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Phthalates and other additives in plastics: human exposure and associated health outcomes

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Concern exists over whether additives in plastics to which most people are exposed, such as phthalates, bisphenol A or polybrominated diphenyl ethers, may cause harm to human health by altering endocrine function or through other biological mechanisms. Human data are limited compared with the large body of experimental evidence documenting reproductive or developmental toxicity in relation to these compounds. Here, we discuss the current state of human evidence, as well as future research trends and needs.

Because exposure assessment is often a major weakness in epidemiological studies, and *in utero* exposures to reproductive or developmental toxicants are important, we also provide original data on maternal exposure to phthalates during and after pregnancy ($n = 242$). Phthalate metabolite concentrations in urine showed weak correlations between pre- and post-natal samples, though the strength of the relationship increased when duration between the two samples decreased. Phthalate metabolite levels also tended to be higher in post-natal samples.

In conclusion, there is a great need for more human studies of adverse health effects associated with plastic additives. Recent advances in the measurement of exposure biomarkers hold much promise in improving the epidemiological data, but their utility must be understood to facilitate appropriate study design.

Keywords: bisphenol A; endocrine disruption; epidemiology; phthalate; polybrominated diphenyl ether; reproductive health

1. INTRODUCTION

Advances in materials science and engineering in recent decades have led to the widespread and diverse use of plastics to provide cheaper, lighter, stronger, safer, more durable and versatile products and consumer goods that serve to improve our quality of life. Plastics can be designed to keep our foods fresher for longer periods of time, can provide therapeutic benefits through timed-release pharmaceuticals and other medical applications, and can prevent electronics and other household items from starting or spreading fires (see Andrady & Neal 2009; Thompson *et al.* 2009*a*,*b*). However, scientific, governmental and public concern exists over the potential adverse human health risks related to ubiquitous exposures to plastic additives among the general population. The leading hypothesis for these growing concerns is that certain chemicals, used in plastics to provide beneficial physical qualities, may also act as endocrine-disrupting compounds (EDCs) that could lead to adverse reproductive and developmental effects (NRC 1999). In men, environmental or occupational exposures to EDCs may be associated with or lead to declined reproductive capacity or possibly increased risk of testicular or prostate cancer (Fleming *et al.* 1999; Pflieger-Bruss *et al.* 2004; Toft *et al.* 2004). In fact, a number of studies have suggested the use of circulating reproductive hormone levels (follicle-stimulating hormone (FSH) and/or inhibin B) as a surrogate measure for semen quality or fecundity in epidemiologic studies (Jensen *et al.* 1997; Uhler *et al.* 2003; Mabeck *et al.* 2005), although other recent studies suggest hormone levels may lack sufficient ability to predict poor semen quality (Dhooge *et al.* 2007; Meeker *et al.* 2007*a*). Endocrine alterations in women resulting from environmental or occupational exposure may represent increased risk for endometriosis, reproductive and other endocrine-related cancers, or impaired oocyte competence, ovarian function or menstrual cycling (Nicolopoulou-Stamati & Pitsos 2001; Pocar *et al.* 2003; Windham *et al.* 2005). Effects of early life exposures to EDCs remain unclear, though it has been suggested that foetal or childhood exposure may lead to altered sex differentiation (Toppari & Skakkebaek 1998), effects on neurological and reproductive development (Tilson 1998; Teilmann *et al.* 2002; Colborn 2004, 2006; Swan *et al.* 2005) and

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increased risk of reproductive problems or cancer later in life (Damgaard *et al.* 2002; Aksglaede *et al.* 2006; Main *et al.* 2006*a*). Programming in early life can determine an individual's future health; therefore, early chemical exposures may have long-term impacts later on in life (Gluckman *et al.* 2008). A leading hypothesis for a collection of linked conditions in human males exposed to EDCs *in utero* is termed 'testicular dysgenesis syndrome (TDS)'. TDS represents a number of reproductive disorders of varying severity that are associated with disturbed gonadal development, including cryptorchidism, hypospadias and smaller reproductive organs (Olesen *et al.* 2007). Later in life, the effects of TDS are hypothesized to manifest as a reduction in semen quality and infertility as well as an increased risk for testicular cancer.

Exposure to plastic additives and other EDCs may cause altered endocrine activity and reproductive development through a number of biological mechanisms, which can target different levels of the hypothalamic–pituitary–gonad/thyroid axis, ranging from effects on hormone receptors to effects on hormone synthesis, secretion or metabolism (Boas *et al.* 2006; Bretveld *et al.* 2006). The purpose of this manuscript is not to discuss the various biological pathways or the hundreds of animal and *in vitro* studies that have been conducted on plastic additives as reproductive and developmental toxicants, but rather to review the existing epidemiologic literature on human exposure to these compounds and the relationship with adverse reproductive or developmental endpoints. Because exposure assessment is a fundamental and frequently weak component in large epidemiological studies due to technical, logistic and financial constraints, and *in utero* exposures are among the exposure periods of greatest concern with regard to EDCs, we also provide original data on human exposure to a class of potential endocrine-disrupting plastic additives during and after pregnancy.

Despite the increasing concern for human health impacts associated with plastic additives, there remains a paucity of human studies that have investigated these relationships. While the clinical significance of some markers of endocrine disruption, reproductive health or altered development that commonly appear in the human research literature remains unclear, such as declines in semen quality or subclinical alterations in circulating hormone levels, there is a limited but growing body of evidence for such changes to be associated with environmental and occupational exposure to plastic additives and other potential EDCs. In addition, these markers may serve as intermediate indicators that altered endocrine function is the pathway linking environmental exposures to clinical reproductive and developmental effects. Also, because such a large number of people are exposed to background levels of a number of proven or suspected EDCs, even seemingly subtle epidemiologic associations may result in large increases in reproductive and other endocrine-related disease among populations and thus should be of great public health concern. The background material presented in this manuscript is meant as an introductory review of human studies conducted in this area to date. The reader is directed to the individual references for additional study detail. We focus here on three types of plastic additives—phthalates, bisphenol A (BPA) and polybrominated diphenyl ethers (PBDEs)—because there is laboratory evidence for reproductive or developmental effects in relation to exposure to these compounds. These chemicals were also chosen to be discussed here based on strong evidence for widespread human exposure (CDC 2005; Calafat *et al.* 2008; Sjodin *et al.* 2008).

2. PHTHALATES

(a) *Exposure*

The diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly known as phthalates, are a group of man-made chemicals widely used in industrial applications. High-molecular weight phthalates (e.g. di(2-ethylhexyl) phthalate (DEHP)) are primarily used as plasticizers in the manufacture of flexible vinyl plastic which, in turn, is used in consumer products, flooring and wall coverings, food contact applications and medical devices (David *et al.* 2001; ATSDR 2002; Hauser & Calafat 2005). Manufacturers use low-molecular weight phthalates (e.g. diethyl phthalate (DEP) and dibutyl phthalate (DBP)) as solvents in personal-care products (e.g. perfumes, lotions, cosmetics), and in lacquers, varnishes and coatings, including those used to provide timed releases in some pharmaceuticals (ATSDR 1995, 2001; David *et al.* 2001).

