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ORIGINAL

The influence of organochlorine compound exposure on the physiological development of children

Influències de les exposicions a composts organoclorats en el desenvolupament fisiològic dels infants

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Abstract

The present study summarizes the advances on the knowledge of the health disturbances associated to fetal exposure to organochlorine compounds in a cohort of children from Menorca. Higher incidence of diverse deleterious health effects at 4 years of age have been observed, e.g. hexachlorobenzene (HCB) and poor social behavior and attention-deficit hyperactivity disorder, 4,4'-DDE and asthma, wheeze, lower respiratory tract infections and alteration of urinary coproporphyrins, HCB, ß-hexachlorocyclohexane and 4,4'-DDE and alteration of thyroid hormones, HCB, 4,4'-DDE and polychlorobiphenyls (PCBs) and overweight, 4,4'-DDT and PCBs and lower neurodevelopment. A protective effect of breastfeeding against decreases of cognitive skills in children due to 4,4'-DDT exposure has also been documented. This protective effect shows that other factors besides pollutant exposure and genetic variability influence on the health effects of environmental pollutants into human populations. These results are important for the understanding of the health implications of exposome studies.

Keywords: Organochlorine compounds, fetal exposures, childhood exposures, DDT, polychlorobiphenyls, neurodevelopment, asthma, obesity, attention-deficit hyperactivity disorder

Resum

Aquest estudi resumeix els avenços en el coneixement dels trastorns de salut associats a l'exposició fetal a compostos organoclorats en una cohort de nens de Menorca. S'ha observat una incidència major de diversos efectes perjudicials per la salut als 4 anys d'edat, per exemple, hexaclorobenzè (HCB) i comportament social pobre i trastorn per dèficit d'atenció amb hiperactivitat, 4,4'-DDE i asma, xiulets pulmonars, infeccions de les vies respiratòries baixes i alteració de coproporfirines urinàries, HCB, ß-hexaclorociclohexà i 4,4'-DDE i alteració de les hormones tiroïdals, HCB, 4,4'-DDE i policlorobifenils (PCBs) i sobrepès, 4,4'-DDT i PCBs i menor desenvolupament neurològic. També s'ha documentat un efecte protector de la lactància materna contra la disminució de les habilitats cognitives dels nens a causa de l'exposició a 4,4'-DDT. Aquest efecte protector mostra que altres factors, a més de l'exposició a contaminants i genètica personal, influeixen en els efectes de salut dels contaminants ambientals en les poblacions humanes. Aquests resultats són importants per comprendre les implicacions per la salut dels estudis d'exposomes.

Palabras clave: Composts organoclorats, exposicions fetals, exposició a la infància, DDT, policlorobifenils, neurodesenvolupament, asma, obesitat, trastorn per dèficit d'atenció amb hiperactivitat

Introduction

The life expectancy of the individuals from western countries has more doubled that of their ancestors two centuries ago. This than great success results from the strong technologic and scientific development generated by the industrial revolution. However, some changes introduced during this period have also generated new risks for human health.

The synthesis, use and environmental spill of organochlorine compounds (OCs) constitute one of these risks. These compounds encompass a series of molecules that are responsible for a large number of deleterious health effects related to chronic exposure to organic chemicals. The most abundant in the environment and human tissues involve pentachlorobenzene (PeCB), hexachlorobenzene (HCB), hexachlorocyclohexanes (α-, β- and γ-HCH isomers), polychlorobiphenyls (PCBs; the main congeners: PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180) and DDT and metabolites.

The history of the past use of these compounds is contradictory. Several of them were considered to be very beneficial at the initial application period but they had to be banned later in view of the observed deleterious health effects in humans and organisms. The most striking example is 4,4'-DDT. In 1948 Paul Hermann Muller was awarded the Nobel Prize in Physiology and Medicine for "its discovery of the high efficiency of DDT as a contact poison against several arthropods". However, in 1962 Rachel Carlson wrote "Silent Spring" describing that this insecticide had also several major effects on the health of warm blood species. Finally, this compound and its metabolites were included in the list of compounds of the Stockholm Convention on Persistent Organic Pollutants for the restriction of its production and use except in the case of disease vector control. In 2005, the World Health Organization recommended the continued use of DDT in limited quantities for public health purposes in situations where potential loss of human life associated with unstable malaria transmission and epidemics is greatest and alternatives were not available[1, 2].

A parallel story could be described for hexachlorobenzene. At the beginning this compound was used as fungicide for the preservation of wheat sowing, which avoided the use of organomercurial compounds for preservation against fungal degradation. However, a major intoxication episode occurred in the Turkish Kurdistan with development of porphyria cutanea tarda as consequence of human consumption of bread manufactured from hexachlorobenzene-treated wheat. This compound was therefore banned after this intoxication episode and now it occurs in the environment because it is generated as by-product in the synthesis of organochlorine solvents.

OCs are very stable from chemical and environmental standpoints. Once released into the environment they remain in organisms, sediments, soils, air and other environmental compartments for decades. After the implementation of the regulations of the Stockholm Convention their concentrations have decreased in some cases but this is not yet a general rule. Furthermore, they have a lipophilic character which enhances their accumulation in organisms, including humans, instead of water dilution.

The strong stability of these compounds is due to the high abundance of chlorine substituents in their molecules. Because of this unique chemical composition, they are unknown to the metabolism of humans and other organisms. No exposure to these compounds occurred in the past. From an evolutionary viewpoint it is now the first time of human OC exposure and bioaccumulation and our metabolism does not know how to treat them. Thus, they accumulate in tissues and fat as consequence of their physical-chemical properties without significant metabolic interaction/degradation and without that membrane barriers may stop their distribution between organs. For instance, they accumulate in maternal tissues, placenta and fetus during pregnancy[3] and children receive an important dose of these compounds during breastfeeding[4]. In consequence, children are exposed at present to these compounds since the earliest stages of their development when their tissues and organs are still in formation. This situation is new in relation to the environmental chemical structures to which humans were exposed earlier than the fifties when these compounds were not in the environment neither in food items.

Now, humans receive inputs of these compounds throughout their life, including the fetal, breastfeeding and toddler periods, infancy, adolescence, maturity and aging. This raises up a new toxicity concept which is not related with the dose but the time of exposure: What are the effects of being exposed to low amounts of one toxicant for very long time periods such as the whole life, including the earliest stages of development?

