# Phthalate Exposure and Human Semen Parameters

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**Background.** There is scientific and public concern about commonly used chemicals, including phthalates, that are associated with reproductive toxicity in laboratory animals and are hormonally active. People are exposed to phthalates through diet, consumer products and medical devices. The present study explored whether environmental levels of phthalates are associated with altered semen quality in humans.
**Methods.** We recruited 168 men who were part of subfertile couples and who presented to the Massachusetts General Hospital andrology laboratory for semen analysis between January 2000 and April 2001. Semen parameters were dichotomized based on 1999 World Health Organization reference values for sperm concentration (<20 million/ml) and motility (<50% motile), as well as Tygerberg Strict criteria for morphology (<4% normal). The comparison group was men for whom these semen parameters were all above the reference values. In urine, eight phthalate metabolites were measured with high-performance liquid chromatography and tandem mass spectrometry. Specific gravity-adjusted phthalate metabolite levels were categorized into tertiles.
**Results.** There was a dose-response relation between tertiles of mono-butyl phthalate and sperm motility (odds ratio per tertile = 1.0, 1.8, 3.0; *P*-value for trend = 0.02) and sperm concentration (1.0, 1.4, 3.3; *P*-value for trend = 0.07). In addition, there was a dose-response relation between tertiles of monobenzyl phthalate and sperm concentration (1.0, 1.4, 5.5; *P*-value for trend = 0.02).
**Conclusions.** There were dose-response relations for monobutyl phthalate and monobenzyl phthalate with one or more semen parameters, and suggestive evidence for monomethyl phthalate with sperm morphology. The lack of a relation for other phthalates may indicate a difference in spermatotoxicity among phthalates.
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**Key words:** semen quality, phthalate, urine, environment, human, epidemiology.



Several commonly used industrial chemicals, including phthalates, are associated with reproductive toxicity in laboratory animals and are hormonally active[1–7] This toxicity is a matter of concern because a large proportion of the U.S. general population is exposed to phthalates.[8,9] Furthermore, several researchers have found declining sperm counts in developed countries[10–13] and there is concern over whether industrial chemicals may be partly responsible.[14]

Phthalates are used in many consumer products such as carpet backing, soaps, shampoos, paints, glues, hairsprays, nail polishes, insect repellents,[15] cosmetics, perfumes[8] and medical products.[16] Di(2-ethylhexyl) phthalate (DEHP), one of the more commonly used phthalates, leaches from blood products, intravenous and dialysate bags and tubing made with PVC (polyvinyl chloride).[16]

Several phthalates, including butyl benzyl phthalate (BBzP), di-n-butyl phthalate (DBP), diisobutyl phthalate (DIBP), diethyl phthalate (DEP) and di-isononyl phthalate (DINP), have demonstrated weak estrogenic activity *in vitro*.[17] However, in *in vivo* bioassays, DBP and DEHP were not shown to be estrogenic.[18] In gestational, lactational or pubertal rodent studies, DBP, BBzP and DEHP produced testicular toxicity as manifested in reduced anogenital distance, reduced testis weights, reten-

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tion of thoracic nipples,[2] malformations of the epididymis and vas deferens, and hypospadias.[8,19] This was consistent with an antiandrogenic mechanism of testicular toxicity, though not at the androgen receptor level.[2,8,19] Most evidence supporting the testicular toxicity of phthalates (particularly DBP, BBzP and DEHP) arises from gestational or lactational phthalate exposures in rodent species.[1,8,20–22] However, limited data from dosing of adult rodents with BBzP and DBP have shown associations with reproductive hormone abnormalities,[3] testicular atrophy and reduced sperm production.[18] Dosing with DEHP in adult rodents did not produce any alteration in reproductive hormones, Leydig cell hyperplasia, seminiferous tubule damage or germ cell degeneration.[4] We know of only one small human study on the possible relation between phthalates and testicular function.[23] However, interpretation of these results is difficult because potential confounders were not considered.

