PHTHALATES AND HUMAN HEALTH

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T he diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly known as phthalates, are a group of man-made chemicals with a wide spectrum of industrial applications (fig 1, table 1). High molecular weight phthalates (for example, di(2-ethylhexyl) phthalate [DEHP], di-isononyl phthalate [DiNP], di-n-octyl phthalate [DnOP]), are primarily used as plasticizers in the manufacture of flexible vinyl which, in turn, is used in consumer products, flooring and wall coverings, food contact applications, and medical devices.[1–3] Manufacturers use low molecular weight phthalates (for example, diethyl phthalate [DEP] and dibutyl phthalate [DBP]) in personal-care products (for example, perfumes, lotions, cosmetics), as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings, including those used to provide timed releases in some pharmaceuticals.[3–5]

In this paper, we review the uses and metabolism of phthalates, and the studies on health effects of phthalates in human populations published between 1973 and June 2005. The references included in this review were searched using the Web of Science database which provides interactive citation and literature searching of the Institute for Scientific Information's Science Citation Index Expanded. The database contains data from more than 5000 scientific journals and covers the period from 1980 to present. We also searched the bibliography cited in the selected references for additional relevant citations.

▶ ROUTES OF EXPOSURE AND METABOLISM

Because phthalates are widely used in many personal care and consumer products, the opportunity is high for non-occupational human exposure (table 1). However, to date, the proportional contribution from the various sources and routes of exposure to phthalates is unknown. Traditionally, ingestion has been considered an important route of exposure. Although phthalates have low volatility, they off-gas and are present in residential indoor air.[6 7] Dermal contact[1 2 4 5] and parenteral exposure from medical devices containing phthalates may also contribute to exposure.[2 8]

On exposure, phthalates are rapidly metabolised and excreted in urine and faeces.[1 2 4 5] During a phase I biotransformation, the relatively polar and low molecular weight phthalates (for example, DEP) primarily metabolise into their hydrolytic monoesters by hydrolysis of one of the ester bonds (fig 1).[4 9] In contrast, the high molecular weight phthalates are first metabolised to their respective hydrolytic monoesters, and then, after enzymatic oxidation of the alkyl chain, to more hydrophilic, oxidative metabolites (fig 2).[1 2 10–14] Monoesters and the oxidative metabolites of phthalates can be excreted in the urine and faeces unchanged or they can undergo phase II biotransformation to produce glucuronide conjugates with increased water solubility and therefore increased urinary excretion.[2 4]

Glucuronidation not only facilitates urinary excretion of phthalate metabolites, but also may reduce their potential for biological activity if the putative biologically active species is the free metabolite. The percentage of free monoester excretion in humans varies depending on the aqueous solubility of the phthalate metabolites.[15–17] Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), two oxidative metabolites of DEHP, are mostly excreted glucuronidated in humans.[16] Most of mono(2-ethylhexyl) phthalate (MEHP), the hydrolytic monoester metabolite of DEHP, is also excreted as a glucuronide, while monoethyl phthalate (MEP), the hydrolytic monoester of DEP, is mostly excreted in its free form.[17] For other phthalates, the percentage of free monoester excretion varies between that of MEP and MEHP.[15 17]

Although the metabolism of the relatively low molecular weight phthalates (for example, DEP) ends with the hydrolytic monoester,[4 5 9] the metabolism of the higher molecular weight phthalates (for example, DEHP, DiNP, di-isodecyl phthalate [DiDP]) continues with transformation of the hydrolytic monoester to oxidative products (fig 2).[1 2 9 11–13 18] Therefore, the use of the hydrolytic monoesters as sole biomarkers for comparing relative exposures to various phthalates in a given study could be misleading. This is especially true when comparing concentrations of the

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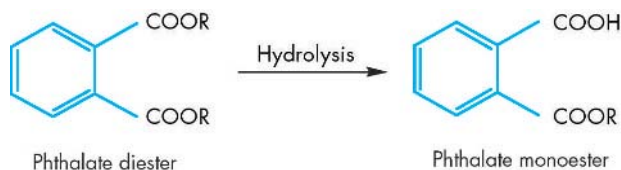
Figure 1 Generic chemical structure of phthalates diesters and phthalate monoester metabolites. R is an alkyl and/or aryl group: CH$_3$ (dimethyl phthalate), CH$_2$CH$_3$ (diethyl phthalate), CH$_2$CH$_2$CH$_2$CH$_3$ (dibutyl phthalate), CH$_2$CH(CH$_2$CH$_3$)CH$_2$CH$_2$CH$_2$CH$_3$ (di[2-ethylhexyl] phthalate), CH$_2$CH$_2$CH$_2$CH$_3$ and CH$_2$C$_6$H$_5$ (butylbenzyl phthalate).

high molecular weight monoester phthalates, such as MEHP, with the lower molecular weight phthalate monoesters, such as MEP, because the metabolism of the higher molecular weight phthalates is more complex and results in more metabolites, thus decreasing the relative amounts of their monoester metabolite. On the other hand, hydrolytic monoesters of high molecular weight phthalates (for example, MEHP) may be used as biomarkers for comparing exposure to their corresponding phthalate diesters (for example, DEHP) among studies, assuming that the hydrolytic monoester is present in detectable concentrations.

However, using the hydrolytic phthalate monoesters as the sole biomarkers of exposure in an epidemiological study could introduce exposure misclassification because the other metabolites are not accounted for. This is particularly important for the hydrolytic monoesters of high molecular weight phthalates, including isomeric phthalates such as DiNP, which represent only a small percentage of all of the urinary metabolites; oxidative phthalate metabolites predominate in urine.

Most of the research on oxidative metabolism of phthalates in humans has been conducted on DEHP.[16] [18–21] The urinary concentrations of two DEHP oxidative metabolites—MEOHP and MEHHP—have been found to be higher than MEHP, the hydrolytic metabolite of DEHP.[16] [19–21] A third oxidative metabolite of DEHP, mono(2-ethyl-5-carboxypentyl) phthalate has also been found at higher concentrations in urine than MEHP.[22] [23] These findings suggest that the relatively low urinary concentrations of MEHP[20] [21] [24–26] compared to the concentrations of the hydrolytic metabolites of other phthalates (for example, DEP, DBP) may result, at least in part, from alternative metabolic pathways[2] [10–12] leading to the formation of oxidative monoester metabolites which are more amenable than MEHP to urinary excretion.

In rodents, DiNP and DnOP, like DEHP, are also initially metabolised into their hydrolytic monoesters, which then form oxidative phthalate monoesters which are the major metabolites detected in the urine.[1] [9] [13–15] Although there may be some differences in the metabolism among species, it is likely that all large molecular weight phthalates undergo similar metabolic pathways in humans.

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MEASURES OF INTERNAL DOSE OF PHTHALATES

Phthalates are widely used in laboratory and medical products and sample contamination is therefore difficult to avoid. Because environmental exposure levels are normally much lower than those resulting from sampling contamination, human studies using the phthalate diesters as biomarkers of exposure were limited to highly exposed populations.[27–31] A different approach uses urinary phthalate monoester metabolites as biomarkers of exposure.[27] [32] By measuring the phthalate monoesters, contamination from the ubiquitous parent compounds is minimised, thus allowing for the study of environmentally exposed populations. Another advantage of using the phthalate monoesters as biomarkers is that the monoesters are generally considered the biologically active molecules.

