RESEARCH REPORT

Association of serum concentrations of persistent organic pollutants with the prevalence of learning disability and attention deficit disorder

Duk-Hee Lee, David R Jacobs, Miquel Porta

Objective: Even though persistent organic pollutants (POPs) are well-known neurotoxicants, there is no previous study, even cross-sectional, on the association between background exposure to POPs and clinically significant developmental disorders, such as learning disability (LD) or attention deficit disorder (ADD), among children from a general population.

Design: Cross-sectional study.

Setting: Study subjects were 278 children aged 12–15 years included in the National Health and Nutrition Examination Survey 1999–2000. The seven most commonly detected POPs (each detectable in ≥20% of children: 3,3',4,4',5-pentachlorobiphenyl; 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HPCDD); 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD); 1,2,3,4,6,7,8-heptachlorodibenzofuran (HPCDF); β-hexachlorocyclohexane; p,p'-dichlorodiphenyltrichloroethane; and trans-nonachlor) were selected.

Main results: Compared with children with non-detectable levels of POPs, adjusted prevalence ORs (95% CIs) of LD among those with detectable levels of HPCDD, OCDD or HPCDF were 2.08 (1.17 to 3.68), 2.72 (1.24 to 5.99) and 2.18 (1.15 to 4.15), respectively. For ADD, the corresponding figures were 3.41 (1.08 to 10.8), 3.33 (0.94 to 11.8) and 2.31 (0.62 to 8.63), respectively.

Conclusions: Associations were observed between serum concentrations of POPs belonging to the categories of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and the prevalence of two clinically significant development problems, LD and ADD. The nature of these associations needs to be clarified by prospective studies.

Among several neurotoxic chemicals measured in NHANES,4 those with long half lives, including persistent organic pollutants (POPs), lead and cadmium, were our target environmental neurotoxicants.5,6 In the case of neurotoxicants with short half lives, current increased levels primarily reflect an increased exposure after developing LD or ADD; thus we did not consider them in our analyses.

POPs include hundreds of different organic chemical compounds with common properties such as long-term persistence, widespread diffusion in the environment and bioaccumulation in fatty tissues of living organisms.7–9 A growing body of scientific evidence associates human exposure to POPs with various health outcomes, including neurodevelopmental impairment through the disruption of the endocrine system.10–12 Even though the most problematic POPs were banned in many developed countries several decades ago, POP residues continue to be commonly found in animal feed and in the fat of fish, meat and dairy products, on a global scale. Humans are mainly exposed to POPs through fatty foods.5,11,12

Serum concentrations of lead and cadmium were measured in participants aged ≥1 year, but POPs were measured only among those aged ≥12 years. Thus, the associations of blood lead or cadmium with the prevalence of LD or ADD were not considered.

Abbreviations: ADD, attention deficit disorder; AhR, Ah receptor; DDE, p,p'-dichlorodiphenyltrichloroethane; HCH, β-hexachlorocyclohexane; HPCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin; HPCDF, 1,2,3,4,6,7,8-heptachlorodibenzofuran; LD, learning disability; LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey; OCDD, 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin; PCB, pentachlorobiphenyl; POP, persistent organic pollutant; TEF, toxic equivalency factor; TNA, trans-nonachlor

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studied among children aged 4–15 years, whereas those of POPs were analysed among children aged 12–15 years.

METHODS
Study population
The 1999–2000 NHANES (public-use dataset, http://www.cdc.gov/nchs/about/major/nhanes/datalink.htm) conducted by the Centers for Disease Control and Prevention was designed to be nationally representative of the non-institutionalised, US civilian population on the basis of a complex, multistage probability sample.\(^\text{11}\)

Measurement
Details of the NHANES protocol and procedures are available elsewhere.\(^\text{11}\) The NHANES standardised home interview was followed by a detailed physical examination in a mobile evaluation clinic or at the participant’s home. A child was considered to have LD if the child or their parents reported that a representative from a school or a health professional ever said that the child had a LD. The child was considered to have ADD if there was a self-report that a doctor or health professional ever said that the child had ADD.

