Bull. Environ. Contam. Toxicol. (2005) 75:952–959

DOI: 10.1007/s00128-005-0842-5

Bulletin of Environmental Contamination and Toxicology

Persistent Organochlorine Pesticides Levels in Blood Serum Lipids in Women Bearing Babies with Undescended Testis

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Received: 22 April 2005/Accepted: 16 August 2005

The organochlorine pesticides have been introduced into agriculture and public health programs against ectoparasites in livestock and humans. Applied in tropical countries, they have provided great benefits in sanitation controlling the spread of vector-borne diseases and ectoparasites. Due to their persistence and lipophility, the pesticides tend to accumulate in lipid-rich compartments of organism (Travis et al. 1988, Charlier and Plomteux 2002, Waliszewski et al. 2003). After entering the organism, they are distributed into corporal lipids, they cross the inner barriers and bioconcentrate in the tissues according to an equilibrium pattern and lipid content (Mussalo-Rauhamaa 1991, Waliszewski et al. 2001 2002). Thus, the adipose tissue acts as depots or reservoirs of persistent chemical substances by virtue of physiochemical interactions of cellular components (Mussalo-Rauhamaa 1991). The analyses of human lipid-rich compartments, such as adipose tissue and blood serum lipids, reflect the rate of environmental exposure which, in turn, permits a quantification of human exposure and its association to human health risks (Waliszewski et al. 2000).

During fetal development, the testicles first appear in the abdomen at about the level of the kidney. They develop at this site and in the seventh month of fetal life they begin to descend the inguinal canal (a small passageway that runs along the abdomen near the groin) into the scrotum. The terms undescended testis and cryptorchidism refer to a relatively common condition in which the testis has not descended into the scrotum and cannot be brought into the scrotum with external manipulation. The undescended testis may be located in the inguinal canal, within the abdominal cavity, or in an ectopic location (unusual), but it is most commonly found (80%) in the inguinal canal. Ten percent of cases are bilateral (involve both testes). Since the testes do not descend from the abdomen to the scrotal sac until the seventh month of fetal development, this condition is more commonly seen in premature infants. It is also associated with hormonal disorders, spina bifida, and other congenital abnormalities.

Several theories have been proposed to explain the mechanism of testicle descent. An endocrinologic effect on the gubernaculum testis, a fetal ligament that connects the testis and the scrotal sac, is the most likely

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explanation. This theory is supported by studies showing androgen receptors on the gubernaculum and the genitofemoral nerve that innervates it. Thus, a disruption of the normal hormonal influence on the gubernaculum or its innervation is believed to be the most likely cause of testicular maldescent (Rajfer 1998).

There is evidence that male reproductive health has suffered impairment over the last decades. In several countries, a possible decline in semen quality has been the focus of considerable controversy, parallels by a well-established increase in the incidence of testicular cancer in young men (Coleman et al. 1993). Increasing incidences of cryptorchidism have likewise been reported (Facemire et al1995, Ankley 1998, Campbell and Hutchinson 1998, Carlsen et al.1992). However, the timing of these malformations may be difficult to interpret due to potential changes in diagnostic criteria and treatment routines (Campbell and Hutchinson 1998). Recently, an association between fetal exposure to environmental estrogens, anti-androgens and the impairment of male reproductive health has been suggested (Sharpe and Skakkebæk 1993). In animal studies, estrogen exposure during pregnancy resulted in the development of cryptorchidism (Grocock et al. 1988). The organochlorine pesticides have been reported to posses estrogenic or antiandrogenic properties (Garcia-Rodríguez et al. 1996). Prenatal exposure to such pesticides may therefore increase the risk of cryptorchidism (Weidner et al. 1998).

The purpose of the present study was to determine the levels of organochlorine pesticides in blood serum lipids in women bearing babies with undescended testis, and compare the results with a control group (women bearing babies with descended testicles).

MATERIALS AND METHODS

In the study, blood samples (approximately 10 ml) were taken post partum from thirty mothers, interned in the IMSS Hospital for delivery, when undescended testicles were diagnosed (UT). The control group (C) consisted of blood samples taken from thirty mothers that bore babies with descended testicles. The blood samples were centrifuged to separate the serum from blood cells, and then total serum lipid contents were determined. The rest of the serum was weighed to determine sample weight, and total lipid content in the sample was calculated.

