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Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study

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ABSTRACT

There is little evidence about exposure to currently used insecticides during early life periods and adverse effects on child neuropsychological development. The aim of this study is to examine the association between residential insecticide use during pregnancy and infancy, and the development of children.

Study subjects were participants in the INMA (Environment and Childhood) Project, a Spanish multicenter birth cohort study. Prenatal and postnatal use of indoor insecticides and other variables were obtained from personal interview during pregnancy and infancy. Mental and psychomotor development was assessed around 14 months using the Bayley Scales of Infant Development. The associations were analyzed by linear regression models.

54% of women used indoor insecticides at home during pregnancy and 47% postnatally. 34% of women used insecticide sprays and 33% used plug-in devices during pregnancy. During infancy, the percentage of women who used insecticide sprays decreased (22%), but the use of plug-in devices was similar to the prenatal period (32%). The use of insecticide sprays during pregnancy was associated with a decrement in psychomotor development ($\beta = -1.9$; 95%CI: -3.4, -0.5) but postnatal use did not associate with mental and psychomotor development. The negative effect was enhanced according to some modifying factors, such as being female, higher levels of prenatal exposure to PCB and mercury and belonging to the lowest social class.

We found certain evidence about the adverse effect of using insecticide sprays during pregnancy on the psychomotor development of children. Some socio-demographic factors and other exposures could enhance that effect.

1. Introduction

Insecticides are very widely used in the domestic setting. Studies conducted in the USA have shown that a high percentage (80–95%) of the population used some type of insecticide at home, even during pregnancy and in dwellings with young children (Adgate et al., 2000; Davis et al., 1992; Whyatt et al., 2002; Wu et al., 2011). The most frequently used were spray insecticides (Wu et al., 2011). In Bristol (UK), results from ALSPAC study (Avon Longitudinal Study of Parents and Children) showed that 93% of parents used domestic insecticides in dwellings with school age children, and 76% used two or more products (Grey et al., 2006). In Spain, 54% of the pregnant women of the INMA (Environment and Childhood) cohort used at least one type of insecticide (Llop et al., 2013).

Human exposure to insecticides may start during the uterine life period by transplacental transfer (Bradman et al., 2003). The developing brain is particularly susceptible to the adverse effects of environmental toxicants, since the blood–brain barrier, which protects the adult brain

Abbreviations: BSID-I, Bayley Scales of Infant Development; CI, Confidence intervals; EPA, Agency of Environmental Protection; RfD, reference dose; Hg, mercury; PCB, Polychlorobiphenyl; ALSPAC study, Avon Longitudinal Study of Parents and Children; CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; CCCEH, Columbia Center for Children's Environmental Health.

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from many toxic agents, is not completely formed until 6 months after birth (Bradman et al., 2003; Grandjean and Landrigan, 2006). Furthermore, fetuses and children are especially vulnerable to insecticide exposure due to their immature detoxification and immune systems. All these factors may increase the risk of developing chronic diseases later in life (Landrigan et al., 2004).

Cognitive delays produced by the exposure to organophosphate during pregnancy have mainly been studied by three birth cohorts conducted in the USA. The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) was a prospective cohort study that investigated the association between long-term pesticide exposure and the health of pregnant women and their children living in the agricultural area of Salinas Valley, California (Castorina et al., 2003). Urinary metabolites of organophosphate pesticides measured during pregnancy associated with mental development and pervasive developmental problems at 24 months of age (Eskenazi et al., 2007), problems of attention at age of 5 years (Marks et al., 2010), and poorer scores in working memory, processing speed, verbal comprehension, perceptual reasoning, and full-scale intelligence quotient at 7 years (Bouchard et al., 2011). Regarding the postnatal exposure to organophosphate pesticides, the authors did not find any significant association with a delay in child neurodevelopment (Young et al., 2005).

The Columbia Center for Children's Environmental Health (CCCEH) examined the effects of prenatal exposure to pesticides on children's neurodevelopment among minority communities in New York City (Perera et al., 2003). They found that higher prenatal levels of chlorpyrifos associated with mental and psychomotor delays, attention problems, and pervasive developmental disorder problems at 3 years of age (Rauh et al., 2006), however they did not observe a relation with prenatal permethrin exposure and adverse neurodevelopment (Horton et al., 2011).

Finally, in the birth cohort of Mount Sinai Hospital in New York City an association between prenatal exposure to malathion and abnormal reflexes in neonates was observed (Engel et al., 2007), as well as between some organophosphate metabolites levels and abnormal reflexes in neonates and a decrement in mental development at 12 months (Engel et al., 2011).

