# Project Progress Summary

## Title of the project
Exposure - outcome relationships in male urogenital malformations with special reference to endocrine disrupters

## Acronym of the project
EXPORED

## Type of contract
Shared cost

## Total project cost
€ 3.223.805 €

## Contract number
QLK4-CT-2001-00269

## Duration
48 Months

## EU contribution
€ 2.292.739

## Commencement date
1 January 2002

## Period covered by the progress report
1 January 2002 - 31 December 2005

## Project Coordinator

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## Key words
exposure, endocrine disrupters, congenital urogenital malformations

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Summary
The objective of this project was to investigate possible associations between male urogenital malformations and exposure to potential endocrine disrupters during pregnancy. The project was based on earlier cohort studies in Denmark and Finland, two countries with different incidences of male urogenital disorders, such as cryptorchidism and hypospadias. Boys with malformations had been diagnosed at birth and followed up to 18 months together with matched controls. Biological samples were systematically collected during these studies for exposure assessment. Comprehensive endocrine evaluation of the children had also been performed and a large database had been created on the basis of questionnaires. Our aim was to provide exposure estimates through direct chemical analysis of biological samples and combine information from these sources to assess the role of exposure to endocrine disrupters in the formation of male urogenital malformations.

To achieve this goal a large range of environmental pollutants with known or suspected endocrine disrupting effects were measured in human placenta and breast milk samples from the joint Danish and Finnish prospective birth cohort studies. Our study revealed significant geographic differences in the levels of many of the compounds measured between the two Nordic countries, with Denmark generally having higher concentrations for most compounds than Finland. These findings suggest that despite close vicinity and comparable life styles, health effects of environmental contaminants may differ between regions. The finding of a generally higher exposure of the Danish boys to environmental pollutants with endocrine disrupting effects supports the hypothesis that exposure to these compounds during fetal development may play a causative role in the higher incidence of cryptorchidism and hypospadias in Danish newborns compared to Finnish newborns.

The evaluation of individual exposure levels and cryptorchidism is still ongoing. Preliminary data evaluation indicates that PBDE exposure is associated with an increased risk of cryptorchidism, whereas dioxin and PCB exposures results are equivocal. However, the ongoing thorough scrutiny of the results involves careful adjustment for possible confounders. Among the halogenated hydrocarbons, persistent pesticides, and phthalates analysed no significant association between cryptorchidism and the individual compounds was evident. However, neither did we in this subgroup find significant differences in birth weight, weight for gestational age, or gestational age between boys with and without cryptorchidism, which is in contrast to our findings in the total baby cohort from which this data set is derived. This indicates that our study groups may be too small to detect subtle changes related to the presence or absence of congenital cryptorchidism. Alternatively, it has been suggested that endocrine disrupting effects in humans are more likely to be due to exposure to mixtures of several environmental compounds simultaneously than to exposure of a specific compound. In that respect it is interesting that a combined statistical analysis of the eight most abundant persistent pesticides show that pesticide levels in breast milk in general are significantly higher in the boys with cryptorchidism than in the healthy boys.

Other end-points may be more sensitive to endocrine disruption than congenital malformations. Although we observed no association between phthalate levels in breast milk and cryptorchidism we did find significant correlations to the endogenous reproductive hormone levels of the boys during the transient activation of the pituitary-gonadal hormone axis at 3 months of age. Increased SHBG is an indirect sign of reduced androgen activity. Elevated LH levels, together with decreased free testosterone and elevated LH:free testosterone ratio, are consistent with an adverse effect on Leydig cell function leading to a reduced biologic androgen effect. Physiologically, there is negative feedback between testosterone levels in serum and pituitary LH secretion. In our study mMP, mEP, mBP, and miNP showed association to changes in reproductive hormone levels that are in accordance with an effect on the Leydig cell function, which is in line with observations in animal studies. mEHP, a phthalate with a higher reproductive toxicity in animal experiments than its parent compound, DEHP, also showed a tendency toward an antiandrogenic effect, which did not reach statistical significance. This may be related to the limited number of samples in our study, the extreme variation of individual exposure levels, or species differences.

Interestingly, in an independent, parallel American study of another mother-child cohort, women with highest excretion of mEP, mBP, mBzP, and mono-isobutyl phthalate (miBP) in urine during pregnancy gave birth to boys who were less virilized, as judged from smaller than expected measurements of anogenital distance. We did not measure anogenital distance in our...
However, our observations on the associations between mBP and mEP and markers of Leydig cell function, in particular, are consistent with the American study in terms of an antiandrogenic effect of phthalate exposure in infant boys, assessed by two different biomarkers. We also found an effect of mEP on SHBG levels and on the ratio between LH and free testosterone, whereas rodent studies did not show any toxicity of its parent compound, DEP. Because mEP was also one of four phthalate metabolites affecting anogenital distance in the American baby study, these observations may indicate a species difference in vulnerability that will have to be studied thoroughly in the future.

Enantiomeric deviations from racemic mixture have been shown to be useful tools for studying pollution processes. Non-racemic proportions of chiral organochlorine pollutants result from enantioselective bioprocesses in the environment, such as metabolism by different enzymes and degradation. In humans enantioselective residues may result from two mechanisms; one is enantioselective exposure enriched along the food chain, the other enantioselective clearing of racemic pollutants by the human body. In our cohorts the enantiomeric ratios (ERs) of hexa-chloro-cyclohexane (HCH) and DDD tended to be non-racemic if the concentration was low but racemic if the concentration was high, indicating that exposure of the women with high concentrations originated from racemic or close to racemic sources. However, even at low total concentrations, some samples showed racemic ERs suggesting that these mothers may recently have been exposed to these compounds but at a low level.

The analysis of the exposure-outcome associations is still ongoing and is expected to provide important data that will improve our understanding of the environmental factors affecting human reproduction and that are essential for a better risk assessment. Thus, the obtained data on dioxin will be used for a better assessment of the risk of dioxin exposure on reproductive health.

The evaluation and scientific reporting of the chemical analytical data in relation to different clinical-outcomes as well as in relation to questionnaire data will continue beyond the closure of the EXPORED consortium. It is anticipated that several papers will be completed for publication over the next 10-12 months. Although the focus of the EXPORED project was to investigate associations between exposures and congenital malformations of the male reproductive organs the chemical data obtained will also be exploited for investigations of possible associations to other clinical out-comes that are available in the Danish and Finnish birth cohorts.

Publications


8. Shen H, Main KM, Virtanen HE, Damgaard IN, Haavisto AM, Kaleva M, Boisen KA, Schmidt IM, Chellakooty M, Skakkebæk NE, Toppari J, Schramm KW: From mother to child: investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere (pending after revisions)