Venous blood and urine samples were collected and shipped every week at 20°C. Blood lead or cadmium levels were determined using graphite furnace atomic absorption spectrophotometry. A total of 53 biologically important POPs were measured as individual chemicals by high-resolution gas chromatography/high-resolution mass spectrometry using isotope dilution for quantification. All analytes were measured in approximately 5 ml of serum using a modified method of Turner et al.\(^\text{2}\) The POPs as provided by NHANES were adjusted for serum total cholesterol and triglycerides.\(^\text{23}\) It should be noted that these analyses have a limit of detection (LOD) partly related to the serum volume available for measuring POPs, which was slightly different depending on how much blood each subject provided. Only the maximum observed LOD for each POP was provided by NHANES. A higher sample volume results in a lower LOD and a better ability to detect lower concentrations of POPs.

We did not study all 53 POPs because most children had serum concentrations of many POPs below the LOD. We therefore selected seven compounds for which at least 20% of study subjects had concentrations above the LOD: 3,3',4,4',5'-pentachlorobiphenyl (PCB126); 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HPCDD); 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD); 1,2,3,4,6,7,8,9-heptachlorodibenzofuran (HPCDF); β-hexachlorocyclohexane (HCH); p,p'-dichlorodiphenyltrichloroethane (DDE); and trans-nonachlor (TNA).

Final samples
Among the 2735 children aged 4–15 years, 2252 had information on lead blood and among the 1251 children aged 12–15 years, 279 had information on all seven POPs because POPs were measured. In the larger sample of 2246 children in whom lead and cadmium were also measured, the adjusted ORs for blood lead (highest vs lowest quintiles) were 0.86, 0.88, 1.09 and 1.25 for LD (p value for trend = 0.12) and 1.04, 0.77, 1.12 and 0.87 for ADD (p value for trend = 0.80). The corresponding figures for blood cadmium were 0.82, 1.02 and 1.02 for LD (p value for trend = 0.96) and 1.13, 1.27 and 1.44 for ADD (p value for trend = 0.27), respectively; this non-significant positive trend disappeared after additional adjustment for POPs.

Among the seven POPs, HPCDD, OCDD and HPCDF showed significant positive associations with the prevalence of LD (table 2). After adjustment for age, race/ethnicity, sex, poverty index ratio, mother’s age at pregnancy, birth weight, body mass index and saturated fat intake, compared with children with non-detectable levels of POPs, ORs (95% CIs) among those with detectable levels of HPCDD, OCDD and HPCDF were 2.08 (1.17 to 3.68), 2.72 (1.24 to 5.99) and 2.18 (1.15 to 4.15), respectively. In contrast, PCB126, DDE and TNA were not associated with LD; HCH was inversely, but not significantly, associated with LD: the adjusted OR (95% CI) was 0.37 (0.13 to 1.02). Additional adjustment for blood lead and cadmium did not materially change the results.

The associations of POPs with ADD were similar to those with LD, although most POPs did not reach statistical significance (table 3); adjusted ORs (95% CIs) for HPCDD, OCDD and HPCDF were 3.41 (1.08 to 10.8), 3.33 (0.94 to 11.8) and 2.31 (0.62 to 8.63), respectively. Similar to LD, the association with HCH was inverse, but not close to statistical significance.

RESULTS
Table 1 shows the distribution of potential confounding variables by LD and ADD among the 278 children. Children with LD or ADD were more likely to be white, with young mothers, who were more likely to have smoked during pregnancy.

Neither blood lead nor cadmium concentrations were associated with LD or ADD in the 278 children in whom POPs were measured. In the larger sample of 2246 children in whom lead and cadmium were also measured, the adjusted ORs for blood lead (highest vs lowest quintiles) were 0.86, 0.88, 1.09 and 1.25 for LD (p value for trend = 0.12) and 1.04, 0.77, 1.12 and 0.87 for ADD (p value for trend = 0.80). The corresponding figures for blood cadmium were 0.82, 1.02 and 1.02 for LD (p value for trend = 0.96) and 1.13, 1.27 and 1.44 for ADD (p value for trend = 0.27), respectively; this non-significant positive trend disappeared after additional adjustment for POPs.