All these studies reported some evidence of adverse effects produced on neurodevelopment by prenatal exposure to organophosphate pesticides. However, there are certain contradictions and inconsistencies with regard to postnatal exposure to these compounds and also to prenatal and postnatal exposure to pyrethroids (Koureas et al., 2012), the group of insecticides most frequently used in domestic settings, so it would be necessary to conduct more epidemiologic studies in humans, and particularly in children.

The aim of the present study is to examine the association between residential insecticide use during pregnancy and infancy, both obtained by personal interview, and a possible delay in the neuropsychological development of children during the second year of life in a multicenter Spanish birth cohort.

2. Material and methods

2.1. Study population

Study subjects were participants in the INMA (Environment and Childhood) Project, a multicenter cohort study which aims to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different geographical areas of Spain (http:www.proyectoinma.org). The study protocol has been reported elsewhere (Guxens et al., 2012; Ribas-Fito et al., 2006). Briefly, the original population in this study consisted of 2644 women recruited at the 1st trimester of pregnancy (November 2003–February 2008) in four regions of Spain: Sabadell (Catalonia), Gipuzkoa (Basque Country), Asturias and Valencia. Excluding women who withdrew from the study, were lost to follow-up, or had induced or spontaneous abortions or fetal deaths, a total sample of 2506 (95%) women were followed up until delivery (May 2004–August 2008). Their children were enrolled at birth, and were followed up until they were 30 months of age (n: 2360, 89%). The final study population consisted of mother–child pairs (n = 1980) with complete data on prenatal and postnatal insecticide use and for whom neuropsychological test scores were available during the second year of child's life. Women participating in the study signed an informed consent form in each phase and the Ethics Committees of the centers involved in the study approved the research protocol.

2.2. Exposure assessment

Information about prenatal and postnatal use of insecticides was obtained using a questionnaire during pregnancy and infancy. These questionnaires were administered by trained interviewers in the reference hospitals or in the primary attention centers.

The use of indoor insecticides during pregnancy (no, yes) was obtained at the third trimester (weeks 28–32). Method of application was also requested (sprays [no, yes], plug-in devices [no, yes] and other types [no, yes]). In a subsequent interview (11–30 months of age of children), we obtained information about the use of indoor insecticides during the first year of life of children (no, yes), as well as the use of insecticide sprays (no, yes), plug-in devices (no, yes) and other types (no, yes). The use of insecticide sprays and plug-in devices are not mutually exclusive and there were some women who used both applications. The prevalence of using other types of insecticides among the women was very low (2%) (Llop et al., 2013), therefore, it has been not used in the analysis.

2.3. Other variables

Socio-demographic, environmental and life-style information was obtained using questionnaires during pregnancy and infancy. The first questionnaire was administered during the first trimester of pregnancy. The variables used in this study were: maternal age, educational level (primary or no education, secondary, university), country of birth (Spain, other), and body mass index before pregnancy (underweight, healthy, overweight, obese). The second questionnaire was administered during the third trimester of pregnancy. We obtained information about: smoking habit during pregnancy (yes, no), maternal employment status (employed, unemployed), parity (0, 1, ≥2), having a garden or yard at home (no, yes) and residential proximity to an agricultural area (no, yes).

In a subsequent interview, when the children were between 11 and 30 months of age, we obtained other covariates: duration of breastfeeding (0, >0–16, >16–24, >24 weeks), main child-care provider (mother, mother with help from father, grandparent or others, other combinations without mother), child care attendance (no, yes), number of siblings (0, 1, ≥2), maternal employment status (employed, unemployed), and parental smoking habit (smoker, non smoker). Breastfeeding was defined as receiving breast milk and allowing supplementation of any food or liquid including nonhuman milk. Information related to the child's gestational age (weeks), sex, anthropometric measures, type of delivery, and the Apgar score at birth was obtained from clinical records. Low birth weight was defined as less than 2500 g, and small for gestational age (SGA) in length was defined as measuring below percentile 10. Preterm birth was considered to be less than 37 weeks of gestation.

We defined parental social class based on maternal or paternal occupation during pregnancy, whichever corresponded to the highest class among them, using a widely used Spanish adaptation of the international ISCO88 coding system (Domingo-Salvany et al., 2000). Class I included managerial jobs, senior technical staff, and commercial managers; class II included skilled non-manual workers; and class III, manual workers.

