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GEMINIS PAPELES DE SALUD

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Ftalato

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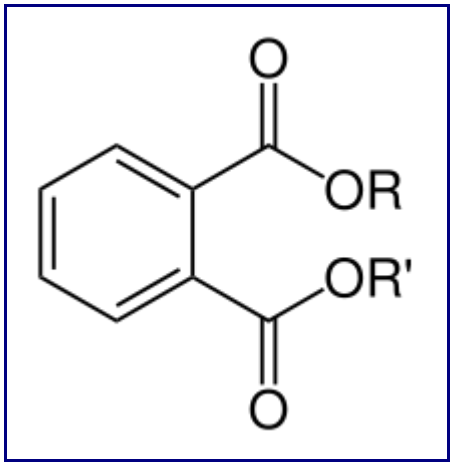

Estructura química general de los ftalatos.

R y R' = C_nH_{2n+1}; n = 4-15.

Los **ftalatos** o **ésteres de ftalato** son un grupo de compuestos químicos principalmente empleados como plastificadores (sustancias añadidas a los plásticos para incrementar su flexibilidad). Uno de sus usos más comunes es la conversión del cloruro de polivinilo (PVC) de un plástico duro a otro flexible.

Los ésteres del ftalato son los ésteres dialquílicos o arílicos del ácido 1,2-bencenodicarboxílico; el nombre *ftalato* deriva de la nomenclatura tradicional de ácido ftálico. Cuando se añade a los plásticos, los ftalatos permiten a las moléculas largas de polivinilo deslizarse unas sobre otras. Los ftalatos presentan una baja solubilidad en agua y alta en aceites, así como una baja volatilidad. El grupo carboxilo polar apenas contribuye a las propiedades físicas de los ftalatos, excepto cuando los radicales R y R' son muy pequeños (tales como grupos etilo y metilo). Son líquidos incoloros e inodoros producidos por reacción del anhídrido ftálico con un alcohol apropiado (normalmente alcoholes de entre 6 y 13 carbonos).

Hasta 2004, los fabricantes produjeron unas 400.000 toneladas de ftalatos al año. Se empezaron a producir en los años 1920, y en grandes cantidades desde los 50, con el nacimiento del PVC. Los ftalatos más empleados son el **DEHP** (di-2-etilhexilftalato), el **DIDP** (diisodecilftalato) y el **DINP** (diisononilftalato). El DEHP es el plastificador más usado con el PVC debido a su bajo coste. El **BBzP** (bencilbutilftalato) se usa en la fabricación de material para suelos basado en PVC. Los ftalatos con radicales R y R' pequeños son usados como disolventes en perfumería y pesticidas.

Los ftalatos se usan también con frecuencia en los esmaltes de uñas, adhesivos, masillas, pigmentos de pintura, juguetes de niños y en la mayoría de los juguetes sexuales.

Los juguetes de niños contienen de entre 20% a 50% de ftalatos del peso total del producto.

Contenido

Efectos sobre la salud

En Argentina, a traves de una resolución del Ministerio de Salud, está prohibida la importación, exportación, comercialización y entrega a título gratuito de juguetes y artículos de puericultura que contengan ftalatos en proporción mayor al 0.1% de volumen en masa.

Tabla de los Ftalatos más comunes

Nombre	Acrónimo	Fórmula estructural	Número de registro CAS
Dimetilftalato	DMP	$C_6H_4(COOCH_3)_2$	131-11-3
Dietilftalato	DEP	$C_6H_4(COOC_2H_5)_2$	84-66-2
Dialilftalato	DAP	$C_6H_4(COOCH_2CH{=}CH_2)_2$	131-17-9
Di-n-propilftalato	DPP	$C_6H_4[COO(CH_2)_2CH_3]_2$	131-16-8
Di-n-butilftalato	DBP	$C_6H_4[COO(CH_2)_3CH_3]_2$	84-74-2
Diisobutilftalato	DIBP	$C_6H_4[COOCH_2CH(CH_3)_2]_2$	84-69-5
Butilciclohexilftalato	BCP	$CH_3(CH_2)_3OOCC_6H_4COOC_6H_{11}$	84-64-0
Di-n-pentilftalato	DNPP	$C_6H_4[COO(CH_2)_4CH_3]_2$	131-18-0
Diciclohexilftalato	DCP	$C_6H_4[COOC_6H_{11}]_2$	84-61-7
Butilbencilftalato	BBP	$CH_3(CH_2)_3OOCC_6H_4COOCH_2C_6H_5$	85-68-7
Di-n-hexilftalato	DNHP	$C_6H_4[COO(CH_2)_5CH_3]_2$	84-75-3
Diisohexilftalato	DIHxP	$C_6H_4[COO(CH_2)_3CH(CH_3)_2]_2$	146-50-9
Diisoheptilftalato	DIHpP	$C_6H_4[COO(CH_2)_4CH(CH_3)_2]_2$	41451-28-9
Butildecilftalato	BDP	$CH_3(CH_2)_3OOCC_6H_4COO(CH_2)_9CH_3$	89-19-0
Di(2-etilhexil)ftalato	DEHP, DOP	$C_6H_4[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$	117-81-7
Di(n-octil)ftalato	DNOP	$C_6H_4[COO(CH_2)_7CH_3]_2$	117-84-0
Diisooctilftalato	DIOP	$C_6H_4[COO(CH_2)_5CH(CH_3)_2]_2$	27554-26-3
n-Octiln-decilftalato	ODP	$CH_3(CH_2)_7OOCC_6H_4COO(CH_2)_9CH_3$	119-07-3
Diisononilftalato	DINP	$C_6H_4[COO(CH_2)_6CH(CH_3)_2]_2$	28553-12-0
Diisodecilftalato	DIDP	$C_6H_4[COO(CH_2)_7CH(CH_3)_2]_2$	26761-40-0
Diundecilftalato	DUP	$C_6H_4[COO(CH_2)_{10}CH_3]_2$	3648-20-2
Diisoundecilftalato	DIUP	$C_6H_4[COO(CH_2)_8CH(CH_3)_2]_2$	85507-79-5
Ditridecilftalato	DTDP	$C_6H_4[COO(CH_2)_{12}CH_3]_2$	119-06-2
Diisotridecilftalato	DIUP	$C_6H_4[COO(CH_2)_{10}CH(CH_3)_2]_2$	68515-47-9
Polietileno Tereftalato	PETE	$[C_{10}H_8O_4]_n$	25038-59-9

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Categorías: Compuestos químicos | Compuestos aromáticos
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RESEARCH ARTICLE

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The Relationship between Environmental Exposures to Phthalates and DNA Damage in Human Sperm Using the Neutral Comet Assay

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Abstract

Phthalates are industrial chemicals widely used in many commercial applications. The general population is exposed to phthalates through consumer products as well as through diet and medical treatments. To determine whether environmental levels of phthalates are associated with altered DNA integrity in human sperm, we selected a population without identified sources of exposure to phthalates. One hundred sixty-eight subjects recruited from the Massachusetts General Hospital Andrology Laboratory provided a semen and a urine sample. Eight phthalate metabolites were measured in urine by using high-performance liquid chromatography and tandem mass spectrometry; data were corrected for urine dilution by adjusting for specific gravity. The neutral single-cell microgel electrophoresis assay (comet assay) was used to measure DNA integrity in sperm. VisComet image analysis software was used to measure comet extent, a measure of total comet length (micrometers); percent DNA in tail (tail%), a measure of the proportion of total DNA present in the comet tail; and tail distributed moment (TDM), an integrated measure of length and intensity (micrometers). For an interquartile range increase in specific gravity-adjusted monoethyl phthalate (MEP) level, the comet extent increased significantly by 3.6 μm [95% confidence interval (95% CI), 0.74-6.47]; the TDM also increased 1.2 μm (95% CI, -0.05 to 2.38) but was of borderline significance. Monobutyl, monobenzyl, monomethyl, and mono-2-ethylhexyl phthalates were not significantly associated with comet assay parameters. In conclusion, this study represents the first human data to demonstrate that urinary MEP, at environmental levels, is associated with increased DNA damage in sperm.

Keywords: comet assay, DNA damage, environmental, human sperm, phthalates, urinary metabolites.

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The authors declare they have no conflict of interest.

Phthalates are multifunctional chemicals used to hold color and scent in consumer and personal care products (Koo et al. 2002); as carpet backing and as solvents in paints, glue, and insect repellents (ATSDR 1999); and to soften a wide range of plastic goods (Bradbury 1996). Di(2-ethylhexyl) phthalate (DEHP), one of the more commonly used phthalates, leaches from blood products, intravenous and dialysate bags, and tubing made with polyvinyl chloride (Nässberger et al. 1987). Phthalates are also present in drinking water, air, and food (ATSDR 1995, 1999, 2000). Despite the rapid metabolism and elimination of most phthalates (Koo et al. 2002; Nässberger et al. 1987; Peck and Albro 1982), theoretically a constant steady state may be reached because of chronic and repetitive, low-level exposures from dietary ingestion and from many commonly used products.

Evidence of widespread exposure of the U.S. population to phthalates comes from two recent studies on the levels of phthalate metabolites in urine samples collected for the Third National Health and Nutrition Examination Survey (NHANES III) (Blount et al. 2000b) and NHANES 1999 (CDC 2001). The NHANES surveys collect biological samples and information about the health and diet of people in the United States (National Center for Health Statistics 2001). Four phthalate metabolites--monoethyl phthalate (MEP), mono-2-ethylhexyl phthalate (MEHP), mono-*n*-butyl phthalate (MBP), and monobenzyl phthalate (MBzP)--were present in more than 75% of U.S. subjects sampled (Blount et al. 2000b; CDC 2001)

Evidence of general population exposure to phthalates (Blount et al. 2000b; CDC 2001), as well as *in vitro* studies suggesting that some phthalates are hormonally active (Harris et al. 1997; Nakai et al. 1999) and animal studies showing associations between some phthalates and testicular toxicity (Gangolli 1982; Li et al. 1998; Parks et al. 2000; Sharpe et al. 1995; Thomas et al. 1982), has generated both public and scientific concern about potential reproductive effects of phthalates. Recent *in vitro* studies using the alkaline comet assay (single-cell gel electrophoresis) found di-*n*-butyl phthalate (DBP) and di-isobutyl phthalate (DiBP) to be genotoxic in human epithelial cells of the upper aerodigestive tract (Kleinsasser et al. 2000a), as well as in mucosal cells and lymphocytes (Kleinsasser et al. 2000b). Additionally, the comet assay was used to detect DNA damage in human lymphocytes induced by *in vitro* exposure to DEHP and MEHP (Anderson et al. 1999)

A lack of consensus on which semen quality tests are the best predictors of human male fertility has led to the development of several new methods to evaluate semen quality. The traditional semen analysis measures sperm concentration, motility, and morphology (World Health Organization 1999). Several laboratory techniques are used to evaluate sperm DNA, such as the sperm chromatin structure assay (SCSA) (Evenson et al. 1991). The SCSA may prove to be a useful clinical test because of its high repeatability and its ability to measure an aspect of fertility that differs from what can be offered by the traditional semen analysis (Evenson et al. 1999). Other DNA tests include fluorescence *in situ* hybridization, used to measure aneuploidy, as well as assays used to measure DNA integrity, including single-cell microgel electrophoresis (comet assay) and the terminal deoxynucleotidyl transferase-mediated dUTP-biotin end-labeling (TUNEL) assay (Lähdetie et al. 1996; Martin 1993; Sun et al. 1997; World Health Organization 1999)

Few published human studies have examined the effect of environmental chemicals on DNA integrity in sperm as measured by the comet assay. In the present study, we used the neutral comet assay to measure DNA integrity in human sperm and investigated whether DNA integrity was associated with urinary concentrations of five phthalate monoesters.

Materials and Methods

Subjects. The study was approved by the Harvard School of Public Health and Massachusetts General Hospital (MGH) Human Subjects Committee, and all subjects signed an informed consent form. Subjects were recruited from an ongoing semen quality study and are male partners of a subfertile couple who presented to the MGH Andrology Laboratory (Boston, MA) between January 2000 and October 2001 for semen analysis as part of an infertility investigation. Eligible men were those between 20 and 54 years old. Men presenting for postvasectomy semen analysis were excluded.

Semen sample collection. Semen was produced on site at MGH by masturbation into a sterile plastic specimen cup after a recommended period of abstinence of 48 hr. After liquefaction at 37°C for 30 min, pH, color, and viscosity measurements were made, and semen was analyzed. Sperm concentration and motility were measured by computer-aided sperm analysis (version 10HTM-IVOS; Hamilton-Thorn, Beverly, MA) using manufacturer instructions, and morphology was measured manually using Kruger strict criteria. Remaining raw semen was then frozen in 0.25-mL cryogenic straws (CryoBiosystem, I.M.V. Division, San Diego, CA) by immersing the straws directly into liquid nitrogen (-196°C). Previous work in our laboratory showed that this freezing method produced results that were highly correlated with results from fresh, unfrozen samples (Duty et al. 2002). The straws were thawed by gently shaking in a 37°C water bath for 10 sec, and the semen was immediately processed for comet assay.

Comet assay. The entire procedure was conducted under low indirect incandescent light (60 W) to minimize light-induced damage to sperm DNA. All chemicals were purchased from VWR Scientific (West Chester, PA) unless otherwise specified. After thawing, semen (with approximately 2×10^5 sperm) was mixed with 400 μL 0.7% agarose (3:1 high resolution; Amresco, Solon, OH). Fifty microliters of this semen/agarose mixture was embedded between two additional 200-μL layers of 0.7% agarose on specially designed, partially frosted, microgel electrophoresis glass slides with a clear central window (Erie Scientific, Portsmouth, NH). Cover glasses were removed before submersion of slides in a cold lysing solution (4°C) of 2.5 M NaCl, 100 mM EDTA tetrasodium salt, 10 mM Tris-base (pH 10), 1% sodium lauroyl sarcosine, and 1% Triton X-100 (Roche Diagnostics Corp., Indianapolis, IN); this step mainly dissolves the cell membrane to make chromatin accessible for the next two enzyme digestion steps. The slides were then transferred to enzyme treatment (2.5 M NaCl, 5 mM Tris, 0.05% sodium lauroyl sarcosine with pH adjusted to 7.4), and 10 mg/mL of RNase (Amresco, Solon, OH). After 4 hr at 37°C, the slides were transferred into enzyme treatment plus 1 mg/mL DNase-free proteinase K (Amresco, Solon, OH) for 18 hr at 37°C. These two steps are crucial for decondensing sperm chromatin and allowing migration of broken DNA out of the nucleus. Slides were then equilibrated in neutral electrophoresis solution (300 mM sodium acetate, 100 mM Tris, pH 9) for 20 min before being electrophoresed under neutral conditions at 12 V and 130 mA for 1 hr at room temperature. This was followed by precipitation and fixation of cells first in absolute alcohol mixed with 10 M ammonium acetate for 15 min, and then in 70% ethanol with 100 mg of spermine for 30 min. The resulting slides were air dried and then stained with YOYO dye (Molecular Probes, Eugene, OR), an intensely fluorescent DNA dye. Fluorescent comet patterns were examined with a Leica fluorescence microscope model DMLB under 400× magnification and fluoroisothiocyanate filter combination.

Image analysis. VisComet image analysis software, kindly donated by Impuls Computergestützte Bildanalyse GmbH (Gilching, Germany) was used to measure comet extent, percent DNA in tail (tail%), and tail distributed moment (TDM) on 100 sperm in each semen sample. Comet extent is a

measure of total comet length from the beginning of the head to the last visible pixel in the tail. This measurement is similar to that obtained by manual analysis using an eyepiece micrometer. Tail% is a measurement of the proportion of the total DNA that is present in the tail. The TDM is an integrated value that takes into account both the distance and intensity of comet fragments. The formula used to calculate the TDM is

$$M_{dist} = \Sigma (I*X) / \Sigma I,$$

where ΣI is the sum of all intensity values that belong to the head, body, or tail, and X is the x-position of intensity value. In addition to these two parameters, cells too long to measure with VisComet (> 300 μm; "long cells") were tallied and used as a third measure of DNA damage. Because of the presence of long cells in most subjects, more than 100 cells may have been screened and scored to allow for the measurement of comet extent, tail%, and TDM on 100 cells per subject.

Urinary phthalate metabolites. We measured the monoester phthalate metabolites because of potential sample contamination from the ubiquitous parent diester and because the metabolites are believed to be the active toxicant, not the parent diester compounds (Li et al. 1998; Peck and Albro 1982). Eight urinary phthalate metabolites--MEP, monomethyl phthalate (MMP), MEHP, MBP, MBzP, mono-*n*-octyl phthalate (MOP), mono-3-methyl-5-dimethylhexyl (isononyl) phthalate (MINP), and monocyclohexyl phthalate (MCHP)--were measured in a single spot urine sample, collected in a sterile specimen cup on the same day as the semen sample. Because more than 75% of the study population had levels of MCHP, MINP, and MOP below the limit of detection (LOD), the results for these metabolites were not informative and are not included in the analysis. The analytical approach has been described in detail elsewhere (Blount et al. 2000a). Briefly, urinary phthalate metabolite determination involved enzymatic deconjugation of metabolites from the glucuronidated form, solid-phase extraction, separation with high-performance liquid chromatography, and detection by tandem mass spectrometry. Detection limits were in the low nanogram per milliliter range. Reagent blanks and $^{13}C_4$-labeled internal standards were used along with conjugated internal standards to increase precision of measurements. One method blank, two quality control samples (human urine spiked with phthalates), and two standards were analyzed along with every 10 unknown urine samples (Blount et al. 2000a). Analysts were blind to all information concerning subjects.

Specific gravity adjustment. We measured urinary specific gravity to identify unreliable urine samples and to normalize phthalate levels for differences in urinary dilution between subjects. We used a hand-held refractometer (National Instrument Company, Inc., Baltimore, MD) that was calibrated with deionized water before each batch of measurements. Phthalate concentrations were corrected for specific gravity by the formula

$$P_c = P[(1.024 - 1)/(SG - 1)],$$

where P_c is the specific gravity-corrected phthalate concentration (nanograms per milliliter), P is the observed phthalate concentration (nanograms per milliliter), and SG is the specific gravity of sample (Boeniger et al. 1993; Teass et al. 1998). Specific gravity-adjusted phthalate levels were used in statistical modeling as a continuous predictor variable without transformation.

Statistical analysis. For data analysis, we used Statistical Analysis Software (SAS), version 8.1 (SAS Institute Inc., Cary, NC), and we performed descriptive analyses of subject characteristics. In separate univariate and multiple regression analyses, the mean of 100 cells per person was used for each of the dependent variables: comet extent, tail%, and TDM. Because mean comet extent and TDM were normally distributed (Shapiro-Wilk test p-values > 0.35), they were used untransformed in the regression analyses. However, because tail% was not normally distributed, analyses using both untransformed and log-transformed tail% were performed. Because the results and their interpretation did not differ, we chose to present only the untransformed tail% results for ease of interpretation. We used regression analysis to explore the relationship between the comet

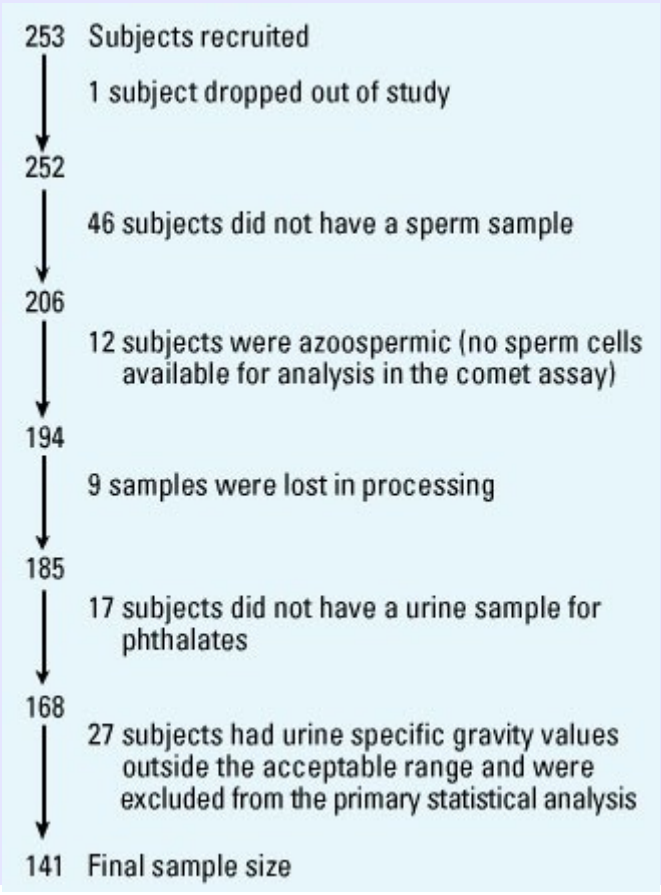
parameters and specific gravity-adjusted urinary phthalate metabolite levels, adjusting for covariates. Covariates for inclusion were based on statistical and biologic considerations (Hosmer and Lemeshow 1989). Because the number of long cells in a semen sample was not normally distributed, it was transformed using the arcsine transformation (Zar 1984) and regressed on urinary phthalates. Spearman correlation coefficients were used to determine correlations among phthalate monoesters and among comet parameters.

In the regression models, age was modeled as a continuous independent variable after checking for appropriateness using a quadratic term. Abstinence time was modeled as an ordinal five-category variable (2 or fewer days, 3, 4, 5, and 6 or more days), and smoking status was used as a dummy variable (current and former vs. never). Race was categorized into four groups: white, African American, Hispanic, and other.

