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REVIEW ARTICLE

Food safety involving ingestion of foods and beverages prepared with phthalate-plasticizer-containing clouding agents

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In May 2011, the illegal use of the phthalate plasticizer di(2-ethylhexyl) phthalate in clouding agents for use in foods and beverages was reported in Taiwan. This food scandal has caused shock and panic among the majority of Taiwanese people and has attracted international attention. Phthalate exposure is assessed by ambient monitoring or human biomonitoring. Ambient monitoring relies on measuring chemicals in environmental media, foodstuff and consumer products. Human biomonitoring determines body burden by measuring the chemicals, their metabolites or specific reaction products in human specimens. In mammalian development, the fetus is set to develop into a female. Because the female phenotype is the default, impairment of testosterone production or action before the late phase may lead to feminizing characteristics. Phthalates disrupt the development of androgen-dependent structures by inhibiting fetal testicular testosterone biosynthesis. The spectrum of effects obtained following perinatal exposure of male rats to phthalates has remarkable similarities with the human testicular dysgenesis syndrome. Epidemiological studies have suggested associations between phthalate exposure and shorter gestational age, shorter anogenital distance, shorter penis, incomplete testicular descent, sex hormone alteration, precocious puberty, pubertal gynecomastia, premature thelarche, rhinitis, eczema, asthma, low birth weight, attention deficit hyperactivity disorder, low intelligence quotient, thyroid hormone alteration, and hypospadias in infants and children. Furthermore, many studies have suggested associations between phthalate exposure and increased sperm DNA damage, decreased proportion of sperm with normal morphology, decreased sperm concentration, decreased sperm morphology, sex hormone alteration, decreased pulmonary function, endometriosis, uterine leiomyomas, breast cancer, obesity, hyperprolactinemia, and thyroid hormone alteration in adults. Finally,

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the number of phthalate-related scientific publications from Taiwan has increased greatly over the past 5 years, which may reflect the health effects from the illegal addition of phthalate plasticizer to clouding agent in foodstuff over the past two decades.

Introduction

In Taiwan, phthalate esters have recently attracted the special attention of the medical community, regulatory agencies and the general public as a result of their widespread illegal use as food and beverage additives. The term phthalates describes a class of chemicals that are dialkyl- or alkylarylesters of 1,2-benzenedicarboxylic acid. Their industrial applications are related to the length of the ester chain. The long-chain or high-molecular-weight phthalates di(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and dipropylheptyl phthalate (DPHP) are primarily used in polyvinyl chloride (PVC) polymer and plastisol applications.[1] They can be found in building and construction materials, cables and wires, floorings, clothing, furnishings, car interiors and car underbody coatings, toys and also food contact materials. DEHP, which had been the most commonly used phthalate for many years, has been substituted, for the most part by DiNP and DiDP/DPHP. Short-chain or low-molecular-weight phthalates, such as dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl-benzyl phthalate (BBzP), di-*n*-butyl phthalate (DnBP) and di-iso-butyl phthalate (DiBP), are often used also in non-PVC applications, for example, personal-care products, paints, adhesives or enteric-coated tablets.[1] As the phthalate plasticizers are not chemically bound to PVC, they can leach, migrate or evaporate into the indoor air and atmosphere, foodstuffs, and other materials.[2] Consumer products containing phthalates can result in human exposure through direct contact and use, through indirect leaching into other products, or via general environmental contamination. Humans are exposed by ingestion, inhalation, and dermal exposure during their whole lifetime, including during intrauterine development.[2]

Phthalate food scandal in Taiwan

In May 2011, the illegal use of the phthalate plasticizer DEHP in clouding agents as additives in foods and beverages was reported in Taiwan (Fig. 1). The Food and Drug Administration discovered a clouding agent that was manufactured and sold by Yu Shen Chem. Co. Ltd. as a food additive containing DEHP, and immediately launched an investigation.[3] Affected businesses were ordered to recall and remove the tainted products from store shelves nationwide. While carrying out a comprehensive island-wide check, the Food and Drug Administration found that a clouding agent produced by Pin Han Perfumery Co. Ltd. contained another plasticizer, DiNP, and immediately detained and sealed the tainted products, as well as identified the marketing channels for the products.[3] Efforts have been made to track down the downstream businesses and prevent plasticizer-tainted products from entering the market.

Clouding agents are legal food additives that have been used for many years. They are normally made of gum arabic, emulsifying agents, palm oil, and other food additives. DEHP and DiNP are categorized as industrial plasticizers, which should not be added to food. After a criminal investigation, it was found that the illegal clouding agents to which DEHP had been added appeared as early as two decades ago, and the color of tainted clouding agents was

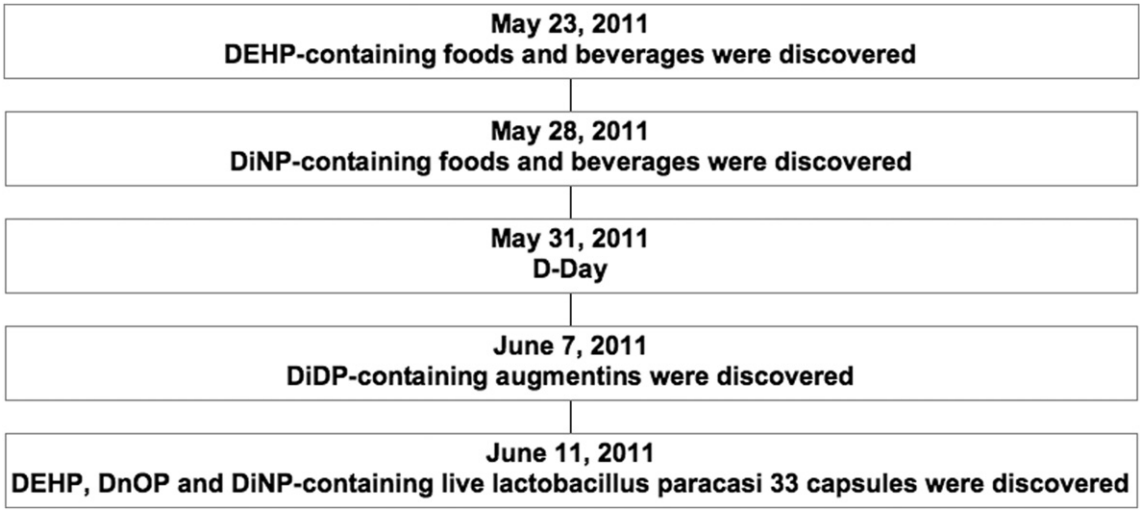

Figure 1 Phthalate food scandal in Taiwan. DEHP = di(2-ethylhexyl) phthalate; DiNP = di-iso-nonyl phthalate; DiDP = di-iso-decyl phthalate; DnOP = di-n-octyl phthalate.