As a result of the ubiquitous use of phthalates in personal-care and consumer products, human exposure is widespread. Exposure through ingestion, inhalation and dermal contact is considered important routes of exposure for the general population (Adibi *et al.* 2003; Rudel *et al.* 2003). For infants and children, added skin contact with surfaces and frequent mouthing of fingers and other objects (e.g. plastic toys) may lead to higher phthalate exposures, as might ingestion of phthalates present in breast milk, infant formula, cow's milk or food packaging (Sathyanarayana 2008). Frequent use of personal-care products may lead to higher exposures to the lower molecular weight phthalates, as increased exposures have been found among men reporting recent use of cologne and aftershave (Duty *et al.* 2005*a*) and among infants whose mothers reported recent use of certain infant-care products (lotions, powders and shampoos) (Sathyanarayana *et al.* 2008). Parenteral exposure from medical devices and products containing phthalates is also an important source of high exposure to phthalates, primarily DEHP (ATSDR 2002; Green *et al.* 2005; Weuve *et al.* 2006), for hospitalized populations.

Upon exposure, phthalates are rapidly metabolized and excreted in urine and faeces (ATSDR 1995, 2001, 2002). Owing to the ubiquitous presence of phthalates in indoor environments and concern for sample contamination when measuring the parent diesters in biological samples, the most common approach for investigating human exposure to phthalates is the measurement of urinary concentrations (biomarkers) of phthalate metabolites. The Centers for Disease Control and Prevention's (CDC) Third National Report on Human Exposure to Environmental Chemicals showed

that the majority of people in the USA have detectable concentrations of several phthalate monoesters in urine (mono-ethyl phthalate (MEP), mono-(2-ethylhexyl) phthalate (MEHP), mono-butyl phthalate (MBP) and mono-benzyl phthalate (MBzP)), reflecting widespread exposure to the parent diester compounds among the general population (CDC 2005). Two oxidative metabolites of DEHP, mono-(2-ethyl-5-hydroxylhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) were present in most subjects at urinary concentrations higher than those of MEHP, the hydrolytic metabolite of DEHP (CDC 2005).

(b) *Exposure assessment for epidemiologic studies*

Exposure assessment is a vital component in environmental epidemiologic studies, yet the details of measuring exposure and the appropriateness of the exposure measures being used are often overlooked. Inadequate attention to exposure estimation can lead to substantial exposure measurement error or misclassification, which in most cases will bias study results towards the null hypothesis and hinder the investigator's ability to see associations between exposure and outcome (Armstrong 2003). Phthalates have short biologic half-lives, from hours to days, and are quickly excreted from the body. Most studies involving human health effects from phthalates are small and include only a single urine sample that may or may not reflect an individual's long-term exposure level. However, because health endpoints of interest are likely associated with windows of exposure over time intervals longer than a few days, information on the temporal variability of urinary levels of phthalates is needed to optimize the design of an exposure assessment in human studies. Temporal variability in exposure can result from changes in exposure sources, such as diet and product use, as well as from changes in xenobiotic metabolism. Therefore, an individual's exposure level may depend on several factors and it is probable that levels would vary considerably over short time periods, such as days. Alternatively, consistent individual time-activity patterns from day-to-day and month-to-month coupled with stable microenvironmental phthalate concentrations (or stable concentrations in food) may lead to 'pseudo-steady state' metabolite concentrations over long periods of time (NRC 2006).

Although urinary phthalate metabolite levels accurately measure a person's exposure at a single point in time, determining exposure over time intervals of weeks or months may require multiple measurements if substantial within-individual variability exists over time. Several recent studies have explored temporal variability of urinary phthalate metabolites, and high within-individual variability has been reported over the course of several days (Fromme *et al.* 2007). In adult men, Hauser *et al.* (2004) reported high day-to-day and month-to-month variability in a person's urinary monoester concentrations, but demonstrated that a single sample may adequately predict average monoester concentrations over a three-month period. However, the predictive values and temporal reliability measures differed between the various monoesters, with a single urinary measure being most predictive for MEP and least predictive for MEHP, and the authors concluded that a second urine sample collected at least 30 days after the first urine sample would serve to assist researchers in more adequately predicting a subject's relative monoester concentration rank within a population (Hauser *et al.* 2004). Results from a recent study among New York City children with repeat urine samples collected over the course of six months supported the findings and recommendations reported by Hauser *et al.* (2004), and suggest that they may extend to other populations and exposure periods of interest (Teitelbaum *et al.* 2008).

Recent studies indicate that certain phthalate diesters and their metabolites are measurable in human breast milk, cord blood and other pregnancy-related specimens (Adibi *et al.* 2003; Latini *et al.* 2003; Main *et al.* 2006*b*). However, despite the ability to quantify phthalate or phthalate metabolite concentrations in a range of biological sample types, the measurement of phthalate monoesters in urine is probably the best approach for estimating phthalate exposures in perinatal epidemiologic studies (Hogberg *et al.* 2008). Owing to the concern for reproductive and developmental effects from *in utero* exposures to phthalates, and the lack of data on the temporal stability of phthalate exposure biomarkers in pregnant women, more information is needed on how phthalate exposure and urinary metabolite concentrations vary during these time periods. We investigated the relationship between maternal pre- and post-natal urine phthalate metabolite concentrations in an effort to better understand temporal variability in concentration that may result from altered exposures or possible changes in phthalate metabolism during and after pregnancy.

Women in our study were originally recruited in the first phase of the Study for Future Families (SFFI), a multicentre pregnancy cohort study. SFFI methods are described in detail elsewhere (Swan *et al.* 2003, 2005). Participants in this portion of the study ($n = 242$) completed a questionnaire and gave a single pre-natal (at median 28.6 weeks gestation) and single post-natal (median time post-delivery 12.6 months) spot urine sample. Urinary phthalate metabolite measurements were carried out by the Division of Laboratory Sciences, National Center for Environmental Health, CDC using high-performance liquid chromatography with isotope-dilution tandem mass spectrometry (Silva *et al.* 2004; Kato *et al.* 2005). This approach allows for the simultaneous quantification in human urine of the following phthalate metabolites: MEP, MBP, mono-methyl phthalate (MMP), mono-3-carboxypropyl phthalate (MCPP), a metabolite of di-*n*-octyl phthalate and a minor metabolite of DBP, mono-isobutyl phthalate (MIBP), MBzP, MEHP and two oxidative metabolites of DEHP, MEHHP and MEOHP.

Most metabolite concentrations were above the limit of detection (LOD), which was between 0.95 and 1.07 $\mu g\, l^{-1}$ for all phthalates. Concentrations below the LOD were assigned the specific metabolite value LOD divided by the square root of 2 for statistical analyses, as has been recommended (Hornung & Reed 1990).

