

Phthalates and Baby Boys

Potential Disruption of Human Genital Development

Epidemiologic research has revealed widespread human exposure to phthalates, a class of chemicals that appear in products as diverse as flexible plastics, industrial solvents, and personal care products. Rodent studies indicate that prenatal exposure to some phthalates can disrupt normal male reproductive tract development, causing effects such as reduced anogenital (anus-to-penis) distance, undescended testicles, and testicular abnormalities affecting function. Although the phthalate concentrations that cause these effects are quite high, changes in gene expression have been seen at much lower levels, and concern exists that human males might be similarly affected. The current study, the first to investigate an association between prenatal phthalate exposure and altered genital formation in humans, finds that these chemicals may indeed contribute to such changes in boys exposed *in utero* [*EHP* 113:1056–1061].

The research team gathered physical data on 134 boys aged 2–36 months who were enrolled with their parents in the Study for Future Families II, a multicenter study to investigate pre- and postnatal phthalate exposure and potential related effects on development. The researchers examined each boy's testes and scrotum, placement and size of each testis, penis size, and anogenital distance; none of the boys had obvious disease or malformation. Urine samples collected during pregnancy were available for 85 of the boys' mothers. Sample analysis quantified concentrations of nine phthalate metabolites, which served as biomarkers of prenatal phthalate exposure.

To investigate correlation between prenatal phthalate exposure and genital development, the researchers calculated an anogenital index (AGI) by dividing each boy's anogenital distance by his weight. After considering how AGI varied as a function of age, they calculated expected values and 25th and 75th percentiles. Each boy's AGI was categorized as either smaller than expected or at least as large as expected. Based on the age-adjusted percentiles for AGI, the boys were further categorized as having a short, intermediate, or long AGI. The researchers also determined the proportion of boys in these three groups who had normal testicular descent, scrotal size, and scrotal appearance.

More than 90% of the mothers had evidence of some phthalate exposure. Urinary metabolite concentrations were categorized as indicating low, intermediate, or high phthalate exposure. The researchers tested whether a boy's exposure level correlated with his odds of having a short AGI while controlling for various confounding factors such as maternal smoking and timing of urine sample collection.

They found that four metabolites—monoethyl phthalate, mono-*n*-butyl phthalate, monobenzyl phthalate, and monoisobutyl phthalate—were significantly associated with short AGI. The association was stronger when high levels of all four metabolites were seen. Phthalate metabolite levels among the mothers of boys classified as having a short AGI were comparable to those measured in one-quarter of the U.S. female population based on data from the National Health and Nutrition Examination Survey published in 2004. The researchers also found that short AGI was associated with incomplete descent of one or both testicles.

Although this study was small, the researchers conclude that, consistent with animal studies, these data provide support for a



What's up down there? New research shows an intriguing correlation between prenatal exposure to phthalates and effects on anogenital distance in baby boys. Although similar results have been shown in mice, this is the first such study in humans.

link between prenatal phthalate exposure and health effects in humans. The researchers suggest that commonly used phthalates may adversely affect male reproductive development, and indicate that this possibility needs to be investigated more thoroughly in a larger, more diverse population. —**Julia R. Barrett**

The Ups and Downs of Thyroid Hormone

PCBs May Reduce Levels in Pregnancy

Maintaining adequate levels of thyroid hormone (TH) during pregnancy is critical for proper placental and fetal development. Environmental contaminants including polychlorinated biphenyls (PCBs), chlorinated pesticides, and mercury have been shown to disrupt the endocrine system in both humans and animals, and experimental studies have shown that these chemicals may decrease circulating TH levels during pregnancy. Now an epidemiologic study by a team of Canadian researchers has revealed that even low-level exposure to some of these chemicals can alter TH status in expectant mothers, with unknown effects [*EHP* 113:1039–1045].