Results

Of the 253 men recruited into an ongoing semen quality study, 1 dropped out and 168 subjects had both phthalate levels and comet analysis results (Figure 1). Because the study initially did not archive semen for future comet assay analyses, the first 46 subjects recruited were excluded even though their urine had been collected and analyzed for phthalates. An additional 17 subjects were excluded from the data analysis because they could not provide a urine sample at the time of semen collection, and 12 subjects with archived semen samples had no sperm (azoospermic), and so the comet assay could not be performed. Nine samples were lost when cryostraws exploded upon thawing.

Figure 1



Subject exclusions. Of the 253 subjects recruited to participate, 168 subjects had semen and urine samples available for analysis. The final sample size for statistical analysis was 141 subjects; 27 subjects were excluded because urine specific gravity was out of the acceptable range (< 1.010 or > 1.030).

Demographic information and semen parameters are given in Table 1. The mean (± SD) of age and body mass index of the 168 subjects was 36.3 ± 5.7 years and 28.2 ± 4.6 years, respectively. About 77% of subjects were white, 7.8% African American, 7.2% Hispanic, and 7.8% other. Most subjects (72.0%) never smoked, and only 9.5% were current smokers (smoked within the past month). The mean (± SD) semen concentration, motility, and strict morphology were 111.1 ± 91.0 million/mL, 52.4 ± 23.6% motile sperm, and 7.1 ± 4.5% normally shaped sperm, respectively. Although the mean values are all larger than the reference values for each semen parameter [World Health Organization (WHO) 1999], 52% of subjects had values for one or more semen parameters below the WHO reference values. Twenty-four subjects (14.3%) had < 20 million sperm/mL, 68 subjects (40.5%) had < 50% motile sperm, and 38 subjects (22.6%) had < 4% normally shaped sperm. Eighty-one subjects (48%) had semen parameters that were above the WHO reference values for all three semen parameters.


Table 1.


Table 2.


Table 3.

The distribution of comet parameters and specific gravity-adjusted urinary phthalate metabolite levels are shown in Table 2. Of the 168 subjects with both comet assay results and urinary phthalate monoester levels, 27 were excluded from the primary data analysis because specific gravity values were outside the acceptable range (< 1.010 or > 1.030) (Boeniger et al. 1993; Teass et al. 1998). MEP was detected in 100% of subjects, MBP and MBzP in at least 95% of subjects, and MEHP and MMP in at least 75% of subjects. The phthalate monoester with the highest concentration was MEP, ranging from 9.8 to 5396.2 (ng/mL) ppb with a geometric mean of 186.8 ppb. The median MEP concentration ranged from 9- to 32-fold higher than any other phthalate metabolite. Interquartile ranges (IQRs) varied considerably among the phthalates, from 443 ppb for MEP to only 9.3 ppb for MMP. The IQR of comet parameters also varied, 44 µm for comet extent and 20 µm for TDM. Fifty percent of comet extents were between 105 and 150 µm, with < 5% of cells longer than 180 µm or shorter than 70 µm. Figure 2A-C demonstrates the heterogeneity of comet tail lengths within an individual; Figure 2D depicts the comet cell referred to as a "long cell," a cell that was too long to measure with image analysis software.

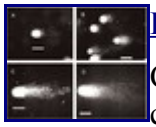


Figure 2.

Comet tail length. (*A*) Cell with a short comet tail. (*B*) Considerable heterogeneity of comet tail lengths within an individual. (*C*) Cell with a long comet tail. (*D*) Comet referred to as a "long cell," a cell with highly fragmented DNA that was too long to measure with image analysis software. Bars = 50 µm.

The mean (± SD) comet extent, tail%, and TDM were 125.3 ± 32.3 µm, 20.9 ± 7.7%, and 59.0 ± 13.7 µm, respectively. Comet extent ranged from 53.4 to 219.2 µm, tail% from 9.9 to 61.6%, and TDM from 29.5 to 91.2 µm. The number of long cells in a semen sample ranged from 0 to 73 cells. We counted the number of long cells in addition to the 100 cells measured with the VisComet software per sample. Comet extent and TDM were highly correlated ($r = 0.90$, $p < 0.0001$); however, tail% was moderately correlated with comet extent ($r = 0.35$, $p < 0.0001$) and weakly correlated with TDM ($r = 0.14$; $p = 0.10$). Moderate correlations existed between the number of long cells and both comet extent and TDM ($r = 0.45$ and $r = 0.44$, respectively; $p < 0.0001$), but the correlation between long cells and tail% was weak ($r = 0.10$, $p = 0.26$). The five phthalate monoesters were only weakly or moderately correlated with each other. The strongest correlation

was found between MBP and MBzP ($r = 0.43$; $p < 0.001$), which is expected because the diester butyl benzyl phthalate (BBzP) gives rise to both MBP and MBzP in a 5:3 ratio (NTP 2000). The weakest correlation was found between MMP and MBzP ($r = 0.015$, $p = 0.9$), suggesting that exposures to these phthalates may come from different sources.

In the univariate linear regression analyses, although not statistically significant, comet extent and TDM were longer in current smokers than in never smokers (127.7 µm vs. 125.5 µm, and 63.0 µm vs. 59.3 µm, respectively). Tail% was higher in former smokers but lower in current smokers compared with never smokers (23.5% and 18.4% vs. 20.1%, respectively; $p = 0.03$ and 0.44, respectively). The relationships between age and both comet extent and TDM were inconsistent and not significant. Comet extent increased 0.007 µm/year [95% confidence interval (CI), -0.92 to 1.06], but TDM decreased 0.14 µm/year (95% CI, -0.56 to 0.27). Tail% significantly increased 0.22%/year (95% CI, 0.00 to 0.44). The number of long cells increased marginally as age increased (< 1 cell/year, $p = 0.07$), but it was not associated with smoking. In contrast to the unstable relationships between age and smoking with comet assay parameters, MEP was significantly associated with comet extent; the regression coefficient was 3.5 µm/IQR (95% CI, 0.73 to 6.33). Tail% was not significantly associated with MEP (-0.11%/IQR; 95% CI, -0.78 to 0.56). The relationship between TDM and MEP and MBzP was less stable and failed to reach statistical significance, with increases of 1.10 µm/IQR (95% CI, -0.10 to 2.30) and 1.18 µm/IQR (95% CI, -0.25 to 2.62), respectively. There were no significant, or even suggestive, univariate relationships between specific gravity-adjusted phthalate levels and the number of long cells. The regression coefficients were close to zero, and the confidence intervals were wide.

Although the relationships between smoking and comet assay parameters were inconsistent, we included smoking as a potential confounder in the multiple regression models because several studies have reported increased DNA damage in smokers (Fraga et al. 1996; Sun et al. 1997; Ündeg(breve)er et al. 1999). Additionally, age was included in the multiple regression models because there is evidence that DNA damage increases with age (Møller et al. 2000; Singh et al. 2001). Generally, the crude and adjusted coefficients in the multiple regression models were similar, indicating that there was minimal confounding by age and smoking status.

The final multiple regression models are summarized in Table 3. After adjusting for age and smoking status, for an IQR increase in specific gravity-adjusted MEP concentrations, the comet extent significantly increased 3.61 µm (95% CI, 0.74 to 6.47), whereas TDM increased 1.17 µm but was of borderline statistical significance (95% CI, -0.05 to 2.38). Tail% decreased marginally with an IQR change in MEP, although it was not significant (-0.17%/IQR; 95% CI, -0.81 to 0.47). In contrast, the coefficients for the relationships between MBP and MEHP and comet extent, tail%, and TDM were near zero and not significant. In addition, the coefficients for the adjusted relationships between phthalate levels and the number of long cells were close to zero and nonsignificant (data not shown).

In a sensitivity analysis, we reanalyzed the data after including the 27 subjects that were excluded from the primary analysis because their urine specific gravity was outside the acceptable range. In the reanalysis, the coefficients for the relationships between MEP and comet extent and TDM, adjusted for age and smoking, were statistically significant and became larger, 3.67 µm/IQR (95% CI, 1.07 to 6.26) and 1.23 µm/IQR (95% CI, 0.12 to 2.34), respectively. The relationship between MEP and tail% remained essentially unchanged (-0.09%/IQR; 95% CI, -0.66 to 0.48). The regression coefficients for the relationship between MBzP and comet extent and TDM increased moderately in magnitude, to 2.89 µm/IQR (95% CI, -0.10 to 5.87) for comet extent and to 1.20 µm/IQR (95% CI -0.07 to 2.46) for TDM. The coefficients and confidence intervals for MBP and MEHP were similar to the results of the initial analysis, and their interpretation remained unchanged. The coefficients for MMP and comet extent and TDM became smaller in magnitude, although the confidence intervals narrowed.

Discussion

The present study represents one of the first human studies to report an association between urinary levels of MEP, at levels found in the general population, and increased DNA migration in sperm, assessed using the neutral comet assay. Specifically, there was a statistically significant positive association between urinary MEP and mean comet extent and a suggestive association with TDM. However, no significant associations were found between comet assay parameters and other urinary phthalate metabolites, including MBP, MBzP, MEHP, and MMP.

Animal data suggest that several phthalates, including butyl benzyl phthalate (BBzP), DBP, DEHP, and MEHP, are associated with damage to the testes and decreased sperm production (Gangolli 1982; Li et al. 1998; Parks et al. 2000; Sharpe et al. 1995; Thomas et al. 1982); however, there are only a few studies on the genotoxicity of these agents. Using the alkaline comet assay, researchers have found evidence of genotoxicity with *in vitro* studies examining lymphocytes and mucosal cells of the upper aerodigestive tract after exposure to DBP and DiBP (Kleinsasser et al. 2000a, 2000b, 2001). In another study using the alkaline comet assay on human leukocytes, an association between MEHP and DEHP and increased tail moments was found (Anderson et al. 1999). In contrast to those studies, in the present study we found no linear association between MEHP or MBP and sperm DNA migration. It is unclear whether the different results derive from the different cell types studied or the use of the neutral assay in the present study, compared with the use of the alkaline assay in the other studies.

In the neutral comet assay, a cell with fragmented DNA has the appearance of a "comet" with a brightly fluorescent head and a fluorescent tail whose intensity represents the relative amount of DNA strand breaks present (Hughes et al. 1997; Singh and Stephens 1998; Singh et al. 1988). The comet assay for human sperm was adapted from methods used on somatic cells, which can be conducted under alkaline or neutral conditions. Neutral conditions were used for human sperm because of the abundance of alkali-sensitive sites in sperm. Alkaline test conditions can induce damage at alkali-labile sites and produce DNA strand breaks (Singh et al. 1989)

In previous studies using the comet assay, changes in DNA migration (comet length) were detected at low levels of x-irradiation, 12.5 centigrays (rads) in human lymphocytes (Singh and Stephens 1997) and 50 centigrays (rads) in human sperm (Duty et al. 2002). Therefore, we considered comet extent and TDM to represent sensitive quantitative measures of DNA damage. However, tail moment is purported to be a more sensitive measure of DNA damage than TDM and comet extent. This increased sensitivity results from observations that with increasing levels of DNA damage, the tail length may not but tail% may continue to increase (McKelvey-Martin et al. 1993). In addition to these traditional comet assay parameters, we also tallied the number of long cells. We hypothesize that the long cell parameter represents an independent measure of DNA damage. This was partially confirmed by the weak correlation with the traditional comet assay parameters. Long cells represent very highly damaged cells. Definitive characterizations of the comet assay parameters and the significance of the long cells remain to be resolved. Although the present study was not designed to investigate this, we felt it was important to quantify long cells as a separate measure because this may prove useful in future studies using the neutral comet assay.

Although the data in the present study suggest an association between MEP and increased DNA migration in the comet assay, they must be interpreted cautiously because the phthalate levels are based on a single urine sample from a limited number of subjects. A recent study documents good reproducibility of urinary phthalate monoester measurements from day to day (Pearson correlation coefficients ranged from 0.5 to 0.8); however, this was in a small number of subjects ($n = 46$), all of whom were women and African American (Hoppin et al. 2002). Because phthalates have short half-lives (Nässberger et al. 1987; Peck and Albro 1982), spot urine samples reflect recent exposure. However, if a steady state of exposure and biologic burden is achieved with chronic repeated exposures to phthalates through the diet and the use of household and personal care products, then the utility of a single specimen is improved.

Urinary phthalate levels were normalized for urine dilution differences by adjusting for specific gravity. There are several methods to adjust for urine volume (Boeniger et al. 1993; Teass et al. 1998), and although creatinine is a frequently used form of adjustment, it is not always appropriate. If a compound is excreted primarily by tubular secretion, it is not appropriate to adjust for creatinine level (Teass et al. 1998). Although the methods of excretion of the phthalate monoesters measured in this study are unknown, terephthalic acid, a dicarboxylic acid phthalate analog, was found to be actively secreted by renal tubules and actively reabsorbed by the kidney (Tremaine and Quebbemann 1985). Furthermore, because organic compounds that are conjugated with glucuronides in the liver, such as phthalates, are eliminated by active tubular secretion (Boeniger et al. 1993), creatinine adjustment may not be appropriate. Additionally, creatinine levels may be confounded by muscularity, physical activity, urine flow, time of day, diet, and disease states (Boeniger et al. 1993; Teass et al. 1998). For these reasons, specific gravity was used to normalize phthalate levels. We excluded samples with specific gravity less than 1.010 or greater than 1.030 (Teass et al. 1998)

Phthalate levels in the present study were compared with levels measured in NHANES III (Blount et al. 2000b) and NHANES 1999 (CDC 2001). Even after limiting the NHANES III data to only men (Barr D. Personal communication; unpublished data), the phthalate levels were on average two to three times higher than those in the present study. The NHANES 1999 phthalate metabolite levels were also twice as high as those in our study. The two exceptions were MEP, which was similar between studies, and MEHP, which was twice as high in our study. MMP was not measured in NHANES data. It is unclear why MEHP levels were high in the present study because few subjects reported recent medical interventions including intravenous infusions, transfusions, or hemodialysis, which might account for higher MEHP levels. Despite the fact that the levels of phthalate monoesters differed between our study and both NHANES studies, the metabolites with the highest levels were similar across studies (Blount et al. 2000b; CDC 2001). In all three studies, the highest phthalate levels were for MEP, followed by MBP and then MBzP.

Although the men in the present study may not be representative of men in Massachusetts, generalizability may not necessarily be limited. It is a misconception that generalization from a study group depends on the study group's being a representative subgroup of the target population (Rothman and Greenland 1998). For generalizability to be limited, the relationship between comet parameters and phthalates in this clinic population would need to differ from the relationship in the population being generalized to. We would need to speculate that men in this andrology clinic differ by some factor that alters their response to phthalates. Currently, there is no reason to suspect that men who visit this andrology clinic are more or less "sensitive" to phthalates than men who visit other clinics or men from the general population. However, until the results of the present study are replicated in larger and different populations, the generalizability of our results will remain unclear.

In summary, although a significant association was seen between MEP and one measure of DNA integrity in sperm, these results need to be duplicated in a larger study. The lack of significant associations between comet assay parameters and the other four phthalate metabolites may indicate a true difference in genotoxicity between monoesters. It may also reflect markedly different exposure distributions of these monoesters when compared with the broad exposure distribution of MEP. Conversely, the comet assay associations found with MEP may reflect conducting multiple comparisons. In conclusion, this is the first epidemiologic study to explore the association between urinary monoester phthalates at general population levels and DNA integrity in sperm. In addition, the present study demonstrates that the neutral comet assay is a potentially useful tool for detecting DNA damage in human sperm in epidemiologic studies.

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ENVIRONMENTAL HEALTH PERSPECTIVES

RESEARCH ARTICLE

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Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure

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Abstract

Prenatal phthalate exposure impairs testicular function and shortens anogenital distance (AGD) in male rodents. We present data from the first study to examine AGD and other genital measurements in relation to prenatal phthalate exposure in humans. A standardized measure of AGD was obtained in 134 boys 2–36 months of age. AGD was significantly correlated with penile volume ($R = 0.27$, $p = 0.001$) and the proportion of boys with incomplete testicular descent ($R = 0.20$, $p = 0.02$). We defined the anogenital index (AGI) as AGD divided by weight at examination [AGI = AGD/weight (mm/kg)] and calculated the age-adjusted AGI by regression analysis. We examined nine phthalate monoester metabolites, measured in prenatal urine samples, as predictors of age-adjusted AGI in regression and categorical analyses that included all participants with prenatal urine samples ($n = 85$). Urinary concentrations of four phthalate metabolites [monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), and monoisobutyl phthalate (MiBP)] were inversely related to AGI. After adjusting for age at examination, *p*-values for regression coefficients ranged from 0.007 to 0.097. Comparing boys with prenatal MBP concentration in the highest quartile with those in the lowest quartile, the odds ratio for a shorter than expected AGI was 10.2 (95% confidence interval, 2.5 to 42.2). The corresponding odds ratios for MEP, MBzP, and MiBP were 4.7, 3.8, and 9.1, respectively (all *p*-values < 0.05). We defined a summary phthalate score to quantify joint exposure to these four phthalate metabolites. The age-adjusted AGI decreased significantly with increasing phthalate score (*p*-value for slope = 0.009). The associations between

male genital development and phthalate exposure seen here are consistent with the phthalate-related syndrome of incomplete virilization that has been reported in prenatally exposed rodents. The median concentrations of phthalate metabolites that are associated with short AGI and incomplete testicular descent are below those found in one-quarter of the female population of the United States, based on a nationwide sample. These data support the hypothesis that prenatal phthalate exposure at environmental levels can adversely affect male reproductive development in humans.

Keywords: anogenital distance, benzylbutyl phthalate, dibutyl phthalate, diethyl phthalate, monobenzyl phthalate, monoethyl phthalate, monoisobutyl phthalate, mono-*n*-butyl phthalate, phthalates, prenatal exposure.

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Diesters of phthalic acid, commonly referred to as phthalates, are widely used in industry and commerce; they are used in personal care products (e.g., makeup, shampoo, and soaps), plastics, paints, and some pesticide formulations. Consistent toxicologic evidence indicates association between several of these phthalate esters and reproductive effects. In particular, dibutyl phthalate (DBP), benzylbutyl phthalate (BzBP), di-2-ethylhexyl phthalate (DEHP), and di-isononyl phthalate have been shown to disrupt reproductive tract development in male rodents in an antian-drogenic manner (Parks et al. 2000). Recent studies have reported significant reductions in anogenital distance (AGD) in Sprague-Dawley rats after prenatal exposure at high doses to BzBP (Nagao et al. 2000; Tyl et al. 2004), DBP (Barlow and Foster 2003; Foster et al. 2000), and DEHP (Gray et al. 2000; Parks et al. 2000).

Despite the growing body of literature on phthalate reproductive toxicity and data demonstrating extensive human exposure (Silva et al. 2004a), few studies have examined the effects of these chemicals on human reproductive development. Colón et al. (2000) reported elevated levels of

several phthalates [including diethyl phthalate (DEP), DBP, and DEHP] in serum samples from young girls with premature breast development. However, the timing of exposure was unknown and high exposure levels may have reflected phthalate contamination of serum samples (McKee and Toxicology Research Task Group 2004). Until recently, the only study of humans to evaluate phthalate exposure and male reproductive toxicity measured phthalate diesters in semen. As with the Colón et al. study, contamination from diesters in laboratory equipment could not be excluded (Murature et al. 1987).

More recent studies have examined phthalate monoester metabolites in urine. Because urinary metabolites are not likely to be present as the result of contamination, these studies avoid this potential source of measurement error. Duty et al. (2003a) reported dose–response relationships between tertiles of monobutyl phthalate and sperm motility and sperm concentration, and between tertiles of monobenzyl phthalate (MBzP) and sperm concentration. They also reported inverse dose–response relationships between monoethyl phthalate (MEP) and sperm DNA damage measured using the neutral single-cell gel electrophoresis (comet) assay (Duty et al. 2003b). In this population of men attending an infertility clinic, increased urinary concentration of MBzP was also associated with decreased follicle stimulating hormone, whereas increases in monobutyl phthalate were marginally associated with increased inhibin-B (Duty et al. 2005).

Newborn male rodents have no scrotum, and the external genitalia are undeveloped; only a genital tubercle is apparent for both sexes. The distance from the anus to the insertion of this tubercle, the AGD, is androgen dependent and about twice as long in males as in females. The AGD has been shown to be a sensitive measure of prenatal antiandrogen exposure (Rhees et al. 1997). Recently, Salazar-Martinez et al. (2004) studied AGD in 45 male and 42 female infants. They measured the distance from the anus to the base of the scrotum in males and from the anus to the base of the genitals (the fourchette) in females. By these measures, AGD was sexually dimorphic and about twice as long in males as in females. No other studies have examined AGD among human males, although two other studies have evaluated AGD in female infants (Callegari et al. 1987; Phillip et al. 1996).

Materials and Methods

Study participants.

Women included in our study were originally recruited into the first phase of the Study for Future Families (SFFI), a multicenter pregnancy cohort study, at prenatal clinics in Los Angeles, California (Harbor-UCLA and Cedars-Sinai), Minneapolis, Minnesota (University of Minnesota Health Center), and Columbia, Missouri (University Physicians), from September 1999 through August 2002. Data collection is still ongoing in Iowa, where a center was added late in SFFI, so Iowa participants are not included in this analysis. Methods are described in detail elsewhere (Swan et al. 2003). Briefly, couples whose pregnancy was not medically assisted were eligible unless the woman or her partner was < 18 years of age, either partner did not read and speak Spanish or English, or the father was unavailable or unknown. All participants completed a questionnaire, most gave blood samples, and after urine collection was added midway through the study, most also gave a urine sample.