Table 1. Distribution of pre- and post-natal phthalate metabolite concentrations in mothers ($\mu g\,l^{-1}$), $N = 242$. Abbreviations: MMP, mono-methyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEP, mono-ethyl phthalate; MBP, mono-butyl phthalate; MBzP, mono-benzyl phthalate; MIBP, mono-isobutyl phthalate.

	pre-/post-natal visit	% above limit of detection	mean ± s.d.	median	90%	max
MMP	pre	44	2.54 ± 4.73	0.71	5.30	42.3
	post	75	3.09 ± 4.33	2.10	5.90	40.7
MCPP	pre	72	2.89 ± 4.05	1.90	5.50	33.2
	post	73	2.95 ± 2.94	1.90	6.80	21.5
MEHP	pre	78	15.4 ± 104	4.00	21.0	1560
	post	92	12.7 ± 19.5	7.15	23.6	173
MEOHP	pre	96	39.6 ± 214	10.2	42.6	2833
	post	99	33.0 ± 73.9	16.0	61.7	728
MEHHP	pre	98	50.8 ± 318	11.3	44.9	4440
	post	98	45.4 ± 106	20.4	83.1	867
MEP	pre	99	816 ± 2839	131	1340	30 528
	post	99	416 ± 1080	133	873	9520
MBP	pre	97	24.7 ± 29.6	17.2	51.8	337
	post	98	30.2 ± 43.3	19.4	68.7	499
MBzP	pre	95	20.9 ± 38.3	9.95	45.8	436
	post	99	26.6 ± 33.8	14.8	64.1	227
MIBP	pre	78	4.16 ± 4.92	2.65	9.00	39.8
	post	85	7.95 ± 17.2	3.60	14.0	179

All phthalate metabolite concentrations were logarithmically transformed to normalize distributions for statistical analysis. Phthalate concentrations were also categorized into quartiles. Using the Pearson correlation coefficient, we examined correlations between maternal pre- and post-natal concentrations of single metabolites and compared quartiles of pre-natal versus post-natal phthalate metabolites. We then compared the differences between pre- and post-natal concentrations within women using a paired Student's *t*-test. Linear regression analyses were then conducted to determine what factors predicted the difference: post-natal phthalate metabolite concentration − pre-natal phthalate metabolite concentration. We considered a number of covariates including difference in (square root) urinary creatinine between pre- and post-natal samples, difference in time between pre- and post-natal study samples, study location, maternal age and race. Results did not differ significantly between creatinine-unadjusted and creatinine-adjusted presentations of the data distribution (data not shown); therefore, we present only creatinine-unadjusted data for all analyses except the regression analyses where creatinine is included as a covariate (Barr *et al.* 2005).

Mothers in the study were primarily Caucasian (77 per cent) with 13 per cent Hispanic/Latina, and 89 per cent reported having health insurance (see supplementary electronic archive table 1). Pre- and post-natal phthalate metabolite concentrations were widely variable with large differences between mean and median concentrations (table 1). Median post-natal concentrations were consistently higher than pre-natal phthalate concentrations for each metabolite except MCPP, for which the median pre- and post-natal concentrations were equal (see supplementary electronic archive figure 1). Pearson correlation coefficients for quartiles of pre- and post-natal phthalate metabolites show that the coefficients increased as the number of days between samples decreased (table 2). Specifically, correlation coefficients for MMP, MEP, MBzP and MIBP were all above 0.40 (coefficient *p*-values <0.05) if samples were collected within 180 days of another. Results of the Student *t*-test comparisons of log pre- and post-natal phthalate metabolites within women showed significant differences ($p < 0.05$; results not shown) between means for each log metabolite except MEP (log pre-natal mean $= 5.03\ \mu g\,l^{-1}$ and log post-natal mean $= 4.83\ \mu g\,l^{-1}$) and MBP (log pre-natal mean $= 2.71\ \mu g\,l^{-1}$ and log post-natal mean $= 2.85\ \mu g\,l^{-1}$). Factors predicting the log difference between maternal pre- and post-natal phthalate concentrations were assessed using multivariate linear regression models (see supplementary electronic archive table 2). The creatinine adjusted values did not differ from the unadjusted values in correlation coefficient and *t*-test analyses, and only creatinine unadjusted values are presented. In linear regression analyses, the difference between pre- and post-natal square root creatinine was a significant predictor for the difference in pre- and post-natal phthalate concentrations for each phthalate metabolite measured. The differences in date of sample and race were not significant predictors of the difference between pre- and post-natal phthalate metabolite concentrations, and maternal age was a significant predictor for pre- and post-natal differences in MEP, MBP and MIBP. There were 19 women with extreme values of metabolite concentration (exceeding six standard deviations of the mean), but results were largely unchanged in analyses excluding these women (results not shown).

To summarize, we found that concentrations of maternal pre- and post-natal phthalate metabolites were widely variable and generally were not significantly related to one another. Pearson correlation coefficients for MEP, MMP, MBzP and MIBP increased as the number of days between sampling decreased, suggesting that pre- and post-natal samples

Table 2. Correlation coefficients between quartile of pre- and post-natal phthalate metabolites.[a] *p-value for coefficient <0.05. Abbreviations are the same as given in table 1.

	all pre- and post-natal samples ($N = 242$)	pre- and post-natal samples taken within 365 days of one another ($N = 84$)	pre- and post-natal samples taken within 180 days of one another ($N = 28$)
MMP	0.17*	0.23*	0.44*
MCPP	0.17*	0.08	−0.04
MEHP	0.06	0.14	0.21
MEOHP	0.04	0.10	0.16
MEHHP	0.08	0.13	0.23
MEP	0.35*	0.31*	0.48*
MBP	0.18*	0.28*	0.10
MBzP	0.28*	0.42*	0.47*
MIBP	0.15*	0.26*	0.63*

[a]Pearson correlation coefficients between pre- and post-natal phthalate quartiles (unadjusted for creatinine).

are better correlated if samples are collected within three months of one another. In examining factors that predict the difference between pre- and post-natal phthalate concentrations, the difference in pre- and post-natal urinary creatinine concentration was consistently a significant factor in regression analyses. This probably represents differences in urinary dilution, metabolism, muscle breakdown and physical activity between pre- and post-natal measurements. Maternal age was a significant predictor for the difference in MEP, MBP and MIBP concentrations, which may reflect different lifestyle choices or physiologic changes due to pregnancy. Race was not a significant predictor of the difference in pre- and post-natal samples, which may reflect a generally homogeneous population, or perhaps suggest that racial factors that affect phthalate concentrations do not change from pre- to post-natal time periods.

Several studies have examined temporal variability in urine phthalate concentrations and show varying results, probably due to differences in study populations and design (Hoppin *et al.* 2002; Hauser *et al.* 2004; Fromme *et al.* 2007; Teitelbaum *et al.* 2008). In a study of 50 adult men and women over eight days, Fromme *et al.* (2007) found significant day-to-day variation, suggesting that several urine samples are needed to characterize exposures over a period of time. Hoppin *et al.* (2002) documented high correlation coefficients for adult female urine phthalate metabolite concentrations measured on two consecutive days, and, as discussed above, Hauser *et al.* (2004) found that a single urine measurement was predictive of a subject's exposure over a three-month period as estimated by tertile classifications determined from nine repeated urine samples. Our study is different in that urine samples were, on average, taken over a year apart with pregnancy and birth occurring between visits. There was also considerable variability in post-natal collection times between individuals. It may be that a single urine measurement is predictive of short-term exposures, as suggested by the current literature, but samples taken before and after major life/physiological events are not related, owing to individual characteristics such as changes in metabolism and lifestyle. Our data suggest that as the interval between samples decreases, agreement between the two increases, and within six months there were moderate correlations for several phthalate metabolites. However, post-natal urinary phthalate metabolite concentrations measured several months to a year following delivery alone would likely serve as a poor surrogate for exposure during pregnancy for most phthalates. We also found that post-natal concentrations exceeded pre-natal concentrations. However, whether this is the result of differing exposures (mother's daily routine and use of phthalate-laden products changing) or metabolism is unknown at this time. Limitations of our study include the single (as opposed to repeated) samples taken during the two different study periods and the inability to measure changes in lifestyle and metabolism in mothers between these periods. Additional research should document phthalate exposures and urine concentrations during and after pregnancy, as well as changes in metabolic processes to determine potential predictors of the difference in phthalate concentrations between pre- and post-natal urine samples.

