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Prenatal exposure to mirex impairs neurodevelopment at age of 4 years

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ABSTRACT

Background: Some studies have suggested that certain organochlorine (OC) compounds may impair neurodevelopment in animals and humans. The objective of this study was to investigate the association between prenatal exposure to an OC pesticide, mirex, and cognitive development in children at age of 4 years.
Methods: A population-based birth cohort in Granada (Spain) recruited between 2000 and 2002 was studied between 2005 and 2006, when the children were 4 years old. Complete data for analyses, including mirex determination in placentas, were gathered on a random sample of 104 children. A standardized version of the McCarthy Scales of Children's Abilities (MSCA) was used to assess children's motor and cognitive abilities. Multivariate analyses were performed to evaluate the relation between MSCA scores and prenatal exposure to mirex, adjusting for potential confounders.
Results: The presence of mirex in placenta was inversely associated with cognitive development at 4 years of age: children with prenatal exposure to mirex (≥limit of quantification: 26%; median: 1.4 ng/g placenta) showed a decrease of 5.15 points in working memory and of 7.33 points in the quantitative area with respect to children of the same age not prenatally exposed to mirex.
Conclusion: The deficit found in intellectual function during early childhood suggests that prenatal exposure to mirex may have a significant impact on school performance.

1. Background

Mirex was used as a pesticide to control insects and as a flame retardant additive in plastics, paper, and electrical goods, among others, and its analogue chlordecone was used to control insects and in household products such as ants and roach traps (ATSDR, 1995). Although both of these organochlorine (OC) compounds were intensively utilized in the past, their use has been heavily restricted or banned in most countries (ATSDR, 1995). In Spain, they were used as pesticides until 1965, their commercialization was banned in 1986, and their importation was prohibited in 1994 (PNA Convenio Estocolmo y Reglamento 850/2004, 2007). The United Nations Environment Program recently identified mirex as 1 of the 12 persistent organic pollutants that most threaten global human and wildlife health (United Nations Environment Programme, 2007), and the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) have classified mirex as a probable human carcinogen (Fisher, 1999; EPA, 2000).

Mirex is a fully chlorinated compound frequently detected in food, soil, and water. This OC pesticide is persistent and lipophilic and has a high potential to accumulate in food chains (ATSDR, 1995). Most organisms cannot metabolize mirex, which is therefore highly resistant to biodegradation, although it can be converted into chlordecone by environmental degradation (Carlson et al., 1976). Once in the body, mirex is accumulated in fatty tissue and may be an important source of exposure for offspring during gestation and *via* breastfeeding, since it can cross the placenta and reach the fetus (ATSDR, 1995).

The immature organism does not have the same capacity as the adult to metabolize and detoxify noxious substances (Charnley and Putzrath, 2001). During the prenatal period, the nervous system

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develops within a tightly controlled time frame (Grandjean and Landrigan, 2006), and animal studies have shown that exposure to low levels of neurotoxic substances during more vulnerable developmental periods can induce permanent functional disturbance in the central nervous system (Andersen et al., 2000). The biological processes involved in the neurogenesis and maturation of the nervous system are similar between humans and animals, therefore analogous neurotoxic effects can be expected if these compounds reach the human developing brain at critical time points, even at low concentrations (Andersen et al., 2000).

Epidemiological studies have shown that *in utero* and lactation exposure to some industrial chemicals (lead, methylmercury, arsenic, toluene, polychlorinated biphenyls [PCBs], and OC pesticides) may cause human neurodevelopmental disorders, subclinical brain dysfunctions, and impaired mental and motor disabilities, including reduced intelligence (Jacobson and Jacobson, 1996; Grandjean et al., 1997; Guillette et al., 1998; Grandjean and Landrigan, 2006; Eskenazi et al., 2006; Ribas-Fito et al., 2006b). Nevertheless, it has also been claimed that the beneficial effects of long-term lactation on neurodevelopment may counterbalance the impact of chemical exposure (Ribas-Fito et al., 2003). These toxins have been implicated in specific learning disabilities, such as intellectual retardation and attention deficit and hyperactivity disorder (ADHD), and in other more subtle effects on attention span, concentration, motor speed, memory, language functions, cognitive skills, and educational and social abilities (WHO, 2002). Regarding the impact of prenatal mirex exposure, there is a good experimental background for the effects on development (Khera et al., 1976; Kavlock et al., 1982; Grabowski and Payne, 1983; El-Bayomy et al., 2002) but not on neurodevelopment (Reiter, 1977).