PCBs and other persistent organohalogens are structurally similar to TH, and are known to have a high affinity for transthyretin, a TH carrier protein. Interference with maternal TH may be one mechanism behind the observed learning and behavioral deficits in children exposed to PCBs in the womb. Most PCB congeners are transferred through the placenta to the fetus such that fetal levels are 30–50% of maternal levels.

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The researchers checked the blood of 149 pregnant women for a range of PCB congeners, several organochlorine pesticides, and mercury. The researchers also measured levels of the major forms of TH— T_4 (the most common circulating form of TH) and T_3 (the form of TH that regulates cellular metabolism)—as well as thyroid-stimulating hormone (TSH), which is released from the pituitary gland and stimulates production of TH. Cord blood samples were collected at birth and analyzed for the same hormones and contaminants to estimate fetal exposure.

Results showed that total maternal T_3 decreased with increasing levels of three PCB congeners, the pesticide *p,p'*-DDE (a persistent metabolite of DDT), the fungicide hexachlorobenzene, and inorganic mercury. No association was found with methylmercury, an organic form of mercury associated with neurologic deficits. In cord blood, the only negative correlation was between free T_4 and inorganic mercury.

The researchers had expected the women to have high PCB and mercury levels because they lived in the polluted St. Lawrence River basin and were likely to have eaten high levels of potentially contaminated fish. But actual serum levels were 3–45 times lower than those previously reported. The authors say this suggests that pregnant women may be more sensitive than the general population to chemicals that appear to reduce TH levels.

Recent epidemiologic research into PCBs' effects on human TH function has been inconsistent, and some studies have found no effect at exposure levels higher than those in this study. But the current finding of a relationship at such low levels indicates that more investigations are needed in pregnant women, including monitoring of even subtle environmental exposures that can disturb maternal and/or fetal thyroid status. For this purpose, the biomarkers should include not only TSH—which currently is the only element of the thyroid system routinely monitored in pregnant women—but all forms of TH.

—Valerie J. Brown

Thimerosal and Animal Brains

New Data for Assessing Human Ethylmercury Risk

Since the 1930s, vaccines have contained thimerosal, a mercury-based preservative that breaks down to ethylmercury and thiosalicylate in the body. By some calculations, children given the usual schedule of vaccines containing thimerosal receive ethylmercury in doses exceeding the U.S. Environmental Protection Agency's guidelines for methylmercury, a known neurotoxicant. Because of the lack of pharmacokinetic and toxicity data for ethylmercury, methylmercury has been used as a reference for ethylmercury toxicity based on the assumption that the two compounds share similar toxicokinetic profiles. However, a new animal study shows that methylmercury is an inadequate reference for ethylmercury due to significant differences in tissue distribution, clearance rates, and ratios of organic to inorganic mercury in the brain [*EHP* 113:1015–1021].

During their first two years, children in the United States may receive more than 20 routine vaccinations. The rise in childhood autism has sparked concerns that thimerosal-derived ethylmercury may be at least partly to blame for some of these cases—concerns that are largely driven by awareness of methylmercury's neurotoxicity. Beginning in 1999 thimerosal-free versions of routine vaccines for children under age 6 started becoming available. However, as of winter 2005, the flu vaccine still contained thimerosal, and the preservative continues to be used in vaccines in other countries.

In the current study, researchers assigned 41 newborn monkeys to one of three exposure groups. Seventeen of the monkeys were injected with vaccines spiked with thimerosal for a total mercury dose of 20 micrograms per kilogram ($\mu\text{g}/\text{kg}$) at ages 0, 7, 14, and 21 days, mimicking the typical schedule of vaccines for human infants. At the same ages, another 17 monkeys received 20 $\mu\text{g}/\text{kg}$ methylmercury by stomach tube to mimic typical methylmercury exposure. A third group of 7 monkeys served as unexposed controls.

The researchers drew blood from all monkeys prior to any exposure and at other points prior to sacrifice, which occurred 2, 4, 7, or 28 days after the last dosing on day 21. Total mercury concentrations were measured in blood samples, and total and inorganic mercury concentrations were measured in brain samples. Organic mercury concentrations were calculated from those values.