Despite the rapid metabolism and elimination of most phthalates,[16,24] a constant steady state concentration may in theory be reached through chronic low-level exposures from dietary ingestion and many commonly used products. Currently, there are no data on the variability of phthalate levels over time periods longer than several days.[25] Evidence of widespread exposure of the general population to phthalates comes from two recent cross-sectional studies on phthalate metabolites in urine collected for the National Health and Nutrition Examination Survey III (NHANES III)[8] and NHANES 1999.[9] The NHANES survey collects biological samples and information about the health and diet of people in the United States.[26] 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.[8,9]

The present study was designed to explore whether urinary phthalate metabolite levels at environmental levels are associated with altered semen quality in adult men.

## Methods

Subjects were 168 men who were part of subfertile couples, and who presented to the Vincent Burnham Andrology lab at MGH between January 2000 and April 2001 for semen analysis as part of an infertility work-up. Men were invited to participate in this study regardless of their prior knowledge of fertility status. Sixty-six percent of eligible men (between 20 and 54 years of age) agreed to participate. Men presenting for postvasectomy semen analysis were excluded. Height and weight were measured, and a questionnaire was used to collect information on medical history and lifestyle factors. The study was approved by the Harvard School of Public Health and Massachusetts General Hospital Human Subjects committees and all subjects signed an informed consent.

Study participants produced a semen sample on-site by masturbation into a sterile plastic specimen cup. The sample was allowed to liquefy at 37°C for 20 minutes before analysis. Subjects had been instructed to abstain from ejaculation for 48 hours before producing the semen sample and to complete a questionnaire on the length of the sexual abstinence period.

Semen analyses were performed without knowledge of subjects' phthalate levels. We analyzed samples for sperm concentration and motion parameters by computer aided semen analysis (CASA) (Hamilton-Thorn Version 10HTM-IVOS). Setting parameters and the definition of measured sperm motion parameters for the CASA were established by Hamilton-Thorn Company (Frames Acquired: 30; Frame Rate: 60 Hz; Straightness (STR) threshold: 80.0%; Medium VAP Cutoff: 25.0 $\mu$m/second; and the duration of the tracking time: 0.5 seconds). To measure both sperm concentration and motility, aliquots of semen samples (5 $\mu$l) were placed into a pre-warmed (37°C) Makler counting chamber (Sefi - Medical Instruments, Haifa, Israel). A minimum of 200 sperm from at least four different fields was analyzed from each specimen. We defined percent motile sperm as World Health Organization (WHO) grade "A" sperm (rapidly progressive with a velocity $\geq$25 $\mu$m/second at 37°C) plus "B" grade sperm (slow/sluggish progressive with a velocity $\geq$5 $\mu$m/second but $<$25 $\mu$m/second).

Using the "feathering" method described in the 1999 WHO manual[27] we made at least two slides for each fresh semen sample. The resulting thin smear was allowed to air dry for 1 hour before staining, which was carried out using a Diff-Quik staining kit (Dade Behring AG, Düdingen, Switzerland). We performed morphologic assessment with a Nikon microscope using an oil immersion 100x objective (Nikon Company, Tokyo, Japan). As the slide was examined from one microscopic field to another, all spermatozoa were assessed and scored as normal or abnormal. Sperm morphology was determined using the strict criteria by Kruger *et al.*[28] A minimum of 200 spermatozoa was counted from two slides for each specimen. Results were expressed as the percentage of normal spermatozoa.

The monoester phthalate metabolites were measured because of potential sample contamination from the parent diester, and because the metabolites (as opposed to the parent diester compounds) are believed to be the active toxicant.[20,24] Eight urinary phthalate monoesters were measured in a single spot urine sample collected in a sterile specimen cup on the same day as the semen sample. The analytical approach has been described in detail elsewhere.[8] 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 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.[8] Analysts at the U.S. Centers for Disease Control and Prevention, Atlanta, Georgia were blind to all information concerning subjects.

We measured eight urinary phthalate metabolites: MEP, monomethyl phthalate (MMP), MEHP, MBP, MBzP, mono-n-octyl phthalate (MOP), mono-3-methyl-5-dimethylhexyl phthalate (isononyl) (MINP), and monocyclohexyl phthalate (MCHP). Because more than 75% of the population had levels of MCHP and MINP below the limit of detection, and more than 50% of subjects had MOP levels below the limit of detection, the results for these metabolites were not informative and are not included in the analysis. MMP was measured on only 92 men; the assay was not performed on the first 76 men. MEP was detected in 100% of subjects, whereas MBP, MBzP and MMP were detected in over 95% of subjects, and 75% had detectable levels of MEHP. These five phthalates were used in the statistical analyses. Twenty-five samples (15%) were excluded from primary analysis because of extreme specific gravity values (less than 1.010 or greater than 1.030).[29] The final sample size for statistical analysis was 143 men (77 for MMP).

