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Hypospadias in offspring is associated with chronic exposure of parents to organophosphate and organochlorine pesticides

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HIGHLIGHTS

- Our aim was to evaluate the possible association between hypospadias and pesticide exposure.
- We determined pesticides in samples from children with hypospadias and their parents.
- Hypospadiac boys and their parents were found to be exposed to pesticides.
- Our data support the hypothesis that organophosphate and organochlorine pesticide exposure may be a potential risk factor for hypospadias.

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ABSTRACT

We have currently evaluated the possible association between hypospadias and exposure to organophosphorus (OP) and organochlorine (OC) pesticides. For this purpose, we measured the dialkyl phosphate metabolites of organophosphate pesticides (DAPs) in the hair and blood, as well as OC pesticides (DDTs, HCHs) in the hair collected from children with hypospadias and their parents.

The concentration of HCHs in the hair samples obtained from mothers was higher than that previously reported for people working in open cultivations, while the concentration of DDTs in the hair samples obtained from mothers, fathers and their children with hypospadias was much higher than that previously reported for occupationally exposed individuals. The DMP concentration in hair samples obtained from mothers was much higher not only from that reported for the general population, but even higher than that reported for occupationally exposed individuals. Furthermore, SUMDEPs and SUMDAPs in the hair samples obtained both from the hypospadiac boys, as well as from their parents were higher than the corresponding values previously reported for the general population.

Our study supports the hypothesis that organophosphate and organochlorine pesticide exposure may be a potential risk factor for hypospadias.

1. Introduction

Hypospadias is a common congenital anomaly that affects boys. It is a condition in which the urethra has failed to completely form and is often associated with a ventral curvature of the penis (chordee). It is classified according to severity. In its mildest form, first degree hypospadias, the urethra opens on the anterior part of the penis (glandular and subcoronal). In the second and third degree, the urethra opens on the shaft of the penis and scrotum or perineum, respectively (Duckett et al., 1996). The prevalence of this anomaly is reported to be 6–31/10,000 live births (Abdullah et al., 2007).

Differences in the reported prevalence can be attributed to ascertainment, geographical or population differences involved in

Abbreviations: IVF, in vitro fertilization; DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; EDCs, endocrine disrupting chemicals; DAPs, non-specific dialkylphosphate metabolites; DMP, dimethyl phosphate; DEP, diethyl phosphate; DETP, ethyl thiophosphate; DEDTP, diethyl dithiophosphate; a-HCH, alpha-hexa-chloro-cyclohexane; SUM DEPs, sum of DEP, DETP, DEDTP; SUM DAPs, sum of DMP, DEP, DETP, DEDTP; PFBBr, 2,3,4,5,6-pentafluoro benzylbromide; DBP, dibutyl phosphate.

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the studies. Although the prevalence has been reported to have an increasing trend in several studies (Kallen et al., 1986; Nassar et al., 2007; Paulozzi, 1999; Toppari et al., 2001), this was not confirmed in others (Abdullah et al., 2007; Ahmed et al., 2004; Aho et al., 2000; Carmichael et al., 2003; Fisch et al., 2009; Porter et al., 2005).

In the pediatric population of the island of Crete, Greece, the prevalence of this anomaly is 47/10,000 live births, as determined by the medical records of the Pediatric Surgery department of the University of Crete.

The etiology of this congenital disease is still unknown, but it is believed to be multifactorial (Fredell et al., 2002). Family clustering clearly shows a pattern of inheritance (Baskin et al., 2001), but mothers and fathers whose sons have hypospadias have been found exposed to pesticides through their occupation or environment. Some environmental pollutants have been recently implicated in the pathogenesis of the disease (Sharpe and Skakkebak, 2008).

Several environmental anti-androgens (endocrine disrupting chemicals—EDCs) have been identified to interfere with male sexual differentiation in rodent models (Gray et al., 2001; Kelce and Wilson, 1997; Tamura et al., 2001; Wolf et al., 1999). The testicular dysgenesis syndrome, hypospadias, cryptorchidism, late testicular cancer and reduced semen quality can all be induced, upon exposure of the parents, in their offspring (Asklund et al., 2004; Sharpe, 2003; Skakkebaek et al., 2001). It has been shown that among the substances that alter the urogenital development in the laboratory are pesticides (Gray et al., 2000; Tamura et al., 2001).

