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Serum concentrations of the endocrine disruptors-organochlorine pesticides (OCPs) in Greek children with Neurodevelopmental Disorders

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Background: The exposure to environmental toxicants has been estimated to contribute directly to 3% of human neurodevelopmental disorders (NDDs)[1]. Organochlorine pesticides (OCPs), which are widespread persistent organic pollutants (POPs), have been implicated mainly because of their endocrine disruptive nature[2]. Previous studies have primarily assessed the association of maternal serum, the placenta barrier and the breast milk levels of OCPs with later developmental or behavioral ability[1]. However, it has not yet clarified to which extent the exposure to OCPs is associated with the clinical spectrum of NDDs, rather than continuous measures of cognitive and behavioral functions[3].

Aim: The aim of the current cross-sectional study was to assess the levels of DDT and its metabolites, HCH and its isomers, cyclodienes and methoxychlor in serum samples from school-aged children diagnosed with High Functioning Autistic Disorder, Attention Deficit Hyperactivity Disorder, and Specific Learning Disorder compared to Typically Developing controls. We hypothesized that children with NDDs would have higher serum concentrations of several OCPs.

Method: A total of 114 schoolchildren of both sexes aged between 6 and 13 years old, took part in the study (Table 1). Children were distributed into four groups: High functioning Autistic disorder [HFA] (n=39), Attention Deficit Hyperactivity Disorder [ADHD] (n=21), Specific Learning Disorder (SLD) (n=32), Typically Developing controls [TD] (n=18). The participants had to be Greek and residing in the city of Athens from the time of their birth. The mother of each child had to reside in Athens more than 5 years prior to the conception's occurrence. Exclusion criteria comprised the co-occurrence of ASD and ADHD, a general IQ below 70 (WISC-III), genetic syndromes or chromosomal abnormalities, comorbid chronic disorders/conditions, comorbid neurological or other psychiatric diseases, taking any kind of medication, prematurity <30 weeks and BMI above the 85th percentile for age and gender. Blood sampling was performed between 8:00 and 10:00 am after an overnight fast. Eighteen OCPs were measured by gas chromatography–mass spectrometry (GC/MS)[4]. OCPs concentrations were adjusted for total serum lipids (TL) [TL = 1.31 * (CHOL + TG) + 0.92] and are presented as nanograms/gram lipid[5].

Table 1. Descriptive characteristics[a] of the study population by group.

	TD	HFA	ADHD	SLD
Age (years)	10.0 ± 2.1	8.6 ± 1.7 **p=0.024**	9.0 ± 1.3 p=0.11	9.3 ± 1.8 p=0.34
Gender, males, n (%)	13 (72.2%)	33 (84.6%) p=0.30	15 (71.4%) p=0.99	19 (59.4%) p=0.54
Birth weight (g)	3301 ± 648	3176 ± 408 p=0.70	3021 ± 496 p=0.22	2936 ± 522 p=0.11
Total cholesterol (g/l)	1.7 ± 0.2	1.9 ± 0.3, **p=0.017**	2.0 ± 0.4, **p=0.015**	1.9 ± 0.3, **p=0.032**
Triglycerides (g/l)	0.69 ± 0.30	0.79 ± 0.47 p=0.80	0.70 ± 0.27 p=0.82	0.71 ± 0.34 p=0.95
Total serum lipids (g/l)	4.1 ± 0.4	4.5 ± 0.8 p=0.074	4.4 ± 0.6 **p=0.042**	4.3 ± 0.6 p=0.34
Wisc General IQ	114.9 ± 14.9	104.5 ± 17.0 **p=0.021**	101.8 ± 12.9 **p=0.0045**	105.9 ± 13.5 **p=0.038**
Wisc Verbal IQ	109.9 ± 16.2	94.6 ± 17.4 **p=0.0039**	96.9 ± 16.3 **p=0.026**	100.7 ± 15.6 p=0.06
Maternal age at birth (years)	33.2 ± 5.0	33.4 ± 5.0 p=0.90	31.3 ± 5.4 p=0.20	31.9 ± 3.2 p=0.42
Gestational age (weeks)	37.4 ± 1.1	38.4 ± 1.3 p=0.076	38.3 ± 2.1 p=0.38	35.9 ± 3.5 p=0.86
Smoking during pregnancy, Yes, n, %	1 (7.1%)	9 (29.0%) p=0.13	2 (9.5%) p=0.99	4 (12.9%) p=0.99

Note: [a] Continuous variables are presented as mean ± standard deviation. P-values refer to comparisons between TD and clinical groups (Mann-Whitney U test for continuous outcomes and Fisher's exact test for categorical variables).

Results: Ten of the 18 OCPs were each detected and quantified in at least one sample. Each clinical group was compared to the TD group. The serum concentrations (SC) of β-HCH (p=0.049), the sum of HCH isomers (∑HCHs) (p=0.025) and o,p´-DDD (p=0.0019) were significantly higher in HFA children. The detection rates (DR) of p,p´-DDT (p=0.037), at least one substance from the group of DDTs detected (ΣDDTs) (p=0.044) and Heptachlor epoxide (p=0.026) were lower in the HFA group (Table 2). No significant differences regarding both the SC and the DR of any OCP were found between the ADHD or SLD groups and the TD group (Table 2). For those OCP-parameters that were found to differ between the clinical groups and the TD group and were, also, associated with demographic, gestational, perinatal and IQ data, multiple regression was applied. In the case of o,p´-DDD levels, inclusion of gestational age in the regression model absorbed statistical significance between TD and HFA groups [TD vs HFA: β-coefficient=-0.53, (-5.7, 4.6), p=0.82, gestational age: β-coefficient=1.5, (-0.46, 3.5), p=0.12].

