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Review

Metabolism of phthalates in humans

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Phthalates are synthetic compounds widely used as plasticisers, solvents and additives in many consumer products. Several animal studies have shown that some phthalates possess endocrine disrupting effects. Some of the effects of phthalates seen in rats are due to testosterone lowering effects on the foetal testis and they are similar to those seen in humans with testicular dysgenesis syndrome. Therefore, exposure of the human foetus and infants to phthalates *via* maternal exposure is a matter of concern. The metabolic pathways of phthalate metabolites excreted in human urine are partly known for some phthalates, but our knowledge about metabolic distribution in the body and other biological fluids, including breast milk, is limited. Compared to urine, human breast milk contains relatively more of the hydrophobic phthalates, such as di-*n*-butyl phthalate and the longer-branched, di(2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP); and their monoester metabolites. Urine, however, contains relatively more of the secondary metabolites of DEHP and DiNP, as well as the monoester phthalates of the more short-branched phthalates. This differential distribution is of special concern as, in particular, the hydrophobic phthalates and their metabolites are shown to have adverse effects following *in utero* and lactational exposures in animal studies.

Keywords: Breast milk / Human excretion / Infant exposure / Metabolism / Phthalates

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1 Introduction

Phthalates belong to the group of endocrine disrupters, which have been shown to affect the male reproductive system in animal studies. Especially di-*n*-butyl phthalate (DBP) and the long-branched di(2-ethylhexyl) phthalate (DEHP) and their metabolites have been shown to cause antiandrogenic effects manifested as decreased anogenital distance (AGD), cryptorchidism, decreased testosterone levels, decreased sperm production and infertility [1 – 5]. These effects are observed in the male offspring of the rodents following *in utero* and lactational exposure. In contrast, similar exposures of adult males result in no or limited effects.

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Abbreviations: AGD, anogenital distance; **AGI,** anogenital index; **FAI,** free androgen index; **FSH,** follicle-stimulating hormone; **LH,** luteinising hormone; **SHBG,** sex hormone-binding globulin; **TDS,** testicular dysgenesis syndrome

During the last four to five decades, the male reproductive health in several Western countries seems to have, in fact, deteriorated and the human fertility rates have declined all over the world [6]. The changes include increased prevalence of hypospadias, cryptorchidism, testicular cancer [7 – 10] and declining semen quality [11 – 13]. The fact that experimental and epidemiological studies have shown correlations between reproductive disorders such as hypospadias, cryptorchidism, testis cancer and infertility or poor semen quality [6, 14, 15] has led to the hypothesis of the existence of testicular dysgenesis syndrome (TDS), which may result from the disruption of gonadal development during foetal life [16]. There are strong indications that environmental factors such as endocrine disrupters could be involved in with the increase in male reproductive health problems [6, 17, 18]. Humans are exposed to phthalates from a wide range of consumer products. Recent studies have shown that infants are exposed to phthalates and their metabolites through breast milk, infant formulae and baby food [19 – 23]. Also prenatal exposure to phthalates through maternal exposure occurs [24 – 26]. Finally, phthalate exposure has also been a consequence of neonatal intensive care [27]. In the light of the TDS-hypothesis and our knowledge from experimental animal stud-

Table 1. Diester phthalates and their metabolites

Phthalates		Metabolites	
Dimethyl phthalate	DMP	Monomethyl phthalate	MMP
Diethyl phthalate	DEP	Monoethyl phthalate	MEP
Di-*n*-butyl phthalate	DBP	Mono-*n*-butyl phthalate	MBP
Di-iso-butyl phthalate	DiBP	Mono-iso-butyl phthalate	MiBP
Butylbenzyl phthalate	BBzP	Monobenzyl phthalate	MBzP
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethylhexyl) phthalate	MEHP
		Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP or 5OH-MEHP
		Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP or 5oxo-MEHP
		Mono(2-ethyl-5-carboxypentyl) phthalate	MECPP or 5cx-MEPP
		Mono(2-carboxy-hexyl) phthalate	MCMHP or 2cx-MMHP
Di-iso-nonyl phthalate	DiNP	Mono-iso-nonyl phthalates	MiNP
		Mono(hydroxy-iso-nonyl) phthalate	MHiNP or OH-MiNP
		Mono(oxo-iso-nonyl) phthalate	MOiNP or oxo-MiNP
		Mono(carboxy-iso-octyl) phthalate	MCiOP or cx-MiNP

ies, the substantial exposure of phthalates to infants is of particular concern.

2 Occurrence and chemistry

Phthalates are a group of synthetic industrial chemicals, which were introduced in the 1920s and since 1933, when DEHP was first synthesised, phthalates have become the most widespread plasticisers. Phthalates are now widely used all over the world, not only as plasticisers but also as additives in industrial products, including food and personal care products [21, 22, 28]. Especially, the addition of DEHP to polyvinyl chlorides (PVC) for flexibility has been an enormous industrial success [29]. However, due to its toxic and endocrine disrupting effects, DEHP has today been replaced by di-iso-nonyl phthalate (DiNP) as the most commonly used plasticiser in PVC in Europe [30]. Phthalates are diesters of 1,2-benzenedicarboxylic acids (phthalic acid). The specific characteristics and the decomposing pattern of the phthalates depend on the length of the dialkyl or alkyl/aryl side chain. The more branched the phthalates are, the more isomeric forms are available and the more hydrophobic the single compound is. Table 1 shows a list of diester phthalates and the most common metabolites, including their abbreviations and Fig. 1 shows the chemical structures of some of the most common phthalates used in Europe [28].