Phthalate monoesters can be measured in biological matrices (for example, urine, serum, breast milk, saliva, ovarian follicular fluid, seminal plasma, and amniotic fluid) by using chromatography coupled with mass spectrometric techniques. High performance liquid chromatography (HPLC) is most common.[18] [32–37] Gas chromatography can also be used to measure phthalate monoesters after conversion to volatile derivatives.[38]

Analytical methods using isotope dilution HPLC coupled with tandem mass spectrometry (MS/MS) for measuring trace levels of selected phthalate monoesters in biological matrices have been reported.[18] [22] [23] [32] [34–37] [39–42] These methods are highly specific, sensitive (detection limits are in the low nanogram per millilitre range), accurate (relative recoveries are ~100%), and precise (inter-day relative standard deviations are generally <15%). In isotope dilution mass spectrometry, a stable isotope of the analyte(s) of interest is added to the matrix before sample preparation; the quantification of the analyte(s) is based on a ratio of the signal from the analyte relative to the signal from the known amount of its stable isotope. Isotope dilution mass spectrometry is considered the most accurate and precise method for trace analysis. Briefly, the determination of phthalate monoesters involves the enzymatic deconjugation of these metabolites

Table 1 Potential sources of exposure and health effects of selected phthalates

Phthalate diester	Potential sources of exposure*	Potential health effects†
Diethyl phthalate	Personal care products (e.g. fragrances), coatings (e.g. pharmaceuticals), dyes, insecticides	Reduced growth rate, food consumption and increased organ weights
Di-n-butyl phthalate	Cellulose acetate plastics, personal care products (e.g. nail polish, cosmetics), lacquers, varnishes, coatings (e.g. pharmaceuticals)	Hepatic and renal effects, developmental and reproductive effects, reduced fetal weight, cryptorchidism, hypospadias, reduced anogenital distance in males.
Butylbenzyl phthalate	Vinyl flooring, adhesives and sealants, car-care products, toys, food packaging, synthetic leather, industrial solvents, personal care products	Testicular toxicity, cryptorchidism, reduced anogenital distance, teratogenic, modulates steroid hormone levels
Di(2-ethylhexyl) phthalate	PVC plastics used in household products (e.g. toys, floor tiles and furniture upholstery, wall coverings), food packaging, blood storage bags and medical devices	Hepatocellular carcinoma, testicular toxicity, anovulation, teratogenic at high doses, affects fetal growth

*List is not inclusive.
†Health effects are in animals, frequently at high dose levels; human health effects are reviewed in the text.
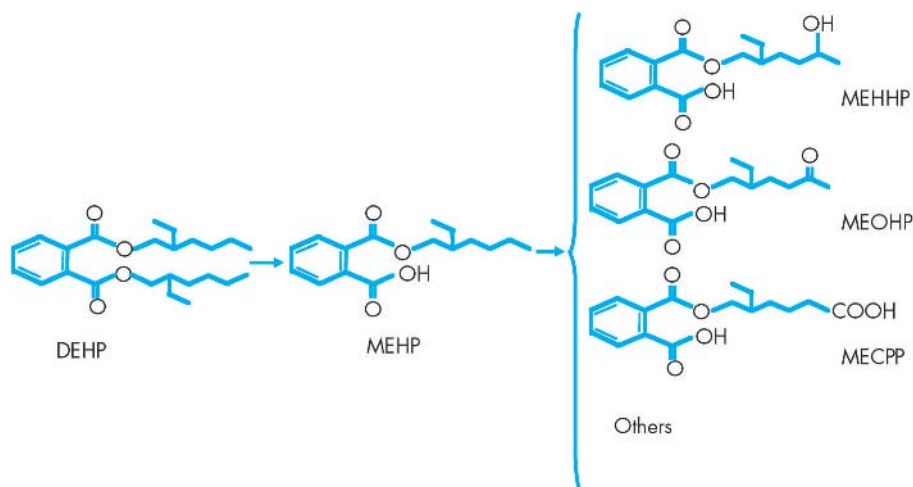
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Figure 2 Selected metabolites of di[2-ethylhexyl] phthalate) (DEHP) in humans: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP).

from their glucuronidated form, followed by solid phase extraction, separation with HPLC, and detection by MS/MS. Analytical methods which do not include mass spectrometric (MS) detection are generally less selective and sensitive than those based on MS detection.

HUMAN EXPOSURE DATA (POPULATION LEVELS)

Because of the potential human toxicities of phthalates, internal dose measurements of phthalates are necessary for exposure and risk assessment. Measuring human exposure to phthalates requires information on their concentrations and/ or their metabolites in biological media as well as on the pharmacokinetics of the phthalates.

Indirect measures of exposure including environmental monitoring and exposure history/questionnaire data can be used to assess human exposure to phthalates. Advances in analytical chemistry have made possible measuring trace levels of phthalates in biological tissues (that is, biomonitoring) and have contributed to increased recent use of biomonitoring in exposure assessment. The most common biomonitoring approach for investigating human exposure to phthalates is the measurement of urinary concentrations of phthalate metabolites. Biomonitoring data combined with indirect measures of exposure are the most appropriate tools for assessing exposure.

Variability in an individual's exposure to phthalates can result from changes in the use of personal care products, diet, or daily activities, and exposure may vary over time. Although phthalate metabolites in urine can be used to accurately measure a person's exposure at a single point in time, determining exposure over weeks or months may require multiple measurements. Therefore, information on the temporal variability of urinary levels of phthalate metabolites is needed to optimise the design of exposure assessment in epidemiological studies. Two studies have addressed this issue. The first study documented relatively good reproducibility of urinary phthalate monoester levels in two first-morning urine specimens collected for two consecutive days from 46 African-American women; day-to-day intra-class correlation coefficients ranged from 0.5 to 0.8.[43] Recently, the temporal variability in urinary phthalate metabolite levels among 11 men who collected up to nine

urine samples each during a three month period was evaluated.[44] Although substantial day-to-day and month-to-month variability in each individual's urinary phthalate metabolite levels existed, a single urine sample was moderately predictive of each subject's exposure over three months. The sensitivities ranged from 0.56 to 0.74. Both between- and within-subject variances and the predictive ability of a single urine sample differed among phthalate metabolites, suggesting that the most efficient exposure assessment strategy for a particular epidemiological study may depend on the phthalates of interest. Furthermore, a cross-sectional study of more than 2540 people in the United States showed variations in the population distributions of several phthalate metabolites depending on the time of day of collection of the urine samples.[26]

These observations, along with the non-persistent nature of phthalates, may reflect differences in the timing of exposure to phthalates during the day. Because collecting 24-hour urine samples is not practical for epidemiological studies, consideration should be given to standardising the time of sample collection. When the goal is to compare relative exposures to various phthalates across populations or among different studies, collecting first-morning urine samples is preferred. However, studies designed to explore potential health risks of phthalates should not restrict sample collection to first-morning urine samples because the data above suggest that relevant exposure opportunities in the course of a day may be missed and thus exposure could be misclassified. At the very least, timing of the urine collection should always be recorded.