This question is even more relevant for children. They are not small adults. They have specific needs and problems because their metabolism and organs are in formation. They have to face development at the physical, cognitive and psychosocial levels. Thus, chemical insults may be more significant in some critical formation time windows than in others. Furthermore, exposure to chemical pollutants in these development stages may lead to clinical deleterious effects later in life[5].

Among the common non-communicable diseases whose incidence may be related, at least in part, to environmental exposure to toxicants, obesity/cardiovascular diseases, diabetes, respiratory disease (including chronic obstructive lung diseases and asthma), cancer and neurological disorders must be considered[6]. Non-com-

municable diseases have long been the major causes of mortality and morbidity in high income countries but low and middle income countries are now also beginning to experience epidemics[6]. The development of these diseases is mostly related to life-time exposures. For example, lung cancer is mainly caused by exposures to tobacco, asbestos and air pollution. However, evidences indicating that an important part of its origin lies in fetal and early life chemical assimilations are increasing. There is a clear need to ascertain what exposures, which individual characteristics, e.g. genetics, life style, and what clinical or non-clinical health disturbances can be related to environmental pollutants at older age.

In order to get progress into this topic, a collaborative network of research focused on children exposure to environmental pollutants was established in Spain[7]. This network involved the study of cohorts of newborns from Menorca, Ribera d'Ebre, Valencia, Sabadell, Granada, Gipuzkoa and Asturias. Among other aspects, the network promoted the measurement of the above mentioned OCs in cord blood serum for assessment of the exposures of newborns during the fetal period. Depending on the cohort, these compounds were also measured in maternal venous cord blood, in breastmilk or in venous cord blood of children at 4 years old.

Among the INMA cohorts, the one from Menorca is the oldest and the one who provided more insight into the consequences of in utero exposure to environmental pollutants and health disturbances in infants during the first years of age. These results have been widely quoted in the international literature. Menorca does not have factories producing OCs but DDT was used for agriculture in the past. The individuals participating in the cohort were therefore exposed to baseline POP levels and can be taken as examples of the regular exposure to POPs in western countries.

In the present paper the advances on the knowledge of the health disturbances resulting from OC exposures in the fetal period is summarized. Impacts on overweight, thryroid function, neurodevelopment, lower respiratory tract infections and asthma are considered. Besides their intrinsic value for the understanding of the etiology of some of the non-communicable diseases, the reported findings illustrate that other aspects besides those related with direct OC exposures or genetic factors are also relevant for the final health outcomes related to OC bioaccumulation.

Methods

The cohort recruited all women presenting for antenatal care over 12 months starting in mid 1997[7]. 482 children were enrolled and 470 (97.5%) provided complete outcome data up to the fourth year visit (**Table I**). Among these, 410 (85%) had OCs measured in cord blood and 285 (59%) in sera collected at four years.

Table I: Characteristics of the study population.

	Number of individuals	%
Participants		
At birth (at four years)[a]	410 (285)[a]	
Sex		
Male	202 (136)[a]	49 (48)[a]
Female	208 (148)	51 (52)
Feeding mode		
Maternal milk	339 (235)[a]	83 (83)[a]
Formula milk	71 (49)	17 (17)
Time of lactation (weeks)		
0.3-10 (0.3-12)[a]	85 (59)[a]	25 (25)[a]
10-20 (12-21.5)	85 (59)	25 (25)
20-28 (21.5-28)	85 (59)	25 (25)
28-100 (28-96)	84 (59)	25 (25)
Time of gestation (weeks)		
27-39	106 (72)[a]	26 (25)[a]
39-40	208 (161)	51 (57)
40-44	96 (50)	23 (18)
Maternal body mass index		
15.3-20.6 (15.3-20.4)[a]	102 (71)[a]	25 (25)[a]
20.6-22.0 (20.4-22.0)	103 (72)	25 (25)
22.0-24.3 (22.0-24.2)	103 (71)	25 (25)
24.3-48.5 (24.2-48.5)	102 (71)	25 (25)
Maternal age		
17-26 (17-26)[a]	102 (71)[a]	25 (25)[a]
26-29 (26-29)	103 (72)	25 (25)
29-32 (29-32)	103 (71)	25 (25)
32-42 (32-41)	102 (71)	25 (25)

[a]*Subset of the same individuals participating in the study at four years old*

OCs were analysed in serum of cord blood and venous blood collected at four years of age. The analytical methods used for these measurements have been described elsewhere[8, 9]

Results

The ages of the participant mothers at delivery represented nearly the whole range of reproductive activity (**Table 1**). Body mass index (BMI) encompassed a large spectrum of cases from underweight (15.3) to obesity (48.5) (**Table 1**). Some cases involved short gestation periods (**Table 1**). 83% of children were breastfed. Time of lactation ranged from very short (2 months or less) to very long (more than one year intervals). No significant biases between the group of participants at birth (n = 410) and 4 years later (n = 285) were observed.

Concentrations of organochlorine compounds

The median concentrations of HCB, HCH, DDTs and PCBs in the cord blood and venous serum collected at 4 years from the Menorca cohort are shown in **Table II**. 4,4'-DDE was the most abundant OCs. Pentachloroben-

zene was generally found above the limit of detection in fewer than 10% of the samples. The median HCB value, 0.68 ng/ml (**Table II**), was high in comparison with those found in studies from other areas except Chukotka (Russia). The high value in Menorca was consistent with the HCB levels of the Spanish general population (not newborns) described in previous studies that are higher than in other European countries[10].

The distributions of HCH were highly dominated by ß-HCH as it is the usual case in human samples from other populations. The α-, γ- and δ-HCH isomers were only found above quantification limit in less than 5% of the total samples (**Table II**). These compounds were therefore not included in the studies. The median ß-HCH in Menorca was lower than those found in Chukotka (Russia[11]), Veracruz (Mexico[12]), Rio de Janeiro (Brazil[13]), New Delhi (India[14]) and Arctic Canada[15].

