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Announcement by the German Federal Environment Agency

Substance monograph: Phthalates – New and updated reference values for monoesters and oxidised metabolites in urine of adults and children

Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency

Content

Introduction

Despite permanent discussions about their toxicity (endocrine disruption), phthalates (alkyl or aryl diesters of 1,2-benzenedicarboxylic acid) continue to represent important industrial chemicals. Total production of phthalates in Western Europe will remain stable over the foreseeable future, at ca. 1 million tonnes annually [1, 2]. However, due to toxicity classifications and use restrictions or bans, the last years have seen a significant change in the spectrum of phthalates used [3]. For instance, di(2ethylhexyl)phthalate (DEHP), on which the Human Biomonitoring (HBM) Commission wrote a substance monograph in 2005, has become less important and been replaced in various industrial processes by phthalates like di-iso-nonyl phthalates (DiNP) and di-iso-decylp hthalates (DiDP). Similar substitution processes have occurred for di-n-butyl phthalate (DBP or DnBP). Advances in HBM for phthalates have entailed that for a growing number of phthalates, the broad range of human body burdens can now be determined and quantified via specific, relevant metabolites. The

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Federal Environment Agency's German Environmental Survey (GerES) on children (3 to 14 years of age) as well as the Human Specimens part of the German Environmental Specimen Bank (ESB) (students 20 to 29 years of age) have offered reliable data on internal phthalate exposure of two populations, which now allow reference values to be derived or updated for concentrations of monoesters and oxidized phthalate metabolites in the urine of adults and children in Germany. Reference values were derived for the following five phthalates (or their metabolites): DnBP, DiBP (di-iso-butylphthalate), BBzP (butylbenzylphthalate), DEHP and DiNP in urine.

Occurrence and usage of phthalates

Phthalates are used industrially as so-called plasticizers, which give plastics (mainly polyvinyl chloride) the required flexibility. Due to their properties as solvents or solubilisers, they are also used in a multitude of other products, mainly direct consumer goods including cosmetics and body-care products. The uses to which phthalates are devoted are dependent upon the physico-chemical properties of these compounds, and these are determined mainly by the length of their alkyl/aryl side chains and the degree of branching (see **Table 1**). A general distinction is drawn between phthalates with short alkyl side-chains (low molecular weight: LMW phthalates) and phthalates with long alkyl side-chains (high molecular weight: HMW phthalates). Phthalates with long alkyl side-chains (4 to 10 carbon (C) atoms) are used as universal plasticizers in plastics (mainly polyvinyl chloride (PVC) whilst phthalates with short alkyl side-chains (1, 2 or 4 carbon atoms) are used also or mainly as solvents in a multitude of applications other than in the plastics sector [4-8].

Global production of plasticizers amounted to ca. 6 million tonnes in 2008 [1]. Phthalates are by far the most important group of compounds in this segment, with a rate of 87% or an annual production of well over 5 million tonnes. Globally, ca. 50% of phthalates are used in Asia, ca. 20% in Western Europe and 16% in North America. In Western Europe, phthalate production amounted to 1 million tonnes in 1998 and fell slightly to 906 thousand tonnes in 2008. What changed very clearly during this period, however, is the spectrum of phthalates (see **Figure 1**). Whilst DEHP still accounted for ca. 47% of all phthalates used in Europe in 1998, its rate fell to 23% in 2008. It was replaced by DINP and DINP, whose rate in 2008 came to 38% and 23%, respectively (up from 17% each in 1998). Among the short-chain phthalates, DBP/DiBP's rate halved from 6% to 3% and BBzP's rate fell from 3% to 1%. This change in production in Europe is assumed to be due to the toxicity classifications, mandatory labelling and use restrictions and bans that have been imposed on certain phthalates. In a global perspective, however, DEHP remained the most important phthalate

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in 2008 (ca. 2.5 million tonnes per year; ca. 50% of total production of phthalates), followed by DiNP (ca. 1.3 million t/year or ca. 25% (see **Figure 2**). Forecasts for 2013 predict stable market rates for these phthalates [1, 3]. Given the global trade in goods (and in particular, the import of many consumer goods from Asian countries), it must be concluded that the evaluation of exposures to DEHP (as well as to other regulated phthalates) will remain relevant in Germany/Europe despite declining usage.

Table 1:
The most important phthalates, in order of increasing length of their alkyl side-chains / molecular weights. The phthalates addressed in this opinion are indicated in bold.

Phthalate			**Chain length** (backbone, without branching)	**Total C content** (of a side-chain)	**Molecular weight** (g/mol)	**CAS no.**
DMP	Dimethyl phthalate	LMW phthalates	1	1	194	131-11-3
DEP	Diethyl phthalate		2	2	222	84-66-2
DiBP	**Di-iso-butyl phthalate**		**3**	**4**	**278**	**84-69-5**
DnBP	**Di-n-butyl phthalate**		**4**	**4**	**278**	**84-74-2**
DPP	Dipentyl phthalate		5	5	306	131-18-0 / 84777-06-0
BBzP	**Butylbenzyl phthalate**		**4/6**	**4/6**	**312**	**85-68-7**
DEHP	**Di(2-ethylhexyl) phthalate**	HMW phthalates	**6**	**8**	**390**	**117-81-7**
DiNP	**Di-iso-nonyl phthalate**		**6-9**	**8-10**	**ca. 419**	**28553-12-0 / 68515-48-0**
DnOP	Di-n-octyl phthalate		8	8	390	117-84-0
DPHP	Di(2-propylheptyl) phthalate		7	10	ca. 447	53306-54-0
DiDP	Di-iso-decyl phthalate		7-9	9-11	ca. 447	26761-40-0 / 68515-49-1

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Figure 1: Production (in 1000t/year) of most important phthalates in Western Europe in the years 1988 to 2008. Separate figures for DnBP and DiBP are not available. Their production is indicated as sum of DnBP and DiBP. Data have been taken from [59, 124].

Figure 2: Production rate (in %) of most important phthalates as a percentage of total production in 2008, by regions. Separate data for DnBP, DiBP and DPHP are not available; these phthalates are subsumed under “Others”. Data have been taken from [59, 77, 124].

HMW phthalates such as DEHP and DiNP as well as di-iso-decyl phthalate (DiDP) and di-(2-propylheptyl) phthalate (DPHP), which are not discussed here) are used almost exclusively as plasticizers for flexible polyvinylchloride plastics (PVC-P) [3, 9]. The content of plasticiser in the final product ranges between 25 and 50%, depending on the desired flexibility of the plastic. These phthalates can be found in numerous PVC products such as flooring, insulating/sealing sheets, wall coverings/textured wallpaper, imitation leather, tubes, electrical and network cables, motor vehicle parts, shoe soles, clothes, and also in toys [10]. In the medical sector, DEHP has been the classical plasticizer in products such as blood bags, infusion bags, tubing, catheters and oxygen masks [11]. Reported non-PVC applications of these phthalates include anti-corrosion paints, anti-fouling paints and fabric dyes. A typical feature of these so-called external plasticizers is that they are not firmly chemically bound to the plastic but are only dispersed in it. As a result, these plasticizers may outgas or leach from the plastic, and in the case of improper use, they can be released from it in relatively large proportions, e.g. when in contact with lipophilic media (such as oil or grease).

Due to their volatility, phthalates with a lower molecular weight such as DBP, DiBP and BBzP (as well as diethyl phthalate (DEP) and dimethyl phthalate (DMP), which are not discussed here) are not normally used as sole plasticiser. Because of their softening and simultaneous solvent-like properties, these phthalates have numerous applications, often in consumer products [10]. Ca. 60% of dibutyl phthalates is used as gelling plasticizers for PVC and non-PVC plastics like polyvinyl acetate, cellulose acetate, polymethylmethacrylates and in rubber production, and ca. 30% is used in such applications as paints, varnishes, dispersions and adhesives [12]. Other applications, like the usage as solvent for plant protection and pest control products or insect repellents, account for 5% of all applications. Among the uses in consumer products mentioned for DiBP is its use as substrate for odour improvers. Due to their universal properties, LMW phthalates have been, and are still, used in cosmetics and body-care products. Cosmetics containing DiBP or DnBP may, however, no longer be marketed in Europe since these substances have been classified as toxic. Despite this ban, dibutyl phthalates have been detected in perfumes and cosmetics in Europe to this day [13]. Its use in dispersion adhesives for paper and packaging means that DiBP may directly reach paper and cardboard packaging via recycling processes and may then transfer to the packaged food [14]. Associations of paper processors have committed to abstain from using DiBP-containing products in future. It can be expected, however, that it will take some time until DiBP concentrations have abated in the recycling cycle [14].

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Exposure sources

Nutrition must generally be assumed to be the main source of population exposure to HMW phthalates (e.g. DEHP and DiNP). For LMW phthalates (like DnBP, DiBP and BBzP), other lifestyle-dependent exposure pathways (cosmetics, body care) seem to be just as relevant as food.