We normalized urinary phthalate levels for dilution by specific gravity adjustment. There are several methods to adjust for urine volume[29,30] 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.[29] Although the methods of excretion of the phthalate monoesters measured in this study are unknown, terephthalic acid, a dicarboxylic acid, was found to be actively secreted by renal tubules and actively reabsorbed by the kidney.[31] Other organic compounds that are conjugated with glucuronides in the liver are known to be eliminated by active tubular secretion.[30] Thus, creatinine adjustment for dilution may not be appropriate for phthalates. Additionally, creatinine levels may be confounded by muscularity, physical activity, urine flow, time of day, diet and disease states.[29,30] For these reasons, we used specific gravity, rather than creatinine, to normalize phthalate levels.

Specific gravity was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD), which was calibrated with deionized water before each measurement. Phthalate concentrations were corrected for specific gravity by the following formula; $P_c = P\ ([1.024-1]/[SG-1])$ where $P_c$ is the specific gravity corrected phthalate concentration (ng/ml), $P$ is the observed phthalate concentration (ng/ml) and $SG$ is the specific gravity of sample.[29,30]

Each subject provided a single semen sample. We chose not to require a second semen specimen because it would have lowered subject participation rates. In keeping with the literature, and to account for the known intraindividual variability in semen parameters, analyses were reported using categorized semen quality parameters. Categorization provides an efficient design for modeling the relation between semen quality and phthalates. Schrader and coworkers[32] demonstrated that the average coefficient of variation for within-subject sperm counts was 44%, indicating that variability within a subject is large relative to the mean. Hence an accurate measurement of sperm count is difficult using one specimen. However, according to the results from Schrader and coworkers, individuals with low sperm counts (below 20 million/ml according to WHO criteria) were unlikely to vary above 20 million/ml on subsequent analyses. Therefore, in the present study, we dichotomized semen parameters based on WHO (1999)[27] reference values for sperm concentration (less than 20 million/mL) and motility (less than 50% motile sperm) and Tygerberg Kruger strict criteria for morphology (less than 4% normal sperm). The comparison group was defined as men with all three semen parameters at or above the reference value.

Phthalate levels were dichotomized using the median for each metabolite. In addition, levels were divided into tertiles to explore dose-response relations. The use of median or tertile phthalate level cutpoints allowed individuals with undetected values to be included in the data analysis. We used chi-square and Fisher's exact tests to assess the relation between the dichotomized phthalate levels and the dichotomized semen parameters. The Mantel-Haenszel chi-square test was used to assess the relation between categoric variables and the categorized semen parameters. We used a multivariate logistic regression model to explore the relation between each semen parameter and each phthalate metabolite. As possible covariates, we considered smoking status, race, age, body mass index and abstinence time; the inclusion of specific covariates in the multivariate models was based on statistical and biological considerations.[33] Age was modeled as a continuous independent variable. Abstinence time was modeled as an ordinal five-category variable (2 or fewer days, 3, 4, 5 and 6 or more days). Smoking status was included as a dummy variable (current and former *vs* never).