In the United States, the National Health Nutrition and Examination Survey (NHANES) is an ongoing survey, conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention (CDC), designed to collect data on the health and nutritional status of the civilian, non-institutionalised US population. The data estimates from NHANES, presented by age group, gender, and race/ethnicity, are probability based, and hence, are representative of the US population. Although the biological specimens collected from NHANES participants are used mostly for clinical and nutritional testing, some of them are

used for assessing exposure to environmental chemicals, including phthalates. In Germany, German Environmental Surveys (GerESs), large scale representative population studies for assessing the exposure of the general population to environmental contaminants, have been conducted since the mid-1980s. Although phthalates were not included in early surveys, DEHP metabolites were measured recently in a subset of samples collected for the 2001/2002 pilot study for the GerES on children.[45]

In the United States, CDC reported the levels of seven urinary phthalate monoester metabolites in two subsets of NHANES participants.[24–26] The first study included the analysis of a non-representative call-back cohort of 289 urine samples, collected from adults during 1988–94 for NHANES III.[24] NHANES 1999–2000[25 26] provided the first nationally representative, population based, phthalate monoester concentrations in urine for selected demographic groups in the United States. Although the two data sets are not directly comparable, the frequencies of detection of the phthalate monoesters were similar. The high molecular weight phthalate monoesters (for example, MEHP, mono-n-octyl phthalate, monoisononyl phthalate) were detected less frequently and at lower levels than the low molecular weight phthalates (for example, MEP, monobutyl phthalate [MBP], monobenzyl phthalate [MBzP]). However, the mean and median concentrations of MEP, MBP, and MBzP were lower in the NHANES 1999–2000 than in the NHANES III subset population while the MEHP concentrations remained essentially unchanged. These findings may be related to the small size and non-representative nature of the sampling for the NHANES III call-back cohort or to reduced exposure to some phthalates for the NHANES 1999–2000 population. The relatively low frequency of detection of the high molecular weight phthalate monoesters may have been due, at least in part, to the fact that these hydrolytic metabolites may not be the most sensitive urinary biomarkers; the oxidative phthalate monoester metabolites are (vide infra). Nonetheless, these two investigations confirmed that human exposure to selected phthalates is widespread. Future NHANES will be useful for monitoring temporal changes in phthalate exposures.

However, even a comprehensive programme such as NHANES has limitations: urine samples from young (that is, <6 years of age) individuals are not collected in the population sampled, the design is cross-sectional, and no data on fetal exposures are collected. Therefore, there is a pressing need for assessing exposure during critical periods of development, periods of increased susceptibility to the potential adverse effects of phthalates.

Few studies have evaluated exposure to phthalates in pregnant women and young children, despite their potential sensitivity to adverse effects of phthalates. MEP, MBP, MBzP, and MEHP were frequently detected in 25 urine samples collected from pregnant African-American and Dominican women living in New York City.[6] The median concentrations of MEP and MEHP were somewhat higher and the median MBzP concentration was similar to that measured in NHANES 1999–2000 for several demographic groups, including adults, women, non-Hispanic blacks, and Mexican Americans. The median concentration of MBP was 1.5–2 times higher than the NHANES 1999–2000 levels, both collectively or when compared within the demographic groups.[25 26] There are only four published studies on the levels of phthalate metabolites in children environmentally exposed to phthalates.[21 45–47] Phthalate monoester concentrations in urine were measured in 19 toddlers aged 12–18 months in Imperial Valley, California.[46] Twelve toddlers provided two urine samples within one month period. Similar to other studies, the metabolites predominantly detected were MEP, MBP, MBzP, and MEHP. The unadjusted mean concentration of MEP and MBP were approximately two and three times higher, respectively, than the geometric mean concentrations in children aged 6–11 years in NHANES 1999–2000,[25 26] whereas the mean concentration of MBzP and MEHP were similar. The concentration of DBP, butylbenzyl phthalate (BBzP), and DEHP metabolites in two groups of German children have been reported recently.[21 45 47] The median urinary concentrations of MBP and MBzP in these German children[21] were about three times higher and two times lower, respectively, than in US children 6–11 years of age.[26] The urinary concentrations of MBP, MBzP, and MEHHP and MEOHP, two oxidative metabolites of DEHP, were significantly higher in 36 children (2–6 years of age) than in 19 adults (including four of their teachers and 15 parents).[21 47] In contrast, the urinary levels of MEHP were significantly higher in the adults than in the children. More importantly, urinary concentrations of the two oxidative metabolites MEHHP and MEOHP were higher than MEHP for both children and adults. The higher concentrations of oxidative metabolites in the children than in the adults might indicate an enhanced oxidative metabolism in children. The higher urinary levels of DEHP oxidative metabolites than of MEHP were confirmed in a larger study of 254 children 3–14 years old.[45] Boys showed higher urinary concentrations than girls for all three DEHP metabolites. The oldest children (13 and 14 years old) had the lowest mean urinary concentrations of DEHP oxidative metabolites. The median urinary concentrations of MEHP in these two groups of German children[21 45] were about 1.5 times higher than in US children 6–11 years of age.[25 26] The urinary concentrations of DEHP oxidative metabolites were not measured in NHANES 1999–2000, but they will be in future NHANES.

The urinary levels of several phthalate monoesters in various populations in Germany have been reported.[20 21 36 45 47] In eight non-occupationally exposed people, the mean urinary levels of MEP, MEHP, and MBP were considerably higher than the levels found in the NHANES 1999–2000 population, but the MBzP levels were about half.[36 47] The same group of investigators also measured the concentrations of these phthalate metabolites in the first-morning voids from 53 females and 32 males, aged 7–64 years, who live in southern Germany.[20] Similar to the NHANES 1999–2000 population, females had higher creatinine adjusted urinary levels of MEP, MBP, MBzP, and MEHP than males. However, the median levels of MBP were eightfold higher and the median levels of MEHP were threefold higher in the German study than in the NHANES 1999–2000 population. Concentrations of MEP were about half those found in NHANES 1999–2000, but levels of MBzP were similar. These data suggest similar sex related differences in exposure to phthalates, both in the United States and in Germany, although differences in the exposure to phthalates may exist geographically. Furthermore, the varying amounts of phthalates used within each country and varying sampling collection times (for example, first-morning voids versus non first-morning voids, seasonal versus throughout-the-year collection) may account for the

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observed differences when comparing the levels of urinary phthalates found in Germany and in the United States.

Another limitation of NHANES is that the survey by its design evaluates the general population, but not specific populations who might be highly exposed.[48–50] These specific populations need to be examined by other studies designed to determine possible associations between high exposures and adverse health effects. Human populations at risk of high exposure to phthalates include individuals receiving medical treatments with polyvinyl chloride (PVC) plastic devices made flexible by the addition of large amounts (up to 40% by weight) of plasticizers, such as DEHP. Specifically, infants born prematurely who often spend their first days or weeks in neonatal intensive care units (NICU) are at especially high risk of exposure to high levels of DEHP.[51] In the first study to evaluate exposure to DEHP in infants undergoing intensive therapeutic interventions, the median urinary concentrations of MEHP in six premature newborns were found to be about 50 times higher than in children between 6 and 11 years of age from the general US population.[49] A second study on urinary levels of MEHP in NICU infants confirmed the higher levels in NICU patients compared to levels in children from the general US population.[52] The researchers assessed the use of DEHP containing products in the care of 54 infants admitted to one of two NICUs, and examined the intensity of use of these products in relation to levels of MEHP in the infants' urine. Before data collection, three DEHP exposure categories (low, medium, and high) were defined based on a review of medical products typically used in both NICUs and information provided by their manufacturers with respect to DEHP content. There was a direct relation between urinary MEHP levels and the extent of medical product use. Urinary MEHP levels among infants considered to be in the highest DEHP exposure category based on the use of high DEHP containing products were five times as high as levels among infants in the lowest DEHP exposure group; urinary MEHP levels among infants in the medium DEHP exposure group were twice as high.

