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Prenatal exposure to DDE and infant's lower respiratory tract infections and wheeze

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ABSTRACT

Objective: to examine whether prenatal exposure to dichlorodiphenyldichloroethylene (DDE) increases the risk of lower respiratory tract infections (LRTI) and wheeze in infants.

Methods: the study is based on a birth cohort of 1455 mother-child pairs. Maternal serum concentrations of DDE, polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) were measured during pregnancy. Parental reports on LRTI and wheeze were obtained when children were 12-14 months old.

Results: 35.4% of children developed at least one LRTI episode and 33.6% at least one wheezing episode during their first 12-14 months of life. Median DDE, PCBs, and HCB concentrations were 116.3, 113.7 and 46.4 ng/g lipid, respectively. DDE concentrations were associated with LRTI risk (relative risk (RR) per 10% increase (95%CI) 1.11 (1.00, 1.22)), also after adjustment for PCBs and HCB. In all quartiles of DDE exposure the risk of LRTI was increased compared to the lowest quartile, but the increase was statistically significant only in the 3rd quartile (RR (95%CI) 1.33 (1.08, 1.62)). No association was observed for PCBs and HCB. Results were similar for wheeze.

Conclusion: This study suggests that prenatal DDE exposure is associated with a higher risk of LRTI and wheeze in infants independently from exposure to other organochlorine compounds.

Keywords: dichlorodiphenyldichloroethylene, children, low respiratory tract infections, wheeze, organochlorine compounds, prenatal exposure.

INTRODUCTION

Acute respiratory infections (ARI) are a worldwide cause of morbidity and mortality in children less than five years old [1]. Lower respiratory tract infections (LRTI), mainly pneumonia and bronchiolitis, are considered to be the major components that account for the global burden of disease from ARI among young children. Several risk factors have been reported to increase vulnerability to LRTI during infancy and childhood, such as tobacco exposure, type and duration of breastfeeding and familiar history of atopy or allergic asthma [2, 3]. Moreover, growing evidence suggests that prenatal exposure to organochlorine compounds (OCs), mainly polychlorinated biphenyl 153 (PCB-153) and dichlorodiphenyldichloroethylene (DDE), may increase the risk of respiratory symptoms during the first years of life, even at low exposure levels [4-9]. In addition, LRTI are one of the major risk factors to develop asthma later in life [2, 10] and prenatal DDE exposure has also been associated with asthma and wheezing in children aged 4 [11] and 6 years [12].

OCs are synthetic persistent organic pollutants worldwide distributed throughout the environment, food and human tissues. Immunologic effects of OCs have been reported in studies conducted both in animals [13-16] and humans [17-20]. However, previous epidemiological studies on the association between OCs and LRTI have not been able to clearly determine which compound (PCBs, DDE, or other OCs) was responsible for these effects due to the high correlation between concentrations of individual compounds [5, 6, 8]. Sunyer et al., in a birth cohort study in Sabadell (Catalonia, Spain), were the first to indicate DDE as the main responsible compound [9], but the study was too small to draw strong conclusions or to examine the role of other risk factors as possible effect modifiers.

In this study we use a larger Spanish birth cohort, including the previous Sabadell study, to: 1) provide more precise estimates for the effect of prenatal DDE exposure on occurrence of LRTI and wheeze in infants, 2) isolate these effects from those of other OCs, including hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs), and 3) explore the role of other risk factors in this association: maternal smoking, maternal history of atopy and allergic asthma, and breastfeeding practices. In addition, since recent studies suggest that a high level of adherence to the Mediterranean diet during

pregnancy protects against the development of asthma and atopy in children [21], maternal diet during pregnancy is also explored as a possible effect modifier.

METHODS

Study population

This study is based in three Spanish regions (Gipuzkoa - Basque Country, Sabadell - Catalonia, and Valencia - Valencia) belonging to the INMA -INfancia y Medio Ambiente (Environment and Childhood) – Project in Spain[22]. All regions followed the same protocol and started recruiting pregnant women into the cohort between 2004 and 2008 (Sabadell N=657, Valencia N=855, Gipuzkoa N=638). Pregnant women coming to the first trimester routine antenatal care visit in the main public hospital or health centre of reference and who fulfilled the inclusion criteria (age above 16 years, intention to deliver in the city, and no problems of communication) were recruited. Protocol details are described elsewhere[22]. This study was conducted with the approval of the hospital ethics committees in the participating regions and written informed consent was obtained from the parents of all children.

Outcomes

Information about physician-confirmed diagnosis of LRTI was obtained from parents through questionnaires when children were one year old (mean (SD) Valencia=12.4 months (1.1), Gipuzkoa=14.3 months (1.2) and Sabadell=14.5 months (0.7)). Occurrence of an LRTI episode was defined as a positive answer to both a general question ("Since the last interview, has the doctor told you that your child has had a chest infection?"), and a specific question on the type of infection (bronchiolitis, bronchitis and pneumonia) determined by the doctor. Children with negative answers to both questions were defined as non-having LRTI, and those reporting positive answers to both questions were defined as having LRTI. Those whose answers to both questions did not match up (N=58) were excluded from the study. Wheezing was defined as a positive answer to the question 'Has your child ever experienced whistling or wheezing from the chest, but not noisy breathing from the nose since birth to 12/14 months?". All these questions were based on the validated ISAAC questionnaire [23].