**TABLE 1. Demographic and Medical History by Semen Parameters (N = 168)**

| | | Semen Parameters Below Reference Values | | |
|---|---|---|---|---|
| | Comparison Subjects* (N = 77) | Sperm Concentration <20 Million/mL (N = 28)† | Sperm Motility <50% Motile (N = 74)† | Sperm Morphology <4% Normal (N = 44)† |
| Age, mean (SD) | 35.6 (5) | 38 (6) | 38 (6) | 37 (5) |
| Abstinence time, N (%) | | | | |
| ≤2 days | 21 (27) | 10 (36) | 21 (28) | 10 (23) |
| 3 days | 26 (34) | 4 (14) | 20 (27) | 12 (27) |
| 4 days | 12 (16) | 5 (18) | 12 (16) | 8 (18) |
| 5 days | 6 (8) | 4 (14) | 8 (11) | 4 (9) |
| 6 or more days | 12 (16) | 5 (18) | 13 (18) | 10 (23) |
| Race, N (%) | | | | |
| White | 61 (79) | 20 (74) | 53 (74) | 30 (70) |
| Black/African-American | 6 (8) | 2 (7) | 6 (8) | 4 (9) |
| Hispanic | 2 (3) | 3 (11) | 10 (14) | 5 (12) |
| Other | 8 (10) | 2 (7) | 3 (4) | 4 (9) |
| Smoking status, N (%) | | | | |
| Never smoker | 55 (71) | 16 (59) | 51 (70) | 31 (72) |
| Ever smoker | 22 (29) | 11 (41) | 22 (30) | 12 (28) |
| Current smoker | 5 (7) | 4 (15) | 6 (8) | 4 (9) |
| Ex-smoker | 17 (22) | 7 (26) | 16 (22) | 8 (19) |
| Previous exam for infertility, N (%) | 10 (13) | 11 (41) | 26 (36) | 18 (42) |

Information on race missing for two men and on smoking for one.
* Subjects with sperm concentration ≥20 million/ml, motility ≥50% motile and morphology ≥4% normal.
† A subject may contribute data to more than one category.

## Results

Of the 168 men in the study population, 28 (17%) had a sperm concentration less than 20 million/ml, 74 (44%) had less than 50% motile sperm, and 44 (26%) had less than 4% normally shaped sperm. There were 77 men (46%) with all three semen parameters above reference values. The semen parameter categories were not mutually exclusive; a man could contribute data to any or all of the below-reference value groups. Subjects were primarily white (77%), with a mean age of 36.4 years (standard deviation = 5.5). Two-thirds had never smoked.

The demographic distribution of study participants, by semen parameter, is summarized in Table 1. Older age and Hispanic ethnic origin were predictors of one or more below-reference semen parameters, whereas current cigarette smoking was weakly associated with low sperm concentration.

There was a wide distribution of the phthalate monoester levels adjusted for specific gravity (Table 2). The median phthalate levels ranged from 153 ng/ml for MEP to 6.3 ng/ml for MEHP, which reflect exposure to DEP and DEHP, respectively. Unadjusted phthalate levels are presented to facilitate comparison with previously published NHANES data. The unadjusted levels also exhibited a wide distribution of individual phthalate levels in a similar rank order.

Phthalate levels were dichotomized using the median for each metabolite. The high-phthalate groups tended to have a higher proportion of nonwhites, with the

**TABLE 2. Total Urinary Phthalate Monoester Concentrations (ng Monoester per mL Urine)* Adjusted for Specific Gravity† and Unadjusted**

| | | Percentile | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phthalate Monoester | N | Min | 5th | 25th | 50th | 75th | 95th | Max | Geometric Mean |
| Adjusted for specific gravity | | | | | | | | | |
| Ethyl (MEP) | 143 | 9.8 | 30 | 60 | 153 | 444 | 1,838 | 11,371 | 180.2 |
| Benzyl (MBzP) | 143 | <LOD | 1.8 | 4.2 | 9.3 | 14.8 | 32.8 | 540 | 8.3 |
| Butyl (MBP) | 143 | <LOD | 3.7 | 10.2 | 16.2 | 23.9 | 58.5 | 434 | 15.7 |
| 2-Ethylhexyl (MEHP) | 143 | <LOD | <LOD | 2.7 | 6.3 | 16.2 | 149.6 | 446 | 7.9 |
| Methyl (MMP) | 77 | <LOD | 1.3 | 3.8 | 6.5 | 12.5 | 30.3 | 33 | 6.6 |
| Unadjusted | | | | | | | | | |
| Ethyl (MEP) | 168 | 8.2 | 26 | 59 | 156 | 454 | 1,937 | 9,476 | 175.5 |
| Benzyl (MBzP) | 168 | <LOD | 1.2 | 4.0 | 10.3 | 18.3 | 49.6 | 450 | 8.7 |
| Butyl (MBP) | 168 | <LOD | 2.3 | 9.6 | 15.9 | 32.5 | 73.1 | 488 | 16.1 |
| 2-Ethylhexyl (MEHP) | 168 | <LOD | <LOD | 2.3 | 5.7 | 17.3 | 154.3 | 520 | 7.6 |
| Methyl (MMP) | 92 | <LOD | 1.1 | 4.0 | 7.5 | 14.9 | 30.3 | 452 | 7.5 |

LOD = limit of detection; Max = maximum; Min = minimum.
* LODs for phthalates (ng/ml) are as follows; MEP, 1.0; MBzP, 0.8; MBP, 0.6; MEHP, 1.2; and MMP, 0.71.
† Excludes 25 subjects with extreme specific gravity (<1.010 or >1.030).