Concerns exist regarding the overall benefits of medical procedures using PVC devices and the potential risks associated with exposure to DEHP.[51] Additional research is needed to establish whether children undergoing intensive therapeutic interventions using DEHP containing devices are at higher risk for altered health outcomes than children undergoing similar treatments but not potentially exposed to DEHP.

HUMAN HEALTH ENDPOINTS
Overview
Although the review on the potential human health effects of phthalates focuses on epidemiological studies, in vitro and experimental animal studies are briefly discussed at the beginning of each health section to provide both background information and guidance in identifying relevant human health endpoints that may be associated with phthalates. Laboratory studies have shown that phthalates exhibit marked differences in toxicity in relation to their structure (table 1). The most well studied structure-activity relationships are for the induction of testicular toxicity in rats. The length of the alkyl side-chain predicted testicular toxicity, with specific phthalates being toxic while others were not.[53 54] Furthermore, the branching of the alkyl side-chain such as that observed within structural isomers (for example, butyl phthalates) resulted in differences in toxicity.[55 56] Laboratory

studies have also shown that the phthalate monoester metabolites are associated with testicular toxicity while the parent diesters are generally not.[57] In addition to the structure of the phthalate ester predicting toxicity, there is also evidence that there is an age dependency in the testicular toxicity. Adult rats were generally less sensitive than young pubertal rats[58] or rats exposed in utero. Administration of some phthalates (for example, DEHP and DiNP) to rodents resulted in adverse liver effects including increased liver weights, elevated liver enzyme levels, histological changes, and, in some cases tumours.[1 13] These effects were associated with peroxisomal proliferation,[59] a process related to metabolism of cholesterol and fatty acids. However, the relevance of these effects to humans is debatable due in part to metabolic differences among species.[60–63]

Developmental outcomes
In experimental animal studies, primarily in rodents, some phthalates induced reproductive tract developmental anomalies that consisted of epididymal malformations or absence of the epididymis, increased incidence of hypospadias, cryptorchidism, decreased anogenital distance (AGD), delayed preputial separation (pubertal milestone), retention of thoracic nipples, and testicular lesions. The testicular lesions were characterised by seminiferous tubule atrophy and Leydig cell hyperplasia. Reproductive tract developmental anomalies were seen in rats dosed during gestation and/or lactation with DBP,[64–66] DEHP,[54] BBzP,[67 68] and DINP.[54] Gray and coworkers failed to find similar effects for DEP or dimethyl phthalate (DMP).[54] Although the effects of DBP, BBzP, DEHP, and DINP were consistent with an antiandrogenic mode of action, in human androgen receptor (AR) transcriptional activation assays, DBP and DEHP or their metabolites did not interact with the AR in vitro.[69] Later evidence suggests that the mechanism may be through reduced fetal testosterone synthesis during sexual differentiation in the male.[70]

Developmental anomalies
Developmental endpoints of interest in humans include hypospadias, cryptorchidism, and AGD. Although there are many published human studies on potential risk factors for hypospadias and cryporchidism, few have explored their relationships with exposure to phthalates. There is currently limited evidence to conclude whether an association between phthalates and male developmental anomalies exists.

Van Tongeren and coworkers in 2002 developed a job-exposure matrix for investigating the association between maternal occupational exposure to potential endocrine disrupting compounds, including phthalates, and hypospadias.[71] Vrijheid and coworkers applied this job exposure matrix in a large study on 3471 cases of hypospadias recorded from 1980 to 1996 by the National Congenital Anomaly System in England and Wales.[72] Overall there was no association of hypospadias with maternal occupational exposure to phthalates. However, for the 1992–96 period there was an increased odds ratio (OR) for "probable" versus "unlikely" exposure to phthalates before adjustment for social class (1.52, 95% CI 1.05 to 2.20) and an OR of 1.26 (95% CI 0.81 to 1.97) after social class adjustment. Hairdressers made up the majority of cases in the probable exposure category. However, in another analysis in which births, rather than all congenital anomalies, were used as the denominator, hairdressers did not show an increased risk of

hypospadias (observed to expected ratio was 0.95, 95% CI 0.66 to 1.32). The authors stated that the association between phthalates and hypospadias should be interpreted cautiously since associations were only seen in one time period and adjustment for social class weakened the association. Furthermore, in addition to potential exposure to phthalates, hairdressers are exposed to several classes of chemicals, including solvents and dye formulations, making it difficult to attribute potential health risks to phthalates in this occupational group.

The job-exposure matrix developed by Van Tongeren and coworkers was also applied by Pierik and coworkers in 2004 in the Netherlands to assess maternal and paternal occupational exposure to endocrine disruptors in relation to cryptorchidism and hypospadias.[73] In this nested case-control study within a cohort of 8695 male newborns in the city of Rotterdam, there were 78 cases of cryptorchidism and 56 cases of hypospadias. Although not reported in the manuscript, maternal and paternal occupational exposures to phthalates were not associated with either abnormality (Pierik, personal communication).

In a recent study, Swan and colleagues explored the relationship between prenatal exposure to phthalates and shortened AGD in 85 male infants from a multicentre study in Los Angeles, CA, Minneapolis, MN, and Columbia, MO.[74] The anogenital index (AGI), defined as AGD (millimetres) divided by weight (kg) at examination, was obtained in 134 boys 2–30 months of age, and the age adjusted AGI was calculated by regression analysis. For 85 of these male infants, a maternal urine sample collected during pregnancy was available. The mean length of gestation at the time of maternal urine sample collection was 28.3 weeks and the mean age at examination of the infants was 12.6 months (interquartile range was 5–16 months). In regression analyses, maternal prenatal urinary concentrations of MEP, MBP, MBzP, and mono-isobutyl phthalate (MiBP) were inversely related to age adjusted AGI. MEHP was unrelated to AGI; however, the DEHP oxidative metabolites, MEOHP and MEHHP, were of borderline significance with AGI. Monomethyl phthalate and mono-3-carboxypropyl phthalate were not associated with AGI. Covariates considered for inclusion in the regression models included mother's ethnicity and smoking status, time of day and season in which the urine sample were collected, and gestational age at urine collection. None of the covariates were retained in the models since their inclusion did not alter the regression coefficients by more than 15%. The authors also performed categorical analyses in which boys with AGI below the 25th centile were classified as having short AGI. The OR for short AGI were 10.2 (95% CI 2.5 to 42.2) for high compared to low MBP concentration, while for medium MBP concentration compared to low the OR was 3.8 (95% CI 1.2 to 12.3). The OR for high compared to low MEP, MBzP, and MiBP concentration were 4.7, 3.8, and 7.3, respectively (all p values <0.05). Interestingly, shorter AGI was associated with an increased proportion of boys with incomplete testicular descent. It is important to note that among the 134 boys in the study for whom genital measurements were available, no frank genital malformations or disease were detected, and 86.6% of them had both testes classified as normal or normal-retractile.

The results of this study are noteworthy because they represent the first human data showing an inverse relationship between prenatal phthalate exposure and shortened AGD. These data are generally consistent with evidence from experimental animal studies on phthalates and shortened male AGD, a sensitive measure of prenatal anti-androgen exposure.[54 70 75 76] Several study limitations include the collection of only a single urine sample late in pregnancy to measure prenatal phthalate exposure, the lack of adjustment for urinary dilution, the relatively small number of boys, and that the reliability of AGD measurements in humans has not been established because the use of AGD in humans is new. However, these limitations would likely introduce random measurement error and thus bias effect estimates to the null. Replication of the AGD results in other study populations is strongly recommended. In addition, the potential reproductive implications of shortened AGD in males are unclear and require further investigation.