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DISCUSSION
A number of prospective studies\(^\text{10–21}\) have reported that prenatal exposure to background POPs could impair intellectual and/or behavioural development among infants and toddlers; however, findings have not been totally consistent whether these effects persist among children. Some studies reported a harmful effect of prenatal exposure to POPs on cognitive and motor abilities even among school-aged children,\(^\text{22–24}\) but other studies reported that the detrimental effects of POPs are only transient
or observed only among children raised in suboptimal environments. Moreover, whether this exposure is associated with clinically significant developmental disorders such as LD or ADD, rather than with continuous scores of intellectual or behavioural functions among school-aged children has never been studied. Slightly decreased scores of cognitive function do not necessarily mean the presence of clinically significant developmental disorders.

In this study, serum concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were positively associated with the prevalence of LD or ADD among children aged 12–15 years. Our findings are basically consistent with those of prospective cohort studies, which showed decreased scores of intellectual or behavioural functions after low-level exposures to some POPs during pregnancy or early infancy. These findings are also biologically 

<table>
<thead>
<tr>
<th>Table 1 Distribution of child- and family-related potential confounding factors according to the presence of learning disability or attention deficit disorder</th>
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<tbody>
<tr>
<td>Learning disability (n = 234)</td>
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<tr>
<td>-----------------------------------------------</td>
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<tr>
<td><strong>Children’s variables</strong></td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Male (%)</td>
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<tr>
<td>White (%)</td>
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<tr>
<td>Birth weight (g)</td>
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<tr>
<td>Body mass index</td>
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<tr>
<td>Saturated fat intake (g)</td>
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<tr>
<td>Blood lead (µg/dl)</td>
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<td><strong>Family and maternal variables</strong></td>
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<td>Poverty:income ratio</td>
</tr>
<tr>
<td>Mother’s age at pregnancy (years)</td>
</tr>
<tr>
<td>Cigarette smoking during pregnancy (%)</td>
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</tbody>
</table>

Values are given as mean (SD) or percentage.

| Table 2 Adjusted prevalence OR and 95% CI of learning disability by category of seven persistent organic pollutants |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| POPs | Detection rate (%) | Non-detectable POPs | Detectable POPs | p Value |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 3,3',4,4',5-penta chlorobiphenyl | 22.4 | Concentration (ng/g of lipid) | 33/208 | 9.1 (6.7–20.6) | 1.26 (0.47 to 3.32) | 0.62 |
| 1,2,3,4,6,7,8-hepta chlorodibenzo-p-dioxin | 30.5 | Concentration (pg/g of lipid) | 24/191 | 38.9 (30.2–50.9) | 20/86 | 0.02 |
| 1,2,3,4,6,7,8,9-octa chlorodibenzo-p-dioxin | 26.8 | Concentration (pg/g of lipid) | 24/199 | 23.0 (18.5–30.2) | 25/78 | 0.02 |
| 1,2,3,4,6,7,8-hepta chlorodibenzofuran | 31.9 | Concentration (pg/g of lipid) | 23/189 | 10.0 (7.8–14.8) | 21/88 | 0.02 |
| j-hexachlorocyclo-hexane | 22.4 | Concentration (ng/g of lipid) | 38/201 | 17.9 (14.6–26.4) | 21/77 | 0.05 |
| Trans-nonachlor | 25.4 | Concentration (ng/g of lipid) | 27/207 | 10.0 (8.4–16.0) | 17/70 | 0.41 |
| p,p’-dichlorodiphenyl trichloroethane | 100 | Concentration (ng/g of lipid) | 75.3 (49.0–98.4) | 278 (186–528) | 0.37 (0.13 to 1.02) | 0.05 |

POP, persistent organic pollutant.
*Adjustment for age, race/ethnicity, sex, poverty index ratio, mother’s age at pregnancy, cigarette smoking during pregnancy, birth weight, body mass index and saturated fat intake.
†Median (interquartile range).
products (e.g., meat, milk, eggs and fish), a positive association
Taken together, we think that neither high body mass index nor
were positively associated with the prevalence of LD or ADD.
only PCDDs and PCDFs, not PCBs or organochlorine pesticides,
adipose tissue would have been positively associated, whereas
between POPs and LD or ADD, all POPs contained in the
weight loss were a problem in analysing the association
However, if the release of POPs from adipose tissue due to
was no information on changes in body weight among children,
index was not associated with the presence of LD or ADD and
reverse causality. However, in this study population, body mass
or ADD, the positive cross-sectional association may be due to
Thus, if changes in body weight occur after development of LD
plausible because POPs are well-known neurotoxicants, even at
low doses. It may seem surprising that there has been no previous study, even cross-sectional, with the outcome of clinically significant developmental disorders. The absence of such studies probably reflects the practical difficulty that measurements of serum concentrations of POPs require such amounts of serum which are large for children.

