlorinated pesticide concentrations. The number of missed responses on the Continuous Performance Test and the two Boston Naming Test measures, in particular, showed decrements associated with increased organochlorine exposures. The addition of maternal age and parity as independent variables changed these results only to a negligible degree. Of note is that CB-118 and CB-153 for some tests were better risk indicators than other congeners, but no clear pattern was apparent, and the calculated total PCB concentration did not show any statistically significant associations with the outcomes. Interestingly, DDE was significantly associated with the continuous performance test and the naming test outcomes, while HCB showed only borderline evidence of neurotoxicity. Addition of these lipophilic contaminants to the latent exposure variable in the structural equation model resulted in only a slight improvement that could be ascribed to better precision of

the exposure estimate due to the availability of two additional exposure variables.

Mercury was associated with deficits on the neuropsychological test measures, several being statistically significant, and with greater effect estimates than those of the organochlorine compounds (Table 3). The mercury regression coefficients reported here are similar to the results for the total cohort previously published (Grandjean et al. 1997). None of the interaction parameters between PCB and methylmercury exposure was significant.

A structural equation model showed that PCB exposure, as modeled from CB-118, CB-138, CB-153, and CB-180, was significantly associated with deficits on the naming test (Table 4). The CB-153 concentration was the best predictor of the latent PCB exposure variable in the model. When adjustment for methylmercury exposure was included in the model for the naming results, mercury remained significant, while PCB exposure did not. The structural models showed excellent fit, with χ^2 test p-values above 0.05 and a upper confidence limits for the RMSEA below 0.05. The only exception was the model for WISC-Block design where the χ^2 test yielded a p-value of 0.01, while RMSEA indicated a good fit (upper confidence limit=0.04).

To examine the overall effect of the exposures on motor and cognitive outcomes, structural equation models were developed to ascertain the effects of PCBs and methylmercury both separately and after mutual adjustment. The results are shown in Table 5. The associations of total PCB with verbally-mediated and motor-related deficits were weak and virtually vanished after adjustment for mercury. The structural models, too, showed excellent fit, with $\chi 2$ test p-values of 0.059 (motor model) and 0.075 (verbal model) and RMSEA upper confidence limits of 0.025 (motor model) and 0.022

(verbal model).

The concurrent PCB concentration (N = 101) was associated with a decrease in the Block Design score (beta = -0.46; p = 0.19) and an increase in the Bender error score (beta = 2.6; p = 0.14). Although seemingly stronger than for prenatal PCB exposure, these tendencies were far from statistical significance. None of the other outcomes showed any clear association with the concurrent PCB exposure, and some were in the opposite direction. Concurrent methylmercury exposure was associated with several outcomes, though not significantly so after covariate adjustment and not as strongly as the prenatal exposure level; only weak tendencies were left after adjustment for the latter.

4. Discussion

The results in this study support our previous conclusion that, in the Faroese population, methylmercury neurotoxicity is a greater hazard than PCB (Grandjean et al. 1997; Grandjean et al. 2001; Steuerwald et al. 2000). Also, the results show that prenatal exposures to methylmercury constitute a greater hazard than lower levels of postnatal exposures at age 7 years. Although robust, these observations need to be evaluated in the light of the validity of the study parameters and the evidence on developmental neurotoxicity available from the Faroes and elsewhere.

Although we analyzed whole blood, rather than serum, the close correlation with concentrations in cord tissue support the validity of this measure. PCB concentrations in cord tissue, cord serum, maternal serum, and milk are also closely correlated (Needham et al., 2011). Thus, given the persistence of the major PCBs (Grandjean et

al., 2008), their concentrations in cord blood are likely to represent a reliable exposure measure of possible developmental neurotoxicity risks.