Pubertal development

Colon *et al* in 2000 conducted a study on the relationship between serum levels of phthalates and premature breast development (thelarche) in young girls in Puerto Rico.[77] Forty one patients with thelarche from the San Juan City Hospital's Pediatric Endocrinology Division (median age 20 months, range 6 months to 8 years) and 35 control subjects (median age 46 months, range 6 months to 10 years) were studied. The controls were seen in the San Juan City Hospital for general paediatric care and did not have thelarche or premature sexual development. High levels of DMP, DEP, DBP, DEHP, and MEHP in serum were detected in 28 thelarche patients (68%) compared to 1 and 5 control subjects who had detectable levels of di-isooctyl phthalate and DEHP, respectively. The largest differences between cases and controls were for DEHP where the average concentrations were 450 and 70 ng/ml (ppb), respectively (p < 0.05). Because of concern with potential sample contamination when measuring the parent diester, each case and control sample was corrected with the set of blanks tested the same day of the analysis.

Although this study is noteworthy since the relationship between environmental chemicals and pubertal development is an understudied area, difficulties surrounding the analytical methods used in this study to analyse serum for phthalate diesters have been raised. McKee explicitly described several inconsistencies between the diester serum levels reported in the Colon study and those found in other studies conducted in both experimental animals and humans.[78] Furthermore, phthalate esters are not oestrogenic in vivo in animal studies,[79] so it is unlikely that phthalate oestrogenicity is a relevant mechanism in humans. Other limitations with the design of the study include the large differences in ages between cases and controls, and assessing phthalate levels at the same time as the assessment of outcome (a cross-sectional design). Despite these limitations, Colon and colleagues' study has generated interest and research on the potential association of phthalates with pubertal development.

A novel, though very small, study explored whether pubertal development was altered in 19 adolescents (13 boys and 6 girls) age 14–16 years who had undergone extracorporeal membrane oxygenation (ECMO) as newborns.[80] ECMO treatments are known to result in high exposure to DEHP.[81] The growth centiles for the ECMO boys and girls were normal for age and sex, and the levels of LH, FSH, testosterone (in boys), and oestradiol (in girls) were within

the normal reference ranges for stage of pubertal development. In ECMO boys in Tanner stage 4–5, the mean phallic length was slightly longer than the reference range (mean for ECMO boys was 11.2 cm, reference range 8.6–9.9 cm). In ECMO boys in Tanner stage 2–3, the mean testicular volume was slightly larger than the reference range (11 ml, range 5–10 ml). The authors concluded that adolescents exposed to significant quantities of DEHP as neonates showed no effects on physical growth and pubertal maturity. However, it is difficult to make definitive conclusions based on this study because it was very small and normal reference ranges for reproductive hormones, physical growth, and sexual maturation are quite wide. Furthermore, the postnatal levels of DEHP or its metabolites in these children were not measured. A larger study would be necessary to detect subtle changes, if they exist.

Male reproductive health

Experimental animal studies have reported associations between pubertal and adult exposure to DBP, BBzP, DEHP, and testicular toxicity.[82–84] Pubertal animals were found to be more sensitive than sexually mature animals to the testicular toxicity of phthalates.[85 86] Testicular toxicity with germ cell loss was primarily induced through effects on Sertoli cells, the cell type responsible for initiation and maintenance of spermatogenesis.[87–90] It is important to note that species differences in sensitivity to DEHP toxicity have been observed, with markedly lower responses in marmosets than in rodents.[91]

Semen quality

The human studies described below provide limited evidence of an association between some phthalates, specifically MBP and MBzP, and poor semen quality. Duty and colleagues have published four manuscripts exploring the relationships between environmental exposure to phthalates and semen characteristics, sperm DNA damage, and serum reproductive hormones.[92–95] Study subjects consisted of male partners of subfertile couples that presented to an infertility clinic in Massachusetts, USA. At the time of the clinic visit, one semen sample, and blood and urine samples were collected. Computer aided sperm analysis (CASA) was used to measure sperm concentration and motility, as well as motion parameters. Strict criteria were used to assess sperm morphology. Sperm DNA damage was assessed with the neutral comet assay. Because the Duty et al study was ongoing, the number of subjects in each publication varies; however, there is overlap of subjects among the publications.

Among 168 men, they found dose-response relationships (after adjusting for age, abstinence time, and smoking status) between MBP and sperm motility (OR per tertile: 1.0, 1.8, 3.0; p for trend = 0.02) and sperm concentration (OR per tertile: 1.0, 1.4, 3.3; p for trend = 0.07).[93] They also found a dose-response relationship between MBzP and sperm concentration (OR per tertile: 1.0, 1.4, 5.5; p for trend = 0.02). There was weak evidence of an association between MBP and sperm morphology, MBzP and sperm motility, and MMP and sperm morphology. Among 220 men, MBP, MBzP, and MEHP had inverse associations, although not significant, with VSL (straight line velocity), VCL (curvilinear velocity), and LIN (linearity = VSL/VCL×100) measured by CASA.[94] Unexpectedly, positive relationships were found between MEP and both VSL and VCL.

To quantify sperm DNA damage in samples analysed using the neutral comet assay, Duty and coworkers used VisComet image analysis software to measure comet extent, a measure of total comet length (µm), 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 (µm).[92] In multiple regression models, after adjusting for age and smoking status, for an interquartile range (IQR) increase in MEP concentration the comet extent increased by 3.6 µm (95% CI 0.74 to 6.47) and TDM increased by 1.2 µm (95% CI −0.05 to 2.38). There were no relationships between MBP, MBzP, MEHP, and MMP and any comet assay parameters.

Duty et al recently published data from 295 men on the relationships between urinary levels of phthalate metabolites and serum levels of FSH, LH, SHBG, testosterone, and inhibin-B.[95] After adjusting for age, body mass index, and time of day that the blood sample was drawn, there was an inverse association between MBzP and FSH, and a positive association between MBP and inhibin B. An IQR increase in MBzP was associated with a 10% decrease (95% CI −16 to −4) in FSH. For an IQR increase in MBP, inhibin B increased 4.8% (95% CI 0 to 10). MEHP was inversely associated with testosterone (−0.47 nmol/ml; 95% CI −1.03 to 0.10; p = 0.1).

In summary, Duty and coworkers found evidence of associations between MBP and MBzP and altered semen quality,[93] MEP and sperm DNA damage,[92] and MBP and MBzP and reproductive hormones.[95] Although there is some consistency of their results with experimental animal studies, there are also inconsistencies. For instance, in studies on laboratory animals, MEHP, MBP, and MBzP have been shown to adversely effect semen production and quality. In the Duty et al study, associations were found with MBP and MBzP, but no associations were found with MEHP.[93] In addition, there is no experimental evidence showing that MEP damages DNA. The changes in the serum levels of inhibin B and FSH in association with MBP and MBzP, respectively, did not change in the expected patterns. Therefore, it is unclear whether these associations represented physiological relevant alterations in these hormones, or whether they represented associations found as a result of conducting multiple comparisons. However, despite these inconsistencies with animal data, this work begins to provide us with human data necessary to assess potential risk and the public health implications related to ubiquitous human exposure to phthalates.