**TABLE 3. Association of Below-Reference Value Semen Parameters with Median Phthalate Monoester Levels* (N = 143 subjects)†**

| | Semen Parameter | | | | | |
|---|---|---|---|---|---|---|
| | Sperm Concentration <20 Million/mL | | Sperm Motility <50% Motile | | Morphology <4% Normal | |
| Phthalate Monoester | Crude OR (CI) | Adjusted OR (CI)‡ | Crude OR (CI) | Adjusted OR (CI)‡ | Crude OR (CI) | Adjusted OR (CI)‡ |
| Ethyl (MEP) | 1.1 (0.4–2.9) | 1.2 (0.4–3.4) | 1.3 (0.6–2.6) | 1.1 (0.6–2.4) | 0.9 (0.4–2.1) | 0.9 (0.4–2.2) |
| 2-Ethylhexyl (MEHP) | 1.1 (0.4–2.9) | 1.0 (0.3–2.9) | 1.4 (0.7–2.8) | 1.4 (0.7–2.9) | 1.2 (0.5–2.6) | 1.2 (0.5–2.8) |
| Butyl (MBP) | 2.2 (0.8–5.8) | 2.4 (0.8–7.2) | 2.3 (1.1–4.6) | 2.4 (1.1–5.0) | 1.6 (0.7–3.5) | 1.7 (0.8–3.9) |
| Benzyl (MBzP) | 1.8 (0.7–4.8) | 2.7 (0.8–8.5) | 1.6 (0.8–3.1) | 1.8 (0.9–3.9) | 1.8 (0.8–4.0) | 2.1 (0.9–5.1) |
| Methyl (MMP) | 2.3 (0.6–8.1) | 1.7 (0.4–7.9) | 1.3 (0.5–3.4) | 1.1 (0.4–3.3) | 2.9 (0.9–9.3) | 3.2 (0.8–12.2) |

Reference category is subjects at or above reference value for sperm concentration (≥20 million/ml), motility (≥50% motile) and morphology (≥4% normal morphology).
* Adjusted for specific gravity.
† Excludes 25 subjects with extreme specific gravity (<1.010 or >1.030). For MMP, N = 77.
‡ Adjusted for age (continuous), abstinence time (5 categories: ≤2 days, 3, 4, 5 and 6+) and smoking (current, former and never).

largest differential for MEP; blacks composed 11% of the high-MEP group, compared with 1% in the low-MEP group (data available with the electronic version of this article at www.epidem.com). Smoking was more prevalent in men with higher MMP and MEHP levels; 50% of men in the high-MMP group were ever-smokers, compared with 23% in the low-MMP group; for MEHP, the numbers were 40% and 20%, respectively. Age was not associated with phthalate metabolites.

When the relations between the dichotomized phthalate levels and dichotomized semen parameters were examined, fewer men in the comparison group were found to have MBP levels above the median (40%) as compared with the low-motility group (60%) (data available with the electronic version of this article at www.epidem.com).

Age and abstinence time were included in each multivariate logistic regression model because they are predictors of semen quality.[34–35] The final models also included smoking status. Crude and adjusted odds ratios for each specific gravity-adjusted phthalate monoester by semen parameter are presented in Table 3. Men with MBP levels above the median were 2.4 times (95% confidence interval [CI] = 1.1–5.0) more likely to have motility below the reference value. Odds ratios were elevated for the associations of MBP with sperm concentration and morphology (OR = 2.4 [CI = 0.80–7.2], and 1.7 [0.8–3.9], respectively). There were also elevated odds ratios for the associations between MMP and sperm morphology (3.2 [0.8–12.2]), and between MBzP and sperm concentration, motility and morphology. The adjusted odds ratios were generally similar to the crude odds ratios.