When evaluating dose-related outcomes, the degree of imprecision of the exposure estimates needs to be taken into account. On average, measurement imprecision and random exposure variability will tend to bias dose-response relationships toward the null. We have estimated the relative imprecision of methylmercury exposure biomarkers to be 25-50%, although the impact of the bias is somewhat dampened by the wide range of exposures in the Faroese birth cohort (Budtz-Jorgensen et al., 2004; Grandjean and Budtz-Jorgensen 2010). In regard to the PCB exposure biomarkers, we assume that the imprecision is less than that for methylmercury due to much longer elimination half-lives of the major PCB congeners (Grandjean et al. 2008). However, if the PCB concentrations considered do not reflect the neurotoxic components of the lipophilic contaminants, this conclusion may not hold. Among possible candidates, DDE is a pesticide metabolite that originates from other sources than PCB, but is also accumulated in whale blubber (Borrell and Aguilar 1993). However, the cord blood DDE concentration correlated closely with PCB and did not reveal any clear indication of neurotoxicity independent of PCB-associated deficits. The same was true for HCB. Inclusion of these substances in the structural equation model did not materially change the results. As the structural equation models contributed adjustment for exposure imprecision, the results do not support any substantial impact of the PCBs or related contaminants, whether measured or unmeasured, unless such exposures are not associated with the analytes determined.

In this population, the naming test appears to be the outcome parameter most

sensitive to PCB, but confounding due to methylmercury makes this finding somewhat ambiguous. The Boston Naming Test is a verbally mediated test that reflects semantic memory and lexicon development, and may differ from most widely used tests. Although applied verbal tasks require the use of language for completion, they may not necessarily reflect naming function, and the evidence available therefore does not allow any judgment concerning plausibility of this functional domain as a potential target of developmental PCB exposure (Boucher et al., 2009). Thus, the present study provides only weak support for PCB neurotoxicity in the presence of elevated methylmercury exposures. Whether this is due to joint effects being less than additive or insufficient statistical power cannot be determined from this study alone.

The structural equation model provides powerful support that the mercury-associated neurodevelopmental deficits seen in the Faroese birth cohort cannot be ascribed to concomitant PCB exposure. In this regard, the conclusion of the present study is in accordance with our previous findings (Budtz-Jorgensen et al. 2002; Grandjean et al. 2001). Also, no important effect modification by PCB exposure was found in regression analyses using a product term between the two exposure biomarkers, in agreement with a previous report (Budtz-Jorgensen et al. 1999). These results indicate that the mercury-associated effect is unlikely to be affected by PCB exposure to any great extent.

Only limited epidemiological support exists that postnatal exposure to PCBs adds to the risks associated with prenatal exposure (Winneke 2011). Due to the small number of serum samples from age 7 analyzed for PCBs, the present study has insufficient power to detect a neurobehavioral effect of the postnatal exposure. In

addition, postnatal neurotoxicity may be obscured by imprecise exposure assessment in regard to vulnerable developmental windows and by the confounding from benefits associated with breast-feeding, which is a main source of postnatal exposure. Likewise, the presence of essential nutrients in maternal seafood diets may cause negative confounding by impacting the outcomes in a direction opposite of the toxicants (Budtz-Jorgensen et al., 2007; Choi et al., 2008). Thus, given the complexity of environmental exposures, and the differences in exposure measures and covariate adjustments, uniform adverse effects of single contaminants should not be expected.

Apparent differences between epidemiological studies in this field (Goodman et al., 2010) should not be interpreted as an indication of disagreement that PCB is neurotoxic to humans (Boucher et al. 2009; European Food Safety Authority 2005). Each study has to be considered in the light of the study setting, the design and the methodology (Stewart et al., 2012). In the present study, the relatively increased exposure to methylmercury contributed a greater neurotoxic risk, and the association with PCB exposure prohibited any assessment of the likely contribution by the latter exposure to the neurobehavioral deficits. We have recently examined the possible interaction between methylmercury and lead (Yorifuji et al., 2011), and shown that the effects may be less than additive. Still, the most serious problem in interpreting research in this field may be that PCB is not a well-defined chemical, and that it may represent a family of persistent, lipophilic compounds, some of which may cause neurotoxicity through interference with thyroid function (Julvez et al., 2011), others possibly through a variety of other means (Hamers et al., 2011). The composition of PCB exposure in different settings and its association with dioxin-like substances, chlorinated pesticides

and other toxicants will vary, thereby introducing a substantial element of uncertainty. The PCB exposure biomarker therefore does not represent a uniform mixture of compounds, and comparison between epidemiological studies must be performed with caution.