This basic insight is supported by a number of independent studies. For example, a controlled study [15] in which three volunteers fasted and only drank mineral water for two days showed that urinary metabolite levels of the HMW phthalates DEHP and DiNP fell drastically and in line with established elimination kinetics to levels near the detection limit. In contrast, metabolite levels for the LMW phthalates DnBP, DiBP and BBzP slightly decreased on average but continued to indicate significant exposures even after 48 hours. In a duplicate study [16] with 50 volunteers, not only urinary levels of phthalate metabolites but also phthalate levels in the ingested food were measured on seven consecutive days. For DEHP, the study showed very good agreement between the daily intake as calculated from measured metabolite levels and DEHP levels in the food ingested. For the LMW phthalates DnBP and DiBP, the median exposure calculated from human biomonitoring data was, respectively, six and three times as high as the calculated exposure via food. However, the authors did find a weak correlation between DiBP levels in the diet and urinary excretion of mono-iso-butyl phthalate (MiBP) ($r=0.25$; $p < 0.001$). BBzP and DiNP were found above detection limit in only a few diet samples, so that no comparison with biomonitoring data could be undertaken although nearly all urine specimens contained these substances. Data from the pilot phase of the German Environmental Survey on Children (GerES IV) already showed that house dust is not a significant exposure pathway for children and adolescents although it contained significant concentrations of DEHP [17].

Scenario-based models, as well, have identified nutrition as the main source of exposure to HMW phthalates [18-21]. In infants and small children, mouthing of phthalate-containing toys or other objects can contribute to the total exposure [6, 22]. The most recent study [19] to calculate the average contributions of a wide range of exposure sources found contaminated food to be the main pathway of exposure to DEHP (>90% of total DEHP exposure of children, adolescents and adults; 50% of total exposure of infants and small children). Exposure pathways different from those of DEHP were derived by the authors for the other HMW phthalates such DiNP. They point out, however, that exposure pathways will likely equalise as DEHP is rapiLODy replaced by DiNP/DiDP, although this substitution could not yet be considered in the model due to a lack of data.

The models also show food to be a significant source of exposure to DnBP and DiBP (its contribution varying between 40% in female adolescents and 90% in male adults), and this result in all its clarity contradicts the HBM data. Dermal uptake (via cosmetics, body-care products) might contribute to significant exposures which are detected in HBM but are difficult to take into account in scenario-based models.

Whilst LMW phthalates are often contained in cosmetics and body-care products as deliberate ingredients or tolerated impurities, foodstuffs (or their constituents) may become contaminated with phthalates, mainly HMW, during their production, transport, processing and storage. In these phases, relatively low but constant levels of contamination may occur during the authorised use of articles which come into contact with foodstuffs (e.g. seals, hoses or conveyor belts). In the EU, specific migration limits (SMLs) exist for those articles, e.g. 1.5 mg/kg of foodstuff for DEHP [23]. Although the use of phthalate-containing articles is strictly regulated, particularly in the case of fat-containing foodstuffs, these very foodstuffs containing fat appear to be the main source of the phthalate exposure, since the lipophilic phthalates accumulate in the fat fraction across all processing steps. There have been a few sporadic reports of significantly elevated phthalate levels in fat-containing foodstuffs (e.g. pesto or olive oil) which were caused by improper use of phthalate-containing articles [24]. Phthalate levels in foodstuffs generally vary considerably, depending on processing and packaging practices, fat content and any unknown or incalculable routes of input. Human breast milk could represent a relevant source of exposure of small children [25-27].

Enteric coatings (on food supplements and on pharmacy-restricted medications) could represent a particular source of high exposures to DnBP. Urinary metabolite concentrations measured after ingestion of such preparations were orders of magnitude higher than the median background exposure of the general population [28-31]. (Voluntary) medical treatments (such as platelet donation) can lead to high DEHP exposures [32-34]. Determination of DEHP metabolites in urine was recently proposed as a screening method to detect illicit blood doping [35] because elevated DEHP metabolite levels may indicate illicit blood transfusions which in the past have been difficult to prove by other means.

Toxicity of phthalates

The phthalates BBzP, DnBP, DiBP, DEHP and DiNP have anti-androgenic effects in animal experiments, and as so-called endocrine disruptors they interfere with the complexly regulated hormonal processes that control sexual differentiation. In rats, they affect the maturation of fetal Leydig cells even at low dosages, reduce or prevent testicular

testosterone production and inhibit *inter alia* formation of the insulin-like 3 (insl-3) peptide hormone [36, 37]. All of these factors manifest themselves in a set of effects called collectively the “phthalate syndrome” [38-40]. Effects include malformations of the testes and epididymides, abnormalities of the gubernacular ligaments, effects on other androgen-dependent tissues, leading to cryptorchism (non-descent of testes), hypospadia (developmental defect of the urethra) and other abnormalities of the reproductive organs but also to a reduced sperm count (as low as to result in infertility) and alteration of the male phenotype towards demasculinization (e.g. reduction of the anogenital distance (AGD), breast development).

Since the “phthalate syndrome” inducible in rodents has many similarities to the “testicular dysgenesis syndrome” which has been described in humans (poor sperm quality, infertility, cryptorchism, hypospadia, testicular cancer, etc.), there has been growing concern in recent years that phthalates could also induce reproduction toxicity and developmental toxicity in humans [41-43].

It will be a major challenge in coming years to study this possible link using human data generated in epidemiological and other studies. The mode of action of endocrine active phthalates presents major problems for any humans-based study approach, for the following reasons: It is known from rat assays (see above) that rats show the most sensitive response within a very short, critical exposure window, namely at the time testosterone-regulated sexual differentiation occurs in male offspring. For rats, this time window is between gestational days 15 and 17 (GD 15-17). Especially in many older studies on prenatal developmental toxicity, the test animals were exposed only up to gestational day 15 [44]. Therefore, the effects subsumed under the “phthalate syndrome”, which are well-described today, did not occur in these studies at all or only at very high dosages. When comparing periods critical for development between humans and rats, one can see that in humans, testosterone-controlled development processes span over a much longer period (pregnancy weeks 8-38 and also after birth). However, the time critical for sexual differentiation in humans is the end of the first trimester of pregnancy, with the process being largely completed at week 16. In rats, the particularly critical time window is towards the end of the gestation period (lasting ca. 21 days), at GD 15-17. Given that over 20 years may elapse between the critical exposure time window(s) (the end of the first trimester of pregnancy) and the diagnosis of certain effects in humans (e.g. reduced sperm count or infertility), it is obvious that establishing a causal relationship between phthalate exposure and manifestations of the testicular dysgenesis syndrome in humans poses problems.

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Several recently-published epidemiological studies describe the relationship between exposure to one or several phthalates and changes in human semen parameters [45-47], DNA damage in human sperm [48, 49], reduced hormone levels in adult men [50], decreased anogenital distance among male infants [51, 52], overweight and insulin resistance 53-55], reduced masculine play in boys [56] and attention deficit hyperactivity disorders [57]. One thing all these studies have in common is that they are based on case numbers that are very small for epidemiological studies and that the exposure route to phthalates (and possible other confounders) is characterised is, in part, very inadequate (e.g. determination of phthalate levels only once during pregnancy).

Since testosterone is decisive for sexual differentiation both in male rodents and in humans, the United States Environmental Protection Agency (U.S. EPA) [58] and the National Research Council [44], among other authors, have proposed to use modulations of fetal testosterone as critical endpoint for derivation of the no observable adverse effect level (NOAEL) and for evaluating toxic potency. Howdeshell et al. [59] derived dose-effect relationships for six phthalates (including DnBP, DFiBP, BBzP and DEHP) by quantifying their influence on testicular testosterone production on gestation day (GD) 18 following maternal exposure (Sprague-Dawley rats) over a period of GD 8 to GD18. BBzP, DnBP, DEHP and DiBP proved to be roughly equipotent, with ED 50 values, derived from dose-effect curves, ranging from 383 mg/kg/day for DEHP to 466 mg/kg/day for DiBP. DPP (dipentyl phthalate) was about threefold more potent, and no effect on fetal testosterone production was found for DEP. **Table 2** lists the potencies of some phthalates relative to that of DEHP, potency referring to the potential for disturbing the testicular function and/or inducing malformations in male offspring [59, 60]. However, the design of the above-mentioned studies with regard to how doses were spaced is inadequate for deriving no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs) for these phthalates with sufficient accuracy.