Table 2. Summary of serum concentrations (SC)[a] and detection rate (DR)[b] of OCPs in the four study groups[c]

Compounds		TD		HFA		ADHD		SLD	
		DR	SC	DR	SC	DR	SC	DR	SC
HCHs	α-HCH	2 (11.1%)	2.9 ± 0.1	6 (15.4%)	7.6 ± 6.2	4 (19.1%)	4.5 ± 2.0	1 (3.1%)	na
	β-HCH	11 (61.1%)	6.1 ± 4.0	30 (76.9%)	10.5 ± 7.7*	11 (52.4%)	8.0 ± 6.1	19 (59.4%)	9.5 ± 7.1
	γ-HCH	0 (0.0%)	na[d]	2 (5.1 %)	5.7 ± 4.9	3 (14.3%)	5.8 ± 5.9	4 (12.5%)	6.2 ± 2.4
	δ-HCH	-	-	-	-	-	-	-	-
	∑HCHs[h]	11 (61.1%)	6.6 ± 4.0	31 (79.5%)	12.0 ± 10.3 *	14 (66.7%)	8.8 ± 7.1	23 (71.9%)	9.4 ± 8.1
DDTs	p,p'-DDT	7 (38.9%)	22.4 ± 11.9	5 (12.8%)*	29.1 ± 20.5	6 (28.6%)	30.1 ± 16.7	9 (28.1%)	32.1 ± 22.4
	p,p´-DDE	3 (16.7%)	48.1 ± 17.2	5 (12.8%)	70.1 ± 27.1	1 (4.8%)	na	3 (9.4%)	61.6 ± 21.7
	o,p´-DDE	0 (0.0%)	na	1 (2.6%)	na	1 (4.8%)	na	2 (6.3%)	5.5 ± 4.4
	p,p´-DDD	2 (11.1%)	39.2 ± 48.9	0 (0.0%)	na	0 (0.0%)	na	0 (0.0%)	na
	o,p´-DDD	14 (77.8%)	2.8 ± 2.3	20 (51.3%)	7.4 ± 6.5 *	14 (66.7%)	5.1 ± 3.6	17 (53.1%)	4.5 ± 3.9
	∑DDTs	17 (94.4%)	24.6 ± 23.4	27 (69.2%)*	24.0 ± 28.4	18 (85.7%)	18.7 ± 24.4	28 (87.5%)	20.0 ± 24.3
Cyclodienes	Heptachlor epoxide	7 (38.9%)	25.0 ± 22.5	4 (10.3%)*	28.4 ± 21.8	5 (23.8%)	40.7 ± 25.3	5 (15.6%)	35.8 ± 22.2
	Endosulfan	1 (5.6%)	na	4 (10.3%)	15.5 ± 4.7	6 (28.6%)	17.4 ± 9.3	5 (15.6%)	14.9 ± 4.8
	Heptachlor,Aldrin, Dieldrin,Endosulfan sulfate,Endrin,Endrin-aldehyde	-	-	-	-	-	-	-	-
	∑Cyclodienes	8 (44.4%)	24.6 ± 20.8	8 (20.5%)	21.9 ± 16.1	10 (47.6%)	30.8 ± 20.1	10 (31.3%)	25.4 ± 18.7
Methoxychlor		-	-	-	-	-	-	-	-
∑OCPs		18 (100%)	38.2 ± 31.5	39 (100%)	30.6 ± 29.4	21 (100%)	36.6 ± 39.4	32 (100%)	32.2 ± 32.6

*Note: [a]SC: total lipids-adjusted serum concentrations in nanograms per gram lipid, presented as mean ± standard deviation; [b]DR: detection rate absolute number and proportion of individuals above the limit of detection; [c]Comparisons were performed between the clinical groups and the TD group (Mann-Whitney U test for continuous & Fisher's exact test for categorical variables); [d]na: Non-applicable; [h]ΣHCHs, ΣDDTs, ΣCyclodienes and ΣOCPs describe regarding the SC: the concentration sum and the DR: at least one chemical from the respective OCPs group detected; *Statistically significant differences*

Conclusion: We demonstrated higher SCs (β-HCH, ΣHCHs) and lower DRs (p,p´-DDT, ΣDDT, heptachlor epoxide) of several OCPs in HFA than TD children. No differences were observed between the ADHD or SLD groups and TD children. Our findings are in accordance with previous, mainly prospective, studies reporting that exposure to environmental toxicants during the fetal period, infancy and early childhood is associated with NDDs in children[1]. The present study provides evidence of the potentially prominent role of HCHs, which have been implicated as a potentially thyroid disrupting compound in children, in the pathophysiology of Autism Spectrum Disorder[6]. Lower DRs found in HFA children may be indicative of a potential dose-response effect of OCPs during development[7]. Even though cross-sectional findings are of limited worth regarding the causal relationship of the observed associations, our results add to potential neurodevelopmental concerns surrounding OCPs.

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