The short-branched low molecular weight phthalates such as dimethyl phthalate (DMP) and diethyl phthalate (DEP) are widely used, especially in cosmetic products. DEP are found in almost all categories of personal care products for infants, children, and adults [31]. Furthermore, DMP and DBP are common in cosmetic products for adults such as perfume, aftershaves, shampoos, make up and nail care products [28]. Not only long-branched high molecular weight phthalates such as butylbenzyl phthalate (BBzP), DEHP and DiNP, but also DBP and di-iso-butyl phthalate (DiBP) are common in many kinds of plastic products, including vinyl flooring, paint and other building materials, toys, plastic bags, gloves, shoes and imitated leather. In addition, DEHP is used as a plasticiser in some medical devices such as blood storage bags and intravenous medical tubing [28]. Some soft plastic material contain up to 40% DEHP [32]. In Europe, most of the food in contact with plastic contain DEHP and DBP. DiBP and DEHP are also found in common food products, such as cereals, bread, biscuits, cakes, nuts, spices, fat and oil in amounts up to about 10 mg/kg [28]. Thus, nearly all groups of industrial consumer products contain phthalates or traces of phthalates. Although phthalates are nonpersistent chemicals that are rapidly metabolised, contamination of the environment is significant due to the widespread use and presence of both low and high molecular weight phthalates in dust, soil, indoor and outdoor air [28, 32, 33]. Therefore, the human exposure to phthalates is ubiquitous.

3 Metabolism of phthalates

Phthalates normally follow a metabolic pathway in at least two steps; a phase I hydrolysis followed by phase II conjugation (Fig. 2). In the first step, the diester phthalate is hydrolysed into the primary metabolite monoester phthalate, in a process catalysed by lipases and esterases in the intestine and parenchyma [34, 35]. Normally this first step in the metabolism would be a detoxification, but *in vitro* and *in vivo* studies have shown that diester phthalates become more bioactive when they are hydrolysed to monoester phthalates [36]. Short-branched phthalates are mainly excreted in urine as its monoester phthalates, while the more long-branched phthalates undergo several biotransformations, including further hydroxylation and oxidation before they are excreted in urine and faeces often as phase

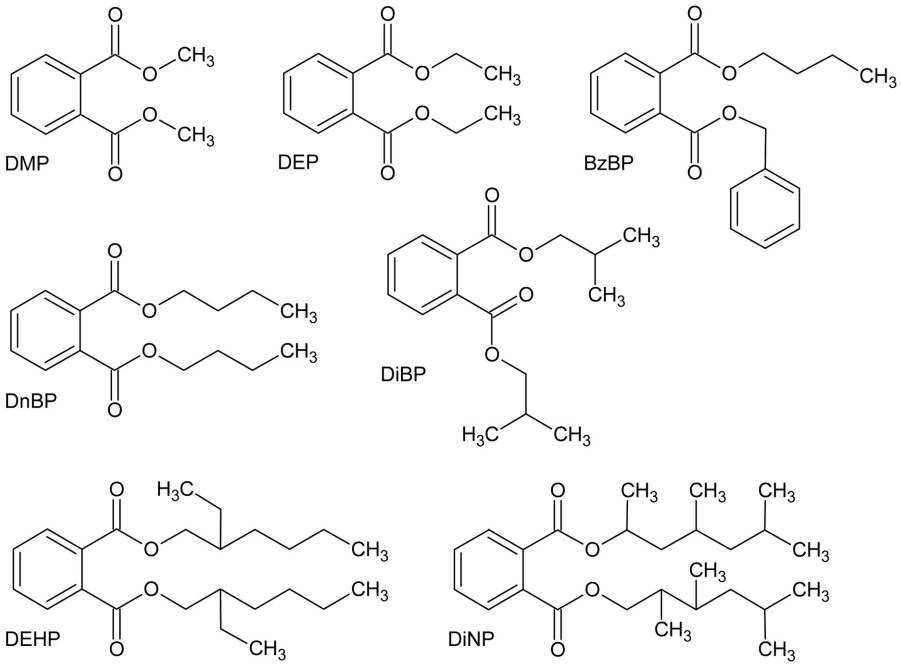

Figure 1. Chemical structure of common used phthalates in Europe.

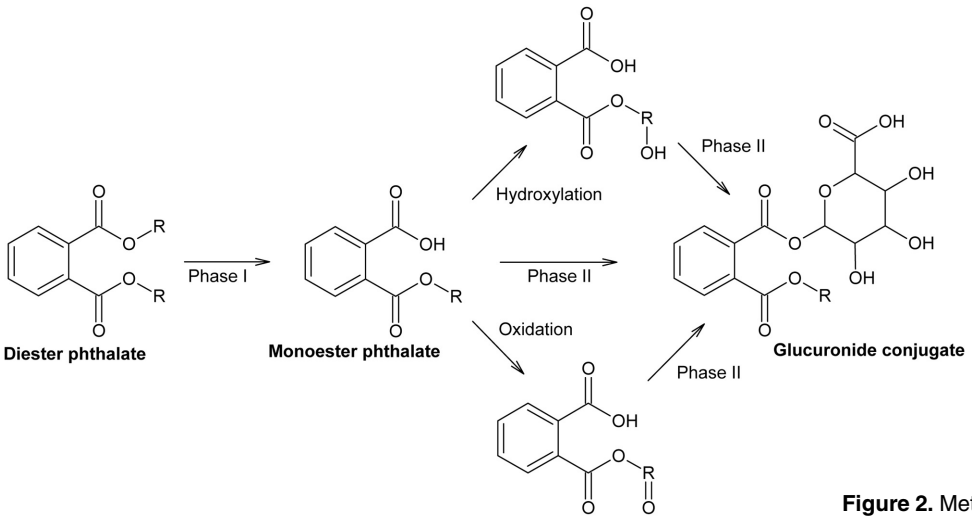

Figure 2. Metabolic pathways for phthalates.