Eighty-five percent of SFFI participants agreed to be recontacted, and we invited these mothers to take part in our follow-up study. The family was eligible for the follow-up study (SFFII) if the pregnancy ended in a live birth, the baby was 2–36 months of age, and the mother lived within 50 mi of the clinic and could attend at least one study visit. Here we report on results from the first study visit only. Human subject committees at all participating institutions approved SFFI and SFFII, and all participants signed informed consents for each study.

Physical examination.

After standard anthropometric measurements (height, weight, head circumference, and skin-fold thickness) were obtained, a detailed examination of the breast and genitals was conducted under the supervision of pediatric physicians who were trained in its administration. Every attempt was made to standardize the examination, which was developed specifically for this study. These methods included training sessions before and during the study and the use of standardized equipment. Neither the pediatric physicians nor the support staff had any knowledge of the mother's phthalate concentrations.

Boys' genital examinations included a description of the testes and scrotum, location and size of each testicle, and measurement of the penis. The placement of each testicle was initially coded in six categories; in the present analysis, boys are dichotomized into those with normal testicular descent (placement of both testes coded as normal or normal retractile) or with incomplete testicular descent (all other cases). The scrotum was categorized as distinct from surrounding tissue or not, and by size (small or not). Penile width and (stretched) length were recorded, and penile volume [proportional to (penile width/2)^2 × penile length] was calculated. We recorded the AGD, measured from the center of the anus to the anterior base of the penis. We also recorded the ano-scrotal distance (ASD), measured from the center of the anus to the posterior base of the scrotum. This latter measurement was used by Salazar-Martinez et al. (2004), who refer to it as AGD.

Phthalate metabolite analysis.

Urinary phthalate metabolite analyses were carried out by the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), which had no access to participant data. The analytical approach for the analysis of urinary phthalate metabolites (Silva et al. 2004b) is a modification of previously published methods (Silva et al. 2003). The analysis involves the enzymatic deconjugation of the phthalate metabolites from their glucuronidated form, automated on-line solid-phase extraction, separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry. This high-throughput method allows for the simultaneous quantification in human urine of the nine phthalate metabolites reported in this work. Limits of detection (LOD) are in the low nanogram per milliliter range. Isotopically labeled internal standards were used along with conjugated internal standards to increase precision and accuracy of the measurements. The method is accurate (spiked recoveries are near 100%), and precise with between-day relative standard deviations of < 10%. Quality control (QC) samples and laboratory blanks were analyzed along with unknown samples to monitor performance of the method. The metabolite concentrations reported here are from 85 prenatal maternal urine samples of a total of 214 that also included postnatal maternal and baby samples from the same mothers and their children. The 214 samples were analyzed for phthalate metabolites in six batches, none of which had to be re-extracted for QC failures. Of the 214 samples, seven were re-extracted using < 1 mL of urine because concentrations of MEP calculated using 1 mL were above the linear range of the method.

Statistical analysis.

After examining descriptive and summary statistics for all study variables, we explored models for AGD. We fit several alternative measures of body size (weight, height, and body mass index) and both additive and multiplicative functions of these. We defined the anogenital index [AGI = AGD/weight (mm/kg)] as a weight-normalized index of AGD.

AGD and AGI were modeled as both linear and quadratic functions of age. For babies born at < 38 weeks, age at examination in the first year was calculated from the estimated date of conception instead of the birth date. Once the best fitting model was identified, we plotted the expected AGI and its 25th and 75th percentiles as a function of age. We categorized boys in two ways: We

dichotomized boys into those with AGI smaller than or at least as large as expected, and we used the difference between observed and expected AGI to define three groups of boys, short (AGI < 25th percentile for age), intermediate (25th percentile ≤AGI < 75th percentile), and long (AGI ≥75th percentile for age) AGI. We also calculated the proportion of boys in these three groups with normal testicular descent (both testes normal or normal retractile) and normal scrotal (scrotum of normal size and distinct from surrounding tissue). We calculated the correlations between AGD and AGI and penile volume, testicular placement and scrotal parameters (size and distinctness from surrounding tissue). Our decision to use AGI as the measure of genital development was made, and cut points for categorical analyses of outcomes were selected, before obtaining phthalate metabolite values.

We used general linear models to explore the relationships between phthalate metabolite concentration (unadjusted for urine concentration) and genital parameters. Most metabolite concentrations were above the LOD; those below the LOD were assigned the value LOD divided by the square root of 2, which has been recommended when the data are not highly skewed, as was the case here (Hornung and Reed 1990). Metabolite concentrations were logarithmically transformed to normalize distributions. We examined several potentially confounding factors including mother's ethnicity and smoking status, time of day and season in which the urine sample was collected, gestational age at sample collection, and baby's weight at examination.

We also categorized metabolite concentrations into low (< 25th percentile), intermediate (between the 25th and 75th percentiles), and high (≥75th percentile) categories and examined the odds ratio (OR) for smaller than expected AGI for babies with high compared with low exposure, and medium compared with low. On the basis of these regression and categorical analyses, we identified the phthalate metabolites most strongly associated with AGI. We refer to these as AGI-associated phthalates.

Because phthalate metabolite concentrations are highly correlated, and because our limited sample size prohibited us from examining multiway interactions, we constructed a summary phthalate score to examine the effect of joint exposure to more than one AGI-associated phthalate. For this purpose, we used quartiles of metabolite concentration; values in the lowest quartile did not contribute to the sum, whereas higher values increased the sum one unit per quartile. We divided this sum into three categories: low (0–1, reflecting little or no exposure to AGI-associated phthalates), intermediate (2–10), and high (11–12, reflecting high exposure to all, or almost all, AGI-associated phthalates). We examined the magnitude of the residual (observed – expected) AGI as a function of this summary phthalate score.

Results

The population for the present analysis was identified from families recruited in California, Minnesota, or Missouri for whom data entry was complete by 17 December 2004, the cutoff date for the present analysis. At that time, 654 participants from these three centers had completed SFFI and given permission to be recontacted. Of these, 477 (72.9%) were eligible for SFFII and 346 (72.5%) participated (Table 1). SFFII participants were demographically similar to nonparticipants except that non-participants were more likely to be Hispanic because of a lower eligibility rate (60%) in CA, where most participants were Hispanic. Of the 172 boys born to these mothers, we excluded 5 boys in twin births, 10 boys with incomplete data, and 23 boys for whom AGD was not recorded [two whose mothers declined the genital exam, with the remainder older boys (mean age, 19.6 months), for whom the study examiner felt the measurement was not reliable, usually because of the boys' activity level]. The remaining 134 boys comprise the sample used for the analysis of AGD and other genital measurements. Among the 134 boys for whom we have genital measurements, no frank genital malformations or disease were detected, and no parameters appeared grossly abnormal. The mean age at first examination was 15.9 months, and mean weight was 10.5 kg (Table 2). Mean (± SD) AGD was 70.3 ± 11.0 mm, with a distribution that was well

approximated by a normal curve. Overall, 86.6% of boys had both testes classified as normal or normal-retractile.

A prenatal urine sample was assayed for phthalate metabolites for mothers of 85 of these boys. These mother–son pairs comprise the data set for the analysis of AGD and phthalate metabolite concentration. Because urine collection began midway through SFFI, mothers with a stored urine sample were recruited later in the study, and their sons tended to be younger at examination (mean age, 12.6 months; interquartile range, 5–16 months). Summary statistics for all boys included in the analysis of physical measurements, and the subset of boys for whom mothers' prenatal phthalate concentrations were also available are shown separately in Table 2.

All phthalate metabolites tested were above the LOD in > 49% of women, and most tested were above the LOD in > 90% of the samples (Table 3). Concentrations spanned four orders of magnitude, from below the LOD (estimated value = 0.71 ng/mL) to 13,700 ng/mL for MEP. Means ranged from 2.68 for mono-3-carboxypropyl phthalate (MCPP) to 629.8 for MEP. Three of the four AGI-associated metabolites (other than MEP) were significantly correlated ($p < 0.005$).

Regression analyses.

We initially modeled AGD as a linear function of age and weight, but this model fit poorly (adjusted $R^2 = 0.22$). We found that using AGI (AGD/weight) as a function of age provided the best fit, as has been shown in rodent models (Vandenbergh and Huggett 1995). The best-fitting model for AGI includes linear and quadratic terms for age and is given by AGI = 10.8835 − 0.3798 (age) + 0.0068 (age^2) (adjusted $R^2 = 0.61$). Using this model, we calculated mean AGI and its 5th, 25th, 75th, and 95th percentiles (Figure 1).

We then examined models that included individual phthalate metabolites. Other than age and age squared, no covariates altered regression coefficients for the phthalate metabolites by > 15%, and none were included in final models. All regression coefficients for individual metabolites (logarithmically transformed to normalize distributions) were negative (Table 4). MEP, mono-*n*-butyl phthalate (MBP), MBzP, and monoisobutyl phthalate (MiBP) were (inversely) related to AGI; *p*-values for regression coefficients were between 0.007 and 0.097. We also measured three metabolites of DEHP. Although the hydrolytic monoester metabolite mono-2-ethylhexyl phthalate (MEHP) was unrelated to AGI [regression coefficient = −0.05; 95% confidence interval (CI), −0.53 to 0.43], regression coefficients for the oxidative monoester metabolites of DEHP, mono-2-ethyl-5-oxo-hexyl phthalate (MEOHP), and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) were of a magnitude comparable with those for MEP and MBzP (*p*-values = 0.114 and 0.145 for MEOHP and MEHHP, respectively). AGI appeared to be independent of the concentrations of monomethyl phthalate (MMP) and MCPP, metabolites of dimethyl phthalate and di-*n*-octyl phthalate, respectively.

Categorical analyses.

The 25 boys with AGI below the 25th percentile for age were classified as having a short AGI. This group had an AGI that was, on average, 18.3% (range, 10–32%) shorter than expected based on the final regression model. Boys with AGI ≥75th percentile of expected were classified as having a long AGI, and boys with AGI between the 25th and 75th percentile of expected were considered intermediate. Boys' weight and age did not differ appreciably among these groups.

Table 5 shows mean and median values for the AGI-associated metabolites for boys in the short, intermediate, and long categories of AGI. We calculated the ORs for short AGI for each monoester metabolite (Table 6). For high compared with low concentration of MBP, the OR for a short AGI was 10.2 (95% CI, 2.5 to 42.2), whereas for medium concentration compared with low the OR was 3.8 (95% CI, 1.2 to 12.3). The corresponding ORs for high compared with low concentration of MEP, MBzP, and MiBP were 4.7, 3.8, and 9.1, respectively (all *p*-values < 0.05).

Other genital parameters.

Degree of testicular descent was associated with AGD (R = 0.20, $p = 0.02$). The proportions of boys with one or both testicles incompletely descended were 20.0, 9.5, and 5.9% for boys classified as having short, intermediate, and long AGI (p-value for short AGI compared with all other boys < 0.001). The proportion of boys with a scrotum categorized as small and/or "not distinct from surrounding tissue" was also elevated for boys with short AGI ($p < 0.001$). AGD was significantly associated with penile volume (R = 0.27, $p = 0.001$), and penile volume divided by weight was correlated with AGI (R = 0.43, $p = 0.001$). Testicular volume, which was measured by orchidometer, is not shown here because participating physicians considered the measurement to be unreliable—a decision made before analyses of phthalate exposure.

ASD was, on average, 47% as long as AGD, and these two measurements were correlated (R = 0.47, $p < 0.0001$). However, the model predicting ASD as a function of baby's age and weight fit poorly (adjusted $R^2 = 0.10$). The fit for the model using ASD/weight as a function of age and age squared was better (adjusted $R^2 = 0.47$) but did not fit as well as the model using AGI ($R^2 = 0.61$). ASD/weight was associated with MEP concentration (regression coefficient = −0.429; 95% CI, −0.722 to −0.137). For the other phthalate metabolites, regression coefficients were less significant (all p-values between 0.11 and 0.97).

Summary phthalate score.

We used the summary phthalate score as defined in "Materials and Methods" to study the effect of joint exposure to more than one AGI-associated phthalate. The summary phthalate score was directly related to the proportion of boys with short AGI ($p = 0.001$). Of the 10 boys whose phthalate scores were high (score = 11–12), all but one had a short AGI. Conversely, of the 11 boys whose scores were low (score = 0 or 1), only one had a short AGI. The ORs for having a short AGI for high summary phthalate score compared with low (OR = 90.0; 95% CI, 4.88 to 1,659), and high compared with medium (29.4; 95% CI, 3.4 to 251) were large and significant, although the confidence intervals were very wide. These data are shown graphically in Figure 1.

Discussion

In the recent National Health and Nutrition Examination Survey (NHANES 1999–2000), most of the general population in the United States had measurable exposure to multiple phthalates (CDC 2003; Silva et al. 2004a). The samples in the present study and in NHANES were both analyzed using comparable methods and standards by the same laboratory, although the specific metabolites that were measured in the two studies differed somewhat. We compared the medians and 75th percentiles of the AGI-associated phthalate metabolite concentrations among two groups of mothers in our study (those whose boys fell in the short AGI group and all others) with those of females in the NHANES sample (Table 7). In the analysis of the NHANES samples, monobutyl phthalate includes both MBP and MiBP, which were measured separately in our study. Metabolite concentrations for mothers of boys with short AGI were consistently higher than those of other mothers. Compared with women in the NHANES sample, metabolite concentrations for our population were somewhat lower. However, our population cannot be directly compared with NHANES: the proportion of pregnant women in the NHANES sample is unknown, and age distributions differ. Nonetheless, these data demonstrate that the four AGI-associated phthalate metabolites are prevalent in the U.S. female population, and levels were not unusually high among mothers whose sons had a short AGI.

Although not identical, AGD in pups is most similar to AGD as we defined it in this study. In rodents, AGD has been shown to be one of the most sensitive end points for phthalates such as DBP (Mylchreest et al. 2000) and other antiandrogens such as flutamide (Barlow and Foster 2003; McIntyre et al. 2001) and finasteride (Bowman et al. 2003). It is difficult to compare the dose to

humans from low-level, ongoing, environmental exposure with that delivered to rodents experimentally in a narrow window of gestation. Nonetheless, it is likely that the doses to which our participants were exposed are lower than those used in toxicologic settings, suggesting that humans may be more sensitive to prenatal phthalate exposure than rodents. This greater sensitivity in humans has been observed for other toxicants. For example, humans are more sensitive to trenbolone by an order of magnitude (Neumann 1976). This greater sensitivity is thought to be a result of rodents' higher metabolic rate and more rapid inactivation of toxicants, both of which have been shown to be inversely related to body size (White and Seymour 2005).

In light of the toxicologic literature for MBP, MBzP, and MiBP (Ema et al. 2003; Foster et al. 1980, 1981; Gray et al. 2000; Nakahara et al. 2003), our data suggest that the end points affected by these phthalates are quite consistent across species. A boy with short AGI has, on average, an AGI that is 18% shorter than expected based on his age and weight as well as an increased likelihood of testicular maldescent, small and indistinct scrotum, and smaller penile size. These changes in AGD and testicular descent are consistent with those reported in rodent studies after high-dose phthalate exposure (Ema et al. 2003; Gray et al. 2000; Mylchreest et al. 2000). The lack of association for MCPP and MMP, which have not been widely studied, is not inconsistent with the toxicologic literature.

With respect to DEP and its metabolite MEP, we note that there are three other human studies suggesting reproductive toxicity (Colón et al. 2000; Duty et al. 2003b; Main KM, unpublished data). It is therefore uncertain whether the absence of data in rodents showing reproductive toxicity is the result of failure to detect it, unmeasured confounding in human studies, or interspecies differences in response to these compounds.

DEHP has been shown to shorten AGD (Gray et al. 2000) and reduce testosterone (Parks et al. 2000). Although MEHP was not associated with AGD in our data, the associations for the oxidative metabolites of DEHP (MEOHP and MEHHP) were of comparable magnitude with those for metabolites of DBP and BzBP, although not statistically significant. Thus, it is unclear whether MEOHP and MEHHP are (inversely) associated with AGI, although associations are of borderline statistical significance because of our sample size, or whether human and rodent responses to this phthalate and its metabolites differ.

Masculinization of external male genitalia, represented by longer AGD, is controlled by dihydrotestosterone (Clark et al. 1990). Ema and Miyawaki (2001) demonstrated that this metabolite of testosterone is markedly decreased by prenatal administration of MBP, suggesting that MBP acts as an antiandrogen. AGD in male rodents is associated with other adverse developmental effects (Foster and McIntyre 2002) and some phthalate-induced changes have been shown to be permanent. For example, Barlow et al. (2004) report that prenatal exposure to 500 mg/kg/day DBP resulted in permanently decreased AGD and testicular dysgenesis. They also report that in utero DBP exposure induced proliferative Leydig cell lesions. Follow-up of exposed children until adulthood will be required to determine whether long-term effects, including testicular dysgenesis, are seen in humans after prenatal phthalate exposure.

Several recent studies of the variability of phthalate monoester concentration in human samples suggest that phthalate concentration in humans is fairly stable, perhaps reflecting habitual use of phthalate-containing household and consumer products (Colón et al. 2000; Hauser et al. 2004; Hoppin et al. 2002). These studies lend support to the use of a single sample for exposure assessment. We obtained only a single prenatal urine sample from each woman, and most samples were obtained quite late in pregnancy (mean = 28.3 weeks). Therefore, the measured phthalate metabolite levels may not reflect exposure during the most sensitive developmental window, resulting in some degree of exposure misclassification. However, unless this misclassification varied systematically with outcome, such errors would bias the effect estimate toward the null. In fact, the categorical analysis, which should be less sensitive to such misclassification, showed stronger associations than did the continuous analysis.

Our analysis is based on a single measure of AGD, and the reliability of this measurement in humans has not been established. During two training sessions, three study physicians each measured AGD in four male infants (mean age, 8.1 months). The mean AGD for these measurements was 58.6 mm, SD was (within infant) 4.2 mm, and coefficient of variation of 7.2%, suggesting that AGD can be measured reliably. Use of this measurement in larger studies in a range of diverse populations, with many more such training sessions, will be needed to obtain normative data.

Although it might have been ideal to examine babies shortly after birth, the timing of grant funding did not allow this. Babies were born to SFFI mothers as early as January 2000, and the first baby visits did not occur until April 2002. To maximize the number of children participating, we allowed recruitment over a range of ages. On the other hand, because the use of AGD in humans is new, the optimal timing for this measurement is not known. Our data suggest that measurements are reliable and informative in young children at least until 18 months, when AGD becomes more difficult to obtain reliably. Its value in adolescents and adults has yet to be determined.

We note that phthalate metabolite levels were highly correlated, and most women were exposed to all metabolites at detectable levels. Gray et al. (2000) suggested that risk assessments for phthalate-induced reproductive toxicity should consider phthalates as a group and include exposures from multiple sources. The score we use reflects joint exposure to the four AGI-associated phthalates, and our results suggest that joint exposure may convey greater than additive risk, but larger sample sizes are needed to confirm this.

Gray and Foster (2003) refer to a “phthalate syndrome” characterized by testicular, epididymal, and gubernacular cord agenesis as well as decreased AGD, and stress the importance of evaluating all components of a syndrome so that affected animals are not misidentified. It has recently been suggested (Fisher 2004) that this “phthalate syndrome” shares many features with the human testicular dysgenesis syndrome proposed by Skakkebaek et al. (2001) to follow chemically induced disruption of embryonic programming and gonadal development during fetal life. The present findings, though based on small numbers, provide the first data in humans linking measured levels of prenatal phthalates to outcomes that are consistent with this proposed syndrome.

This is the first study to look at subtle patterns of genital morphology in humans in relation to any prenatal exposure. It was motivated by toxicologic studies showing that genital morphology is altered by antiandrogens, including some phthalates. We report that AGD, the most sensitive marker of antiandrogen action in toxicologic studies, is shortened and testicular descent impaired in boys whose mothers had elevated prenatal phthalate exposure. These changes in male infants, associated with prenatal exposure to some of the same phthalate metabolites that cause similar alterations in male rodents, suggest that commonly used phthalates may undervirilize humans as well as rodents.

Figures and Tables

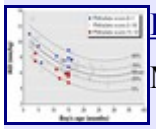

Figure 1.

Mean AGI (mm/kg) in relation to boys’ age at examination (months).

Table 1.

Participants included in present analysis.

Table 2.

Characteristics of boys with complete physical examination.

Table 3.

Percentiles of phthalate monoester metabolites.

Table 4.

Regression analyses of AGI on $\log_{10}$ monoester metabolite concentration, controlling for age and age squared.

Table 5.

Mean (median) phthalate monoester metabolite levels by AGI category.

Table 6.

OR (95% CI) for AGI less than expected from regression model, by monoester metabolite level.

Table 7.

Concentrations of four phthalate metabolites in three groups of women (ng/mL).

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FDA Public Health Notification: PVC Devices Containing the Plasticizer DEHP

July 12, 2002

Dear Colleague:

This is to inform you that FDA's Center for Devices and Radiological Health completed its safety assessment of Di(2-ethylhexyl)phthalate (DEHP) released from polyvinyl chloride (PVC) medical devices in September, 2001, and to advise you of steps that you can take to reduce the risk of exposure in certain populations.