The median values of 4,4'-DDE and 4,4'-DDT were 1.0 ng/ml and 0.08 ng/ml, respectively (**Table II**). The dominance of 4,4'-DDE over 4,4'-DDT is consistent with the old origin of this mixture of pollutants because the latter is the one used as pesticide and the former is a transformation compound. The concentrations of 4,4'-DDE observed in Menorca were lower than those found in areas that have recently used this compound for malaria control such as Veracruz (Mexico[12]), Chukotka (Russia[11]) and New Delhi (India[14]) but higher than those found in other areas such as the Faroe Islands (Denmark[16]), Arctic Canada[15] and Rio de Janeiro (Brazil[13]) (**Table II**).

The distributions of PCBs were dominated by PCB138, PCB153 and PCB180 which corresponds to the congeners with more hydrophobic properties from this group of pollutants. The median of total PCB concentrations for the seven congeners analyzed was 0.51 ng/ml. These concentrations were lower than those reported in Michalovce[17], the Faroe Islands[16] and Chukotka[11] and higher than those found in the newborn populations of Rotterdam[18] and the Canadian Arctic[15].

Maternal determinants of OC concentrations in children.

Cord blood OC concentrations showed significant correlations with the age of the mother at delivery for HCB, ß-HCH, 4,4'-DDE, 4,4'-DDT, PCB118, PCB153, PCB138 and total PCBs[4]. According to these results, older mothers transferred higher OC concentrations into newborns. The compounds exhibiting these correlations were those found in higher concentration in cord blood. These results are in agreement with maternal age dependences of the concentrations of PCBs and HCB in newborns from Germany[19], 4,4'-DDE in newborns from Ribera d'Ebre[20] and 4,4'-DDE, PCBs and HCB in newborns from Quebec[19]. The concentrations in sera collected at four years only showed significant correlation with age of the mother for HCB ($p < 0.01$) and 4,4'-DDT ($p < 0.05$)[4]. The incorporation of new OC inputs through diet (e.g. breastfeeding) probably decreased significantly the relevance of the initial *in utero* intake, except in the case of the two aforementioned compounds.

OC concentrations in cord blood showed significant correlations with the BMI of the mother at delivery for HCB, 4,4'-DDE and 4,4'-DDT[4]. Higher BMI corresponded to higher OC concentrations in cord blood. No significant association between the concentrations of these compounds and cord blood lipids was observed. For HCB and 4,4'-DDE the degree of significance was very high ($p < 0.0001$ and $p < 0.001$, respectively). These two compounds were those present in the highest average concentration in the newborns (**Table II**). The concentrations in sera of four year old children only showed significant correlation with maternal BMI for HCB ($p < 0.05$). These data from the cohort of Menorca showed for the first time a direct relationship between maternal BMI and the concentration of some OCs in children at birth and at four years old.

Table II: Comparison of the median concentrations of organochlorine compounds in cord serum between Menorca and other world areas.

Area of study	N	Period of delivery	∑PCBs[a,b] ng/ml	HCB[a] ng/ml	β-HCH[a] ng/ml	4,4'- DDE[a] ng/ml	4,4´-DDT[a] ng/ml	Reference
Menorca (cord blood)	410	1997-1998	0.51[c] 0.64[d]	0.68[c] 0.76[d]	0[c] 0.22[d]	1.0[c] 1.6[d]	0.08[c] 0.18[d]	(4)
Menorca (four years of age)			0.73[c] 1.0[d]	0.31[c] 0.42[d]	0.21[c] 0.29[d]	0.81[c] 1.6[d]	0[c] 0.081[d]	(4)
Rotterdam (Netherlands)	382	1990-1992	0.45	NA	NA	NA	NA	(18)
Michalovce (Slovakia)	92	2002-2004	1.21	NA	NA	NA	NA	(17)
Faroe Islands	316	1986-1987	1.8	NA	NA	1.3	NA	(16)
Chukotka (Russia)	48	2001-2002	6.6[c]	4.0[c]	5.6[c]	6.4[c]	0.66[c]	(11)
Arctic Canada	400	1994-1999	0.23[c]	0.07[c]	0.03[c]	0.34[c]	0.03[c]	(15)
Veracruz (Mexico)	60	1997-1998	NA	0.8	0.7	6.0	0.8	(12)
Rio de Janeiro (Brazil)	10	1997-1998	NA	0.13	0.54	0.76	ND	(13)
New Delhi (India)	23	2006-2008	NA	NA	3.59	1.98	0.93	(14)

[a]The concentrations are reported in the same units as given in the referenced studies. [b]The concentrations are reported according to the number of congeners analyzed by the authors. [c]Median. [d]Mean. [e]Geometric mean. NA: Not analyzed; ND: Not detected.

Influence of milk feeding

The average concentrations of HCB, 4,4'-DDE, 4,4'-DDT, PCB153, PCB138 and PCB180 and total PCBs in sera collected at four years exhibited significantly higher values in breastfed than artificially fed children[4]. The degree of significance of the differences was very high ($p < 0.0001$) for most of these compounds. Accordingly, breastfeeding was very significant for the concentrations of OC in four year old children despite they stopped breastfeeding 2.3-3.5 years before being tested. The period of lactation was also correlated with the concentrations of HCB, ß-HCH, 4,4'-DDE, PCB118, PCB153, 4,4'-DDT, PCB138, PCB180 and total PCBs accumulated in four year old breastfed children.

In all cases, longer lactation corresponded to higher concentrations in serum. These results were consistent with studies on ß-HCH, HCB, 4,4'-DDE and PCBs in newborns from Germany (7 years[21]) and PCBs and 4,4'-DDT in Michigan (4 years[22]) and in Groningen (18 months[23]). In the Menorca cohort, the nursing period encompassed a very wide time range (0.3-100 weeks) and nearly all OCs examined showed significant correlation with this determinant.

The magnitudes of change varied between compounds, breastfed children showed concentration increases of 0.5 ng/ml of total PCBs in the blood serum content at 4 years when compared to birth. 4,4'-DDE increases were of about 0.2 ng/ml and ß-HCH increased by 0.1 ng/ml. Conversely, volatile compounds such as PeCB and HCB decreased, 0.1 ng/ml and 0.22 ng/ml, respectively. In the case of children fed with formula only concentration decreases in the blood serum collected at four years with respect to birth were observed, involving decreases of 0.3 ng/ml for total PCBs, 1.4 ng/ml for 4,4'-DDE, 0.6 ng/ml for ß-HCH and between 0.1 and 0.2 ng/ml for 4,4'-DDT, ß-HCH and PeCB[4].