Cross-sectional findings may reflect reverse causality. Thus, it is important to evaluate whether serum concentrations of POPs can increase after development of LD or ADD. Children with low body mass index or weight loss may experience an increase in serum concentrations of POPs due to bioconcentration. Thus, if changes in body weight occur after development of LD or ADD, the positive cross-sectional association may be due to reverse causality. However, in this study population, body mass index was not associated with the presence of LD or ADD and body mass index was adjusted for in the final analyses. As there was no information on changes in body weight among children, it was impossible to directly evaluate the effect of weight loss. However, if the release of POPs from adipose tissue due to weight loss were a problem in analysing the association between POPs and LD or ADD, all POPs contained in the adipose tissue would have been positively associated, whereas only PCDDs and PCDFs, not PCBs or organochlorine pesticides, were positively associated with the prevalence of LD or ADD. Taken together, we think that neither high body mass index nor weight loss explain our findings. As POPs in the general population, except breastfeeding infants, come from animal products (e.g., meat, milk, eggs and fish), a positive association might also be observed if intake of animal products increased sharply after development of LD or ADD. However, the current intake of saturated fat (used as a marker of intake of animal products) was not different between children with LD or ADD and those without either condition. This suggests that the exposure to POPs did not increase due to more intake of animal products after development of LD or ADD, thereby further reducing the possibility of reverse causality. In addition, some studies have reported that serum POPs levels were most strongly determined by breast feeding even in school-aged children, not by their diet.

As serum concentrations of some POPs have been decreasing over several decades in the US general population, the current increase in LD or ADD may seem to be contradictory. However, similar to our earlier observation concerning the influence of obesity and POPs on the risk of type 2 diabetes, other factors that have recently increased may interact with POPs on the risk of LD or ADD. Alternatively, other POPs which were not measured in NHANES dataset, like the brominated flame retardants, perfluorinated compounds or chlorinated paraffins, which are increasingly used in the US and also show neurotoxicity in experimental studies, may be important and correlated with POPs measured in NHANES.

Most POPs were non-detectable among the children included in this study, and even common POPs were detected only in about 20% of children. We suspect that this limited detection is primarily due to limited amounts of serum used for POPs measurement. Most available evidence indicates that POPs would have been detectable in almost all children if sufficient

**Table 3** Adjusted** prevalence OR and 95% CI of attention deficit disorder by category of seven persistent organic pollutants

<table>
<thead>
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<td>9.1 (6.7–20.6)</td>
<td>6/69</td>
<td>0.96</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-hepta chlorodibenzo-p-dioxin</td>
<td>30.5</td>
<td>12/191</td>
<td>38.9 (30.2–50.9)</td>
<td>14/86</td>
<td>0.04</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin</td>
<td>26.8</td>
<td>12/199</td>
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POP, persistent organic pollutant.

*Adjustment for age, race/ethnicity, sex, poverty index ratio, mother’s age at pregnancy, birth weight, cigarette smoking during pregnancy, body mass index and saturated fat intake.

†Median (interquartile range).
amounts of serum had been used. In fact, concentrations of different POPs probably fluctuate differentially over time in different geographical areas. Most epidemiological studies have studied the extent of postnatal exposure through indirect measurements of POPs in breast milk among breastfeeding infants. Unlike prenatal exposures, postnatal exposures to POPs through breast milk were mostly not associated with cognitive function among infants or children, with some exceptions. Exposure through breast milk may differ from that through other foodstuffs because breast milk is believed to have many beneficial effects for infant development. POPs concentrations in children aged 12–15 years may be influenced by animal products consumption as well as breast milk. Postnatal exposure to environmental neurotoxicants through animal products may be harmful. Or, the current study might suggest that exposure to POPs contained in breast milk may present adverse effects in the long term. Although the most serious effects of neurotoxicants seem to be on cell migration and adverse effects in the long term. Although the most serious effects of neurotoxicants seem to be on cell migration and adverse effects in the long term, brain development continues at a slower pace for years postnatally.