Devices Affected

PVC is a plastic polymer that is used in a wide array of products. Unplasticized PVC is hard and brittle at room temperature. A plasticizer (softener) is typically added to increase the flexibility of the polymer. DEHP is the plasticizer for most PVC medical devices.

Devices that may contain DEHP-plasticized PVC include:

- intravenous (IV) bags and tubing
- umbilical artery catheters
- blood bags and infusion tubing
- enteral nutrition feeding bags
- nasogastric tubes
- peritoneal dialysis bags and tubing
- tubing used in cardiopulmonary bypass (CPB) procedures
- tubing used in extracorporeal membrane oxygenation (ECMO)
- tubing used during hemodialysis

Nature of the Problem

Everyone is exposed to small levels of DEHP in everyday life. However, some individuals can be exposed to high levels of DEHP through certain medical procedures. DEHP can leach out of plastic medical devices into solutions that come in contact with the plastic. The amount of DEHP that will

leach out depends on the temperature, the lipid content of the liquid, and the duration of contact with the plastic. Seriously ill individuals often require more than one of these procedures, thus exposing them to even higher levels of DEHP.

Exposure to DEHP has produced a range of adverse effects in laboratory animals, but of greatest concern are effects on the development of the male reproductive system and production of normal sperm in young animals. We have not received reports of these adverse events in humans, but there have been no studies to rule them out. However, in view of the available animal data, precautions should be taken to limit the exposure of the developing male to DEHP.

Risk determinants

Two factors determine the degree of risk posed by exposure to DEHP in a medical setting. The first is the patient's sensitivity to DEHP. Based on the evidence cited above, the male fetus, male neonate, and peripubertal male would appear to be high-risk groups. The second factor is the dose of DEHP received by the patient. This is determined largely by the type of procedure performed, as well as the frequency and duration of these procedures.

Highest risk procedures

We examined the potential risk of exposure posed to patients by comparing the dose of DEHP that patients might receive during various procedures to a "Tolerable Intake" (TI) value for the compound.

The following procedures have been identified as posing the highest risk of exposure to DEHP:

- exchange transfusion in neonates
- ECMO in neonates
- total Parenteral Nutrition (TPN) in neonates (with lipids in PVC bag)
- multiple procedures in sick neonates (high cumulative exposure)
- hemodialysis in peripubertal males
- hemodialysis in pregnant or lactating women
- enteral nutrition in neonates and adults
- heart transplantation or coronary artery bypass graft surgery (aggregate dose)
- massive infusion of blood into trauma patient
- transfusion in adults undergoing ECMO

In contrast, there is little or no risk posed by patient exposure to the amount of DEHP released from PVC IV bags during the infusion of crystalloid fluids (e.g., normal saline, D5W, Ringer's Lactate). Further, there is little risk posed by exposure to the amount of DEHP released from PVC bags used to store and administer drugs that require a pharmaceutical vehicle for solubilization, when label instructions are followed.

Recommendations

Most importantly, you should not avoid the procedures cited above simply because of the possibility of health risks associated with DEHP exposure. The risk of not doing a needed procedure is far greater than the risk associated with exposure to DEHP.

For some of the above procedures, PVC devices that do not contain DEHP can be substituted, or devices made of other materials (such as ethylene vinyl acetate (EVA), silicone, polyethylene or polyurethane) can be used, if available. If PVC devices containing DEHP must be used, you may be able to minimize exposure to DEHP by, for example, using the freshest possible blood products stored at the lowest possible temperature, or by using heparin-coated ECMO circuits.

We recommend considering such alternatives when these high-risk procedures are to be performed on male neonates, pregnant women who are carrying male fetuses, and peripubertal males. One source for identifying alternative devices that do not contain DEHP-plasticized PVC is http://www.sustainablehospitals.org[1], associated with the University of Massachusetts Lowell.

For other patient groups, who are presumably at lower risk, the decision to use DEHP alternatives must take into account the medical advantages and drawbacks of the substitute materials and their availability.

Reporting Adverse Events to FDA

The Safe Medical Devices Act of 1990 (SMDA) requires hospitals and other user facilities to report deaths and serious injuries associated with the use of medical devices, including the devices cited above. We request that you follow the procedures established by your facility for such mandatory reporting.

We also encourage you to report other adverse events associated with the use of medical devices. You can report these directly to the device manufacturer. You can also report to MedWatch, the FDA's voluntary reporting program. You may submit reports to MedWatch one of four ways: online at http://www.accessdata.fda.gov/scripts/medwatch[2] , by telephone at 1-800-FDA-1088; by FAX at 1-800-FDA-1078; or by mail to MedWatch, Food and Drug Administration, HF-2, 5600 Fishers Lane, Rockville, Maryland 20857.

Getting More Information

If you have questions about this Notification, please contact FDA's Office of Surveillance and Biometrics by e-mail at phann@fda.hhs.gov or by phone at 301-796-6640.

FDA Medical Device Public Health Notifications[3] are available on the Internet. You can also be notified through email each time a new Public Health Notification is added to our web page. To subscribe, visit: http://service.govdelivery.com/service/subscribe.html?code=USFDA_39[4].

Sincerely yours,

David W. Feigal, Jr., MD, MPH
Director
Center for Devices and Radiological Health
Food and Drug Administration

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Page Last Updated: 02/11/2011
Note: If you need help accessing information in different file formats, see Instructions for Downloading Viewers and Players.

Ftalatos y PVC, el plástico venenoso

PVC: The Poison Plastic

The Campaign for Safe, Healthy Consumer Products

Home | About PVC | Safe Products | Companies | Media Center | About Us

Take Action!

PVC: Unhealthy for Our Nation's Children and Schools

TOXIC Toys R Us

In 2008, Toys "R" Us promised to reduce PVC plastic, phthalates, and lead in children's and infant's toys. However, Toys "R" Us has broken its promise The company has neglected to label toxic components in their toys and has failed to remove PVC, the poison plastic, from the toys it sells.

Independent product testing has confirmed that Toys "R" Us is selling toys made with PVC. Chemicals released during PVC's lifecycle have been linked to chronic diseases in children, impaired child development and birth defects, cancer, disruption of the endocrine system, reproductive impairment and immune system suppression.

There is no safe way to manufacture, use or dispose of PVC products. As the largest specialized toy retailer in America, with more than 800 stores nationwide, Toys" "R Us has the economic power to eliminate toxics from the toy supply chain entirely.
Check out the **Toxic Toys "R" Us** report for more details about how Toys "R" Us'' broken promise may impact the health of you and your family. View the report

Find safe PVC-free products for your family!

Pass Up the Poison Plastic - the PVC-Free Guide for Your Family & Home lists the most common consumer products made out of PVC and safer PVC-free products including baby products, children's toys, electronics, and more.
Download **CHEJ's Pass Up the Poison Plastic guide** now!

If you found these resources helpful, please consider making a donation to CHEJ. Your donation will support our continued efforts to keep you and your family safe from environmental health threats.

1/14/10
Major Green Purchasing Victory!

New York consumers and the environment got a green holiday gift on December 29th when the state approved the nation's most comprehensive Green Purchasing policy to avoid "bad actor" chemicals in products. CHEJ was successful in convincing the state to approve a policy requiring all state agencies to consider avoiding 85 chemicals in products purchased by the state, such as carcinogens. This is the most comprehensive chemical avoidance purchasing list in the country and it will have a major impact on greening the marketplace with NY's annual buying power of $9 billion. For more information, go to CHEJ's blog at http://www.chej.org/blog/

11/18/10
New Report Finds Toxic PVC Toys Still Widely Sold at Toys "R" Us

A new report by CHEJ and the Teamsters found that Toys "R" Us has broken its promises to rid their shelves of toxic PVC toys. The report, Toxic Toys R Us – PVC Toxic Chemicals in Toys and Packaging was released just as the 2010 holiday shopping season begins. Over 70% of toys tested contained PVC, the most toxic plastic for our health and environment.

Download **The Report**
Read the press release

10/29/10
PVC-Free Film Screenings at New York Colleges!
All across New York State, college students are launching campaigns at their schools to get PVC **off of their campuses**! Over the next the next two weeks, CHEJ is hitting the road on our PVC-Free Campus Tour with our 25 foot tall inflatable rubber ducky and three short films to raise awareness about the hazards of PVC.

Here's the tour schedule.

10/25/10
CHEJ and 50+ Organizations Call on White House OMB to Act on Dioxin

In 1985, the EPA released its first health assessment of Dioxin. For nearly 25 years, the chemical industry has fought the release of this report. Now the EPA is closer than ever to releasing this important report. CHEJ, the Breast Cancer Fund and others are calling on the Office of Management & Budget to move the process forward. **Read the full letter sent to the OMB.**

10/22/10
Major New Study Shows Vinyl Flooring's Threat to School Children

HealthyStuff.org's new study finds PVC flooring seven times more likely to contain hazardous chemical additives than non-vinyl alternatives. **Full press release**.

[MORE CAMPAIGN NEWS]

Resumen de Salud Pública
Di(2-etilhexil) ftalato (DEHP) *[Di(2-ethylhexyl) phthalate]*

CAS#: 117-81-7

septiembre de 2002

Este Resumen de Salud Pública es el capítulo sumario de la Reseña Toxicológica para el di(2-etilhexil ftalato). El mismo forma parte de una serie de Resúmenes de Reseñas Toxicológicas relacionados a sustancias peligrosas y sus efectos sobre la salud. Una versión más breve, ToxFAQs™, también está disponible. Esta información es importante para usted debido a que esta sustancia podría causar efectos nocivos a su salud. Los efectos a la salud de la exposición a cualquier sustancia peligrosa van a depender de la dosis, la duración, la manera de exposición, las características y hábitos personales, y si están presentes otras sustancias químicas. Si desea información adicional, puede comunicarse con el Centro de Información de la ATSDR al 1-888-422-8737.

Trasfondo

Este resumen de salud pública le informa acerca del di(2-etilhexil) ftalato (DEHP) y de los efectos de la exposición a esta sustancia.

La Agencia de Protección del Medio Ambiente de EE. UU. (EPA, por sus siglas en inglés) identifica los sitios de desechos peligrosos más serios en la nación. Estos sitios constituyen la Lista de Prioridades Nacionales (NPL, por sus siglas en inglés) y son los sitios designados para limpieza a largo plazo por parte del gobierno federal. El DEHP se ha encontrado en por lo menos 737 de los 1,613 sitios actualmente en la NPL o que formaron parte de la NPL en el pasado. Sin embargo, el número total de sitios de la NPL en los que se ha buscado DEHP no se conoce. A medida que se evalúan más sitios, el número de sitios en que se encuentre DEHP puede aumentar. Esta información es importante porque la exposición a esta sustancia puede perjudicarlo y estos sitios pueden constituir fuentes de exposición.

Cuando una sustancia se libera desde un área extensa, por ejemplo desde una planta industrial, o desde un recipiente como un barril o botella, la sustancia entra al ambiente. Esta liberación no siempre conduce a exposición. Usted está expuesto a una sustancia solamente cuando entra en contacto con ésta. Usted puede estar expuesto al inhalar, comer o beber la sustancia, o por contacto con la piel.

Si usted está expuesto al DEHP, hay muchos factores que determinan si le afectará adversamente. Estos factores incluyen la dosis (la cantidad), la duración (por cuánto tiempo) y de la manera como entró en contacto con esta sustancia. También debe considerar las otras sustancias químicas a las

que usted está expuesto, su edad, sexo, dieta, características personales, estilo de vida y condición de salud.

1.1 ¿Qué es el DEHP?

DEHP es la abreviación de di(2-etilhexil) ftalato. El DEHP es una sustancia química manufacturada que se añade comúnmente a los plásticos para hacerlos más flexibles. Otros nombres por los cuales se conoce a esta sustancia son dioctil ftalato (DOP) y bis(2-etilhexil) ftalato (BEHP). (Nótese que di-n-octil ftalato es el nombre de una sustancia química diferente.) Algunos nombres registrados para el DEHP incluyen Platinol DOP®, Octoil®, Silicol 150®, Bisoflex 81® y Eviplast 80®. El DEHP es un líquido incoloro casi sin olor. No se evapora fácilmente y muy poco se encontrará presente en el aire aun cerca de fuentes de producción. Se disuelve más fácilmente en materiales como gasolina, removedores de pintura y aceites que en agua. Se encuentra presente en muchos plásticos, especialmente en materiales fabricados de vinilo, los que pueden contener hasta cerca de 40% de DEHP, aunque es más común encontrar niveles más bajos. El DEHP se encuentra presente en productos de plástico tales como revestimiento de murallas, manteles, baldosas, tapices de muebles, cortinas de baño, mangueras, forros de piscinas, ropa para la lluvia, calzones para bebés, muñecas, ciertos juguetes, zapatos, tapices y techos de automóviles, papel y láminas transparentes para envolver, cubierta de alambres y cables, tuberías para uso médico y bolsas para almacenar sangre.

1.2 ¿Qué le sucede al DEHP cuando entra al medio ambiente?

El DEHP puede entrar al ambiente desde fábricas que manufacturan o usan DEHP y desde productos domésticos que lo contienen. Con el tiempo, el DEHP puede escapar al ambiente desde los materiales plásticos. Por lo tanto, el DEHP está ampliamente distribuido en el ambiente. Cerca de 291,000 libras fueron liberadas por industrias el año 1997. A menudo se encuentra cerca de industrias, vertederos y sitios de desechos. Una gran cantidad de plásticos que contienen DEHP se encuentran enterrados en vertederos. El DEHP se ha encontrado en el agua subterránea cerca de plantas para la disposición de desechos.

Cuando el DEHP se libera al suelo, generalmente se adhiere firmemente al suelo y no se moviliza muy lejos del lugar en que fue liberado. El DEHP se disuelve muy lentamente en el agua subterránea o en el agua superficial con la que entra en contacto. Tarda varios años para que el DEHP presente en materiales desechados o enterrados desaparezca del ambiente. Debido a que el DEHP no se evapora fácilmente, generalmente muy poca cantidad pasa al aire. El DEHP en el aire se adherirá a partículas de polvo y será transportado nuevamente a la tierra por la gravedad y la lluvia o la nieve. Aunque pequeñas, las liberaciones de DEHP desde materiales plásticos, revestimientos y pisos en el interior de hogares y lugares de trabajo, pueden producir niveles de DEHP más altos que los que se encuentran al aire libre.

En la presencia de productos químicos, el DEHP puede degradarse a mono(2-etilhexil) ftalato (MEHP) y 2-etilhexanol. Muchas de las propiedades del MEHP son similares a las del DEHP y por lo tanto, su comportamiento en el ambiente es similar. En la presencia de oxígeno, el DEHP en el agua puede ser degradado por microorganismos a dióxido de carbono y a otros productos químicos más sencillos. El DEHP no se degrada muy fácilmente en la profundidad del suelo o en el fondo de lagos o ríos donde hay poco oxígeno. El DEHP se encuentra en pequeñas cantidades en peces y en otros animales, y también se ha descrito incorporación en plantas.

1.3 ¿Cómo podría yo estar expuesto al DEHP?

Usted puede estar expuesto al DEHP a través del aire, el agua o el contacto de la piel con plásticos que contienen DEHP. Los alimentos también pueden contener DEHP, pero no se sabe cuanto.

Aunque no está claro, es probable que una pequeña cantidad de DEHP se transfiera a través del contacto de la piel con vestimentas de plástico o con otros artículos que contienen DEHP. La exposición a través de esta ruta parece ser de poca importancia debido a que las vestimentas, como por ejemplo los impermeables, no hacen contacto con la piel directamente y además, aun si hubiera contacto directo, la cantidad transferida sería muy pequeña.

Usted puede estar expuesto al DEHP a través del agua potable, sin embargo no se sabe si esto es común. Si usted toma agua de un pozo localizado cerca de un vertedero o de un sitio de desechos, puede exponerse a cantidades de DEHP por sobre lo normal.

Usted puede respirar DEHP que ha sido liberado al ambiente. El nivel promedio de DEHP en el aire de ciudades y áreas industriales es menos de 2 partes de DEHP por trillón de partes de aire (ppt). Los niveles de DEHP en el aire dentro de una habitación donde se ha instalado el piso recientemente pueden ser más altos que los niveles al aire libre. Los trabajadores en fábricas que manufacturan o usan DEHP también respiran niveles de esta sustancia más altos que el promedio.

El DEHP también puede entrar al cuerpo durante ciertos procedimientos médicos. Este tipo de exposición es probablemente más importante que cualquier exposición ambiental. La sangre que se almacena en bolsas de plástico y que se usa para transfusiones contiene entre 4.3 y 1,230 partes de DEHP por millón de partes de sangre (ppm). Otros productos médicos almacenados en envases de plástico también liberan DEHP. Los tubos flexibles usados para administrar líquidos o medicamentos pueden transferir DEHP al paciente. Los tubos plásticos usados para diálisis renal frecuentemente contienen DEHP y liberan DEHP a la sangre del paciente. El DEHP también está presente en los tubos plásticos de respiradores y de esta forma entra a los pulmones.

1.4 ¿Cómo puede el DEHP entrar y abandonar mi cuerpo?

El DEHP entra a su cuerpo cuando usted come alimentos o toma agua que contiene esta sustancia, o cuando respira aire contaminado. Pequeñas cantidades de DEHP pueden entrar a su cuerpo a través de contacto de la piel con plásticos, aunque los científicos están bastante seguros de que muy poco DEHP penetra de esta forma. La mayor parte del DEHP que entra a su cuerpo en los alimentos, el agua, o el aire pasa de los intestinos y los pulmones a la sangre. El DEHP se puede introducir directamente a la sangre si usted recibe una transfusión o si recibe medicamentos a través de tubos plásticos o si se somete a tratamientos de diálisis.

Después de que el DEHP se ingiere, la mayor parte es degradada rápidamente en el intestino a MEHP y 2-etilhexanol. La degradación es mucho más lenta si el DEHP entra a la sangre directamente a través de una transfusión. Aunque cierta cantidad de MEHP es transportada a la sangre desde el intestino, esta cantidad es muy pequeña, de manera que la mayor parte del DEHP que se ingiere es eliminada del cuerpo en las heces. Los compuestos que entran a la sangre viajan en la corriente sanguínea al hígado, los riñones, los testículos y a otros tejidos, y pequeñas cantidades pueden ser almacenadas en la grasa y podrían ser secretadas en la leche materna. La mayor parte del DEHP, MEHP y 2-etilhexanol abandona el cuerpo en la orina y las heces dentro de las primeras 24 horas.

1.5 ¿Cómo puede afectar mi salud el DEHP?

Para proteger al público de los efectos perjudiciales de sustancias químicas tóxicas, y para encontrar maneras para tratar a personas que han sido afectadas, los científicos usan una variedad de pruebas.

Una manera para determinar si una sustancia química perjudicará a una persona es averiguar si la sustancia es absorbida, usada y liberada por el cuerpo. En el caso de ciertas sustancias químicas puede ser necesario experimentar en animales. La experimentación en animales también puede usarse para identificar efectos sobre la salud como cáncer o defectos de nacimiento. Sin el uso de animales de laboratorio, los científicos perderían un método importante para obtener información

necesaria para tomar decisiones apropiadas con el fin de proteger la salud pública. Los científicos tienen la responsabilidad de tratar a los animales de investigación con cuidado y compasión. Actualmente hay leyes que protegen el bienestar de los animales de investigación, y los científicos deben adherirse a estrictos reglamentos para el cuidado de los animales.

Es improbable que los niveles de DEHP que se encuentran en el ambiente causen efectos perjudiciales a la salud en seres humanos. Un hombre que tragó voluntariamente 10 gramos (aproximadamente 0.4 onzas) de DEHP sufrió irritación del estómago y diarrea. La mayoría de la información que tenemos acerca de los efectos a la salud del DEHP proviene de estudios en ratas y ratones a los que se les administró DEHP en los alimentos, o se introdujo en el estómago por la boca con la ayuda de un tubo. En la mayoría de estos estudios, las cantidades de DEHP que se les dio a los animales fueron mucho más altas que las cantidades que se encuentran en el ambiente. Las ratas y los ratones parecen ser especialmente sensibles a ciertos efectos del DEHP. Por lo tanto, es más difícil predecir algunos efectos a la salud en seres humanos basado en la información en estos estudios.

Respirar DEHP no parece tener serias consecuencias de salud. Los estudios en ratas han demostrado que el DEHP en el aire no afecta la mortalidad o la capacidad para reproducirse. Como se mencionó anteriormente, casi nada de DEHP se evapora al aire. Es improbable que usted sufra efectos a la salud a raíz de contacto de la piel con DEHP porque esta sustancia no penetra la piel muy fácilmente.

Las exposiciones orales breves a niveles de DEHP muchos más altos que los encontrados en el ambiente interfieren con la formación de espermatozoides en ratones y en ratas. Estos efectos fueron reversibles, pero los animales alcanzaron la madurez sexual más tarde cuando fueron expuestos antes de la pubertad. Las exposiciones breves a niveles bajos de DEHP no parecieron afectar la fertilidad de los machos.

Los estudios de exposiciones prolongadas en ratas y ratones han demostrado que dosis orales altas de DEHP afectan principalmente el hígado y los testículos. Estos efectos se produjeron a niveles de DEHP mucho más altos que los que se reciben a través de exposiciones ambientales. La toxicidad del DEHP en otros tejidos no está muy bien caracterizada, aunque en algunos estudios en animales se han descrito efectos de la tiroides, los ovarios, los riñones y la sangre. La posibilidad de que afecte el riñón causa especial preocupación porque en seres humanos este órgano está expuesto al DEHP durante diálisis y porque se han observado alteraciones estructurales y funcionales del riñón en algunas ratas expuestas al DEHP. Debido a que las alteraciones de los riñones en pacientes sometidos a diálisis por largo tiempo pueden deberse a la enfermedad renal existente, y regularmente no se han observado alteraciones renales en animales expuestos al DEHP, el significado de las alteraciones renales en la rata no está claro.