Blood serum samples were analyzed according to previously described method (Waliszewski and Szymczynski 1991). The qualitative and quantitative determinations were performed by gas chromatography on a Varian 3400 CX apparatus, equipped with a ^{63}Ni electron capture detector. Using US EPA Method 608 for pesticides separation, a fused silica column SPB 608 30 m X 0.32 mm ID, 0.5 µm film was employed with the following temperature program: 193°C (for 7 min) rising to 250°C at 6°C/min, hold for 20 min. The carrier gas was nitrogen at 25 cm/min, and split/splitless sample injection of 1 µL in splitless mode was employed.

All samples were analyzed for: HCB, β-HCH, pp'DDE, op'DDT and pp'DDT. The minimum detection limits expressed on fat basis for the organochlorine pesticides studied were: 0.001 mg/kg for HCB and β-HCH, 0.002 mg/kg for pp'DDE, op'DDT and pp'DDT. To determine the quality of the method, the recovery study was performed on ten overspiked replicates of a blank cow blood sample, which revealed contamination levels below detection limits. The fortification study, done at 0.010 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 90 to 95% of recovery. The standard deviation and coefficients of variation were below 10, indicating excellent repeatability of the method. The concentrated sulfuric acid used in the clean-up step of serum extracts, permits quantitative fat precipitation and degrades the ubiquitous phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides (Waliszewski and Szymczynski 1990).

Total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Wiener Lab for clinical laboratories (Anonymous 1996).

To compare variability of blood serum organochlorine pesticide concentrations between UT and C groups, the following test were calculated applying statistical softwares: Minitab version 12 and SAS System version 8: paired t-test, Pearson correlation coefficients (r), coefficient of determinations (r^2), Mann-Whitney test and relative risk (RR).

RESULTS AND DISCUSSION

Exposure to environmental hazards is not limited to the environment. Potential sources of exposure include food, air, water, soil, and hobbies. Individuals may have multiple exposures that in many cases occur chronically and at low doses. The reproductive health implications of chronic exposures to reproductive toxicants are not well documented and, in general, the mechanisms of toxicity are either poorly understood or unknown.

Reports of declining sperm counts over the past 50 years and other disturbing trends alerted scientists to the possibility that exposure to chemicals in the environment may damage male reproductive health. Testicular cancer, the most common malignancy in men 15-44 years of age (Ekbom and Akre 1998), has increased markedly in incidence in the 20th century in virtually all countries studied. The incidence of hypospadias, a developmental malformation of the male urethra, appears to be increasing worldwide. Undescended testicle, another developmental defect, may have increased in some human populations and appears to be increasing in wildlife (Anonymous 1986, Guillette et al. 1999). Environmental xenoestrogens and antiandrogens such as organochlorine pesticides may contribute to abnormal or subnormal reproductive system development, and

appear to act on the males specifically during embryonic development (Kelce et al. 1995, Facemire et al.1995).

Table 1. Serum organochlorine pesticide levels (mg/kg on fat basis) in the UT group (n=30) and controls (n=30).

Pesticide	X ± SD	SEM	Median	Ranges
HCB	0.210 ± 0.142	0.026	0.189	0.060 – 0.522
HCB-control	0.178 ± 0.105	0.019	0.131	0.031 – 0.383
β-HCH	0.212 ± 0.121	0.022	0.202	0.025 – 0.536
β-HCB-control	0.279 ± 0.147	0.027	0.261	0.094 – 0.889
pp'DDE	2.562 ± 0.804	0.147	2.650	1.274 – 4.139
pp'DDE-control	2.357 ± 0.859	0.157	2.307	0.888 – 4.608
op'DDT	0.108 ± 0.044	0.008	0.100	0.030 - 0.202
op'DDT-control	0.087 ± 0.043	0.050	0.088	0.005 - 0.181
pp'DDT	0.427 ± 0.045	0.029	0.429	0.186 - 0.742
pp'DDT-control	0.352 ± 0.211	0.034	0.278	0.057 - 0.838
Σ-DDT	3.042 ± 0.929	0.170	3.193	1.622 – 4.887
Σ-DDT-control	2.799 ± 0.926	0.169	2.841	1.221 – 5.392

During the study, blood serums taken from mothers after deliveries were analyzed to determine the organochlorine pesticide residue concentrations in two groups: undescended (UT) and descended testicles (C). The samples were paired to determine possible differences between concentrations. The results obtained, expressed on fat basis (mg/kg) are presented in Table 1 as mean values (X) ± standard deviation of means (SD), standard error of mean (SEM), median and ranges. The comparison of mean and standard deviation values for all organochlorine pesticides between both sample groups indicates higher values in blood serum from the UT group, compared to the controls (HCB 0.210 vs 0.178, pp'DDE 2.562 vs 2.357, op'DDT 0.108 vs 0.087, pp'DDT 0.427 vs 0.352 and Σ-DDT 3.012 vs 2.799). However, β-HCH levels were lower in the UT group (0.212 vs 0.279).