II conjugated compounds, Fig. 2 [37, 38]. The phase II conjugation is often catalysed by the enzyme uridine 5′-diphosphoglucuronyl transferase to form the hydrophilic glucuronide conjugate. Conjugates are easily excreted in urine.

With the development of a multiple quantitative method using SPE followed by HPLC isotope dilution MS/MS, it became possible to detect monoester phthalates in human urine in the low ng/mL range [39–41]. An advantage of measuring the monoester phthalates as a biomarker for exposure was that the contamination of samples with the ubiquitous diester phthalates, which is prone to give high background level, was eliminated. The method has since been modified several times. Currently several selective, sensitive and fast methods are advisable for quantitative analyses of a wide range of monoester phthalates and secondary metabolites in several different fluids and tissues, such as of urine, blood, milk, amniotic fluid, saliva, semen, meconium, liver and placenta [42–47].

3.1 DMP, DEP, DBP and BBzP

Simple short-branched phthalates, such as DMP and DEP are mostly excreted in urine as the unconjugated monoesters; monomethyl phthalate (MMP) and monoethyl phthalate (MEP). In human urine, about 70% of the excreted MEP was excreted unconjugated and a similar glucuronidation pattern was found in plasma [38, 48].

The metabolism of DBP (or DnBP) in rats has been known for many years [48, 49]. Of a single oral dose administrated to rats, 80–90% of DBP was metabolised and excreted in

urine within 48 h and, in addition, about 5% was excreted in faeces. In urine, one major and three minor metabolites were identified; monobutyl phthalate (MBP) account for 88% of total excreted amount in urine, while mono(3-hydroxybutyl) phthalate, mono(4-hydroxybutyl) phthalate and phthalic acid only account for about 8, 2 and 2%, respectively [49]. Other studies have shown that the glucuronide conjugate of MBP is the major metabolite in urine of both rats and hamsters [43, 49, 50] and that both MBP and its glucuronide form were present in all tissue including placenta, foetus and amniotic fluid in pregnant rats 30 min after dosing, while unchanged DBP accounted for less than 1% [51]. Similarly, in a human study it was found that only about 6% of MBP was excreted in urine in its unconjugated form, the majority was excreted as glucuronide conjugate [38]. The very simple metabolic pattern and the fact that MBP is the major metabolite of DBP might be the reason why almost all studies since have used MBP as the biomarker for DBP exposure. DiBP and BBzP have metabolic patterns similar to that of DBP. For instance, in human urine, only 7% of the total amount of monobenzyl phthalate (MBzP) was excreted in its unconjugated form [38]. Furthermore, exposure to BBzP has been shown to result in the excretion of small amounts of MBP in urine [52].

3.2 DEHP

DEHP has been the most used and probably also the most studied phthalate. Because of the branched chain of DEHP the metabolic pattern is complex and several metabolites of DEHP are identified and characterised. The first step in the metabolism of DEHP is a very rapid hydrolysis of DEHP to mono(2-ethylhexyl) phthalate (MEHP) catalysed by unspecific lipases [53]. In rodents, MEHP is further metabolised into a wide range of secondary metabolites (diacids and ketoacids), which in some species such as in mice, are primarily excreted in urine as glucuronide conjugates, while rats primarily excrete unconjugated metabolites [54]. Also, the metabolic distribution of the secondary metabolites is very different in rats and mice, and between young and old animals [36, 54–56].

Today more than 15 metabolites of DEHP, many of them identical with metabolites previously identified in rodents, have been identified in human urine [57, 58]. In recent studies, secondary metabolites such as mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) along with the primary metabolite MEHP have been used to predict exposure to DEHP [59, 60]. Recently, two other major metabolites were identified and characterised in human urine as mono(2-ethyl-5-carboxypentyl) phthalate (MECPP or 5cx-MEPP) and mono(2-carboxymethylhexyl) phthalate (MCMHP or 2cx-MMHP) [57, 61, 62]. In a human single oral dose study, it was found that about 71% of the DEHP dose was excreted after 24 h and further 4% of dose was excreted in the next 20 h. Besides the two newly identified metabolites MECPP and MCMHP, accounting for 19 and 4% of the given dose, respectively, the three previously known metabolites were identified in the urine samples; MEHP (6%), MEHHP (23%) and MEOHP (15%). In urine, the peak value of MEHP was observed only 2 h after dosing and after approximately 4 h for MEHHP, MEOHP and MECPP, while MCMHP peaked after more than 8 h [37, 57, 61].

Table 2. DEHP metabolites excreted in urine

	German population[a)]		US population[b)]	
	ng/mL[c)]	%[d)]	ng/mL[c)]	%[d)]
MEHP	9.8	4.5	3.3	6.9
MEHHP	47.5	21.7	15.1	31.7
MEOHP	39.7	18.1	7.8	16.4
MECPP	85.5	39.0	16.2	34.0
MCMHP	36.6	16.7	5.2	10.9

a) German population, $n = 19$, randomly selected [62].
b) US population, $n = 129$, randomly selected [63].
c) Geometric mean.
d) The sum of the five major metabolites was set to be 100%.

