INTRODUCTION: Prenatal exposure to some pesticides can adversely affect male reproductive health in animals. We investigated a possible human association between maternal exposure to 27 organochlorine compounds used as pesticides and cryptorchidism among male children.

DESIGN: Within a prospective birth cohort, we performed a case–control study; 62 milk samples from mothers of cryptorchid boys and 68 from mothers of healthy boys were selected. Milk was collected as individual pools between 1 and 3 months postpartum and analyzed for 27 organochlorine pesticides.

RESULTS: Eight organochlorine pesticides were measurable in all samples (medians: nanograms per gram lipid) for cases/controls: 1,1-dichloro-2,2-bis(4-chlorophenyl)ethyene (p,p'-DDE): 97.3/83.8; β-hexachlorocyclohexane (β-HCH): 13.6/12.3; hexachlorobenzene (HCB): 10.6/8.8; t-endo-endosulfan: 7.0/6.7; oxychlordane: 4.5/4.1; 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT): 4.6/4.0; dieldrin: 4.1/3.1; cis-heptachloroepoxide (cis-HE): 2.5/2.2. Five compounds [octachlorostyrene (OCS); pentachlorobenzene, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT); p,p'-DDE; mirex] were measurable in most samples (detection rates 90.8–99.2%) but in lower concentrations. For methoxychlor, cis-chlordane, pentachloroanisole (PCA), γ-HCH, 1,1-dichloro-2-(2-chlorophenyl)-2,4(5-HCB); trans-chlordane, α-HCH, and (α,γ)-endosulfan, (+)-endosulfan, and trans-heptachloropoxide were detected at negligible concentrations and low detection rates and were not analyzed further. Seventeen of 21 organochlorine pesticides (p,p'-DDE, p,p'-DDT, p,p'-DDD, o,p'-DDT, α-HE, and dieldrin) were measured in higher median concentrations in case milk than in control milk. Apart from trans-chlordane (p = 0.012), there were no significant differences between cryptorchid and healthy boys for individual chemicals. However, combined statistical analysis of the eight most abundant persistent pesticides showed that pesticide levels in breast milk were significantly higher in boys with cryptorchidism (p = 0.032).

CONCLUSION: The association between congenital cryptorchidism and some persistent pesticides in breast milk as a proxy for maternal exposure suggests that testicular descent in the fetus may be adversely affected.

KEY WORDS: cryptorchidism, human breast milk, infants, persistent organochlorine pesticides.
29 Danish and 33 Finnish cases defined as boys with cryptorchidism (unilateral or bilateral) at birth. Four Danish and 25 Finnish boys were still cryptorchid at 3 months of age, whereas the others (25/8) had spontaneous descent. Danish/Finnish controls (36/32), defined as boys without cryptorchidism at birth or 3 months, were included. In Denmark, the controls were selected randomly from the entire birth cohort of healthy boys. In Finland, the boys were selected prospectively by a case–control design in which the boys with cryptorchidism were matched to controls at birth for maternal parity, smoking (yes/no), diabetes (yes/no), gestational age (± 7 days), and date of birth (± 14 days). This design was chosen in Finland because of lack of sufficient funding to collect and store biologic samples from all. To ensure that all prospectively planned chemical analyses could be performed, only breast milk samples with a volume > 125 mL were included.

The study was conducted according to the Helsinki II Declaration (World Medical Association 2004) and was approved by the local ethical committees in both countries (Finland: 7/1996, Denmark: KF01-030/97) and the Danish Data Protection Agency (registration no. 1997-1200-074). The families were included after oral and written informed consent had been obtained from the parents.

Human breast milk samples were collected from 1 to 3 months postpartum in Denmark and from 1 to 2 months postpartum in Finland as successive aliquots. All mothers were given oral and written instructions to feed the baby first and then sample milk aliquots (hind milk) by manual expression into a glass or porcelain container, avoiding the use of mechanical breast pumps. The aliquots were frozen consecutively in a glass bottle (250-mL Pyrex glass bottle with Teflon cap (1515/06D, Bibby Sterilin, Staffordshire, England) and stored in household freezers. The samples were delivered frozen to the hospital at the 3 months’ examination and stored at −20°C.