(c) *Human developmental studies*

Human studies that have investigated associations between phthalate exposure and adverse pregnancy outcomes or developmental effects are presented in table 3. As compared with the large body of evidence in laboratory animals documenting phthalate reproductive or developmental toxicity, these associations have been immensely understudied in humans to date. In one Italian study of 84 newborns that examined gestational age in relation to phthalate exposure (Latini *et al.* 2003), MEHP in the cord blood of the newborns was associated with shorter gestational age at delivery (odds ratio for absence of detectable MEHP in cord blood associated with a 1-week increase in gestational age = 1.50, 95% CI 1.01–2.21). To date, most human studies investigating reproductive or developmental health outcomes associated with foetal or infant exposure to phthalates have been limited to males, and there is indirect evidence suggesting that certain phthalates may impart anti-androgenic effects in the perinatal period (see §2(d) below). In a study of phthalate concentrations in breast milk and serum hormone levels in three-month-old male offspring, Main *et al.* (2006*b*) identified a significant inverse association between MBP, the active metabolite of DBP and free testosterone levels (determined by total testosterone: sex hormone binding globulin ratio) in male offspring. They also reported positive associations between MEP, MMP and MBP with luteinizing hormone (LH): free testosterone ratio, which is a measure of Leydig cell function. There were also positive relationships between mono-isononyl phthalate (MiNP) and LH, and between MEP, MBP and sex hormone binding globulin (SHBG). However, phthalate levels were

Table 3. Health outcomes in infants and children associated with phthalate concentrations in biological or environmental samples.[a] Adapted from Swan (2008). Abbreviations: BBzP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; MMP, mono-methyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEP, mono-ethyl phthalate; MBP, mono-butyl phthalate; MBzP, mono-benzyl phthalate; MiNP, mono-isononyl phthalate.

system	timing of exposure	sex	outcome	phthalate or metabolite (measured in urine unless otherwise noted)	references
reproductive	pre-natal	males/ females	shorter gestational age at birth	MEHP (in cord blood)	Latini *et al.* (2003)
	pre-natal (mean age 12.6 months at exam)	males	shorter anogenital distance	MEHP, MEOHP, MEHHP, MEP, MBP	Swan (2008)
			reduced penile size	MEHP	
			incomplete testicular descent	MEHP, MEHHP, MEOHP	
	lactation (mean age 3 months)	males	increased SHBG	MEP, MBP	Main (2006*b*)
			increased LH/free T	MMP, MEP, MBP	
			increased LH	MiNP	
			decreased free T	MBP	
	early childhood	females	premature thelarche	DEHP (in serum)	Colon *et al.* (2000)
respiratory, allergy and asthma	childhood	males/ females	rhinitis and eczema	BBzP (in house dust)	Bornehag *et al.* (2004)
			asthma	DEHP (in house dust)	Bornehag *et al.* (2004)
			wheezing, rhinitis and eczema	DEHP (in house dust)	Kolarik (2008)

[a]Associations significant at $p < 0.05$.

not associated with the occurrence of cryptorchidism in a nested case–control analysis.

Earlier evidence for DEHP and/or MEHP as an endocrine disruptor was reported in a study of 41 Puerto Rican girls (aged six months to 8 years; median age 20 months) with premature thelarche, which is defined as premature breast development in girls younger than 8 years of age with the absence of other physical signs of puberty onset. In the study, Colon *et al.* (2000) compared serum levels of phthalate diesters (and MEHP) between the thelarche cases and 35 paediatric controls (median control age 46 months). Among the phthalates measured, the largest differences between cases and controls were found for serum DEHP concentration, which were 450 and 70 ng ml^{-1}, respectively ($p < 0.05$). However, there are concerns regarding several aspects of the study design, including potential sample contamination by phthalate diesters during the collection, transport, storage, processing or analysis of the serum samples.

Other developmental effects of phthalate exposures may include pulmonary system effects: allergies, rhinitis, asthmatic reactions and direct toxicity. Bornehag *et al.* (2004) conducted a nested case–control study comparing phthalate concentrations in indoor dust in 198 children (ages 3–8 years) with allergic/asthmatic symptoms to 202 controls without symptoms in Sweden. They found significantly higher concentrations of butyl benzyl phthalate (BBzP) in the house dust of cases as compared with that of controls. Dust concentrations of BBzP were also associated with allergic and eczematous symptoms in a dose-dependent manner, as were DEHP dust concentrations and asthmatic symptoms (Bornehag *et al.* 2004). Another study in Bulgarian children produced similar results, where increased house dust DEHP concentrations were related to increased wheezing symptoms in a dose–response manner (Kolarik *et al.* 2008). These authors also found increased wheezing and rhinitis associated with BBzP in house dust, but these findings were not statistically significant (Kolarik *et al.* 2008). Because of the cross-sectional nature of these studies, we cannot determine if concentrations of phthalates in the home are also involved in the development of atopic diseases. In a study conducted in pre-term infants with polyvinyl chloride (PVC) respiratory tubing, Roth *et al.* (1988) found these infants to have clinically and radiologically confirmed hyaline membrane disease, which was hypothesized to be caused by the DEHP exposure. Di(2-ethylhexyl) phthalate was also found in the lung tissue of one of the patients who died of a pneumothorax soon after birth (Roth *et al.* 1988). These findings have not been replicated, but several authors have suggested that PVC medical tubing may be a likely source of respiratory health effects in young neonates (Hill *et al.* 2001, 2003; Tickner *et al.* 2001). One study of adults found an inverse relationship between urinary MEP and MBP and pulmonary function (Hoppin *et al.* 2004), but because urine markers reflect all routes of exposure and may not be a good measure of inhaled phthalate concentrations, it is difficult to interpret these results. Given these findings, further research needs to examine route- or target-specific phthalate biomarkers of exposure and potential pathophysiologic

changes relating to the development of allergic and asthmatic diseases and pulmonary function in children.