For DEHP, there are sound studies which all put the NOAEL and LOAEL for androgen-deficiency-induced malformations at 5 and 10 mg/kg/day, respectively [61-63]. For DnBP, an LOAEL or NOAEL of ca. 50 mg/kg body weight/day can be derived for embryotoxic effects and effects on male fertility in the F1 generation [64, 65]. In a relatively recent rat developmental toxicity study [66], reduction of spermatocyte development and mammary gland changes were found at lower doses than those established in earlier studies, the lowest being 1.5 to 3 mg/kg BW/day (LOAEL). For DiBP, BBzP and the DiNP isomers, the

data base for deriving NOAEL/LOAELs for the above anti-androgenically induced endpoints (at doses administered in the critical time window during sexual differentiation) is insufficient.

Table 2:

Relative potencies of some phthalates compared to DEHP. The estimated potencies refer to the potential of the relevant phthalate for disturbing the fetal testicular function and/or inducing malformations of androgen-regulated reproductive organs in male offspring of rats [44, 75-79]. The phthalates addressed in this opinion are indicated in bold.

Phthalate	Estimated relative potency*	Reference
DEHP	1	[60]
DnBP	1	[59, 75, 124]
DiBP	1	[59, 75, 125]
BBzP	1	[59, 126]
DiNP	0,15	[59, 127-129]
DPP	3	[37, 127]
DEP	0	[59, 130]

* normalized to DEHP

The European Chemicals Agency (ECHA) has recently included four phthalates (BBzP, DEHP, DiBP and DnBP) in the candidate list of substances of very high concern for authorisation (www.echa.europa.eu). Also, the European Union has classified these phthalates as reprotoxic substances of categories 2 or 3 with respect of their effects on both fertility and development (see **Table 3**). Category 2 substances and preparations containing such substances must be labelled with a skull-and-crossbones symbol. In addition, category 2 substances may not be used in cosmetics and body-care products [67] (Directive 2004/93/EC). Directive 2005/84/EC [68] restricts the use of certain phthalates in toys and childcare articles. It should be noted that the phthalates covered by the restrictions include phthalates which are classified as reprotoxic and not classified as well (see **Table 3**).

The European Food Safety Authority (EFSA) has derived tolerable daily intakes (TDI values) for certain phthalates (based on data from animal experiments) as listed in **Table 4** [69-74]. One has to note several points. First, no TDI has been derived for DiBP to date. Second, the TDIs for DnBP, DEHP and BBzP, all based on anti-androgenic effects, differ by a factor of 50. This reflects the status in the studies used for their derivation but contradicts the large-scale studies by Gray et al. for the US EPA [75] (see **Table 2**), which attribute to the above three phthalates higher potencies (for testosterone-induced endpoints) in the range of the NOAEL/LOAEL of DEHP. Third, the TDIs for DiNP and DiDP were derived on the basis of

liver changes and not on the basis of anti-androgenic effects. The TDI set for DiNP, at least, ought to be within the range of a possible TDI for endocrine/anti-androgenic effects [60].

Table 3:
Classification of major phthalates according to Annex I to Council Directive 67/548/EEC [131] in regard to their reproduction toxicity and restrictions according to Directive 2005/84/EC [68]. The phthalates addressed in this opinion are indicated in bold.

Phthalate	Year	Reproduction	Development	Restricted under 2005/84/EC [68]
DMP	-	-	-	-
DEP	-	-	-	-
DiBP	2009 [132]	Cat. 3 (R 62)	Cat. 2 (R 61)	-
DnBP	2001 [133]	Cat. 3 (R 62)	Cat. 2 (R 61)	X
BBzP	2004 [67]	Cat. 3 (R 62)	Cat. 2 (R 61)	X
DPP	2004 [67]	Cat. 2 (R 60)	Cat. 2 (R 61)	-
DEHP	2001 [133]	Cat. 2 (R 60)	Cat. 2 (R 61)	X
DnOP	-	-	-	X
DiNP	-	-	-	X
DPHP	-	-	-	-
DiDP	-	-	-	X

R 60: May impair fertility.
R 61: May cause harm to the unborn child.
R 62: Possible risk of impaired fertility.

Table 4:
Tolerable daily intake (TDI) values set by the European Food Safety Authority (EFSA). The phthalates addressed in this opinion are indicated in bold.

Phthalate	TDI µg/kg/dag	Primary target organ, effect
DiBP	-*	-
DnBP	10	Reproductive cells
DEHP	50	Testes, development
BBzP	500	Reduced anogenital distance
DiNP	150	Liver
DiDP	150	Liver

-*: no TDI discussed/derived to date

The current discussions about phthalates also revolve around their cumulative toxicity, i.e. the dose-additivity of effects both among phthalates themselves and in combination with other anti-androgens [44, 75-81]. Various studies in the USA and Europe have demonstrated dose-additivity of phthalates between themselves, but also with other anti-androgens whose mode of action differs from that of the phthalates (e.g. via the androgen receptor) but which

have the same target tissue. EFSA's attempt to formulate a group-TDI [69], which failed due to lacking knowledge about the mode of action and lacking data on comparable endpoints, will likely be repeated sometime soon on the basis of new findings from these large-scale studies and this time have a positive outcome, i.e. derivation of a group-TDI for phthalates.

Human biomonitoring parameters to estimate internal exposure and adverse effects

About a decade ago, work began as part of human biomonitoring studies to quantify phthalate exposure of the general population by determining urinary concentrations of specific phthalate metabolites [82-85]. There is so much literature on phthalates today that it cannot be discussed here exhaustively. Comprehensive reviews of phthalate HBM were published by Koch and Calafat (2009) [86] and Wittassek et al. (2010)[15]. The reader is also referred to the Federal Environment Agency's substance monograph on DEHP of 2005 [87].

In general, blood (or its constituents) and urine are the matrices commonly used in human biomonitoring studies to characterise exposure. For non-persistent chemicals such as the phthalates – with relatively short excretion half-lives – urine is the matrix of choice because these chemicals or their metabolites are present in urine in higher concentrations than in blood. In addition, urine is a non-invasive matrix which is relatively easy to hanLODe and is easy to collect.

Blood as a possible matrix for phthalate HBM presents another key problem. Phthalates, which are ubiquitous environmental chemicals as well as commonly used in clinical and laboratory supplies, readily accumulate in blood during sampling and storage, due to its lipophilicity. They are then quickly metabolised to the relevant monoesters by lipase enzymes in the blood. The analytical result, regarded as correct from the viewpoint of laboratory analysis, gives no clue as to whether the monoester levels result from an actual intake or from contamination during the pre-analytical phase (sampling/storage) [88-90]. Therefore, in general, the use of any matrix other than urine (not only blood, but also blood from the umbilical cord, placenta tissue, human breast milk, amniotic fluid, meconium, saliva) in phthalate human biomonitoring is not advisable [91-94]. Oxidised metabolites are less affected by these contamination impacts, but are quantitatively of minor relevance in blood and the other body matrices mentioned above (which is not true for their presence in urine).

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Human metabolism and exposure biomarkers

Metabolism and elimination of phthalates is very complex and therefore, so is the selection of appropriate biomarkers and their interpretation. In a first rapid metabolic step, phthalate diesters (parent phthalates) are cleaved into their respective monoesters. This can occur at different sites in the body (e.g. in the mouth or on the skin, in the stomach, in the intestines and/or in blood). In a second step, the alkyl side-chain of the monoester may undergo phase I oxidation and be modified with functional groups such as hydroxyl, keto or a carboxy group or shortened by ß-oxidation. In a third step, both the hydrolytic monoester and the oxidized secondary metabolites can be conjugated with glucoronic acid. Urine will then contain the monoester and the oxidized secondary metabolites in varying proportions, depending on the parent phthalate, and in more or less conjugated form (see **Figure 3**).