Exposure measurements in biologic samples from boys with congenital cryptorchidism at birth and controls were prospectively planned to include persistent and nonpersistent chemicals. Twenty-seven organochlorine compounds were selected by the following criteria: previous worldwide use, suspicion of endocrine-disrupting activity from animal and/or in vitro studies, and highly sensitive analytic methods available. As part of other substudies, the same breast milk samples were planned to be analyzed for other compounds with suspected endocrine-disrupting activity.

The selected breast milk samples were thawed at room temperature for 12 hr, heated, and shaken for 30 min at 37°C to homogenize the samples, and then divided into smaller aliquots and refrozen at −20°C until further chemical analysis. Extraction, cleanup, and analysis of organochlorine pesticides in the milk samples were based on a method that has been described in detail elsewhere (Shen et al. 2005). Milk samples (10 mL) were extracted with 250 mL of a mixture consisting of acetone and n-hexane (2:1 v/v) (Beck 2000). The milk extracts were collected in flasks weighed in advance and evaporated using a rotary vacuum evaporator (water bath at 45°C). After evaporation, the flasks were placed into an excitor until stable weight was achieved. The lipid content was calculated on wet weight basis. The residual was dissolved in toluene, and gel permeation chromatography followed by sandwich cartridge cleanup was used to remove lipids and other interferences from the extract. Finally, the organochlorine pesticides were measured by high-resolution gas chromatography/high-resolution mass spectrometry quantified by an isotopic dilution method.

Statistical Analysis

Descriptive data of the mothers and the boys (anthropometric measurements) are reported as mean ± SD or number (percentage) (Table 1). We tested differences between boys with and without cryptorchidism by unpaired t-tests or chi-square tests. Descriptive statistics of pesticide concentrations are given as medians and ranges (minimum and maximum) because of skewed distributions. The sum of DDT metabolites was calculated as the sum of all six metabolites. We calculated the DDE(dichlorodiphenylchloroethylene)/DDT ratio and the enantiomeric median ratio (ER) by simple division: p,p’-DDE/1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p’-DDT and (+)-isomer concentration)/(−)-isomer concentration for the individual compounds, respectively. We tested the differences between cryptorchid and healthy boys using the Mann-Whitney U-test. We tested the differences in pesticide levels between cryptorchid and healthy boys using a logistic regression model in which the pesticide level (log-transformed) and, in some analyses, country and other potential confounders were entered as covariates. This approach had the advantage of allowing the inclusion of cases and controls based on their pesticide levels in the analysis. This selection does not introduce bias in the estimation. p-Values were not corrected for multiple testing.

To determine whether a given measurement of a sample indicated the presence of a pesticide, both the limit of detection (LOD) and the limit of quantification (LOQ) were determined for every sample. We defined the LOD as three times the background noise of the analytic instrument. Samples with values below the LOD were nondetectable. Samples above LOD, in which the pesticide concentration could not be reliably quantified, were assigned a value below the LOQ; the LOQ represents the level at which a compound concentration was determined with sufficient precision. We defined the LOQ as three times the value of a blank sample, which was the blank matrix used in sample preparation. Detection rate was defined as percentage of samples with a detectable and quantifiable value. Because statistical handling of measurements below the LOD or below the LOQ may influence results, we tried different selection schemes to test whether conclusions were sensitive to the actual values of the unquantifiable measurements. We performed three analyses to compare the exposures of cryptorchid and normal boys. The first included all data using the LOD for samples with nondetectable values and the detected value for unquantified samples. The second included all data except for samples with values below the LOQ. The third analysis excluded all nondetectable samples. Exposure levels reported in Table 2 are based on the third analysis excluding samples with values below the LOD or below the LOQ. Exposure patterns between cases and controls using the other two statistical approaches did not substantially differ from those in Table 2 (data not shown).