Our research group has investigated the presence of mirex in placenta, breastmilk, and children's adipose tissue samples in Southern Spanish populations (Moreno et al., 2004; Lopez-Espinosa et al., 2007, 2008; Fernandez et al., 2007b). We have also previously reported an association between prenatal exposure to mirex in placenta samples and small-weight-for-gestational-age (SGA) birth (Lopez-Espinosa et al., 2007) or a greater risk of urogenital malformations in newborns (Fernandez et al., 2007b), suggesting that current mirex levels in placenta may affect fetal development in Southern Spain. The main aim of this study was to examine the relationship between prenatal mirex exposure and neurodevelopment in children aged 4 years, controlling for potential physical, demographic, and psychological confounders, and other potential neurotoxicants.

2. Materials and methods

2.1. Study population

In 2000–2002, a cohort of mother–son pairs was established at the San Cecilio University Hospital of Granada with the initial aim of investigating exposure to endocrine disrupting chemicals and male urogenital malformations (Fernandez et al., 2007b). This cohort is part of the INMA (Environment and Childhood) study, a population-based cohort study in Spain that focuses on prenatal environmental exposures in relation to growth, development and health from early fetal life until childhood (Ribas-Fito et al., 2006a; Fernandez et al., 2007c). Written informed consent was obtained from parents of children before the study, which was approved by the Ethics Committee of the San Cecilio University Hospital. The inclusion and exclusion criteria were published elsewhere (Lopez-Espinosa et al., 2007). In the INMA study protocol, the medical follow-up of these children at the age of 4 years included a neuropsychological evaluation. Briefly, between 2005 and 2006, 1 out of 3 mothers ($n = 255$) from the whole cohort were randomly contacted by phone and invited to participate in the neurocognitive testing of their children. The parents agreed to complete several self-reported questionnaires on breastfeeding history, social and working environment, parent-to-infant attachment, and parental mental health, considered as effect modifiers on infant mental developmental (Jacobson and Jacobson, 2005). We have information on placenta mirex levels in 104 mother–son pairs (Lopez-Espinosa et al., 2007), who were therefore selected for the present study. Data from questionnaires at delivery and at 4 years of age were available for all 104 children on maternal age, parental education, occupation status, parity, intrauterine tobacco exposure, marital status, alcohol/drug consumption, breastfeeding, parent-to-infant attachment and mental health scores, school trimester at evaluation and child age. Information on birth length, weight and gestational age at birth was gathered from medical records by pediatricians responsible for the recruitment of the children. Maternal drug consumption was not considered for the present analysis because none of the women declared any history of drug consumption during pregnancy. All women were Caucasians. No differences in characteristics were found between this subsample ($n = 104$) and the remainder of the 255 children with available neuropsychological test results (data not shown).

2.2. Sample collection

We collected placentas at delivery in the maternity unit of the San Cecilio University Hospital of Granada. Each placenta was examined and weighed, and a triangular portion was taken that included maternal and fetal sides as well as central and peripheral parts. This portion was cut, homogenized, coded, frozen at −86 °C, and confidentially and anonymously stored until its processing.

2.3. Extraction and purification of mirex from placenta tissue

Mirex was extracted by using a previously reported method (Lopez-Espinosa et al., 2007; Fernandez et al., 2007a). Briefly, an aliquot of 400 mg of placenta homogenate was dissolved in hexane (20 mL) and separated from natural estrogens by high performance liquid chromatography (HPLC). Further chemical analyses were applied to fractions collected in the first 11 min, during which the most lipophilic compounds (including OC pesticides) were eluted. Mirex was determined in placental tissue by gas chromatography and electron-capture detection (GC/ECD) using a Varian-3350 gas chromatograph with ECD (63Ni) and was confirmed by GC and mass spectrometry (MS) with a Saturn 2000 ion-trap mass spectrometer from Varian, as previously described (Rivas et al., 2001; Cerrillo et al., 2005). The GC limit of quantification (LOQ) and limit of detection (LOD) for mirex were 1 and 0.5 ng/mL, respectively.

2.4. Cognitive assessment

The children were interviewed by trained psychologists at the Early Attention Department of the San Cecilio University Hospital of Granada for completion of the Spanish version of the McCarthy Scales of Children's Abilities (MSCA), which provides information on cognitive and motor abilities (McCarthy, 1972). A strict protocol was applied to avoid inter-observer variability (Julvez et al., 2007b), including inter-observer training and three sets of quality controls (inter-observer reliability tests) during the field work. The inter-observer variability was <5%. The psychologists were blind to the child's exposure to OC compounds and to the type and duration of breastfeeding.