white and esthetically pleasing.[4] Furthermore, the clouding agents made with DEHP could be preserved for up to half a year longer than those made using palm oil.[4] Diluting DEHP-laced clouding agents could allow it to be added to more drinks, and thus, increase profits.[4]

After discovering that clouding agents from Yu Shen Chem. Co. Ltd. and Pin Han Perfumery Co. Ltd. contained DEHP and DiNP, respectively, the Food and Drug Administration immediately launched investigations to track down where the clouding agents had been sold; all products from the above two companies containing clouding agents were detained, while those found in sales outlets were taken off the shelves for public health protection. The Department of Health[3] announced that starting on May 31, 2011 (D-Day), all ingredients, semi-finished products, and products containing clouding agents confirmed by the Department of Health as containing plasticizers must be taken off the shelves and recalled; this included sports drinks, fruit beverages, tea beverages, jam, fruit pulp and fruit jelly, and food supplements in capsules, tablets or powders. The products will be allowed back on the shelf after the businesses provide product safety certifications verifying that the products contain no plasticizers. During its ongoing investigations of DEHP- and DiNP-containing clouding agents from Yu Shen Chem. Co. Ltd. and Pin Han Perfumery Co. Ltd., respectively, the Food and Drug Administration notified the World Health Organization and the European Union Rapid Alert System of Food and Feed of such products and the relevant companies.[3] Simultaneously, countries that have imported the tainted products have also been notified to take responsive measures. Nevertheless, this scandal has caused shock and panic among the majority of Taiwanese people and has attracted international attention.

Metabolism of phthalates

The metabolism and elimination of phthalates are complex.[5] In a first rapid step, which can occur at various stages/sites in the body (e.g., mouth or skin, stomach, intestines, or blood), the phthalate diester is cleaved into the respective hydrolytic monoester. In a second step, the alkyl chain of the resulting hydrolytic monoester can be modified by various oxidation reactions. In a third step, both the hydrolytic monoester and the oxidized secondary metabolites can be conjugated with glucuronic acid and finally excreted in urine. The extent of oxidative modification increases relative to the alkyl chain length of the phthalate monoester. Oxidative metabolites are more water soluble than the corresponding hydrolytic monoesters, which, in turn, have decreased water solubility when the length of the alkyl chain increases. Therefore, low-molecular-weight phthalates are mostly metabolized to their hydrolytic monoesters.[5] By contrast, high-molecular-weight phthalates with $\geq$8 carbons in the alkyl chain metabolize to their hydrolytic monoesters, which are extensively transformed to oxidative products.[5] Selected phthalates and their respective metabolites (as biomarkers of exposure) that are currently being investigated in epidemiological studies are summarized in Table 1.

Phthalate exposure assessment

Ambient monitoring relies on measuring chemicals in environmental media, foodstuff and consumer products.[5] Based on these data, together with survey/questionnaire data on personal lifestyle, product use, and food consumption, scenarios representing realistic exposure situations are

Table 1 Commonly used phthalate esters and their primary (hydrolytic monoester) and secondary (oxidized monoester) metabolites.

Compound	Primary metabolite	Secondary metabolite
Low molecular weight phthalate		
Dimethyl phthalate	Mono-methyl phthlate	
Diethyl phthalate	Mono-ethyl phthalate	
Di-cyclohexyl phthalate	Mono-cyclohexyl phthalate	
Di-n-pentyl phthalate	Mono-n-pentyl phthalate	
Butyl-benzyl phthalate	Mono-benzyl phthalate	
Di-iso-butyl phthalate	Mono-iso-butyl phthalate	3OH-Mono-methylpropyl phthalate
Di-n-butyl phthalate	Mono-n-butyl phthalate	3OH-Mono-n-butyl phthalate
		3carboxy-Mono-propyl phthalate
High molecular weight phthalate		
Di(2-ethylhexyl) phthalate	Mono-(2-ethylhexyl) phthalate	5OH-Mono-(2-ethylhexyl) phthalate
		5oxo-Mono-(2-ethylhexyl) phthalate
		5carboxy-Mono-(2-ethylhexyl) phthalate
Di-n-octyl phthalate	Mono-n-octyl phthalate	3carboxy-Mono-propyl phthalate
Di-iso-nonyl phthalate	Mono-iso-nonyl phthalate	7OH-Mono-methyloctyl phthalate
		7oxo-Mono-methyloctyl phthalate
		7carboxy-Mono-methyheptyl phthalate
Di-iso-decyl phthalate and Dipropylheptyl phthalate	Mono-iso-decyl phthalate	6OH-Mono-propylheptyl phthalate
		6oxo-Mono-propylheptyl phthalate
		Mono(2,7-methyl-7-carboxyheptyl) phthalate (mono-carboxyisooctyl phthalate)

generated to calculate the range in daily exposure through these pathways. Combining these external exposure estimates with organ- and situation-specific uptake rates, the daily internal exposure in (mg/kg/day) can be calculated. The main aim of these models, however, is to estimate possible contributions of different pathways to the total exposure and not to estimate reliably the overall or average extent of exposure of the general population. However, the ubiquitous presence of phthalates in the environment poses an analytical challenge known as the phthalate blank problem.[5]

Another way to perform an exposure assessment is human biomonitoring.[5] Human biomonitoring determines internal exposure or body burden by measuring the chemicals, their metabolites or specific reaction products in human specimens. Most of the biomarkers used in human plasticizer biomonitoring are specific metabolites generated in the human body (Table 1), therefore, they are not prone to environmental phthalate contamination.[5] Although phthalate monoester metabolites can be measured in many biological samples such as urine, serum, breast milk, saliva, seminal fluid, and amniotic fluid, they are most commonly measured in urine using high-performance liquid chromatography with tandem mass spectrometry. Serum concentrations of phthalates have been measured, but phthalates have been measured in much greater quantities in urine as compared with serum.[6] This may be due to the fact that serum contains enzymes that convert diesters into monoesters. Therefore, phthalate body burden can be estimated through measurement of phthalate metabolites in the urine.