Exposure assessment

Concentrations of OCs (HCB, p,p'-DDE, and PCB congeners 28, 118, 138, 153 and 180) in maternal serum extracted between the 7th week and the 26th week of pregnancy (median=12.9 weeks) from peripheral veins, were stored in crystal tubes at 20ºC and analyzed with gas chromatograph using methods described elsewhere [24]. The limits of detection (LOD) were 0.071 ng/ml in Sabadell and Gipuzkoa and between 0.01 and 0.071 ng/ml in Valencia. International intercalibration exercises showed that differences of levels between regions were not due to lab differences. For comparison purposes, values in Valencia below 0.071 ng/ml were set as non-detected. Samples with non-detectable levels were then set at a value of half the LOD. The sum of PCBs (ΣPCBs) was calculated by summing the concentrations of all individual congeners except PCB28, which was detectable in less than 1% of samples. PCB138, 153 and 180 were the predominant congeners. All exposures are expressed on a lipid basis in ng/g lipid using the method described elsewhere [25]. Correlations between lipid adjusted and not adjusted values were high (0.97 for p,p'-DDE and 0.95 for ΣPCBs).

Other variables

Information on co-variables was extracted from the questionnaires answered by the mothers during the 3rd trimester of pregnancy and at age 12-14 months. Covariables of interest for the current study included: maternal age, social class (based on International Standard Classification of Occupations), education and country of origin of the mother, maternal smoking during pregnancy, maternal smoking during the year after birth, parity (first child or not), day care attendance, duration of predominant breastfeeding (never breastfeeding, breastfeeding 1-16 weeks, 17-24 weeks, >24 weeks), maternal history of atopy and/or allergic asthma, and maternal consumption of meat, fish and vegetables during pregnancy (divided into tertiles). Since maternal atopy and allergic asthma were highly correlated ($p<0.001$), we combined them into a new single variable: "atopic-asthmatic mother". Pre-pregnancy weight of the mother, gestational age and weight at birth were collected from clinical records or reported by mothers.

Statistical methods

Out of the initial population of recruited mother-child pairs (N=2150) 279 were lost to follow up at the time of the age 12-14 month's visit and 416 had missing information for exposure to OCs, one of the outcomes or country of origin, resulting in 1455

mother-child pairs with complete exposure-outcome information. Because of their very different exposure profiles, analyses were performed separately for Spanish mothers (N=1342) and Latin-American mothers (N=79); mothers of other origin were not included (N=34).

Some of the covariables of interest for our analysis had missing information (between 0.1% and 3.4%). These missing values were imputed by multiple imputation[26]. This method is based on conditioning the missing variables density to given predictor variables, which in our case were country of origin, parity, gestational age, maternal age, maternal pre-pregnancy weight, maternal social class and maternal education, sex and birth weight, duration of predominant breastfeeding, day care attendance, smoking during pregnancy or one year after birth, being an atopic-asthmatic mother, maternal consumption of meat, fish and vegetables during pregnancy and lipid and OCs levels in maternal blood. These imputations were done separately by region of study.

A log-binomial regression model was used to analyze the relationship between concentrations of DDE, HCB and ΣPCBs with LRTI and wheezing. Generalized Additive Models (GAMs) were used to graphically examine the shape of relationships between OCs exposure and outcome variables. These did not show statistically significant evidence for a departure from (log)linear relationships (p-values for gain in linearity between 0.12 and 0.23) (Figure 1). However, since graphically the evidence was not very strong, especially for PCBs, we performed analyses using OCs concentrations both as (log-transformed) continuous exposure variable and as exposure categories using quartiles as cut-offs. Potential confounder variables were included one by one in the model. Variables were retained in the final model if they were related to the outcome ($p<0.2$), or changed the β-coefficient for the relationship between exposure and outcome by more than 10%. Variables that did not meet these criteria, but which were considered important risk factors for LRTI or wheezing were included in the final model as well (maternal smoking during pregnancy, maternal smoking during the year after birth, social class and duration of predominant breastfeeding). Co-variables included in the final models for each outcome are indicated in the results tables (Table 4 and 5).

Figure 1. Generalized Additive Models to examine the shape of relationships between OCs exposure and lower respiratory tract infections: a) DDE (p-value for gain in linearity=0.23), b) HCB (p-value=0.12), c) ΣPCBs (p-value=0.13).

DDE= dichlorodiphenyldichloroethylene, HCB= hexachlorobenzene, PCBs= polychlorinated biphenyls.

The influence of multi-pollutants on the relationship between DDE, HCB or ΣPCBs and LRTI was examined by including these compounds in one model together. Given that some characteristics of the participants and the mixture of OCs varied by region, sensitivity analyses were carried out stratifying by region. Analyses with the one pollutant model were further stratified by potential effect modifiers such as atopic-asthmatic mother, maternal smoking during pregnancy, maternal smoking 1 year after pregnancy, duration of predominant breastfeeding, consumption of vegetables and fruit, and fish consumption during pregnancy. Wald tests were used to test the statistical significance of interaction terms. Since a similar analysis of the Sabadell cohort has been published [9] we performed a sensitivity analysis excluding subjects from Sabadell.