The initial absorption rate and tissue distribution of mercury was similar in both exposed groups. However, total mercury progressively accumulated in the blood of methylmercury-exposed monkeys and remained detectable 28 days after the last dose. Among thimerosal-exposed monkeys, total mercury in blood declined rapidly between doses, and the researchers estimated clearance to be 5.4-fold higher than in the methylmercury group. In the thimerosal group, the half-life of total mercury in blood was 6.9 days, compared to 19.1 days for the methylmercury group.

Brain concentrations of total mercury were approximately 3–4 times lower in the thimerosal group than in the methylmercury group, and total mercury cleared more rapidly in the thimerosal group (with a half-life of 24.2 days versus 59.5 days). However, the proportion of inorganic mercury in the brain was much higher in the thimerosal group (21–86%

A sticky situation. New data show that using methylmercury as a reference for calculating risk from ethylmercury in vaccines may be fraught with problems.



of total mercury) compared to the methylmercury group (6–10%). Brain concentrations of inorganic mercury were approximately twice as high in the thimerosal group compared to the methylmercury group. Inorganic mercury remains in the brain much longer than organic mercury, with an estimated half-life of more than a year. It's not currently known whether inorganic mercury presents any risk to the developing brain.

Given these findings, the researchers caution that risk assessments for thimerosal based on studies using blood mercury measurements may not be valid, depending on the design of the study. Further, the observed differences in distribution and breakdown of mercury compounds between exposed groups indicate that methylmercury is not a suitable model for thimerosal toxicity.

The researchers emphasize, however, that the risks associated with low-level exposures to inorganic mercury in the developing brain are unknown, and they describe other research linking persistent inorganic mercury exposure with increased activation of microglia in the brain, an effect recently reported in children with autism. They recommend further research focused specifically on the biotransformation of thimerosal and its neurotoxic potential.

–Julia R. Barrett

Pancreatic Effects of EDCs

Low Doses Can Impair Glucagon Secretion

Endocrine-disrupting chemicals (EDCs) mimic naturally occurring hormones such as estrogen by occupying hormone receptors and triggering a reaction in the body. Interactions of EDCs with the classical (nuclear) estrogen receptors ER- α and ER- β have been well characterized, and there is also growing knowledge regarding interactions with nonclassical receptors (found elsewhere, as on the cell membrane). Both classical and nonclassical estrogen receptors occur throughout the body, reflecting the many roles played by estrogen in regulating the body's functions. This widespread presence also translates to myriad ways that EDCs could potentially interfere with health. New research now suggests that pancreatic cells are affected by EDC exposure, with potential health consequences [*EHP* 113:969–977].

Although the pancreas might seem an unlikely estrogen target, it bears classical and nonclassical receptors on both α - and β -cells in the islet of Langerhans. These cells secrete glucagon and insulin, respectively, hormones that regulate blood glucose levels, among other functions. In α -cells, low blood glucose causes increased calcium oscillations—or fluctuations in intracellular calcium concentrations—via a transmembrane channel; these oscillations trigger glucagon secretion.

Glucagon regulates functions in fat tissue and in the liver, brain, kidney, intestine, and pancreas. The primary role of glucagon is to enhance glucose synthesis and release in the liver. Secondary roles include increased fatty acid release from fat cells and appetite control in the central nervous system. These responses to low glucose can be