The MSCA scale comprises 18 subsets derived from five different areas (perceptual-performance, verbal, memory, quantitative, and motor), yielding a standardized test score for these five domains and an overall summary score, the general cognitive

index (GCI), which is highly correlated with the Standford–Binet intelligent scale (Jacobson et al., 1990). The GCI is an estimation of the child's global intellectual function and is formed by combining scores for three areas (perceptual-performance, verbal, and quantitative) that do not overlap in content.

The Condon questionnaire (Condon and Corkindale, 1998) consists of 19 items that assess the mother's and father's emotional response to her infant along a number of dimensions relating to parent-to-infant attachment. The questionnaire was translated into Spanish by standardized methods and adapted to children of 4 years old with good psychometric results (data not shown). In our analysis, we obtained a global paternal attachment score ranging from 19 to 95. A higher score in the parent-to-infant attachment scale indicates a closer bond of affection.

Maternal mental health was assessed using the short 12-item version of the General Health Questionnaire (GHQ-12), which is designed to identify psychological distress and short-term changes in mental health in community and primary care settings (Goldberg and Williams, 1998). In our analysis, we obtained a global mental health score ranging from 0 to 36. A higher score in the general mental health scale indicates greater psychological distress.

In accordance with previous Spanish studies (Ribas-Fito et al., 2006b; Julvez et al., 2007b; Freire et al., 2009), MSCA items were grouped by neuropsychological function as follows: verbal memory (MSCA items 3 and 7II), working memory (MSCA items 5 and 14II), memory span or short-term memory (MSCA items 6, 7I and 14I), and executive functions (MSCA items 2, 5, 6, 14II, 15, 17 and 18).

2.5. Statistical analysis

An analysis between MSCA scale scores (outcome variables) and prenatal exposure to mirex (exposure variable) was conducted. Except for working memory function, neurodevelopment scores followed a normal distribution and were treated as continuous variables. Working memory was transformed into a normally distributed variable by applying the formula $1/(x)^3$, inversely transforming the outcome coefficient after the univariate and multivariate models were fitted. Neurodevelopment outcomes were standardized to a mean of 100 points with a standard deviation (SD) of 15 in order to homogenize areas and functions. Because of the number of samples with levels of mirex below the LOQ, this variable was categorized for the statistical analysis, using the LOQ as cut-off point. The relationship between GCI or mirex ($\geq$ or <LOQ) and characteristics of the study population was examined by simple linear or logistic regression analyses, respectively.

We performed simple and multivariate linear regression analyses between McCarthy scores and the presence/absence of mirex in placenta. Potential confounding factors (Table 1) were selected on the basis of previous studies (Ribas-Fito et al., 2006b; Julvez et al., 2007a) with the addition of the emotional bond between parents and the infant and the mental health of the parents (Freire et al., 2009). We also considered the potential confounding effect of environmental exposures to other OC compounds such as DDT and metabolites or hexachlorobenzene (HCB), which were determined in the same placenta samples (Lopez-Espinosa et al., 2007). Variables were included and retained in the final model if $p \leq 0.20$ in univariate analyses and their inclusion altered the mirex coefficient by $\geq$10%. Multivariate models were also adjusted for some other variables, including maternal educational level, age at evaluation, and the psychologist performing the test, even if $p > 0.20$ in univariate analyses and/or they did not change the mirex coefficient by $\geq$10%. A model was constructed for the GCI, and the same model was constructed for each area and function. The normality and homoscedasticity of regression residuals were also assessed. SPSS Version 15 and STATA version 9.0 software packages were used for the data analyses.

3. Results

3.1. Study population

Table 1 shows the prenatal mirex exposure and GCI scores as a function of study population characteristics. Mean maternal age was 33.5 years, 42% of the mothers had completed primary schooling, and 53% were primiparous. More than 20% of them smoked during pregnancy. Mothers with placental mirex levels $\geq$ LOQ had higher gestational age and higher maternal age, although these associations did not reach statistical significance. Placenta samples with mirex levels $\geq$ LOQ had lower maternal mental health scores and higher mother/father-to-infant attachment scores, although the only significant association was with mother-to-infant attachment. GCI scores were significantly higher in older boys and in those whose mothers had more schooling and were non-significantly higher in those with better mother-to-infant attachment test results. Finally, GCI scores were lower in the children of mothers with a previous pregnancy.

3.2. Concentrations of mirex in placenta

Mirex at a concentration $\geq$ LOQ was found in 26% (27/104) of the placenta samples. The median level in these 27 placentas was 1.4 (range: 0.5–19.1) ng/g placenta.

