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Prenatal exposure to hexachlorobenzene (HCB) and reproductive effects in a multicentre birth cohort in Spain ☆,☆☆

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HIGHLIGHTS

- This is a birth cohort study carried out in three different areas of Spain.
- The median concentration of HCB in maternal serum was 45.45 ng/g lipids.
- HCB levels in maternal serum are not associated with birth size.
- HCB levels in maternal serum are not associated with gestational length.

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ABSTRACT

Objective: To investigate the possible association between birth size or gestational length and maternal serum concentrations of hexachlorobenzene (HCB) in a population exposed to background levels.
Methods: A total of 1568 mother–child pairs recruited in three Spanish areas (INMA Project) from 2004 to 2008 participated in the study. Multivariate analysis was performed between birth weight and length, weeks of gestation, preterm birth or small for gestational age and HCB concentrations in maternal serum.
Results: The median concentration of HCB was 45.45 ng/g lipids. No association was found between HCB exposure levels and birth weight (β: −50.42 [−109.88; 9.04]), birth length (β: −0.07 [−0.32; 0.18]), gestation age (HR: 1.07 [0.94; 1.22]), small for gestational age (OR: 0.95 [0.56; 1.61]) and preterm birth (OR: 0.60 [0.29; 1.28]). Results remain similar after adjustment for other organochlorines.
Conclusion: Our findings support the idea that exposure to low levels of HCB does not affect the intrauterine growth nor the duration of gestation.

Abbreviations: HCB, hexachlorobenzene; INMA, Infant and the Environment; OCs, organochlorine compounds; PCB, polychlorinated biphenyls; p,p′-DDE, 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene; GC-ECD, gas chromatograph–electron capture detector; GC-MSD, gas chromatograph–mass spectrometer detector; GC-NICI-MS, gas chromatograph–negative ion chemical ionization–mass spectrometry; SGA, small for gestational age; LMP, last menstrual period; BMI, body mass index.

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☆☆ Ethics approval: This study was conducted with the approval by an appropriated committee for each of the three cohorts. In Valencia the study was approved by the Clinical Ethical Committee of the Universitary Hospital of La Fe in Valencia 29 October 2004. In Sabadell the study was approved by the Clinical Research Ethical Committee of the Medical Assistance Municipal Institute in Barcelona 20 July 2005. In Gipuzkoa the study was approved by the Clinical Research Ethical Committee of the Donostia Hospital in San Sebastian 13 July 2005.

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1. Introduction

Organochlorine compounds (OCs) were widely used through most of the 20th century, until they were banned or their use tightly restricted in the seventies and eighties. As a consequence, the concentration of these compounds has fallen, both in the environment and in food and other biological samples (Hardell et al., 2010; Mikes et al., 2012; Schade and Heinzow, 1998). In Spain HCB was forbidden for agricultural use in 1986 and for environmental uses in 1994. Polychlorinated biphenyls (PCBs) and DDT were banned in 1986 and 1975 respectively. Given their capacity to persist in the environment and their lipophilic nature, however, these compounds tend to accumulate in the food chain and the body (bioaccumulation), being found in adipose tissue, maternal milk and blood, and umbilical blood, indicating that these compounds are transferred from mother to foetus (Bergonzi et al., 2009; Cioroiu et al., 2010; Ibarluzea et al., 2011; Patayová et al., in press; Sala et al., 2001).

The effects of intrauterine exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) or their derivatives such as p,p′-dichlorodiphenyl dichloroethene (p,p′-DDE) in terms of foetal growth and gestational age have been explored in multiple studies, with different results (Weselak et al., 2007; Wigle et al., 2008; Windham and Fenster, 2008). The results of a meta-analysis of the data from various European cohorts, including ours, indicating an association between the levels of PCB 153 exposure and birth weight have been recently published (Govarts et al., 2012).

The effects on health of HCB exposure have been less well studied. The first description of toxic effects of HCB on health was related to extreme exposure due to consumption of contaminated cereals in Turkey in the nineteen fifties, acute and chronic effects being observed (Jarrell et al., 1998, 2002). On the other hand, toxic effects of HCB on reproduction have been reported in animals (Alvarez et al., 2000). Indeed, the reproductive effects of exposure to background levels of HCB have been investigated in various epidemiological studies but the results have been inconsistent. While some authors have reported a relationship with infant length at birth (Lopez-Espinosa et al., 2011; Ribas-Fito et al., 2002) or a decrease in length of gestation (Dallaire et al., 2013; Fenster et al., 2006; Ribas-Fito et al., 2002), others have found no significant associations (Bjerregaard and Hansen, 2000; Gladen et al., 2003b; Khanjani and Sim, 2006; Sagiv et al., 2007; Torres-Arreola et al., 2003). Three studies have found an association with various measurements of foetal growth in subsets of the study population: weight and length at birth of infants of smoking mothers (Eggesbo et al., 2009), length of male newborns (Dewailly et al., 1993b) and weight of female newborns (Schade and Heinzow, 1998).