Table 2. Statistical test (two-tailed t-test (p), Pearson correlation coefficient (r), coefficient of determination (r^2) and Mann-Whitney test applied to identify differences in organochlorine pesticide levels between UT and control groups.

Pesticide	p	r	r^2	Mann-Whitney
HCB	0.320	-0.143	0.020	0.712
β-HCH	0.059	-0.121	0.015	0.057
pp'DDE	0.345	0.137	0.019	0.258
op'DDT	0.089	0.457	0.209	0.107
pp'DDT	0.128	0.119	0.014	0.059
Σ-DDT	0.226	0.228	0.052	0.196

To determine variability between means (Table 2), a two-tailed comparison test was applied, indicating $p>0.05$ values for all organochlorine pesticide

studied, marking no significant differences between means of their concentrations in blood serum lipids. The calculated results of Pearson correlation coefficients are lower then 0.5 revealing a bad correlation for all organochlorine pesticide concentrations in both sample groups. The same goes for the following coefficients of determinations (r^2) stating differences in organochlorine pesticide concentrations between UT and control groups. The applied Mann-Whitney test, to compute the equality of two population medians from independent random samples that have the same shape, reveals no significant differences for median organochlorine pesticide concentrations.

Table 3. Relative Risk (RR) values and 95% confidence intervals (95% CI) calculated for organochlorine pesticide concentrations in UT and control groups.

Pesticide	RR	95% CI
HCB	1.16	1.07 - 1.26
β-HCH	0.73	0.66 - 0.79
pp'DDE	1.11	1.07 - 1.15
op'DDT	1.49	1.16 - 1.82
pp'DDT	1.43	1.26 - 1.61
Σ-DDT	1.12	1.09 - 1.15

The relative risk (RR) is calculated by comparing the incidence of disease in exposed (undescended testis) population with the control. To examine the relative risk (RR) in relation to organochlorine pesticide levels, the multivariate logistic regression model was calculated. Table 3 presents the relative risk values (RR) and their 95% of confidence intervals (95%CI) that establish the boundaries of the relative risk calculated for all organochlorine pesticides. If the relative risk is 1.0, the risk in exposed individuals is the same as unexposed individuals. If the relative risk is greater than 1.0, the risk in exposed individuals is greater than in those not exposed. For the result to indicate a doubling of the risk, the relative risk must be greater than 2.0. In the study, the RR value does not reach 2.0, revealing no significant differences in relative risk values. These results lead us to reject the null hypothesis and state that organochlorine pesticides do not cause undescended testicle illness.

The confidence level of 95% means, that if a study were repeated, it would produce a relative risk in 95% of the repetitions. For β-HCH, the value of RR was below 1 and the CI below 1, indicating insignificant relative risk at the 95% level. For all remaining pesticides the RR values exceeded 1, but did not exceed 1.5, revealing that if the study were repeated, it would produce a relative risk below 1.5. The confidence intervals (CI) shows a range of values (1.07 to 1.82) within which the results of a study sample would be likely to fall if the study were repeated numerous times. Based on a confidence level of 95%, a study showed a relative risk no more than 1.49 and had a confidence interval of 1.07 to 1.82, which means that if the study were repeated, it would produce a relative risk between 1.07 and 1.82 in

95% of the repetitions. The narrower the confidence interval, the greater the confidence in the relative risk estimate found in the study. The relative risk values not exceed 2.0 and the confidence intervals could not include 1.0. The results no indicate more than a doubling of the risk nor any statistical differences between mean and median organochlorine pesticide lipid serum levels and undescended testicles presented in new born babies.

In light of these results, it can be assumed that no correlation exists between concentrations of persistent organochlorine pesticides in maternal blood serum lipids and problems of testicle descent in their babies. The results agree with conclusions previously reported by Hosie et al. (2000), Longnecker et al. (2002), Flores-Luevano et al. (2003), Bhatia et al. (2005).

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