3.3. MSCA areas and functions

Table 2 shows the median MSCA scores for the whole group and for those with placental mirex levels $\geq$ and <LOQ. The mean (SD) GCI for the whole group was 101.4 (15.1). Higher scores in most MSCA areas and functions were found in placenta samples with mirex levels <LOQ versus $\geq$LOQ. Briefly, mean (SD) scores for working memory and quantitative area were 93.8 (8.8) and 94.4 (12.1), respectively, in newborns with placental mirex levels $\geq$ LOQ versus 102.1 (15.8) and 102.5 (15.6), respectively, in those with mirex levels < LOQ.

3.4. Association between mirex and MSCA

Table 3 shows the simple and multivariate linear regression analyses between MSCA scores and placental exposure to mirex. Children with placental mirex $\geq$ LOQ had a poorer performance in working memory ($\beta = -6.23$; $p = 0.01$) and in the quantitative area ($\beta = -8.08$; $p = 0.02$), and these associations remained significant after adjustment for potential confounders (working memory function: $\beta = -5.15$; $p = 0.02$; quantitative area: $\beta = -7.33$; $p = 0.04$). No significant associations were found for the other MSCA areas and functions.

Mirex presence in placenta was still negatively associated with working memory ($\beta = -5.48$; $p = 0.01$) and quantitative area ($\beta = -7.61$; $p = 0.04$) after adjusting for potential confounders, including levels of $\sum$DDTs (sum of *o*,*p*′-DDT, *p*,*p*′-DDT, *p*,*p*′-DDE and *o*,*p*′-DDD) and HCB in the same placenta samples. The association between the presence of mirex in placenta and the remaining functions and areas remained non-significant after adjustment for these OC pesticides.

4. Discussion

In this study, 4-year-old children prenatally exposed ($\geq$LOQ) to mirex (26%; median: 1.4 ng/g placenta) showed a decrease of 5.15

Table 1
Prenatal mirex exposure and general cognitive index scores by characteristics of the study population ($n = 104$) in the INMA-Granada cohort ($n = 104$), 2000–2006.

Covariates	Mean or %	Mirex (≥ or <LOQ)			General cognitive index		
		OR	95% CI	p	β	95% CI	p
Child							
Birth weight (g)	3343	1.00	0.99, 1.00	0.98	0.00	−0.01, 0.00	0.81
Birth length (cm)	50.8	0.94	0.79, 1.11	0.46	0.54	−0.60, 1.69	0.35
Gestational age (weeks)	39.5	1.39	0.99, 1.95	0.06	0.85	−1.21, 2.92	0.41
Age (years)	4.3	0.49	0.04, 6.28	0.58	18.35	1.78, 34.92	0.03
School season at evaluation (%)							
3rd year, 3rd trimester	37.5	R			R		
4th year, 1st trimester	26.9	1.21	0.42, 3.47	0.73	1.58	−5.77, 8.94	0.67
4th year, 2nd and 3rd trimester	35.6	0.59	0.20, 1.75	0.34	7.00	0.19, 13.81	0.04
Mother							
Maternal age (years)	33.5	1.08	0.98, 1.19	0.11	0.11	−0.53, 0.74	0.74
Educational level (%)							
To primary school	42.3	R			R		
Secondary school	39.4	0.54	0.20, 1.43	0.22	9.00	2.67, 15.34	0.01
University	18.3	0.36	0.09, 1.44	0.15	6.07	−1.94, 14.08	0.13
Occupation status (%)							
Employed	65.0	R			R		
Unemployed	35.0	1.67	0.66, 4.21	0.28	−4.31	−10.67, 2.05	0.18
Number of siblings (%)							
0	52.9	R			R		
1	35.6	1.20	0.46, 3.11	0.71	−11.01	−17.08, −4.95	<0.01
2 or more	11.5	1.61	0.41, 6.24	0.48	−6.82	−15.90, 2.27	0.14
Marital status (with child at 4 years) (%)							
With stable partner	97.1	R			R		
Single, divorced or widowed	2.9	1.44	0.12, 16.57	0.77	−11.41	−28.96, 6.12	0.20
Smoking during pregnancy (%)							
No	79.8	R			R		
Yes	20.2	2.07	0.74, 5.74	0.16	0.01	−7.38, 7.38	0.99
Alcohol during pregnancy (%)							
No	88.5	R			R		
Yes	11.5	0.20	0.05, 1.99	0.21	−1.46	−10.72, 7.80	0.76
Breastfeeding (%)							
No	16.9	R			R		
<16 weeks	36.0	6.36	0.73, 55.30	0.09	2.06	−7.38, 11.49	0.67
≥16 weeks	47.1	4.97	0.58, 42.31	0.14	−2.35	−11.41, 6.71	0.61
Mental health (mean score)[a]	10.4	0.90	0.80, 1.01	0.09	0.21	−0.47, 0.89	0.53
Mother-to-infant attachment (mean score)[b]	75.3	1.10	1.01, 1.19	0.02	0.39	−0.08, 0.86	0.10
Father							
Educational level (%)							
To primary school	53.4	R			R		
Secondary school	21.4	1.67	0.58, 4.83	0.34	1.09	−6.45, 8.62	0.78
University	25.2	0.53	0.16, 1.81	0.31	5.71	−1.39, 12.82	0.11
Mental health (mean score)[a]	9.5	0.93	0.83, 1, 06	0.29	−0.04	−0.79, 0.71	0.92
Father-to-infant attachment (mean score)[b]	75.8	1.08	1.00, 1.17	0.05	0.10	−0.32, 0.59	0.65