In two human studies of German ($n = 19$) and US populations ($n = 129$), respectively, both with unknown exposure to DEHP, similar metabolic distribution pattern were found with a few exceptions. The major metabolites in both studies were MECPP followed by MEHHP, MEOHP and MCMHP. Only low amounts were characterised as MEHP in the German (4.5%) and American (6.9%) studies, Table 2 [62, 63]. Minor amounts of metabolites such as mono(2-ethyl-4-carboxybutyl) phthalate (MECBP, 1.2 ng/mL), mono(2-ethyl-3-carboxypropyl) phthalate (MECPrP, 1.3 ng/mL) and mono-2-(1-oxoethylhexyl) phthalate (MOEHP, 0.7 ng/mL) were also measured in the American samples [58, 63]. The level of excreted metabolites in the German population was three- to five-fold higher than in the US population; the same tendency has been shown in previous studies of urine excretion of MEHP, MEHHP and MEOHP in German [64, 65] and US populations [40, 60, 66]. However, in another US population study ($n = 62$, randomly selected), the level of MEHHP and MEOHP were higher and nearly comparable to the German level of these metabolites; 35.9 and 28.3 ng/mL, respectively [59].

The five metabolites of DEHP found in human urine were also found in serum, but in serum the major metabolite 2 h after dosing was MEHP (5.0 μg/mL), while the other metabolites at this time point were measured in the range of 0.05–0.6 μg/mL. MEHP, MEHHP, MEOHP and MECPP peaked 2 h after dosing and MCMHP peaked 4 h after dosing. Furthermore, the half-life for elimination of MCMHP was estimated to be ≥5 h, which was at least twice that of the other four metabolites measured in serum [37, 61].

According to half-life, distribution pattern and major metabolites of DEHP, the recent human metabolism studies

of DEHP indicates that secondary metabolites such as MECPP in urine and MCMHP in serum are much stronger biomarkers for DEHP exposure than the previously used biomarker MEHP. Furthermore, when analysing exposure to DEHP, it is necessary to deconjugate DEHP metabolites; for instance, it has been shown that only about 15.7% of MEHP is excreted in urine in its unconjugated form, and a similar conjugation pattern was found in plasma [38].

3.3 DiNP

DiNP are a group of isomeric form of dinonyl phthalates with varying length and branching of the nonyl chains; an example is shown in Fig. 1. Compared to DEHP, few studies on *in vivo* metabolism of DiNP exist. Like other high molecular weight phthalates DiNP has been shown to metabolise into several secondary metabolites before it is excreted in urine [67, 68]. In the urine from rats treated with one single dose of commercial DiNP, only about 0.04% of excreted metabolites was the monoester phthalate, mono-iso-nonyl phthalate (MiNP), while about 81% of the excreted metabolites were identified as the secondary metabolite, mono(carboxy-iso-octyl) phthalate (MCiOP or cx-MiNP). Several other secondary metabolites have also been identified in rat urine; among these mono(hydroxy-iso-nonyl) phthalate (MHiNP or OH-MiNP), mono(oxo-iso-nonyl) phthalate (MOiNP or oxo-MiNP) and mono(carboxy-iso-heptyl) phthalate (MCiHpP) account for about 8, 3 and 5% of the excreted metabolites, respectively [67].

Also, in humans, DiNP is excreted in urine as secondary metabolites. In a study of 129 urine samples from human adult volunteers with unknown DiNP exposure, MiNP, MCiOP, MOiNP and MHiNP were analysed. The primary metabolite, MiNP, was not detectable at all. In contrast, the secondary metabolites MCiOP, MOiNP and MHiNP were detectable in nearly all the urine samples. The major metabolites; MHiNP was excreted as both unconjugated compound and conjugated glucuronide, MCiOP was primarily excreted in its unconjugated form and the minor metabolite MOiNP was primarily excreted in its glucuronidated form. The three metabolites accounted for about 55, 38 and 6% of the excreted metabolites, respectively [68]. A comparable study of the German population ($n = 25$) also found these three metabolites of DiNP excreted in urine [69], but the total level was about two-fold higher in the German population than in the US population, and the metabolic pattern was different from the US population [68, 69]. In the German study, the content of the three metabolites was very similar; MCiOP accounted for about 40%, MHiNP for 37% and MOiNP for 22% of the measured DiNP excretion in human urine (Table 3). Although the urinary excretion of DiNP metabolites have shown differences in the metabolic pattern between populations, in both human studies, a high positive correlation between metabolites was observed [68, 69]. The recent studies of DiNP excretion in human and rat urine show that the primary metabolite MiNP might not be the best urinary biomarker for DiNP exposure, and when MiNP is used as a marker, the exposure probably would be underestimated [68].

Table 3. DiNP metabolites excreted in urine

	German population[a)]		US population[b)]	
	ng/mL[c)]	%[d)]	ng/mL[c)]	%[d)]
MHiNP	14.9	37.1	11.4	55.6
MOiNP	8.9	22.1	1.2	5.9
MCiOP	16.4	40.8	7.8	38.2

a) German population, $n = 25$, randomly selected [69].
b) US population, $n = 129$, randomly selected [68].
c) Geometric mean.
d) The sum of the five major metabolites was set to be 100%.

3.4 Metabolic distribution in other matrices than urine

Methods for the analysis of phthalate metabolites in other matrixes than in urine have been developed, but not many studies have been conducted yet. One study showed that the glucuronide distribution pattern of the monoester phthalates in serum was similar to that of urine [38], but the content of phthalate metabolites in serum is in general lower compared with the excretion of metabolites in urine. For instance, MBzP was not detectable in human serum, while it was found in urine, and only a few percent of MEP was found in serum compared to the excretion of MEP in urine. In the same study, the content of MEHP was similar in serum and urine [38]. This is in accordance with the human single dose study [37], where it was also shown that MEHP was a minor metabolite of DEHP and that the other metabolites, MEHHP, MEOHP, MECPP and MCMHP were found in much higher amounts in urine than in serum [37].