As expected, dilution resulting from children growth tended to reinforce the decreases and counterbalance the increases. In the Menorca cohort the average growth involved changes from ca. 3.2 kg at birth to ca. 16.2 kg at four years of age corresponding to approximate blood volumes of 0.24 L and 1.2 L, respectively. Accordingly, the observed changes involved increases of total POPs in all cases but these were much higher in breastfed children than in formula fed children. Total concentrations of 4,4'-DDE, PCBs, HCB, ß-HCH and 4,4'-DDT in the venous system increased by 1.9, 1.3, 0.4, 0.3 and 0.1 µg in the former and by 0.1, 0.6, 0.05, 0.25 and 0.1 µg in the latter.

Methods

Overweight

Overweight at 6.5 years was defined as a BMI z-score ≥85th percentile of the World Health Organization reference. The OC concentrations in cord blood were measured and treated as categorical variables (tertiles). Children's diet was assessed by a food frequency questionnaire. No statistically significant associations between OC and height were found.

Children in the highest cord blood HCB group (> 1.0 ng/ml) had higher weight and BMI at age 6.5, ß = 1.92 kg (0.64) and 0.95 kg/m^2 (0.31), respectively[24]. The highest prevalence of overweight (20%) and obesity (17%) was also found in this group. Increased relative risks of overweight in the highest group of prenatal exposure to PCBs (> 0.9 ng/mL) was also found, 1.7 (95% confidence interval 1.9-2.64) (**Table III**[25]). Significant results were observed for the second tertiles of 4,4'-DDE exposure (0.7-1.5 ng/ml) showing a Relative Risk of 1.67 with a confidence interval of 1.10-2.55[25]. These associations were stronger in girls than in boys. Adjustment for birth

Table III: Crude and adjusted estimated effects (RR, 95%CI) of prenatal PCBs, DDE and DDT concentrations on overweight at 6.5 y in the cohort of Menorca (n = 344; Valvi et al., 2012).

	OC concentrations (ng/ml)	N	Crude model	Multivariable adjusted model[a]	Multi-pollutant adjusted model[a,b]
PCBs					
RR (95% CI)	<0.6	110	Ref.	Ref.	Ref.
	0.6-0.9	117	0.82 (0.51,1.32)	0.97 (0.58,1.62)	0.92 (0.54,1.56)
	>0.9	117	1.30 (0.86,1.96)	1.70 (1.09,2.64)	1.54 (0.95,2.49)
DDE					
RR (95% CI)	<0.7	113	Ref.	Ref.	Ref.
	0.7-1.5	116	1.52 (0.97,2.40)	1.67 (1.10,2.55)	1.45 (0.93,2.24)
	>1.5	115	1.41 (0.88,2.25)	1.28 (0.81,2.03)	0.94 (0.58,1.54)
RR per each ng /mL lnDDE increase		344	1.19 (0.99,1.43)	1.15 (0.95,1.39)	1.13 (0.91,1.42)
DDT					
RR (95% CI)	<0.06	108	Ref.	Ref.	Ref.
	0.06-0.18	124	1.32 (0.84,2.10)	1.19 (0.76,1.87)	1.12 (0.73,1.71)
	>0.18	112	1.42 (0.90,2.26)	1.17 (0.73,1.88)	1.11 (0.68,1.81)
RR per each ng/mL lnDDT increase		344	1.09 (0.95,1.25)	1.04 (0.91,1.19)	1.01 (0.87,1.15)

[a]Adjusted for birth weight, previous parity, maternal pre-pregnancy BMI, maternal education and social class at pregnancy, maternal smoking in pregnancy, maternal age at delivery and breastfeeding. [b]Additionally adjusted for HCB and the other OCs shown in this table (all OCs in tertiles).

weight, other OCs and diet did not modify the model. Prenatal exposure to HCB, PCBs and DDE was therefore likely associated with an increase in BMI and weight at age 6.5 years.

Thyroid function

Thyroid hormones are essential for normal brain development. At birth, examination of associations between levels of thyrotropin (TSH, thyroid-stimulating hormone) and OCs showed a positive association with cord blood serum concentrations of ß-HCH in the cohort of Menorca[26]. High ß-HCH levels were paralleled with high TSH concentrations. Studies of four-year-old children in the cohort of Menorca[26, 27] have shown that higher prenatal levels of 4,4'-DDT, ß-HCH and PCB congeners PCB138, PCB180, PCB153 and PCB118 were related to lower total triiodothyronine (T_3) levels (**Table IV**). In addition, free thyroxine (T_4) was found to be inversely related with PCB118 concentrations (**Table IV**), while no association was observed between TSH and any of the OCs measured.

Asthma, wheeze and risk of lower respiratory tract infections

Early life exposure to OCs is also suspected to increa-

Table IV: Unadjusted association (coefficient and standard error) between thyroid hormones and TSH concentrations and quartiles of organochlorine compounds. (n=259)[27].