We also studied the modifying effect of other neurotoxic pollutants, such as polychlorobiphenyl (PCBs) and mercury since prenatal exposure to mercury and PCB congeners has been related previously to a deleterious effect on neuropsychological development (Forns et al., 2012; Grandjean and Landrigan, 2006). PCBs congeners 138, 153, and 180 (available for the Valencia, Gipuzkoa and Sabadell regions) levels were analyzed in maternal blood samples taken at the first trimester of gestation. Total mercury levels were analyzed in cord blood samples taken at delivery in all regions included in this study. Reference dose of mercury proposed by the Environmental Protection Agency (EPA RfD: 6.4 μ/L in cord blood) and the 1st tertile of total PCBs (0.48 ng/mL) were taken into account as cut-off points in order to build dichotomous variables.

Other possible modifying factors taken into account were sex of children, breastfeeding, and social class. Sex of children has been identified as a modifying factor when we studied the effect of prenatal exposure to mercury on neuropsychological development in the same birth cohort (Llop et al., 2012). Breast milk has a high content of long-chain polyunsaturated fatty acids and other micronutrients that may also mitigate the deleterious effect of pesticides (Ribas-Fito et al., 2007). Finally, socio-economic status could also be influencing the association between insecticide exposure and neuropsychological development, since some socio-economic characteristics, such as maternal employment and educational level, have been related to children's neurodevelopment in other studies (Koutra et al., 2012), and also some conditions of deprivation have been associated with higher residential insecticide use (Whyatt et al., 2002).

2.4. Child neurodevelopment test

Neuropsychological development of the children was assessed at around age 14 months (range 11–23 months) using the Bayley Scales of Infant Development (BSID-I) that assess age-appropriate mental and psychomotor development, including performance abilities, memory, early language skills, psychomotor skills and coordination. The Bayley Scales are composed of the Mental Scale (163 items) and the Psychomotor Scale (81 items). All testing was done in the corresponding health care center in the presence of the mother, by a total of twelve specially trained psychologists. To limit inter-observer variability, we applied a strict protocol, including training sessions where inter-observer differences were quantified and the use of three sets of quality controls (inter-observer-reliability-tests). The inter-rate reliability estimated by intra-class correlation was 0.90 for mental test scores, and 0.91 for psychomotor test scores.

The Bayley tests were performed on 2360 children who attended the visit. Raw scores were standardized for child's age in days at the time of the test administration. Standardized residuals were then typified as having a mean of 100 points with a 15 standard deviation.

2.5. Statistical analysis

Multivariate linear regression models were built to assess the relation between prenatal and postnatal self-reported use of indoor insecticides, separately, and both mental and psychomotor development scales. Beta coefficients and 95% confidence intervals were obtained. A 2-step procedure was used for multivariate model building. In the first step, we built a core model for both mental and psychomotor scales. The variables included in the core models have been related to neuropsychological development in the literature or in our previous analysis (Llop et al., 2012). The core model for the mental scale included: body mass index before pregnancy, maternal age, educational level, country of birth, social class, sex, low birth weight, weeks of gestation, main child-care provider, and psychologist. In the case of the psychomotor scale the core model included maternal age, country of birth, social class, body mass index before pregnancy, weeks of gestation, number of siblings, SGA in length, nursery attendance, paternal smoking during the child's first year, and psychologist.

Additional potential confounders (having a garden or yard with plants at home, residential proximity to an agricultural area) related to the use of insecticides among the women in a previous study (Llop et al., 2013), were included if they changed the magnitude of the main effects by more than 10%.

Region was not introduced as a variable at this phase because of its high correlation with the psychologist variable. In these adjusted models, both mental and psychomotor, we included the exposure variables obtained during pregnancy: use of indoor insecticides, use of insecticide sprays and use of plug-in devices. The use of insecticide sprays and use of plug-in devices were introduced jointly into the models. The same procedure was used in order to evaluate postnatal exposure to insecticides on neuropsychological development.

Table 1
Socio-demographic and life style characteristics of study population and the original cohort, INMA Project, Spain, 2003–2008.