To explore possible dose-response relations between semen parameters and MBP, MBzP and MMP, we categorized these phthalate levels into tertiles (Table 4). We found dose-response relations (OR per tertile adjusted for age, abstinence and smoking) between MBzP and sperm concentration (1.0, 1.4 and 5.5; *P* for trend = 0.02) and between MBP and sperm concentration (1.0, 1.4 and 3.3; *P* for trend = 0.07). We also found a dose-response relation between MBP and sperm motility (1.0, 1.8 and 3.0; *P* for trend = 0.02). Weaker evidence for dose-response relations was found with MBP and morphology and MBzP with sperm motility, as well as between MMP and morphology.

Sensitivity analyses were conducted to test the robustness of the data. We performed a reanalysis after excluding five men who had azoospermia or diabetes mellitus. Azoospermic men were excluded to prevent undue statistical influence from their extreme value and also because the mechanism responsible for azoospermia may be related to an obstructive mechanism or Y-chromosome deletions. Men with diabetes were excluded because diabetes may alter metabolism and excretion of phthalates. In the reanalysis, the odds ratios and their confidence intervals remained essentially unchanged (data available with the electronic version of this article at www.epidem.com).

We also analyzed the data including the 25 men whose urine samples were deemed unreliable based on extreme specific gravity. Using the median cutpoints, odds ratios generally decreased slightly (10%–20%), although confidence intervals narrowed (data available with the electronic version of this article at www.epidem.com). The relation between MBP and sperm motility remained stable, whereas the associations of MBzP with sperm concentration and motility and of MMP with morphology remained suggestive, although with smaller odds ratios.

We reanalyzed the data using all 168 phthalate monoester measurements unadjusted for specific gravity. The associations of high MMP with sperm morphology and of high MBzP with sperm concentration became stronger and more stable, with odds ratios of 4.1 (CI = 1.1–15.6) and 3.1 (1.2–8.4), respectively (data available

**TABLE 4. Associations of Below-Reference Value Sperm Concentration, Sperm Motility and Sperm Morphology with Tertiles of Phthalate Monoester Levels (N = 143)***

| Specific-gravity adjusted phthalate tertile | Sperm Concentration | | | | | Sperm Motility | | | | | Sperm Morphology | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Crude OR | 95% CI | Adj. OR† | 95% CI | N | Crude OR | 95% CI | Adj. OR† | 95% CI | N | Crude OR | 95% CI | Adj. OR† | 95% CI |
| Butyl phthalate (MBP), tertile | | | | | | | | | | | | | | | |
| 1‡ | 6 | 1.0 | | 1.0 | | 15 | 1.0 | | 1.0 | | 12 | 1.0 | | 1.0 | |
| 2 | 6 | 1.3 | 0.4–4.6 | 1.4 | 0.3–6.0 | 21 | 1.8 | 0.8–4.3 | 1.8 | 0.7–4.6 | 12 | 1.3 | 0.5–3.4 | 1.5 | 0.5–4.3 |
| 3 | 10 | 2.7 | 0.8–8.6 | 3.3 | 0.9–12.6 | 27 | 2.9 | 1.2–6.9 | 3.0 | 1.2–7.6 | 15 | 2.0 | 0.8–5.3 | 2.2 | 0.8–6.1 |
| P for trend | | | 0.10 | | 0.07 | | | 0.02 | | 0.02 | | | 0.2 | | 0.1 |
| Benzyl phthalate (MBP), tertile | | | | | | | | | | | | | | | |
| 1‡ | 5 | 1.0 | | 1.0 | | 18 | 1.0 | | 1.0 | | 14 | 1.0 | | 1.0 | |
| 2 | 6 | 1.1 | 0.3–4.1 | 1.4 | 0.3–6.0 | 21 | 1.1 | 0.5–2.5 | 1.1 | 0.5–2.8 | 6 | 0.4 | 0.1–1.2 | 0.4 | 0.1–1.4 |
| 3 | 11 | 3.0 | 0.9–10.2 | 5.5 | 1.3–23.9 | 24 | 1.8 | 0.8–4.3 | 2.1 | 0.8–5.3 | 19 | 1.8 | 0.7–4.7 | 2.2 | 0.8–6.0 |
| P for trend | | | 0.07 | | 0.02 | | | 0.2 | | 0.1 | | | 0.2 | | 0.1 |
| Methyl phthalate (MMP), tertile | | | | | | | | | | | | | | | |
| 1‡ | 4 | 1.0 | | 1.0 | | 9 | 1.0 | | 1.0 | | 4 | 1.0 | | 1.0 | |
| 2 | 5 | 1.6 | 0.3–7.4 | 1.7 | 0.3–11.1 | 13 | 1.8 | 0.6–5.9 | 2.1 | 0.6–7.7 | 6 | 1.9 | 0.4–8.5 | 1.5 | 0.3–7.4 |
| 3 | 5 | 1.6 | 0.3–7.4 | 0.7 | 0.1–5.3 | 11 | 1.6 | 0.5–5.1 | 1.2 | 0.3–4.6 | 10 | 3.2 | 0.8–12.9 | 2.4 | 0.5–11.7 |
| P for trend | | | 0.6 | | 0.8 | | | 0.5 | | 0.8 | | | 0.1 | | 0.3 |