In view of the above, we studied the possible association between exposure to pesticides and hypospadias. For the aim of this study, we determined the concentrations of non-specific dialkylphosphate metabolites (DAPs) of organophosphorus (OP) pesticides. Dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) were measured in hair and blood samples obtained from children with hypospadias and their parents. Moreover, the organochlorine (OC) pollutants, alpha-hexa-chloro-cyclohexane (a-HCH), hexachlorobenzene (HCB), lindane (HCHs), as well as opDDE, ppDDE, opDDD, ppDDD, opDDT and ppDDT (DDTs) were determined in hair samples of the same children and their parents.

Throughout the manuscript, SUM DEPs refers to the sum of diethyl phosphates (DEP, DETP, DEDTP) and SUM DAPs refers to the sum of diethyl phosphates and DMP (DEP, DETP, DEDTP, DMP).

2. Material and methods

2.1. Sample collection and storage

A total of 29 young boys (aged 3–7 years old) diagnosed with hypospadias (of several degrees) and 49 parents (26 mothers and 23 fathers) participated in this study. All the boys underwent surgery and hair and blood samples were collected from them and their parents. For the boys, hair sampling was performed prior to the surgical operation. Approximately 200 g or more of hair were collected by cutting the hair close to the skull and marking the root of the hair. Blood samples were collected from the boys under general anesthesia. Following centrifugation, the serum was stored at −70 °C until analysis. All ethical issues on working with humans were adhered to, written informed consent was obtained from all parents and the study was approved by the ethics committee.

2.2. Materials

Diethyl ether (95.5%), toluene (99.5%), hydrochloric acid (37%), sodium metabisulfite (98%) and potassium carbonate were obtained from Merck (Darmstadt, Germany). DMP (98%) was purchased from Acros Organics (Geel, Belgium). DEP (98.9%) was obtained from Chem Service (West Chester, USA), DETP (98%) and DEDTP salts (95%) from Sigma–Aldrich (Steinheim, Germany). Methanol and acetonitrile, both HPLC-grade, were purchased from Roth (Karlsruhe, Germany). Sodium chloride (NaCl) was from Riedel-de Haen (Seelze, Germany). The derivatization agent 2,3,4,5,6-pentafluoro benzylbromide (PFBBr, 99%) was purchased from Sigma–Aldrich (Steinheim, Germany) and water (LC–MS grade) from Sigma–Aldrich (Buchs, Switzerland).

All individual standards of DDTs and HCHs were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). Hexane and dichloromethane were of HPLC grade (Merck, New Jersey, USA). Hydrochloric acid 37%, concentrated sulfuric acid 95–97%, were analytical grade reagents (Merck). Anhydrous sodium sulfate for residue analysis, basic aluminium oxide 70–230 Mesh and silica gel 60–200 Mesh (Merck) were used after heating overnight at 120 °C. Empty cartridges (5 ml) were purchased from Supelco (Bellefonte, PA, USA).

2.3. Stock solutions

Stock solutions (1 mg/ml) of each individual DAP (DMP, DEP, DETP and DEDTP) and organochlorine (a-HCH, HCB, lindane, opDDE, ppDDE, opDDD, ppDDD, opDDT and ppDDT) were prepared in methanol and stored at −20 °C. Mixed working solutions of both analytes' groups were prepared and stored at 0 °C, in the dark, covering concentration range from 0 to 1000 ng/ml for DAPs and from 0 to 100 ng/ml for organochlorines.

2.4. Spiked samples

Spiked hair samples: Pooled blank samples of hair (analytes concentration < LOQ) were used for the preparation of spiked hair samples in concentrations of 0, 50, 100, 250, 500 and 1000 pg/mg for DAPs and 0, 10, 25, 50 and 100 pg/mg for HCHs and DDTs. In all spiked solutions dibutyl phosphate (DBP) and (1,2,3,4-tetrachloronaphthalene) (TCN) was added as internal standards in a concentration of 1 pg/mg and 0.1 pg/mg, respectively.