β: regression coefficient; CI: confidence interval; LOQ: limit of quantification; mean: arithmetic mean; OR: odds ratio; R: category of reference.
Smoking during pregnancy: at least one cigarette/day in the third trimester.
[a] Mean score for the general mental health scale; a higher score indicates greater psychological distress.
[b] Mean score for the parent-to-infant attachment scale; a higher score indicates a closer bond of affection.

in working memory and 7.33 points in quantitative area with respect to children of the same age not prenatally exposed to mirex. These findings suggest that prenatal exposure to mirex may affect cognitive performance.

Mirex and its analogue chlordecone are OC compounds with a high C/Cl atomic ratio that degrade very slowly and are therefore concentrated thousands of times in the food chain (Ahlborg et al., 1995). Both OCs are structurally highly similar, only differing in the replacement of two bridgehead chlorine atoms on the mirex molecule with a carbonyl oxygen atom on the chlordecone molecule. Much more abundant toxicological information is available on chlordecone, including its role as a neurotoxicant (ATSDR, 1995), than on mirex, although a few publications have recorded mirex neurotoxicity. For example, Reiter (1977) showed that breastfed juvenile rats had high sensitivity to acute exposure to mirex, since ingestion of the milk of dams treated with 2.5 mg/kg/day on lactation days 1–4 produced no behavioral abnormalities at the time of the exposure but caused increased activity in the animals when they reached adulthood. More recently, El-Bayomy et al. (2002) observed neuronal tube defects on gestational day 9.5 after feeding pregnant rats with mirex.

To our best knowledge, this is the first epidemiological study on the influence of prenatal mirex exposure on neurodevelopment. Children who were prenatally exposed to mirex showed impairment in the quantitative area and working memory, which require high-level functions of transformation, reorganization and storage of the information. However, prenatal exposure to mirex did not appear to affect motor skills, indicating that this compound might

Table 2
McCarthy Scale scores by prenatal exposure to mirex ($n = 104$) in the INMA-Granada cohort, 2000–2006.

	Exposure to mirex Mean (SD)		
	<LOQ	≥LOQ	All
McCarthy areas			
General cognitive index	101.6 (15.5)	100.9 (14.4)	101.4 (15.1)
Perceptual-performance	102.3 (13.9)	100.2 (14.5)	101.8 (14.0)
Verbal	100.1 (16.1)	103.3 (13.6)	100.9 (15.5)
Memory	101.6 (17.3)	99.7 (11.8)	101.1 (16.0)
Quantitative	102.5 (15.6)	94.4 (12.1)	100.4 (15.2)
Motor	101.0 (14.0)	101.7 (14.2)	101.2 (14.0)
McCarthy functions			
Executive function	102.0 (15.4)	100.9 (13.6)	101.7 (14.9)
Memory Span	100.3 (16.0)	100.2 (11.9)	100.3 (15.0)
Working memory	102.1 (15.8)	93.8 (8.8)	99.9 (14.8)
Verbal memory	101.6 (16.1)	102.6 (12.6)	101.8 (15.2)

LOQ: limit of quantification; SD: standard deviation.
A higher score indicates a better performance.

not interfere with early motor development. Some recent studies may assist our understanding of the consequences of the impairments found. For instance, an association was reported between working memory (the capacity to store and combine information over time) and mathematical ability (Mazzocco, 2008). Poor performance in working memory was also found to be a characteristic of children who fail at school (Alloway and Archibald, 2008). The poorer performance in the quantitative area found in children prenatally exposed to mirex at birth is closely related to working memory function and may therefore predict future mathematical difficulties.