		Original cohort (n = 2360)[b]		Study population (n = 1980)[c]	
		N	%	N	%
Region	Gipuzkoa	475	20.1	506	25.6
	Sabadell	594	25.2	530	26.8
	Asturias	583	24.7	254	12.8
	Valencia	708	30.0	690	34.8
Maternal age[a] (years)		30.8	4.2	30.7	4.2
Social class	I	769	32.6	641	32.4
	II	592	25.1	516	26.1
	III	998	42.3	823	41.6
Maternal job situation during pregnancy	Unemployed	376	15.9	291	14.7
	Employed	1984	84.1	1689	85.3
Smoking during pregnancy	No	1928	83.5	1657	83.8
	Yes	380	16.5	321	16.2
Country of birth	Other	184	7.8	159	8.1
	Spain	2169	92.2	1814	91.9
Educational level	Primary or without education	542	23.0	457	23.1
	Secondary	976	41.4	830	42.0
	University	837	35.5	690	34.9
Body mass index before pregnancy	Underweight	99	4.2	87	4.4
	Healthy	1646	69.7	1372	69.3
	Overweight	437	18.5	369	18.6
	Obese	178	7.5	152	7.7
Low birth weight	No	2225	95.0	1880	95.5
	Yes	116	5.0	89	4.5
Preterm birth	No	2237	95.6	1894	96.3
	Yes	102	4.4	72	3.7
Small for gestational age in length	No	2053	90.5	1740	90.9
	Yes	215	9.5	175	9.1
Sex	Female	1136	48.2	962	48.6
	Male	1219	51.8	1017	51.4
Chile care attendance	No	1426	64.3	1320	67.0
	Yes	791	35.7	650	33.0
Main child care provider	Mother	1296	58.8	1165	59.5
	Mother with help from father, grandparent or others	712	32.3	621	31.7
	Other combinations without mother	196	8.9	173	8.8
Breastfeeding (weeks)	0	345	15.3	274	13.9
	>0–16	564	24.9	480	24.4
	>16–24	358	15.8	302	15.3
	>24	994	44.0	914	46.4
Number of siblings	0	1348	57.4	1118	56.8
	1	870	37.0	738	37.5
	≥2	132	5.6	113	5.7

[a] Mean and standard deviation.
[b] Original cohort: children followed up until 30 months of age.
[c] Children with completed data on insecticide exposure and available neuropsychological test scores.

Sensitivity analyses were performed in order to evaluate the robustness of the multivariate models and these were repeated after eliminating certain population subgroups: preterm birth (n = 72), low birth weight (n = 89), and children with an underlying pathology (such as very preterm, epilepsy, hypotonia, plagiocephaly, and fetal suffering, n = 16).

Finally, we stratified the study population according to potential modifying factors, such as sex (girl, boy), breastfeeding (≤16 weeks, >16 weeks), social class (I, II, III), prenatal exposure to total mercury (≤US EPA RfD, >US EPA RfD), and maternal total PCBs levels (≤1st tertile, >1st tertile) in order to assess the effect on coefficients of prenatal and postnatal use of insecticides.

The main analyses were carried out using the SPSS version 15 and Stata version 11 (StataCorp LP, College Station, Texas) statistical packages.

3. Results

Characteristics of the study population are shown in Table 1. Nearly half of the women included in the present study belonged to a lower social class, 85.3% worked during pregnancy, 16.2% smoked during pregnancy, most of them were Spanish, and secondary school was the most frequently reported level of educational attainment among the women. The only three differences found between original cohort and study population were according to region (p < 0.001), child-care attendance (p = 0.045) and maternal country of birth (p < 0.001). There were more non-Spanish women and children who attended child-care services among the study population than in the original cohort.

Fifty-four percent of women used indoor insecticides at home during pregnancy. Differences in percentages according to the habit of using insecticides between the different regions were observed, the percentage of women who used insecticides at home was the highest in Sabadell (67%) and the lowest in Gipuzkoa (29%) (Chi^2 p-value < 0.001). Thirty-four percent of women used insecticide sprays and 33% used plug-in devices. During the postnatal period 47% of women used indoor insecticides. Compared to the prenatal period, insecticide spray was less used (22%). The percentage of using plug-in devices was similar in both periods (32%) (Table 2).

Prenatal use of indoor insecticides was not associated either with the mental or psychomotor development of children, however, when this use was broken down into different application methods, we found that the use of insecticide sprays during pregnancy was associated with a decrement in psychomotor development (β = −1.9; 95%CI: −3.4, −0.5) (Table 3). Postnatal use of indoor insecticides was not associated with mental and psychomotor development.

The sensitivity analyses performed, excluding from analysis preterm children (β = −1.9; 95%CI: −3.3, −0.4), low birth weight (β = −2.4; 95%CI: −3.8, −0.9), and children with underlying pathology (β = −1.9; 95%CI: −3.3, −0.5) confirmed the stability of our results. Additionally, the prenatal and postnatal use of both sprays and plug-in devices were introduced jointly into the adjusted models and the result did not change (β = −1.8; 95%CI: −3.3, −0.3 for psychomotor development and use of prenatal insecticide sprays).