Murature and coworkers recruited 21 university students to explore the relationship between sperm concentration and DBP concentrations in the cellular fractions of ejaculates.[96] The geographical location from which the subjects were recruited was not indicated. Furthermore, the statistical analyses performed were not traditional—that is, they did not treat the subjects as a single population. Instead, the authors assumed that there were two populations that differed in their ability to metabolise DBP. It is not entirely clear, but it seems that the two populations were defined by a visual inspection of DBP concentrations. Based on DBP concentrations, the subpopulations were defined as those with a lower ability to metabolise and those with a greater ability to metabolise DBP. Within each subpopulation, they explored the relationship between DBP and sperm concentration. In the subpopulation with a lower ability to metabolise DBP, there was an inverse relationship between sperm concentration and DBP (r = −0.4; slope of regression −0.7). In the subpopulation with a greater ability to

metabolise DBP, there was also an inverse correlation of −0.4 (slope of regression −0.6) between DBP and sperm concentration. The study did not measure or adjust for potential confounders.

In India, Rozati and coworkers studied 53 men: 21 infertile men with poor semen quality and 32 control men with normal semen parameters.[97] Phthalate esters were measured in seminal plasma and results were reported as the sum of a mixture of DMP, DEP, DBP, BBzP, DEHP, and DnOP. The concentration of phthalates was inversely correlated with sperm morphology (r = −0.77, p < 0.001) and positively correlated with the percentage of single stranded DNA in sperm (r = 0.86, p < 0.001) assessed with the sperm nuclear chromatin condensation test. The concentration of phthalates was not correlated with ejaculate volume, sperm concentration, or motility. The authors measured total phthalate diesters and did not report results for individual phthalates. The results are noteworthy because they demonstrate the presence of phthalates in seminal plasma. However, because diesters were measured, sample contamination is a potential concern.

Time to pregnancy

In Sweden, Modigh and colleagues conducted a well designed retrospective study on the relationship between time to pregnancy among partners of men occupationally exposed to DEHP.[98] They recruited couples in which the male partner was employed at plants producing or using DEHP (that is, plants processing PVC plastic); 227 couples participated and contributed 397 pregnancies. Exposure was estimated using air sampling measurements combined with questionnaires assessing work location and work tasks. Men were assigned to three exposure groups (none, low [<0.1 mg/m^3], and high [>0.1 mg/m^3]). No associations were found between exposure to DEHP and time to pregnancy. Statistical methods were used in which they also considered the time lag for sperm production and maturation, as well as restricting the analyses to the first recorded pregnancy. The results remained unchanged. Although maximum exposure was 2.1 mg/m^3, paternal exposure exceeded 0.5 mg/m^3 for only four pregnancies.

Testicular cancer

Although exposure to phthalates was not directly measured, the relationship between testicular cancer and occupational exposure to PVC plastics has been explored in two studies. Hardell *et al* used the Swedish Cancer Registry to identify both cases of testicular cancer (n = 148) and controls (n = 315).[99] A self-administered questionnaire was used to assess occupational histories. Potential occupational exposure to PVC plastics were confirmed by the employers. Six cases with seminoma and two controls reported exposure to PVC (OR = 5.6, 95% CI 1.1 to 196). Exposures to other types of plastics were not associated with increased risk. In a subsequent larger study, Hansen in 1999 did not find an association (OR ∼1.0) between occupational exposure to plastics, mainly PVC, and testicular cancer.[100] Hansen's study was a nationwide case-control study (3745/7212) with objective assessment of work histories using the national Supplementary Pension Fund in Denmark. In conclusion, based on these two studies there is inadequate evidence of an association between occupational exposure to PVC plastics and testicular cancer.

Female reproductive health

Recent studies in experimental animals have found associations between DEHP and its metabolite MEHP and ovarian toxicity in sexually mature rats.[101–103] Effects of DEHP include prolonged oestrous cycles, suppressed or delayed ovulation, smaller preovulatory follicles due to reduced granulosa cell size, and decreased circulating serum oestradiol. Suppression of oestradiol production by granulosa cells secondarily resulted in increased serum FSH levels and an absence of the LH surge necessary for ovulation. DEHP also suppressed serum progesterone levels. Thus, DEHP and MEHP resulted in hypoestrogenic, hypoprogestinic, anovulatory cycles in adult female rats. MMP, MEP, monopropyl phthalate, MBP, and monopentyl phthalate had no effects on oestradiol production.[103] Studies in pregnant and pseudopregnant rats have also found associations between DBP and BBzP and impaired implantation in mated females and decreased decidualisation in the pseudopregnant animals.[104 105] Based on the experimental studies, endpoints of interest in humans should include ovulatory function, which may include reduced fertility or prolonged time to pregnancy, and serum levels of oestradiol, progesterone, LH, and FSH.

Endometriosis

Cobellis and colleagues in Italy published in 2003 a case-control study on the relationship between plasma and peritoneal fluid levels of DEHP and endometriosis.[106] Study subjects were fertile women that underwent diagnostic laparoscopy for ovarian cysts or chronic pelvic pain and dysmenorrhoea. Patients with histological confirmation of endometriosis were enrolled as cases (n = 35). Controls (n = 24) were healthy age matched subjects without known infertility or reproductive diseases. Blood samples were obtained the day before the surgical procedure or immediately before anaesthesia for laparoscopy. Peritoneal fluid was obtained by culdocentesis during laparoscopy. Women with endometriosis had higher plasma DEHP concentrations (median 0.57 μg/ml, IQR 0.06–1.23) than control women (0.18 μg/ml, IQR 0–0.44) (p = 0.0047). Plasma MEHP concentrations did not differ between groups and were generally very low except for two cases. For cases, the median MEHP was 0.38 μg/ml (IQR 0.1–0.97) and for controls the median was 0.58 μg/ml (IQR 0.34–0.71). Cases and controls had similar levels of peritoneal DEHP and MEHP concentrations. The correlation between serum DEHP and MEHP concentrations was weak (r = 0.16, p ⩾ 0.3). The weak relationship between serum levels of DEHP and MEHP raises questions regarding the utility of the DEHP measurements. Because peritoneal fluid may contain esterases capable of hydrolysing DEHP to MEHP, further exploration into why the relationships between DEHP and MEHP with endometriosis differed is warranted.

Pregnancy outcomes (gestational age, complications of pregnancy)

Latini and coworkers measured serum DEHP and MEHP in the cord blood of 84 newborns born at the general practice Brindisi Hospital, Brindisi, Italy in 2003.[107] The mean gestational age was 38.4 weeks (SD 2.2, range 27–44 weeks) and the mean birth weight was 3220 g (SD 680, range 1150–4350 g). Eleven of the newborns were preterm births and three were very low birth weight babies. All cord blood specimens were collected and handled with glass devices to avoid contamination with DEHP. DEHP and MEHP were

each present in 65 of the samples (77%). MEHP positive newborns (n = 65) had a younger gestational age than MEHP negative newborns (n = 19) (38.16 weeks (SD 2.34) compared to 39.35 weeks (SD = 1.35), respectively, p = 0.033). In logistic regression analyses on gestational age, the OR for absence of MEHP was 1.50 (95% CI 1.013 to 2.21). Gestational age was not associated with DEHP positive newborns compared to DEHP negative newborns. There were no differences in birth weight when DEHP or MEHP positive newborns were compared to DEHP or MEHP negative newborns, respectively. In addition, DEHP or MEHP did not predict sex of the infant, delivery mode, maternal smoking, premature rupture of the membranes, presence of cord loops, neonatal jaundice, or small size for gestational age. Potential study limitations include the lack of information on whether all the deliveries were uncomplicated vaginal births, whether any pregnancies required medical interventions during delivery that may have lead to exposure to DEHP, and the relatively low selectivity and sensitivity of the analytical method used for measuring DEHP and MEHP. The authors hypothesise that the shorter pregnancy duration may be due to phthalates playing a role in inducing an intrauterine inflammatory process, a risk factor for preterm birth.