(d) *Anogenital distance and the phthalate syndrome*

Numerous animal studies report that several phthalates (particularly DEHP and DBP) induce a marked reduction in foetal testosterone and insulin-like growth factor-3, resulting in a syndrome of male reproductive abnormalities (Foster 2006; Gray *et al.* 2006). In addition to shortened anogenital distance (AGD), which is a sensitive and non-invasive measure of potential androgen deficiency during foetal development in rodents and humans, other abnormalities include hypospadias, cryptorchidism and malformations of the epididymis, vas deferens, seminal vesicles and prostate; together they comprise the 'phthalate syndrome' (Gray *et al.* 2006). Prior to 2005, only a single study had evaluated AGD in human males (Salazar-Martinez *et al.* 2004), and none had examined AGD in relation to environmental chemical exposure. Swan *et al.* recently examined AGD and other genital parameters in male human infants in relation to their mother's phthalate exposure. The details of study methods are presented in a 2005 publication (Swan *et al.* 2005) that includes an analysis of the first 85 boys in this population, among whom genital measurements (including AGD) and concentrations of phthalate metabolites in maternal pre-natal urine samples were available. Follow-up analysis was subsequently conducted on a larger sample of boys, including 166 exams on 106 boys for whom genital measurements and pre-natal phthalate exposure measures were available (Swan 2008). We summarize those results here.

The male infants in the study were examined initially 2–24 months after birth (mean 12.8 months of age) using a detailed examination of breast and genitals developed specifically for this study. Boys' examinations included a description of the testes and scrotum, location of testes, measurement of the penis and AGD. AGD was measured in boys from the centre of the anus to the cephalad (towards the head) base of the penis and averaged 72.4 mm. For each infant, the expected weight for age (weight percentile) was determined by standard curves (http://www.cdc.gov/growthcharts/). AGD was then adjusted for age and weight percentile and examined in general linear models and categorical analyses. In these 106 mother–son pairs, maternal urinary concentrations of the three measured metabolites of DEHP were significantly and inversely related to AGD (p-values = 0.001, 0.002 and 0.017 for MEOHP, MEHHP and MEHP, respectively), as were MEP (p-value = 0.005) and MBP (p-value = 0.049).

For each boy, testicular descent was classified as incomplete if one or both testicles were not graded as normal (fully descended) or normal retractile. Each of the three measured DEHP metabolites were related to risk of incomplete testicular descent (p-values = 0.040, 0.054 and 0.048 for MEOHP, MEHHP and MEHP, respectively). AGD was correlated strongly with penile width and, to a lesser extent, with penile length ($p = 0.0003$ and 0.059, for width and length, respectively). In a mixed model controlling for age and weight percentile, penile width (but not length) was significantly associated with concentration of the sum of DEHP metabolites and the monoester metabolite (MEHP). No other metabolites were associated with penile size.

This study demonstrates inverse associations between AGD, penile width and testicular descent and one or more metabolites of DEHP, as well as between AGD and both MEP and MBP. These findings suggest that AGD is a marker for insufficient foetal androgenization and suggest that low-dose phthalate exposure may affect several markers of human male genital development.

(e) *Human studies in adults*

(i) *Semen quality*

Studies that have explored phthalate effects in adults are presented in table 4. A substantial proportion of the limited human studies on phthalates and reproductive effects in adults have been focused on investigating associations with altered semen quality. In an early study, Murature *et al.* (1987) measured DBP concentrations in the cellular fractions of ejaculates from 21 university students and found an inverse relationship with sperm concentration. The study had a number of limitations, including a small number of participants, the failure to adjust for potential confounders and measurement of the parent compound, which may be subject to sample contamination. In another small study, conducted in India, Rozati *et al.* (2002) studied 21 infertile men with poor semen quality and 32 'control' men with normal semen parameters. Phthalate esters were measured in seminal plasma and the results were reported as the sum of a mixture of dimethyl phthalate, DEP, DBP, BBzP, DEHP and di-*n*-octyl phthalate. The concentration of phthalates was inversely correlated with sperm morphology but not correlated with ejaculate volume, sperm concentration or motility. In this study, as in the Murature study, the measurement of phthalate diesters raises concern with sample contamination from the ubiquitous presence of the diester in the environment.

More recently, a larger study using urinary levels of phthalate metabolites was conducted by Duty *et al.* (2003*a*), with follow-up analysis reported by Hauser *et al.* (2006). 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 sample of semen and urine were collected. In the initial study, there were dose–response relationships (after adjusting for age, abstinence time and smoking status) between MBP and below World Health Organization (WHO) reference value sperm motility and sperm concentration among 168 men (Duty *et al.* 2003*a*). There was also a dose–response relationship between MBzP (the primary hydrolytic metabolite of BBzP) and below WHO reference value sperm concentration. In a recent follow-up study including these 168 men, plus an additional 295 men newly recruited into the study, Hauser *et al.* (2006) confirmed the associations between MBP and increased

Table 4. Health outcomes in adults associated with phthalate concentrations in biological samples.[a] Adapted from Swan (2008). Abbreviations are the same as given in table 3.

system	sex	outcome	phthalate or metabolite (measured in urine unless otherwise noted)	references
reproductive	males	increased sperm DNA damage	MEP, MEHP	Hauser *et al.* (2007)
		increased sperm DNA damage	MEP	Duty *et al.* (2003*b*)
		decreased sperm motility	MBP	Duty *et al.* (2003*a*); Hauser *et al.* (2006)
		decreased sperm concentration	MBP, MBzP	Duty *et al.* (2003*a*); Hauser *et al.* (2006)[b]
	males	decreased sperm motility	DBP and DEHP in semen samples	Zhang (2006)
	males	decreased free T and increased LH/free T	MBP	Pan *et al.* (2006)
	males	decreased FSH	MBzP	Duty *et al.* (2005*b*)[c]
	males	decreased motility, reduced LH	MEP	Jonsson *et al.* (2005)
respiratory, allergy and asthma	males	decreased pulmonary function	MEP, MBP	Hoppin *et al.* (2004)
metabolic	males	increased waist circumference	MBzP, MEHHP MEOHP, MEP	Stahlhut *et al.* (2007)
thyroid	males	altered thyroid hormone levels (decreased T3 and T4)	MBP, MBzP, MEP MEHP	Meeker *et al.* (2007*b*)

[a]Associations significant at $p < 0.05$.
[b]In Hauser *et al.* (2006), p-value for MBzP association was 0.13.
[c]In Duty *et al.* (2005*b*), p-value for association between MEHP and testosterone was 0.10.

odds of below-reference sperm concentration and motility. The relationships appeared to follow dose-dependent patterns, where greater odds ratios were calculated among increasing phthalate metabolite quartiles. However, there was only a suggestive association between the highest MBzP quartile and low sperm concentration ($p = 0.13$), which was not fully consistent with the results of the preliminary analysis (Duty *et al.* 2003*a*).

In a recently published study from Sweden, Jonsson *et al.* (2005) recruited 234 young Swedish men at the time of their medical conscript examination. Each man provided a single urine sample used to measure concentrations of MEP, MEHP, MBzP, MBP and phthalic acid. In contrast to the US study, in the Swedish study there were no relationships of MBP or MBzP with any of the semen parameters. MEHP was also not associated with any of the semen parameters, but men in the highest quartile for MEP had fewer motile sperm and more immotile sperm than men in the lowest MEP quartile. Contrary to their hypothesis, phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm. Phthalic acid is a non-specific marker of phthalate exposure, formed as the result of the hydrolysis of any of the phthalates measured. Interactions between urinary phthalate levels and PCB 153 (measured previously in serum samples from these men) were assessed by including an interaction term in the models. There was no evidence of multiplicative interactions between PCB 153 and any of the phthalates with the reproductive markers (data were not shown). This is in contrast to a previous study by Hauser *et al.* (2005), where they found interactions of MBP and MBzP with congener PCB 153 in relation to sperm motility.