Tolerable daily intake/reference dose level/ minimal risk level

Table 2 shows risk assessments of phthalates, performed by different expert panels in Europe and America.[2] Based on the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) derived from animal studies, the Scientific Committee on Toxicity, Ecotoxicity and the Environment obtained tolerable daily intake (TDI) values for phthalates. Phthalates, which are commonly used such as DEHP and DINP, are those with the lowest calculated TDI values.[2] The minimal risk level (MRL), derived by the Agency for Toxic Substances and Disease Registry are obtained by consideration of the most sensitive endpoint and the most sensitive species, using NOAEL or LOAEL and taking uncertainty factors into account. Thus, the lowest MRL of 0.06 mg/kg/day has been calculated for chronic exposure to DEHP, based on a NOAEL of 5.8 mg/kg for adverse testicular effects, and an intermediate duration oral MRL based on an LOAEL of 140 mg/kg bodyweight for reduced fertility.[2] The US Environmental Protection Agency has derived a chronic reference dose level of 0.02 mg/day for DEHP based on an LOAEL of 19 mg/kg/day for hepatic effects in guinea pigs.[2]

Toxicity of phthalates in animal models

In mammalian development, the fetus is set to develop into a female (Fig. 2).[7] This is not always the default pathway,

Table 2 Risk assessment of phthalates (reprinted by permission from Elsevier: *Int J Hyg Environ Health*[2]).

	Country	Committee	mg/kg/d	MRL/TDI/RfD
DEP	USA	ATSDR	7	MRL acute oral exposure
			5	MRL chronic oral exposure
		US-EPA	0.8	RfD chronic exposure
DnBP	USA	ATSDR	0.5	MRL acute oral exposure
		US-EPA	0.1	RfD chronic exposure
	Canada	Health Canada	0.06	TDI
	EU	CSTEE	0.1	TDI
BBzP	USA	US-EPA	0.2	RfD chronic exposure
	EU	CSTEE	0.2	TDI
DEHP	USA	US-EPA	0.020	RfD chronic exposure
		ATSDR	0.100	MRL intermediate duration exposure
			0.060	MRL chronic exposure
	Canada	Health Canada	0.044	TDI
	EU	CSTEE	0.050	TDI
		ECB/EU	0.020	TDI for newborns <3 mo, women in childbearing age
			0.025	TDI infants 3 to <12 mo
			0.048	TDI population except newborns, infants, women in childbearing age
DnOP	USA	ATSDR	3	MRL acute oral exposure
			0.4	MRL intermediate duration exposure
	EU	CSTEE	0.37	TDI
DiNP	EU	CSTEE	0.15	TDI
DiDP	EU	CSTEE	0.25	TDI

ATSDR = Agency for Toxic Substances and Disease Registry; BBzP = butyl benzyl phthalate; CSTEE = Scientific Committee on Toxicity, Ecotoxicity and the Environment; DEP = diethyl phthalate; DnBP = di-n-butyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DnOP = di-n-octyl phthalate; DiNP = di-iso-nonyl phthalate; DiDP = di-iso-decyl phthalate. ECB = European Chemicals Bureau; EPA = Environmental Protection Agency; MRL = minimal risk level; TDI = tolerable daily intake; RfD = reference dose.

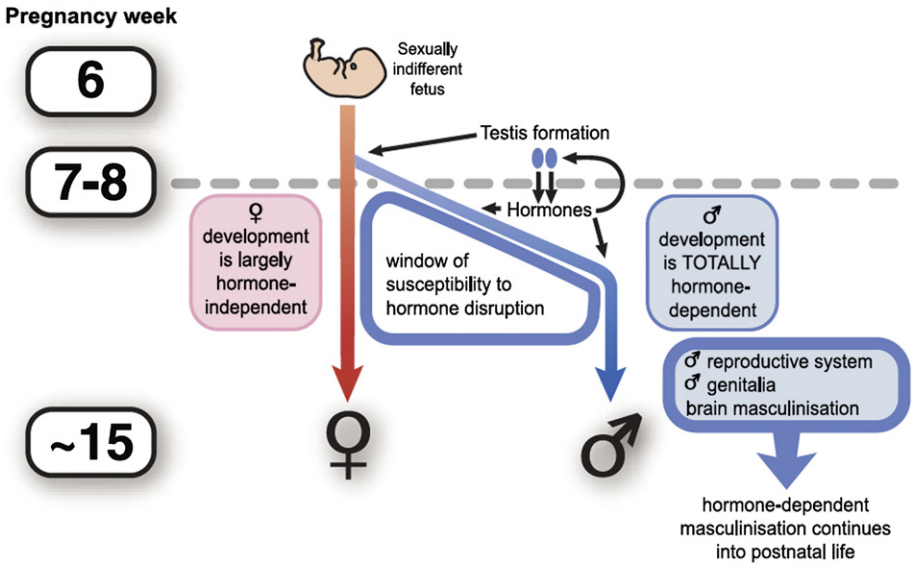

Figure 2 Sex determination and sexual differentiation in humans (reprinted by permission from Elsevier: *Best Pract Res Clin Endocrinol Metab*[7]). The preset program is for the sexually undifferentiated fetus to follow the female development pathway; diversion from this pathway is achieved by formation of the testes. Hormone production by the testes is then responsible for the masculinization process that is responsible for most of the sexual differentiation. As a consequence, male development, but not female development, is completely hormone-dependent and is, thus, inherently more susceptible to endocrine disruption.

but in general, the external genitalia and internal reproductive tract develop into female organs. Thus, the Mullerian ducts persist internally and develop into the fallopian tubes, uterus and upper part of the vagina, whereas the Wolffian ducts, which otherwise develop into the epididymis, vas deferens, and seminal vesicles (in the male), spontaneously degenerate.[7] For a fetus to develop into a male, the female development pathway must be disrupted. This intervention is initiated by activation of the Sry gene on the Y chromosome. Through processes that are incompletely understood, but which involve activation of factors such as WT1, SF1, and in particular Sox9, the undifferentiated gonad begins to become a testis via differentiation of the Sertoli cells. This is followed by a number of events that result in the formation of seminiferous cords containing premeiotic germ cells and outside of and between the seminiferous cords, hormonally active Leydig cells. In this way, a testis is formed. Completion of testis formation does not, however, lead automatically to development along the male development pathway. For this to happen, hormonal intervention is required on three separate but interrelated fronts, involving the production and secretion by the testis of anti-Mullerian hormone, testosterone, and insulin-like factor 3. Therefore, it should be apparent that becoming a phenotypic male is not just about forming a testis; it is very largely a hormone-dependent process, with each of the hormones playing a role to divert development from the female pathway.[7] In rats, for example, the programming window for testis development occurs during the last week of gestation (gestational days 15.5—21.5). This programming window is divided into three distinct phases: early, middle, and late.[8] The early phase, between gestational days 15.5 and 17.5, is the period when testosterone production is initiated. The middle phase, gestational days 17.5—19.5, is immediately before the period of morphological differentiation of the male reproductive system, and corresponds to the period when testosterone production in the male fetus peaks (at gestational day 19). The late phase between gestational days 19.5 and 21.5 is the period when reproductive cells morphologically differentiate (prostatic budding and Wolffian duct differentiation).[8]