To investigate a combined effect of persistent pesticides, we used eight pesticides for which all individuals had measurable and

| Characteristic | Denmark Cases (n = 29) | Controls (n = 36) | p-Value | Denmark Cases (n = 33) | Controls (n = 32) | p-Value |
|----------------|------------------------|-----------------|---------|------------------------|-----------------|---------|
| Maternal age (years) | 31.0 ± 3.9 | 30.8 ± 4.4 | 0.908 | 29.8 ± 4.9 | 28.8 ± 4.7 | 0.408 |
| Maternal height (cm) | 170.3 ± 5.1 | 170.1 ± 6.0 | 0.896 | 165.9 ± 5.4 | 165.5 ± 4.5 | 0.634 |
| Progesterone weight (kg) | 67.9 ± 8.5 | 67.2 ± 8.9 | 0.739 | 65.0 ± 10.7 | 61.1 ± 6.4 | 0.083 |
| Progesterone BMI | 23.4 ± 3.3 | 23.2 ± 3.0 | 0.860 | 23.7 ± 4.0 | 22.1 ± 2.3 | 0.056 |
| Parity [n (%)] | 4 (13.8) | 4 (11.1) | 0.705 | 9 (27.3) | 9 (28.1) | 0.828 |
| Smoking [n (%)] | 9 (28.1) | 11 (30.6) | 0.863 | 5 (15.6) | 5 (15.6) | 1.000 |
| Gestational age (days) | 276 ± 15 | 273 ± 8 | 0.029 | 278 ± 11 | 280 ± 9 | 0.688 |
| Birth weight (kg) | 3.6 ± 0.7 | 3.8 ± 0.5 | 0.135 | 3.6 ± 0.5 | 3.6 ± 0.4 | 0.506 |
| Birth length (cm) | 52.3 ± 3.8 | 53.1 ± 2.1 | 0.246 | 51.2 ± 1.9 | 51.1 ± 1.9 | 0.956 |

Values are given as mean ± SD or number (%). The p-value describes differences between cases and controls.
quantifiable levels ($p,p^\prime$-DDE, $p,p^\prime$-DDT, $\beta$-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), $\alpha$-endoendosulfan, cis-heptachloroepoxide (cis-HC), oxychlorodane, and dieldrin). A statistical test of the null hypothesis that there were no differences in the median exposure levels of cases and controls was carried out as a Monte Carlo permutation test. In the permutations, cases and controls were randomly assigned exposure profiles from the observed profiles, and median levels between cases and controls were compared within countries. We then compared the observed test statistic (the number of median exposures, which were higher among cases than controls) with the permutation distribution. The permutation scheme of the test takes into account the within-individual association structure between different persistent organochlorine pesticides—that some individuals tend to have high exposure to many pesticides, whereas others have low exposures.

### Results

Study population characteristics are given in Table 1. We found no significant differences between the mothers giving birth to a cryptorchid boy versus a healthy boy. Gestational age of cryptorchid boys was slightly lower than in normal boys (Denmark: $p = 0.029$; Finland: $p = 0.688$). No systematic difference between cases and controls with regard to year of birth was observed ($p = 0.543$). In both countries, the selected controls did not differ from the healthy mothers and boys in the entire cohorts with respect to maternal age, parity, smoking, gestational age, and birth weight (data not shown).

Table 2 shows the results of pesticide measurements in breast milk samples of mothers with cryptorchid and normal boys. The lipid content (percent weight per weight) did not differ significantly ($p = 0.707$) between cases [3.7 (range, 1.1–7.9)] and controls [3.8 (range, 0.4–10.1)]. Concentrations of persistent pesticides (nanograms per gram lipid) in breast milk showed large individual variations and skewed distributions as well as differences in absolute levels.