Although the Swedish study had some similarities to the US study, in that they were both cross-sectional studies in which a single urine and semen sample were collected, there were also many important differences. One of the primary differences was in the age of the study population and the method of recruitment. The Swedish study population consisted of young men (median age 18 years, range 18–21 years) undergoing a medical examination before military service. Since approximately 95 per cent of young men in Sweden undergo the conscript examination, these young men reflected the general population of young Swedish males. In contrast, in the US study, the median age of the men recruited from an infertility clinic was 35.5 years and ranged from 22 to 54 years. None of the men from the infertility clinic were 21 years of age or younger. The differences across studies in the ages and source of the men may account for some of the differences in results between studies. For instance, it is unclear whether men presenting to an infertility clinic are more 'susceptible' to reproductive toxicants, including phthalates, than men from the general population. Furthermore, it is also unclear whether middle-aged men, as compared to young men, are more susceptible to reproductive toxicants because of an age-related response to the toxicant. Other differences across studies include major differences in participation rates (14% in the Swedish

study and 65% in the US study) and differences in the analytical methods used to measure urinary phthalate metabolites, where the method in the US study was much more sensitive than that used in the Swedish study.

In the Massachusetts (USA) study, semen samples from 379 men were also cryogenically frozen and sperm cells later analysed for DNA damage using the neutral comet assay (Hauser *et al.* 2007). Sperm DNA damage measurements included comet extent (CE), percentage of DNA in tail (tail%) and tail distributed moment (TDM). In multivariate linear regression models adjusted for age and smoking, significant positive associations were found for at least one of the three DNA damage measures with MEP (CE, TDM), MBP (tail%), MBzP (CE, TDM) and MEHP (tail%). For MEP, the significant association with CE and TDM confirmed previous findings among an earlier and smaller subset from the same study population (Duty *et al.* 2003*b*). Another interesting finding was that MEHP was strongly associated with all three DNA damage measures after adjustment for the oxidative DEHP metabolites, which may serve as phenotypic markers of DEHP metabolism to 'less toxic' metabolites and lower susceptibility to exposure-related effects compared with those individuals with low concentrations of oxidative DEHP metabolites relative to MEHP concentration (Hauser 2008). Metabolism of phthalates depends on the size and structure of the diester, and can occur via two steps: phase I (e.g. hydrolysis, oxidation) followed by phase II (conjugation) (Frederiksen *et al.* 2007). Since the monoester metabolite may be the more bioactive form of the phthalate, individuals who are predisposed to form and retain more monoester may have a heightened sensitivity to phthalate exposure.

In summary, the epidemiologic data on semen quality and/or sperm cell integrity in relation to phthalate exposure remain limited and inconsistent. Additional studies are critically needed to help elucidate possible explanations for differences across studies, and most importantly, to address whether phthalate exposure alters semen quality, sperm function and male fertility.

(ii) *Other reproductive/endocrine effects*

Several human studies have investigated associations between exposure to phthalates and circulating hormone levels. In a study of workers producing PVC flooring with high exposure to DEHP and DBP, urinary concentrations of metabolites of these phthalates were inversely associated with free testosterone levels (Pan *et al.* 2006). A report on 295 men from the Massachusetts (USA) infertility clinic study found a suggestive inverse association between MEHP and testosterone, along with a positive association between urinary MBP and inhibin B (a glycoprotein hormone produced by the gonads that has an inhibitory effect on pituitary FSH production), and an inverse association between urinary MBzP and FSH (Duty *et al.* 2005*b*). However, the significant results for MBP and MBzP and hormone levels were in patterns inconsistent with the authors' hypotheses. It is interesting to note that although MEHP concentrations in the Massachusetts study were several orders of magnitude lower than those measured in the exposed Chinese workers (Pan *et al.* 2006), the evidence for decreased testosterone in relation to DEHP/MEHP was consistent between the two studies. It is also interesting to note that the inverse association between MBP and testosterone in the study of exposed Chinese workers (Pan *et al.* 2006) appears to be consistent with the male infant studies described earlier, where MBP concentrations were inversely associated with anogenital index (a measure of androgen activity) and free testosterone (Swan *et al.* 2005; Main *et al.* 2006*b*). On the other hand, the study of 234 young Swedish men found an inverse association between urinary MEP and LH but no association between MEP, MBP, MEHP or other phthalate metabolites in urine and FSH, testosterone, oestradiol or inhibin B (Jonsson *et al.* 2005).

Owing to the documented anti-androgenic effects of certain phthalates in animal models, and recent observations that low testosterone in adult males may be associated with an increased prevalence of obesity and type 2 diabetes (Ding *et al.* 2006; Selvin *et al.* 2007), Stahlhut *et al.* (2007) explored the relationship between phthalate exposure and waist circumference in a large cross-sectional study carried out among a subset of adult male participants in the 1999–2002 US National Health and Nutrition Examination Survey (NHANES). The authors reported significant associations between urinary phthalate monoester concentrations (MBzP, MEHHP, MEOHP and MEP) and increased insulin resistance (measured through homeostatic model assessment), and positive associations between MBP, MBzP and MEP and waist circumference. These findings provide preliminary evidence of a potential contributing role for phthalates in the overall population burden of insulin resistance, obesity and related clinical conditions, but additional studies are needed.

The potential for phthalates to affect thyroid function has been demonstrated in animal studies, but human studies are limited to two recent investigations: one within the Massachusetts (USA) male infertility clinic study (Meeker *et al.* 2007*b*) and another among pregnant Taiwanese women in their second trimester (Huang *et al.* 2007). In the Massachusetts study, phthalate metabolite concentrations were measured in urine and thyroid hormones were measured in serum from 408 men. MEHP was inversely associated with free T4 and total T3, but was not associated with thyroid-stimulating hormone (TSH). The inverse association between MEHP and free T4 became stronger when also taking into account the concentrations of oxidative DEHP metabolites that were positively associated with free T4. As with the findings from the study of sperm DNA damage (Hauser *et al.* 2007), these results may reflect metabolic susceptibility to the adverse effects of MEHP among individuals who less efficiently oxidize DEHP and/or MEHP (Meeker *et al.* 2007*b*; Hauser 2008). Among 76 pregnant Taiwanese women, Huang *et al.* (2007) reported an inverse association between MBP and both total and free levels of T4. Unlike the study among US men, they did not find an inverse association with MEHP, but there were considerable

differences between the design of the two studies. In addition to having a smaller study size and a vastly different study population, the Taiwanese study also did not take into account concentrations of oxidative DEHP metabolites, which served to strengthen the associations between MEHP and thyroid hormones in the US study. More study is needed on the association between phthalate exposure and thyroid function, which plays an important role in many human systems including reproduction and foetal neurodevelopment.