Our results suggest some specificity of the areas associated with mirex prenatal exposure. A similar pattern was reported by other authors who investigated the association between other OC compounds and cognitive deficit. For instance, Jacobson et al. (1990) found that prenatal exposure to PCBs worsened memory performance, implying a cognitive deficit, and the performance of two MSCA tasks related to numerical and verbal memory. These activities require transformation and reorganization of information, which depend on attentional control processes. More recently, the same group showed that *in utero* exposure to PCBs and other contaminants (DDE, polybrominated biphenyls, lead, and mercury) were associated with lower intellectual function in school-age children (Jacobson and Jacobson, 1996). Spanish research into the association between OC pesticides in cord serum and neurodevelopment found that DDT levels were associated with decreased cognitive skills (Ribas-Fito et al., 2006b) and HCB levels with lower behavioral competence in preschoolers (Ribas-Fito et al., 2007). However, other authors failed to find an association between prenatal exposure to OC compounds and impairment of neurodevelopment (Fenster et al., 2007).

The mechanism responsible for intrauterine vulnerability to this compound is not known, although migratory cells and cells undergoing mitosis are especially sensitive to toxic insults (Annau and Eccles, 1986; Jacobson and Jacobson, 1996). Thyroid hormone disruption has been proposed as a potential mechanism of action for the neurodevelopment effects of some OCs (Porterfield, 2000), since they are needed to stimulate neuronal and glial proliferation and differentiation (Lavado-Autric et al., 2003). Thus, PCB and OC pesticide exposure *in utero* has been linked to reduced serum concentrations of thyroid hormones (Chevrier et al., 2008; Lopez-Espinosa et al., 2009). Mirex appears on the European Commission list of compounds with evidence of endocrine disruption in wildlife and humans (European Commission, 2001) and chlordecone is a well-known endocrine disruptor (Soto et al., 1995). However, there are few data on prenatal exposure to mirex and thyroid hormone status, and no association has been found (Takser et al., 2005). Nevertheless, higher thyroid stimulating hormone (TSH) levels in 4-year-old children, although within normal ranges, were recently reported to be associated with impaired neurodevelopment (Alvarez-Pedrerol et al., 2007), especially with inattention symptoms, which are closely related to a poor working memory function.

A potential limitation of the present study is the small sample size, although the similar effect estimates found in unadjusted and adjusted models indicate that residual covariates had little impact on results. A further study limitation is that the simultaneous exposure of individuals to multiple chemical compounds may have confounded our results, and identification of the chemical or combination of chemicals that may impair neurodevelopment remains a challenge. However, our adjustment for other potential neurotoxicants, such as DDTs or HCB, did not confound any of the associations.

A strength of the present study is that account was taken of potential confounding factors, which are especially important in studies of the potential health effects of children's exposure to environmental neurotoxins, because the effects of covariates are often stronger than the effects of primary interest (Mink et al., 2004). Thus, a child's intelligence quotient can be especially influenced by family (parental mental health, feelings of attach-

Table 3
Simple and multivariate regression analyses between McCarthy Scale scores and prenatal exposure to mirex ($n = 104$) in the INMA-Granada cohort, 2000–2006.

	Exposure to mirex					
	Simple regression analyses			Multivariate regression analyses[a]		
	β	95% CI	p	β	95% CI	p
McCarthy areas						
General cognitive index	−0.71	−7.46, 6.04	0.84	−0.62	−6.09, 7.33	0.85
Perceptual-performance	−2.08	−8.31, 4.14	0.51	−0.29	−6.60, 6.03	0.93
Verbal	3.19	−3.68, 10.05	0.36	3.87	−2.97, 10.71	0.26
Memory	−1.95	−9.07, 5.16	0.59	−1.60	−8.74, 5.55	0.66
Quantitative	−8.08	−14.65,−1.51	0.02	−7.33	−14.36,−0.30	0.04
Motor	0.70	−5.55, 6.94	0.83	1.27	−5.44, 7.97	0.71
McCarthy functions						
Executive function	−0.77	−5.51, 3.96	0.75	−0.68	−6.10, 7.47	0.84
Memory Span	−0.12	−6.79, 6.55	0.97	−0.21	−6.57, 6.99	0.95
Working memory	−6.23	−10.30,−2.15	0.01	−5.15	−9.47,−0.83	0.02
Verbal memory	0.97	−5.93, 7.86	0.78	0.84	−6.23, 7.90	0.82

β: regression coefficient; CI: confidence interval.
[a] Models adjusted for age at evaluation, gestational age, maternal educational level, maternal mental health, maternal emotional bond of affection towards children, and psychologist performing the test.

ment, level of education) and non-family (school) factors (Jacobson and Jacobson, 1997). Furthermore, unlike many previous studies, we included assessments of psychometrical covariates in our analysis (Freire et al., 2009). Finally, previous studies in the same cohort reported that prenatal exposure to mirex was associated with the risk of having a SGA birth or a newborn with chryptorchidism (Fernandez et al., 2007b; Lopez-Espinosa et al., 2008) suggesting a role for exposure to mirex through placenta and fetal development.