	lnTSH Coefficient	p	Free T4 Coefficient	p	Total T3 Coefficient	p
HCB (ng/ml)						
0.00 - 0.193 (reference)	0.46 mU/l		1.06 ng/dl		155 ng/dl	
0.194- 0.304	-0.02 (0.08)		0.00 (0.02)		-6.1 (3.8)	
0.305 - 0.506	0.02 (0.08)		0.00 (0.02)		-8.8 (3.8)*	
0.507 - 4.52	0.13 (0.08)	0.079	-0.02 (0.02)	0.602	-5.3 (3.8)	0.120
p,p'-DDE (ng/ml)						
0.00 - 0.435 (reference)	0.50 mU/l		1.05 ng/dl		151 ng/dl	
0.436 - 0.807	-0.05 (0.08)		0.00 (0.02)		0.1 (3.8)	
0.808 - 1.75	-0.06 (0.08)		0.01 (0.02)		-1.4 (3.8)	
1.76 - 43.9	0.09 (0.08)	0.280	-0.02 (0.02)	0.379	-5.0 (3.8)	0.166
p,p'-DDT (ng/ml)						
10.00 - 0.025 (reference)	0.42 mU/l		1.06 ng/dl		156 ng/dl	
0.026 - 0.049	0.04 (0.08)		0.00 (0.02)		-7.5 (3.8)*	
0.050 - 0.103	0.11 (0.08)		0.00 (0.02)		-9.0 (3.8)*	
0.104 - 0.657	0.12 (0.08)	0.101	-0.03 (0.03)	0.360	-7.9 (3.8)*	0.40
β-HCH (ng/ml)						
0.00 - 0.107 (reference)	0.57 mU/l		1.08 ng/dl		155 ng/dl	
0.108 - 0.190	-0.15 (0.08)		-0.03 (0.02)		-5.0 (3.8)	
0.191 - 0.304	-0.16 (0.08)*		-0.04 (0.02)		-7.1 (3.8)	
0.305 - 5.65	-0.01 (0.08)	0.833	-0.05 (0.02)	0.070	-9.0 (3.8)*	0.015
PCB-138 (ng/ml)						
0.00 - 0.104 (reference)	0.50 mU/l		1.06 ng/dl		153 ng/dl	
0.105 - 0.174	-0.09 (0.08)		0.00 (0.02)		-0.8 (3.8)	
0.175 - 0.276	0.05 (0.08)		0.00 (0.02)		-4.8 (3.8)	
0.277 - 8.71	0.02 (0.08)	0.382	-0.01 (0.02)	0.674	-6.2 (3.8)	0.061
PCB-180 (ng/ml)						
0.010 - 0.063 (reference)	0.44 mU/l		1.07 ng/dl		151 ng/dl	
0.064 - 0.115	-0.01 (0.08)		-0.04 (0.02)		2.6 (3.8)	
0.116 - 0.211	0.14 (0.08)		-0.01 (0.02)		-4.4 (3.8)	
0.212 - 7.20	0.09 (0.08)	0.097	-0.02 (0.02)	0.694	-4.3 (3.8)	0.097
PCB-153 (ng/ml)						
0.014 - 0.140 (reference)	0.49 mU/l		1.06 ng/dl		152 ng/dl	
0.141 - 0.250	-0.07 (0.08)		0.00 (0.02)		2.0 (3.8)	
0.251 - 0.410	0.03 (0.08)		0.01 (0.02)		-4.9 (3.8)	
0.411 - 10.88	0.07 (0.08)	0.208	-0.02 (0.02)	0.482	-6.3 (3.8)	0.032
PCB-118 (ng/ml)						
0 - 0.069 (reference)	0.39 mU/l		1.09 ng/dl		155 ng/dl	
0.069 - 0.098	0.17 (0.08)*		-0.03 (0.02)		-3.2 (3.8)	
0.099 - 0.128	0.09 (0.08)		-0.06 (0.02)*		-4.8 (3.8)	
0.129 - 1.824	0.12 (0.08)	0.247	-0.07 (0.02)**	0.003	-11.5 (3.8)**	0.003
sum of PCBs (ng/ml)						
0.148-0.546	0.41 mU/l		-0.04 ng/dl		155 ng/dl	
0.547-0.775	0.08 (0.08)		-0.01 (0.02)		-4.7 (3.8)	
0.776 - 1.171	0.09 (0.08)		-0.05 (0.02)		-7.4 (3.8)	
1.172 - 41.17	0.16 (0.08)	0.046	1.08 (0.02)	0.193	-8.3 (3.8)*	0.021

** p- value <0.05 ** p-value <0.01 (in comparison to the reference category)*

se the risk of lower respiratory tract infections (LRTIs) and wheeze in infants. The effects of these pollutant exposures have been documented at 14 months, 4 and 6.5 years.

Children in the ages of 4 and 6.5 years were examined for increased risk of asthma and atopy upon exposure to 4,4'-DDE[28, 29]. Asthma was defined on the basis of wheezing at 4 and 6.5 years of age, persistent wheezing or doctor-diagnosed asthma. Specific immunoglobulin-E (IgE) against house dust mite, cat, and grass in sera extracted at 4 years of age was measured.

Wheezing at 4 years of age was found to increase with 4,4'-DDE concentration, particularly in the highest quartile (9% in the lowest quartile (<0.57 ng/ml) versus 19% in the highest quartile (1.90 ng/ml); relative risk = 2.63 (95% CI: 0.96–7.20), adjusting for maternal asthma, breast feeding, education, social class, or other OCs; **Table V**). The association was not modified by IgE sensitization and occurred with the same strength among non-atopic subjects and among those with persistent wheezing or diagnosed asthma. 4,4'-DDE was not associated with atopy alone. No association was found for 4,4'-DDE concentrations in these infants at 4 years and the pulmonary or atopy indicators. The results were consistent with contributions of prenatal exposure to 4,4'-DDE residues to asthma development.

The relevance of epigenetic changes in the association between 4,4'-DDE and asthma was investigated in 122 children of the cohort of Menorca[30]. DNA methylation of the CpG site in the arachidonate 12-lipoxygenase (ALOX12) gene was identified as a possible epigenetic biomarker for the risk of asthma-related phenotypes.

Wheezing phenotypes were defined between 4 and 6 years. Cytosine-guanine (CpG) dinucleotide site DNA methylation differences associated with wheezing phenotypes were screened using the Illumina GoldenGate Panel I. ALOX12 DNA methylation was strongly determined by underlying genetic polymorphisms. The findings were validated and replicated using pyrosequencing.

Information on maternal smoking and folate supplement use was obtained through questionnaires. The genotypes were extracted from genome-wide data. The screening identified lower DNA methylation at a CpG site in the ALOX12 gene in children having persistent wheezing compared with those who never wheezed (p = 0.003). DNA hypomethylation at ALOX12 loci was associated with higher risk of persistent wheezing (odds ratio per 1% methylation decrease, 1.13; 95% CI: 0.99–1.29; p = 0.077). Higher levels of prenatal 4,4'-DDE were associated with DNA ALOX12 hypomethylation (p = 0.033).

Table V: Adjusted associations between 4,4'-DDE in cord serum and wheezing at age 4 (risk ratio and 95% confidence interval)[28].