The female phenotype is the default pathway, therefore, impairment of testosterone production or action before the late phase may lead to feminizing characteristics such as reduced anogenital distance and hypospadias.[8] Unlike other antiandrogens, which act by binding to the androgen receptors, and to inhibit their ability to respond to androgens, phthalates disrupt the development of androgen-dependent structures, mainly by inhibiting fetal testicular testosterone biosynthesis.[9—11] This effect is mediated by changes in gene expression of enzymes and proteins involved in testosterone production by fetal Leydig cells, including the steroidogenic acute regulatory protein, which participates in the transport of cholesterol to the inner mitochondrial membrane, which is the rate-limiting step in the steroidogenic pathway.[12,13] It has recently been shown that the expression of another product of the fetal Leydig cells, insulin-like factor 3, is reduced in phthalate-exposed animals.[10] Such an effect might explain the incidence of retention of testes in the abdomen (cryptorchidism) following phthalate exposure, because insulin-like factor 3 is involved in the initial stages of testicular descent into the scrotum.[10,14]

Some phthalates, such as DnBP,[15,16] DiBP,[17—19] DEHP,[9,20,21] BBzP[22,23] and DiNP,[20,24] are developmental and reproductive toxicants. They modulate the endogenous production of fetal testicular testosterone and influence insulin-like factor 3 and follicle-stimulating hormone production.[25] Critical effects are related to functional and

structural impairment of male reproduction and development,[14,20,26] and manifest in malformations of the epididymis and the external genitalia (hypospadias), undescended testes (cryptorchidism), impaired spermatogenesis, and a general reduction of male fertility.[15] Phthalates also cause signs of feminization (retention of nipples/areolae in male rodents) and a reduced anogenital distance as a first indication of general demasculinization.[16,23] This group of symptoms in animals is called phthalate syndrome.[14,27]

Phthalates exhibit low acute toxicity with lethal dose 50 values of 1–30 g/kg body weight or with even higher concentrations.[2] In short- and long-term rodent studies, dose-related adverse effects have been found in liver, kidney and, for selected phthalates, thyroid gland and testis. All phthalates have been tested negative for mutagenicity or genotoxicity. With regard to carcinogenicity, the activity of DEP is questionable; for DiNP, no hints for carcinogenicity have been observed; DBP seems to be associated with tumor promoting activity; and exposure to DEHP produces hepatocellular carcinoma in rodents, along with a variety of other hepatocellular effects such as proliferation of peroxisomes and mitochondria, increased Cyp4A1 and peroxisomal palmitoyl CoA (PCoA) activity, liver tissue proliferation, and suppression of apoptosis.[2] Most of these effects are mediated by an induction of the peroxisome proliferator-activated receptor (PPAR)-α.[28] In PPAR-α knockout mice, administration of DEHP does not result in hepatocellular effects. For several reasons, for example, differences in PPAR-α density, regulation and signaling pathways, adverse effects associated with PPAR activation in rodents do not occur in humans. Nevertheless, the US Environmental Protection Agency has determined that DEHP is a probable human carcinogen. Furthermore, DEHP is included in the list of Group 2B carcinogens by the International Agency for Research on Cancer,[29] and listed by the Environmental Protection Administration Executive Yuan, R.O.C. (Taiwan) as a Category 4 toxic chemical.

Toxicity of phthalate in human infants and children

The spectrum of effects obtained following perinatal exposure of male rats to phthalates has remarkable similarities with human testicular dysgenesis syndrome. According to Skakkebaek et al,[30] human testicular dysgenesis syndrome is characterized by low sperm counts, cryptorchidism, hypospadias, and testicular cancer, and the clinical expression of these symptoms may vary with the severity of the syndrome. Accordingly, less severe manifestations would result in impaired spermatogenesis, while other symptoms, such as testicular cancer, may be present in more severely affected individuals.[30]

Several epidemiological studies have examined the relationship between phthalate exposure and adverse outcomes in infants and children (Table 3).[31,53–58,60–64] In one of the pioneering studies, Swan et al[32] have found that urinary concentrations of four phthalate metabolites [mono-ethyl phthalate (MEP), mono-n-butyl phthalate (MBP), monobenzyl phthalate (MBzP) and mono-iso-butyl phthalate (MiBP)] were inversely related to the anogenital index. The anogenital index was defined as anogenital distance divided by weight at examination. After adjusting for age, p values for regression coefficients ranged from 0.007 to 0.097. Comparison between boys with a prenatal MBP concentration in the highest quartile with those having a prenatal MBP concentration in the lowest quartile yielded an odds ratio (OR) for a shorter than expected anogenital index of 10.2 [95% confidence interval (CI) = 2.5–42.2]. The corresponding ORs for MEP, MBzP and MiBP were 4.7, 3.8 and 9.1, respectively ($p < 0.05$). Furthermore, the age-adjusted anogenital index decreased significantly as the phthalate score increased (p value for slope = 0.009). The association between male genital development and observed phthalate exposure is consistent with the phthalate-related syndrome of incomplete virilization. The median concentrations of phthalate metabolites that are associated with a short anogenital index and incomplete testicular descent are below the concentrations found in one-quarter of the female population in the United States, on the basis of a nationwide sample. These data support the hypothesis that prenatal phthalate exposure at environmental levels can adversely affect male reproductive development in humans.[32] Main et al[33] have investigated whether phthalate monoester contamination of human breast milk had any influence on the postnatal surge of reproductive hormones that signals testicular dysgenesis in newborn boys. All phthalate monoesters were found in breast milk with large variations: mono-methyl phthlate (MMP) 0.10 (< 0.01–5.53 μg/L); MEP 0.95 (0.07–41.4 μg/L); MBP 9.6 (0.6–10,900 μg/L); MBzP 1.2 (0.2–26 μg/L); mono-(2-ethylhexyl) phthalate (MEHP) 11 (1.5–1410 μg/L); and mono-iso-nonyl phthalate (MiNP) 95 (27–469 μg/L). Finnish breast milk had higher concentrations of MBP, MBzP and MEHP, and Danish breast milk had higher values for MiNP (p = 0.0001–0.056). No association was found between phthalate monoester levels and cryptorchidism. However, MEP and MBP showed positive correlations with sex-hormone binding globulin (r = 0.323, p = 0.002 and r = 0.272, p = 0.01, respectively); MMP, MEP, and MBP with luteinizing hormone:free testosterone ratio (r = 0.21–0.323, p = 0.002–0.044) and MiNP with luteinizing hormone (r = 0.243, p = 0.019). MBP was negatively correlated with free testosterone (r = −0.22, p = 0.033). The findings were in line with other human data showing incomplete virilization in infant boys prenatally exposed to phthalates.[33]