Further research is warranted to explore the effects of prenatal mirex exposure on intellectual function reported here, which may have serious implications for school performance, studying the impact of this exposure on specific components of cognitive development.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Ahlborg UG, Lipworth L, Titus-Ernstoff L, Hsieh CC, Hanberg A, Baron J, et al. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. Crit Rev Toxicol 1995;25:463–531.

Alloway TP, Archibald L. Working memory and learning in children with developmental coordination disorder and specific language impairment. J Learn Disabil 2008;41:251–62.

Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Julvez J, Ferrer C, Sunyer J. TSH concentration within the normal range is associated with cognitive function and ADHD symptoms in healthy preschoolers. Clin Endocrinol (Oxf) 2007;66:890–8.

Andersen HR, Nielsen JB, Grandjean P. Toxicologic evidence of developmental neurotoxicity of environmental chemicals. Toxicology 2000;144:121–7.

Annau Z, Eccles CU. Prenatal exposure. In: Annau Z, editor. Neurobehavioral toxicology. Baltimore: Johns Hopkins University Press; 1986153–67.

ATSDR. Toxicology profile for mirex and chlordecone. 1995.

Carlson DA, Konyha KD, Wheeler WB, Marshall GP, Zaylskie RG. Mirex in the environment: its degradation to kepone and related compounds. Science 1976;194:939–41.

Cerrillo I, Granada A, Lopez-Espinosa MJ, Olmos B, Jimenez M, Cano A, et al. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk. Environ Res 2005;98:233–9.

Charnley G, Putzrath RM. Children's health, susceptibility, and regulatory approaches to reducing risks from chemical carcinogens. Environ Health Perspect 2001;109:187–92.

Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. Am J Epidemiol 2008;168:298–310.

Condon JT, Corkindale CJ. The assessment of parent-to-infant attachment: development of a self-report questionnaire instrument. J Reprod Infant Psychol 1998;16:57–76.

El-Bayomy AA, Smoak IW, Branch S. Embryotoxicity of the pesticide mirex in vitro. Teratog Carcinog Mutagen 2002;22:239–49.

EPA US. Guidance for assessing chemical contaminant data for use in fish advisories. F-4. 2000.

Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, et al. In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. Pediatrics 2006;118:233–41.

European Commission. European Commission Environment COM document. Candidate list of substances as a basis for priority setting. Annex 13. 2001.

Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. In utero exposure to DDT and performance on the Brazelton neonatal behavioral assessment scale. Neurotoxicology 2007;28:471–7.

Fernandez M, Molina-Molina JM, Lopez-Espinosa MJ, Freire C, Campoy C, Ibarluzea J, et al. Biomonitoring of environmental estrogens in human tissues. Int J Hyg Environ Health 2007a;210:429–32.

Fernandez MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez JM, et al. Human exposure to endocrine disrutping chemicals and prenatal risk factors for cryptorchidism and hypospadias. A nested case-control study. Environ Health Perspect 2007b;115(Suppl. 1):8–14.

Fernandez MF, Sunyer J, Grimalt J, Rebagliato M, Ballester F, Ibarluzea J, et al. The Spanish environment and childhood research network (INMA study). Int J Hyg Environ Health 2007c;210:491–3.

Fisher BE. Most unwanted. Environ Health Perspect 1999;107:A18–23.

Freire C, Ramos R, Puertas R, Lopez-Espinosa MJ, Julvez J, Aguilera I, Cruz F, Fernandez MF, Sunyer J, Olea N. Association of traffic-related air pollution with cognitive development in children. J Epidemiol Community Health; 2009; jech.2008.084574v1-jech.2008.084574.

Goldberg D, Williams P. A user's guide to the General Health Questionnaire. UK: NFER-Nelson, Windsor; 1998.

Grabowski CT, Payne DB. The causes of perinatal death induced by prenatal exposure of rats to the pesticide, mirex. Part I: Pre-parturition observations of the cardiovascular system. Teratology 1983;27:7–11.

Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet 2006;368:2167–78.

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.

Guillette EA, Meza MM, Aquilar MG, Soto AD, Garcia IE. An anthropological approach to the evaluation of preschool children exposed to pesticides in Mexico. Environ Health Perspect 1998;106:347–53.

Jacobson JL, Jacobson SW. Evidence for PCBs as neurodevelopmental toxicants in humans. Neurotoxicology 1997;18:415–24.