	All	Non-atopic
4,4'-DDE in quartile (ng/ml)*		
< 0.57	1	1
0.57 – 1.03	1.00 (0.41-2.43)	1.32 (0.37-4.70)
1.03 – 1.90	1.62 (0.70-3.74)	2.63 (0.96-7.20)
> 1.90	2.36 (1.19-4.69)	2.49 (1.00-6.19)

** adjusted for the socio-economic variables*

Poor social behavior and Attention-Deficit Hyperactivity Disorder

Poor social behavior and attention-deficit hyperactivity disorder (ADHD) was examined in infants as early indicator of developmental neurotoxicity. Positive associations of these disturbances in pre-schoolers of 4 years and prenatal exposure to HCB were identified in Menorca (**Table VI**[31]). The California Preschool Social Competence Scale and the ADHD were scored by each 4-year-old-child's teacher. Children's diet and parental sociodemographic information was obtained through a questionnaire. All prenatal HCB exposure categories were associated with an increase in the risk of having a poorer Social Competence and ADHD, but only those children with HCB concentrations above 1.5 ng/ml at birth had a statistically significant increased risk of having a poor Social Competence = 4.04 (1.76–9.58) and ADHD = 2.71 (1.05–6.96) (relative risk (standard error); **Table VI**). No association was found between prenatal HCB and the cognitive and psychomotor performance of these children. No association was found for HCB concentrations of these infants at 4 years and the test scores.

Cognitive skills

Early life exposure to OCs is suspected to have deleterious effects on neurodevelopment which may involve decreases in cognitive or psychomotor skills. Studies on 4-year-old children from the cohort of Menorca in which OCs were measured at delivery and at 4 years were used for further assessment of DDT impact of the critical exposure age of infant development. Examination on the neuropsychological development using the McCarthy Scales of Children's Abilities (MCSA) at 4 years of age showed that 4,4'-DDT cord serum concentration at birth was inversely associated with verbal, memory, quantitative, and perceptual-performance skills[32]. Children whose 4,4'-DDT concentrations in cord serum were >0.20 ng/ml had mean decreases of 7.86 points in the verbal scale (standard error, 3.21) and 10.86 points in the memory scale (standard error, 4.33) when compared with children whose concentrations were <0.05 ng/ml (**Table VII**). These associations were stronger among girls. Prenatal exposure to higher concentrations of 4,4'-DDT was associated with a decrease in preschoolers' cognitive skills. No association was found for 4,4'-DDT concentrations of these infants at 4 years.

Further examination of prenatal exposure to OCs and impaired neurodevelopment at 4 years of age showed no statistically significant effects of the sum of prenatal

PCBs on MCSA scores. Nevertheless, individual congener analyses yielded significant detrimental effects of prenatal PCB153 on the majority of MCSA scores, while no effects were observed for other PCB congeners. The levels of PCBs at 4 years of age were not associated with neurodevelopment. Thus, prenatal exposure to low-level concentrations of PCBs, particularly PCB153, was observed to be associated with an overall deleterious effect on neuropsychological development at 4 years of age, including negative effects on the executive and verbal functions and on visuo-spatial abilities, but not on motor development[33].

Examination of the possible influence of genetic variability in the 4-year-old infants from this cohort showed a significant relation with GST genes (*GSTP1*, *GSTM1*, and *GSTT1*[34]). Genotyping was conducted for the coding variant Ile[105]Val from *GSTP1* and for null alleles from *GSTM1* and *GSTT1*. Linear regression models were used to measure the association between OCs and neurodevelopment scores by GST polymorphisms. In children having any *GSTP1* Val-105 allele DDT cord serum concentration was observed to be inversely associated with general cognitive, memory, quantitative, and verbal skills, executive function and working memory (**Table VIII**). *GSTP1* polymorphisms and prenatal DDT exposure showed a statistically significant interaction for general

Table VI: Crude and adjusted relative risk of having Poor social behaviour (< 80 points in the Social Competence Scale) and Attention-Deficit Hyperactivity Disorder at age 4 in relation to in utero exposure to HCB (95% CI)[31]

	Unadjusted n=377	Adjusted † n=377	Adjusted for other OCs ‡ n=377	Menorca cohort ‡ n=329
	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)	Coefficient (SE)
SOCIAL COMPETENCE				
HCB category				
Reference¶	1	1	1	1
0.5-0.99 ng/ml	1.16 (0.62-2.18)	1.40 (0.68-2.87)	1.77 (0.83-3.79)	1.84 (0.82-4.11)
1-1.49 ng/ml	1.04 (0.48-2.29)	1.47 (0.59-3.62)	1.83 (0.72-4.69)	1.51 (0.52-4.35)
≥ 1.5 ng/ml	2.88 (1.39-5.97)*	4.04 (1.76-9.58)*	5.63 (2.13-14.88)*	6.18 (2.06-18.50)*
HCB, ng/ml#	1.52 (1.05-2.22)*	1.79 (1.15-2.76)*	2.10 (1.30-3.40)*	2.18 (1.28-3.74)*
ATTENTION-DEFICIT HIPERACTIVITY DISORDER				
Reference¶	1	1	1	1
0.5-0.99 ng/ml	1.19 (0.58-2.42)	1.23 (0.54-2.78)	1.47 (0.63-3.46)	1.38 (0.57-3.32)
1-1.49 ng/ml	1.73 (0.77-3.91)	2.28 (0.88-5.96)	2.74 (1.01-7.45)*	2.17 (0.73-6.49)
≥ 1.5 ng/ml	2.05 (0.90-4.67)**	2.71 (1.05-6.96)*	3.43 (1.24-9.51)*	3.11 (1.01-9.55)*
HCB, ng/ml#	1.49 (0.99-2.24)**	1.63 (1.02-2.63)*	1.88 (1.13-3.14)*	1.77 (1.00-3.11)*

¶ Reference group: <0.5 ng/ml. # Natural logarithmic transformed HCB concentration. * p<0.05; ** p<0.10. † Adjusted for age, cohort, gender, maternal education, paternal education, tobacco and alcohol exposure, maternal age in years and type and duration of breastfeeding (see methods). ‡ Adjusted for same variables above and PCBs, 4,4'-DDE and 4,4'-DDT

Table VII: Adjusted¶ associations between DDT and the general cognitive, the verbal and memory McCarthy areas according to gender[32]