Latini et al[34] have measured serum DEHP and MEHP concentrations in the cord blood of 84 newborns and found detectable cord blood DEHP and/or MEHP concentrations in 88.1% of the samples. Either DEHP or MEHP was present in 65 of 84 (77.4%) of the examined samples. Mean concentrations of DEHP and MEHP were 1.19 ± 1.15 μg/mL (95% CI = 0.93–1.44) and 0.52 ± 0.61 μg/mL (95% CI = 0.39–0.66), respectively. MEHP-positive newborns showed a significantly lower gestational age compared with MEHP-negative infants (p = 0.033). Logistic regression analysis results indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery (OR = 1.50, 95% CI = 1.013–2.21; p = 0.043). These findings confirmed that human exposure to DEHP can begin *in utero* and suggest that phthalate exposure is significantly associated with a shorter pregnancy duration.[34] Colon et al[35] have attempted to identify pollutants in the serum of Puerto Rican girls with premature breast

Table 3 Health outcomes in infants and children associated with phthalate concentration in biological or environmental samples (reprinted by permission from Elsevier: *Environ Res*[31]).

System	Timing of exposure	Sex	Outcome	Phthalate or metabolite (measured in urine unless otherwise noted)	Reference
Reproductive	Prenatal	Male/female	Shorter gestational age at birth	MEHP (in cord blood)	[34]
	Prenatal (mean age: 12.6 mo at examination)	Male	Shorter anogenital distance	MEHP, MEOHP, MEHHP, MEP, MBP	[31,32]
			Reduced penile size	MEHP	
			Incomplete testicular descent	MEHP, MEHHP, MEOHP	
	Lactation (mean age 3 mo)	Male	Increased sex hormone binding globulin	MEP, MBP	[33]
			Increased luteinizing hormone/ free testosterone	MMP, MEP, MBP	
			Increased luteinizing hormone	MiNP	
			Decreased free testosterone	MBP	
	Early childhood	Female	Premature thelarche	DEHP (in serum)	[35]
Respiratory, allergy and asthma	Childhood	Male/female	Rhinitis and eczema	BBzP (in house dust)	[36]
			Asthma	DEHP (in house dust)	
			Wheezing, rhinitis and eczema	DEHP (in house dust)	[37]

BBzP = butyl benzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; MBP = mono-butyl phthalate; MEHHP = mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEHP = mono-(2-ethylhexyl) phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl) phthalate; MEP = mono-ethyl phthalate; MiNP = mono-isononyl phthalate; MMP = mono-methyl phthalate.

development or thelarche. A total of 41 serum samples from thelarche patients and 35 control samples were analyzed. No pesticides or their metabolite residues were detected in the serum of the study or control subjects. Significantly high levels of phthalates (DMP, DEP, DBP and DEHP) and its major metabolite MEHP were identified in 28 (68%) samples from thelarche patients. Of the control samples analyzed, only one showed significant levels of mono-iso-nonyl phthalate. This study suggests a possible association between plasticizers with known estrogenic and antiandrogenic activity and the cause of premature breast development in a human female population.[35]

Bornehag et al[36] have investigated possible associations between persistent allergic symptoms in children and the concentration of phthalates in dust collected from their homes. Out of a cohort of 10,852 children, 198 cases with persistent allergic symptoms and 202 controls without allergic symptoms were selected. Higher median concentrations of BBzP in dust were found among cases than among controls (0.15 *vs*. 0.12 mg/g dust). Analysis of the case group by symptoms showed that BBzP was associated with rhinitis ($p = 0.001$) and eczema ($p = 0.001$), whereas DEHP was associated with asthma ($p = 0.022$). Furthermore, dose–response relationships for these associations were supported by trend analyses. This study has shown that phthalates, within the range of what is normally found in indoor environments, are associated with allergy symptoms in children.[36] Finally, Kolarik et al[37] have investigated the associations between allergy symptoms in children and the concentration of phthalate esters in settled dust collected from children's homes in Sofia and Burgas, Bulgaria. A total of 102 children (2–7 years of age) had symptoms of wheezing, rhinitis, and/or eczema in the preceding 12 months (cases), and 82 were nonsymptomatic (controls). A higher concentration of DEHP was found in the homes of case children than in those of controls (1.24 *vs*. 0.86 mg/g dust). The concentration of DEHP was significantly associated with wheezing in the preceding 12 months ($p = 0.035$). A dose–response relationship was found between DEHP concentration and case status and between DEHP concentration and wheezing in the preceding 12 months. The study established a potential association between the concentration of DEHP in indoor dust and wheezing among preschool children in Bulgaria.[37]

Apart from the list in Table 2, recent epidemiological studies also have suggested possible associations between phthalate exposure and shorter gestational age,[38,39] shorter anogenital distance,[40,41] precocious puberty,[42] pubertal gynecomastia,[43] premature thelarche,[44] low birth weight,[45] attention deficit hyperactivity disorder,[46,47] low intelligence quotient,[48] thyroid dysfunction and growth retardation,[49] and hypospadias[50] in infants and children.