Spiked serum samples: Blank serum samples (analytes concentration < LOQ) were used for the preparation of spiked serum samples in concentrations of 0, 1, 1.25, 2.5, 5 and 10 ng/ml for DAPs. In all spiked solutions DBP was added as internal standard in a concentration of 1 μg/ml.

2.5. Sample treatment

The samples were analyzed for the compounds of interest by gas chromatography–mass spectrometry (GC–MS), as described in previously published studies (Kanavouras et al., 2011; Tsatsakis et al., 2008a,b, 2010; Ueyama et al., 2006) with slight modifications as described below:

DDTs and HCHs extraction from hair: The head hair sample (200 mg) was washed with water, methanol and hexane and cut in small pieces (approximately 2–3 mm). The sample was extracted twice by incubation with 2 ml of hexane at 40 °C in an ultrasonic bath for 3 h and the joined extract (4 ml) was passed through SPE cartridges, packed from the bottom with 250 mg deactivated alumina (10% water), 500 mg of acidified silica and 250 mg anhydrous Na_2SO_4. The cartridges were activated by the addition of 2 ml of hexane:dichloromethane (4:1, v/v). The solvent used for elution was 2 ml of hexane: dichloromethane (1:1, v/v). The final eluent was dried under a gentle nitrogen stream and reconstituted in 100 μl TCN 0.1 μg/ml in hexane (external standard) (Tsatsakis et al., 2008a,b).

DAPs extraction from hair: Washed hair samples were dried and pulverized in a ball mill homogenizer. Two (2) ml of methanol containing 100 ng dibutyl phosphate (DBP) as internal standard were added and hair was incubated at room temperature in an ultrasonic bath for 4 h. Following centrifugation, the supernatant was transferred to a clean vial. Two (2) more milliliters of methanol were added to the solid residue, followed by ultrasound-assisted solid–liquid extraction (30 min). The joined extract (4 ml) was centrifuged at 2500 × *g* for 5 min, filtered and transferred to a test-tube containing 15 mg of K_2CO_3 and methanol was evaporated to dryness. Fifteen (15) mg of K_2CO_3 was added again to the residue, before it was reconstituted in 0.5 ml of acetonitrile and 0.1 ml solution of pentafluorobenzylbromide (PFBBr) in acetonitrile (1:3, v/v) and incubated at 80 °C in a water bath for 30 min. The acetonitrile was evaporated to dryness and the residue was dissolved in 100 μl of toluene (Tsatsakis et al., 2010).

DAPs extraction from serum: The serum sample (1 ml) was transferred to a clean 15 ml screw-top glass vial containing 4 g of NaCl and 50 mg of $Na_2S_2O_5$, 0,25 mL of HCl (6 M) and 100 ng of DBP were added. The mixture was incubated for 5 min at 40 °C. Liquid–liquid extraction was performed by adding 1.5 ml of diethyl ether–acetonitrile (1:1, v/v) followed by mechanical shaking for 5 min. After the extraction, the samples were centrifuged at 2000 g (5 min) at 4 °C. The supernatant was collected in another vial containing 15 mg of K_2CO_3 and the liquid–liquid extraction step was repeated. The two extracts were combined and evaporated to dryness under a stream of nitrogen at 30 °C. The residue was reconstituted in 0.5 ml of acetonitrile and 0.1 ml of PFBBr in acetonitrile (1:3, v/v) and incubated in a water bath at 80 °C for 30 min with occasional shaking (Ueyama et al., 2006). After incubation, the mixture was brought to room temperature and acetonitrile was evaporated to dryness under a stream of nitrogen at 35 °C. The residue was dissolved in 100 μl of toluene.