Accordingly, the present study is based on a population exposed to a mixture of persistent organochlorine contaminants from seafood as well as to methylmercury. While most studies on developmental PCB exposure have not taken methylmercury exposure into account as a confounder or effect modifier, the present study provides evidence that such adjustment may be necessary. Although no clear-cut PCB-associated effects could be identified in this birth cohort at early school age, this finding does not mean that organochlorine contaminant exposure should be regarded as being innocuous. Subtle effects may be difficult to detect given the impact of concomitant methylmercury exposures.

5. Conclusions

The present report examines the potential developmental neurotoxicity associated with prenatal exposure to PCBs based on comprehensive data on neurotoxicant exposures in a Faroese birth cohort born in 1986-1987. Several measures of PCB exposure, including a structural equation model, showed only weak associations with test deficits at age 7 years. Adjustment for methylmercury exposure substantially weakened the PCB effects. Although a statistically significant PCB effect was observed for verbal test deficits in the structural equation model, it virtually disappeared after adjustment for methylmercury exposure, while the latter remained statistically significant. Thus, in the

presence of elevated methylmercury exposure, PCB neurotoxicity may be difficult to document, and exposure to PCBs and related lipophilic compounds does not explain or confound the methylmercury neurotoxicity previously reported in this cohort.

Conflict of interest statement

The authors have no competing interests to declare.

Acknowledgments

This research was supported by the U.S. National Institute of Environmental Health Sciences (ES09797). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH or any other funding agency.

References

Barr DB, Weihe P, Davis MD, Needham LL, Grandjean P. Serum polychlorinated biphenyl and organochlorine insecticide concentrations in a Faroese birth cohort. Chemosphere 2006;62:1167-82.
Bollen KA. Structural equations with latent variables. New York: John Wiley; 1989.
Borrell A, Aguilar A. 1993. Pollution by DDT and PCB in blubber and muscle of long-finned pilot whales from the Faroe Islands. In: Biology of northern hemisphere pilot whales (Donovan GP, Lockyer CH, Martin AR, eds). Cambridge: International Whaling Commission, 351-67.
Boucher O, Muckle G, Bastien CH. Prenatal exposure to polychlorinated biphenyls: a neuropsychologic analysis. Environ Health Perspect 2009;117:7-16.
Budtz-Jorgensen E, Keiding N, Grandjean P, White RF. Methylmercury neurotoxicity independent of PCB exposure. Environ Health Perspect 1999;107:A236-A7.
Budtz-Jorgensen E, Keiding N, Grandjean P, Weihe P. Estimation of health effects of prenatal methylmercury exposure using structural equation models. Environmental health : a global access science source 2002;1:2.
Budtz-Jorgensen E, Keiding N, Grandjean P. Effects of exposure imprecision on estimation of the benchmark dose. Risk Anal 2004;24:1689-96.
Budtz-Jorgensen E, Grandjean P, Weihe P. Separation of risks and benefits of seafood intake. Environ Health Perspect 2007;115:323-7.
Budtz-Jorgensen E, Debes F, Weihe P, Grandjean P. Structural equation models for meta-analysis in environmental risk assessment. Environmetrics 2010;21:510-27.
Choi AL, Cordier S, Weihe P, Grandjean P. Negative confounding in the evaluation of toxicity: the case of methylmercury in fish and seafood. Crit Rev Toxicol 2008;38:877-93.
Coccini T, Randine G, Castoldi AF, Grandjean P, Ostendorp G, Heinzow B, et al. Effects of developmental co-exposure to methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) on cholinergic muscarinic receptors in rat brain. Neurotoxicology 2006;27:468-77.
Dahl R, White RF, Weihe P, Sorensen N, Letz R, Hudnell HK, et al. Feasibility and validity of three computer-assisted neurobehavioral tests in 7-year-old children. Neurotoxicol Teratol 1996;18:413-9.
Debes F, Budtz-Jorgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. Neurotoxicol Teratol 2006;28:536-47.
Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test - Children's Version (CVLT-C). San Antonio: Psychological Corporation; 1994.
Dietrich KN, Bellinger D. 1994. The assessment of neurobehavioal development in studies of the effects of prenatal exposure to toxicants. In: Prenatal exposure to toxicants : developmental consequences (Needleman HL, Bellinger D, eds). Baltimore: Johns Hopkins University Press, 57-85.
European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain on a request from the Commission related to the presence of non-dioxin-like polychlorinated biphenyls (PCB) in feed and food. The EFSA Journal 2005;284:1 - 137.
Fischer C, Fredriksson A, Eriksson P. Neonatal co-exposure to low doses of an ortho-

PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 2008;244:157-65.
Goodman M, Squibb K, Youngstrom E, Anthony LG, Kenworthy L, Lipkin PH, et al. Using systematic reviews and meta-analyses to support regulatory decision making for neurotoxicants: lessons learned from a case study of PCBs. Environ Health Perspect 2010;118:727-34.
Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, Videro T. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Archives of Environmental Health 1992;47:185-95.
Grandjean P, Weihe P, Needham LL, Burse VW, Patterson DG, Jr., Sampson EJ, et al. Relation of a seafood diet to mercury, selenium, arsenic, and polychlorinated biphenyl and other organochlorine concentrations in human milk. Environ Res 1995;71:29-38.
Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 1997;19:417-28.
Grandjean P, Budtz-Jorgensen E, White RF, Jorgensen PJ, Weihe P, Debes F, et al. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. American Journal of Epidemiology 1999;150:301-5.
Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, et al. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 2001;23:305-17.
Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet 2006;368:2167-78.
Grandjean P, Budtz-Jorgensen E, Barr DB, Needham LL, Weihe P, Heinzow B. Elimination half-lives of polychlorinated biphenyl congeners in children. Environ Sci Technol 2008;42:6991-6.
Grandjean P, Budtz-Jorgensen E. An ignored risk factor in toxicology: The total imprecision of exposure assessment. Pure Appl Chem 2010;82:383-91.
Hamers T, Kamstra JH, Cenijn PH, Pencikova K, Palkova L, Simeckova P, et al. In vitro toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl congeners and their relative toxic contribution to PCB mixtures in humans. Toxicol Sci 2011;121:88-100.
Julshamn K, Andersen A, Ringdal O, Morkore J. Trace elements intake in the Faroe Islands. I. Element levels in edible parts of pilot whales (Globicephalus meleanus). Sci Total Environ 1987;65:53-62.
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.
Kaplan E, Goodglass H, Weintraub S. The Boston Naming Test. 2nd ed. Philadelphia: Lea & Febiger; 1983.
Kline RB. Principles and practice of structural equation modeling. 3rd ed. New York: Guilford Press; 2011.
Letz R, Baker EL. NES2, neurobehavioral evaluation system manual. 4th ed. Winchester, MA: Neurobehavioral Systems, Inc.; 1988.
Murata K, Weihe P, Araki S, Budtz-Jorgensen E, Grandjean P. Evoked potentials in Faroese children prenatally exposed to methylmercury. Neurotoxicol Teratol 1999;21:471-2.

Needham LL, Grandjean P, Heinzow B, Jorgensen PJ, Nielsen F, Patterson DG, Jr., et al. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 2011;45:1121-6.
Petersen MS, Halling J, Damkier P, Nielsen F, Grandjean P, Weihe P, et al. Polychlorinated biphenyl (PCB) induction of CYP3A4 enzyme activity in healthy Faroese adults. Toxicology and applied pharmacology 2007;224:202-6.
Raven J. Standard progressive matrices. London: H. K. Lewis; 1958.
Schlange H, Stein B, von Boetticher I, Taneli S. Göttinger Formreproduktions-Test. Göttingen: Verlag für Psychologie; 1977.
Skrondal A, Rabe-Hesketh S. Generalized latent variable modeling : multilevel, longitudinal, and structural equation models. Boca Raton: Chapman & Hall/CRC; 2004.
Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, et al. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J Pediatr 2000;136:599-605.
Stewart PW, Reihman J, Lonky E, Pagano J. Issues in the interpretation of associations of PCBs and IQ. Neurotoxicology and teratology 2012;34:96-107.
Vestergaard T, Zachariassen P. Dietary survey 1981–82, in Faroese. Fróðskaparrit 1987;33:5-18.
Wechsler D. Wechsler intelligence scale for children revised. New York: Psychological Corp.; 1974.
Winneke G. Developmental aspects of environmental neurotoxicology: Lessons from lead and polychlorinated biphenyls. J Neurol Sci 2011;308:9-15.
Yorifuji T, Debes F, Weihe P, Grandjean P. Prenatal exposure to lead and cognitive deficit in 7- and 14-year-old children in the presence of concomitant exposure to similar molar concentration of methylmercury. Neurotoxicol Teratol 2011;33:205-11.