Eight compounds—$p,p^\prime$-DDE, $\beta$-HCH, HCB, $\alpha$-endoendosulfan, oxychlorodane, $p,p^\prime$-DDT, dieldrin, and cis-HC (listed with decreasing concentrations)—were quantifiable in all samples. The concentrations of these eight compounds were higher than those of any of the remaining 19 compounds, which were all measured in very low concentrations. The sum of all DDT metabolites was slightly higher for cases than controls, but the difference did not reach statistical significance. The ratio between $p,p^\prime$-DDE and $p,p^\prime$-DDT was higher among controls than cases, but not significantly. Seventeen of 21 organochlorine pesticides [$p,p^\prime$-DDT, $p,p^\prime$-DDE; 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene ($\alpha$-DDT), HCH ($\alpha$, $\beta$, $\gamma$), HCB, pentachloroanisole (PCA); $\alpha$-endoendosulfan; cis-HC; chlordane (cis-, trans-); oxychlorodane; methoxychlor; octachlorostyrene (OCS); and dieldrin] were measured in slightly higher median concentrations in milk from mothers giving birth to cryptorchid boys than in mothers giving birth to healthy boys with only trans-chlordane reaching statistical significance ($p = 0.012$) (Table 2).

Table 3 presents the overall exposure pattern of cryptorchid and healthy boys depending on inclusion of samples with values below the LOD and/or LOQ. The overall exposure pattern in the three analyses was comparable, showing that most compounds were measured in higher concentrations in milk from case mothers than in milk from control mothers.

A combined analysis (a Monte Carlo permutation test) of the eight most prevalent organochlorine pesticides revealed that the difference between milk from case mothers and control mothers was not likely due to chance ($p = 0.032$).

### Table 2: Pesticide concentrations in human breast milk samples from mothers of 62 boys with cryptorchidism and 68 healthy boys.

| Pesticide              | Detection rate (%) | Samples < LOD (n) | Samples < LOQ (n) | Lipid-based concentrations (ng/g lipid) | $p$-Value adjusted | $p$-Value adjusted | $p$-Value adjusted |
|------------------------|--------------------|-------------------|-------------------|----------------------------------------|--------------------|--------------------|--------------------|
|                        | Cases (n = 62)     | Controls (n = 68) |                   |                                        |                    |                    |                    |
| Lipid (% w/w)          | Lipid (% w/w)      |                   |                   |                                        |                    |                    |                    |
| $\alpha$-HCH           | 69.2               | 0                 | 40                | 0.20 (0.04–3.45)                       | 0.07               | 0.36               | 0.47               |
| $\beta$-HCH            | 100                | 0                 | 0                 | 13.64 (7.46–26.53)                     | 0.12               | 0.06               | 0.26               |
| $\gamma$-HCH           | 54.5               | 0                 | 71                | 0.36 (0.06–4.05)                       | 0.55               | 0.93               | 0.77               |
| HCB                    | 100                | 0                 | 0                 | 0.19 (0.04–2.36)                       | 0.02               | 0.19               | 0.16               |
| PCA                    | 43.1               | 1                 | 73                | 0.08 (0.03–0.28)                       | 0.03               | 0.50               | 0.36               |
| PerCB                  | 91.5               | 0                 | 11                | 0.08 (0.03–0.28)                       | 0.07               | 0.93               | 0.93               |
| $\alpha$-endoendosulfan| 100                | 0                 | 0                 | 0.08 (0.03–0.28)                       | 0.03               | 0.50               | 0.36               |
| cis-HC                 | 100                | 0                 | 0                 | 0.19 (0.04–2.36)                       | 0.02               | 0.19               | 0.16               |
| cis-Chlordane          | 30.8               | 66                | 24                | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| trans-Chlordane        | 61.5               | 22                | 28                | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| Oxychlorodane          | 100                | 0                 | 0                 | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| Methoxychlor           | 25.9               | 1                 | 94                | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| OCs                    | 90.8               | 0                 | 12                | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| Dieldrin               | 100                | 0                 | 0                 | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |
| Mirex                  | 99.2               | 0                 | 1                 | 0.06 (0.02–0.35)                       | 0.06               | 0.05               | 0.01               |

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*Pesticide concentrations (nanograms per gram lipid) are given as medians (for compounds with detection rate > 50%) and range (minimum to maximum), detection rates as percent-

| Detection rates did not differ significantly.