Separate analyses for the Latin American population followed the same methodology as those for the Spanish population. Tertiles of DDE concentrations were created instead of quartiles, because of the small population. HCB and the ΣPCBs were not analyzed due to the very low concentrations detected within this population (below the LOD in 54.4% and 78.5% of samples). All analyses were conducted using STATA 10.

RESULTS

Spanish population (main analysis)

There were significant differences between Spanish mother-child pairs included in the main analyses and those excluded (Appendix 1); mothers included were older, had a higher education level, a higher pre-pregnancy weight and smoked less. They also ate more fruit, vegetables and fish and breastfed their children for longer. Among included subjects, there were less preterm and low birth weight children and a higher percentage of wheezing cases. Maternal prenatal concentrations of HCB and ΣPCBs were significantly higher among included participants, but DDE concentrations and prevalence of LRTI did not significantly differ from excluded subjects (p=0.21 and p=0.32, respectively).

Table 1. Characteristics of the Spanish study population by low respiratory tract infection (LRTI) and by wheezing status during the 12-14 months of life (N=1342).

Characteristics[#]	LRTIs			Wheeze		
	Never (N=867)	**Ever (N=475)**	**p-value**	**Never (N=891)**	**Ever (N=451)**	**p-value**
Characteristics of the children						
Male Sex (%)	48.3	58.2	0.00	48.1	58.4	0.00
Preterm (<37 weeks, %)	3.5	4.4	0.38	4.0	3.6	0.73
Low birth-weight (<2500g, %)	5.7	4.7	0.42	5.8	4.5	0.32
Predominant breastfeeding (%)			0.56			0.06
0 weeks	18.4	20.4		17.8	21.7	
1-16 weeks	33.1	32.1		31.5	35.4	
17-24 weeks	36.8	34.4		38.1	31.6	
>24 weeks	11.7	13.1		12.6	11.4	
Day-care attendance	30.3	41.6	<0.001	30.3	42.2	<0.001
Region (%)			0.01			0.00
Gipuzkoa (N=500)	36.7	38.3		35.9	39.9	
Sabadell (N=455)	31.7	37.9		32.2	37.3	
Valencia (N=387)	31.6	23.8		31.9	22.8	
Characteristics of the mother						
Age, years (mean, SD)	30.7 (4.1)	31.1 (3.7)	0.13	30.8 (4.1)	30.9 (3.7)	0.74
Pre-pregnancy weight, kg (mean, SD)	62.3 (11.3)	63.4 (12.2)	0.12	62.6 (11.7)	62.9 (11.6)	0.34
Social class (%)			0.92			0.08
Non-manual jobs	76.6	75.6		77.7	73.4	
Manuals jobs	22.6	23.6		21.3	26.2	
Unknown or unclassifiable	0.8	0.8		1.0	0.4	
Education (%)			0.87			0.41
Primary school	23.3	24.3		22.6	25.8	
Secondary	39.7	39.9		40.4	38.5	
University	37.0	35.8		37.0	35.7	
Smoking during pregnancy (%)	16.5	17.4	0.70	14.6	21.2	0.00
Smoking during the first year (%)	25.9	28.1	0.43	24.6	30.8	0.02
Maternal allergy and/or asthma (%)	24.5	32.3	0.00	25.6	30.4	0.07
Parity (First child, %)	61.3	43.8	0.00	60.6	44.4	0.00
Diet of the mother (g/day, mean, SD)						
Meat	113.2 (42.3)	113.1 (41.3)	0.96	112.5 (41.9)	114.4 (42.0)	0.44
Fish	66.1 (29.4)	68.0 (31.0)	0.27	66.1 (29.9)	68.0 (30.2)	0.27
Vegetables & fruit	516.7 (211.2)	515.6 (216.0)	0.93	520.1 (213.3)	508.7 (211.8)	0.36

[#]Percentages or mean and standard deviation (sd) are presented based on imputed data. Missing data in this population (N=1342) varied between 0 and 0.7% for most of the co-variables, except for smoking during pregnancy (1.4%) and during the first year of life (1.3%) and breastfeeding duration (3.5%).

A total of 35.4% and 33.6% of the children had at least one episode of LRTI or wheezing, respectively, during their first year of life (Table 1). LRTI and wheeze were highly correlated, with 76% of children with LRTI also reporting wheezing symptoms. Boys, children attending to day care attendance, with maternal atopy-asthma and smoking, with multiparous mothers and breastfeeding for a shorter period had a higher risk of ever having LRTI and/or wheezing symptoms (Table 1).

Maternal levels of DDE were higher among preterm, low birth weight children and not attending to day care services (Table 2). Mothers with higher levels of DDE were older, had a higher pre-pregnancy weight and had lower education levels. Higher consumption of meat was related to higher DDE maternal levels as well. In general, concentrations of HCB were much lower (46.4 ng/g lipid) than DDE (116.3 ng/g lipid) or ΣPCBs (113.7 ng/g lipid) (Table 3). Correlation coefficients were: 0.43 (DDE and ΣPCBs), 0.49 (DDE and HCB) and 0.40 (ΣPCBs and HCB), all with $p<0.001$.