Table 1
Major characteristics of birth cohort members in regard to prenatal PCB exposure.

Parameter	Mean ± SD
Maternal age (yrs)	27.4 ± 5.4
Parity (none/one/at least two in %)	33.2 / 35.3 / 31.5
Smoking (no/yes in %)	60.3 / 39.7
Alcohol consumption during pregnancy (never/ever in %)	76.1 / 23.9
Whale meat dinners per month (none/one/two/more in %)	19.5 / 28.4 / 24.6 / 27.5
Fish dinners per week (none or one/two/three/more in %)	15.2 / 36.0 / 29.1 / 19.7
Birth weight (g)	3677 ± 536
Cord-blood PCB (µg/L)*	1.86 (1.16-3.16)
Cord-blood mercury (µg/L)*	23.0 (13.6-42.2)
Age at examination (yrs)	6.86 ± 0.32
Age-7 serum-PCB (µg/g lipid)*	1.71 (1.06-2.64)
Age-7 blood-mercury (µg/L)*	8.36 (4.38-18.0)

*Geometric mean with interquartile range in parenthesis.

Table 2

Regression coefficients (Betas) for the logarithmic transformation of cord blood concentrations of PCB congeners (µg/L) Betas indicate the change for a 10-fold increase in exposure.*

		CB-118		CB-138		CB-153		CB180		Sum-PCB	
Outcome	N	Beta	p	Beta	P	Beta	p	Beta	p	Beta	p
NES2 Finger Tapping											
Preferred hand	854	-0.512	0.258	-0.240	0.637	-0.648	0.230	-0.147	0.760	-0.481	0.394
Non-preferred hand	854	-0.179	0.682	-0.179	0.710	-0.478	0.357	-0.018	0.970	-0.332	0.539
Both hands	849	-1.290	0.157	-1.140	0.262	-1.102	0.310	-0.339	0.726	-0.960	0.396
NES2 Hand-Eye Coordination											
Error score	851	0.005	0.826	0.037	0.109	0.037	0.135	0.030	0.185	0.041	0.117
NES2 Continuous Performance Test											
Reaction time (s)	401	7.620	0.329	9.413	0.242	13.313	0.134	10.271	0.207	14.083	0.135
Missed responses (ln)	404	0.202	0.015	0.040	0.641	0.174	0.066	0.106	0.224	0.149	0.138
Wechsler Intelligence Scale for Children-Revised											
Digit span forward	847	-0.013	0.910	0.053	0.667	0.023	0.864	0.086	0.469	0.033	0.810
Similarities	708	0.153	0.627	0.414	0.257	0.203	0.591	0.133	0.691	0.358	0.363
Block design	846	-0.018	0.833	-0.209	0.025	-0.110	0.279	-0.012	0.895	-0.113	0.282
Bender Visual Motor Gestalt Test											
Error score	853	0.139	0.721	0.746	0.081	0.604	0.191	0.345	0.401	0.590	0.220
Reproduction	801	-0.197	0.119	-0.235	0.093	-0.248	0.101	-0.087	0.519	-0.230	0.145
Boston Naming Test											
Score without cues	824	-0.721	0.0743	-0.669	0.133	-0.787	0.104	-0.747	0.081	-0.872	0.083
Score with cues	823	-0.995	0.0125	-0.586	0.183	-0.981	0.040	-0.814	0.054	-0.964	0.052
California Verbal Learning Test - Children											
Learning	839	0.980	0.148	0.961	0.193	1.187	0.140	0.801	0.261	1.293	0.122
Short-term recall	827	0.035	0.860	0.120	0.585	0.188	0.429	0.077	0.717	0.176	0.477
Long-term recall	799	-0.050	0.824	-0.225	0.366	-0.164	0.544	-0.191	0.429	-0.214	0.447
Recognition	792	0.032	0.844	-0.020	0.913	-0.053	0.785	-0.016	0.926	-0.031	0.878