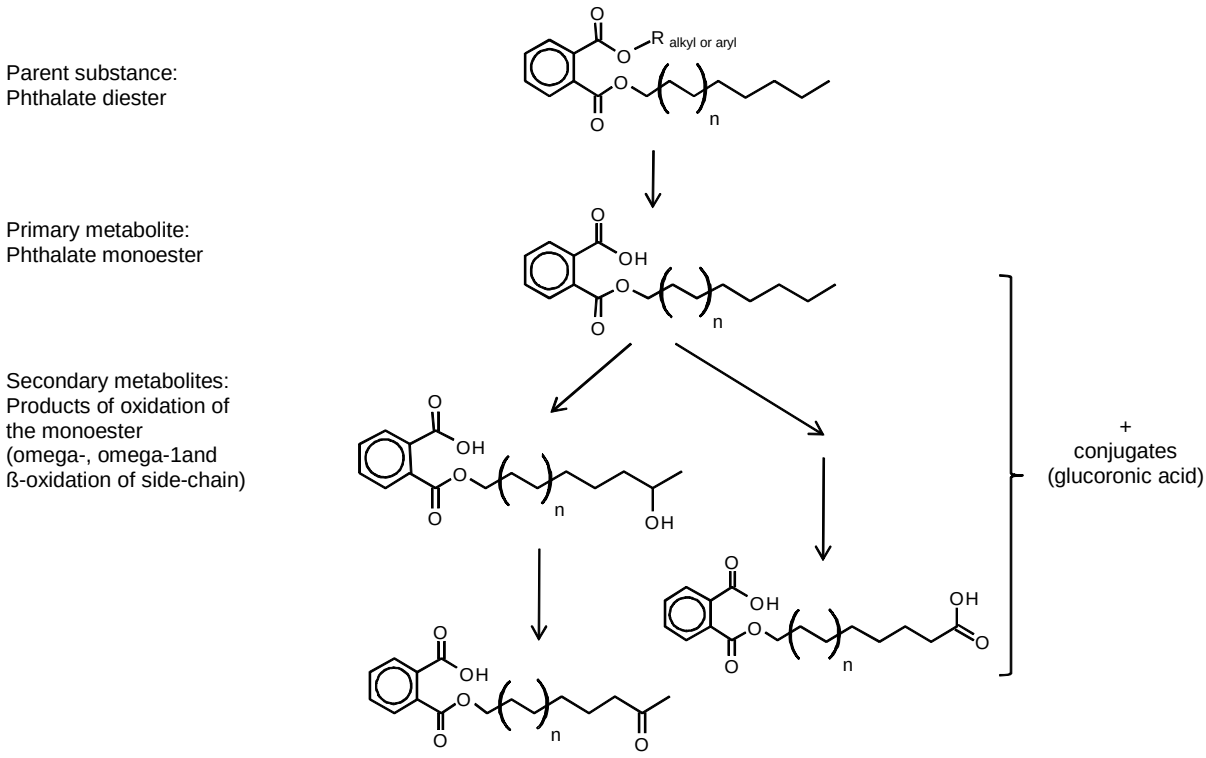

Figure 3: General scheme on phthalate metabolism

The extent to which the alkyl side-chain of a phthalate monoester is modified by oxidation increases with the length of the alkyl side-chain. The metabolites so modified are more water-soluble and therefore easier for the body to excrete via urine than the simple monoesters, whose water solubility decreases with increasing length of their alkyl side-chains. Therefore, LMW phthalates (like DEP, DiBP or DnBP) will be excreted via urine mostly in the form of their hydrolytic monoesters. With growing length of the alkyl side-chain

(DEHP), the metabolites modified by oxidation will become more dominant, the most extreme case being DiNP, whose hydrolytic monoester is not excreted in urine in any relevant amounts. This must be taken into account in the interpretation of human biomonitoring data [15, 85]. Using hydrolytic monoester metabolites as the sole biomarkers to compare phthalate exposures can be misleading, especially when comparing LMW phthalates vs. HMW phthalates. Ca. 70% of an oral dose of DnBP is excreted in urine as the hydrolytic monoester [96], while less than 10% of DEHP [96] and less than 2% of DiNP [97] are excreted as the hydrolytic monoesters. In recent years, research on the oxidative metabolism of phthalates has made considerable progress. In addition to the studies on DEHP, there now exist detailed studies on the (oxidative) human metabolism of DiNP. Besides the increased oxidative metabolism (omega-, omega-1 and ß-oxidation) of HMW phthalates, there is another fact adding to the complexity: Whilst DEHP is a substance whose side-chain consists of a defined isomer (2-ethyl-hexyl side-chain), DiNP (as well as DiDP) is a complex mixture of isomers whose composition varies depending on the nature of the mixture of alcohols used for its synthesis. This is also why there are several DiNP products on the plasticizer market, depending upon the manufacturer or the manufacturing process. The isomeric alkyl side-chains provide for a broad spectrum of hydrolytic and oxidised monoesters. A major DiNP isomer first had to be identified before suitable oxidative metabolites could be derived, synthesised and used for valid human biomonitoring of DiNP exposure [98-100]. **Table 5** lists the relevant metabolites of the five phthalates which are used as exposure biomarkers and for which reference values for their excretion in urine are presented here. **Table 6** lists the fraction corresponding to the excretion of the relevant metabolite via urine (within 24 hours) relative to the oral dose of the parent phthalate. This table illustrates, firstly, the large difference in metabolism between LMW and HMW phthalates and, seconLODy, that human biomonitoring by measuring these metabolites in urine covers a very large part of the dose and is therefore very informative with regard to the actual exposure. The metabolites listed for DiNP account for only ca. 40% of the dose, but it is known that further metabolites (e.g. twice oxidised side-chains or side-chains shortened by ß-oxidation) are formed and excreted, which cannot currently be determined quantitatively.

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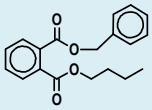
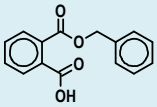
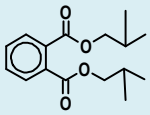
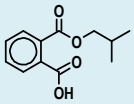
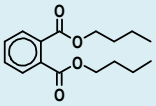
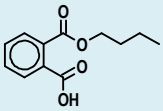
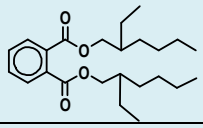
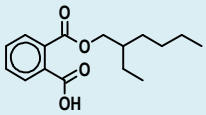
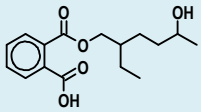
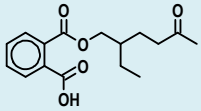
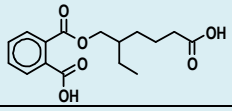
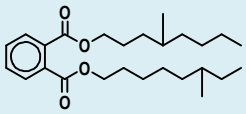
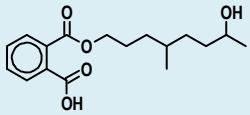
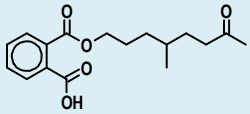
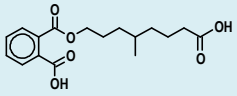
Table 5:

The phthalates and phthalate metabolites for which reference values are derived in this opinion

Parent phthalate	Primary metabolite (hydrolytic monoester)	Secondary metabolite (oxidised monoester)
Butyl-benzyl phthalate (BBzP) 	Mono-benzyl phthalate (MBzP) 	*n.a.*
Di-iso-butyl phthalate (DiBP) 	Mono-iso-butyl phthalate (MiBP) 	*n.a.*
Di-n-butyl phthalate (DnBP) 	Mono-n-butyl phthalate (MnBP) 	*n.a.*
Di(2-ethylhexyl) phthalate (DEHP) 	Mono(2-ethylhexyl)phthalate (MEHP) 	5OH-mono(2-ethylhexyl)phthalate (5OH-MEHP) 
		5oxo-mono(2-ethylhexyl) phthalate (5oxo-MEHP) 
		5carboxy-mono(2-ethylhexyl) phthalate (5cx-MEPP) 
Di-iso-nonyl phthalate (DiNP)* 	n.a.	7OH-mono-methyloctyl phthalate (OH-MiNP) 
		7oxo-mono-methyloctyl phthalate (oxo-MiNP) 
		7carboxy-mono-methylheptyl phthalate (cx-MiNP) 

n.a.: not analysed/not applicable
* many different side-chain isomers possible

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Table 6:
Fraction f_{ue} [*1] of the oral dose of the parent phthalate excreted in urine (within 24 hours) through the metabolite indicated.

Parent phthalate	Metabolite		f_{ue}	Reference
DnBP	MnBP		69%	[95]
DiBP	MiBP		69%[*2]	[95]
BBzP	MBzP		73%	[95]
DEHP	MEHP	5.9%	Total: 62.7%	[96]
	5OH-MEHP	23.3%		
	5oxo-MEHP	15.0%		
	5cx-MEPP	18.5%		
DiNP	OH-MiNP	18.4%	Total: 37.5%	[97]
	oxo-MiNP	10.0%		
	cx-MiNP	9.1%		

f_{ue}: Fraction of urinary excretion
[*1] The fraction f_{ue} indicated here may not be confused with the total fraction of the dose which is excreted via urine. In fact, other metabolites, not listed here, are also excreted via urine. Furthermore, excretion is not yet completed after 24 hours. Consequently, the total fraction excreted via urine will be significantly higher than the fraction excreted through the metabolites listed here.
[*2] For MiBP no fraction has been determined to date. The same fraction as for MnBP was assumed.

Polymorphisms or other, individual factors influencing the elimination of phthalate metabolites in qualitative or quantitative terms are not described in the current literature. A slight change in the range of metabolites - away from monoesters towards oxidised metabolites - was found in the morning urine samples (first morning void) of children and small children [17, 101]. It has not yet been clarified conclusively whether this is due to enzymatic differences or whether kinetic effects come into play (in the case of children's morning void, it can be assumed that more time has elapsed between analysis and last exposure compared to adults [102]). This effect is taken into account, however, if both the monoester and the oxidised metabolites are determined simultaneously when determining exposure.