2.6. GC–MS analysis

The analysis of organochlorine (a-HCH, HCB, lindane, opDDE, ppDDE, opDDD, ppDDD, opDDT, ppDDT) and DAPs was performed on an electron ionization mass spectrometric GC-QP2010 Shimadzu system equipped with an Equity TM-5 (30 m × 0.20 mm × 0.20 μm) capillary column supplied by Supelco (Supelco, 595 North Harrison Road, Bellefonte, PA 16823-0048, USA). Pure helium with a column

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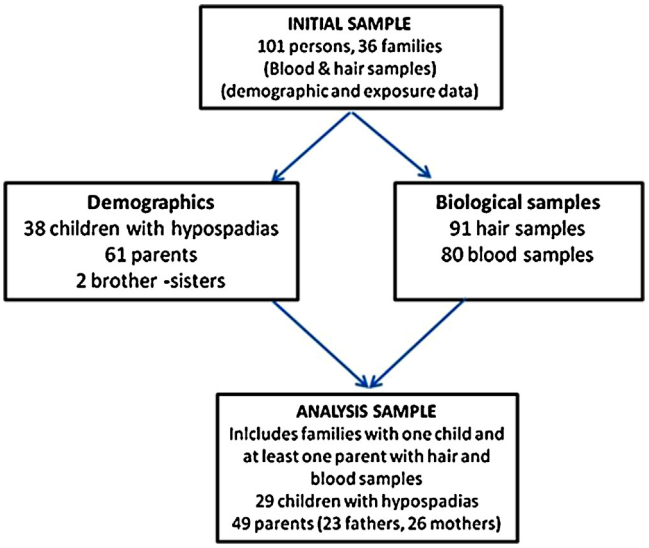

Fig. 1. Study design: schematic description of the samples collected and analyzed.

flow of 1 ml/min was used as a carrier gas. The solutions (1 μl) were injected into the system in the splitless mode and analyzed under the following conditions:

For DDTs and HCHs: The column temperature was initially held at 60 °C for 1 min, raised to 180 °C at 15 °C/min, held for 1 min, raised to 250 °C at 4 °C/min, held for 1 min and was finally raised to 300 °C, at 30 °C/min, where it remained stable for 2 min. The target (quantitation) and qualifier ions (*m*/*z*) for a-HCH were 181, 109, 219, for HCB 284, 294, for Lindane 181, 111, 219, for opDDE and ppDDE 246, 176, 318, for opDDD, ppDDD, opDDT and ppDDT 235, 165.

For DAPS: The column temperature was initially held at 60 °C for 1 min, raised to 180 °C at 20 °C/min, held for 1 min, raised to 250 °C at 4 °C/min, held for 1 min and was finally raised to 300 °C, at 25 °C/min, where it remained stable for 2 min. The injector, interface and ion source temperatures were set at 270 °C, 300 °C and 230 °C, respectively. The target (quantitation) and qualifier ions (m/z) for each DAP were 306 and 110 for DMP, 258 and 334 for DEP, 350 and 274 for DETP and 366 and 185 for DEDTP.

Table 1
Demographic characteristics of the parents.

	Parent		
	Father	Mother	*p*
Age (years)	32.7 ± 4.5	27.7 ± 3.9	
Height (m)	1.76 ± 0.04	1.64 ± 0.05	
Weight (kg)	78.8 ± 10.1	61.6 ± 10.0	
Occupation			
Possible exposure	13 (50.0%)	23 (79.3%)	0.022
No exposure	13 (50.0%)	6 (20.7%)	
Relatives with hypospadias			
Yes	0 (0.0%)	1 (4.0%)	
No	13 (61.9%)	13 (52.0%)	
No information	8 (38.1%)	11 (44.0%)	
Relatives with Cryptorhidism			
Yes	1 (4.8%)	2 (8.0%)	0.793
No	12 (57.1%)	12 (48.0%)	
No information	8 (38.1%)	19 (41.3%)	
Smoking			
Yes	9 (45.0%)	10 (40.0%)	0.736
No	11 (55.0%)	15 (60.0%)	
Alcohol consumption			
Yes	7 (35.0%)	1 (4.0%)	0.015
No	13 (65.0%)	24 (96.0%)	
Exposure to organophosphates			
Yes	7 (33.3%)	4 (16.0%)	0.170
No	14 (66.7%)	21 (84.0%)	
Exposure to other chemicals			
Yes	5 (23.8%)	4 (16.0%)	0.506
No	16 (76.2%)	21 (84.0%)	
Level of education			
Primary	4 (18.2%0	3 (12.0%)	0.555
Secondary	8 (36.4%)	7 (28.0%)	
Lyceum	8 (36.4%)	9 (36.0%)	
University	2 (9.1%)	6 (24.0%)	

3. Statistical analysis

Continuous variables were expressed as mean ± standard deviation, while the concentrations of organochlorine pollutants and DAPs were presented as medians and quartiles. The association between two variables was examined using Spearman's rho. Comparisons between groups were performed using the Mann–Whitney and the Kruskal–Wallis tests. The IBM SPSS 20.0 software was used for data analysis.