*Regression coefficients are adjusted for confounders, but not for methylmercury exposure

Table 3
Regression coefficients (Betas) for the logarithmic transformation of cord blood concentrations of chlorinated pesticides (µg/L). Betas indicate the change for a 10-fold increase in exposure.*

		DDE		HCB		Mercury	
Outcome	N	Beta	p	Beta	p	Beta	p
NES2 Finger Tapping							
Preferred hand	854	-0.455	0.330	-0.365	0.560	-1.147	0.035
Non-preferred hand	854	-0.596	0.183	-0.933	0.120	-0.511	0.328
Both hands	849	-0.977	0.298	-1.581	0.211	-1.721	0.117
NES2 Hand-Eye Coordination							
Error score	851	0.028	0.189	-0.005	0.860	0.031	0.212
NES2 Continuous Performance Test							
Reaction time (s)	401	16.886	0.036	20.729	0.104	44.375	<0.001
Missed responses (ln)	404	0.221	0.010	0.192	0.154	0.297	0.001
Wechsler Intelligence Scale for Children-Revised							
Digit span forward	847	-0.106	0.358	-0.051	0.738	-0.222	0.101
Similarities	708	0.252	0.438	0.975	*0.027*	0.077	0.839
Block design	846	-0.140	0.109	-0.056	0.634	-0.123	0.228
Bender Visual Motor Gestalt Test							
Error score	853	0.499	0.213	0.212	0.691	0.587	0.201
Reproduction	801	-0.158	0.229	-0.255	0.141	-0.252	0.095
Boston Naming Test							
Score without cues	824	-0.945	0.023	-0.868	0.119	-1.658	<0.001
Score with cues	823	-1.040	0.011	-1.043	0.058	-1.809	<0.001
California Verbal Learning Test - Children							
Learning	839	1.113	0.109	0.056	0.952	-0.816	0.314
Short-term recall	827	0.176	0.390	0.356	0.193	-0.488	0.042
Long-term recall	799	-0.123	0.597	0.070	0.824	-0.461	0.089
Recognition	792	-0.114	0.498	0.200	0.377	-0.225	0.254

*Regression coefficients are adjusted for confounders, but not for other exposures

Table 4
Regression coefficients (Betas) for the joint effects of logarithmic transformations of major PCB congeners after adjustment for covariates in a structural equation model.

Outcome	Beta	95% CI		P
NES2 Finger Tapping				
Preferred hand	-0.310	-1.411	0.791	0.58
Non-preferred hand	-0.190	-1.247	0.867	0.72
Both hands	-1.144	-3.359	1.072	0.31
NES2 Hand-Eye Coordination				
Error score	0.043	-0.009	0.094	0.10
NES2 Continuous Performance Test				
Reaction time (s)	12.49	-5.47	30.44	0.17
Missed responses	0.166	-0.030	0.363	0.097
Wechsler Intelligence Scale for Children-Revised				
Digit span forward	0.098	-0.175	0.371	0.48
Similarities	0.296	-0.477	1.068	0.45
Block designs	-0.124	-0.331	0.083	0.24
Bender Visual Motor Gestalt Test				
Error score	0.593	-0.362	1.549	0.22
Reproduction	-0.190	-0.498	0.119	0.23
Boston Naming Test				
Score without cues	-1.100	-2.098	-0.103	*0.031*
Score with cues	-1.185	-2.170	-0.201	*0.018*
California Verbal Learning Test-Children				
Learning	0.773	-0.891	2.437	0.36
Short-term recall	0.157	-0.336	0.649	0.53
Long-term recall	-0.183	-0.738	0.373	0.52
Recognition	-0.066	-0.481	0.348	0.75

Table 5
Regression coefficients (Betas) for the joint effects of logarithmic transformations of major PCB congeners, and mercury exposure before and after mutual adjustment in a structural equation model with confounder adjustment

Outcome	PCB				Mercury			
	Beta	95% CI		p	Beta	95% CI		p
	Separate analysis							
Verbal	-0.609	-1.550	0.332	0.20	-1.762	-2.766	-0.758	<0.001
Motor	-0.431	-1.310	0.448	0.34	-1.130	-2.079	-0.181	0.020
	Mutual adjustment							
Verbal	0.181	-0.909	1.271	0.74	-1.895	-3.094	-0.696	0.002
Motor	0.097	-0.934	1.128	0.85	-1.252	-2.415	-0.089	0.035