Tabacova and coworkers in 1999 published an abstract describing the results of a cross-sectional study in Bulgaria on 93 pregnant women residing in proximity to a plastics production plant.[108] Urinary levels of non-specified phthalates were measured and compared in women with normal pregnancies to those complicated by anaemia, toxaemia (includes conditions such as preeclampsia, hyperemesis), and spontaneous abortion. They controlled for demographic characteristics, reproductive history, and lifestyle factors. The overall mean phthalate level for women with any complications was 217 µg/100 ml urine compared to 81 µg/ 100 ml for women with normal pregnancies (p = 0.02). Although these results are intriguing, because only an abstract was published, the potential strengths and limitations of the methods used in the study are unknown. Therefore, this study is only presented for completeness of the review.

Respiratory health

Interior materials used in the home environment include plasticised PVC products used as wall and floor covering materials. These PVC products are potential emission sources of chemicals including, among others, DEHP widely used as a PVC plasticizer. Other sources of emissions include viscosity modifiers and stabilisers used in the PVC products. Phthalates have been measured in residential indoor environments in both house dust and indoor air.[109] The emission chemicals from these PVC products may cause airway inflammation and increase the risk of bronchial obstruction and asthma. MEHP has been shown to induce bronchial hypersensitivity in rats,[110] and preterm infants exposed to respiratory PVC tubing materials had a higher risk of bronchial asthma.[81] MEHP may also have properties similar to prostaglandins, potent mediators of inflammation.[111]

Airway obstruction and lung function

Several epidemiological studies have explored the relationship between interior materials in the indoor environment and airway obstruction and asthma. These studies used questionnaires or experts' observation to identify and categorise residential indoor surface materials.[112][113] In

another study, researchers explored the relationship between wheeze in childhood and synthetic bedding.[114]

Jaakkola and coworkers conducted a matched pair case-control study (251 cases and controls) within a birth cohort of 3754 children born in Oslo, Norway from 1992 to 1993.[112] Information on the children's health and environment was obtained at 6, 12, 18, and 24 months of follow up. The interior surfaces were categorised by trained experts and exposure was categorised based on the materials used; in addition, a plasticizer exposure index was calculated. Physician or medical records or information form questionnaires was used to identify bronchial obstruction, defined as two or more episodes with symptoms and signs of bronchial obstruction or one episode lasting more than one month. In both the crude analysis and in the adjusted analysis, the risk of bronchial obstruction was greater in the presence of PVC in the floors (adjusted OR = 1.89; 95% CI 1.14 to 3.14) and of textile wallpaper in one or more rooms (adjusted OR = 1.58; 95% CI 0.98 to 2.54) compared with the homes with wood or parquet flooring and painted walls and ceilings. Bronchial obstruction was also associated with the plasticizer exposure index (adjusted OR = 2.72; 95% CI 1.50 to 4.91, comparing the highest quartile of exposure to the reference category). The study was well designed and a variety of potential confounders were collected and adjusted for. Among the same cohort of children, Oie and coworkers in 1999 found that the relation of bronchial obstruction to plasticizer exposure index was stronger in homes in the low air change category than in the high air change category.[115]

Jaakkola and coworkers in 2000 conducted a population based cross-sectional study on 2568 Finnish children aged 1–7 years to assess the relationship between the presence of plastic wall materials in the home and respiratory health.[113] A questionnaire was used to assess the child's health and home environment, specifically focusing on the presence of plastic wall materials. The outcomes of interest were doctor diagnosed asthma, allergic rhinitis, persistent wheezing, persistent cough, persistent phlegm, weekly nasal congestion/excretion, and respiratory infections. There were associations between the presence of plastic wall materials and lower respiratory tract symptoms: persistent wheeze (OR = 3.42, 95% CI 1.13 to 10.36), cough (OR = 2.41, 95% CI 1.04 to 5.63), and phlegm (OR = 2.76, 95% CI 1.03 to 7.41). Upper respiratory symptoms were not associated with plastic wall materials. Although there was an association with asthma, the confidence interval was wide (OR = 1.52, 95% CI 0.35 to 6.71).

Ponsonby and coworkers in 2003 published the results of a prospective study on a birth cohort of infants in Tasmania.[114] Parental report of bedding data at 1 month of age was available for 863 infants (of 6378 children) who were participating in a seven year follow up survey. Synthetic bedding included synthetic overlying quilts or synthetic pillows which included foam, sponge, tontine, polyester, and Dacron. One outcome measure was frequency of wheeze defined as more than 12 episodes in the past year. Synthetic pillow use at 1 month of age was associated with frequent wheeze at 7 years of age (adjusted relative risk (aRR) = 2.5, 95% CI 1.2 to 5.5) independent of later childhood exposure to synthetic bedding. In addition, current synthetic pillow and quilt use was associated with frequent wheeze (aRR = 6.4, 95% CI 1.2 to 35). Among children with asthma, the age of onset was earlier if synthetic bedding was used in infancy.

Although this study was well designed and the associations between synthetic bedding and wheeze were consistent and strong, it is difficult to determine the potential role, if any, of phthalates emitted from the synthetic bedding. There are many other potential explanations for the observed associations because synthetic bedding may lead to increased exposure to house dust mite, volatile organic compounds, and reduced exposure to endotoxin, all potential risk factors for childhood asthma and wheeze.

Hoppin and coworkers in 2004 explored the association between urinary levels of phthalate monoesters and pulmonary function parameters (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and maximum mid-expiratory flow (MMEF)) among 240 adults from NHANES III call-back cohort.[116] After controlling for race, age, height, body mass index, smoking history, and current smoking, there was an association between MBP urinary levels and three measures of pulmonary function (FVC, FEV_1, PEF) in males but not females. Among men there were large decrements in lung function; for an IQR change (25th to 75th centile) in urinary MBP levels, FEV_1 decreased 112 ml (SE = 51, p = 0.04), FVC decreased 131 ml (SE = 63, p = 0.04), and PEF decreased 367 ml (SE = 181, p = 0.05). MEP was associated with lower FVC and FEV_1 in men. MEHP was not associated with any of the lung function measures. In analyses limited to non-smoking men, point estimates remained essentially unchanged. Among non-smoking women, MEHP was positively associated with FEV_1 and MMEF. In this cross-sectional study, it is unclear why associations were only found in men and why MEHP, a metabolite of DEHP which is found in PVC plastics and implicated in the studies on residential use of PVC plastic interior materials,[111–113] was not associated with lung function decrements. Because the Hoppin *et al* study was cross-sectional it was also not possible to determine whether the decrements in lung function were associated with long term phthalate exposure or associated with recent exposure and thus represent short term decrements that may be reversible. However, despite these uncertainties, the study had several strengths including objective measures of lung function and the use of biological measures of phthalate exposure.