The clinical significant of anogenital distance has been explored in two recent studies. Mendiola et al[51] have found that anogenital distance (measured from anus to the posterior base of the scrotum) is associated with sperm concentration, motility, morphology, total sperm count and total motile count ($p = 0.002$–0.048). Men with anogenital distance below (compared to above) the median were 7.3 times more likely (95% CI = 2.5–21.6) to have a low sperm concentration ($<20 \times 10^6$/mL).[51] Eisenberg at al[52] also have found that infertile men possess significantly shorter mean anogenital distance and penile length compared to fertile controls (anogenital distance: 31.8 *vs*.44.6 mm, penile length: 107.1 *vs*.119.5 mm, $p < 0.01$). On both unadjusted and adjusted linear regression, anogenital distance was significantly correlated with sperm density and total motile sperm count. After adjusting for demographic and reproductive variables, for each 1-cm increase in male anogenital distance, the sperm density increases by 4.3 million/mL (95% CI = 0.53–8.09 million/mL, $p = 0.03$) and the total motile sperm count increases by 6.0 million sperm (95% CI = 1.34–10.58 million, $p = 0.01$).[52]

Toxicity of phthalate in adults

Clinical studies that have explored phthalate adverse effects in adults are presented in Table 4.[31] Duty et al[53] have explored whether environmental levels of phthalates are associated with altered semen quality in humans. A dose–response relationship was found between tertiles of MBP and sperm motility (OR per tertile = 1.0, 1.8, 3.0; p value for trend = 0.02) and sperm concentration (1.0, 1.4, 3.3; p value for trend = 0.07). In addition, there was a dose–response relationship between tertiles of MBzP and sperm concentration (1.0, 1.4, 5.5; p value for trend = 0.02).[53] In another study, Duty et al[54] have investigated whether environmental levels of phthalates were associated with altered DNA integrity in human sperm. One hundred and sixty-eight subjects were recruited from the Massachusetts General Hospital. For an interquartile range increase in specific gravity-adjusted MEP level, the comet extent of the sperm increased significantly by 3.6 μm (95% CI = 0.74–6.47); the tail distributed moment also increased by 1.2 um (95% CI = −0.05 to 2.38) but was of borderline significance. The data demonstrated that urinary MEP was associated with increased DNA damage in sperm.[54] Duty et al[55] have also investigated the association between environmental levels of phthalates and altered reproductive hormone levels in adult men. An interquartile range change in MBzP exposure was significantly associated with a 10% (95% CI = −16 to −4) decrease in follicle-stimulating hormone concentration. In addition, an interquartile range change in MBP exposure was associated with a 4.8% (95% CI = 0–10) increase in inhibin B, but this was of borderline significance. Although the researchers found associations between MBP and MBzP urinary concentrations and altered levels of inhibin B and follicle-stimulating hormone, the hormone concentrations did not change in the expected patterns. Therefore, it is unclear whether these associations represent physiologically relevant alterations in these hormones, or whether they represent associations found as a result of conducting multiple comparisons.[55]

In a later study, Hauser et al[56] recruited 463 male partners of subfertile couples who presented for semen analysis to the Massachusetts General Hospital. There were dose–response relationships of MBP with low sperm concentration (OR per quartile adjusted for age, abstinence time, and smoking status = 1.00, 3.1, 2.5, 3.3; p value for trend = 0.04) and motility (1.0, 1.5, 1.5, 1.8; p value for trend = 0.04). There was suggestive evidence of an

Table 4 Health outcomes in adults associated with phthalate concentration in biological samples (reprinted by permission from Elsevier: *Environ Res*[31]).

System	Sex	Outcome	Phthalate or metabolite (measured in urine unless otherwise noted)	Reference
Reproductive	Male	Increased sperm DNA damage	MEP, MEHP	57
		Increased sperm DNA damage	MEP	54
		Decreased sperm motility	MBP	53, 56
		Decreased sperm concentration	MBP, MBzP	53, 56
	Male	Decreased sperm morphology	DEHP in semen samples	60
	Male	Decreased free testosterone and increased luteinizing hormone/free testosterone	MBP	61
	Male	Decreased follicle-stimulating hormone	MBzP	55
	Male	Decreased motility and reduced luteinizing hormone	MEP	58
Respiratory and immune (including allergy and asthma)	Male	Decreased pulmonary function	MEP, MBP	62
Metabolic	Male	Increased waist circumference	MBzP, MEHHP MEOHP, MEP	63
		Increased insulin resistance	MBP, MBzP MEP	
Thyroid	Male	Thyroid (decreased triiodothyronine and thyroxine)	MEHP	64

DEHP = di(2-ethylhexyl) phthalate; MBP = mono-butyl phthalate; MBzP = mono-benzyl phthalate; MEHP = mono-(2-ethylhexyl) phthalate; MEHHP = mono-(2-ethyl-5-hydroxylhexyl) phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl) phthalate; MEP = mono-ethyl phthalate.

association between the highest MBzP quartile and low sperm concentration (1.00, 1.1, 1.1, 1.9; p value for trend = 0.13). There were no relationships between MEP, MMP and DEHP metabolites and the semen parameters. Therefore, the study confirmed previous results that showed a relationship of altered semen quality and exposure to MBP at general population levels.[56] In another study involving 379 men from an infertility clinic,[57] urinary concentrations of phthalate metabolites were measured. Sperm DNA damage measurements included comet extent, percentage of DNA in tail, and tail distributed moment. MEP, a metabolite of DEP, was associated with increased DNA damage. MEHP, a metabolite of DEHP, was associated with DNA damage after adjustment for the oxidative DEHP metabolites. After adjustment for mono-(2-ethyl-5-hydroxylhexyl) phthalate (MEHHP), for an interquartile range increase in urinary MEHP, comet extent increased by 17.3% (95% CI = 8.7–25.7%), tail distributed moment increased by 14.3% (95% CI = 6.8–21.7%) and percentage of DNA in tail increased by 17.5% (95% CI = 3.5–31.5%). Therefore, sperm DNA damage was associated with MEP and MEHP after adjusting for DEHP oxidative metabolites.[57]

In a Swedish study,[58] urine, serum and semen samples were collected from 234 young men at the time of their medical conscript examination. Semen volume, sperm concentration, and motility were measured, together with sperm chromatin integrity (sperm chromatin structure assay) and biochemical markers of epididymal and prostatic function. For MBP, MBzP and MEHP, no clear pattern of associations was observed with any of the reproductive biomarkers. Subjects within the highest quartile for MEP had fewer motile sperm (mean difference = 8.8%; 95% CI = 0.8–17), more immotile sperms (8.9%; 95% CI = 0.3–18), and lower luteinizing hormone values (0.7 IU/L; 95% CI = 0.1–1.2), but there was no suggestion of harmful effects for most other endpoints. Phthalic acid actually was associated with improved reproductive function, as measured by several markers. The observed weak associations between one phthalate biomarker and impairment of a few aspects of reproductive function biomarkers[58] were not consistent with the results from a study in the United States.[59]