The content of phthalates in amniotic fluid has been analysed in rodent studies and a method for the analysis of human amniotic fluid has also been developed [43, 47, 70]. One study of pregnant rats dosed with DBP showed that MBP and its glucuronide were present in maternal plasma, foetal plasma and amniotic fluid [70]. The major metabolite was MBP in both maternal and foetal plasma and with increase in oral dose (five-fold), a nonlinear increase in MBP in the maternal (ten-fold) and foetal plasma (eight-fold) was found. At low dose, it was also shown that the concentration of MBP and MBP glucuronide was about three-fold higher in the maternal plasma than in the foetal plasma, but at a five-fold higher dose, the concentration of both metabolites was nearly similar in both maternal and foetal plasma. In amniotic fluid, MBP was initially the major metabolite but 24 h after dosing, the major metabolite was MBP glucuronide, which was further transformed to several isomers of the glucuronide compound. The half

life of MBP and MBP glucuronide was very different in the three matrix; about 3 h for both metabolites in the maternal plasma, about 4.2–6.5 h in foetal plasma and about 6–11 h for MBP in amniotic fluid, while a half life of up to 64 h was shown for MBP glucuronide in the amniotic fluid after high dose of DBP [70]. Another rat study confirmed the distribution pattern of phthalate metabolites. After dosing of pregnant rats with DBP and DEHP, it was shown that increased dose resulted in increased concentration of metabolites in the amniotic fluid compared to the concentration in the maternal urine. In this study, MEHP and MBP were primarily unconjugated in the amniotic fluid the day after the last dosing, while the MEHP was conjugated in the maternal urine and MBP was both unconjugated and conjugated in the maternal urine [43].

Human amniotic fluids have been analysed for ten common phthalate metabolites, including two secondary metabolites of DEHP [47]. MBP was detected in nearly all samples (~93%). MEP and MEHP could only be detected in 39 and 24% of the samples, respectively, whereas the level of the other metabolites was below the detection limit. The levels of MBP in human amniotic fluid were two- to three-folds lower than in serum from population studies. This is in accordance with the ratio between MBP in maternal blood and amniotic fluids in low-dose rat studies [70]. The level of MBP in amniotic fluid was about seven- and four-fold lower than the levels in urine in children and adults, respectively [47].

Breast milk has also been a subject for investigations and methods to analyse the content of phthalates and their metabolites in human breast milk, cow milk and infant formulae have been developed [19, 23, 44, 71, 72]. Few human studies have yet been reported, which univocally show that breast milk is contaminated with phthalates [19, 20, 23, 73]. In a recent study DEP, DBP and DEHP were determined in 86 milk samples collected during six month from 21 North American breast-feeding mothers. It was found that DEHP was the absolute major contaminant and that the levels of phthalates fluctuated during the collection period [23]. By method validation using three pools of human breast milk it was shown that monoester phthalates were in general unconjugated in breast milk [19] unlike in serum [38]. In a prospective Danish–Finnish cohort study, the unconjugated form of six common monoesters phthalate, MMP, MEP, MBP, MBzP, MEHP and MiNP, were measured in individual breast milk samples collected as additive aliquots 1–3 months postnatally [20, 44]. The data of the three studies are summarised in Table 4. The level of MBP in the pooled milk sample was similar to that found in serum and lower than the levels in urine, while the concentrations of both MEHP and MiNP in the pools were higher than both serum and urine levels [19, 38]. The unconjugated form of both MBP and MiNP were detected in about four- to ten-fold higher concentrations in the Danish–Finnish cohort study [20] than in the pooled milk samples [19], whereas equal concentrations of MEHP were found in the two studies [19, 20]. The level of MiNP found in the Danish–Finnish breast milk samples was also two- to four-fold higher than the level of secondary DiNP metabolites measured in urine in both German- and US-population studies (Table 3). Taken together, these new reports on the content of phthalates in human breast milk indicate that the long-branched phthalates such as DBP, DEHP and DiNP are excreted unmetabolised or as its primary monoester phthalates in relatively high levels in human breast milk and maybe this reflects an alternative metabolic pathway of phthalates in the breast-feeding women.

Table 4. Phthalates and monoester phthalates in human breast milk

Phthalate	American[a)]	Monoester	Danish[b)] ng/L	Finnish[c)] ng/L	Pooled milk[d)] ng/L
DEP	0.14	MMP	0.10	0.09	
		MEP	0.93	0.97	
DBP	0.51	MBP	4.3	12	1.1
		MBzP	0.9	1.3	
DEHP	109	MEHP	9.5	13	7.7
		MiNP	101	89	16.1

a) Breast milk samples ($n = 86$) collected from North American mothers ($n = 21$), geometric mean [23].
b) Danish breast milk samples ($n = 65$), values are median concentrations [20].
c) Finnish breast milk samples ($n = 65$), values are median concentrations [20].
d) Mean of three pooled breast milk samples used for method validation [19].

Methods for the analysis of phthalate metabolites in saliva, semen and meconium have also been developed, but during the validation of the methods, it was found that among 14 common metabolites, only seven were found in human saliva samples ($n = 39$) and only MECPP and a few other DEHP metabolites were found in the meconium samples ($n = 5$). In a pool of semen samples, only MECPP was found [42, 46]. However, the LOD in these methods are in the same range as for the urine methods, indicating that phthalates are excreted in very low amounts in semen, meconium and saliva.