Available data on the internal exposure of the general population

Studies from across the world exist on urinary levels of phthalate metabolites in the general population. Reviews on phthalate exposure of the non-occupationally exposed population can be found in [15, 86, 103] and in **Table 7**.

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Table 7:

Concentrations of phthalate metabolites in urine (in µg/l) of the general population

			MnBP		MiBP		MBzP		5OH-MEHP		5oxo-MEHP		5cx-MEHP		OH-MiNP		oxo-MiNP		cx-MiNP	
Country, study period	**Age** [years]	**N**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**	**P 50**	**P 95**
USA 2003-2004 [134]	6-11	342	36.7	191	7.0	40.6	35.0	255	36.5	318	25.8	197	51.6	391	-		-		-	
USA 2003-2004 [134]	12-19	729	28.2	134	5.6	22.7	24.9	152	29.8	317	20.3	212	42.7	448	-		-		-	
USA 2003-2004 [134]	≥ 20	1534	20.7	108	3.9	19.9	12.1	79.5	18.4	225	12.4	139	29.2	312	-		-		-	
Germany 2002 [83]	7-63	85	181	825	-		21.0	146	46.8	224	36.5	156	-		-		-		-	
Germany 2005 [135]	14-60	399	46.9	172	44.9	183	7.2	45.6	19.2	81.8	14.7	56.0	26.2	93.6	3.0		5.5		-	
USA 2005 [136]	≥ 18	129	-		-		-		-		-		-		13.2	43.7	1.2	6.6	8.4	46.2
Germany 1988-2003 [111]	20-29	634	112	604	34.5	176	7.4	50.4	21.0	77.2	16.7	57.5	26.9	98.8	2.0	11.9	1.0	5.6	-	
Germany 2002, 2004, 2006, 2008 [112]	20-29	229	32.8	124	28.2	100	5.0	21.2	14.4	41.1	9.7	33.5	14.5	49.6	3.13	16.5	2.16	11.3	3.69	22.6
Germany 2003-2006 [103]	3-14	599	93,4	310	88,0	308	18,1	76,2	46,0	164	36,3	123	61,4	209	11,0	50,6	5,4	28,9	12,7	58,9

Representative data on phthalate exposure of children in Germany were generated by the German Environmental Survey on Children 2003-2006 (GerES IV) [103, 104]. Like the earlier German Environmental Surveys, GerES IV is a population-representative, cross-sectional study for which subjects were selected in a multi-stage stratified random procedure. GerES IV is the environmental module of the German Health Interview and Examination Survey for Children and Adolescents (German acronym: KiGGS) conducted by the Robert Koch Institute (RKI) [106-108], which also select the sample and carried out the field work for GerES IV. GerES IV was conducted between May 2003 and May 2006 on randomly selected children 3 to 14 years of age from 150 locations. The methodologies applied (selection of the random sample, questionnaires, collection of samples, analysis, statistics) have been described by Becker et al. (2009) [109] and Schulz et al. (2004, 2008) [102, 110]. Morning void, i.e. the full amount of urine discharged after getting up in the morning, were collected by all children other than those who still wore diapers at night. For urine collection, the parents of the children 3 to 4 years of age (or in the case of girls, up to 6 years of age, in consultation with the parent) were given decontaminated 750 ml “toilet insets” (Tyco Healthcare Deutschland GmbH, Neustadt/Donau) and the parents of the older children were given decontaminated 1 l square bottles (Kautex, Bonn-Holzlar) along with written instructions on how to collect the sample. The urine samples were transferred into Saarstedt tubes and stored at -20℃ until analysis. Due to limited fina ncial resources, phthalate metabolites were determined in a subsample consisting of 600 randomly selected samples [103].

Data from the German Environmental Specimen Bank (ESB) are available to characterise phthalate exposure of adults in Germany. Specimens are taken annually from living persons in order to ascertain normal body burdens and their time-dependency. The specimens are stored in the ESB. The sample chosen for the ESB are student volunteers (from the universities of Münster, Halle/Saale, Greifswald and Ulm), about half of them are female and 90% belong the age group of 20 to 29 years. They thus mainly represent 20 to 29 year olds in Germany with average body burdens (without any detectable specific exposure). The urine samples collected for the ESB are 24-h samples. In two retrospective studies, samples archived in ESB were analysed for phthalate metabolites. One time series focused on the levels in samples from students from the years 1988-2003 [111], the second study covered samples from students from the years 2002-2008 [112]. Both studies investigated over 60 samples per year from students (half of them were male, half female) from the University of Münster.

Reference values

Reference values characterise the basic exposure of a population group which is not subject to any recognisable specific exposure. Reference values are determined if possible on the basis of data from a suitable reference population [113]. According to the IUPAC guideline [114] the reference value is defined within the 95% confidence interval of the 95th population percentile of the distribution of concentrations of a specific substance in a body fluid of a representative population.

Data from the population-representative GerES IV (2003-2006) were available to derive reference values for phthalate metabolites in the urine of children in Germany. Data from the German ESB were used to derive reference values for adults. Out of the data set spanning from 1988 to 2008, only the data from the years 2006 and 2008 were used to have the most up-to-date data base (see **Table 8**).

The statistical parameters needed for derivation of a reference value – the estimated 95th population percentile and the attendant 95% confidence interval – were calculated for each analyte according to the parametric procedure assuming a log normal distribution or according to the bootstrapping procedure in cases where a log normal distribution did not exist.

Table 8:

Data used as a basis for derivation of reference values for phthalate metabolites in urine (µg/l) of the general population in Germany; children 3 to 14 years of age in Germany[1] – GerES 2003-2006, morning void [137]; students 20 to 29 years of age from Münster, 2006 and 2008, 24-h urine [137]

Phthalate / Metabolite Population	N	%>LOD	P50	P95	Range	CI-PP95
DEHP / ∑ 5OH-MEHP + 5oxo-MEHP						
3 – 14 years	592	100	83.3	277	12.0 - 6130	249 – 296 [a]
20 – 29 years	112	100	17.4	43.1	3.91 -72,1	41.4 – 58.2 [a]
DEHP / 5OH-MEHP (LOD: 0.25 µg/l)						
3 – 14 years	592	100	45.9	153	6.14 - 3640	141 – 169 [a]
20 – 29 years	112	100	10.5	27.2	2.42 – 39,0	24.6- 34.5 [a]
DEHP / 5oxo-MEHP (LOD: 0.25 µg/l)						
3 – 14 years	592	100	36.2	120	4.56 - 2490	109 – 130 [a]
20 – 29 years	112	100	6.84	20.2	1.49 – 33.1	17.0 – 24.3[a]
DEHP / 5cx-MEPP (LOD: 0.25 µg/l)						
3 – 14 years	592	100	61.4	202	9.18 - 4490	180 - 214 [a]
20 – 29 years	112	100	11.1	29.2	2.25 – 36.7	26.8 – 38.5 [a]
DnBP / MnBP (LOD: 1.00 µg/l)						
3 – 14 years	592	100	93.4	304	12.6 - 1090	269 - 318 [a]
20 – 29 years	112	100	23.0	69.7	4.25 - 250	64.2 – 97.5 [a]
DiBP / MiBP (LOD: 1.00 µg/l)						
3 – 14 years	592	100	88.0	297	13.6 - 1243	257 - 302 [a]
20 – 29 years	112	100	26.8	147	6.32 - 317	89.8 – 143 [a]
BBzP / MBzP (LOD: 0.25 µg/l)						
3 – 14 years	592	99.9	18.1	73.0	<0.25 - 468	67.7 – 84.3 [a]
20 – 29 years	112	99	3.66	14.5	<0.25 – 31.8	11.5 – 18.2 [a]
DiNP / ∑ OH-MiNP+oxo-MiNP+cx-MiNP						
3 – 14 years	592	98	30.5	135	1.18 – 468	114 - 140 [a]
20 – 29 years	112	88	9.76	67.4	0.39 - 105	40.2 – 71.5 [a]
DiNP/ OH-MiNP (LOD: 0.25 µg/l)						
3 – 14 years	592	100	11.0	50.9	0.93 – 198	42.9 – 53.2 [a]
20 – 29 years	112	98	3.46	20.4	<0.25 – 36.0	13.5 – 23.7 [a]
DiNP / oxo-MiNP (LOD: 0.25 µg/l)						
3 – 14 years	592	98	5.40	28.9	<0.25 – 86.7	25.6 – 33.0 [b]
20 – 29 years	112	92	2.19	16.0	<0.25 – 27.2	7.7 – 21.7 [b]
DiNP / cx-MiNP (LOD: 0.25 µg/l)						
3 – 14 years	592	99,8	12.7	57.6	<0.25 – 195	50.4 – 62.1 [b]
20 – 29 years	112	93	3.82	28.0	<0.25 – 41.8	13.8 – 37.2 [b]

N = size of sample; % > LOD = percentage above the limit of detection (LOD); values < LOD were set to LOD/2; P50, P95 = percentiles; range = minimum to maximum; CI-PP95 = 95% confidence interval for the 95. population percentile (PP95);
[1] = when calculating CI-PP95 for concentrations in urine of children, only samples with a creatinine concentration between 0.3 and 3.0 g/l urine were taken into account; as a result, these evaluations are based on 592 out of 599 samples; [a] = parametric procedure; [b] = bootstrapping procedure

Based on the data, the HBM Commission has derived the reference values listed in **Table 9**. In applying these reference values, it must be borne in mind that the data used to derive the reference values for phthalate metabolites in the urine of children date from past periods (2003-2006) and therefore reflect the situation existing at that time. The data for derivation of reference values for phthalate metabolites in the urine of 20 to 29 year olds are not based on a population-representative sample, but come from a student sample in Münster contributing to the German ESB for Human Specimens (ESBhum). It should also be noted that the samples from GerES IV are morning urine samples whereas the ESBhum samples are 24-h urines.