Table 2. Geometric mean (GM) and Geometric standard deviation (GSD) of the concentrations[#] of DDE, HCB and ΣPCBs (ng/g lipids) by characteristics of the Spanish study population (N=1342[*†]).

	%[ǂ]	DDE GM (GSD)	HCB[*] GM (GSD)	ΣPCBs[†] GM (GSD)
Characteristics of the children				
LRTIs				
Never	64.6	117.9 (2.1)	44.2 (2.3)	113.4 (1.7)
Ever	35.4	122.1 (2.2)	44.5 (2.3)	110.6 (1.8)
Wheezing				
Never	66.4	117.9 (2.2)	44.5 (2.3)	113.2 (1.8)
Ever	33.6	122.2 (2.2)	43.9 (2.3)	110.7 (1.7)
Sex				
Male	51.8	121.0 (2.2)	44.3 (2.3)	112.8 (1.8)
Female	48.3	117.6 (2.1)	44.3 (2.3)	112.0 (1.7)
Preterm (<37 weeks)				
Yes	3.8	156.1 (2.2)**	48.6 (2.5)	141.2 (1.7)***
No	96.2	118.1 (2.2)	44.1 (2.3)	111.4 (1.7)
Low birth-weight (<2500g)				
Yes	5.3	142.7 (2.2)**	44.9 (2.6)	115.0 (1.8)
No	94.7	118.2 (2.6)	44.3 (2.3)	112.2 (1.7)
Predominant breastfeeding				
0 weeks	18.6	122.9 (2.1)	51.3 (2.3)***	111.3 (1.7)
1-16 weeks	33.5	118.4 (2.3)	46.0 (2.4)	109.4 (1.7)
17-24 weeks	35.7	117.4 (2.1)	40.3 (2.2)	112.5 (4.7)
>24 weeks	12.2	122.8 (2.0)	42.1 (2.2)	122.3 (1.8)
Day-care attendance				

Yes	34.3	109.8 (2.1)***	42.0 (2.2)*	121.4 (1.7)***
No	65.7	124.6 (2.2)	45.5 (2.4)	107.9 (1.8)
Region				
Gipuzkoa (N=500)	37.3	92.8 (2.1)***	34.6 (2.1)***	130.2 (1.6)***
Sabadell (N=455)	33.9	112.6 (2.0)	41.9 (2.0)	82.5 (1.6)
Valencia (N=387)	28.8	176.8 (2.1)	65.0 (2.6)	134.6 (1.8)
Characteristics of the mother				
Age, years				
<29	26.5	98.7 (2.1)***	33.2 (2.2)***	79.8 (1.8)***
29-31	31.7	115.2 (2.0)	42.6 (2.2)	109.1 (1.6)
32-33	17.4	123.6 (2.2)	50.8 (2.3)	128.8 (1.6)
>34	24.4	149.7 (2.3)	57.6 (2.3)	153.5 (1.6)
Pre-pregnancy weight, kg				
<57	36.1	110.4 (2.2)**	37.2 (2.4)***	120.0 (1.7)***
57-65	33.3	123.3 (2.1)	42.5 (2.3)	115.1 (1.7)
>65	30.6	126.3 (2.2)	56.8 (2.2)	101.3 (1.8)
Social class				
Non-manual occupation	76.2	117.7 (2.1)	44.3 (2.3)	113.8 (1.7)
Manuals occupation	23.0	126.5 (2.2)	44.3 (2.4)	107.5 (1.8)
Unknown or unclassifiable	0.8	87.6 (4.1)	46.7 (3.2)	124.0 (2.3)
Education				
Primary school	23.7	132.5 (2.3)**	44.1 (2.6)	104.0 (1.8)***
Secondary	39.7	117.1 (2.2)	45.0 (2.3)	105.6 (1.8)
University	36.6	113.9 (2.1)	43.6 (2.2)	126.4 (1.6)
Smoking during pregnancy				
Yes	16.8	125.4 (2.1)	44.5 (2.6)	113.8 (1.7)
No	83.2	118.3 (2.2)	44.2 (2.3)	112.1 (1.7)
Smoking during the first year				
Yes	26.7	124.6 (2.2)	45.0 (2.4)	107.1 (1.8)*
No	73.3	117.4 (2.1)	44.0 (2.3)	114.4 (1.7)
Maternal allergy and/or asthma				
Yes	27.2	122.0 (2.1)	45.1 (2.2)	112.4 (1.8)
No	72.8	118.4 (2.2)	44.0 (2.3)	112.4 (1.7)
Parity‡				
Nulliparous	55.1	122.4 (1.0)	47.2 (1.0)***	116.6 (1.0)***
Multiparous	44.9	115.7 (1.0)	40.9 (1.0)	107.4 (1.0)
Diet of the mother (g/day)				
Meat				
<94.14	33.4	110.9 (2.1)**	39.9 (2.3)***	119.5 (1.7)***
94.14-128.8	33.3	118.6 (2.2)	46.5 (2.2)	114.3 (1.8)
>128.8	33.3	129.2 (2.2)	46.8 (2.4)	103.9 (1.7)
Fish				
<51.6	33.4	116.3 (2.2)	44.7 (2.4)	104.5 (1.8)***
51.6-75.5	33.3	118.9 (2.1)	45.2 (2.2)	115.9 (1.7)
>75.5	33.3	123.0 (2.2)	43.0 (2.3)	117.2 (1.7)
Vegetables & fruit				
<411.0	33.4	113.9 (2.2)	42.2 (2.4)	104.2 (1.7)***
411.0-582.6	33.3	118.0 (2.0)	46.1 (2.2)	117.6 (1.7)