In spite of the development of analytical methods and studies of the metabolism of phthalates in urine and several other biological matrixes, the knowledge in this area is still insufficient, and more investigations on the metabolic distribution and phamacokinetics are needed.

4 Endocrine disrupting effects of phthalates in humans

Several animal studies have demonstrated that perinatal exposure to DBP and the more long-branched phthalates

such as BBzP, DEHP and DiNP and their metabolites results in altered sexual differentiation in male rats. The effects observed includes cryptorchidism, decreased testosterone levels, testicular atrophy, Sertoli cell abnormalities, decreased weight of the androgen-dependent organs, reduction in daily sperm production and lower epididymal sperm counts [2, 32, 74–79]. Exposure to the short-branched phthalates, DMP and DEP, has not shown similar effects [75]. In female offspring *in utero* and lactational exposure to high dose of DEHP results in delay of the time of puberty onset [80]. Many of the effects on the development of the male reproductive system observed in rats following maternal phthalate exposure are similar to the conditions observed in men with TDS [1, 2, 6].

Whether phthalate exposure also represents a risk for human health is still unclear as human studies have not yet shown a clear causative link between phthalate exposure and human health problem [81]. However, recent research in this area has indicated that phthalate exposure may also be harmful to humans. In adult men, a relationship between DNA damage in human sperm and the level of MEP in urine was observed, while MMP, MBP, MBzP were not significantly associated with increased DNA damage [82, 83]. This association between sperm DNA damage and urinary MEP could not be confirmed in a similar Swedish study [84]. Also no association between urinary MEHP and sperm DNA damage was observed when the secondary, oxidative metabolites of DEHP were not considered [82]. However, when MEHP levels were adjusted for the levels of the secondary metabolites, a significant association between urinary MEHP and sperm DNA damage was observed [83]. Thus, higher the ratio of MEHP/MEHHP or MEHP/MEOHP greater the sperm DNA damage, indicating that further metabolism of MEHP into the secondary metabolites is protective for sperm DNA damage. Accordingly, it was suggested that the ratio between MEHP and the secondary metabolites could be a phenotypic marker of DEHP metabolism to less toxic metabolites [83]. In a recent study, a dose–response relationship of urinary concentration of MBP with low sperm concentration and motility and suggestive evidence between the highest MBzP quartile and low sperm concentration was found. On the other hand, no relationships between low semen quality and MMP, MEP and the major metabolites of DEHP were found in this study [85]. The reason for the inconsistency in which the monoester phthalates show associations to sperm DNA damage (associated with MEP) and low sperm concentration (associated with MBP), respectively, is unclear. The finding of an association is clearly not the same as showing a causal relationship. In the above studies on sperm parameters, it is possible that the phthalate metabolite that showed association to sperm parameters is a proxy for other factors that may affect sperm production. Also, it can not be excluded that the observed statistical associations may be chance findings due to mass significance.

Other studies have looked at phthalate exposure and reproductive hormone levels in adult men. A modest reduction in serum free testosterone has been found in a group of men with high occupational exposure to DBP and DEHP as compared to a control group [86]. Both urinary MBP and MEHP levels were significantly negatively correlated to the serum free testosterone levels; however, in this study group, urinary MBP and MEHP were also highly positively correlated to each other. In contrast, no difference in follicle-stimulating hormone (FSH), luteinizing hormone (LH), or estradiol serum levels was observed between the high-exposure and the control group [86]. This is in contrast to another study, which found associations between the urinary concentration of MBP and MBzP and serum concentrations of the reproductive hormones inhibin B and FSH [87]. However, the associations observed in this study were not easy to explain from a biological point of view and again, could be a chance finding.

The above-mentioned human studies all reported exposures to adult men, but based on animal studies, the most susceptible time point for effects of phthalate exposures on male reproductive health is clearly the prenatal and perinatal development of the male reproductive tracts. Two human studies have related health outcomes to foetal and neonatal exposures to phthalates [20, 26, 88]. In the first study, an anogenital index (AGI) based on the anogenital distance (AGD), a measure commonly used in toxicology as marker of masculinisation, was used in a human study. AGI was measured in boys 2–30 month of age and compared with their mother's exposure to common phthalates, measured as metabolic excretion in urine during pregnancy. An association between decreased AGD (corresponding to demasculinisation) and elevated prenatal exposure to MEP, MBP, MiBP and MBzP was reported. No associations between AGD and MMP, mono-3-carboxypropyl phthalate (MCPP) and the metabolites of DEHP (MEHP, MEHHP and MEOHP) were seen [26]. Using different methods for the estimation of the daily phthalate exposure of mothers of male infants exhibiting reduced AGI, similar results were obtained [88]. The second study was a case-control study of male infants with or without cryptorchidism. In this study, the reproductive hormones of male infants at the age of three month were measured as biomarkers for early testicular function, and they correlated with the content of six phthalate monoesters measured in breast milk samples from their respective mothers [20]. Serum samples were analysed for testosterone, LH, FSH, inhibin B and sex hormone-binding globulin (SHBG) and free androgen index (FAI) was calculated as the ratio between testosterone and SHBG. In the breast milk samples, the content of the phthalate monoesters MMP, MEP, MBP, MBzP, MEHP and MiNP were measured [20, 44]. No association was seen between the levels of phthalate monoesters and cryptorchidism, but a correlation was present between the levels of reproductive hormones of the infants and their exposures to