Table 9:
Reference values for metabolites of different phthalates in urine of children and adults in Germany

Phthalate	Metabolite in urine	Children 3 to14 years of age, living in Germany, 2003 to 2006	Adults 20 to 29 years of age from Münster, 2006 and 2008
DnBP	**MnBP**	**300 µg/l**	**70 µg/l**
DiBP	**MiBP**	**300 µg/l**	**140 µg/l**
BBzP	**MBzP**	**75 µg/l**	**15 µg/l**
DEHP	**∑ 5OH-MEHP + 5oxoMEHP**	**280 µg/l**	**50 µg/l**
	5OH-MEHP	**160 µg/l**	**30 µg/l**
	5oxoMEHP	**120 µg/l**	**20 µg/l**
	5cx-MEPP	**200 µg/l**	**30 µg/l**
DiNP	**∑ 3 DiNP-Metabolite**	**140 µg/l**	**60 µg/l**
	OH-MiNP	**50 µg/l**	**20 µg/l**
	oxo-MiNP	**30 µg/l**	**15 µg/l**
	cx-MiNP	**60 µg/l**	**25 µg/l**

When applying reference values, the uncertainty of analytical measurements must generally be taken into account, i.e. it must be ensured when evaluating HBM data that the analyses were performed under internal and external quality assurance conditions [115]. This has been shown by the experience gained in interlaboratory comparisons conducted by the Deutsche Gesellschaft für Arbeits- und Umweltmedizin (German occupational and environmental medicine association) [116]. Another criterion to be applied when evaluating a

possible exceedance of the reference value for a substance in urine is that the creatinine concentration in the urine must be within a range of 0.5 and 2.5 g/l [117].

The HBM Commission emphasises that reference values are purely statistically derived values which per se provide no indication of the health relevance of the exposure. In other words, an exceedance of a reference value does not necessarily imply a health risk; by the same token, a measured concentration lower than the reference value cannot be construed as evidence that no health risk exists.

Measures to be taken when the reference value is exceeded

In cases where the reference value is exceeded, control measurements should be carried out. Extremely diluted or concentrated urine samples should be excluded from such measurements. Exceedances of reference values, when reliably established and confirmed, should give cause for efforts to find the exposure sources and for measures to reduce the exposure where reasonably achievable.

Scientific studies to identify and evaluate the sources of exposure to phthalates are still ongoing. The results available so far suggest that contaminated foodstuffs may be responsible for the broad background exposure to high molecular weight phthalates like DEHP and DiNP. Fat-containing foodstuffs are particularly susceptible to high contamination with phthalates as the latter accumulate in the fat phase across all processing steps. The Federal Institute for Risk Assessment (BfR) has published several opinions [24, 118-120] pointing out that contaminated foodstuffs (e.g. pasta sauces, cooking oils, food in oil, pesto) may be a significant source of exposure to phthalates. In general, it appears that neither the consumption of organic food nor the consumption of little-packaged or conventionally packaged food (e.g. in jars) has a significant influence on phthalate exposure [19, 121]. Likewise, indoor parameters (e.g. house dust, swipe samples), which partly exhibit considerable phthalate concentrations, do not currently seem to contribute significantly to phthalate exposure of the general population [15, 17]. Therefore, it is recommended to avoid hasty cleanup measures (e.g. exchange of PVC flooring or textured wallpaper).

The sources of exposure to low molecular weight phthalates present a much more heterogeneous picture. In their case, food and other sources seem to have comparable relevance. For instance, some studies have traced exposure to certain phthalates to the use of body-care products and cosmetics. Despite the ban imposed, some phthalates can be detected in perfumes/cosmetics to this day. However, these phthalates are not normally to be regarded as ingredients, but as unintentional impurities [13]. For some low molecular

weight phthalates, GerES IV has found correlations between body burdens and concentrations in house dust samples [122]. What this finding means still has to be clarified. The use of phthalate-containing building products, body-care products or other products might lead not only to elevated body burdens but also, at the same time, to elevated phthalate levels in the indoor environment. It is generally recommended that indoor spaces be aired out regularly and surfaces be wiped regularly with a damp cloth.

As already mentioned, enteric coatings and capsules may be a particular source of high exposures to DnBP. Whether a medication or food supplement contains dibutyl phthalate as auxiliary substance is indicated in the patient information leaflet under “Other ingredients”. Medical treatments (e.g. platelet donation) can lead to high exposures to DEHP.

HBM values

The Commission is currently considering the derivation of toxicology-based HBM values for the phthalates addressed in this opinion. The Commission regards the data base as sufficient and is planning to derive HBM values as derived for DEHP in 2007 [123]. Furthermore, the Commission is currently discussing the possibility of deriving a cumulative HBM value for phthalates, taking into account the similar mode of action of the phthalates addressed in this opinion as well as the fact that the general population is exposed to nearly all of them at the same time.

Summary

A multitude of HBM studies in recent years have shown that the general population is broaLODy exposed to a whole range of phthalates. Advances in the analytical methods have been such that an ever growing number of phthalates can be determined reliably and without contamination via their specific metabolites in urine. Due to toxicity classifications and use restrictions in Europe, the phthalate market is in flux. Use of phthalates like DEHP and DnBP has decreased markeLODy in Europe over the last years whilst other phthalates (like DiNP or DiBP) have become more dominant as substitution products. Data from the ESB show that the development of internal exposure to the phthalates concerned reflects this change. Consequently, the DEHP reference values derived in 2007 for children and adults in Germany (5OH-MEHP: 220 µg/L urine; 5oxo-MEHP: 150 µg/L urine) were replaced by new - lower reference - values, and reference values for DiBP, DnBP, BBzP and DiNP were derived for the first time.

The derivation is based on representative data on phthalate exposure of children (3-14 years of age) in Germany, which were generated as part of the GerES IV (2003-2006), as well as

on data from the German ESB from 2006 and 2008 which come from a cohort of students (20-29 years of age) not representative of the population (see **Table 8**). The samples from GerES IV are morning voids, whereas the ESB samples are 24-h urines. The reference values derived separately on the basis of these two study populations are presented in **Table 9**. As the phthalate market is changing rapidly, it may be assumed that these reference values will be applicable for a limited time only. The Commission emphasises once again that these reference values are purely statistically derived values which per se provide no indication of the health relevance of the exposure.

A health-related assessment of HBM results or data is possible only for DEHP so far, using the HBM I value which has been derived for DEHP. The HBM I value describes the concentration of a substance in a body matrix below which, according to the Commission's current assessment, no adverse health effects must be expected and therefore no action needs to be taken. The HBM I value for the sum of the DEHP metabolites 5OH-MEHP and 5oxo-MEHP was set (based on analogy considerations to TDI values) at 500 µg/L for children (6-13 years of age), at 300 µg/l for women of childbearing age and at 750 µg/L for men from age 14 upwards and for the rest of the population. HBM values for the other phthalates or a HBM value for the sum of endocrine active phthalates are currently being discussed by the Commission.

Acknowledgement

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References

1. Chemical Economics Handbook Marketing Research Report: 'Plasticizers' (2009) SRI Consulting. www.sriconsulting.com.
2. 'Plasticizers Market Data' (2006) Arbeitsgemeinschaft PVC und Umwelt e.V. Bonn. www.agpu.de.
3. European Council for Plasticizers and Intermediates - Saykali M (2010) Phthalates and their future in PVC applications - Current status and path forward. 'PVC Plasticizers 2010'. 10. Feb. 2010. Brüssel, Belgien.
4. 'European Union Risk Assessment Report for 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP) (Final Report 2003)' (2003) European Chemical Bureau. Ispra, Italy.
5. 'European Union Risk Assessment Report for 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich and di-"isodecyl" phthalate (DIDP) (Final Report)' (2003) European Chemicals Bureau. Ispra, Italy.
6. 'European Union Risk Assessment Report for Bis(2-ethylhexyl) phthalate (Final Report 2008)' (2008) European Chemicals Bureau. Ispra, Italy.
7. 'European Union Risk Assessment Report for dibutyl phthalate (with addendum 2004)' (2004) European Chemicals Bureau. Ispra, Italy.
8. 'European Union Risk Assessment Report for Benzyl Butyl Phthalate (Final Report 2007)' (2007) European Chemicals Bureau. Ispra, Italy.