4. Results

A total of 101 individuals, from 36 families, were initially recruited for the study. Out of these 101 individuals, analysis was performed only for families which included one child with hypospadias and at least one parent for whom hair and blood samples were available. Therefore, the individuals that finally participated in the study included 29 children with hypospadias and 49 parents (Fig. 1).

The demographic characteristics of the parents that were included in the study are shown in Table 1. A case of cryptorchidism and hypospadias among relatives was rare (3 and 1 report respectively), while approximately 40.0%, of the participants lacked information on this subject. The self-reported occupational exposure was low, 11 parents involving OP and 9 parents involving other chemicals. On the other hand, the suspicion of being occupationally exposed to chemicals (in occupations such as building workers, farmers and cleaners) was high, namely 50.0% in males and 79.3% in females. As far as smoking is concerned, 45.0% of fathers and 40.0% of mothers were smokers (Table 1).

The 29 children that participated in the study had a mean age of 5.0 ± 4.1 years. Most of the children were delivered after a full term pregnancy ($n = 24$, 82.8%). Fourteen children (48.3%) and eleven children (37.9%) were the first or second child in the family respectively.

The methods' linearity, accuracy and precision, as well as the limits of detection and qualification for the analytes in hair were validated in previous studies for both DAPs (Tsatsakis et al., 2010) and HCHs and DDTs (Tsatsakis et al., 2008a,b).

The level of exposure to pesticides, expressed as their concentration in hair samples, is presented in Table 2. The median values of HCHs were significantly greater (38.1 pg/mg) in mothers, compared to fathers (15.3 pg/mg) and their children with hypospadias (17.8 pg/mg) ($p = 0.037$). The same was observed for DDTs. Their concentration in hair was 144.4 pg/mg for mothers, 24.9 pg/mg for fathers and 77.5 pg/mg for children with hypospadias ($p = 0.009$). On the other hand, no significant difference was observed for DMP, SUMDEPs, or SUMDAPs between the different members of a family.

The level of exposure to pesticides, expressed as their concentration in blood, is presented in Table 3. No significant difference in DMP, SUMDEPs or SUMDAPs was found between family members (*p* values are 0.311, 0.654 and 0.367 respectively).

The association of blood and hair levels between children with hypospadias and their parents was estimated using Spearman's rho. The DMP concentration in hair of children with hypospadias, correlated significantly with the DMP level in hair of their parents ($r_s = 0.506$, $p = 0.001$) (Fig. 2). On the other hand, the SUMDEPs and SUMDAPs in hair did not correlate between children and their parents ($r_s = 0.085$, $p = 0.686$ and $r_s = 0.067$, $p = 0.837$, respectively) (data not shown).

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Table 2
Level of exposure, expressed as concentration (pg/mg) in hair samples, in children with hypospadias and their parents.

		Mean	SD	1st quartile	Median	3rd quartile	*p* Kruskal–Wallis
DMP	Children with hypospadias	327.1	566.2	<LOD	31.5	534.8	0.071
	Father	315.3	423.1	<LOD	102.3	536.7	
	Mother	453.7	511.2	110.5	249.9	526.2	
SUMDEPs	Children with hypospadias	1060.9	1610.6	<LOD	521.2	1511.0	0.675
	Father	1508.6	2970.1	<LOD	585.9	1473.7	
	Mother	1285.8	2256.9	396.2	528.4	996.3	
SUMDAPs	Children with hypospadias	1388.0	2066.4	<LOD	593.9	2057.9	0.328
	Father	1824.0	3234.6	<LOD	829.7	1832.6	
	Mother	1739.5	2341.7	577.2	1050.0	1887.4	
HCHs	Children with hypospadias	89.9	225.6	<LOD	17.8	46.5	0.037
	Father	108.1	394.6	<LOD	15.3	40.3	
	Mother	81.7	106.3	17.4	38.1	89.5	
DDTs	Children with hypospadias	233.1	742.6	<LOD	77.5	166.4	0.009
	Father	789.2	3531.5	<LOD	24.9	57.6	
	Mother	467.2	1167.5	42.0	144.4	263.3	

Table 3
Level of exposure, expressed as concentration (ng/ml) in blood samples, in children with hypospadias and their parents.