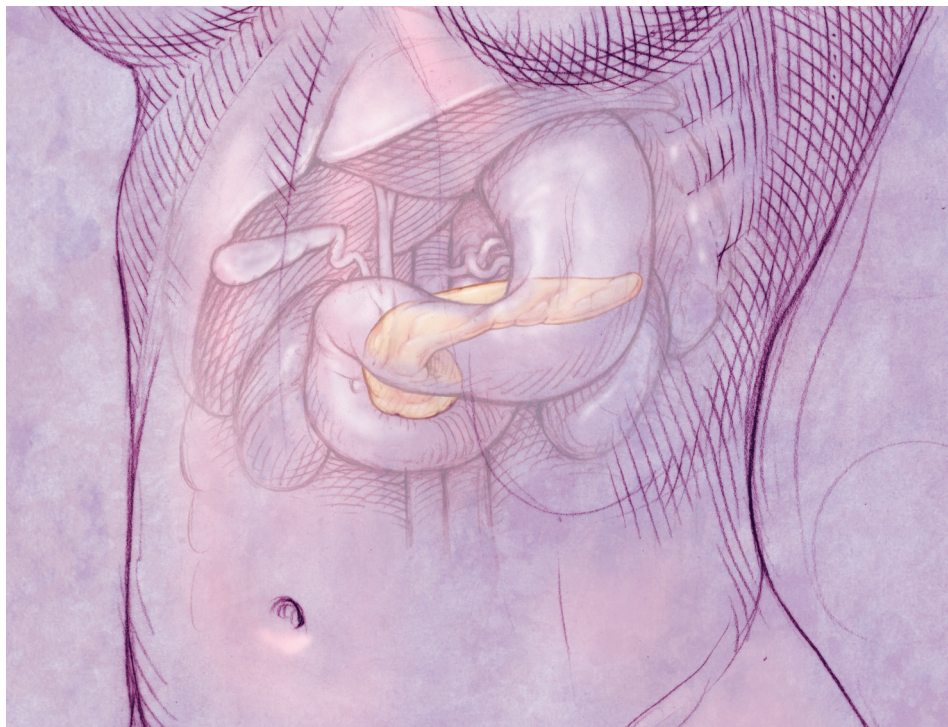
suppressed by the endogenous estrogen 17 β -estradiol and, as demonstrated in the current report, by EDCs as well.

The research team focused on two EDCs: bisphenol A, a component of such products as polycarbonate plastic and dental sealants, and diethylstilbestrol, a synthetic estrogen used from the 1940s to the 1970s to prevent miscarriage. Based on previous research, the researchers hypothesized that 17 β -estradiol and the EDCs would bind to nonclassical estrogen receptors on the membrane of glucagon-producing α -cells and activate a sequence of secondary messengers within the cell, leading to control of the transmembrane calcium channel and related calcium oscillations.

To test the hypothesis, they examined freshly isolated mouse pancreatic islets and subjected samples to physiological assays. Competitive binding assays indicated that 17 β -estradiol, bisphenol A, and diethylstilbestrol shared a common membrane-binding site. This binding was unaffected in competitive assays using the pure antiestrogen ICI182,780, which inhibits only classical ER-mediated effects. This result indicated that the common binding site was a nonclassical membrane estrogen receptor. Immunocytochemical assays confirmed that 17 β -estradiol and the EDCs bound to glucagon-producing α -cells.

Further assays of bisphenol A alone used compounds known to inhibit steps along the suspected pathway. These assays provided evidence that 17 β -estradiol and bisphenol A affected the sequence of cellular reactions that ultimately regulates calcium oscillations. These oscillations were tracked by laser scanning confocal microscopy.

The researchers note that there is some debate regarding the EDC concentrations necessary to produce biological effects in humans and animals. Their study indicates that EDC doses in the nanomolar range are sufficient to suppress calcium oscillations, potentially affecting secretion of glucagon. The possible consequences of this suppression could include changes in glucose and lipid metabolism and reduced use of stored glucose and fat, which could contribute to obesity. –Julia R. Barrett



Organ at risk? Low doses of the endocrine-disrupting chemicals diethylstilbestrol and bisphenol A were shown to affect pancreatic cells, potentially suppressing secretion of glucagon. This hormone regulates blood glucose levels and plays a role in appetite control and release of fatty acids from fat cells.

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