Stratified analysis taking alternately into account sex of children, prenatal exposure to Hg, prenatal exposure to PCBs, and social class showed some changes in the beta coefficients of psychomotor development of children whose mothers used prenatal insecticide sprays. Female sex, children with cord blood Hg levels > EPA RfD and children with maternal levels of total PCBs >1st tertile showed a larger decrement in psychomotor development if their mothers used prenatal insecticide sprays during pregnancy. Additionally, children whose mothers belonged to the lowest social class and used insecticide sprays during pregnancy had a significant decrement in their psychomotor test scores (β = −3.3; 95%CI: −5.6, −1.1) (Fig. 1C). The interaction was not significant for Hg and PCBs levels (p = 0.193 and 0.206, respectively) and significant for children's sex and social class (p = 0.029 and 0.002, respectively).

Regarding the use of plug-in devices, children whose mothers belonged to the second social class and used plug-in devices postnatally had a significant increase (β = 2.9; 95%CI: 0.1, 5.6) in their

Table 3
Multivariate linear regression analysis between prenatal and postnatal use of indoor insecticides and mental and psychomotor Bayley's scales in infants participating in the INMA Project, Spain, 2003–2010.

	Mental development			Psychomotor development		
	Beta	95%CI		Beta	95%CI	
Pregnancy						
Use of insecticides at home	0.1	−1.4	1.6	−0.7	−2.1	0.7
Use of insecticide spray[a]	−0.5	−2.0	1.0	**−1.9**	**−3.4**	**−0.5**
Use of plug-in device[a]	0.1	−1.4	1.7	0.6	−0.8	2.0
Infancy						
Use of insecticides at home	0.3	−1.2	1.8	−0.2	−1.6	1.2
Use of insecticide spray[b]	−0.5	−2.3	1.2	−1.0	−2.7	0.6
Use of plug-in device[b]	0.7	−0.9	2.2	0.9	−0.6	2.4

95%CI: 95% confidence intervals.
Bold data are significant at p = 0.009.
Mental development adjusted by body mass index before pregnancy, maternal age, educational level, country of birth, social class, sex, low birth weight, weeks of gestation, main child-care provider, and psychologist.
Psychomotor development adjusted by maternal age, country of birth, social class, body mass index before pregnancy, weeks of gestation, number of siblings, small-for-gestational-age SGA in length, nursery attendance, paternal smoking during the child's first year, and psychologist.
Reference categories: not use of insecticides at home, not use of insecticide sprays and not use of pug in devices.
[a] These variables were included jointly in the models.
[b] These variables were included jointly in the models.

Table 2
Prenatal and postnatal use of insecticides among participants in the INMA Project, Spain, 2003–2008.

	Whole cohort		Asturias		Gipuzkoa		Sabadell		Valencia	
	N	%	N	%	N	%	N	%	N	%
Pregnancy										
Use of insecticides at home	1077	54	135	53	147	29	355	67	440	64
Use of spray[a]	676	34	72	28	77	15	198	37	329	48
Use of plug-in device[a]	650	33	95	37	84	17	234	44	237	34
Infancy										
Use of insecticides at home	942	48	139	55	72	14	321	61	410	59
Use of spray[a]	435	22	42	16	37	7	98	18	258	37
Use of plug-in device[a]	639	32	108	42	43	8	249	47	239	35
Mental development scores (Mean ± sd)	98.5	16	101.0	16	96.9	16	98.5	16	98.7	17
Psychomotor development scores (Mean ± sd)	98.9	16	101.9	16	96.3	17	98.2	13	100.3	16

sd: standard deviation.
[a] The use of sprays and plug-in devices are not mutually exclusive. There were some women who used both applications.

