Guest Editorial
Phthalate Exposure during Pregnancy and Lower Anogenital Index in Boys: Wider Implications for the General Population?

The article by Swan et al. (2005) in this issue of *Environmental Health Perspectives* reignites the issue of the role that phthalate exposure during pregnancy may play in the etiology of reproductive disorders in human males. It does so by providing the first evidence of an association between phthalate exposure of mothers during pregnancy and attenuation of androgen action in their male babies. If this association is indicative of cause and effect, it will join together three key areas of research, namely, testicular dysgenesis syndrome (TDS) disorders in human males, induction of TDS-like disorders by phthalate administration to laboratory animals during pregnancy (experimental studies), and the widespread exposure of humans to various phthalates.

TDS refers to a collection of disorders of newborn or young adult males thought to have a common origin in fetal life as a result of testicular dysgenesis (Skakkebaek et al. 2001). These disorders include cryptorchidism (testis maldescent) and hypospadias, the two most common congenital malformations in newborns (2–4% and 0.3–0.7% incidence, respectively), testicular germ cell cancer, and low sperm counts. Low sperm count affects approximately 20% of young men in many European countries (Jørgensen et al. 2002), whereas testicular cancer is the most frequent cancer of young men, and its incidence has been increasing progressively in Caucasian men for the past 50 years or more (Richiardi et al. 2004). An integral feature of the TDS hypothesis is hormonal dysfunction of the fetal testis, in particular, reduced production of testosterone (Sharpe and Skakkebaek 2003). Because testosterone is largely responsible for transforming the fetus into a male, TDS can be viewed as a sexual differentiation (i.e., masculinization) disorder. But where do phthalates come in?

Studies by several groups have shown that exposing pregnant rats to certain phthalates (di-n-butyl, diethylhexyl phthalate or butyl benzyl phthalate) during the period of sexual differentiation of the pups results in a collection of disorders in the male offspring similar to TDS disorders in men (Fisher et al. 2003; Mylchreest et al. 1998). More tellingly, the phthalate exposure results in focal testicular dysgenesis (Barlow et al. 2004; Fisher et al. 2003; Mahood et al. 2005) and associated suppression of hormone production (testosterone, insulin-like factor 3) by the fetal testis (Parks et al. 2000; Thompson et al. 2004; Wilson et al. 2004). One indication that the phthalate-exposed pups are “undermasculinized” is that they exhibit a reduction in anogenital distance (AGD) (Ema and Miyawaki 2001; Gray et al. 2000; Mylchreest et al. 2000; Zhang et al. 2004). AGD in male rats is normally about twice that in females, and a similar sex difference is evident in humans (Salazar-Martinez et al. 2004). This difference is a direct reflection of growth-stimulating actions of androgens, such as testosterone, on the perineum in fetal life; it is therefore an indicator of the level of androgen action in the fetus and thus of the masculinization process. Reduced AGD = reduced androgen levels or action.

Reduction in AGD in rats occurs after dosing the pregnant mothers with phthalates at concentrations >250 mg/kg/day (Ema and Miyawaki 2001; Mylchreest et al. 2000; Zhang et al. 2004). Humans are exposed to a range of phthalates (Koch et al. 2003; Silva et al. 2004), with urinary concentrations of the primary metabolites of key phthalates in the range of 5–40 μg/L (150–1,000 μg/L for monoethyl phthalate). Although the phthalate dose administered to rats and the concentration of urinary phthalate metabolites in humans is difficult to compare directly, the consensus view has been that humans are not exposed to phthalates at levels capable of affecting fetal testis hormone production and masculinization in rats. Swan et al. (2005) challenge this view by showing a negative relationship between the anogenital index (AGI) in boys 2–30 months of age and the level of phthalate metabolites in maternal urine during the index pregnancy. At face value, this suggests that phthalates have similar adverse effects on fetal testosterone production in humans as they do in rats. However, this effect must occur at lower levels of phthalate exposure in humans, because reduced AGI was found in boys born to mothers with phthalate levels similar to those found in about 25% of the U.S. population (Silva et al. 2004).

If this interpretation is correct, it provides a strong likelihood that phthalate exposure in humans is one potential cause of TDS disorders, a conclusion that has widespread public health implications. So how certain can we be about this interpretation?

Swan et al. (2005) show an association between maternal phthalate exposure and AGI in boys—they do not show that one caused the other or that the phthalates caused reduced testosterone production. They also do not show that phthalate exposure caused abnormalities (all boys were “normal”), although the collateral finding that boys with a lower AGI had a higher incidence of cryptorchidism and reduced penis size, when compared with boys with a higher AGI, is consistent with an abnormal reduction in testosterone levels. Measurement of urinary, salivary, or blood (e.g., cord blood) testosterone levels in relation to AGI in future studies should help to clarify this issue, as should establishment of more detailed normative data for AGI in boys and girls and the evaluation of AGI in boys with established evidence of deficient androgen action, such as those with hypospadias.