Bornehag and colleagues in 2004 explored the relationship between allergic symptoms in children and the concentration of phthalates in house dust.[117] A nested case control study was conducted within a cohort of 10 852 children 1–6 years of age in Varmland, Sweden. The cases consisted of 198 children with persistent allergic symptoms and 202 controls without symptoms. Persistent symptoms were defined as eczema, wheezing, or rhinitis without a cold during the preceding 12 months on the initial questionnaire and at least two of three symptoms reported 18 months later on the follow up questionnaire. Controls did not report symptoms on either questionnaire. In addition to questionnaire assessment of symptoms, physicians performed examinations and obtained a medical history to assess asthma, rhinitis, and eczema. Apart from 23 children, case-control assignment based on the questionnaire agreed with the physician diagnosis. Separate statistical analyses were presented for case control status based on the questionnaire and for physician diagnosis of disease. PVC flooring materials in the child's bedroom were associated with questionnaire case status (OR = 1.59, 95% CI 1.05 to 2.41). Children with physician diagnosed asthma, rhinitis, or eczema had higher BBzP concentrations in bedroom dust than children without disease. Higher DEHP dust concentrations were also found in the bedrooms of children with physician diagnosed asthma. The BBzP relationships remained after restricting the analysis to homes with PVC flooring in the child's bedroom.

In dose-response analyses in which phthalates were categorised into quartiles, BBzP was associated with case status in the crude and adjusted analyses. Adjustments were made for the following covariates: environmental tobacco smoke, gender and age of child, type of building, construction period of building, self reported water leakage, and exposure to other phthalates. In addition, associations between BBzP and doctor diagnosed rhinitis and eczema, and between DEHP and doctor diagnosed asthma were found.

The authors hypothesised that in addition to the different toxicological and pharmacokinetic properties of the phthalates, the physical and chemical properties of the different phthalates may partially account for the differences noted between BBzP and DEHP with regard to the type of symptom. BBzP would likely have higher concentrations in the gas phase while DEHP would likely have higher concentrations in the condensed phase (that is, dust and airborne particles). This may account for the association of DEHP with lower airway symptoms compared to the association of BBzP primarily with skin and mucosal irritation. Potential confounding by socioeconomic status (SES) was considered unlikely in the study because of a more homogeneous distribution of SES in Sweden. Furthermore, when the analyses were restricted to single family houses the results were unchanged.

In conclusion, there is limited epidemiological evidence of an association between some phthalate esters and allergic and airway symptoms. A cross-sectional study found associations between urinary levels of MBP and lower pulmonary function in adults,[116] while a case-control study of children found associations between allergic symptoms and house dust levels of BBzP and DEHP.[117] Several studies have also reported relationships between bedding materials and PVC building materials in the indoor environment and airway obstruction or asthma.[112–114]

OCCUPATIONAL HEALTH STUDIES: GENERAL HEALTH EFFECTS

Nielsen and coworkers conduced a small study on 44 workers at a PVC processing plant in which DiDP, DEHP, and BBzP were used as plasticizers.[118] Workers were employed for an average of 8 years (range 1–21). The controls were five healthy men who were members of the laboratory staff. Exposure was assesses by phthalate diester measurements in air and phthalate monoester metabolites in urine. Air exposures varied and in specific job titles exceeded 2 mg/m^3. In workers, they did not find an increased prevalence of peripheral neuropathy, obstructive lung disease, or decreased lung function measures. However, the study was small and underpowered to detect changes in the prevalence of these outcomes.

Among 147 workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins, Milkov and coworkers in 1973 reported a moderately increased prevalence of toxic polyneuritis.[119] It was difficult to relate findings to specific phthalates or phthalates in general as there were other workplace chemical (for instance, vinyl

chloride, carbon monoxide, and hydrogen chloride) and physical exposures.

Occupational studies on potential associations with health risks are very limited. Further research among occupationally exposed workers is needed to better define potential risk. Occupations with potential exposure include, among others, producers of phthalates, PVC plastic manufacturers, fragrance industry, and workers in beauty care, such as nail technicians and hairdressers.

CONCLUSIONS

Depending on the health endpoint of interest, there is currently only limited or inadequate human data on the relationships between exposure to phthalates and human health effects. Although phthalates have been in commercial use for over 50 years, human studies using biological measures of exposure (that is, monoester) have generally been conducted within the last five years. Further epidemiological studies are needed to advance our understanding of potential human health risks of phthalates. The following are the most common limitations of the published studies on associations between exposure to phthalates and health outcomes: (1) small human populations;[74 77 92–95 106–108 118 119] (2) sensitivity, selectivity, or contamination issues related to some analytical methods;[77 96 97 106–108] and (3) lack of either

Key points

► Phthalates are a family of compounds that exhibit marked differences in toxicity in relation to their structure. In laboratory animal studies, some phthalates have been associated with developmental and reproductive toxicity. These studies show that the most sensitive life stages are: fetal > peri-pubertal > adult (mature).

► Most of the US population is exposed to phthalates. Data suggest that phthalate exposure is also prevalent in other countries (e.g. Germany and Denmark). Exposure is believed to be primarily through ingestion and inhalation, although dermal exposure may be important for some phthalates (e.g. diethyl phthalate). Special populations, such as neonates in intensive care units, may be highly exposed to di(2-ethylhexyl phthalate, DEHP) through the use of medical devices.

► Phthalates have short biological half-lives, metabolise quickly, do not accumulate, and are primarily excreted in the urine. Therefore, the urinary concentrations of phthalate metabolites provide an excellent biomarker of exposure.

► The metabolism and excretion of phthalates varies based on their chemical structure. Oxidative metabolism is prevalent for high molecular weight phthalates (e.g. DEHP, di-isononyl phthalate). Therefore, biomonitoring strategies should be designed with this consideration in mind.

► Exposure to high doses of some phthalates causes reproductive and developmental toxicities in both male and female animals. Although several human studies have explored possible associations between phthalate esters and altered semen quality, shortened gestation, reduced anogenital distance in baby boys, and premature breast development in young girls, data are limited and further study is recommended.

► There is limited epidemiological evidence of an association between some phthalate esters, specifically DEHP, dibutyl phthalate, and butylbenzyl phthalate, and allergic and airway symptoms.

environmental[72 80 99 100 112–114 119] or biological measures of phthalates.[72 80 98–100 112–114 117 119] In contrast, toxicological studies provide sufficient evidence that some phthalate esters and their metabolites, specifically MBP, MBzP, and MEHP, are reproductive and developmental toxicants in rats.[54 64–68]

Based on our review of the literature and our understanding of the toxicity of phthalates from experimental studies, the following recommendations for future research are put forward. Studies need to be conducted to identify the phthalate metabolites relevant to human health and the relative contribution of the pathways through which humans are exposed to phthalates. A few outstanding questions and issues related to these needs include the following. What oxidative metabolites of high molecular weight phthalates (for example, DEHP, DiNP, DiDP) are the best biomarkers of exposure to these phthalates? To assess DEHP exposure, is it sufficient to measure only urinary levels of MEHP or should the oxidative metabolites also be measured? If MEHP and oxidative metabolites are measured, how do we use these measures in epidemiological studies? What is the proportional contribution of ingestion, inhalation, and dermal exposure to phthalate body burden?

Human health effects studies are needed to explore: (1) the relationship of gestational exposure to phthalates with male reproductive tract development (AGD, hypospadias, cryptorchidism); (2) the relationship of both early life (that is, gestational and pubertal) and adult exposure with male and female reproductive function (that is, menstrual cycle and semen quality assessments, as well as measures of fertility and fecundity); and (3) the relationship of early life and adult exposure with asthma and obstructive airway disease. Another area of interest is the relationship, if any, between early life exposure and testicular cancer, part of the triad of the testicular dysgenesis syndrome.[120]

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