	Concentration of 4,4'-DDT 0.05-0.10 Reference † ng/ml β (SE)		0.10-0.20 ng/ml β (SE)	> 0.20 ng/ml β (SE)
All infants	*n=203*	*n=86*	*n=74*	*n=112*
General Cognitive	104.03	1.45 (2.72)	-2.01 (2.95)	-5.87 (2.60)*
Verbal	98.38	1.80 (3.36)	-4.02 (3.65)	-7.86 (3.21)*
Memory	88.93	1.64 (4.53)	-4.46 (4.92)	-10.86 (4.33)*
Girls	*n=101*	*n=48*	*n=33*	*n=55*
General Cognitive	104.67	-1.37 (3.95)	-0.44 (4.47)	-8.89 (3.89)*
Verbal	97.22	-2.26 (4.86)	-2.58 (5.51)	-12.79 (4.80)**
Memory	88.22	-2.46 (6.61)	-4.76 (7.47)	-17.19 (6.51)**
Boys	*n=102*	*n=38*	*n=41*	*n=57*
General Cognitive	102.64	3.39 (4.09)	-5.15 (4.06)	-3.74 (3.63)
Verbal	101.99	5.66 (5.05)	-6.65 (5.01)	-3.41 (4.47)
Memory	96.54	2.47 (6.82)	-6.30 (6.77)	-5.63 (6.04)

Each row is a different multivariate model. Adjusted for gender, scholar trimester at examination, psychologist, breastfeeding, maternal social class and maternal consumption of alcohol and tobacco during pregnancy. Neurodevelopmental scores are centered to the mean.
† Infants in the lowest quartile of DDT exposure (<0.05 ng/ml)

cognitive (p = 0.051) and quantitative (p = 0.018) skills, executive function (p = 0.009) and working memory (p = 0.017; **Table VIII**).

According to *GSTM1* and *GSTT1* polymorphisms, there were no significant associations between DDT and cognitive functioning at the age of 4 years. The results indicated that children with *GSTP1* Val-105 allele were at a higher risk of the adverse cognitive functioning effects of prenatal 4,4'-DDT exposure.

These studies on neurodevelopment were extended to 11 years by use of the continuous performance test-II (CPT-II) which was administered to 393 11-year-old children[35]. The results showed that a number of socio-environmental factors during prenatal life and early childhood, such as socio-demographic characteristics, breast feeding, maternal nutritional supplementation with folic acid and vitamins and OC exposure, e.g. 4,4'-DDE and PCB levels at 4 years, may influence inattentive and hyperactive/impulsive symptomatology during preadolescence[35]. This study was the first reporting some relationships between low neuropsychological development at 11 years and OC exposure at preschooler ages instead of *in utero*. Confirmation from other independent studies is needed.

Influence of breastfeeding on neurodevelopment

Breastfeeding was associated with increases of the preschoolers cognition performance in the McCarthy Scale while DDT was associated with lower performance[36]. Children who were breastfed for more than 20 weeks had a better cognitive performance regardless of their in utero DDT exposure. A linear dose response between breastfeeding and cognition was observed in all DDT groups (adjusted ß for high exposed to DDT (SE) = 0.30 (0.12) per week breastfed) (**Table IX**[36]).

As described in multiple studies[37-40], breastfeeding alone is beneficial for children's neurodevelopment which may be due to the occurrence of superior nutrients in breast milk than in formula milk[41, 42] or to the high frequency of physical and psychological contact between mothers and their infants during the breastfeeding process[43]. Children that are breastfed have a continuous exposure to OCs that in formula fed infants is much lower[4]. As a result, the concentrations of DDT and DDE at 4 years are higher among the longer-term breastfed children than in those with short or no breastfeeding (**Table IX**). However, the 4-year old children from the cohort of Menorca show that long term breastfeeding was beneficial for child development regardless the concentration of DDT in cord serum (**Table IX**). These results suggest that breastfeeding did not increase the neurotoxicological risk through higher potential DDT exposure. Maybe breast milk nutrients could counterbalance the negative effect of DDT but other possibilities should also be investigated.

Correspondences between OC exposures and health effects

The repeated analysis of OCs in the Menorcan children allows to discriminating between effects of environmental OC exposure in utero and at 4 years of age. As shown in **Table X**, in all cases the diverse health effects observed at 4 years were related to OC exposure in utero and not

Table VIII: Adjusted associations (ß(standard error)) between concentrations of DDT† in cord serum (ng/ml) and neurodevelopment at age 4 years by GSTP1 polymorphisms[34].

	GSTP1 genotype		
	Ile/Ile N=149	**Ile/Val or Val/Val N=177**	**p for interaction**
McCarthy areas			
General cognitive	7.125 (6.163) p = 0.250	-8.410 (4.208) p = 0.047	0.051
Perceptual-performance	4.670 (5.751) p = 0.418	-3.805 (4.147) p = 0.360	0.207
Memory	0.898 (6.389) p = 0.888	-6.748 (4.314) p = 0.120	0.350
Quantitative	8.959 (7.228) p = 0.217	-3.584 (1.457) p = 0.015	0.018
Verbal	0.619 (6.484) p = 0.924	-8.234 (4.298) p = 0.057	0.341
Motor	10.326 (5.621) p = 0.068	2.941 (4.084) p = 0.472	0.751
Executive function	10.166 (6.434) p = 0.117	-10.145 (4.243) p = 0.018	0.009
Working memory	7.360 (6.912) p = 0.289	-2.754 (1.162) p = 0.019	0.017

†p,p´-DDT.
‡ Each cell is a different multivariate model. Adjusted for sex, school trimester at examination, psychologist, breastfeeding, maternal social class, and maternal consumption of alcohol and use of tobacco during pregnancy.

to the concentrations of these pollutants at 4 years, the age in which health disturbances were measured. The effects of exposure to OCs are therefore more critical during the prenatal than the postnatal periods. Accordingly, the period of highest human formation and growth is also the most critical to chemical insults by environmental pollution. Studies devoted to assess health diseases related to environmental pollutants should take into account this temporal perspective since no associations between OC intake and deleterious effects at 4 years would be identified from the sole analyses of the concentrations of these compounds at this age. This observation is relevant for the use of new concepts such as the exposome, which is devoted to evaluate all accumulated exposures to environmental pollutants in humans to correlate this information with the health clinical history of the individuals and their genetic characteristics. Failure to correctly assess what were the contributions to the exposome in the growth periods may lead to trivial non-significant results.