The findings of Swan et al. (2005) need to be confirmed independently. This is not a criticism of the quality of the study (which is excellent) but a standard requirement of good science. Better understanding of the comparative fetal exposure of humans to phthalates and those in the experimental studies in rats, for example, by comparison of phthalate metabolite levels in amniotic fluid, would also be instructive. What is also urgently needed is improved knowledge about how humans, in particular pregnant women, are exposed to phthalates (Silva et al. 2004). What are the most important routes: air, water, diet, or personal care products? One sensible, precautionary response to the present findings is for women who are planning a pregnancy to minimize their exposure to phthalates, but they cannot be guided how to do so unless we know their primary routes of exposure.

Two final notes of caution for readers who consider it wise to assume that phthalates cause TDS in humans. First, the association of AGI with phthalate exposure could be fortuitous; for example, the same lifestyle practices that expose a woman to phthalates might themselves cause the reduction in testosterone/AGI or expose her to other factors that cause this effect. Second, the TDS hypothesis argues that any factor that causes testicular dysgenesis (mal-development) is likely to result in TDS disorders, and this includes well-established genetic disorders (Skakkebaek et al. 2001); it is also likely to be affected by maternal lifestyle. Therefore, phthalates will not be the only cause of TDS. For the moment, we do not even know they are a cause, but it is undoubtedly a possibility that deserves urgent further study.
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The author declares he has no competing financial interests.

REFERENCES

Barlow NJ, McIntyre BS, Foster P.M.D. 2004. Male reproductive tract lesions at 6, 12, and 18 months of age following in utero exposure to di(n-butyl) phthalate. Toxicol Pathol 32:79–90.

Ema M, Miyawaki E. 2001. Adverse effects on development of the reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthalate, during late pregnancy. Reprod Toxicol 15:189–194.

Fisher JS, Macpherson S, Marchetti N, Sharpe RM. 2003. Human ‘testicular dysgenesis syndrome’: a possible model based on in-utero exposure of the rat to dibutyl phthalate. Hum Reprod 18:299–305.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DNR, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350–365.

Jorgensen N, Carlsson E, Nermoen I, Punab M, Suominen J, Andersen AG, et al. 2002. East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod 17:2199–2206.

Koch HM, Rossbach B, Drexler H, Angerer J. 2003. Internal exposure of the general population to DEHP and other phthalates—determination of secondary and primary phthalate monoester metabolites in urine. Environ Res 93:177–185.

Mahood IK, McKinnell C, Fisher JS, Walker M, Hallmark N, Sharpe RM. 2005. Abnormal Leydig cell aggregation in the fetal testis of rats exposed to di(n-butyl) phthalate and its possible role in testicular dysgenesis. Endocrinology 146:613–623.

Mylchreest E, Cattley RC, Foster P.M.D. 1998. Male reproductive tract malformations in rats following gestational and lactational exposure to di(n-butyl) phthalate: an anti-androgenic mechanism? Toxicol Sci 42:47–50.

Mylchreest E, Wallace DG, Cattley RC, Foster P.M.D. 2000. Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. Toxicol Sci 55:143–151.

Parks LG, Ostby J, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, et al. 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 58:339–349.

Richardi L, Bellocco R, Adams HO, Torranga A, Barlow L, Hakulinen T, et al. 2004. Testicular cancer incidence in eight northern European countries: secular and recent trends. Cancer Epidemiol Biomarkers Prev 13:2157–2166.

Salazar-Martinez E, Romano-Queru F, Yanes-Marquez E, Longnecker MP, Hernandez-Avila M. 2004. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. Environ Health 3:8; doi: 10.1186/1476-069X-3-8 [Online 13 September 2004].

Sharpe RM, Skakkebaek NE. 2003. Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research. Pure Appl Chem 75:2023–2038.

Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caufield SP, et al. 2004. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey [NHANES] 1989–2000. Environ Health Perspect 112:331–338.

Skakkebaek NE, Raipert-De Meets E, Main KM. 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972–978.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113:1056–1061.

Thompson CJ, Ross SM, Guido KW. 2004. Di(n-butyl) phthalate impairs cholesterol transport and steroidogenesis in the fetal rat testis through a rapid and reversible mechanism. Endocrinology 145:1227–1237.

Wilson VS, Lambright C, Furr J, Ostby J, Wood C, Held G, et al. 2004. Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. Toxicol Lett 146:207–215.

Zhang Y, Jiang X, Chen B. 2004. Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate in utero and during lactation and determination of its NOAEL. Reprod Toxicol 16:689–705.