Los seres humanos absorben y degradan el DEHP en el cuerpo de manera diferente que las ratas y ratones. Por lo tanto, muchos de los efectos observados en ratas y en ratones luego de la exposición al DEHP puede que no ocurran en seres humanos y en animales como por ejemplo los monos (primates).

Ningún estudio ha evaluado el potencial del DEHP para producir cáncer en seres humanos. La ingestión de altas dosis de DEHP por un tiempo prolongado produjo cáncer del hígado en ratas y en ratones.

El Departamento de Salud y Servicios Humanos (DHHS) ha determinado que es razonable predecir que el DEHP es carcinogénico en seres humanos. La EPA ha determinado que el DEHP es probablemente carcinogénico en seres humanos. Estas determinaciones fueron basadas totalmente en el hallazgo de cáncer del hígado en ratas y en ratones. La Agencia Internacional para la Investigación del Cáncer (IARC, por sus siglas en inglés) recientemente cambió su clasificación del DEHP de "posiblemente carcinogénico en seres humanos" a "no clasificable en cuanto a carcinogenicidad en seres humanos," basado en la manera diferente como responde el hígado de

seres humanos y primates comparado al hígado de ratas y ratones.

1.6 ¿Cómo puede el DEHP afectar a los niños?

Esta sección discute los posibles efectos sobre la salud en seres humanos expuestos durante el período desde la concepción a la madurez a los 18 años de edad.

Los niños pueden estar expuestos al DEHP si comen alimentos o toman agua contaminada con DEHP o si respiran esta sustancia en el aire. Los niños pequeños también pueden estar expuestos al chupar o tocar objetos de plástico (juguetes) o chupetes que contienen DEHP, como también al ingerir leche materna que contiene DEHP. Los niños también pueden estar expuestos al DEHP si se someten a ciertos procedimientos médicos que requieren el uso de tubos flexibles como los que se usan para administrar líquidos o medicamentos al paciente. Sin embargo, no hay evidencia definitiva de efectos adversos en niños expuestos al DEHP de esta forma.

En estudios de ratas y ratones preñados expuestos oralmente a grandes dosis de DEHP se observaron efectos sobre el desarrollo del feto, incluso defectos de nacimiento y aun muerte de fetos. En animales jóvenes se observaron alteraciones de la estructura de los huesos y de partes del cerebro, del hígado, riñón y los testículos. Estos efectos adversos sugieren que el DEHP o algunos de sus productos de degradación cruzaron la placenta y alcanzaron el feto. Por lo tanto, seres humanos expuestos durante el embarazo a niveles de DEHP suficientemente altos podrían dar a luz a bebés de bajo peso y/o con alteraciones en el desarrollo del esqueleto o el sistema nervioso, aunque no es seguro que esto ocurra. Los estudios en animales también han demostrado que el DEHP o algunos de sus productos de degradación pueden pasar de la madre a los bebés a través de la leche materna y alterar el desarrollo de los animales que lactan. Esto también podría ocurrir en seres humanos porque el DEHP se ha detectado en la leche materna.

No sabemos si los niños difieren de los adultos en su susceptibilidad a los efectos del DEHP. Sin embargo, hay estudios que sugieren que los animales machos jóvenes son más susceptibles a los efectos adversos del DEHP sobre los órganos sexuales que los adultos.

1.7 ¿Cómo pueden las familias reducir el riesgo de exposición al DEHP?

Si su doctor encuentra que usted ha estado expuesto a cantidades significativas de DEHP pregunte si sus niños también podrían haber estado expuestos. Puede que su doctor necesite pedir que su departamento estatal de salud investigue. Como se menciona en la Sección 1.8, los exámenes para el DEHP sólo dan una medida de exposición reciente a esta sustancia.

El DEHP es usado en muchos productos de plástico, pero especialmente en un plástico conocido como cloruro de polivinilo (PVC) o vinilo. En productos manufacturados que lo contienen, el DEHP se encuentra en niveles más altos cuando el producto es nuevo. Los productos viejos contienen cantidades más bajas de DEHP. Artículos fabricados de PVC incluyen a muchos juguetes plásticos, algunos muebles de plástico, tapices de automóviles y de muebles, cortinas de baño, algunas mangueras, manteles y ciertos pisos (pisos de vinilo). No todos los productos de PVC contienen DEHP. Debido a que el DEHP puede encontrarse en algunos juguetes, existe preocupación porque los niños que chupan esos juguetes podrían estar expuestos al DEHP. Un estudio demostró que el DEHP puede pasar de los plásticos a saliva artificial producida en el laboratorio.

1.8 ¿Hay algún examen médico que demuestre que he estado expuesto al DEHP?

El examen más específico que puede usarse para determinar si usted ha estado expuesto al DEHP es medir el MEHP y otros productos de degradación en la sangre y la orina. Este examen sólo es útil para medir exposición reciente, debido a que el DEHP es degradado rápidamente a otras sustancias que son eliminadas de su cuerpo. También se puede medir otro producto de degradación (el ácido ftálico), pero este examen no es específico para el DEHP. Uno ó 2 días después de la exposición sus heces podrían ser analizadas para determinar la presencia de metabolitos del DEHP. Estos exámenes no están disponibles rutinariamente en los consultorios médicos.

1.9 ¿Qué recomendaciones ha hecho el gobierno federal para proteger la salud pública?

El gobierno federal desarrolla reglamentos y recomendaciones para proteger la salud pública. Los reglamentos **pueden** ser impuestos por ley. Las agencias federales que desarrollan reglamentos para sustancias tóxicas incluyen a la EPA, OSHA y la Administración de Drogas y Alimentos de EE. UU. (FDA, por sus siglas en inglés). Las recomendaciones proveen instrucciones valiosas para proteger la salud pública, pero **no pueden** imponerse por ley. Las organizaciones federales que desarrollan recomendaciones para sustancias tóxicas incluyen a la Agencia para Sustancias Tóxicas y el Registro de Enfermedades (ATSDR) y el Instituto Nacional de Salud y Seguridad Ocupacional (NIOSH, por sus siglas en inglés) .

Los reglamentos y recomendaciones pueden ser expresados como 'niveles que no deben excederse' en el aire, agua, suelo o alimentos y se basan generalmente en niveles que afectan a los animales. Estos niveles luego se ajustan para la protección de seres humanos. En ciertas ocasiones estos 'niveles que no deben excederse' difieren entre organizaciones federales debido a las diferentes duraciones de exposición (una jornada de 8 horas al día o de 24 horas al día), el uso de diferentes estudios en animales o a otros factores.

Las recomendaciones y los reglamentos son actualizados periódicamente a medida que se dispone de información adicional. Para obtener la información más reciente, consulte a la organización o agencia federal que la otorga. Los siguientes son algunos reglamentos y recomendaciones para el DEHP:

Existen varios reglamentos federales que regulan la cantidad de DEHP en productos de consumo, el agua potable y el ambiente del trabajo. La FDA limita los tipos de materiales de empaque para alimentos que pueden contener DEHP. La EPA limita la cantidad de DEHP en el agua potable a 6 partes de DEHP por billón de partes de agua (6 ppb). La EPA requiere que derrames de 100 libras o más de DEHP al ambiente sean reportados a la Agencia. La OSHA limita la concentración de DEHP en el aire del trabajo a 5 miligramos de DEHP por metro cúbico (mg/m^3) de aire durante una jornada de 8 horas diarias. El límite de exposición de corta duración (15 minutos) es de 10 mg/m^3. Las recomendaciones establecidas por la Conferencia Americana de Higienistas Industriales de Gobierno (ACGIH, por sus siglas en inglés) para el DEHP en el trabajo son las mismas que los reglamentos establecidos por la OSHA.

1.10 ¿Dónde puedo obtener más información?

Si usted tiene preguntas o preocupaciones, por favor comuníquese con el departamento de salud y calidad ambiental de su comunidad o estado o con la ATSDR a la dirección y número de teléfono que aparecen más abajo.

La ATSDR también puede indicarle la ubicación de clínicas de salud ocupacional y ambiental. Estas

clínicas se especializan en la identificación, evaluación y el tratamiento de enfermedades causadas por la exposición a sustancias peligrosas.

Las Reseñas Toxicológicas también están disponibles (en inglés) en *Internet* en www.atsdr.cdc.gov y en *CD-ROM*. Usted puede solicitar una copia del *CD-ROM* que contiene las Reseñas Toxicológicas de la ATSDR llamando libre de cargos al número de información y asistencia técnica al 1-800-*CDCINFO* (1-800-232-4636), a través de correo electrónico al cdcinfo@cdc.gov o escribiendo a:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mail Stop F-62
Atlanta, GA 30333
Fax: 1-770-488-4178

Las organizaciones con fines de lucro pueden solicitar copias de las Reseñas Toxicológicas finalizadas a:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Website: http://www.ntis.gov/

Referencias

Agencia para Sustancias Tóxicas y el Registro de Enfermedades. (*ATSDR*). 2002. Reseña Toxicológica de los Di(2-etilhexil) ftalato (DEHP) (en inglés). Atlanta, GA: Departamento de Salud y Servicios Humanos de EE. UU., Servicio de Salud Pública.

Agencia para Sustancias Tóxicas y el Registro de Enfermedades
4770 Buford Hwy NE, Atlanta, GA 30341
Tel: CDC Contact Center: 800-CDC-INFO • 888-232-6348 (TTY)
cdcinfo@cdc.gov

UN VENENO MEDIOAMBIENTAL

EL PVC: UN VENENO MEDIOAMBIENTAL

(Textos y gráficos extraídos de distintas publicaciones de Greenpeace)

El PVC es un plástico que lleva cloro en su composición (el 57% del plástico virgen es cloro).
Su fabricación, al igual que otros procesos industriales que utilizan cloro, implica la formación y emisión al medio ambiente de sustancias organocloradas tóxicas, persistentes y bioacumulativas.
Los gases, aguas residuales y residuos emitidos y vertidos por las fábricas de este plástico contienen cloruro de vinilo, hexaclorobenceno, PCBs, dioxinas y otras muchas sustancias organocloradas extremadamente tóxicas.

* El plástico clorado PVC (policloruro de vinilo) ocasiona graves riesgos al medio ambiente y a la salud pública, durante todo su ciclo de vida. Los principales están asociados con la generación y emisión de dioxinas durante el proceso de fabricación del cloruro de vinilo y la incineración de productos de PVC, y la migración de los aditivos, como es el caso de los plastificantes que necesariamente contienen los productos de este plástico blando. Por todo ello, el PVC puede denominarse "veneno medioambiental".
(Sentencia dictada por el Tribunal Superior de Viena, Austria el 31 de Marzo del 94)

La fabricación de este plástico también requiere mucha energía, necesaria para separar el cloro del sodio, al que se encuentra fuerte y establemente unido formando sal común.
Los vendedores de PVC no tienen en cuenta esta etapa de la fabricación cuando comparan el consumo energético de este producto con el de otros materiales.

INDICE :

PRODUCTOS DE PVC Y ADITIVOS

Un producto de PVC puede contener hasta un 60% de aditivos, que le confieren estabilidad, plasticidad o rigidez, color, etc., convirtiéndolo en un cóctel de compuestos químicos, muchos de ellos tóxicos.

Si el producto de PVC es blando, como las mangueras y tuberías flexibles, tapicerías, suelos o papeles pintados de vinilo, entonces contienen plastificantes. Las sustancias que se utilizan como plastificantes del vinilo son los **ftalatos**, unos compuestos que han resultado cancerígenos en animales de laboratorio y que además son estrogénicos, esto es, pueden alterar el sistema hormonal. Los plastificantes se liberan de los productos de PVC blando.

Metales pesados tóxicos, como el plomo y el cadmio se utilizan también como aditivos del PVC y se pueden
encontrar en ventanas, persianas y revestimientos de este material. Recientemente ha dejado de utilizarse en Europa el cadmio. Su legado tóxico perdura en los productos que se fabricaron con anterioridad y que aún se encuentran en nuestros edificios.

RESIDUOS DE PVC

Los materiales de construcción de PVC tienen una vida media de 5 a 30 años, según el producto de que se trate. Una vez que se convierten en residuos, estos materiales van a parar a las escombreras, vertederos de RSU (Residuos Sólidos Urbanos) o incineradoras.

En los vertederos, los aditivos del PVC se liberan poco a poco de los materiales que los contienen, contaminando el suelo y el agua. Si se queman los residuos, ya sea en vertederos o incineradoras, el cloro que contienen se convierte en ácido clorhídrico (un gas corrosivo) y en sustancias organocloradas tóxicas, incluyendo dioxinas.

MATERIALES DE C0NSTRUCCION DE PVC E INCENDIOS

Los riesgos del PVC ante incendios han llevado a numerosos municipios y empresas europeas, como el Metro
de Londres o el de Bilbao, e incluso a la Armada de los EE.UU. a sustituir el uso de productos clorados. Cuando se queman materiales que contienen cloro, se forma ácido clorhídrico y compuestos organoclorados. El ácido clorhídrico es un gas muy corrosivo que produce graves daños materiales y humanos. Este ácido reacciona también con los aditivos que contiene el PVC, creando así un volumen mayor de humos tóxicos.

Entre las sustancias organocloradas que se forman durante la combustión del PVC se encuentran las dioxinas, que contaminan las cenizas y escombros de los incendios, convirtiéndolos en residuos tóxicos. En definitiva, el PVC convierte un incendio en un accidente químico, multiplicando los daños materia'fes, ambientales, humanos y económicos.

**La decisión de eliminar este plástico ya ha sido tomada por muchas autoridades locales, instituciones y
arquitectos europeos y de todo el mundo.**

* La construcción del estadio olímpico de Sidney, minimizó el uso de PVC, en concreto, utilizaron alternativas en los materiales de fontanería, drenaje y pavimentación.

* La ciudad austríaca de Linz ha conseguido eliminar progresivamente hasta un 85% del PVC en los edificios públicos y seis de los nueve gobiernos regionales de Austria han aprobado restricciones a su uso en obras públicas.

* Bonn, la capital alemana, acordó prescindir al máximo del uso de PVC en los edificios públicos: escuelas, guarderías, residencias de ancianos y estaciones de metro. Desde 1989 se

han construido en Berlín unos 130 edificios públicos que han limitado su utilización. De hecho, en Alemania, más de 200 ayuntamientos y seis estados federales han decidido restringir su uso.

* Los Gobiernos de Dinamarca y Suecia también están considerando en la actualidad restricciones a este material.

* Bergen, la segunda ciudad en población de Noruega, tomó en 1991 la decisión de eliminar el PVC de sus edificios públicos. Desde entonces, numerosos edificios nuevos y proyectos de reforma se han llevado a cabo con una mínima utilización de este producto.

* El Metro de Londres prohibió la utilización de cables halogenados en sus estaciones entre los que se incluyen los fabricados con PVC, a raíz de un compromiso que adoptaron sobre seguridad ante incendios.

* Las instalaciones de metro de Viena, Berlín, Dússeldorf y Bilbao, tampoco utilizan este tipo de cables.

* El ayuntamiento de Barcelona decidió sustituir progresivamente el uso de productos clorados, incluyendo PVC, de todas las actividades, obras o servicios que se lleven a cabo con participación municipal. Con esta decisión se unía a la veintena de municipios españoles que ya han aprobado medidas para reducir el uso de este plástico.

CIUDADES QUE HAN APROBADO MEDIDAS CONTRA EL PVC EN ESPAÑA

Badía del Vallés (Barcelona), Barcelona (Barcelona), Carmona (Sevilla), Casas-Ibáñez (Albacete), Catilleja de la Cuesta (Sevilla), Coria del Río (Sevilla), Ecija (Sevilla), Fene (A Coruña), Guadalcanal (Sevilla), Illescas (Toledo), Jumilla (Murcia) (en moratoria), Mairena de Aljarafe (Sevilla), Mancor de la Vall (Mallorca), Mislata (Valencia), Montcada i Reixac (Barcelona), Mugardos (A Coruña), Narón (A Coruña), Neda (A Coruña), Novelda (Alicante), Rinconada (Sevilla), Ripollet (Barcelona), Terradillos (Salamanca), Tossa de Mar (Girona), Utrera (Sevilla).

PARLAMENTOS

Andalucía y Cataluña.

MATERIALES DE CONSTRUCCION ALTERNATIVOS AL PVC

En el mercado español se pueden encontrar alternativas más respetuosas con el medio ambiente a todos los usos de este plástico. Las alternativas son, en algunas ocasiones, más caras que el PVC, pero sus ventajas ambientales, técnicas y su mayor duración compensan, en nuestra opinión, la mayor inversión inicial. Además, el incremento de la demanda de estos materiales alternativos reducirá a medio plazo su coste. Estas alternativas muestran que es posible reducir, e incluso evitar, el uso de PVC en la construcción o renovación de nuestros bogares.

Puedes conseguir el intorme "Construyendo el futuro: una guía para construir sin PVC" y el disquete "Alternativas al PVC", que contiene una base de datos de fabricantes y distribuidores en España, dirigiéndote a Greenpeace España.

ALTERNATIVAS AL PVC EN LA CONSTRUCCIÓN

PRODUCTO	MATERIALES ALTERNATIVOS
Tuberías de Distribución	Cerámica, Arcilla, Acero inoxidable, Cobre, Polietileno (PE),

	Polipropileno (PP)
Tuberías evacuación y alcantarillado	Cerámica vitrificada, Arcilla, Fundición, PE, PP
Ventanas	Madera (mejor procedente de sistemas de gestión forestal sostenible)
Cables e instalaciones eléctricas	Poliolefinas (PE, PP y copolímeros), Baquelita, Cerámica
Revestimientos	Linóleo, Corcho, Madera, Piedra, Cerámica
Cubiertas impermeabilizantes	Caucho (EPDM), PE

EL PVC EN LOS JUGUETES DE LOS NIÑOS

"La utilización de PVC en juguetes infantiles representa un riesgo inaceptable y evitable, debido a la evidencia científica que demuestra los efectos tóxicos de los plastificantes en animales de experimentación. El elevado potencial de exposición, la vulnerabilidad infantil a sustancias tóxicas y los recientes estudios científicos sobre los posibles efectos en el sistema hormonal, desaconsejan totalmente su uso».

En 1996, Greenpeace inició una investigación sobre los juguetes blandos de PVC, con el fin de determinar la presencia de plastificantes tóxicos, llamados ftalatos. Los resultados fueron alarmantes. El equipo de investigación de Greenpeace Internacional, en la Universidad de Exeter (Reino Unido), analizó 71 juguetes de diferentes países, de los cuales 63 eran de PVC o tenían piezas de este material, y todos ellos contenían una cantidad considerable de ftalatos, entre un 10% y un 40% en peso.

Los resultados de los análisis efectuados sobre liberación de ftalatos en los artículos que eran de PVC, revelaron que la dosis a la que estarían expuestos los bebés, a partir de estos productos, sería de 5 a 10 veces superior a la proveniente de los alimentos. Además, se excedía la dosis diaria aceptable para el DINP (el ftalato más empleado en juguetes).

"Es inaceptable que nuestros hijos estén
expuestos a sustancias químicas en esas cantidades, especialmente
cuando son tan jóvenes."

Lars Carisen, Director de Investigación del
Departamento de Química Ambiental, Instituto Nacional de Investigación
Ambiental, Dinamarca.

"¿Deberían los niños chupar o morder juguetes de PVC?
No.
Contienen sustancias tóxicas que pueden absorverse fácilmente.
Sabemos que aparecen en la corriente sanguínea de los niños."

Mientras que la etiqueta de algunos de los juguetes analizados por Greenpeace que contenían grandes cantidades de ftalatos, llevaban la etiqueta de "no tóxico", la de un frasco de DINP, para uso en laboratorio, advierte en los países de la Unión Europea (de acuerdo con la Directiva sobre Sustancias Peligrosas):

"Peligroso por inhalación, contacto con la piel e ingestión"

"Puede causar cáncer"
"Posible riesgo de efectos irreversibles"
"Evitar la exposición a esta sustancia. Obtener instrucciones especiales antes de su utilizacion"
"Llevar ropa, guantes, mascarillas y gafas de protección adecuadas".

Así mismo, las hojas de seguridad que acompañan el DINP contienen la siguiente información:

"Peligro para el sistema reproductor, posible teratógeno"

Aún hay empresas envasadoras de agua en España que siguen utilizando el PVC para vendernos el agua, si bién muchas de ellas han hecho gala de su responsabilidad cambiando este tipo de envase.
(Para más información dirigirse a información@greenpeace.es)

¿QUÉ PODEMOS HACER NOSOTROS?

* Elige juguetes Fabricados con materiales naturales: caucho, tejidos no sintéticos (como algodón o Lana), madera, corcho... etc.

* Antes de comprar un juguete comprueba la composición en la etiqueta: El PVC viene identificado con la palabra "vinilo", el número "3" o la letra "v". Si la etiqueta no incluye la composición pregunta al comerciante y en caso de duda no compres el juguete.

* Escribe o llama a la oficina de atención al consumidor de los fabricantes de juguetes. Encontrarás su dirección y el teléfono en las etiquetas o preguntando a los comerciantes.