		Mean	SD	1st quartile	Median	3rd quartile	*p* Kruskal–Wallis
DMP	Children with hypospadias	21.0	53.9	<LOD	6.6	22.2	0.311
	Father	29.8	55.4	<LOD	3.9	29.4	
	Mother	27.7	36.4	1.4	16.6	39.8	
SUMDEPs	Children with hypospadias	45.7	66.6	12.4	22.9	64.7	0.654
	Father	62.0	91.2	<LOD	23.6	90.9	
	Mother	61.9	80.2	19.0	22.9	114.0	
SUMDAPs	Children with hypospadias	66.7	81.0	19.5	32.7	89.7	0.367
	Father	91.8	126.5	<LOD	31.9	127.4	
	Mother	89.6	84.2	30.4	42.1	159.9	

The concentration of DMP in blood did not correlate between children with hypospadias and their parents ($r_s = 0.224$, $p = 0.294$) (data not shown). On the other hand, the SUMDEPs (Fig. 3) and SUMDAPs (Fig. 4) correlated significantly ($r_s = 0.558$, $p < 0.001$ and $r_s = 0.348$, $p = 0.035$, respectively).

5. Discussion

There is currently extensive scientific evidence pointing towards an association of pesticide exposure and different chronic diseases, among which are birth defects and reproductive disorders. For these various diseases, different mechanisms have been proposed until today such as oxidative stress, mitochondrial dysfunction, genetic damages, epigenetic phenomena, endocrine disruption and unfolded protein response (Mostafalou and Abdollahi, 2013).

We have currently presented results that support the hypothesis that there is a connection between exposure to pesticides and the pathogenesis of hypospadias. Four parents reported that they had relatives with hypospadias or cryptorchidism, while relevant information was not provided by a large number of parents. Therefore, an increased congenital risk cannot be excluded in the participants

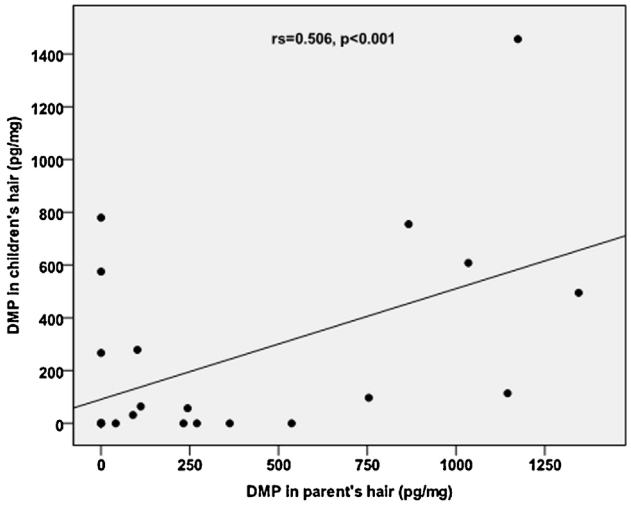

Fig. 2. Scatter plot of the DMP concentrations in hair between parents and children with hypospadias.

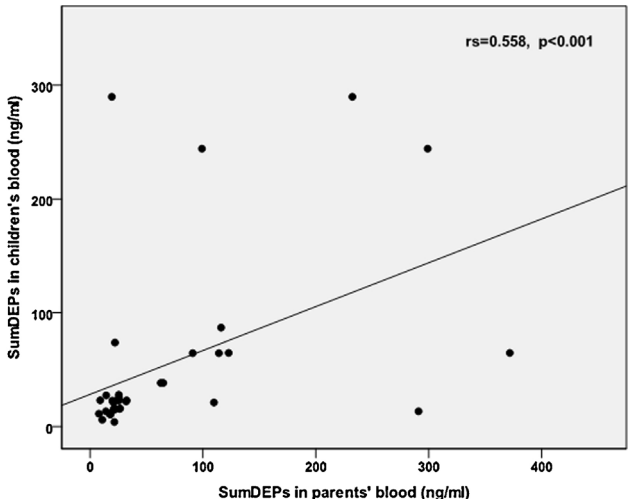

Fig. 3. Scatter plot of the sumDEPs in blood between parents and children with hypospadias.