9. Lorz PM, Towae FK, Enke W, Jäckh R, Bhargava N, Hillesheim W (2007) Phthalic acid and derivatives. In 'Ullmann's Encyclopedia of Industrial Chemistry'. (Ed. Wiley-VCH) (Wiley-VCH:
10. 'Datenblätter Gefahrstoffe' (2010) BG RCI - Berufsgenossenschaft Rohstoffe und chemische Industrie. http://www.gischem.de/.
11. 'Opinion on Medical Devices Containing DEHP Plasticised PVC; Neonates and Other Groups Possibly at Risk from DEHP Toxicity.
Adopted by The Scientific Committee on Medicinal Products and Medical Devices on 26 September 2002.' (2002) DG SANCO-European Commission, Health & Consumer Protection Directorate-General.
12. Umweltforschungsplan des Bundesministers für Umwelt, Naturschutz und Reaktorsicherheit - Forschungsbericht 106 01 076: 'Stoffströme wichtiger endokrin wirksamer Industriechemikalien (Bisphenol A; Dibutylphthalat/Benzylbutylphthalat;
Nonylphenol/Alkylphenolethoxylate)' (1997)
13. 'Opinion on Phthalates in Cosmetic Products - adopted by the Scientific Committee on Consumer Products at its 11th plenary meeting of 21 March 2007' (2007) DG SANCO-European Commission, Health & Consumer Protection Directorate-General.
14. 'Di-isobutylphthalat in Papieren und Kartons für den Kontakt mit Lebensmitteln - Kurzprotokoll einer außerordentlichen Sitzung der Arbeitsgruppe „Papier, Karton und Pappe" vom 5. Juli 2007 im BfR' (2007) Bundesinstitut für Risikobewertung.
15. Wittassek M, Koch HM, Angerer J, Bruning T (2010) Assessing exposure to phthalates - The human biomonitoring approach. Mol Nutr Food Res
16. Fromme H, Gruber L, Schlummer M, Wolz G, Bohmer S, Angerer J, Mayer R, Liebl B, Bolte G (2007) Intake of phthalates and di(2-ethylhexyl)adipate: results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. Environ Int 33: 1012-1020
17. Becker K, Seiwert M, Angerer J, Heger W, Koch HM, Nagorka R, Rosskamp E, Schluter C, Seifert B, Ullrich D (2004) DEHP metabolites in urine of children and DEHP in house dust. Int J Hyg Environ Health 207: 409-417
18. Clark K, Cousins I, MacKay D (2003) Assessment of Critical Exposure Pathways. In 'The Handbook of Environmental Chemistry, 3Q. Phthalate Esters'. (Ed. C Staples) pp. 227-262. (Springer: New York)
19. Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K (2006) What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Analysis 26: 803-824
20. Meek ME, Chan PKL (1994) Bis(2-Ethylhexyl)Phthalate - Evaluation of Risks to Health from Environmental Exposure in Canada. J Environ Sci Health, Part C: Environ Carcinog Ecotoxicol Rev 12: 179-194
21. Tertiary Human exposure to selected phthalates in Denmark: 'Human exposure to selected phthalates in Denmark' (2003) Søborg, Denmark.
22. Babich MA, Chen SB, Greene MA, Kiss CT, Porter WK, Smith TP, Wind ML, Zamula WW (2004) Risk assessment of oral exposure to diisononyl phthalate from children's products. Regul Toxicol Pharmacol 40: 151-167
23. 'Commission Directive 2007/19/EC of 30 March 2007 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs' (2007)
24. Stellungnahme des Bundesinstitutes für Risikobewertung (BfR): 'Übergang von Phthalaten aus Twist off-Deckeln in Lebensmittel - Aktualisierte Stellungnahme Nr. 025/2007' (2007) Berlin, Germany.
25. Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, Schmidt IM, Suomi AM, Virtanen HE, Petersen JH, Andersson AM, Toppari J, Skakkebaek NE (2006) Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect 114: 270-276
26. Mortensen GK, Main KM, Andersson AM, Leffers H, Skakkebaek NE (2005) Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS). Anal Bioanal Chem 382: 1084-1092
27. Latini G, Wittassek M, Del Vecchio A, Presta G, De Felice C, Angerer J (2009) Lactational exposure to phthalates in Southern Italy. Environ Int 35: 236-239
28. Koch HM, Muller J, Drexler H, Angerer J (2005) Dibutylphthalate (DBP) in medications: are pregnant women and infants at risk? Umweltmed Forsch Prax 10: 144-146
29. Hernandez-Diaz S, Mitchell AA, Kelley KE, Calafat AM, Hauser R (2009) Medications as a Potential Source of Exposure to Phthalates in the US Population. Environ Health Perspect 117: 185-189
30. Hauser R, Duty S, Godfrey-Bailey L, Calafat AM (2004) Medications as a source of human exposure to phthalates. Environ Health Perspect 112: 751-753
31. Seckin E, Fromme H, Volkel W (2009) Determination of total and free mono-n-butyl phthalate in human urine samples after medication of a di-n-butyl phthalate containing capsule. Toxicol Lett 188: 33-37
32. Weisbach V, Koch HM, Angerer J, Eckstein R (2006) Di(2-ethylhexyl)phthalate exposure of apheresis donors is procedure-related. Transfusion 46: 1457-1458
33. Koch HM, Angerer J, Drexler H, Eckstein R, Weisbach V (2005) Di(2-ethylhexyl)phthalate (DEHP) exposure of voluntary plasma and platelet donors. Int J Hyg Environ Health 208: 489-498
34. Koch HM, Bolt HM, Preuss R, Eckstein R, Weisbach V, Angerer J (2005) Intravenous exposure to di(2-ethylhexyl)phthalate (DEHP): metabolites of DEHP in urine after a voluntary platelet donation. Arch Toxicol 79: 689-693

35. Monfort N, Ventura R, Latorre A, Belalcazar V, Lopez M, Segura J (2010) Urinary di-(2-ethylhexyl)phthalate metabolites in athletes as screening measure for illicit blood doping: a comparison study with patients receiving blood transfusion. Transfusion 50: 145-149
36. Wilson VS, Lambright C, Furr J, Ostby J, Wood C, Held G, Gray LE (2004) Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. Toxicol Letters 146: 207-215
37. Borch J, Ladefoged O, Hass U, Vinggaard AM (2004) Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reprod Toxicol 18: 53-61
38. Fisher JS, Macpherson S, Marchetti N, Sharpe RM (2003) Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate. Hum Reprod 18: 1383-1394
39. Gray LE, Ostby J, Furr J, Price M, Veeramachaneni DNR, Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58: 350-365
40. Foster PM, Mylchreest E, Gaido KW, Sar M (2001) Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 7: 231-235
41. Skakkebaek NE (2002) Endocrine disrupters and testicular dysgenesis syndrome. Horm Res 57 Suppl 2: 43
42. Wohlfahrt-Veje C, Main KM, Skakkebaek NE (2009) Testicular dysgenesis syndrome: fetal origin of adult reproductive problems. Clin Endocrinol (Oxf) 71: 459-465
43. Sharpe RM, Irvine DS (2004) How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? Br Med J 328: 447-451
44. Committee on the Health Risks of Phthalates (2008) 'Phthalates and Cumulative Risk Assessment - The Task Ahead.' (The National Academies Press: Washington)
45. Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM (2006) Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology 17: 682-691
46. Duty SM, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, Overstreet J, Hauser R (2004) The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. J Androl 25: 293-302
47. Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen ZY, Herrick RF, Christiani DC, Hauser R (2003) Phthalate exposure and human semen parameters. Epidemiology 14: 269-277
48. Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, Calafat AM (2007) DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Hum Reprod 22: 688-695
49. Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, Herrick RF, Christiani DC, Hauser R (2003) The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. Environ Health Perspect 111: 1164-1169
50. Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R (2005) Phthalate exposure and reproductive hormones in adult men. Hum Reprod 20: 604-610
51. Swan SH (2008) Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res 108: 177-184
52. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113: 1056-1061
53. Stahlhut RW, Van Wijngaarden E, Dye TD, Cook S, Swan SH (2007) Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult US males. Environ Health Perspect 115: 876-882
54. Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, Webster TF (2008) Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environ Health 7: 27
55. Hatch EE, Nelson JW, Stahlhut RW, Webster TF (2010) Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl
56. Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, Sparks A, Weiss B (2009) Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl
57. Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, Wolff MS (2010) Prenatal Phthalate Exposure is Associated with Childhood Behavior and Executive Functioning. Environ Health Perspect
58. 'IRIS Toxicological Review and Summary Documents for Dibutyl Phthalate (External Peer Review)' (2006) U.S. EPA.
59. Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK, Gray LE (2008) A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105: 153-165
60. 'Phthalates and Bisphenol A (BPA): written Testimony before the House Energy & Commerce Committee; Commerce, Trade, and Consumer Protection Subcommittee; United States House of Representatives' (2008)
61. 'Multigeneration reproduction toxicity study in rats: Diethylhexylphthalate: Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet.' (2003) TherImmune Research Corporation (Gaithersburg, Maryland). No. TRC Study No 7244-200,