Tertile cut-points (ng/ml): butyl = 0–11.64, 12.24–20.13, 20.16–433.93; benzyl = 0–5.50, 5.54–12.94, 13.04–540.24; methyl = 0–4.38, 4.43–10.18, 10.29–32.46.
* Comparison group is subjects at or above reference value for sperm concentration (≥20 million/ml), motility (≥50% motile) and morphology (≥4% normal morphology. Excludes 25 people with extreme specific gravity (<1.010 or >1.030).
† Adjusted for age (continuous), abstinence time (5 categories: ≤2 days, 3, 4, 5 and 6 days) and smoking (current, former and never).
‡ Reference category.

with the electronic version of this article at www.epidem.com). The association between high MBP and sperm motility also remained strong and stable, and the association with sperm concentration became slightly stronger, with narrower confidence intervals (OR = 2.5 [1.0–6.6]).

## Discussion

Our study suggests that some phthalate monoesters, at environmental levels, are associated with lower sperm concentration, lower motility and increased percentage of sperm with abnormal morphology in humans. Specifically, we found dose-response relations of MBP with sperm motility and sperm concentration. There was also a dose-response relation between MBzP and sperm concentration. We also found limited evidence for an association of higher MMP with poor sperm morphology.

Our sensitivity analyses confirmed the robustness of the data. The analyses were not sensitive to the presence of extreme semen parameter values (*ie*, azoospermic men) or the exclusion of the 25 urine samples with extreme specific gravity. In addition, the analysis of unadjusted urinary measures of phthalate monoesters strengthened associations between MBP, MBzP and MMP with one or more of the semen parameters.

Our data are consistent with the animal data suggesting that several phthalates are testicular toxins and decrease sperm production.[1,18] Rats exposed to DEHP, DBP and the main urinary metabolite, MBP, demonstrated reduced testicular weights and histologic changes in the seminiferous tubules.[1]

The strengths of the present study include a reliable biomarker of exposure rather than self-reported exposures. Biomarkers have the potential to integrate exposures to chemicals from all routes of exposure including oral, dermal, inhalation and ingestion.[30] Furthermore, by measuring the monoester phthalate (*ie*, the metabolite), we avoided any confusion from postcollection contamination by plastic products (such as the urine specimen collection cup).[36]

These results must be interpreted cautiously because the phthalate levels are based on a single spot urine sample from a limited number of subjects. Although one recent study documents good reproducibility (Pearson correlation coefficients ranging from 0.5 to 0.8) of urinary phthalate monoester measurements from day to day, this was based on a small number of African-American women (N = 46).[25] Because phthalates have short half-lives,[16,24] urine samples reflect recent exposure. However, if a steady state of exposure is achieved with chronic repeated exposures to phthalates through diet and through household and personal care products, then the use of a single specimen is improved.

We compared unadjusted geometric mean phthalate levels from the present study with results from NHANES III[8] and NHANES 1999.[9] Even after limiting the NHANES III data to men (Dana Barr, personal communication, 13 November 2001), phthalate levels in NHANES were 2–3 times higher than those in the present study. The NHANES 1999 phthalate metabolite levels were also twice as high as those in the present study. The two exceptions were MEP, which was similar between studies, and MEHP, which was twice as high in the present 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 procedures (such as IVs, transfusions or hemodialysis) that might account for higher MEHP levels.