3. BISPHENOL A

(a) *Exposure*

BPA is used in a variety of consumer products, including epoxy resins that are used to line food cans (Kang *et al.* 2003), polyester–styrene (Factor 1998) and polycarbonate plastics used for baby bottles and other containers (Brede *et al.* 2003). BPA-containing resins and plastics are also used in some dental sealants (Sasaki *et al.* 2005) and fillings (Joskow *et al.* 2006), adhesives, protective coatings, flame retardants (Samuelsen *et al.* 2001) and water storage tanks and supply pipes (Bae *et al.* 2002). BPA is a polymer, but degrades into its monomeric form over time, a process that can be accelerated by heat exposure (Brotons *et al.* 1995). The monomeric form can leach from its source into adjacent materials such as into water or into the food products from the lining of the can. Several studies have demonstrated detectable levels in packaged foods that were contained in wrapping or cans coated with BPA (Lopez-Cervantes & Paseiro-Losada 2003). The majority of human exposure is via ingestion of food-containing BPA (Kang *et al.* 2006; Vandenberg *et al.* 2007).

Widespread exposure to BPA among the US population has recently been documented. In a subset of the 2003–2004 NHANES, 93 per cent of urine samples had detectable levels of BPA (Calafat *et al.* 2008). The subset included 2517 participants aged 6 years and older, with a mix of urban and rural residences. Human studies have also measured detectable levels of BPA in a variety of human body fluids and some tissues. Of potential concern to reproductive and developmental health endpoints is the presence of BPA in follicular fluid and amniotic fluid (Ikezuki *et al.* 2002), umbilical cord blood (Schonfelder *et al.* 2002) and breast milk (Sun *et al.* 2004).

Adult humans metabolize BPA via the hepatic glucuronidation pathway, and the biologic half-life of BPA is approximately 6 h, with nearly complete urinary excretion in 24 h (Volkel *et al.* 2002). Therefore, urinary BPA levels primarily reflect exposures that occurred $\leq$1 day preceding the collection of the urine specimen, and, like with phthalates, information on the ability of urinary biomarkers to predict individual exposure over longer periods of time is needed to properly design human studies. In a recent study investigating temporal variability of urinary BPA concentrations among 82 men and women, Mahalingaiah *et al.* (2008) reported high within-individual variability but concluded that a single sample may be relatively predictive of BPA concentration over a period of weeks or months in an epidemiologic study that categorizes subjects into broad BPA exposure groups (e.g. tertiles) (Mahalingaiah *et al.* 2008). However, if feasible, a second urine sample from individuals in a study would likely improve the sensitivity of exposure categories.

(b) *Human health effects*

BPA has been shown to have oestrogenic properties in studies published as early as 1936 (Dodds & Lawson 1936). These findings have been confirmed in a large number of subsequent *in vitro* and animal studies, along with evidence for other biological activities such as effects on thyroid function (Richter *et al.* 2007; Wetherill *et al.* 2007). Despite the high level of concern for health effects related to BPA exposure, and documented widespread human exposure to BPA, human epidemiologic studies of adverse health outcomes in association with BPA exposure are severely limited. BPA levels in blood have been associated with a variety of conditions in women, but studies have been small in size and have provided limited details on subject selection criteria. In one epidemiologic study, serum BPA levels were reported to be associated with recurrent miscarriage (Sugiura-Ogasawara *et al.* 2005). Compared with 32 non-parous women without fertility problems, the study reported that the mean serum BPA concentration was three times higher in 45 women with a history of three or more consecutive first-trimester miscarriages. Among 35 of the women who became pregnant there was some suggestive evidence of higher BPA concentrations among the women who subsequently miscarried again compared with women who went on to have a successful pregnancy, though the median BPA concentrations were the same between the two groups. Another Japanese study reported higher maternal serum BPA concentrations among 48 women carrying foetuses with an abnormal karyotype compared to 200 women carrying foetuses with a normal karyotype (Yamada *et al.* 2002).

Two small human studies have investigated exposure to BPA and hormone levels, where statistically significant positive correlations were found between BPA concentrations in serum and circulating total and free testosterone levels in both men and women (Takeuchi & Tsutsumi 2002; Takeuchi *et al.* 2004). These investigators also reported that women with polycystic ovary syndrome (PCOS) had higher serum levels compared with women without PCOS (Takeuchi & Tsutsumi 2002; Takeuchi *et al.* 2004). Another small study among men occupationally exposed to BPA during the application of epoxy resins found an inverse association between BPA and FSH (Hanaoka *et al.* 2002). Finally, a study among 172 adults reported a positive association between urinary BPA concentration and frequency of sister chromatid exchange measured in peripheral lymphocytes, but not between urinary BPA and self-diagnosed endocrine disorders (Yang *et al.* 2006). To summarize, suggestive but limited evidence exists for an association between BPA exposure and adverse human health endpoints, and human studies that have appeared in the literature thus far have suffered from

a number of limitations. Given the widespread human exposure to BPA and the increasing concern for its potential to adversely affect health through various mechanisms, there is a clear need for more epidemiological research.

4. POLYBROMINATED DIPHENYL ETHERS

(a) *Exposure*

PBDEs are flame-retardant chemicals that are added to a variety of consumer products, including textiles, thermoplastics used in electronics (e.g. televisions, computers) and products containing polyurethane foam (e.g. mattresses, upholstered furniture) to make them difficult to burn (ATSDR 2004). Additive flame retardants like PBDEs are not chemically but physically combined with polymers at levels ranging from 5 to 30 per cent by weight, creating the possibility for them to leach out of the treated materials into the surrounding environment (EU 2001). In 2001, the worldwide demands for technical grade PBDEs were estimated to be 67 000 metric tonnes, with the Americas accounting for about half of that amount (ATSDR 2004). Despite bans or voluntary discontinuation of the production of certain commercial PBDE formulations in Europe and the USA, the general population continues to be exposed due to their persistence in the environment. PBDEs are highly hydrophobic and thus are stored in biological systems and bioaccumulate up the food chain, although bioaccumulation is thought to be inversely related to the degree of PBDE bromination (ATSDR 2004). The primary routes of exposure to humans are probably ingestion of contaminated foods and inhalation of indoor air or ingestion or dermal uptake of house dust containing PBDEs released from electrical appliances and furniture (ATSDR 2004; Webster *et al.* 2005; Allen *et al.* 2007; Lorber 2008). Recent evidence suggests that exposure to PBDE among women during pregnancy likely results in foetal exposure, though the rate of placental transport may vary by congener (Gomara *et al.* 2007; Schecter *et al.* 2007). For infants, consumption of breast milk is probably a primary source of exposure to the lower brominated PBDE congeners due to their lipophilicity (Schecter *et al.* 2003). Human biomonitoring studies of blood, breast milk or adipose tissue samples have shown high geographic variability in exposure levels, with concentrations among individuals in the US orders of magnitudes higher than those found among European and other populations studied throughout the world (ATSDR 2004; Schecter *et al.* 2005). In addition, there is much greater variability in PBDE levels measured in biological matrices compared with other persistent organic pollutants (Birnbaum & Cohen Hubal 2006), which may reflect continuing and direct exposures among subsets of the population.