>582.6	33.3	126.5 (2.3)	44.6 (2.3)	115.8 (1.8)

DDE= dichlorodiphenyldichloroethylene, HCB= hexachlorobenzene, PCBs= polychlorinated biphenyls.
‡Percentages are presented based on imputed data.
#As concentrations of all compounds were not normally distributed, these were log-transformed before calculating differences of exposure between groups of each characteristic.
*One child was excluded from the analysis with HCB because it was an outlier (N=1341).
†Two children had no information for PCBs within the Spanish population and one was excluded from the analysis with ΣPCBs because it was an outlier (N=1339).
‡ Adjusted for maternal age at delivery.
*p-value<0.10, **p-value<0.05, ***p-value<0.01

Table 3. Percentage of samples below de limit of detection (LOD) and concentrations (ng/g lipid) of DDE, HCB and PCBs in the Spanish (N=1342*†) and the Latin-American (N=79) populations.

	Spanish population†		Latin-American population	
	% < LOD	**Median (25^{th}, 75^{th})**	**% < LOD**	**Median (25^{th}, 75^{th})**
DDE	0.8	116.3 (72.6, 191.7)	0.0	385.0 (146.2, 953.8)
HCB*	5.8	46.4 (26.4, 79.0)	60.8	6.9 (5.8, 18.8)
PCB180	5.2	32.5 (21.7, 47.7)	65.8	6.3 (5.3, 9.3)
PCB153	2.2	45.1 (31.5, 63.2)	54.4	7.0 (5.8, 15.2)
PCB138	10.0	27.1 (17.7, 39.2)	72.2	6.3 (5.3, 12.2)
PCB118	76.5	6.4 (5.6, 7.8)	78.5	6.0 (5.3, 7.2)
Σ PCBs†	NA	113.7 (79.4, 158.6)	NA	27.9 (23.4, 45.2)

DDE= dichlorodiphenyldichloroethylene, HCB= hexachlorobenzene, PCBs= polychlorinated biphenyls.
*One child was excluded from the analysis with HCB because it was an outlier (N=1341).
†Two children had no information for PCBs within the Spanish population and one was excluded from the analysis with ΣPCBs because it was an outlier (N=1339).
NA= not applicable.

Associations between the co-variables in the final regression models and the outcomes are presented in the appendix section (Appendix 2). Risk of LRTI increased with increasing DDE exposure and was statistically significant after adjustment for potential confounders (crude RR for 10% increase in DDE concentration (95%CI)= 1.04 (0.95, 1.14) and adjusted RR (95%CI)= 1.11 (1.00, 1.22)). Adjustment for other pollutants gave a RR of borderline statistical significance (RR (95%CI)= 1.11 (0.99, 1.24)). In all quartiles of DDE the risk of LRTI was increased compared to the lowest quartile, but the increase was statistically significant only in the 3rd quartile (adjusted relative risk (RR) (95%CI)= 1.33 (1.08, 1.62)). This increase remained after adjustment for other OCs (RR (95%CI) for 3rd quartile DDE= 1.40 (1.13, 1.73)) (Table 4). Prenatal HCB levels did not increase the relative risk of LRTI. In the multi-pollutant model, the risk of LRTI in the highest quartile of PCB exposure was statistically significantly lower

than in the lowest quartile (Table 4). Risk estimates for wheezing were very similar to those found for LRTI (Table 5).

Table 4. Number of total children (N), cases of LRTI during the first 12-14 months of life (%) and crude and adjusted relative risk (RR (95% Confidence Interval)) for continuous exposure and for each quartile of DDE, HCB and ΣPCBs exposure within the Spanish study population (N=1342[*†]).