A class of hexachlorocyclohexane is a well-known neurotoxin, although most experimental studies have been performed with γ-hexachlorocyclohexane. Thus, the inverse associations of HCH with LD or ADD may be strange. We think that these inverse associations may be due to complicated interactions among chemical mixtures with similar pathways when simultaneous exposure occurs. Toxicological studies have reported that potentiation, inhibition, antagonism, synergism or other interactions of chemicals in an organism may significantly affect the toxicity of chemical mixtures. Alternatively, they may be false positive findings.

The concept of toxic equivalency factors (TEFs), a measure of the ability to bind to the Ah receptor (AhR), has been developed to facilitate risk assessment and regulatory control of exposure to complex POPs mixtures. However, we did not use TEFs to calculate the cumulative effect of POPs because the strength of association of the POPs observed in this study did not seem to be correlated with the respective TEFs, leading us to hypothesise that binding to AhR may not be the critical pathway. For example, the TEFs of HPCDD and OCDD, 0.01 and 0.0001, were not concordant with their LD ORs of 2.08 and 2.72, respectively (table 2). Supporting this speculation, there is evidence that PCB congeners have differential effects on end points of neurotoxicity depending on their chemical structure: specifically, the ortho-substituted congeners which have low affinity for the AhR are neurotoxic, whereas coplanar (dioxin-like) congeners are relatively inactive in producing neurotoxic effects.

In contrast, even though many cross-sectional or prospective studies have reported associations between blood lead levels and neurodevelopmental deficits in school-aged children, blood lead levels were not associated with the presence of LD or ADD in this study. The more important point may be that the 97% of the study subjects had blood lead levels <5 μg/dl. Even though these levels of blood lead could be related to subtle intellectual impairment as shown in previous studies, they may not lead to the development of definite developmental disorders. Besides lead, there are only a few studies on the association between cadmium exposure and cognitive function in children. In this study, blood cadmium levels showed a weak positive trend with ADD, but it disappeared after adjusting for POPs. Similar to our findings, a Dutch study also reported a positive association of cognitive abilities with POPs, but not with lead or cadmium.

This study has several limitations. First, the cross-sectional study design. Second, LD or ADD was self-reported, probably including children who were having any of a broad range of problems. Use of objective diagnostic criteria for LD or ADD may be feasible in clinical settings. However, in epidemiological studies among general populations, self-reported disease outcomes may be informative, even if misclassification leads to the underestimation of true relationships. In addition, in this study, the results were not different for LD and ADD. These points suggest that background exposure to POPs may be associated with a broad range of developmental disorders among children. Third, although some subjects with a higher value of POP but a lower sample volume might have been classified in the reference group, and vice versa, such misclassifications would be unrelated to self-reported LD or ADD, again weakening the true strength of association. Fourth, we could only adjust for variables that were examined in NHANES and there could be residual confounding. Fifth, we could not evaluate other pollutants such as methylmercury as it was not measured among children aged 4–15 years.

In summary, there were positive relationships between serum concentrations of selected POPs and the prevalence of two clinically relevant developmental problems, LD and ADD. A possible role of background exposure to POPs in future risk of developmental disorder needs to be clarified in large prospective studies, conducted in the general population.

What this paper adds

- Persistent organic pollutants (POPs) are well-known neurotoxicants. Prospective cohort studies have reported lower scores for continuous markers of intellectual or behavioural functions after low-level exposures to some POPs.
- No previous study, not even of cross-sectional design, has examined whether there is an association between background exposure to POPs and clinically significant developmental disorders, such as learning disability (LD) or attention deficit disorder (ADD), in the general population.
- This cross-sectional study found that children who had high serum concentrations of POPs belonging to the categories of polychlorinated dibenzo-\(p\)-dioxins and polychlorinated dibenzofurans showed about twice the prevalence of LD or ADD as those with non-detectable levels.

Policy implications

Developmental disorders such as learning disabilities or attention deficit disorders have a significant impact on community resources. As consumption of animal products is the main source of background exposure to PCDDs or PCDFs in general population, POPs may be a preventable and treatable cause of developmental disorders.
REFERENCES