In a Chinese study, Zhang et al[60] monitored the level of phthalates in human semen samples and analyzed the relationship between phthalate levels and semen parameters. Three phthalates (DEP, DBP and DEHP) were detected in most of the biological samples, with median levels of 0.30 mg/L (0.08–1.32 mg/L) in semen specimens. There was a significant positive association between liquefied time of semen and phthalate concentrations in semen. The correlation coefficients were 0.456 for DEP, 0.475 for DBP, and 0.457 for DEHP. There was no significant difference between phthalate concentrations in semen and sperm density or livability, although the correlation coefficients were negative. These results suggest that people who reside in Shanghai are exposed to phthalates, especially to DBP and DEHP. Although the level of phthalates is relatively mild, an association of phthalate levels and reduced quality of human semen has been demonstrated in the Chinese study.[60] In another study, Pan et al[61] examined urine and blood samples from 74 male workers who worked at a factory that produced unfoamed PVC flooring, and were

exposed to DBP and DEHP, and compared the samples to control samples from 63 male construction workers. Compared to the unexposed workers, the exposed had substantially and significantly elevated concentrations of MBP (644.3 *vs.* 129.6 μg/g creatinine, $p < 0.001$) and MEHP (565.7 *vs.* 5.7 μg/g creatinine, $p < 0.001$). Free testosterone was significantly lower (8.4 *vs.* 9.7 μg/g creatinine, $p = 0.019$) in exposed workers than in unexposed. Free testosterone was negatively correlated to MBP ($r = -0.25$, $p = 0.03$) and MEHP ($r = -0.19$, $p = 0.095$) in the group of exposed workers. Regression analyses revealed that free testosterone decreased significantly with increasing total phthalate ester score (the sum of quartiles of MBP and MEHP; $r = -0.26$, $p = 0.002$). The data confirmed a modest and significant reduction of serum-free testosterone in workers with higher levels of urinary MBP and MEHP compared to unexposed workers.[61]

Hoppin et al[62] have assessed the association between phthalate exposure and four pulmonary function parameters [forced vital capacity (FVC), forced expiratory volume at 1 second (FEV1), peak expiratory flow (PEF), and maximum mid-expiratory flow] among the 240 adult participants of the Third National Health and Nutrition Examination Survey (NHANES III) with urinary phthalate data. MBP was significantly associated with decrements in three measures of pulmonary function (FVC, FEV1 and PEF) in men but not in women. Between the 25th to the 75th percentile in the MBP level among men, the FEV1 decreased to 112 mL (standard error = 51, $p = 0.03$). MEP levels were associated with lower FVC and FEV1 values in men. MEHP levels were not adversely associated with any of the pulmonary function parameters evaluated. The results suggested that MBP and MEP, but not MEHP, might influence pulmonary function among adult men.[62]

Stahlhut et al[63] have investigated phthalate exposure and its associations with abdominal obesity and insulin resistance. Subjects were US adult male participants of the National Health and Nutrition Examination Survey (NHANES) 1999–2002. Six phthalate metabolites were modeled with prevalent exposure and known or suspected antiandrogenic activity as predictors of waist circumference and log-transformed homeostatic model assessment (HOMA; a measure of insulin resistance) using multiple linear regression; adjusted for age, race/ethnicity, fat and total calorie consumption, physical activity level, serum creatinine, and urine creatinine (model 1); and were adjusted for model 1 covariates plus measures of renal and hepatic function (model 2). In model 1, four metabolites were found to be associated with increased waist circumference (MBzP, MEHHP, MEOHP and MEP; $p \leq 0.013$) and three with increased HOMA (MBP, MBzP and MEP; $p \leq 0.011$). When also adjusted for renal and hepatic function, parameter estimates declined, but all significant results were confirmed, except for HOMA-MBP. The findings suggest that concentrations of several prevalent phthalate metabolites have significant correlations with abdominal obesity and insulin resistance.[63] Meeker et al[64] have collected concurrent samples of urine and blood from 408 men in the United States. An inverse association was found between MEHP urinary concentrations and free thyroxine and triiodothyronine serum levels, although the relationships did not appear to be linear when MEHP concentrations were categorized by quintiles. There was evidence of a plateau at the fourth quintile, which was associated with a 0.11 ng/dL decrease in free thyroxine (95% CI: −0.18 to −0.03) and a 0.05 ng/mL decrease in triiodothyronine (95% CI: −0.10 to 0.01) compared with the first (lowest) MEHP quintile. The inverse relationship between MEHP and free thyroxine remained when adjusted for oxidative metabolite concentrations; this simultaneously demonstrated a suggestive positive association with free thyroxine. Therefore, urinary MEHP concentrations may be associated with altered free thyroxine and/or total triiodothyronine levels in adult men.[64]

Apart from the list in Table 3, recent epidemiological studies have also suggested possible associations between phthalate exposure and endometriosis,[65–70] uterine leiomyomas,[66] breast cancer,[71] obesity,[72] poor semen quality,[73] hyperprolactinemia,[74] and low circulating sex steroid hormone levels[75] in adults.