* Asegúrate de que tus hijas e hijos no juegan con este tipo de juguetes en sus guarderías, colegios o parques infantiles. Pide a los profesores, asociación de padres y/o dirección, que los retiren y eviten comprarlos.

* Pide a los responsables de sanidad y de guarderias y escuelas infantiles de tu ayuntamiento o
comunidad autónoma que tomen medidas para evitar la exposición de los niños a estos juguetes peligrosos.

* Advierte a tus familiares y amigos con niñas y niños pequeños de este riesgo.

* Evita las botellas de agua de PVC. Puedes beber agua del grifo, instala un filtro si es muy caliza. Si necesitas agua envasada este es el orden de preferencia de materiales ambientalmente aceptables: vidrio retornable, vidrio no retornable (acuérdate de reciclarlo), PE (polietileno), PP (polipropileno) y PET (polietilentereftalato).
Las garrafas grandes son preferibles a las botellas pequeñas. Para distinguir el PVC de los otros plásticos da un pellizco a la botella, si queda una marca blanca, es PVC.

* Evita también las botellas de aceite y vinagre de PVC. Para distinguirlas sigue el procedimiento anterior.

* Apoya la campaña de boicot de Greenpeace a los envases de PVC.

* Antes de comprar cualquier envase plástico, comprueba su composición. Rechaza todos los que sean de PVC, vendrán marcados con "3". Si no viene indicada la composición pregunta al comerciante y recházalos si no te informa adecuadamente.

* No compres mordedores para bebés ni juguetes de PVC. Suelen venir indicados como "vinilo".

* Denuncia la publicidad engañosa de los fabricantes de PVC. Escribe cartas de protesta a los directores de las publicaciones donde aparezcan anuncios de productos de PVC en los que se afirme que son ecológicos".

* Utiliza materiales de construcción alternativos al PVC, cuando reformes o construyas tu casa.

* Consigue en Greenpeace el listado de materiales de construcción alternativos y muéstraselo a tu constructor, arquitecto, aparejador, decorador, albañil, fontanero, electricista, etc.

* Propón a tu ayuntamiento que se declare "libre de PVC", puedes conseguir más información sobre esta iniciativa en Greenpeace.

* Escribe a los Ministerios de Sanidad y de Medio Ambiente pidiéndoles que prohiban el uso de productos de PVC.

José Manuel Romay Beccaría. Ministro de Sanidad
Ministerio de Sanidad
Paseo del Prado 18 y 20. 28071 Madrid
Tfn.: (91) 596 10 00

Isabel Tocino
Ministra de Medio Ambiente. Ministerio de Medio Ambiente.
Plaza San Juan de la Cruz s/n. 28071 Madrid
Tfn.: (91) 597 70 00

Mr. Martin Bangemann:
Commissioner for Industrial Affairs European Commission
Rue de la Loi 200
B- 1049 Bruxelles. Bélgica.

Estás en Página de Medioambiente: PVC. ¿Ir a? --> Agua Basura Bosques Consumo Energía Transporte Vivienda

(Volver a Página de Medioambiente)

ENVIRONMENTAL HEALTH PERSPECTIVES
RESEARCH ARTICLE

The Association between Asthma and Allergic Symptoms in Children and Phthalates in House Dust: A Nested Case-Control Study

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Abstract

Global phthalate ester production has increased from very low levels at the end of World War II to approximately 3.5 million metric tons/year. The aim of the present study was to investigate potential associations between persistent allergic symptoms in children, which have increased markedly in developed countries over the past three decades, and the concentration of phthalates in dust collected from their homes. This investigation is a case-control study nested within a cohort of 10,852 children. From the cohort, we selected 198 cases with persistent allergic symptoms and 202 controls without allergic symptoms. A clinical and a technical team investigated each child and her or his environment. We found higher median concentrations of butyl benzyl phthalate (BBzP) in dust among cases than among controls (0.15 vs. 0.12 mg/g dust). Analyzing the case group by symptoms showed that BBzP was associated with rhinitis ($p = 0.001$) and eczema ($p = 0.001$), whereas di(2-ethylhexyl) phthalate (DEHP) was associated with asthma ($p = 0.022$). Furthermore, dose-response relationships for these associations are supported by trend analyses. This study shows that phthalates, within the range of what is normally found in indoor environments, are associated with allergic symptoms in children. We believe that the different associations of symptoms for the three major phthalates—BBzP, DEHP, and di-*n*-butyl phthalate—can be explained by a combination of chemical physical properties and toxicologic potential. Given the phthalate exposures of children worldwide, the results from this study of Swedish children have global implications.

Keywords: allergy, asthma, BBzP, children, DEHP, homes, phthalates.

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Airborne phthalate esters are present at detectable levels across the surface of Earth. They were first identified in outdoor urban air (Cautreels and Van Cauwenberghe 1976a, 1976b) and subsequently have been recognized as global pollutants (Atlas and Giam 1981; Giam et al. 1978) and major constituents of indoor air (Weschler 1980, 1984). Their presence in outdoor and indoor environments reflects their large emission rates coupled with moderate atmospheric lifetimes. The total global consumption of phthalate esters is estimated to exceed 3.5 million metric tons/year, with di(2-ethylhexyl) phthalate (DEHP) constituting roughly 50% of the market share (Cadogan and Howick 1996). Consumption of di-*n*-butyl phthalate (DnBP) and *n*-butyl benzyl (BBzP) phthalate is smaller but still quite large (> 100,000 metric tons/year each) (Cadogan and Howick 1996). Although DEHP plasticizes numerous products, roughly 95% of the current production is used in polyvinyl chloride (PVC) (National Toxicology Program 2003), where it typically constitutes 30% of PVC by weight (Cadogan and Howick 1996; Kavlock et al. 2002b). DnBP is used in latex adhesives, in nail polish and other cosmetic products, as a plasticizer in cellulose plastics, as a solvent for certain dyes, and, to a lesser extent than DEHP, as a plasticizer in PVC (Kavlock et al. 2002c). BBzP is a plasticizer for vinyl tile, carpet tiles, and artificial leather and is also used in certain adhesives (Kavlock et al. 2002a).

Research groups have assessed the exposures of various populations to phthalate esters by using their metabolites in human urine as biomarkers [Barr et al. 2003; Blount et al. 2000; Centers for Disease Control and Prevention (CDC) 2003; Koch et al. 2003]. The biomarker results translate to daily exposures for DnBP, BBzP, and DEHP of 1.5, 0.88, and 0.71 µg/kg/day in the United States (Kohn et al. 2000); 0.95, 0.71, and 0.84 µg/kg/day in the United States (derived from data from Barr et al. 2003, their Table 1, using the procedure outlined by Kohn et al. 2000); and 5.22, 0.60, and 13.8 µg/kg/day in Germany (Koch et al. 2003). These findings confirm the relatively large daily exposure to phthalates in industrialized countries. Although the dominant route of exposure to DnBP, BBzP, and DEHP is thought to be via ingestion (Fromme et al. 2004; Kavlock et al. 2002a, 2002b, 2002c), few if any population-based data are available to support this statement. Indeed, a recent study has demonstrated associations between phthalate concentrations in inhaled air and urinary monoester metabolites (Adibi et al. 2003).

The incidence of asthma and allergy has increased throughout the developed world over the past 30 years (Beasley et al. 2003). The short interval over which it has occurred implies that the increase is caused by changes in environmental exposures rather than genetic changes (Etzel 2003; Strachan 2000). Changes in indoor environments warrant special attention because indoor air constitutes a dominant exposure route. Increased exposures to allergens and/or adjuvants (enhancing factors) may each be partially responsible for the increase. Multidisciplinary reviews of the scientific literature on associations between indoor exposures and asthma and allergies (Ahlbom et al. 1998; Andersson et al. 1997; Bornehag et al. 2001; Schneider et al. 2003; Wargocki et al. 2002) indicate that the underlying causal factors responsible for these increases remain unknown.

The use of plasticized products and, consequently, exposures to phthalate esters have increased dramatically since the end of World War II. Phthalate esters have been suggested to act as either allergens or adjuvants (Jaakkola et al. 1999; Oie et al. 1997). Several recent studies have examined the ability of different phthalate esters to function as adjuvants in BALB/c mice injected with a known antigen. DEHP displayed an adjuvant effect with immunoglobulin G1 at a concentration of 2,000 mg/mL after both one and two boosters (Larsen et al. 2001b). In contrast, DnBP only showed an adjuvant effect with immunoglobulin G1 after the second booster (Larsen et al. 2002), and BBzP showed no adjuvant effect (Larsen et al. 2003). Consistent with these results, the monoester of DEHP showed an adjuvant effect whereas the monoesters of DnBP and BBzP did not (Larsen et al. 2001a).

The present study is a nested case-control study on 198 symptomatic children and 202 healthy controls, including detailed clinical examinations by physicians in parallel with extensive inspections and measurements within the subjects' homes. The cases and controls were selected from the first phase (Dampness In Buildings and Health, phase I), which was a cross-sectional questionnaire soliciting health and environmental information regarding all 14,077 children 1-6 years of age in the county of Värmland, Sweden; responses were obtained for 10,852 (Bornehag et al. 2003).

The aim of the present study was to investigate potential associations between persistent allergic symptoms in children and the concentrations of different phthalates in dust collected from their homes.

Materials and Methods

Inclusion criteria for cases and controls. The selection criteria for the cases (Dampness In Buildings and Health, phase II) were as follows: *a*) in the initial questionnaire, reports of at least two incidents of eczema, or wheezing or rhinitis without a cold, during the preceding 12 months; and *b*) in the follow-up questionnaire 1.5 years later, at least two of three possible symptoms reported. Inclusion criteria for the controls were *a*) no symptoms in the first questionnaire and *b*) no symptoms in the follow-up questionnaire. For both groups they had to *c*) not have rebuilt their homes because of moisture problems and *d*) not have changed residence since the first questionnaire. All children with at least two symptoms in the first questionnaire were invited to participate in the case-control study (n = 1,056, corresponding to 9.7% of the total population). In the first questionnaire, 5,303 (48.9%) reported no airway, eye, nose, or skin symptoms. Of these, 1,100 children were randomly selected and invited to participate in the case-control study. This process ultimately yielded 198 cases and 202 controls.

Families were more inclined to participate if the child was reported to have more symptoms, if there was no smoking in the family, and if they belonged to a higher socioeconomic group.

Medical examination. The medical examination of the 400 children (3-8 years of age) was performed during the same 2 weeks that the technical investigations of the homes, including dust collection, were carried out. Medical doctors examined the children and took a detailed history of each child. Blood samples were drawn from 387 children and screened for common allergens (Phadiatop, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), timothy, birch, mugworth, cat, horse, dog, house dust mites (*Dermatophagoidespteronyssinus* and *Dermatophagoidesfarinae*), and one mold (*Cladosporium*).

Physicians' diagnoses of the children agreed well with the case-control status as reported in the questionnaire. All children with obvious asthma were found among cases, whereas 10 cases were found among controls (two children with rhinitis and eight children with eczema). Furthermore, 13 cases were found to be misclassified. In the analyses regarding case-control status, the study design has been used; that is, the 23 (10 plus 13) misclassified children have not been reclassified.

Building investigations. There were 10 pairs of siblings among the 400 children; hence, they lived in 390 buildings. Between October 2001 and April 2002, six professional inspectors performed visual inspections and indoor air quality assessments, including dust sampling, in these 390 dwellings. During these investigations, a preestablished checklist was followed regarding building characteristics, mold and water damages, surface materials, and other building-related items.

Phthalates in dust. Samples of dust from 390 homes were collected from molding and shelves in the children's bedroom. The dust was collected on 90-mm membrane filters in holders made of styrene-acrylonitrile polymer mounted on a sampler made of polypropylene (VacuuMark disposable nozzle; Petersen Bach, Bjerringbro, Denmark) connected to a vacuum cleaner. The filter was weighed before and after sampling under controlled conditions. Conditioning the filters before weighing (23°C, 50% relative humidity) was critical to obtaining reproducible filter weights. From

the 390 homes there were 9 missing samples, 13 samples with errors in the laboratory analysis, and 6 samples with a negative dust weight. Consequently, there were 362 valid samples. Only filters with a reliably measurable net increase in weight (≥ 25 mg) were included in the present analysis; 346 of the 362 dust samples met this criterion.

The dust samples were extracted in precleaned 10-mL glass vials for 30 min using 2 mL dichloromethane. This procedure was repeated, and the two extracts were then combined and transferred to 3-mL autosampler vials. Aliquots from these vials were injected into either a gas chromatograph/mass selective detector (GC/MSD) for phthalate identification or a GC/flame ionization detector for quantitation. GC was performed using a 25-m capillary column (HP 1C; Agilent, Folsom, CA, USA; inner diameter, 0.2 mm; stationary phase, polydimethyl siloxane). The injector temperature was 280°C; column temperature started at 100°C for 3 min and then increased at 8°C/min to 300°C, which was maintained for 20 min. The detector temperature and transfer line to the MSD were maintained at 280°C. The analytical and field sampling techniques were tested in a preliminary study that found only limited influence from background contributions to the analyzed samples. In the present study, field blanks have indicated no significant background contributions. The dust concentrations (milligram per gram dust) of six phthalates were determined: diethyl phthalate (DEP), diisobutyl phthalate (DIBP), DnBP, BBzP, DEHP, and diisononyl phthalate (DINP).

Statistical method. The concentrations of phthalates in the dust were log-normally distributed. Hence, analyses of potential associations between concentrations of phthalates in dust and health outcomes have been conducted using nonparametric tests (Mann-Whitney *U*-test). Log-transformed, normally distributed concentrations were tested with parametric tests (*t*-test). The concentrations are reported as medians, as arithmetic means, and as geometric means with 95% confidence intervals (CIs). The CIs were calculated with a back-transform of mean log ± 2 × SE. Dose-response relationships were tested by factoring the phthalate concentrations into quartiles and using both uni- and multivariate logistic regression analyses. Adjustments have been made for environmental tobacco smoke as well as sex and age of the child, because these have been associated with asthma and allergic symptoms. Adjustments for type of building were made, because living in a privately owned single-family house was a selection factor for both cases and controls (Bornehag et al., unpublished data). Indeed, cases and controls lived mainly in single-family houses (88.7%). Furthermore, the frequency of PVC as flooring material was lower in single-family houses than in multifamily houses (51.6 vs. 71.8%). Adjustments for the construction period of the building and self-reported water leakage in the home during the previous 3 years were made because these are associated with the concentrations of phthalates in dust. Finally, adjustments were made for exposure to other phthalates. Multiple logistic regressions were performed by a backward elimination technique where only significant variables were included in the final model. The analyses were considered statistically significant when $p < 0.05$.

The study was approved by the local ethics committee.

Results

Compared with other types of flooring materials, PVC flooring in the child's bedroom was positively associated with case status [adjusted odds ratio (OR), 1.59; 95% CI, 1.05-2.41].

Phthalates in dust. Results are presented in Tables 1-3 and Figure 1. In Tables 1 and 2, median phthalate dust concentrations are reported for data sets that include all valid samples with a reliably measurable net increase in weight (346 of 390 homes), and geometric mean concentrations are reported for data sets that exclude samples with phthalate dust concentrations less than the detection limit. (If, instead, nondetects were assigned concentrations of one-half the detection limit, then for phthalates with a large number of nondetects, their dust concentrations would no longer be log-normally distributed.) The geometric mean concentrations of BBzP and DEHP were higher in

bedrooms with PVC flooring than in bedrooms without such flooring [BBzP: 0.208 (n = 164) vs. 0.147 (n = 107) mg/g dust; DEHP: 0.994 (n = 186) vs. 0.638 (n = 155) mg/g dust; both $p < 0.001$ by t-test]. DEP, DIBP, DnBP, and DINP were not associated with PVC flooring.

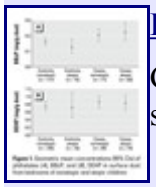

Figure 1.

Geometric mean concentrations (95% CIs) of phthalates (*A*), BBzP, and (*B*), DEHP in surface dust from bedrooms of nonatopic and atopic children.

Table 1.

Table 2.

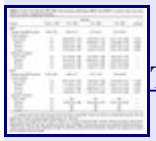

Table 3.

Association between phthalates in dust and health effects. Cases had a higher concentrations of BBzP in the dust samples from the children's bedrooms than did the controls in parametric as well as in nonparametric tests (Table 1). Cases with physician-diagnosed rhinitis or eczema had higher BBzP concentrations in the bedroom dust compared with controls (Table 2). Furthermore, cases with doctor-diagnosed asthma had higher DEHP concentrations in the dust compared with controls. In analyses restricted to single-family and row houses, the same associations were found (data not shown).

In an analysis restricted to homes with PVC flooring in the child's bedroom (n = 189), the median BBzP concentration was significantly higher among cases compared with controls (0.21 vs. 0.16 mg/g dust, respectively; Mann-Whitney U-test, p = 0.042), and BBzP was associated with rhinitis and eczema (Table 2). Such differences between cases and controls were not observed for DEHP.

BBzP concentrations in the highest quartile were associated with an increased risk of being a "case child" (Table 3). The same association was found after adjusting for possible confounders. Table 3 also shows associations between phthalates in dust and doctor-diagnosed asthma, rhinitis, or eczema. A dose-response relationship was found between concentrations of BBzP in dust and doctor-diagnosed rhinitis and eczema in both crude and adjusted analyses. For DEHP, a dose-response relationship was found for asthma in both crude and adjusted analyses, as well as in analysis restricted to single-family houses (data not shown for the latter).

Specific immunoglobulin E in blood. Figure 1 presents the concentration of phthalates in dust among cases and controls with and without specific immunoglobulin E in blood (i.e., atopics and nonatopics). Within the group of cases, the highest geometric mean concentrations of BBzP were found in dust from the bedrooms of atopics. However, when comparing cases with and without atopy, the difference was not statistically significant (p = 0.564).

Discussion

In the present study we found associations between dust concentrations of specific phthalate esters and asthma, rhinitis, and eczema. As shown in Tables 2 and 3, BBzP is significantly associated with doctor-diagnosed rhinitis and eczema, whereas DEHP is significantly associated with doctor-

diagnosed asthma. Interestingly, no such associations are found for DnBP despite the fact that the median concentrations of BBzP and DnBP in the settled dust were comparable (0.150 vs. 0.135 mg/g; Table 1). Hence, these three phthalates display strikingly different associations between their dust concentrations and the health outcomes monitored in this study. From a physical chemistry viewpoint, DnBP, BBzP, and DEHP are significantly different from one another; they possess different vapor pressures, polarities, water solubilities, and octanol/air partition coefficients. For example, the vapor pressures of DnBP and BBzP are two orders of magnitude greater than that of DEHP. This means that greater fractions of DnBP and BBzP are in the gas phase as opposed to the condensed phase (i.e., associated with dust and airborne particles). We estimate that, for a particle concentration of 20 $\mu g/m^3$, > 80% of airborne DnBP and > 80% of airborne BBzP are in the gas phase, whereas > 85% of airborne DEHP is associated with airborne particles (Weschler 2003). The deposition of a compound in the respiratory tract is strongly influenced by whether it is present in the gas phase or associated with airborne particles. Furthermore, as a consequence of their inherent chemical differences, DnBP, BBzP, and DEHP, as well as their monoester metabolites, produce different effects in a mouse model (Larsen et al. 2001a, 2001b, 2002, 2003). Furthermore, each of these phthalates has its distinct human metabolic pathway (Barr et al. 2003). We suspect that the different relative distributions between the gas and condensed phases, coupled with different toxicologic and pharmacokinetic behaviors, contribute to the fact that DnBP, BBzP, and DEHP are associated with different health outcomes (i.e., DnBP, no associations; BBzP, skin and mucosa symptoms; DEHP, lower airway symptoms).

In the present study there is a general association between PVC flooring and case status (OR, 1.59). Both BBzP and DEHP correlate with the amount of PVC flooring in the subjects' homes. However, these two phthalates are not associated with health effects simply because they are associated with PVC flooring. This conclusion is supported by a number of observations: First, specific associations between BBzP and DEHP dust concentrations and doctor-diagnosed diseases (Table 3) are more pronounced than associations between PVC flooring and such diseases. Second, although BBzP and DEHP dust concentrations do correlate, the correlation is weak ($R = 0.52$), and they are associated with different health effects. Third, in a restricted analysis, including only homes with PVC flooring, higher concentrations of BBzP were found in dust from case homes than in that from control homes.

The reported concentrations of phthalates in the bedroom dust (Table 1) are consistent with those reported in other studies. In dust samples from 120 U.S. homes located on Cape Cod, Massachusetts (Rudel et al. 2003), the median concentrations were 0.34, 0.045, and 0.020 mg/g dust for DEHP, BBzP, and DnBP, respectively. In a study of 59 apartments in Berlin, Germany (Fromme et al. 2004), the median concentrations were 0.70, 0.030, and 0.047 mg/g dust for DEHP, BBzP, and DnBP. Clausen et al. (2003) measured mean DEHP concentrations of 3.2 mg/g dust in 15 Danish schools and 0.86 mg/g dust for 23 Danish homes. Oie et al. (1997) reported mean concentrations of 0.64 mg DEHP/g dust and 0.11 mg BBzP/g dust for 38 homes in Norway. Pohner et al. (1997) reported a 95th percentile DEHP concentration of 2.0 mg/g dust for 272 German homes, whereas another German study on 286 homes reported a 95th percentile DEHP concentration of 2.6 mg/g dust (Butte et al. 2001).