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between cases and controls for single pesticides (data not shown).

For oxychlordane and cis-HE, the enantionic concentrations were available. The absolute concentrations of the enantiomeric isomers were higher, but not significantly, for cryptorchid boys than for controls (data not shown). The ER for oxychlordane (cases/controls) was 1.36/1.28. For cis-HE, the corresponding figures were 2.48/2.19 (p = 0.103 and p = 0.467, respectively).

**Discussion**

Most persistent organochlorine pesticides were found in higher concentrations in boys with cryptorchidism than in controls, although no individual compound was significantly correlated with cryptorchidism. For eight chemicals (p,p'-DDE, p,p'-DDT, β-HCH, HCB, α-endosulfan, cis-HE, oxychlordane, and dieldrin), which were measurable in all samples, the differences between cases and controls were unlikely to be due to chance. Although we cannot exclude the possibility that individual chemicals alone may cause cryptorchidism, our study suggests that exposure to more than one chemical at low concentrations represents a risk factor for congenital cryptorchidism. There may also be simultaneous coexposure to other environmental chemicals that contribute to the effect on testicular descent; the pesticide measurements may represent a proxy marker (sentinel) for other exposures, such as brominated flame retardants.

We were able to quantify most organochlorine pesticides at low levels in breast-milk samples. This indicates that these chemicals are still relevant despite the fact that most have been banned or restricted in the study areas for many years. High DDE/DDT levels support the assumption the current exposure level primarily originates from previous contamination, environmental persistence, and long-range atmospheric dissipation, and less from imported food products and increased traveling activity to areas with ongoing use of pesticides such as DDT for malaria control.

Levels of the investigated pesticides have generally declined in the study area, whereas the birth prevalence of cryptorchidism appears to have increased (Boisen et al. 2004). This may explain why no single compound was strongly correlated with cryptorchidism. However, low concentrations of a mixture of chemicals over time may still be harmful to the fetus. In addition, the overall exposure to other chemicals with endocrine-disrupting activity may have increased in the same period. Thus, the persistent pesticides investigated here may reflect current overall exposure: Women with the highest levels of persistent pesticides may also be the ones with the highest concentrations of other endocrine-disrupting chemicals. This hypothesis is supported by a previous study that found the concentrations of HCB, p,p'-DDE, p,p'-DDT, and β-HCH in individual samples increased with increasing polychlorinated biphenyl concentrations (Andersen and Orbaek 1984).