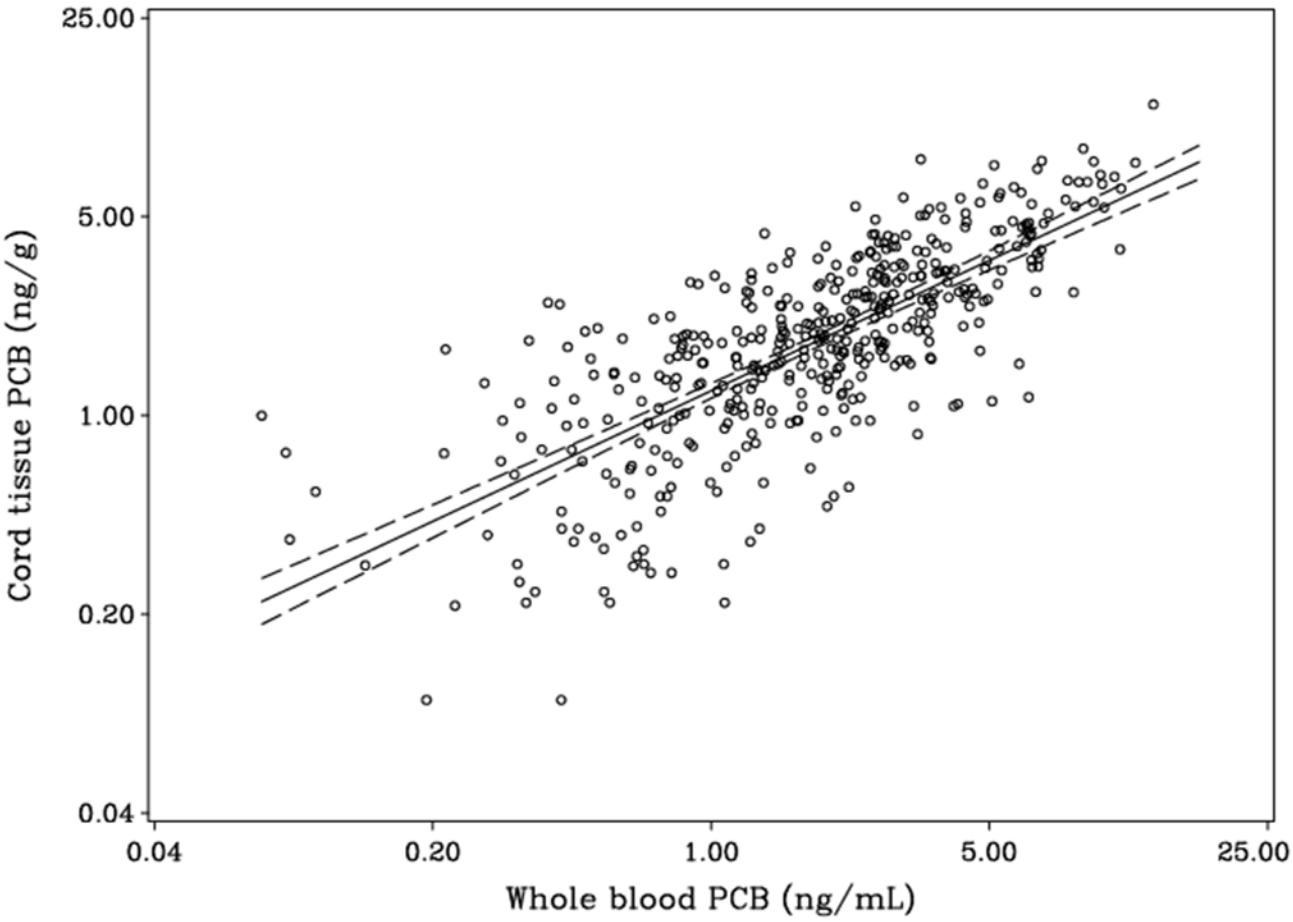

Figure 1. Association between total PCB concentrations in cord tissue (ng/g wet weight) and in whole blood from the cord (ng/mL) from 432 Faroese births.