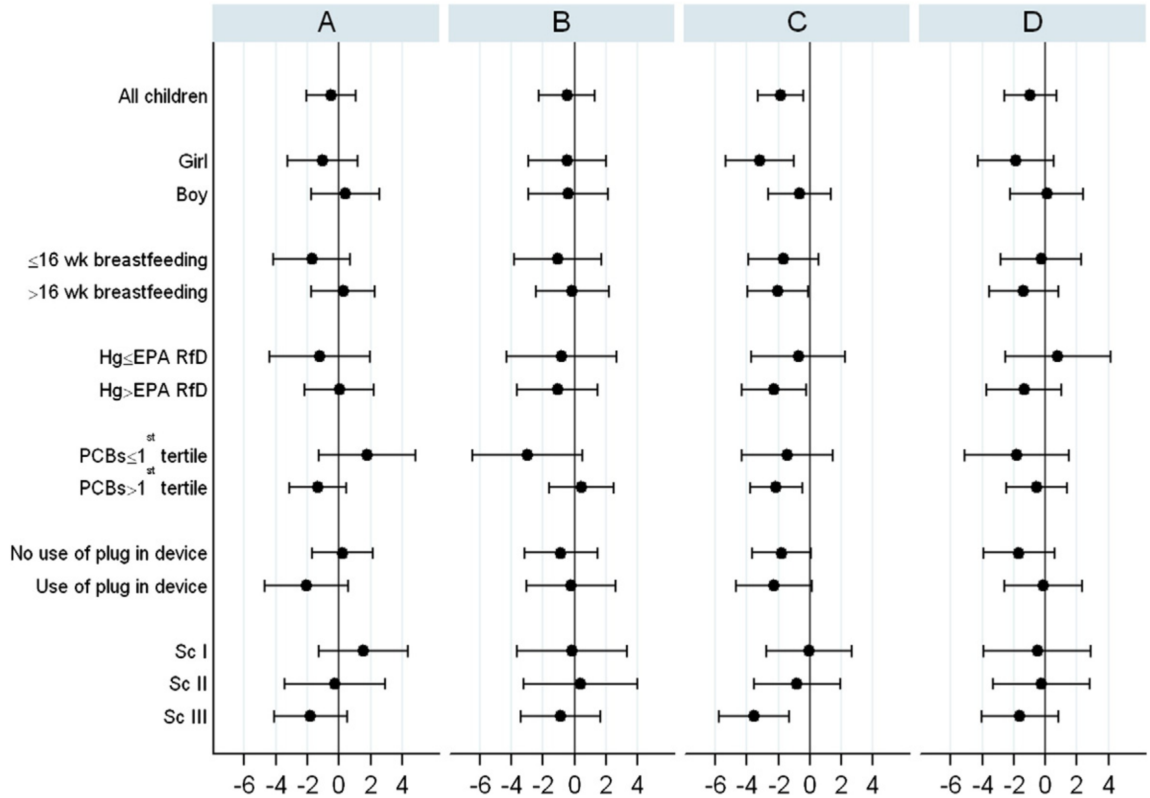

Fig. 1. Effect of potential modifiers on coefficients for associations between the use of insecticide sprays and children's neuropsychological development, INMA Project, Spain, 2003–2010. A: Use of insecticide sprays during pregnancy and effect on mental development. B: Use of insecticide sprays during infancy and effect on mental development. C: Use of insecticide sprays during pregnancy and effect on psychomotor development. D: Use of insecticide sprays during infancy and effect on psychomotor development (mental development is adjusted by body mass index before pregnancy, maternal age, educational level, country of birth, social class, sex, low birth weight, weeks of gestation, main child-care provider, and psychologist. Psychomotor development is adjusted by maternal age, country of birth, social class, body mass index before pregnancy, weeks of gestation, number of siblings, small-for-gestational-age SGA in length, nursery attendance, paternal smoking during the child's first year, and psychologist. wk: week; EPA RfD = 6.4 μ/L;1st tertile of total PCBs = 0.48 ng/mL; Sc: Social class).

psychomotor test scores (Fig. 2D). The interaction term was significant ($p = 0.017$).

4. Discussion

This is one of the first studies in Europe exploring the association between early life exposures to insecticides and infant neurodevelopment. Results from this birth cohort study conducted in Spain have shown a negative association between reported use of residential insecticide sprays during pregnancy and psychomotor development in infants. Nevertheless, postnatal use of insecticide sprays did not associate with a delay in the infant's neuropsychological development.

Results from the CHAMACOS, CCCEH and Mount Sinai cohorts suggest that prenatal exposure to organophosphates associated negatively with intellectual development during childhood. This association became less consistent when they studied the effects of postnatal exposure. However, very few epidemiologic studies have evaluated the potential neurological effects due to exposure to pyrethroids, the family of insecticides more commonly used in the domestic environment in Spain (Moreno et al., 2003), and their results are not conclusive (Koureas et al., 2012). Horton et al. (2011) observed a negative association between prenatal exposure to piperonyl butoxide (a pyrethroid synergist) and cognitive development at 36 months, but not with permethrin (a common pyrethroid). In another study, no association was found between prenatal exposure to a group of pyrethroids (bioallethrin, transfluthrin, cyfluthrin and cypermethrin) and delayed cognitive development at 24 months (Ostrea et al., 2012).