To our knowledge, no previous studies have compared levels of persistent organochlorine pesticides in breast milk with the birth prevalence of cryptorchidism. Breast milk was chosen as a surrogate biomarker of previous maternal exposure to persistent pesticides because these compounds accumulate in lipid-rich tissue and thereby in breast milk (Beek 2000; Jensen and Slorach 1991). There is a dynamic equilibrium between levels of persistent compounds in maternal adipose tissue and breast milk (Cerrillo et al. 2005; Kanja et al. 1992; Rogan et al. 1986; Skaare et al. 1988; Waliszewski et al. 2001); therefore, daily intake of pesticides during lactation has little influence on the levels measured in milk. Both human and animal studies have demonstrated that pesticides during pregnancy can be transferred to the fetus by crossing the placenta (Foster et al. 2000; Lange et al. 2002; Waliszewski et al. 2000). Levels measured in breast milk were positively correlated to levels measured in umbilical cord samples (Cerrillo et al. 2005; Kanka et al. 1992; Skaare et al. 1988; Waliszewski et al. 2001); therefore, daily intake of pesticides during lactation has little influence on the levels measured in milk. Both human and animal studies have demonstrated that pesticides during pregnancy can be transferred to the fetus by crossing the placenta (Foster et al. 2000; Lange et al. 2002; Waliszewski et al. 2000). Levels measured in breast milk were positively correlated to levels measured in umbilical cord samples (Cerrillo et al. 2005; Kanka et al. 1992; Skaare et al. 1988; Waliszewski et al. 2001). Concentrations of compounds in breast milk are a suitable proxy for fetal exposure during pregnancy. As persistent pesticides are accumulated in the lipid fraction of the breast milk, any variations in the lipid content may affect the levels measured. In our study, the women were carefully instructed to collect only hind milk, and they collected many small aliquots that were pooled over time. Thus, the breast milk samples in this study represent an average content over a long period. Because we found no differences in mean lipid content between cases and controls, the potential bias induced by lipid variation due to collection is negligible. In both countries, the selected controls did not differ from the healthy mothers and boys in the entire cohorts with respect to maternal age, parity, smoking, gestational age, and birth weight, and therefore we believe that the samples are representative.

Because of matching for the most common confounders, such as parity, in the Finnish population, there were no significant differences between cases and controls. In the Danish group, only gestational age differed slightly (p = 0.029). Primiparae, slim women, and smokers tend to have higher pesticide levels (Jensen and Slorach 1991). As more mothers of healthy boys were primiparae and nonsmokers and had lower mean body mass index (BMI) than mothers of cryptorchid boys, our study may underestimate the effect of organochlorine pesticide exposure on cryptorchidism. No definitive relation between maternal age and the level of organochlorine pesticides in breast milk has been reported; some studies have found that older mothers have higher levels, whereas others have not (Jensen and Slorach 1991). In our study, case mothers were slightly older than control mothers, but fewer of them were primiparae. Older mothers had higher concentrations (significant difference in three compounds (p,p'-DDE, oxychlordane, and α-endosulfan)). However, older primiparae mothers (n = 7:3 cases (4.8%); 4 controls (5.9%)) especially contributed to this difference. Because they were equally distributed in the two groups, this cannot explain the difference between cases and controls. Milk from mothers giving birth to premature babies may be different in composition. One Danish study described higher levels of HCB in milk from mothers giving birth to premature infants (Jensen and Slorach 1991). In our study, levels were higher in milk from mothers giving birth to premature infants. The differences were not significant, and the number of premature infants was low (five cases and two controls) and cannot therefore explain the difference we found.

The sensitivity of the analytic method allowed the detection of traces of organochlorine pesticides below the LOQ. This phenomenon was, as expected, most frequent for pesticides detected in generally low concentrations. Because the statistical handling of these measurements can profoundly change the results, we evaluated our data carefully with different approaches and found that the overall findings remained unchanged.

Few other studies have investigated the possible relationship between persistent pesticide levels in biologic samples and the prevalence of cryptorchidism. Hosie et al. (2000) compared levels of pesticides [DDT and metabolites, toxaphene, HCH (α, β, γ), HCB, PCA, PeCB, and several chlorinated cyclodienes such as heptachlor] in fat biopsies from 18 cryptorchid boys and 30 controls. Their findings were comparable with ours: Pesticide concentrations

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**Table 3. Exposure pattern of cryptorchid versus healthy boys described by three statistical approaches.**

| Type of samples included in the analysis | No. of pesticides included | No. of compounds for which the median pesticide concentration (ng/g lipid) is: |
|----------------------------------------|---------------------------|---------------------------------------------------------------------|
|                                        |                           | Cases > controls | Cases = controls | Cases < controls |
| Ia                                     | 23                        | 14              | 5               | 4               |
| Ib                                     | 23                        | 15              | 5               | 3               |
| IIC                                    | 21                        | 17              | 2               | 2               |

*All samples are included, samples below the LOD as the LOD values and samples below the LOQ with the measured value. **Samples below the LOD included as the LOD values, samples < LOQ excluded. *Only measurements above the LOD and above the LOQ are included. δHeptachlor and δ-HCH not included.*
were higher among cases than controls, but reaching significance for only a few (HCB and heptachloroepoxide). However, the relatively limited total number of samples and especially the broad age range of boys (0.1–15 years) limits the interpretation of the data. For some participants, biomonitoring was conducted a long time after the relevant prenatal exposure window for testicular maldescent.