Table 5. Correlation between the level of maternal phthalate excretion and markers for infant reproductive health

	MMP	MEP	MBP	MiBP	MBzP	MEHP	MiNP
LH [20]	nc	nc	nc		nc	nc	+
SHBG [20]	nc	+	+		nc	nc	nc
LH/FAI ratio [17, 20]	+	+	+		nc	+[a)]	+[a)]
FAI [20]	nc	nc	–		nc	nc	nc
AGD [26]	nc	+	+	+	+	nc	

+, Data with positive correlation; –, data with negative correlation; nc, no correlation was found.
a) Only boys without cryptorchidism.

some of the common monoesters phthalate through breast milk feeding. MEP and MBP showed positive correlation with SHBG; MMP, MEP and MBP with LH/FAI ratio; MiNP with LH and MBP was negatively correlated with FAI. In the group of infants without cryptorchidism, MEHP and MiNP also correlated positively with the LH/FAI ratio [17, 20]. All in all, these correlations pointed to a decreased androgen action in the boys with the highest maternal exposures. Data from the two human studies are summarised in Table 5.

Phthalate exposure has also been linked to human adverse health effects other than male reproductive effects. Thus, human studies have indicated associations between the exposure of DEHP and endometriosis [89] and shortening of the duration of human pregnancy have been reported [25].

5 Foetal and infant exposure

The human exposure to phthalates has been estimated from known contents of phthalates in food, personal care products and other consumer products and from the known excretion of phthalate metabolites present in human urine [4, 28, 32, 33, 90]. It is very difficult to make such estimates, as there are many different sources of exposure. Most of the studies of excreted urinary metabolites are based on single spot urine samples. However, a new study of an adult German population demonstrate a significant day-to-day within-subject variability, suggesting that the exposure assessment should not be based on single urine measurements [91]. Furthermore, previously published exposure estimates based on excretion in urine are probably underestimated. In particular, the estimates of the exposure to the long-branched phthalates (DEHP and DiNP) based on urine excretion of their primary metabolites MEHP and MiNP might result in too low estimates, because the monoester phthalates are, in fact, not the major excreted metabolites of these compound. As mentioned, secondary metabolites of the long-branched phthalates have been identified and characterised during the last years [37, 62, 68, 69, 92], and co-measurement of these will in the future give more precise estimates of human exposures to phthalates.

Recent studies on urine excretion of phthalates in populations seem to conclude that children are more exposed to phthalates than adults [28, 32, 33]. For instance, the urinary excretion of MBP and MBzP in children (median age 4.7 years) was two- to four-fold higher than in adults [94]. The intake seemed to be correlated to younger age of the child; thus, the intake of 1-year-old infants of BBzP and DEHP was estimated to be about four-fold higher and the intake of DBP was estimated to be three-fold higher than the intake calculated for a representative US population. For children (6–11 years), the estimated intake of BBzP was about twice the intake of the general population, while the intake of DBP and DEHP in older children was shown to be similar to the general population [4, 94, 95]. The impact of age on the metabolic pattern is known from animal studies where the distributions of primary and secondary metabolites in urine from young and old rats were significantly different [36, 55, 56]. However, there are so far no systematic studies on the metabolic pattern of phthalates in children compared to adults. Thus, theoretically the relatively higher levels of monoester phthalates in the younger children could be due to the different metabolic pathways compared to adults, who mainly excrete the long-branched phthalates as secondary metabolites. To clarify this hypothesis, systematic studies of the human phthalate metabolism in different age groups are highly needed. Knowledge about the metabolic excretion pattern of the long-branched secondary phthalate metabolites of children compared to adults would be very valuable in future risk assessment of phthalates.

Considering that phthalates may be important endocrine disrupters for humans, particularly in foetal life and early childhood, it seems most important to identify the metabolic pathways of phthalates from the first host, the mother, to the second host, the foetus and infant through placenta and breast milk (Fig. 3). Little is known about these pathways, but recent studies have confirmed that unborn and infants are in fact highly exposed to phthalates. In a model system, it was shown that monoester phthalates get distributed in cord, placenta, maternal and foetal perfusate in accordance with the physical-chemical properties of the compounds [96]. In an Italian study, the prenatal exposure to DEHP and MEHP has been investigated in the cord blood from 84 newborns and in the blood from their respective mothers. The mean concentration of DEHP in cord and maternal blood were 2.05 and 1.15 μg/mL, respectively, and in both cord and maternal blood the mean concentration of MEHP was 0.68 μg/mL [97], indicating that the foetus shares the mother's exposure to MEHP. The difference found in the levels of DEHP in cord blood and maternal blood was not discussed, but in general it is difficult to measure unmetabolised phthalates in biological matrices, because environmental contamination of samples is a risk.

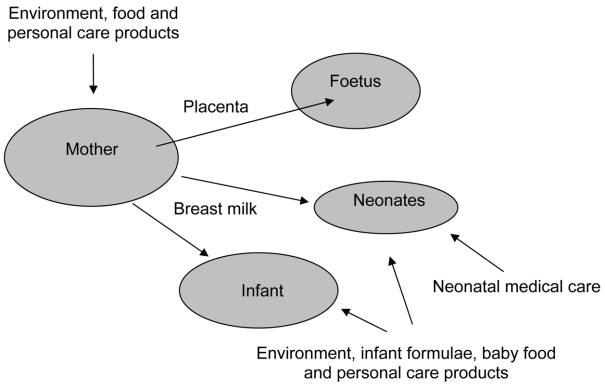

Figure 3. Exposure route of phthalates.