These previous studies used bio-monitoring techniques to assess insecticide exposure. Biomarkers of exposure to insecticides in urine or plasma may determine the dose of active ingredient absorbed by the study population; however, they only provide values that reflect acute or short-term exposure. Questionnaires are useful tools for assessing exposure to residential insecticides since they enable us to determine the exposure during a period of time greater than the half life of these substances. This is important since when residential insecticides are used they have a short half life of a few hours or days, especially pyrethroids (Egeghy et al., 2011). This fact is relevant regarding effects related to a more prolonged exposure, such as during pregnancy or during infancy. Nevertheless, the use of questionnaires to assess insecticide exposure has certain limitations since they do not give information on other routes of exposure such as diet, exposure in other places such as leisure establishments or other people's homes or the active substances used.

Questionnaires have been used in epidemiologic studies to assess occupational exposure to insecticides. Handal et al. (2008) reported that 3–23 months aged children whose mothers worked in the flower industry during pregnancy presented a delay in fine motor skills and poor visual acuity in a rural region of Ecuador with intensive use of insecticides. However, among the same population, postnatal maternal employment in the flower industry was associated with better development scores in children aged 24–61 months (Handal et al., 2007). Residential use of insecticides, both during pregnancy and childhood, was associated with children's better gross motor scores. The authors attributed this inconsistency to the complexity of the relation between risk factors and social characteristics, since female

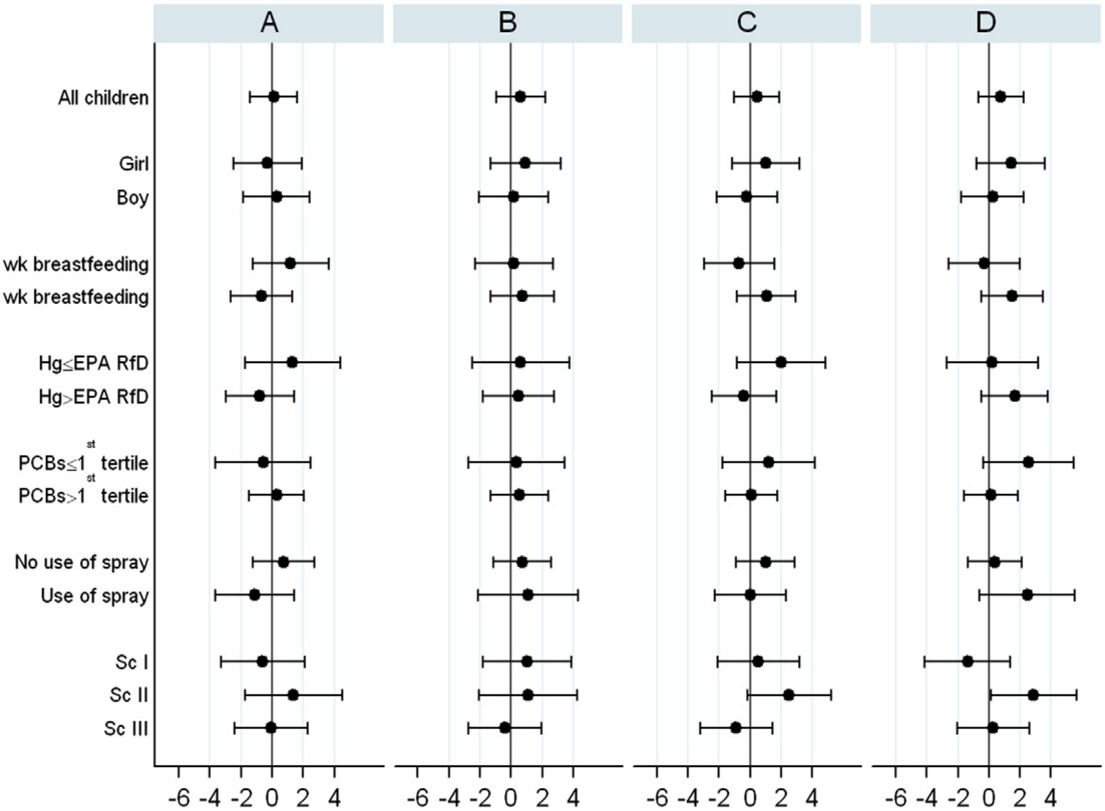

Fig. 2. Effect of potential modifiers on coefficients for associations between the use of plug-in devices and children's neuropsychological development, INMA Project, Spain, 2003–2010. A: Use of plug-in devices during pregnancy and effect on mental development. B: Use plug-in devices during infancy and effect on mental development. C: Use of plug-in devices during pregnancy and effect on psychomotor development. D: Use of plug-in devices during infancy and effect on psychomotor development (mental development is adjusted by body mass index before pregnancy, maternal age, educational level, country of birth, social class, sex, low birth weight, weeks of gestation, main child-care provider, and psychologist. Psychomotor development is adjusted by maternal age, country of birth, social class, body mass index before pregnancy, weeks of gestation, number of siblings, small-for-gestational-age SGA in length, nursery attendance, paternal smoking during the child's first year, and psychologist. wk: week; EPA RfD = 6.4 μ/L;1st tertile of total PCBs = 0.48 ng/mL; Sc: Social class).

workers in the flower industry had higher salaries and better access to health care than women working at home or in other occupations. The use of insecticides at home may imply an added expense for the family, which would only be available for those with economic security.