Two studies have been published comparing levels of DDE and DDT in maternal blood and cryptorchidism and hypospadias in the offspring based on two large birth cohorts conducted in the United States in the 1960s (Bhatia et al. 2005; Longnecker et al. 2002). Although both studies were based on biologic samples collected in a period during which DDT was still being used, neither study found firm associations. Bhatia et al. (2005) did not find any association. Longnecker et al. (2002) found adjusted odd ratios to be elevated, although not significantly, and concluded that the results were consistent with a modest to moderate association between DDE/DDT and cryptorchidism. Both studies were performed as nested case–control studies within large prospective birth cohort studies; the women were recruited during pregnancy and the diagnosis of cryptorchidism was well ascertained. Although these studies applied a different biologic matrix (blood) than ours (breast milk), the concentrations in ours were lower. This would also be expected, as the samples in both studies were collected in the period from 1959 to 1966, when DDT was still permitted.

Other studies without access to biologic samples have also reported associations between cryptorchidism and parental pesticide exposure (García-Rodríguez et al. 1996; Garry et al. 1996; Kristensen et al. 1997; Pierik et al. 2004; Restrepo et al. 1990; Weidner et al. 1998). Most of these studies were register-based and retrospective. One was performed as a nested case–control study within large prospective birth cohort studies; the women were recruited during pregnancy and the diagnosis of cryptorchidism was well ascertained. Although these studies applied a different biologic matrix (blood) than ours (breast milk), the concentrations in ours were lower. This would also be expected, as the samples in both studies were collected in the period from 1959 to 1966, when DDT was still permitted.

Persistent pesticides and cryptorchidism

In this study we focused on pesticides with suspected endocrine-disrupting activity or pesticides that have been used worldwide (European Commission Directorate General Environment 2000; Toppari et al. 1996). The relative potency of each pesticide is often much lower than that of natural hormones. However, several of the included compounds act as both estrogen and antiandrogens (Andersen et al. 2002; Kelce et al. 1995), which might increase their possible adverse effect on testicular descent. Furthermore, different compounds may interact, thereby enhancing their effects (Andersen et al. 2002; Kortenkamp and Altenburger 1998; LeBlanc et al. 1997; Silva et al. 2002). Existing data on possible mixture effects of the specific organochlorine pesticides in vitro are limited. A combination of 10 compounds including endosulfan, dieldrin, methoxychlor, and some DDT metabolites demonstrated a cumulative effect (Soto et al. 1994, 1995). Similarly, a number of studies have found indications of additivity for some of the pesticides included in our study (Arnold et al. 1997; Merritt et al. 1999; Payne et al. 2001; Shekhar et al. 1997; Sumpter and Jobling 1995; Vonier et al. 1996). Others have not been able to demonstrate additivity between dieldrin and endosulfan (Ashby et al. 1997; Wade et al. 1997) or methoxychlor (Ashby et al. 1997). Investigating the possible effects of mixtures is complicated because mechanisms of actions for individual compounds are often poorly known, and some chemicals may act through different routes depending on dosage.

In conclusion, our study suggests an association between congenital cryptorchidism and some persistent organochlorine pesticides present in mothers’ breast milk. Although our study cannot provide proof for a causal relationship, our data are in line with results from animal studies. Thus, prenatal exposure to persistent organochlorine pesticides may adversely affect testicular descent in boys.