Regarding atopic status and its association with phthalate dust concentrations, the chosen study design is not optimal. Because there were only 16 atopic controls, the power of the analysis on atopic children is limited. On the other hand, our findings could be interpreted to mean that the mechanism is of a nonimmunologic nature (e.g., exposure increases the risk for irritation).

To identify potential selection biases in the study group, we obtained information for all invited families from the first cross-sectional questionnaire. This revealed that the final study group contained significantly more single-family houses than the eligible population. Adjusting and restricting the analyses have addressed this problem. There was no selection bias regarding PVC flooring because included and nonincluded cases and controls reported about the same frequency of

occurrence of PVC flooring in the child's bedroom (Bornehag et al., unpublished data). Furthermore, 10 controls and 13 cases were misclassified when comparing self-reported symptoms and doctors diagnoses. However, when these children were excluded from the analyses, the reported associations remained. Finally, to be included as a "case," a child was required to have at least two symptoms. Consequently, this study was not fine-tuned to examine associations between building factors and single symptoms (i.e., asthma, rhinitis, or eczema). However, even if the design is suboptimal, meaning it was more difficult to find associations between single symptoms and exposures, the association between selected building factors and single symptoms is meaningful and possibly underestimates true associations.

The reported analyses are based on samples with a weight > 25 mg. However, when including all samples (n = 362), the reported associations between exposure and symptoms remained or became stronger (data not shown).

Koo et al. (2002) present weak associations between exposure estimates for different phthalate esters, based on their urinary biomarkers, and the level of education, family income, and residency (urban or rural) in a reference U.S. population. Given that study, one might speculate that the associations reported in the present study are driven by demographic factors. However, in contrast to the United States, where 22.4% of the children live in households with incomes < 50% of the national median, in Sweden only 2.6% of the children live in such households (Unicef 2000). Additionally, the association in our study holds when the analysis is restricted to single-family houses; such homes have an even more homogeneous socioeconomic status. Hence, different demographic factors between cases and controls appear to be an unlikely explanation for the associations observed in the present study. Furthermore, given that the dust concentrations of DnBP, BBzP, and DEHP display quite different associations with different symptoms, the associations reflect a biologic response rather than just lifestyle or demographic factors associated with an increased use of plasticized materials.

This study demonstrates associations between BBzP and DEHP concentrations in dust and selected allergies and asthma. Although multiple factors likely are responsible for the increases in allergies and asthma that have been documented in developed countries over the past 30 years, it is striking that these increases have occurred during a period when plasticized products have become ubiquitous in the homes, schools, and workplaces of the developed world.

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Productos químicos 'cambia-sexo' que 'feminizan' niños, *New Scientist*, 27 de mayo de 2005.

'Gender-bending' chemicals found to 'feminise' boys

17:17 **27 May 2005** by **Andy Coghlan**

"Gender-bending" chemicals mimicking the female hormone oestrogen can disrupt the development of baby boys, suggests the first evidence linking certain chemicals in everyday plastics to effects in humans.

The chemicals implicated are phthalates, which make plastics more pliable in many cosmetics, toys, baby-feeding bottles and paints and can leak into water and food.

All previous studies suggesting these chemicals blunt the influence of the male hormone testosterone on healthy development of males have been in animals. "This research highlights the need for tougher controls of gender-bending chemicals," says Gwynne Lyons, toxics adviser to the WWF, UK. Otherwise, "wildlife and baby boys will be the losers".

The incriminating findings came from a study of 85 baby boys born to women exposed to everyday levels of phthalates during pregnancy. It was carried out by Shanna Swan at the University of Rochester School of Medicine and Dentistry, New York, US, and colleagues.

As an index of feminisation, she measured the "anogenital distance" (AGD) between the anus and to the base of the penis. She also measured the volume of each boy's penis. Earlier studies have shown that the AGD is twice in boys what it is in girls, mainly because in boys the hormone testosterone extends the length of the perineum separating the anus from the testicles.

Undescended testicles

In animals, AGD is reduced by phthalates - which mimic oestrogen - which keep testosterone from doing its normal job. At higher doses, animals develop more serious abnormalities such as undescended testicles and misplaced openings to the urethra on the penis - a group of symptoms called "phthalate syndrome" in animals.

When Swan's team measured concentrations of nine phthalate metabolites in the urine of pregnant women, they found that four were linked with shorter AGD in sons born to women showing high exposure levels.

Although none of the boys developed abnormal genitals, the quarter of mothers who were exposed to the highest concentrations of phthalates were much more likely to have had boys with short AGDs compared with the quarter of mothers who had the lowest exposures to the chemicals.

And although all the boys had genitals classified as "normal", 21% of the boys with short AGDs had incomplete testicular descent, compared with 8% of other boys. And on average, the smaller the AGD, the smaller the penis.

Changing masculinisation

Swan believes that at higher exposures, boys may suffer from testicular dysgenesis syndrome - the human collection of more serious abnormalities which corresponds to "phthalate syndrome".

"We're not exactly seeing testicular dysgenesis syndrome, but a cluster of endpoints consistent with it," said Swan on at an international conference on Endocrine Disrupting Chemicals in San Diego, US.

"If you see this, you're very likely to see every other aspect of masculinisation changed too," says Fred vom Saal, professor of reproductive biology at the University of Missouri-Columbia, US.

Vom Saal says this could include behavioural changes like those seen in animals, including an aversion to "rough-and-tumble" play and a reduction in aggressiveness.

Criticising methods

Environmentalists say the results strengthen the case for a ban or restriction on some phthalates in baby toys, as has been proposed in Europe and California.

But phthalate manufacturers maintain that the chemicals have been thoroughly tested and are safe. They are also critical of aspects of the study. David Cadogan, director of the European Council for Plasticisers and Intermediates, points out that just one urine sample was taken from each pregnant woman, which cannot rule out drastic variations in exposure over time.

Also, he says that all AGD measurements should have been taken in babies exactly the same age, not in babies ranging from three to 24 months in age as in the study. The disparity in ages meant that complicated mathematical analyses had to be applied which may have made it more difficult to distinguish genuine differences in AGD from differences accounted for by age or weight.

Swan's results will appear in the journal *Environmental Health Perspectives*.

Productos químicos ubicuos asociados al desarrollo anormal de la reproducción humana, *Scientific American*, 27 de mayo de 2005

SCIENTIFIC AMERICAN™

Ubiquitous Chemical Associated with Abnormal Human Reproductive Development

By Sarah Graham | May 27, 2005 |

Researchers have identified a link between exposure in the womb to widely used chemicals known as phthalates and adverse effects on genital development in male babies, according to a new report. Previous toxilogical studies had suggested that fetal exposure to the chemicals can affect reproductive development in rodents, but the new results indicate that developing humans could be vulnerable as well.

Phthalates are common components of items ranging from plastics to paints to personal care products such as nail polish and shampoo. In the first study of prenatal exposure to phthalates in humans, Shanna H. Swan of the University of Rochester and her colleagues studied 85 mother-son pairs. Prior to giving birth, the mothers supplied urine samples, which were analyzed for the presence and quantity of nine phthalate metabolites. The doctors also examined the children, who were between the ages of two and 30 months, for genital characteristics used as markers of normal sexual development.

When the researchers correlated the results with the mothers' level of exposure, they found that higher levels of four metabolites in their urine were correlated with a higher-than-expected number of changes to genital development in the baby boys. These changes include smaller scrotum and penis size and a smaller measurement known as the anogenital distance (AGD). None of the children were grossly abnormal, however, and the scientists detected no complete malformations or definite markers of disease. The results are consistent with the findings of animal studies, the authors note, but they caution that the use of AGD as a marker of sexual development in people is relatively new. Nevertheless, they conclude that the findings "support the hypothesis that prenatal phthalate exposure at environmental levels can adversely affect male reproductive development in humans." A report detailing the study will be published in an upcoming issue of the journal *Environmental Health Perspectives.*

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Prenatal phthalate exposure and reduced masculine play in boys

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Summary

Other Sections Summary Introduction Materials and methods Results Discussion References

Foetal exposure to antiandrogens alters androgen-sensitive development in male rodents, resulting in less male-typical behaviour. Foetal phthalate exposure is also associated with male reproductive development in humans, but neurodevelopmental outcomes have seldom been examined in relation to phthalate exposure. To assess play behaviour in relation to phthalate metabolite concentration in prenatal urine samples, we recontacted participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples. Mothers completed a questionnaire including the Pre-School Activities Inventory, a validated instrument used to assess sexually dimorphic play behaviour. We examined play behaviour scores (masculine, feminine and composite) in relationship to ($\log_{10}$) phthalate metabolite concentrations in mother's urine separately for boys ($N = 74$) and girls ($N = 71$). Covariates (child's age, mother's age and education and parental attitude towards atypical play choices) were controlled using multivariate regression models. Concentrations of dibutyl phthalate metabolites, mono-*n*-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) and their sum, were associated with a decreased (less masculine) composite score in boys (regression coefficients −4.53,−3.61 and −4.20, $p = 0.01$, 0.07 and 0.04 for MnBP, MiBP and their sum respectively). Concentrations of two urinary metabolites of di(2-ethylhexyl) phthalate (DEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-

5-hydroxyhexyl) phthalate (MEHHP) and the sum of these DEHP metabolites plus mono(2-ethylhexyl) phthalate were associated with a decreased masculine score (regression coefficients −3.29,−2.94 and −3.18, $p = 0.02$, 0.04 and 0.04) for MEHHP, MEOHP and the sum respectively. No strong associations were seen between behaviour and urinary concentrations of any other phthalate metabolites in boys, or between girls' scores and any metabolites. These data, although based on a small sample, suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys. Our findings suggest that these ubiquitous environmental chemicals have the potential to alter androgen-responsive brain development in humans.
Keywords: di(2-ethylhexyl) phthalate, dibutyl phthalate, play behaviour, prenatal exposure phthalates, Pre-School Activities Inventory, sex-dimorphism

Introduction

Phthalate esters are pervasive environmental chemicals. Although several of these are now banned for use in toys and some other products designed for young children (Kamrin, 2009), this legislation does not limit prenatal exposure. Moreover, phthalates are present in so many other products and manufactured in such quantity, that exposure is virtually universal (CDC, 2005). A large body of work in laboratories around the world has demonstrated that, in experimental animals, when exposure occurs during the period of foetal sexual differentiation, some phthalates, notably di(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP), inhibit the synthesis of testosterone by Leydig cells, thereby reducing foetal testosterone concentration (Welsh *et al.*, 2008). As a result, male pups exhibit a cluster of altered androgen-dependent anatomical features that reflect disordered sex differentiation, including a reduced – that is, a less masculine – anogenital distance (AGD), impaired testicular descent and reduced genital size. This cluster of alterations has been referred to as the 'phthalate syndrome' (Foster, 2006). Some phthalate-related changes have also been identified in adult female rodents, but no significant changes have been reported in female neonates. In male rodents, the phthalate syndrome, which is initially identified neonatally, has been shown to have adverse consequences for later sexual development. We recently reported data demonstrating an association between prenatal exposure in humans, particularly to DEHP and its urinary metabolites, and a similar cluster of reproductive developmental outcomes in male infants (Swan *et al.*, 2005; Swan, 2008). In addition, free serum testosterone in human male infants has been negatively correlated with levels of some phthalate metabolites in breast milk (Main *et al.*, 2006). However, the long-term consequences of these findings for humans are uncertain.

In particular, the potential for these antiandrogens to influence the course of brain sexual differentiation has only recently been addressed (Engel *et al.*, 2009). This question is rooted in our understanding of how gonadal hormones influence mammalian neural development. Testosterone exposure during early development produces a masculine neural phenotype by influencing cell survival, neural growth and neurochemical specification. In rodents, this process involves the enzyme aromatase, which, by a biological irony, converts testosterone into oestradiol, which then shapes the male structure. In rats, the critical programming window for genital tract development occurs in gestational days 18–21 (Welsh *et al.*, 2008), a period that corresponds to a testosterone surge in the developing male. In humans, the testes begin to function at about week 8 of gestation and, while dates are uncertain, testosterone appears to be elevated in the male foetus from about weeks 8 to 24 of gestation (Reyes *et al.*, 1973; Smail *et al.*, 1981). The critical period for brain sexual differentiation is unknown and may not be the same as that for reproductive tract development. Whatever the critical period, testosterone is an essential mediator; if the antiandrogenic actions of phthalates reduce its secretion by the foetus, brain sexual differentiation may be altered.

Data from Swan *et al.* (2005) and Swan (2008) support the hypothesis that in humans, maternal exposure to phthalates, particularly DEHP, lowers foetal testosterone production and results in incomplete masculinization of the genital tract, resulting in a shortened AGD as well as incomplete testicular descent and smaller penile size. These data suggest that the same process might plausibly influence brain sexual differentiation and its expression in sexually dimorphic behaviours. Play behaviours offer themselves as a test of the hypothesis that phthalate exposures during gestation

may alter brain sexual differentiation and its behavioural outcomes.
Young male and female humans, rats and non-human primates all show sex differences in play behaviours. Young male rats and non-human primates, for example, engage in more play-fighting or rough-and-tumble play than their female counterparts (Pellis, 2002; Wallen, 2005). Young male rhesus monkeys, like boys, also show distinct preferences for toys with wheels (Hassett *et al.*, 2008) and vervet monkeys show sex differences in toy preferences similar to those shown previously in children (Alexander & Hines, 2002). Finally, and more central to our hypothesis, standardized inventories of sex differences in play behaviours have been constructed, such as the Pre-School Activities Inventory (PSAI; Golombok & Rust, 1993), which has been shown to be sensitive to early androgen exposure (Hines *et al.*, 2002; Auyeung *et al.*, 2009) and to reflect the endocrine-disrupting properties of dioxins and Polychlorinated Biphenyls (PCBs) (Vreugdenhil *et al.*, 2002). We chose to use a slightly modified version of the PSAI (which we refer to as PSAI-M) to investigate changes in sex-typical play behaviours in a subsample of the population described in Swan *et al.* (2005) and Swan (2008).

Materials and methods

Study population

The Study for Future Families (SFF) is a multi-centre pregnancy cohort study in which women and their partners were recruited at prenatal clinics. Initial recruitment took place at clinics affiliated with university hospitals in Los Angeles, CA (Harbor-UCLA and Cedars-Sinai), Minneapolis, MN (University of Minnesota Health Center) and Columbia, MO (University Physicians) between September 1999 and December 2002 (Swan *et al.*, 2003). Recruitment in Iowa City, IA (University of Iowa) was conducted during 2002–2005. Couples who were at least 18 years old, who spoke English or Spanish and whose pregnancy was conceived without medical treatment were eligible. If the couple agreed to participate, both partners completed questionnaires and gave a serum sample (at mean 28.6 weeks of pregnancy) and, for those recruited after September 2000, a urine sample. In 2000, we initiated a follow-up study (SFFII) to measure genital parameters in human infants in relation to their mother's prenatal phthalate exposure. Eighty-five per cent of SFFI participants had agreed to be recontacted, and we invited these mothers to take part in SFFII if the pregnancy ended in a live birth, the baby was 2–36 months of age at the time of recontact, the mother lived within 50 miles of the clinic, and had provided a urine sample during pregnancy. In 2006, we initiated a second follow-up of SFF children with the goal of examining play behaviour in relation to prenatal phthalate metabolite concentration, results of which are reported here. For this study, children born during 2000–2003 who had a physical exam in SFFII and whose mothers had provided a urine sample were eligible. Human subject committees at all participating institutions approved SFF-I and SFF-II and all subjects signed informed consents for each study. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was limited and determined not to constitute engagement in human subjects' research.

Sample collection and measurement of phthalate metabolites

Urine samples were collected during mid-pregnancy at the time of the mother's prenatal visit. Staff at the Division of Laboratory Sciences of the CDC, which had no access to subject data, carried out the analyses. The uri-nary concentrations of nine urinary phthalate metabolites were measured using an analytical approach that involves the enzymatic deconjugation of the metabolites from their glucuronidated form, automated on-line solid-phase extraction, separation with high performance liquid chromatography and detection by isotope-dilution tandem mass spectrometry (Silva *et al.*, 2004). Isotopically labelled internal standards were used along with conjugated internal standards to increase precision and accuracy of the measurements. Along with the samples, each analytical run included calibration standards, reagent blanks and quality control materials of high and low concentration to monitor for accuracy and precision. Limits of detection (LOD) were in the low nanogram per millilitre (ng/mL) range. Most metabolite concentrations were above the LOD; those below the LOD were assigned the value LOD divided by the square root of 2, which has been recommended when, as in this case, the data are not highly skewed (Hornung & Reed, 1990).

Pre-School Activities Inventory

Parents were asked to complete the PSAI (Golombok & Rust, 1993) as well as a brief questionnaire that included questions on other relevant covariates (such as age, number and age of siblings, parental education and questions on parental attitudes towards sex-atypical toy choice). The PSAI is designed to discriminate play behaviour both within and between the sexes, and has been standardized on children in the UK, the Netherlands and the US (Golombok *et al.*, 2008). It consists of 24 items (12 considered 'feminine' and 12 'masculine') addressing three aspects of play behaviour: type of toys, activities and child characteristics. Answers are given on a 5-point, Likert-type scale ranging from 'never' to 'very often'. A total score is computed based on the sum of scores for masculine items, minus the sum of scores for feminine items following Golombok & Rust (Golumbok & Rust 1993). A higher total (composite) score implies more male-typical play behaviour and a lower score implies more female-typical play behaviour. We also looked at the masculine and feminine subscale totals separately. For these subscales, a higher score on the feminine scale indicates more feminine play behaviour, whereas a higher score on the masculine scale indicates more masculine play behaviour.

Parental Attitude Scale

As the child's choice of a toy might depend on the availability of that toy in the household, or the parents' views about the child's play with that toy, we attempted to assess the parental attitudes towards sex-atypical play. The mother was asked, 'what would you do if you had a boy who preferred toys that girls usually play with?' The five possible responses ranged from 'strongly encourage' (him to play in this way) to 'strongly discourage'. She was also asked how she thought the father of a boy would respond to these questions (see Appendix). The five possible responses for each parents' attitude were coded 1–5 and summed for each parent. This resulted in a score, which we called parental attitude-boys (PAB), reflecting the combined responses of both parents towards boys' play, for which a value of 2 reflects the strongest encouragement of a boy to play with toys 'girls usually play with' and 10 the strongest discouragement of such play, whereas six indicates neutrality. A similar scale (PAG) was constructed for girls. PAB was included when modelling boys' PSAI-M in relation to phthalate metabolite concentrations and PAG when modelling girls'. As these variables have not been used previously and remain to be validated, we also analysed our data without including them to assess their influence on our results.

Statistical analysis

Phthalate metabolite distributions were reviewed. As these distributions were extremely skewed, and because the relationships between metabolites concentrations and PSAI-M scores were markedly more linear on the logarithmic scale, metabolite concentrations were $\log_{10}$-transformed in all analyses. Prior to examining associations between phthalate metabolite concentrations and outcomes, we reviewed the data for extreme values (e.g. those that were more than 1.5 times the interquartile range above the 75th percentile, after log transformation) and excluded three subjects whose DEHP metabolite concentrations were extreme, and one whose MnBP concentration was extreme. One girl whose mother was missing all phthalate metabolite concentrations was also excluded. In addition, prior to examining associations between phthalate metabolite concentrations and outcomes, we conducted an item analysis of each of the 24 PSAI questions and examined their distribution in boys and girls. After examining unadjusted correlations between phtha-late metabolites and the PSAI-M scores, we conducted multiple regression analyses to examine the relationships between these variables. Covariates initially considered in these analyses were: creatinine concentration, sex and age of child, maternal age, parental education, number of the same and opposite sex siblings, ethnicity, clinic location and parental attitude. Covariates that altered the effect estimates for at least one metabolite-play behaviour score by at least 10% (maternal age, boy's age, mother's education, father's education, parental attitude and the interaction of mother's education and parental attitude) were retained in the model. Regression analyses were performed using Generalized Linear Models (SAS Institute Inc, 1999).

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Summary

Foetal exposure to antiandrogens alters androgen-sensitive development in male rodents, resulting in less male-typical behaviour. Foetal phthalate exposure is also associated with male reproductive development in humans, but neurodevelopmental outcomes have seldom been examined in relation to phthalate exposure. To assess play behaviour in relation to phthalate metabolite concentration in prenatal urine samples, we recontacted participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples. Mothers completed a questionnaire including the Pre-School Activities Inventory, a validated instrument used to assess sexually dimorphic play behaviour. We examined play behaviour scores (masculine, feminine and composite) in relationship to ($\log_{10}$) phthalate metabolite concentrations in mother's urine separately for boys ($N = 74$) and girls ($N = 71$). Covariates (child's age, mother's age and education and parental attitude towards atypical play choices) were controlled using multivariate regression models. Concentrations of dibutyl phthalate metabolites, mono-*n*-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) and their sum, were associated with a decreased (less masculine) composite score in boys (regression coefficients −4.53,−3.61 and −4.20, $p = 0.01$, 0.07 and 0.04 for MnBP, MiBP and their sum respectively). Concentrations of two urinary metabolites of di(2-ethylhexyl) phthalate (DEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and the sum of these DEHP metabolites plus mono(2-ethylhexyl) phthalate were associated with a decreased masculine score (regression coefficients −3.29,−2.94 and −3.18, $p = 0.02$, 0.04 and 0.04) for MEHHP, MEOHP and the sum respectively. No strong associations were seen between behaviour and urinary concentrations of any other phthalate metabolites in boys, or between girls' scores and any metabolites. These data, although based on a small sample, suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys. Our findings suggest that these ubiquitous environmental chemicals have the potential to alter androgen-responsive brain development in humans.