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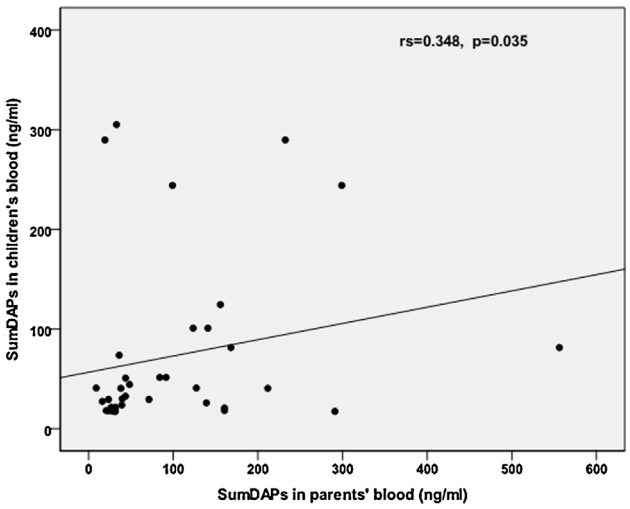

Fig. 4. Scatter plot of the sumDAPs in blood between parents and children with hypospadias.

of our study, since familial clustering for male external genital malformations has been already reported (Brouwers et al., 2007; Gaspari et al., 2011).

Other limitations of our study include the lack of a control group and the limited sample size, as well as the long time interval between the onset of the disease (upon birth) and the measurements conducted, since the children had a mean age of 5 years.

In 2005, in a study similar to ours, researchers measured ppDDT and ppDDE in the serum of mothers of children with hypospadias, as well as of mothers of children with no malformations and reported no association of DDT or DDE with hypospadias (Bhatia et al., 2005). Furthermore, in 2011 scientists used data from the National Birth Defects Prevention Study (NBDPS) in USA and concluded that low intensity maternal periconceptional occupational pesticide exposure is not a risk factor for hypospadias (Rocheleau et al., 2011). In another study, where researchers examined the relation between maternal serum DDE levels during pregnancy and odds of hypospadias, the results were reported as inconclusive (Longnecker et al., 2002).

On the other hand, it was recently shown that fetal contamination by endocrine-disrupting chemicals (EDCs) such as DDT, secondary to parental exposure, before or during pregnancy, is associated with the development of genital malformations (Gaspari et al., 2012). Nevertheless, in that study, exposure was documented through questionnaires answered by the parents and not through the determination of EDCs in biological samples. Furthermore, a meta analysis on the risk of hypospadias resulting from parental exposure to pesticides revealed that elevated risks of hypospadias were associated with maternal and paternal occupational exposure (Rocheleau et al., 2009). Last but not least, scientists provided evidence that maternal exposure to EDCs, and in particular elevated plasma hexachlorobenzene concentration, is associated with the development of hypospadias in the offspring (Giordano et al., 2010).

We have currently showed that in children diagnosed with hypospadias, both the children and their parents were exposed to OP and OC pesticides, as demonstrated through the analysis of blood and hair samples.

HCHs and DDTs were not analyzed in blood, despite the fact that OCs, which are lipophilic chemicals stored in maternal adipose tissue, can be transferred to the blood stream. The reason for choosing hair over blood samples is the fact that blood samples provide information only of recent exposure. On the contrary, hair samples provide reliable information for past exposure, systematic, chronic and accumulated exposure. Especially hair samples of long length, such as those collected from the women of the study, can provide information for the last months or even the last year (Tsatsakis et al., 2008a,b).

In the current study, DMTP was not included in the analysis in spite of being very often found as an OP metabolite. DMTP was exclusively and in detail studied in previously published articles (Margariti and Tsatsakis, 2009a,b). Moreover, both DMP and DMTP are referred to as derivatives of *O,O*-dimethyl-substituted OPs. Furthermore, they both provide similar information for the burden exposure to these OPs. In addition, as shown in a previous study (Tsatsakis et al., 2009), DMP derives from a bigger number of *O,O*-dimethyl-substituted OPs compared to DMTP.