This temporal perspective may also be relevant for exposure effects in the early stages of postnatal growth and health disturbances later on age. Thus, the use of the CPT-II test have shown that exposure to 4,4'-DDE and PCBs at 4 years may be associated to low neuropsychological development at 11 years[35]. These results are preliminary and should be confirmed with further research. However, in what concerns neurodevelopment they are consistent with the process of progressive brain development during childhood and adolescence that if not completed adequately may lead in some cases to diverse psychiatric disturbances[45, 46].

On the other hand, the positive effects of breastfeeding involve some interesting questions concerning the current modes of association between exposure to environmental pollutants and health diseases. Breastfeeding time is one of the life periods in which humans incorporate most OCs. In Menorca, we have observed very significant differences in serum OC composition among 4 year-old children who breastfed and who did not, des-

Table IX: Adjusted change¶ (coefficient and 95% confidence intervals) in the general cognitive, verbal and memory McCarthy areas scoring by breastfeeding according to exposure to DDT at birth[36]

	All population n=391	Low exposed < 0.05 ng/ml n=162	Mid exposed 0.05-0.20 ng/ml n=138	High exposed > 0.20 ng/ml n=91
General Cognitive				
Reference†	103.98	109.04	100.32	91.91
Short-term Breastfeeding	1.90 (2.57)	2.11 (4.21)	0.35 (4.74)	3.79 (5.09)
Long-term Breastfeeding	7.66 (2.66)*	5.69 (4.53)	6.90 (4.86)	13.04 (5.83)*
Verbal				
Reference†	48.26	50.97	46.74	38.61
Short-term Breastfeeding	0.28 (1.54)	0.48 (2.54)	-0.91 (2.80)	2.27 (3.07)
Long-term Breastfeeding	3.10 (1.60)	2.21 (2.74)	2.41 (2.70)	5.92 (3.53)
Memory				
Reference†	21.26	23.46	19.33	15.73
Short-term Breastfeeding	0.37 (1.01)	0.87 (1.63)	-0.24 (1.89)	1.15 (2.06)
Long-term Breastfeeding	2.03 (1.05)	0.85 (1.75)	2.22 (1.81)	3.13 (2.37)

* p-value < 0.05 ¶ Adjusted for gender, scholar trimester at examination, psychologist, maternal social class, maternal education and maternal consumption of alcohol and tobacco during pregnancy. † Scoring in infants who were formula fed

Table X: Observed effects of exposure to persistent organic pollutants in humans

	Exposure[a]	Effect	Reference
HCB	*In utero*	Poor social behavior	(31)
HCB	*In utero*	ADHD	(31)
HCB	*In utero*	Alteration of thyroid hormones	(26, 27)
HCB	*In utero*	Overweight	(24)
DDE	*In utero*	Asthma, wheeze and lower respiratory tract infections, including genetic variability	(28-30)
DDE	*In utero*	Alteration of thyroid hormones	(26, 27)
DDE	*In utero*	Alteration of urinary coproporphyrins	(44)
DDE	*In utero*	Overweight	(25)
DDE	4 years	Neuropsychological development	(33)
DDT	*In utero*	Decrease of cognitive skills and influence of genetic variability	(32, 34)
ß-HCH	In utero	Alteration of thyroid hormones	(26)
PCBs	In utero	Overweight	(25)
PCBs	In utero	Neuropsychological development	(35)
PCBs	4 years	Neuropsychological development	(33)

[a]Exposure refers to the time at which the pollutants were measured.

pite that breastfeeding only encompassed three months in most cases and six months at the most[4]. The OC input associated to breastfeeding is therefore very significant.

In this same cohort, children whose 4,4'-DDT concentrations in cord blood serum were above 0.2 ng/ml showed significant decreases in the verbal and memory scales at four years of age in comparison with those having 4,4'-DDT concentrations below 0.05 ng/ml (**Table VII**). An association between higher exposure to 4,4'-DDT and lower cognitive performance is observed. However, this association is not found when comparing the 4,4'-DDT concentration of the four-year-old children with their cognitive skills at this age. One main reason for this lack of association appears to be the beneficial effects of breastfeeding on neurodevelopment (**Table IX**). According to these results, an univocal association between exposure to OCs and deleterious effects on neurodevelopment cannot be established. Besides differences of the impact of these pollutants at different growing periods, activities such as breastfeeding have beneficial effects despite they involve an important increase of these pollutants in breast feeders. This observation must be considered in scientific approaches trying to establish direct associations between integrated life exposure to pollutants (the exposome), genetic variability and health disturbances, the models may not be univocal such as in the case of breastfeeding.

Conclusions

The analysis of OCs in cord and 4-year-old blood serum in children from the cohort of Menorca has shown higher incidence of several health disturbances at 4 years of age that are related to in utero exposure and not to concentrations at four years. Some of these health effects are associated to exposure to one specific pollutant, such as HCB and poor social behavior and ADHD or 4,4'-DDE and asthma, wheeze, lower respiratory tract infections and alteration of urinary coproporphyrins. In other cases, the associations are observed for more than one compound such as HCB, ß-HCH and 4,4'-DDE and alteration of thyroid hormones, HCB, 4,4'-DDE and PCBs and overweight, 4,4'-DDT and PCBs and lower neuropsychological development.

The present results show that chemical insults at some critical growth periods may generate health disturbances later in age. This time-delayed correspondence has also been observed for chemical exposure to 4,4'-DDE and PCBs at four years and higher incidence of delays in neuropsychological development in eleven-year-old children. This is the first case of identification of OC exposure at childhood and effects at 11 years. These results should be confirmed with further studies.

According to these results, full understanding of the possible health effects of this type of chemical insults in children requires a follow up of their health status along time. Crosssectional studies only considering pollutant body burden concentrations and health status at one specific time window may miss causal associations. A protective effect of breastfeeding against decreases of cognitive skills due to 4,4'-DDT exposure has also been documented. This result, besides its obvious nutritional interest, shows that other factors than pollutant exposure and genetic variability are relevant for the incidence of the deleterious health effects of environmental pollutants into human populations.

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