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Results

As questionnaires were mailed to families 4–7 years after their initial enrollment in the study, many had moved and could no longer be contacted. Of the 334 eligible families to whom questionnaires were mailed, 128 were returned undeliverable, presumably because the family had moved. Of the 206 questionnaires that were not returned undeliverable, all but 56 were returned completed (72.8%). Mothers who returned completed questionnaires were somewhat more likely to be Caucasian (88% vs. 78.6%) and to have completed college (73.3% vs. 68.4%) than those who could not be contacted, or who failed to return a completed questionnaire.

We conducted an item analysis of the 24 questions (available upon request). Q5F ('avoids taking risks') had been classified as a 'feminine' question. However, the mean (2.6) and median (3.0) were equal in boys and girls in our sample. Similarly, the mean (3.2) and median (4.0) for Q5A ('likes to explore new surroundings'), which was classified as a masculine question, were the same in boys and girls. As these items were not sex-dimorphic in our sample, they were dropped, reducing the total number of items to 22 and forming the modified instrument (PSAI-M), which we used in all

analyses. As shown in Table 1, the mean and standard deviations of the composite scores obtained using PSAI-M in our population were in close agreement with those for several thousand children obtained using the original PSAI (Golombok *et al.*, 2008).

Table 1

Summary statistics for modified Pre-School Activities Inventory (PSAI) compared with published data on PSAI[a]

	PSAI-M (current study)	**PSAI (Golombok *et al.*, 2008)**
Boys		
N	74	2726
Mean age (months)	60	57
Mean composite score (SD)	65.9 (8.6)	64.2 (8.8)
Girls		
N	71	2775
Mean age (months)	59	57
Mean composite score (SD)	31.4 (8.7)	35.1 (9.4)

[a]PSAI-M excludes two items that were equally distributed in boys and girls in the current study.

None of the urinary concentrations of phthalate metabolites other than those of DEHP and DBP were associated with play behaviour scores in boys or girls in either univariate or initial multivariate analyses (*p*-values were between 0.23 and 0.99 for all metabolites other than those of DEHP and DBP; data not shown). Nor were the concentrations of any phthalate metabolites associated with play behaviour in girls. Therefore, the remaining analyses were limited to metabolites of DEHP: mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) as well as their sum (denoted RDEHP), and metabolites of DBP: mono-*n*-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP), as well as their sum (denoted ΣDBP) in relation to play behaviour scores in boys.

Summary statistics for the three PSAI-M scores and other covariates are shown in Table 2. Mean parental attitude scores (PAB and PAG) were close to neutral, with parents of girls somewhat more likely to discourage sex-atypical play (A value of 3 indicates the parent would neither encourage nor discourage such play. The sum of these, combining attitudes of both parents, was included in multivariate models, for which a value of 6 indicates neutrality).

Table 2

Summary of Pre-School Activities Inventory (PSAI) scores and covariates

Variable	Boys ($N = 74$)[a] Mean/SD/range	Girls ($N = 71$)[a] Mean/SD/range
PSAI-M Scores		
Composite	65.9/8.6/37.3–83.5	31.4/8.7/13.0–51.6
Masculine	40.8/5.9/26–53	27.9/5.7/16.0–43.0
Feminine	24.7/6.1/13–43	43.2/5.5/30.0–54.0
Parental Attitude Scores		
PAB (Boys' play)	5.6/1.5/2.0–8.0	5.9/1.6/ 2.0–10.0
PAG (Girls' play)	5.0/0.6/3.0–9.0	6.9/1.3/3.0–10.0
Mother's age (years)	30.5/5.2/18.3–40.3	30.3/5.4/18.7–41.3
[illegible] age (years)	5.0/0.6/3.6–6.4	4.9/0.7/3.6–6.0

Phthalate metabolite concentrations in our sample (shown in Table 3), are consistent with those reported in a national sample (CDC, 2005). We initially included creatinine concentration in these models to adjust for urinary dilution, However, creatinine concentration was not retained in final models, as point estimates were close to zero (p-values = 0.52–0.90) and removing this variable had little influence on the effect estimates.

Table 3

Mean and percentiles for DEHP and DBP metabolite concentration in prenatal urine by sex

		Boys ($N = 74$)[a]					Girls ($N = 71$)[a]				
Phthalate	Metabolite (ng/mL)	Mean	25th	50th	75th	% > LOD	Mean	25th	50th	75th	% > LOD
DEHP	MEHP	5.2	1.4	2.9	6.2	78.3	8.7	1.2	4.3	11.3	77.4
	MEHHP	16.0	5.2	9.8	17.3	98.6	18.3	6.3	12.1	21.5	98.5
	MEOHP	14.3	4.7	9.0	17.9	94.5	15.5	5.4	10.8	20.7	97.1
	ΣDEHP	35.6	11.7	22.6	40.3		42.5	16.0	28.0	58.2	
DBP	MnBP	19.4	6.9	12.5	28.3	97.2	23.0	9.0	18.0	32.3	98.5
	MiBP	4.0	0.7	2.4	5.1	72.9	4.1	1.5	2.8	5.0	80.2
	ΣDBP	23.4	8.3	14.8	34.0		27.0	10.0	20.5	37.1	
Creatinine (mg/dL)		79.3	33.0	59.0	124		94.3	51.7	76.4	130	

LOD, limit of detection.

[a]Excludes four samples with extreme phthalate metabolite concentration and one with missing phthalate concentrations.

The two metabolites of DBP, as well as their sum, were associated with a decreased (less masculine) composite score in boys. Regression coefficients were −4.53, −3.61 and −4.20 (p = 0.01, 0.07 and 0.04) for MiBP, MnBP and their sum (denoted ΣDBP) respectively (Table 4). Of the DBP metabolites, only MiBP was associated (positively) with the feminine score (coefficient 2.48, p = 0.07). The (weak) negative associations between DBP metabolites and the masculine score were unremarkable.

Table 4
Regression coefficients (95% CI) for boys' Pre-School Activities Inventory (PSAI) scores on concentration of ($\log_{10}$) phthalate metabolite concentration in prenatal urine

Phthalate	Metabolite	Composite	Masculine	Feminine
DEHP	MEHP	–1.04 (–4.72 to 2.63)	–0.95 (–3.85 to 1.95)	–0.01 (–2.66 to 2.66)
	MEHHP	–2.24 (–5.95 to 1.46)	–3.29 (–6.14 to –0.43)	–1.25 (–3.93 to 1.44)
	MEOHP	–2.44 (–6.10 to 1.22)	–2.94 (–5.78 to –0.10)	–0.72 (–3.39 to 1.95)
	ΣDEHP	–2.64 (–6.60 to 1.32)	–3.18 (–6.26 to –0.10)	–0.78 (–3.67 to 2.11)
DBP	MnBP	–3.61 (–7.48 to 0.26)	–2.21 (–5.29 to 0.87)	1.07 (–1.77 to 3.92)
	MiBP	–4.53 (–8.12 to –0.94)	–1.65 (–4.57 to 1.28)	2.48 (–0.16 to 3.92)
	ΣDBP	–4.20 (–8.18 to –0.23)	–2.32 (–5.50 to 0.86)	1.50 (–1.43 to 4.43)

Using Generalized Linear Models controlling for boy's age, mother's age, mother's education, parents' attitude towards boy's play, and interaction of mother's education and attitude towards boy's play.

Concentrations of two urinary metabolites of DEHP, MEOHP and MEHHP, as well as the sum of concentrations of MEHHP, MEOHP and MEHP (denoted ΣDEHP), were associated with a decreased masculine score. Regression coefficients were −3.29, −2.94 and −3.18 (p = 0.02, 0.04 and 0.04 for MEHHP, MEOHP and ΣDEHP respectively), as seen in Table 5. Associations between DEHP metabolites and the composite and feminine scores were weak (all p-values >0.36).

Regression coefficients (95% CI) for girls' Pre-School Activities Inventory (PSAI) scores on concentration of ($\log_{10}$) phthalate metabolite concentration in prenatal urine

Phthalate	Metabolite	Composite	Masculine	Feminine
DEHP	MEHP	–0.01 (–3.37 to 3.37)	0.07 (–2.15 to 2.29)	0.07 (–2.26 to 2.40)
	MEHHP	–1.08 (–5.13 to 2.97)	–0.56 (–3.23 to 2.11)	0.42 (–2.38 to 3.23)
	MEOHP	–1.39 (–5.57 to 2.78)	–0.82 (–3.57 to 1.93)	0.45 (–2.45 to 3.34)
	ΣDEHP	–0.81 (–4.94 to 3.31)	–0.42 (–3.14 to 2.30)	0.32 (–2.53 to 3.17)
DBP	MnBP	–1.07 (–5.46 to 3.32)	0.21 (–2.69 to 3.10)	1.18 (–1.85 to 4.20)
	MiBP	0.38 (–3.86 to 4.63)	1.04 (–1.75 to 3.82)	0.69 (–2.24 to 3.62)
	ΣDBP	–0.87 (–5.41 to 3.67)	0.21 (–2.78 to 3.20)	1.01 (–2.13 to 4.14)

Using Generalized Linear Models controlling for boy's age, mother's age, mother's education, parents' attitude towards boy's play, and interaction of mother's education and attitude towards boy's play.

While not all associations between DEHP and DBP metabolite concentrations and play behaviour reached statistical significance at p = 0.05, all regression coefficients for the masculine and composite scores and these metabolites were negative for boys, suggesting that these metabolites are (to various degrees) associated with less masculine play behaviour. To express these in terms that are easier to interpret than the regression coefficients, we show the per cent change in the composite and masculine scores the model would predict, if the mothers' metabolite concentration was increased from the 10th to the 90th percentile. These predicted changes are shown in Fig. 1.

Those for which the significance probability of the underlying effect estimates reached $p = 0.05$ are starred.

Figure 1

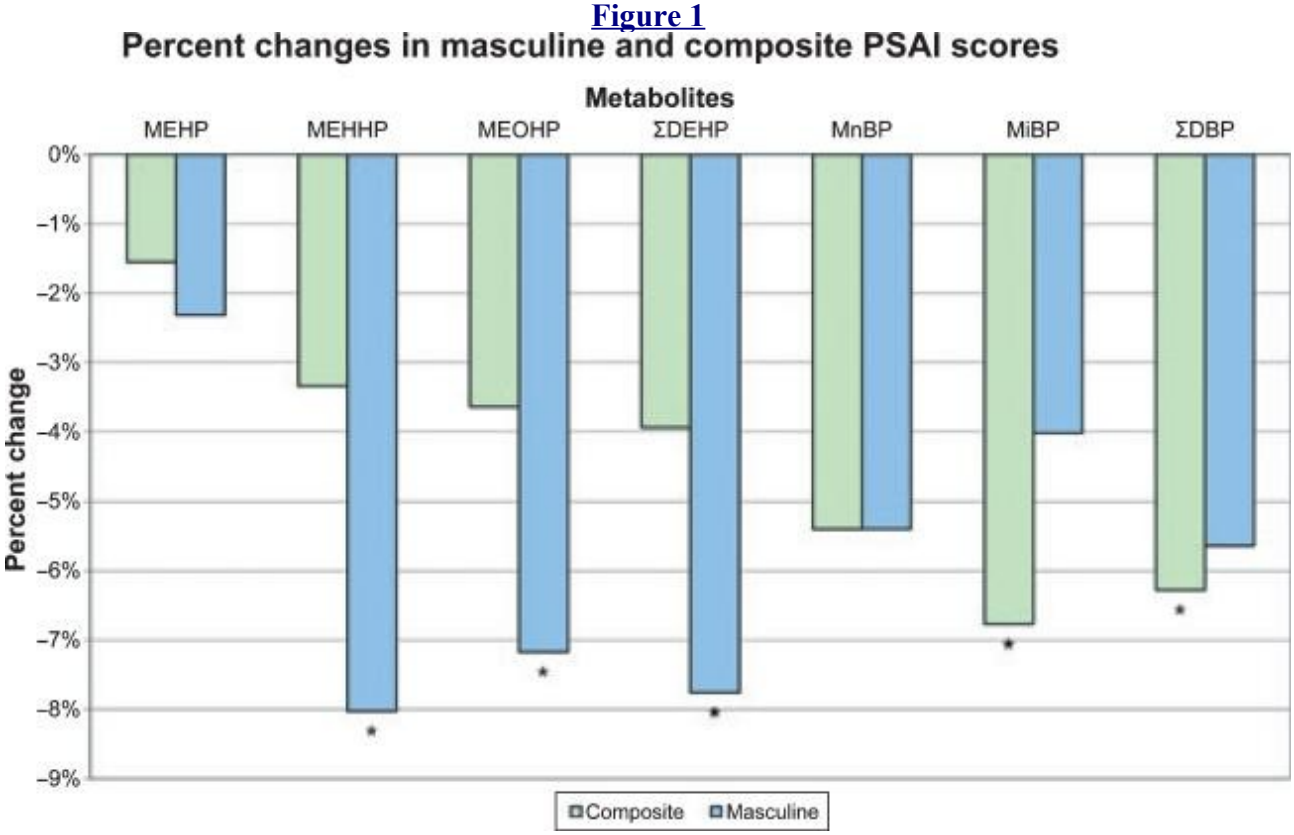

Figure 1
Percent change in PSAI-M score expected if the phthalate metabolite concentration in boy's mother's prenatal urine was increased from the 10th percentile to the 90th percentile. Stars indicate *p*-values of <0.05 for regression coefficients in multivariate model.

We reran these analyses excluding the Parental Attitude Scores (and their interaction with maternal education). We found that questions on parental attitude were important for some associations, but not all. Removing these questions resulted in changes to the effect estimates of between 0 and 12.5%. In particular, adding these parental attitude questions strengthened the associations between DEHP metabolites and the masculine score. On the other hand, when PAB is not included, the association between MiBP concentrations and boys' feminine score is somewhat stronger (coefficient = 2.79, *p*-value = 0.048), implying more feminine play with higher DBP exposure. However, after adjusting for parental attitude this association is reduced somewhat (coefficient 2.48, $p = 0.07$), suggesting some negative confounding by PAB.

Discussion

Even with a relatively small pool of subjects, the message emerging from this study is consistent with the hypothesis that animated its design. Namely, that the antiandrogenic properties of some phthalate esters, documented in scores of papers on the developing male rodent reproductive tract, and more recently by studies in humans (Swan, *et al.*, 2005; Swan, 2008) possess the potential to modify male behaviour, potentially reflecting changes to the developing brain. If replicated in a larger sample, it would be a finding with implications that extend far beyond the scope of children's play preferences, as noted below.

How large were the changes we observed? Given the non-intuitive nature of the outcome scores and the fact that they were modelled in relation to the logarithm (base 10) of the urinary metabolite concentrations, interpretation of the regression coefficients is not straightforward. As an example, consider the coefficient of −3.29 for ($\log_{10}$) MEHHP in relation to the masculine score as an

example, which implies that a one-unit change in the $\log_{10}$ MEHHP metabolite concentration was associated with a decrease of 3.29 in the boys' masculine score, conditional on fixed values of all other model covariates. The 10th and 90th percentiles of $\log_{10}$ MEHHP differ by 1.0, so the predicted change in the boys' masculine score for an increase in MEHHP concentration from the 10th to the 90th percentile is a decrease of −3.29. If a boy had a typical (median) masculine score (40.5) and his mother's MEHHP metabolite concentration increased from the 10th to 90th percentile, his score would be expected to decrease to 37.5 (a decrease of 8%), which would bring his masculine score down to the 27th percentile. Other predicted decreases in boy's PSAI masculine and composite scores associated with unit increases in DEHP and DBP metabolite concentrations are shown in Fig. 1.

As noted in the introduction, there are critical periods for hormonal influences on brain development in mammals, beginning in utero. Sex differences in patterns of behaviour relate to differences in brain organization that develop under the influence of testosterone and its metabolites during this period, although societal expectations and contingencies, beyond the period of early development, also exert significant influences. It is understandable, therefore, that antiandrogenic chemicals, a class that includes phthalates, are capable of influencing the development of male-typical behaviour, much as they impair masculinization of genital structures. Two other antiandrogenic chemicals, vinclozolin (Hotchkiss *et al.*, 2003; Colbert *et al.*, 2005) and flutamide (Casto *et al.*, 2003), also reduce male-typical play behaviour in male rats that are exposed prenatally.

Our subject population consisted of children 3.6–6.4 years of age. Many investigations of children's behaviours seem to accord sex differences a minor role, providing no standards for boys and girls separately. Many standard behavioural assessments do not provide information on sex differences in young children. For example, the Ages and Stages questionnaire (Squires *et al.*, 1997) is standardized on the basis of age and not distinguished by sex. Norms for the Parents' Evaluation of Developmental Status questionnaire (Brothers *et al.*, 2008) are also not differentiated by sex (Brothers *et al.*, 2008).

Sex differences in play preferences, however, are detectable early in development. Toy choices in girls and boys have been found to differ in infants as young as 12–13 months of age (Servin *et al.*, 1999; Van De Beek *et al.*, 2009), and may be manifest even earlier, as reflected in visual attention. For instance, Alexander *et al.* (2009) found that 3- to 8-month-old girls and boys showed different patterns of visual fixations to images of dolls and trucks. Similar sex differences in toy preferences have been seen in non-human primates (Alexander & Hines, 2002; Hassett *et al.*, 2008). These differences may be based on perceptual features of the stimuli that arouse different responses in males and females governed by how the brain was organized by androgens during development. In a finding somewhat related to ours, gestational phthalate exposure was reported to be related to different patterns of response in male and female neonates on the Brazelton Neonatal Behavioural Assessment Scale (Engel *et al.*, 2009).

Phthalates are endocrine-disrupting chemicals (EDCs). Sex-specific influences of environmental chemicals on endpoints such as reproductive tract anomalies are comparatively straightforward to investigate, which accounts for the substantial literature on such measures. A comprehensive evaluation of how development is altered by EDCs must include neurobehavioural endpoints. Here, appropriate tools for assessment are particularly crucial because they may reveal important effects whose nature (e.g. cognitive style, social behaviours such as play, temperament) makes them easy to overlook in conventional toxicity assessment.

We obtained only a single prenatal urine sample from each woman and most were obtained quite late in pregnancy (mean 28.3 weeks). Therefore, the phthalate metabolite concentrations reported here may not reflect exposure during the most sensitive developmental window. However, a recent study of the variability of phthalate metabolite concentrations in men of reproductive age found that a single urine sample was reasonably predictive of the subject's exposure to the parent phthalate over 3 months (Hauser *et al.*, 2004). This may reflect habitual use of phthalate-containing household and consumer products.

Our analysis took a simple approach to the link between phthalate exposure and play behaviour by examining one phthalate at a time. As we hypothesized based on anti-androgenic activity reported in many rodent (as well as human) studies, metabolites of DEHP and DBP were most strongly associated with play behaviour in males, whereas other phthalate metabolites (MEP, MCPP, MMP and MBzP) were not, when examined individually. However, our environment, in contrast, exposes us to many phthalates, to a variety of other antiandrogenic agents, and to other exposures with unknown effects and interactions. Of our children examined at a mean age of 12 months, 81% had detectable concentrations of at least seven phthalate metabolites in their urine (Sathyanarayana *et al.*, 2008). As shown by Rider *et al.* (2008), Sharpe *et al.* (1995) and Howdeshell *et al.* (2008), antiandrogens with diverse mechanisms of action, including phthalates, exert similar effects on male reproductive development in a dose-additive fashion. These findings suggest that exposure assessments should be based on exposure to multiple agents that act on common endpoints if we are not to underestimate their combined effects. Our own data indicate that, even where not statistically significant, associations for all five of the DEHP and DBP metabolites suggested less male-typical play behaviour. A more sophisticated model of joint action of these metabolites is warranted.
This study is the first to relate complex sexually dimorphic behaviour to phthalate exposure. Although our results are based on a relatively small sample, their internal consistency and their compatibility with current knowledge about how gonadal hormones mould sex differences in brain and behaviour support their plausibility. Their implications warrant extensive investigation.

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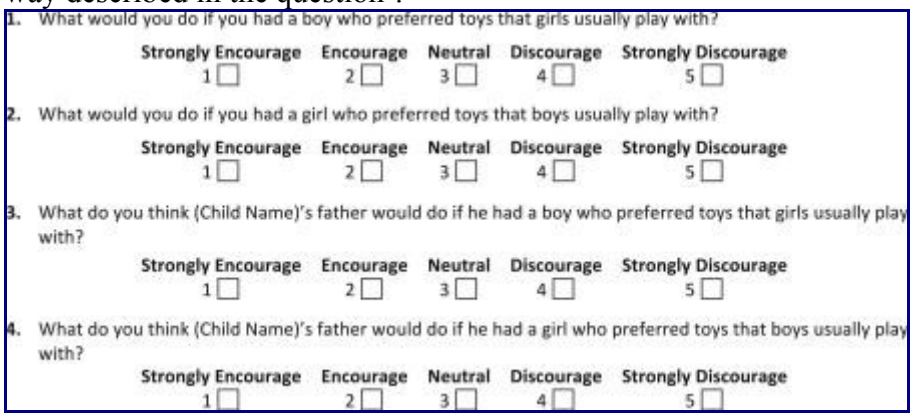
Appendix: Parental Attitude Questions

In the following four questions, ‘Encourage’ means ‘Encourage him (or her) to play in the way described in the question’ and ‘Discourage’ means ‘Discourage him (or her) from playing in the way described in the question’.



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