The concentration of HCHs in the hair samples obtained from mothers (38.1 pg/mg) was higher than that previously reported for people working in open cultivations (24.1 pg/mg) (Tsatsakis et al., 2008a). Furthermore, the concentration of DDTs in the hair samples obtained from mothers (144.4 pg/mg), fathers (24.9 pg/mg) and their children with hypospadias (77.5 pg/mg) was much higher than that previously reported for occupationally exposed individuals (8.9 pg/mg for greenhouse workers, 3.3 pg/mg for animal breeders and 5.2 pg/mg for people working in open cultivations) (Tsatsakis et al., 2008a) (Fig. 5). When DMP was determined in hair samples, we found that its concentration in the hair samples obtained from mothers (249.9 pg/mg) was much higher not only from that reported for the general population (165.0 pg/mg), but even higher than that reported for occupationally exposed individuals (181.7 pg/mg) (Tsatsakis et al., 2010). Furthermore, SUMDEPs

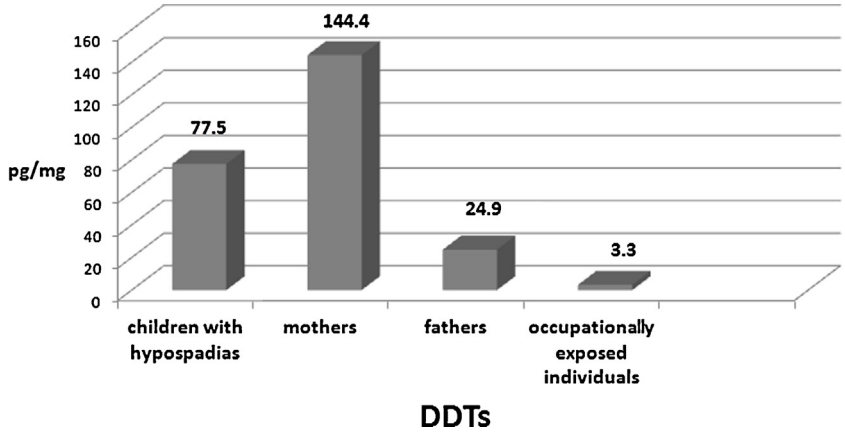

Fig. 5. Concentration of DDTs in hair samples (pg/mg) obtained from children with hypospadias, their fathers and mothers, and occupationally exposed individuals.

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and SUMDAPs in the hair samples obtained both from the hypospadiac boys, as well as from their parents were higher than the corresponding values previously reported for the general population (119.5 pg/mg for SUMDEPs and 301.5 pg/mg for SUMDAPs) (Tsatsakis et al., 2010).

Our results are in agreement with another study (Fernandez et al., 2007) in which opDDT, ppDDT and lindane were measured in placenta tissues and concluded that high concentrations of those chemicals were associated with an increased risk for urogenital malformations in the offspring.

These results were reinforced a year later when ppDDE, b-HCH, HCB and ppDDT were measured in human milk and placental tissues. It was found that the levels of these compounds were higher in Danish, compared to Finnish samples and correlated with a higher prevalence of hypospadias in Danish men compared to Finnish men (Shen et al., 2008). Last but not least, it was recently shown that hypospadiac boys have higher ppDDE and b-HCH blood levels, compared to control boys (Shekharyadav et al., 2011).

Our results support the hypothesis that there is a connection between exposure to pesticides and the pathogenesis of the disease. All hypospadiac boys examined, as well as their parents, were found to be chronically exposed to pesticides, as demonstrated through hair analysis.

6. Conclusions

Hypospadias is a congenital disease of unknown etiology. The implication of multiple factors in the pathogenesis of the disease cannot be excluded.

Our findings support the hypothesis that organophosphate and organochlorine pesticide exposure may be a potential risk factor for hypospadias, since all hypospadiac boys and their parents were found, upon analysis of hair and blood samples, to be exposed to pesticides.

Conflict of interest

None.

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