Jacobson JL, Jacobson SW. Methodological issues in research on developmental exposure to neurotoxic agents. Neurotoxicol Teratol 2005;27:395–406.

Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N Engl J Med 1996;335:783–9.

Jacobson JL, Jacobson SW, Humphrey HE. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr 1990;116:38–45.

Julvez J, Ribas-Fito N, Forns M, Garcia-Esteban R, Torrent M, Sunyer J. Attention behaviour and hyperactivity at age 4 and duration of breast-feeding. Acta Paediatr 2007a;96:842–7.

Julvez J, Ribas-Fito N, Torrent M, Forns M, Garcia-Esteban R, Sunyer J. Maternal smoking habits and cognitive development of children at age 4 years in a population-based birth cohort. Int J Epidemiol 2007b;36:825–32.

Kavlock RJ, Chernoff N, Rogers E, Whitehouse D, Carver B, Gray J, et al. An analysis of fetotoxicity using biochemical endpoints of organ differentiation. Teratology 1982;26:183–94.

Khera KS, Villeneuve DC, Terry G, Panopio L, Nash L, Trivett G. Mirex: a teratogenicity, dominant lethal and tissue distribution study in rats. Food Cosmet Toxicol 1976;14:25–9.

Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe MC, Escobar del RF, Berbel P, et al. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 2003;111:1073–82.

Lopez-Espinosa MJ, Granada A, Carreño J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from Southern Spain and some related factors. Placenta 2007;28:631–8.

Lopez-Espinosa MJ, Lopez-Navarrete E, Rivas A, Fernandez MF, Nogueras M, Campoy C, et al. Organochlorine pesticide exposure in children living in southern Spain. Environ Res 2008;106:1–6.

Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, et al. Association between thyroid hormone levels and 4,4′-DDE concentrations in pregnant women (Valencia, Spain). Environ Res 2009;109:479–85.

Mazzocco MM. Introduction: mathematics ability, performance, and achievement. Dev Neuropsychol 2008;33:197–204.

McCarthy D. Manual for the McCarthy Scale of Children's Abilities. New York, NY: Psychological Corporation; 1972 (Spanish adaptation: Madrid TEA. Ediciones, S.A. (1996).

Mink PJ, Goodman M, Barraj LM, Imrey H, Kelsh MA, Yager J. Evaluation of uncontrolled confounding in studies of environmental exposures and neurobehavioral testing in children. Epidemiology 2004;15:385–93.

Moreno FM, Jimenez TM, Garrido FA, Martinez Vidal JL, Olea-Serrano F, Olea N. Determination of organochlorine compounds in human biological samples by GC-MS/MS. Biomed Chromatogr 2004;18:102–11.

PNA Convenio Estocolmo y Reglamento 850/2004. Plan Nacional de Aplicación del Convenio de Estocolmo y el Reglamento 850/2004, sobre Contaminantes Orgánicos Persistentes. Subdirección General de Calidad del Aire y Prevención de Riesgos del Ministerio de Medio Ambiente; 2007.

Porterfield SP. Thyroidal dysfunction and environmental chemicals—potential impact on brain development. Environ Health Perspect 2000;108(Suppl. 3):433–8.

Reiter L. Behavioral toxicology: effects of early postnatal exposure to neurotoxins on development of locomotor activity in the rat. J Occup Med 1977;19:200–4.

Ribas-Fito N, Cardo E, Sala M, Eulalia de MM, Mazon C, Verdu A, et al. Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants. Pediatrics 2003;111:e580–5.

Ribas-Fito N, Ramon R, Ballester F, Grimalt J, Marco A, Olea N, et al. Child health and the environment: the INMA Spanish Study. Paediatr Perinat Epidemiol 2006a;20:403–10.

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;115:447–50.

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 2006b;164:955–62.

Rivas A, Fernandez MF, Cerrillo I, Ibarluzea J, Olea-Serrano MF, Pedraza V, et al. Human exposure to endocrine disrupters: standardisation of a marker of estrogenic exposure in adipose tissue. APMIS 2001;109:185–97.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 1995;103(Suppl. 7):113–22.

Takser L, Mergler D, Baldwin M, de GS, Smargiassi A, Lafond J. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environ Health Perspect 2005;113:1039–45.

United Nations Environment Programme. Stockholm Convention on Persistent Organic Pollutants (POPs). 2007 Available: http://www.pops.int 2007 (accessed 2 April 2007).

WHO. In: Children's health and environment: a review of evidence. Third Ministerial Conference on Environment and Heath in London in 1999, WHO regional office for Europe, Copenhagen; 2002.