For assessing human PBDE exposure in epidemiological studies, measuring the various PBDE congeners in blood is the preferred method (with the exception of certain study designs or populations, such as breast milk in studies of breast-feeding infants), and it provides a rather temporally stable exposure measure due to the persistent and bioaccumulation properties of PBDEs. Estimated half-lives for PBDEs in human serum range from weeks to months, depending on the congener, and half-lives in adipose tissue and for terminal elimination from the body are of the order of several years (Birnbaum & Cohen Hubal 2006). Thus, unlike urinary BPA and phthalate metabolite measures, a single blood PBDE measure is likely to be highly predictive of an individual's average PBDE level over a span of months or years.

(b) *Human health effects*

While a number of brominated flame retardants, including PBDE, are considered pervasive endocrine-disrupting environmental contaminants of concern, very few human studies have explored the associations between exposure and evidence for reproductive or developmental effects. Only one human study on data (Hagmar *et al.* 2001) has explored associations between non-occupational PBDE exposure and thyroid function or thyroid hormone levels despite a growing body of evidence for these effects in animal and *in vitro* studies (Zhou *et al.* 2002; Costa & Giordano 2007; Kuriyama *et al.* 2007; Tseng *et al.* 2008). In a study of 110 adult males from Sweden and Latvia, plasma concentrations of PBDE 47 were inversely and significantly associated with TSH, but not with T3 or T4, after adjusting for age (Hagmar *et al.* 2001). Conversely, a longitudinal study among a very small number of workers ($n = 11$) at an electronics recycling facility who were thought to be exposed to low levels or PBDEs reported no associations between exposure to specific congeners or the sum of PBDE congeners measured in the study and T3, T4 or TSH (Julander *et al.* 2005). However, PBDE 153 was suggestively associated with T4 (p-value $= 0.08$), and several statistically significant relationships between specific PBDE congeners and thyroid hormones were observed within individual workers over the study period. An earlier workplace study reported four cases of hypothyroidism among 35 exposed workers involved with the manufacture of PBDE 209 compared with no cases among 89 unexposed workers (Bahn *et al.* 1980).

A small number of recent human studies have assessed other reproductive and developmental outcomes in association with PBDE exposure, as animal studies have demonstrated PBDEs are anti-androgenic (Stoker *et al.* 2005) and that the foetal period may be one of high sensitivity to PBDE exposure (Lilienthal *et al.* 2006). Main *et al.* (2007) recently compared PBDE concentrations in placenta and breast milk between Danish and Finnish boys with and without cryptorchidism, and also explored associations between PBDE and reproductive hormone levels in the boys. They reported that PBDE concentrations in breast milk, but not placenta, were significantly higher in boys with cyrptorchism than in controls. They also reported a positive correlation between the sum of 14 PBDE congeners and LH. In a Swedish case–control study of testicular cancer among men and their mothers who were recruited in the years 1999–2000, there was an increased odds (odds

ratio = 3.5; 95% CI 1.1–11) for testicular cancer among men whose mothers were above the 75th percentile for blood PBDE concentrations (sum of congeners 47, 99 and 153) (Hardell *et al.* 2006). However, this association was not found when basing exposure on PBDE concentrations measured in the men. This discrepancy suggests either a chance finding when using the mother's PBDE levels or perhaps a biological mechanism traced back to *in utero* exposures. Finally, despite a small study size ($n = 20$), Chao *et al.* (2007) recently reported statistically significant associations between concentrations of PBDE congeners in breast milk and adverse birth outcomes, including reduced birth weight, birth length, chest circumference and Quetelet's index (kg m^{-2}), as well as suggestive associations between PBDE levels and altered menstrual cycle characteristics. The PBDE levels measured in breast milk in this study and in the study of cryptorchidism (Main *et al.* 2007) were similar to levels reported in Europe and East Asia, but much lower than those measured in the USA (Schecter *et al.* 2003; Chao *et al.* 2007).

5. CONCLUSIONS AND FUTURE RESEARCH NEEDS

A number of chemicals used in plastics for property enhancement are emerging environmental contaminants of concern (see also the discussion in Oehlmann *et al.* (2009), Koch & Calafat (2009), Thompson *et al.* (2009*b*) and this paper). Although the epidemiological data on the plastic additives described here suggest that there may be associations with altered endocrine function and reproductive or developmental effects, the number of human studies is currently limited and the quantity and quality of the data available for the different compounds are varied. Also, for some of the more studied associations, such as between phthalates and semen quality, the data across studies are not consistent. This may be due to small study sizes and lack of statistical power or differences in study design, study populations, exposure assessment strategies, exposure levels, exposure sources, exposure routes, multiple/competing physiologic mechanisms, analytical approaches and potential confounding variables considered in the statistical analysis (e.g. age, BMI, season). The limited human data, and in certain instances inconsistent data across studies, highlight the need for further epidemiological research on these classes of chemicals. Most studies to date have been cross-sectional in nature. Future longitudinal studies are needed to explore the temporal relationship between exposure to plastic additives and adverse reproductive and developmental outcomes to provide more information on whether these relationships may be causal in nature. Owing to the complex nature of the endocrine system, studies should evaluate not only individual hormone levels but also the ratios between relevant hormones (e.g. LH : testosterone ratio in males as a marker for Leydig cell function) that may help provide clues to the biological mechanisms of xenobiotic activity in humans.

Researchers face a number of challenges that need to be addressed to further our understanding of the relationship between plastic additives and adverse human health effects. One future challenge includes the shifts in exposure levels among populations over time caused by the ever-changing patterns of production and use of these compounds. Another challenge is to understand how simultaneous co-exposures to these chemicals may affect endocrine function. It is well known that humans are exposed to all these compounds simultaneously, and to many other chemicals. However, most studies to date have only addressed single chemicals or classes of chemicals, and there are limited data on the interactions between chemicals within a class or across classes. Chemicals may interact additively, multiplicatively or antagonistically in what is commonly referred to as the 'cocktail effect'. The human health risks of exposure to chemical mixtures are much understudied. Despite these challenges, evolving and innovative technologies designed to improve the assessment of human exposure and intermediate biological markers of effect should provide enhanced opportunities for improving our understanding of the relationship between these environmental chemicals and reproductive and developmental health. Innovations include improved biomarkers of exposure, more sophisticated statistical methods that deal with multiple exposures simultaneously and sensitive new measures of intermediate alterations in human endocrine function, reproductive health and foetal/child development.

More information is required on biological mechanisms of plastic additives in humans as well as the clinical and public health consequences of changes of intermediate markers of effect observed in human studies. For example, to date, in most studies that have reported statistically significant hormone alterations attributed to environmental and occupational exposures, the actual degree of hormone alteration has been considered subclinical. However, much remains unknown as to whether hormone changes currently considered subclinical may be associated with increased risk of adverse systemic effects in the long term. Furthermore, although seemingly subtle, small changes in hormone levels resulting from exposure may be of public health importance when considering the prevalence of exposure to plastic additives and EDCs among entire populations. Finally, human research is needed on potential latent and transgenerational effects (e.g. epigenetic modifications) of exposure to plastic additives and other EDCs, including the possibility of environmentally linked foetal origins of adult diseases, as well as genetic, metabolic, demographic or environmental characteristics resulting in increased individual susceptibility to adverse health effects following exposure.

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