The high level of MEHP found in human cord blood is in contrast to previous pharmacokinetic studies of pregnant rats dosed with radiolabelled DBP. The radioactivity in dam plasma was found to be about twice as high as in foetus plasma after administration of ^{14}C-DBP to pregnant rats [70]. Another rat study showed that the concentration of ^{14}C-DBP was about 2–12-fold higher in other tissues such as placenta and maternal liver and kidney than in the embryo at gestation day 14, 2 h after one single oral dose ^{14}C-DBP [51]. This discrepancy may be due to species differences or could also reflect the difference between exposure to a single oral dose compared to the presumably constant exposure of the human population.

An American study of samples from 54 anonymous donors has shown very low excretion of phthalate metabolites into amniotic fluids. In fact, out of ten measured phthalate metabolites only MEP, MPB and MEHP were found in amniotic fluid and the maximum levels of these three compounds were 9.0, 264 and 2.8 ng/mL, respectively [47]. Similarly, the concentrations of both MBP and MEHP in the maternal rat urine were much higher than the concentrations of the compounds measured in amniotic fluid. Furthermore, the degree of glucuronidation of the metabolites was different in the two matrices. Both MEHP and MBP were primarily excreted as unconjugated compounds in amniotic fluid and in concentrations up to about 25 and more than 1000-fold lower, respectively, than the concentration of the compound excreted in the maternal rat urine [43]. Thus, the animal studies indicate that the foetus is less exposed to phthalates than the dams, but there are still gaps in our knowledge about the metabolic pathways of phthalates in pregnant animals and humans. More information on the distribution of primary as well as secondary metabolites in placenta, cord blood and amniotic fluids, compared to the level of the metabolites in maternal serum and urine, is warranted.

Neonates in need of intensive medical treatment have been exposed to very high amount of, especially, DEHP from medical devices. Not only DEHP was primarily excreted as MECPP (10.2 mg/mL ~66%), MEHHP (2.4 mg/mL ~15%) and MEOHP (2.1 mg/mL ~14%), but also other metabolites were excreted, and among them only about 0.6% was excreted as MEHP [63, 98]. This metabolic pattern was different from the metabolic pattern found in general population (Table 2) [62, 63]. Whether this reflects that neonates and infants have a different metabolic pathway than adults or whether it is a consequence of the health status of neonates in intensive care remains to be studied. The median daily intake of DEHP by infants treated with high-intensiveness products were estimated to be 233–352 μg/kg bw/day, which was about two-fold higher than the exposure estimated for a typical adult [27]. Infants are also exposed to phthalates especially MiNP and MEHP during breast feeding (Table 4) [19, 20, 23]. In infant formulae, the level of MBP and MEHP was found to be 0.6–3.9 and 5.6–9.1 ng/mL, respectively [44], and also baby food, cow milk and other consumer milk products contain phthalates and monoester phthalates [21, 44, 72]. Furthermore infants and small children are exposed to phthalates from personal care products, toys and environment.

6 Conclusion and perspective

The recent identification of secondary metabolites of particularly the long-branched phthalates in human urine and to some extent also in other body fluids have shown that the pattern of human metabolism of phthalates is complex. In future, multiple analyses quantification of all known major metabolites should preferably be included to provide data for more valid estimations of exposures of our populations. Possible differences in metabolic patterns between different age groups need to be further characterised in order to estimate more accurately the daily exposures based on the concentration and distribution of the different metabolites in urine; inclusive in children, where metabolism may be different.

In general, our knowledge on individual patterns of excretion of phthalate metabolites in urine is limited to a few, relatively small studies. However, individual variation in excretion patterns may turn out to be an important tool to identify vulnerable segments of the population. In this respect, it is intriguing that it was the ratio between the primary and secondary metabolites of DEHP rather than the actual concentration of the individual metabolites that has been shown to be associated to human sperm characteristics [83]. Thus, it needs to be tested whether ratios between different metabolites could be markers of individuals with 'poor' *versus* 'good' ability to metabolise phthalates into less harmful metabolites, based on populations studies of associations with clinical or biochemical outcomes. It would also be interesting to investigate whether different exposure routes results in distinct patterns of metabolites, *e.g.*, whether dermal uptake results in a different metabolic pattern than oral uptake.

The distribution of phthalates and their metabolites to other biological matrixes, fluids or tissues are so far only investigated in a few studies. For the nursing woman, breast-feeding is an alternative route of excretion, which may have implications not only for the estimation of daily exposures of the mother but also for the exposure of the child. The distribution of metabolites in breast milk seems to be very different from that of urine with higher amounts of long-branched phthalates, such as DBP, DEHP and DiNP and their monoesters being excreted *via* breast milk. Considering that animal studies have clearly shown that permanent adverse effects of phthalate exposure is the consequence of foetal and neonatal exposures *via* the mother, special attention should be given to the possible routes of transfer of phthalates and their metabolites from the mother to the foetus *via* placenta and to the newborn child *via* breast milk. Thus, it is essential that the methods developed for future analysis of breast-milk, cord-blood and amniotic fluid include all the major primary and secondary metabolites.

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7 References

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Dr. Hanne Frederiksen (MSc, PhD), senior scientist and responsible for the chemical laboratory at Department of Growth and Reproduction, Rigshospitalet, Copenhagen University Hospital, has present scientific research priority in the field of human metabolic distribution and excretion of endocrine disruptors. Previous research has been focused on bioavailability, metabolism, DNA adducts formation and excretion of food borne heterocyclic amines and polyphenols and the chemical analysis of these compounds in biological matrices.

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