	Exposure level (ng/g lipid)	N (% LRTI cases)	Crude RR (95%CI)	p-value	Adjusted RR[#] (95%CI)	p-value	Multipollutant adjusted RR[‡](95%CI)	p-value
DDE								
Continuous		1342 (35.4)	1.04 (0.95, 1.14)	0.43	1.11 (1.00, 1.22)	0.05	1.11 (0.99, 1.24)	0.07
Q1	<72.6	336 (32.4)	1		1		1	
Q2	72.6-115.9	335 (35.5)	1.10 (0.89, 1.35)	0.40	1.16 (0.94, 1.43)	0.16	1.20 (0.97, 1.48)	0.09
Q3	115.5-191.7	336 (39.9)	1.23 (1.00, 1.51)	0.05	1.33 (1.08, 1.62)	0.01	1.40 (1.13, 1.73)	<0.01
Q4	>191.7	335 (33.7)	1.04 (0.84, 1.29)	0.72	1.20 (0.96, 1.51)	0.11	1.28 (1.00, 1.64)	0.04
HCB[*]								
Continuous		1341 (35.4)	1.00 (0.92, 1.10)	0.87	1.06 (0.95, 1.17)	0.30	1.03 (0.92, 1.15)	0.63
Q1	<26.4	336 (35.4)	1		1		1	
Q2	26.4-46.4	335 (34.9)	0.98 (0.80, 1.21)	0.89	0.96 (0.79, 1.18)	0.72	0.95 (0.78, 1.17)	0.65
Q3	46.4-79.0	335 (37.0)	1.04 (0.85, 1.28)	0.67	1.08 (0.88, 1.32)	0.47	1.03 (0.84, 1.29)	0.74
Q4	>79.0	335 (34.3)	0.97 (0.79, 1.19)	0.77	1.06 (0.84, 1.33)	0.63	1.03 (0.80, 1.33)	0.80
ΣPCBs[†]								
Continuous		1339 (35.3)	0.95 (0.83, 1.08)	0.44	1.00 (0.86, 1.18)	0.93	0.92 (0.77, 1.11)	0.39
Q1	<79.4	335 (37.9)	1		1		1	
Q2	79.4-113.7	335 (36.1)	0.95 (0.78, 1.16)	0.63	0.98 (0.80, 1.20)	0.83	0.90 (0.73, 1.11)	0.34
Q3	113.7-158.6	335 (35.5)	0.94 (0.77, 1.14)	0.52	0.94 (0.75, 1.17)	0.56	0.83 (0.66, 1.05)	0.13
Q4	>158.6	334 (31.7)	0.84 (0.70, 1.03)	0.10	0.84 (0.65, 1.08)	0.17	0.73 (0.56, 0.95)	0.02

DDE= dichlorodiphenyldichloroethylene, HCB= hexachlorobenzene, PCBs= polychlorinated biphenyls.
[#]Adjustment: region, sex of the child, age and pre-pregnancy weight of the mother, allergic or asthmatic mother, parity (first child) and social class, predominant breastfeeding, maternal smoking status (during pregnancy and during the 1st year of life of the child) and day-care attendance during the first year of life.
[‡] Also adjusted for DDE, HCB and ΣPCBs (N=1338).
[*]One child was excluded from the analysis with HCB because it was an outlier (N=1341).
[†]Two children had no information for PCBs within the Spanish population and one was excluded from the analysis with ΣPCBs because it was an outlier (N=1339).

Table 5. Number of total children (N), cases of wheezing during the first 12-14 months of life (%) and crude and adjusted relative risk (RR (95% Confidence Interval)) for continuous exposure and for each quartile of DDE, HCB and ΣPCBs exposure within the Spanish study population (N=1342[†]).

Exposure quartile	Exposure level (ng/g lipid)	N (% Wheezing cases)	Crude RR (95%CI)	p-value	Adjusted RR[#] (95%CI)	p-value	Multipollutant adjusted RR[‡](95%CI)	p-value
DDE								
Continuous		1342 (33.6)	1.04 (0.94, 1.15)	0.42	1.09 (0.99, 1.21)	0.08	1.14 (1.01, 1.28)	0.03
Q1	<72.6	336 (31.9)	1					
Q2	72.6-115.9	335 (32.5)	1.02 (0.82, 1.27)	0.85	1.10 (0.88, 1.36)	0.41	1.12 (0.90, 1.40)	0.31
Q3	115.5-191.7	336 (38.4)	1.21 (0.98, 1.48)	0.08	1.30 (1.06, 1.59)	0.01	1.37 (1.10, 1.70)	<0.01
Q4	>191.7	335 (31.6)	0.99 (0.80, 1.24)	0.96	1.11 (0.89, 1.40)	0.35	1.20 (0.94, 1.55)	0.15
HCB[*]								
Continuous		1341 (33.6)	0.99 (0.90, 1.08)	0.78	1.02 (0.93, 1.12)	0.69	1.00 (0.90, 1.13)	0.91
Q1	<26.4	336 (33.3)	1		1		1	
Q2	26.4-46.4	335 (34.0)	1.02 (0.83, 1.26)	0.85	0.98 (0.80, 1.21)	0.86	0.98 (0.79, 1.21)	0.84
Q3	46.4-79.0	335 (34.3)	1.03 (0.83, 1.27)	0.79	1.05 (0.86, 1.30)	062	1.03 (0.83, 1.29)	0.77
Q4	>79.0	335 (32.8)	0.99 (0.79, 1.22)	0.89	1.05 (0.85, 1.31)	0.64	1.06 (0.83, 1.35)	0.65
ΣPCBs[†]								
Continuous		1339 (33.5)	0.95 (0.83, 1.09)	0.49	0.94 (0.81, 1.09)	0.41	0.86 (0.72, 1.02)	0.70
Q1	<79.4	335 (34.3)	1		1		1	
Q2	79.4-113.7	335 (35.2)	1.03 (0.83, 1.26)	0.80	1.02 (0.84, 1.25)	0.83	0.95 (0.77, 1.18)	0.66
Q3	113.7-158.6	335 (34.3)	1.00 (0.81, 1.23)	1.00	0.96 (0.78, 1.19)	0.73	0.86 (0.68, 1.09)	0.21
Q4	>158.6	334 (30.2)	0.88 (0.71, 1.10)	0.26	0.85 (0.67, 1.07)	0.17	0.75 (0.58, 0.97)	0.03