We observed a relation between the type of insecticide application and the socio-economic class of women; women who belonged to a lower social class had higher odds of using insecticide sprays than those from the upper classes. We controlled by socio-economical class in the analysis but we cannot exclude some residual confounding mainly related to the socio-family environment. The stratified analysis showed that children whose mothers belonged to the lowest social class and use insecticide sprays during pregnancy had worse scores in the psychomotor development test. This could be related to the habit of using insecticide sprays (higher among the most deprived families) and also to poor neighborhood conditions (old houses with more presence of crops and poor ventilation). Other studies have found an association between socio-economic class and housing conditions with increased pest levels and subsequent increases in insecticide usage (Rauh et al., 2002; Whyatt et al., 2002).

Regarding other possible modifying factors that could influence the relation between exposure variables and neuropsychological development of children, we found that female sex of children, higher cord blood mercury levels and maternal PCBs levels during pregnancy may enhance the negative effect of insecticide exposure by sprays application during pregnancy. Girls whose mothers used insecticide sprays during pregnancy had lower scores in the psychomotor development evaluation than boys. We observed the same modifying effect of sex in previous studies in the INMA cohort, specifically when we studied the adverse effect of prenatal exposure to mercury on children's psychomotor development (Llop et al., 2012). However, these results should be interpreted with caution and must be confirmed in further studies.

Two known neurotoxic substances, mercury and polychlorinated biphenyl (PCBs), seem to enhance the deleterious effects of insecticide sprays; children with cord blood total mercury levels above the reference dose proposed by the EPA from the USA and whose mothers had used insecticide sprays during pregnancy obtained the worst scores in psychomotor tests. The same negative effect was found among children whose mothers had total PCBs levels above 1st tertile during pregnancy, although in both cases p of interaction was not statistically significant. Deleterious effect of prenatal exposure to mercury and PCBs on cognitive development has been reported before (Forns et al., 2012; Grandjean and Landrigan, 2006; Grandjean et al., 1997), and we hypothesized that there could be a neurotoxic synergistic effect among these two pollutants and insecticides exposure, however this must be confirmed in further studies.

Postnatal use of plug-in devices showed a positive coefficient with psychomotor development of children when the study population was stratified according to social class. The reason for that unexpected tendency may be a misclassification of the exposure variable.

Experimental laboratory studies using model compounds suggest that many insecticides currently used in Europe could cause neurodevelopmental toxicity (Shafer et al., 2005). These studies suggest that the toxic mechanisms of pyrethroids are related to the disturbance of kinetics of voltage-gated sodium channels and that perturbation during development of the nervous system could affect its structure and function. The main pathway of carbamates and organophosphates is related to the inhibition of acetylcholinesterase enzyme, which hydrolyses the neurotransmitter acetylcholine. Acetylcholine and other neurotransmitters play unique trophic roles in the development of the central nervous system (Bjorling-Poulsen et al., 2008).

There are some limitations to our questionnaire; i.e., the lack of information about ventilation or cleaning surfaces after the treatment, the presence of children, and pet applications. Some studies have found a good correlation between the information obtained from a questionnaire and the information obtained by monitoring biological samples (Colt et al., 2004; Lu et al., 2006). However, other studies did not (Berkowitz et al., 2003; Sexton et al., 2003). A future goal in the INMA study will be to evaluate this type of association, since we have biological samples available that were obtained during pregnancy. The longitudinal character of this study made it possible to obtain sufficient information concerning maternal and child characteristics that may affect mental and psychomotor development, as well as those that may act as confounders of exposure.

5. Conclusion

This study provides some evidence of adverse effects of using insecticide sprays during pregnancy on the psychomotor development of children in the INMA cohort. The findings suggest that current use of insecticides in Europe, probably at low exposure levels, and particularly spray application, is deleterious for the developing nervous system. Postnatal uses do not show any adverse effect such as plug-in devices at any exposure period. Further research is needed to assess long-term neuropsychological delays and extended measures of the exposure. Additionally, more investigation is required in order to understand the possible modifiers of such toxicity.

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