Although we compared our phthalate levels with the published NHANES datasets, these comparisons are not strictly appropriate because there are several important differences between our dataset and the NHANES datasets. The NHANES datasets included women, who in the NHANES III dataset had higher levels of several phthalates than men.[9] Because our population was comprised entirely of men it was therefore not unexpected that the phthalate levels in our data were lower. A breakdown of NHANES 1999 phthalate levels by age and gender is not publicly available for comparison. Although we were able to subset the NHANES III data by gender, we have several reservations about the validity of this comparison. The NHANES III samples analyzed for phthalate monoesters were not a random sample of the overall population, but rather a nonrepresentative call-back sample of 289 volunteers, of whom 127 were men. Furthermore, the NHANES III samples were collected 10–15 years earlier than our samples, and there may be temporal changes in phthalate levels attributable to changes in use patterns and sources of phthalates, as suggested by the observation that NHANES 1999 levels were lower than NHANES III. Thus, because of both temporal trends and differences in study populations, our data are not strictly comparable with NHANES III or 1999 data, and so the comparisons should not be used to determine quantitative differences among populations. Because the NHANES datasets and our data represent some of the first human data on phthalate levels, we conclude that the distribution of phthalate levels in men of reproductive age remains unclear.

Although the levels of phthalate monoesters differed between the present study and both NHANES studies, the ranking of metabolite levels was similar across studies.[8,9] In all three studies, the highest phthalate levels were for MEP, followed by MBP and then MBzP.

Our study was a cross-sectional study conducted within an andrology clinic. Although exposure and outcome were measured simultaneously (*ie*, semen and urine samples were collected on the same day), the relatively short time interval for spermatogenesis (3 months) lowers concern about the use of a contemporaneous urine sample. Furthermore, temporal variability of phthalate levels is likely to result in nondifferential misclassification of exposure because the variability is unlikely to be dependent on semen parameters. This would bias our results towards the null, making it more difficult to detect a dose-response relation rather than accounting for the relation observed. A better understanding of the temporal variability in urinary phthalate levels is needed before the implications of using a single spot urine sample can be fully explored.

Although the men in the present study may not be representative of men in Massachusetts, generalizability of the results is not necessarily 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.[37] For generalizability to be limited, the relations between semen quality and phthalates in this clinic population would have to differ from the relations in the larger population. We would need to speculate that men in this andrology clinic differ by some factor that alters their testicular response to phthalates. Currently, there is no reason to suspect that men who visit this andrology clinic are more or less "sensitive" to phthalates as compared with men who visit other clinics or men from the general population. However, until the results of the present study are replicated in larger and more diverse populations, the generalizability of our results will remain unclear.

In general, semen studies are challenging to conduct because participation rates are traditionally low.[38] General population semen studies are the most challenging and may have very low participation rates. This challenge makes it difficult to define the distribution of semen parameters in men from the general population. One subsample of the general population that has been studied is men attempting to conceive, which is presumably a less selected group of men than infertility clinic patients. In comparison with two recent studies on men attempting to conceive, the percent of men with sperm concentration below WHO reference value in our study (17%) was higher than Finnish men (5%) but the same as Danish men (17% and 18%).[39–40] In a study on an unselected population, 25% of men 18–22 years old who participated in a compulsory examination for military service had sperm concentrations below the reference value.[41] Comparisons across studies and across countries are difficult because of differences in semen analysis techniques and because the role of geography on semen parameters remains unclear.

We recently published preliminary results from a similar group of men on the relation between urinary levels of phthalate monoesters and DNA damage in human

sperm as measured with the neutral comet assay.[42] We found a positive dose-response association between urinary levels of MEP and increased DNA damage in human sperm. The relations between DNA damage and the other phthalate monoesters were inconsistent.

In conclusion, our data presented here on the association of MBP and MBzP with impairments of semen parameters are consistent with animal studies on testicular toxicity. However, our data on MMP are not consistent with animal studies, which have shown that MMP is not a testicular toxicant.[43–44] In addition, our data on MEHP are also consistent with animal studies, which have shown that, although MEHP is a testicular toxicant after gestational or lactational exposures, adult exposures are not associated with testicular toxicity.[4] Further studies on phthalate monoesters in humans are needed to confirm these preliminary associations between several phthalates and a decrease of one or more semen parameters.

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