DDE= dichlorodiphenyldichloroethylene, HCB= hexachlorobenzene, PCBs= polychlorinated biphenyls.
[#]Adjustment: region, sex of the child, allergic or asthmatic mother, parity (first child) and social class, predominant breastfeeding, maternal smoking status (during pregnancy and during the 1st year of life of the child) and day-care attendance during the first year of life.
[‡] Also adjusted for DDE, HCB and ΣPCBs (N=1338).
[*]One child was excluded from the analysis with HCB because it was an outlier (N=1341).
[†]Two children had no information for PCBs within the Spanish population and one was excluded from the analysis with ΣPCBs because it was an outlier (N=1339).

The association between DDE and LRTI did not differ between strata defined by region (Appendix 3), duration of predominant breastfeeding, maternal smoking during pregnancy or the first year of life, atopic-asthmatic mother or by maternal consumption of vegetables and fruit, meat or fish (data not shown). Sensitivity analysis adjusting for

preterm births or excluding these children (N=51) from the model did not modify the results. Analysis without imputed data also provided similar results.

Latin-American population

Among mothers of Latin-American origin, DDE was detected in all samples, with a median concentration of 385.0 ng/g lipid (Table 3). The risk estimate for continuous DDE exposure in Latin American mothers was very similar to those in Spanish mothers, but did not reach statistically significance (adj RR (95%CI)= 1.14 (0.92, 1.42). When analyzing by tertiles, estimates became statistically significant after adjustment (2nd tertile of DDE, RR (95%CI)= 2.59 (1.00, 6.66) and the 3rd tertile (RR (95%CI)=2.89 (1.10, 7.55)). Results were similar for wheezing (Table 6).

Table 6. Number of total children (N), cases of LRTIs and wheezing during the first 12-14 months of life (%) and crude and adjusted relative risk (RR (95% Confidence Interval)) for continuous exposure and for each tertile of DDE within the Latin-American population (N=79).

	DDE	Levels (ng/g lipid)	N (%LRTI)	Crude RR (95%CI)	p-value	Adjusted RR[#†] (95%CI)	p-value
LRTIs							
	Continuous		79 (29.1)	1.10 (0.89, 1.37)	0.37	1.14 (0.92, 1.42)	0.23
	T1	<197.9	27 (22.2)	1		1	
	T2	197.9-595.9	26 (30.8)	1.38 (0.55, 3.47)	0.49	2.59 (1.00, 6.66)	0.05
	T3	> 595.9	26 (34.6)	1.56 (0.64, 3.79)	0.33	2.89 (1.10, 7.55)	0.03
Wheezing							
	Continuous		79 (26.6)	1.16 (0.94, 1.42)	0.17	1.20 (0.98, 1.48)	0.08
	T1	<197.9	27 (14.8)	1		1	
	T2	197.9-595.9	26 (30.8)	2.08 (0.70, 6.12)	0.19	2.34 (0.73, 7.55)	0.15
	T3	> 595.9	26 (34.6)	2.34 (0.81, 6.71)	0.12	3.54 (1.54, 8.12)	<0.01

DDE= dichlorodiphenyldichloroethylene.
[#]LRTIs model adjustment: region, sex of the child, low birth weight, predominant breastfeeding, age of the mother, parity, maternal smoking during pregnancy, being atopic-asthmatic mother and fish and vegetable maternal consumption during pregnancy.
[†]Wheezing model adjustment: region, sex of the child, low birth weight, predominant breastfeeding, age and pre-pregnancy weight of the mother, studies of the mother, smoking during the first year of life and maternal consumption of meat during pregnancy.

DISCUSSION

The present study suggests that prenatal exposure to DDE is associated with a higher risk of LRTI and wheeze in infants. The DDE effect was independent of HCB or PCBs exposure and was not clearly modified by other risk factors, including maternal smoking, maternal medical history of atopy/asthma, maternal dietary habits in

pregnancy or breastfeeding practises. These results confirm our previous findings in a subsample of the present study (using a different definition of the outcome)[9].