The ban on HCB as a fungicide led to a significant decrease in its environmental concentration, but it is still being used in the chemical industry and is a by-product of chlorinated solvent production; moreover, some of the HCB in the soil evaporates into the air, so it is likely that people are going to continue to be exposed to this compound at levels similar to the current concentrations for some time (Bailey, 2001; Barber et al., 2005).

Birth weight and length of gestation reflect the progression of foetal development from conception to birth, and preterm babies or those with lower birth weight have greater morbidity and mortality. Although exposure to HCB in the general population has decreased in recent years, the fact that there is some evidence of an association between exposure and foetal growth abnormalities and/or reduction in the length of gestation, makes it very important from a public health of view to thoroughly investigate these possible effects.

The objective of this study was to assess the effect of maternal blood levels of HCB exposure during the first trimester of pregnancy, on various indicators of foetal growth and pregnancy duration, taking into account the maternal exposure to other OCs, in the framework of the INMA multi-centre study (Environment and Childhood).

2. Methods

2.1. Study population

This study was based on the data from three cohorts of pregnant women (Gipuzkoa, Sabadell and Valencia) from the INMA project, a prospective follow-up study of mother–child pairs recruited in various different hospitals during the first trimester of pregnancy. The Gipuzkoa study area, located in the Basque Country, covers 519 km^2, including three narrow valleys. The population of the area is approximately 89,000 inhabitants, spread out between 25 small towns. The main activity in the area is the iron and steel industry. Sabadell is a city situated in the metropolitan area of Barcelona, with a population of nearly 200,000 inhabitants. The Valencia study area covers around 1372 km^2 including 34 municipalities, and is composed of a typically urban zone (city of Valencia), a metropolitan semi-urban area dedicated to industrial and agricultural activity, and a typically rural zone (a total of 300,000 inhabitants) (Fig. 1). Details of the study design and protocols used have been described previously (Fernandez et al., 2007). The recruitment period ran from February 2004 to February 2008. The inclusion criteria were: maternal age ≥ 16 years old, single pregnancy, recruitment during the first trimester of pregnancy, no use of assisted reproduction techniques, planned birth in the referral hospital and no communication problems. All participants gave written informed consent and the study protocol was approved by the Ethics Committees of the participating centres. A total of 2122 women were recruited during their first trimester of pregnancy. In this study we excluded mothers who were not born in Spain (n = 211). A total of 84 cases were lost for follow-up: 29 due to miscarriages, 7 due to still births and 48 because the woman withdrew from the study. HCB exposure and birth outcome data were collected for 1568 participants (26 and 233 cases with missing data on birth or HCB exposure respectively).

2.2. Exposure assessment

Blood samples were collected in the recruitment period (1st trimester of pregnancy). Samples were processed, separated into 1-ml aliquots, and then frozen to −80 °C until analyzed. Concentrations of HCB, PCBs (28, 118, 138, 153 and 180), β and γ-hexachlorocyclohexane, and p,p'-DDE in serum were measured. Samples collected in Gipuzkoa and Sabadell were analyzed in the Gipuzkoan Basque Government Laboratory while samples collected in Valencia were analyzed in the Department of Environmental Chemistry (IDAEA-CSIC) in Barcelona.

Two improved methods for the measurement of organochlorine pesticides (OCPs) and PCBs in human serum were used in this study. The method used in the Basque Government Laboratory (Goni et al., 2007) required 500 μl of serum per sample. Initial extraction was performed using 96-well solid-phase extraction disc plates and was followed by a clean-up with silica gel/sulfuric acid. Quantification was carried out by gas chromatography with electron capture detector (GC-ECD). Gas chromatography coupled to a mass spectrometer detector (GC-MSD) was used for quantitative and qualitative confirmation. Relative standard deviation (precision) was <15% for OCPs and PCBs. In the method used in Barcelona Laboratory (Grimalt et al., 2010), compounds were liquid–liquid extracted with hexane from 1 ml of serum and extracts cleaned up with sulfuric acid prior to quantification by GC-ECD. Quantitative and qualitative confirmation was performed by gas chromatography coupled to negative ion chemical ionization mass spectrometry (GC-NICI-MS). Precision measured as relative standard deviation was <14% for all the compounds. Both laboratories were in compliance with the Arctic Monitoring and Assessment Program (AMAP) Ring Test Proficiency Program for persistent organic pollutants in human serum (Centre de Toxicologie Institut National de Santé Publique du Québec). Limits of detection (LOD) were 0.071 ng/ml for Sabadell and Gipuzkoa samples and between 0.010 and 0.071 ng/ml