Phthalate exposure-related studies in Taiwan

The number of phthalate-related scientific publications from Taiwan has increased greatly over the past 5 years.[76–82] It is not certain if this reflects the adverse health effects following illegal addition of DEHP and DiNP to clouding agents in foods and beverages over the past two decades. In 2007, Huang et al[76] examined the association between phthalate exposure and thyroid hormones in pregnant women. Serum and spot urine samples were collected from 76 pregnant women at second trimester. Urinary MBP, MEP and MEHP (median levels = 81.8, 27.7 and 20.6 ng/mL, respectively) were the predominant substances. Significant mild negative correlations were found between thyroxine and urinary MBP ($r = -0.248$, $p < 0.05$), and between free thyroxine and urinary MBP ($r = -0.368$, $p < 0.05$). After adjusting for age, body mass index and gestation, urinary MBP levels showed negative associations with free thyroxine and thyroxine (free thyroxine: beta = −0.110, $p < 0.001$; thyroxine: beta = −0.112, $p = 0.003$). Therefore, exposure to DBP might affect thyroid activity in pregnant women, but how DBP affects thyroid function remains unclear.[76] Chen et al[77] have examined the migration level of phthalate from PVC films by simulating food handling. To estimate a worst-case scenario of phthalate migration, food was covered with PVC films and then microwaved. The DEHP levels in food increased significantly after heating. The calculated intake of phthalates after heating and the percentage of the TDI (based on a 60 kg body weight) from eating a 400-g meal were 1705.6 μg and 92.2% for DEHP. Determinations of urinary metabolites from 60 subjects also revealed that MMP, MBP and MEHP were detectable in >90% of samples. The results reflected the extensive use of plastic materials in Taiwan.[77] Huang et al[78] also have evaluated the possible association between maternal urine excretion, exposure of the fetus to phthalates in amniotic fluid, and health of newborns. The median levels of three phthalate monoesters in urine and amniotic fluid were 78.4 and 85.2 ng/mL for MBP; 24.9 and 22.8 ng/mL for MEHP; 19.8 and not detected for MEP. A significant positive correlation was

found only between creatinine-adjusted urinary MBP and amniotic fluid MBP ($r^2 = 0.156$, $p < 0.05$) in all infants. In female infants, a significant negative correlation was found between amniotic fluid MBP, anogenital distance ($r = -0.31$, $p < 0.06$), and the anogenital index adjusted for birth weight ($r = -0.32$, $p < 0.05$). The data showed that *in utero* exposure to phthalates, in general, had antiandrogenic effects on the fetus.[78] In another study, Chou et al[79] recruited girls in early puberty: 30 with premature thelarche, 26 with central precocious puberty, and 33 normal controls. The mean urine levels of MMP were significantly higher in the premature thelarche group (96.5 ± 134.0 ng/mL) than in the control group (26.4 ± 30.0 ng/mL; $p = 0.005$). The levels of MBP correlated with the intake of seafood and drink, and the use of plastic cups. The levels of MEHP correlated with the intake of seafood and meat and exposure to plastic wrapping. The significantly higher MMP concentrations in the premature thelarche girls suggested that phthalate might be one of the environmental causes of early puberty in Taiwan.[79] Huang et al[80] have conducted another case–control study to determine whether estrogen-dependent diseases are associated with phthalate exposure and how the glutathione S-transferase M1 (GSTM1; a major detoxification enzyme) genotype might modulate the risk. Patients with leiomyoma had significantly higher level of total urinary MEHP (52.1 *vs.* 18.9 μg/g creatinine, $p < 0.05$) than the controls, whereas patients with endometriosis had an increased level of urinary MBP (94.1 *vs.* 58.0 μg/g creatinine, $p < 0.05$). Subjects with the GSTM1 null genotype had significantly higher risk for adenomyosis relative to those with the GSTM1 wild-type genotype (OR = 5.30; 95% CI = 1.22–23.1), even after adjustment for age and phthalate exposure. Subjects who carried the GSTM1 null genotype and had a high urinary level of MEHP showed a significantly increased rate of adenomyosis (OR = 10.4; 95% CI = 1.26–85.0) and leiomyoma (OR = 5.93; 95% CI = 1.10–31.9) after adjustment for age, compared to those with the GSTM1 wild-type genotype and low urinary levels of MEHP. These results suggest that both the GSTM1 null genotype and phthalate exposure are associated with adenomyosis and leiomyoma.[80] Lin et al[81] have assessed the possible association between maternal phthalate exposure and cord sex steroid hormones in pregnant women and their newborns among the general population. A total of 155 maternal and infant pairs were recruited and analyzed. No significant correlation was found between steroid hormones and phthalate metabolites for male newborns, although there was a marginally significant correlation between MMP and estradiol. After adjusting for maternal age, estradiol levels in cord serum from male newborns were not correlated with maternal urinary phthalate metabolites. In female newborns, the maternal urinary levels of MEHP and 5OH-MEHP were negatively correlated with the free testosterone and free testosterone/estradiol levels in cord serum, with Pearson correlation coefficients ranging between −0.24 and −0.29 ($p < 0.05$). In addition, after the gestational age was adjusted, the maternal urinary level of DEHP negatively correlated with the free testosterone and free testosterone/estradiol levels in cord serum. The data suggest that maternal exposure to phthalates affect the status of sex steroid hormones during the fetal and newborn stages of development.[81] In a recent study,[82] 45 workers were divided into low- and high-DEHP-exposed groups in accordance with the median levels of DEHP (23.7 $\mu g/m^3$) in personal air. In the high-DEHP-exposed group, significant increases were found in the tendency for sperm DNA denaturation, DNA fragmentation index, and propensity for coffee drinking. After adjusting for coffee drinking, cigarette smoking, and age, personal air concentrations of DEHP showed positive associations with DNA denaturation ($\beta = 0.038$) and DNA fragmentation index ($\beta = 0.140$) and a negative associations with sperm motility ($\beta = -0.227$). Therefore, the study demonstrated a link between the DEHP concentration in ambient air and adverse effects on sperm motility and chromatin DNA integrity.[82]

Conclusions

High exposure to phthalates can occur via chronic ingestion of foods and beverages prepared with phthalate plasticizer-containing clouding agents. Nevertheless, there are several basic steps that can help to reduce the burden of phthalates on the body: (1) drink more water to flush out more phthalates from the body; (2) eat a high-fiber diet to increase stool bulk and thereby increases phthalate excretion from the colon; (3) eat more fresh fruits and vegetables to ingest more vitamin C, a well-known antioxidant, which may help to overcome the increase in oxidative stress induced by phthalate exposure that leads to some adverse health conditions[83]; (4) eat less fatty food, in which levels of some phthalates are particularly high because of their attraction to fat molecules[84]; (5) eat more fresh foods and less prepackaged food because DEHP exposure has been demonstrated to be substantially reduced in diets restricted to food with limited packaging[85]; (6) wash hands before eating and drinking to help reduce oral ingestion of phthalates; (7) do not reheat food in plastic containers or plastic wrapping in the microwave because high temperatures may cause phthalates to leach out of the plastics; (8) avoid using a highly concentrated personal care product, because phthalates are used to add texture and luster to hair spray, deodorant, nail polish, lipstick, perfumes and many other baby products; and (9) in the case of chronic ingestion of phthalate-contaminated foods, especially when dietary exposure estimates exceed the TDI/reference dose level/MRL, stop consumption of the foods immediately and seek medical advice when in doubt.

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