Only three other cohort studies, in Canada [5, 6], Menorca [11, 12], and Sweden [9], assessed respiratory infections or wheezing in young children in relation to prenatal DDE exposure, and results are inconsistent. In the Canadian cohort, with higher levels of DDE (GM=294 ng/g lipid), there was no clear increase of LRTI risk with DDE concentrations at age 6-12 months [5], but at age 5 years exposure to OCs (DDE and others assessed using PCB-153 concentrations as surrogate) did increase risk of LRTI [6]. The same study did find increased risks of upper respiratory tract infections (URTI) and/or otitis in both age groups. The Swedish study found that DDE exposure was related to a non-statistically significant decrease in LRTI risk, but DDE levels were lower to those in our study (median=88 ng/g lipid) and children were assessed at age 3 months, providing little time to develop infections[8]. In the Menorca birth cohort, with prenatal DDE median levels of around 170 ng/gr lipid, DDE was associated to a higher risk of wheeze and asthma at age 4 and 6.5 years, but not to earlier wheezing or LRTI during the first year of life [11, 12]. This somewhat conflicts our results but the number of children in the Menorca cohort (N<400) may have been too small to detect early effects. It will be important to assess the DDE effects in our present cohort at older ages. Most of the previous studies could not separate the effects of DDE on LRTI from those of other OCs [5, 6, 8], whereas our study clearly identifies DDE as the main responsible compound. This could be partly explained by the different correlations between DDE and other OCs across studies; in the Swedish [8] and the Canadian cohorts [5, 6] correlations between DDE and other OCs were between 0.66 and 0.89, whereas correlations among the non-migrant population of the present Spanish cohort were below 0.49. In our study risk of LRTI and wheezing decreased in the highest quartile of PCBs exposure; this decrease was statistically significant only after adjustment for DDE and HCB. We do not have a real explanation for these results, but this may be a chance result, unexplained confounding or a problem of multicollinearity between OCs. Our quartile results (with significant increase only in the 3rd quartile) indicate that the association between DDE and LRTI may not be strictly monotonic, even though we do observe a linear trend with continuous exposure and additional linear spline analyses did not show statistical evidence for differences between spline slopes (Appendix 4). Non-monotonic functions have also been reported by others [5, 6].

In our cohort, mothers from Latin American origin showed very different patterns of OCs exposure from Spanish mothers, with very high DDE levels, low levels of PCBs and HCB, and low correlations between DDE and these other OCs. Diet, other life-style factors, and differences in industrial development, may explain part of these differences [27], together with the fact that in Latin-America the use of dichlorodiphenyltrichloroethane (DDT), the parent compound of DDE, lasted until the late 90's for agricultural and malaria vector control purposes [28]. The similarity of our DDE effect in both Spanish and Latin American children indicates that this effect may apply widely to populations with different OCs exposure patterns. This is of especial interest since nowadays the practice of using DDT for malaria vector control is still present or planned to be introduced in many developing countries with endemic malaria [29]. However, our results are limited by the small size of Latin-American population and further studies in areas with high levels of DDE and low levels of other OCs are indicated.

The mechanisms by which DDE and other OCs may produce LRTI and wheeze are not fully understood. However, some studies have shown an association between DDE exposure levels and the uncontrolled production of cytokines and the increase of nitric oxide (NO) production in macrophages, contributing to inflammatory reactions, cytokine imbalance and immune-dysregulation [16, 17, 20]. Also, DDE has been associated to altered levels and a reduced viability and proliferation capacity of immune system cells (macrophages, lymphocytes and monocytes) [8, 17, 18], mainly through apoptosis (programmed cell death) [13, 30], which seems to be caused by oxidative stress [30]. Although apoptosis plays a very important role under normal physiological conditions, when not regulated, apoptosis can contribute to immune-dysregulation and immunodeficiency [31]. Moreover, recent studies suggest that apoptotic cells actively regulate the immune response by releasing immunosuppressive cytokines (e.g. TGFβ1) and by suppressing the secretion of pro-inflammatory cytokines (e.g. TNFα), indicating an immunosuppressive response [30, 32], which could lead to an increased risk of contracting infections. Further studies are needed to understand better the mechanisms by which DDE interferes with the immune system [31]; although cell counts serve as a general indicator of the immune status, future research should focus on the performance

of cytokine assays, since they can provide a more mechanistic examination of the effect of exposure [32].

A limitation of our study is the lack of serology or culture to confirm the lower respiratory tract infection diagnosis. However, we used repeated questionnaire items to define lower respiratory tract infection and our results were consistent whichever definition criteria was selected (data not shown). The fact that we found similar results for wheezing, a related outcome to LRTI at this age, and that co-variables in the final model were associated to the outcomes as expected, also provide strength to our results. Our cohort is somewhat selective as loss to follow-up and incompleteness of the questionnaires occurred more in younger mothers with lower educational levels [27]; this is also reflected in the differences between our included study population and the excluded mothers. It is unlikely that this has led to spurious associations, but it means that these groups of the population are underrepresented in our sample. We observed somewhat inconsistent results between the regions of our cohort (Appendix 3), but there was no evidence of heterogeneity between regions (p-value for interaction between 0.30 and 0.82) and inconsistencies may have resulted from small comparison groups, especially in the quartile analyses. Meta-analyses of the estimates of each region were performed and results were similar to those of the pooled analysis (Appendix 4). Any small differences between regions might be due to the percentage of mothers reporting LRTI or wheezing during the first year of life of their children, which was lower in Valencia than in Gipuzkoa or Sabadell. This is probably because in Valencia respiratory questionnaires were administered at around 12 months of age, instead of at 14 months in the other regions. Strengths of the current study are its prospective study design and large population size. Also, in a sensitivity analysis we were able to show that the DDE effect was not only due to the influence of the one region for which data had already been published (data not shown) [9].

CONCLUSION

The present study reinforces the hypothesis that prenatal exposure to DDE is associated with higher risk of LRTI and wheeze in infants. Since LRTI cause substantial morbidity in infancy and LRTI and wheeze are possible risk factors for subsequent childhood asthma, especial attention should be paid in countries where DDT is nowadays used for malaria control.

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figure 1

Figure 1