62. Gray LE, Barlow NJ, Howdeshell KL, Ostby JS, Furr JR, Gray CL (2009) Transgenerational effects of Di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter. Toxicol Sci 110: 411-425
63. Blystone CR, Kissling GE, Bishop JB, Chapin RE, Wolfe GW, Foster PM (2010) Determination of the di-(2-ethylhexyl) phthalate NOAEL for reproductive development in the rat: importance of the retention of extra animals to adulthood. Toxicol Sci 116: 640-646
64. Wine RN, Li LH, Barnes LH, Gulati DK, Chapin RE (1997) Reproductive toxicity of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. Environ Health Perspect 105: 102-107
65. Gray LE, Wolf C, Lambright C, Mann P, Price M, Cooper RL, Ostby J (1999) Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol Ind Health 15: 94-118
66. Lee KY, Shibutani M, Takagi H, Kato N, Takigami S, Uneyama C, Hirose M (2004) Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology 203: 221-238
67. European Parliament and Council (2004) Directive 2004/73/EC adapting to technical progress for the twenty-ninth time Council Directive 67/548/EEC on the approximation oft he laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:152:0001:0314:EN:PDF
68. European Parliament and Council (2005) Directive 2005/84/EC amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and baby articles) http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:EN:PDF
69. 'Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on the possibility of allocating a group-TDI for Butylbenzylphthalate (BBP), di-Butylphthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP), di-Isononylphthalate (DINP) and di-Isodecylphthalate (DIDP)' (2005) European Food Safety Authority, Parma, Italy.
70. EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Di-Butylphthalate (DBP) for use in food contact materials. The EFSA Journal 242: 1-14
71. EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. The EFSA Journal 243: 1-20
72. EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Di-isononylphthalate (DINP) for use in food contact materials. The EFSA Journal 244: 77-83
73. EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Butylbenzylphthalate (BBP) for use in food contact materials. The EFSA Journal 241: 1-14
74. EFSA (2005) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Di-isodecylphthalate (DIDP) for use in food contact materials. The EFSA Journal 245: 1-14
75. Gray LE, Wilson VS, Stoker T, Lambright C, Furr J, Noriega N, Howdeshell K, Ankley GT, Guillette L (2006) Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. Int J Androl 29: 96-104
76. Rider CV, Furr J, Wilson VS, Gray LE, Jr. (2008) A mixture of seven antiandrogens induces reproductive malformations in rats. Int J Androl 31: 249-262
77. Howdeshell KL, Furr J, Lambright CR, Rider CV, Wilson VS, Gray LE, Jr. (2007) Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. Toxicol Sci 99: 190-202
78. Rider CV, Wilson VS, Howdeshell KL, Hotchkiss AK, Furr JR, Lambright CR, Gray LE, Jr. (2009) Cumulative effects of in utero administration of mixtures of "antiandrogens" on male rat reproductive development. Toxicol Pathol 37: 100-113
79. Christiansen S, Scholze M, Dalgaard M, Vinggaard AM, Axelstad M, Kortenkamp A, Hass U (2009) Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ Health Perspect 117: 1839-1846
80. Kortenkamp A, Faust M (2010) Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Int J Androl 33: 463-474
81. Rider CV, Furr JR, Wilson VS, Gray LE, Jr. (2010) Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. Int J Androl 33: 443-462
82. Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW (2000) Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 108: 979-982

83. Koch HM, Rossbach B, Drexler H, Angerer J (2003) Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. Environ Res 93: 177-185
84. Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, Sadowski M, Needham LL, Calafat AM (2003) Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ Health Perspect 111: 1148-1151
85. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, Brock JW, Needham LL, Calafat AM (2004) Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ Health Perspect 112: 331-338
86. Koch HM, Calafat AM (2009) Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond B Biol Sci 364: 2063-2078
87. Stellungnahme der Kommission Human-Biomonitoring des Umweltbundesamtes (2005) Stoffmonographie Di(2-ethylhexyl)phthalt (DEHP) - Referenzwerte für 5oxo-MEHP und 5OH-MEHP im Urin. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 48: 706-722
88. Kessler W, Phokha W, Csanady GA, Filser JG (2001) No background concentrations of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in blood of rats. Arch Toxicol 75: 62-64
89. Kessler W, Numtip W, Grote K, Csanady GA, Chahoud I, Filser JG (2004) Blood burden of di(2-ethylhexyl) phthalate and its primary metabolite mono (2-ethylhexyl) phthalate in pregnant and nonpregnant rats and marmosets. Toxicol Appl Pharmacol 195: 142-153
90. Koch HM, Bolt HM, Angerer J (2004) Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol 78: 123-130
91. Kato K, Silva MJ, Brock JW, Reidy JA, Malek NA, Hodge CC, Nakazawa H, Needham LL, Barr DB (2003) Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass Spectrometry. J Anal Toxicol 27: 284-289
92. Calafat AM, Slakman AR, Silva MJ, Herbert AR, Needham LL (2004) Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci 805: 49-56
93. Kato K, Silva MJ, Needham LL, Calafat AM (2006) Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line SPE and isotope-dilution high-performance liquid chromatography--tandem mass spectrometry. Anal Chem 78: 6651-6655
94. Mose T, Mortensen GK, Hedegaard M, Knudsen LE (2007) Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod Toxicol 23: 83-91
95. Anderson WAC, Castle L, Scotter MJ, Massey RC, Springall C (2001) A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Additives and Contaminants 18: 1068-1074
96. Koch HM, Bolt HM, Preuss R, Angerer J (2005) New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol 79: 367-376
97. Koch HM, Angerer J (2007) Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. International Journal of Hygiene and Environmental Health 210: 9-19
98. Silva MJ, Kato K, Wolf C, Samandar E, Silva SS, Gray EL, Needham LL, Calafat AM (2006) Urinary biomarkers of di-isononyl phthalate in rats. Toxicology 223: 101-112
99. Kato K, Silva MJ, Wolf C, Gray LE, Needham LL, Calafat AM (2007) Urinary metabolites of diisodecyl phthalate in rats. Toxicology 236: 114-122
100. Koch HM, Muller J, Angerer J (2007) Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers. J Chromatogr B Analyt Technol Biomed Life Sci 847: 114-125
101. Koch HM, Drexler H, Angerer J (2004) Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). Int J Hyg Environ Health 207: 15-22
102. Lorber M, Angerer J, Koch HM (2010) A simple pharmacokinetic model to characterize exposure of Americans to Di-2-ethylhexyl phthalate. J Expo Sci Environ Epidemiol 20: 38-53
103. Becker K, Goeen T, Seiwert M, Conrad A, Pick-Fuss H, Muller J, Wittassek M, Schulz C, Kolossa-Gehring M (2009) GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int J Hyg Environ Health 212: 685-692
104. Schulz C, Seiwert M, Becker K, Conrad A, Kolossa-Gehring M (2008) Der Kinder-Umwelt-Survey (KUS) 2003-2006: Stichprobe und Studienbeschreibung. Umweltmed Forsch Prax 13: 379-390
105. Schulz C, Conrad A, Becker K, Kolossa-Gehring M, Seiwert M, Seifert B (2007) Twenty years of the German Environmental Survey (GerES): human biomonitoring - temporal and spatial (West Germany/East Germany) differences in population exposure. Int J Hyg Environ Health 210: 271-297
106. Kurth BM (2007) Ein Überblick über Planung, Durchführung und Ergebnisse unter Berücksichtigung von Aspekten eines Qualitätsmanagements. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 50: 533-546
107. Kurth BM, Bergmann KE, Hölling H, Kahl H, Kamtsiuris P, Thefeld W (2002) Der bundesweite Kinder- und Jugendgesundheitssurvey – Das Gesamtkonzept. Gesundheitswesen 64: 3-11
108. Kamtsiuris P, Lange M, Schaffrath Rosario A (2007) Der Kinder- und Jugendgesundheitssurvey (KiGGS): Stichprobendesign, Response und Non-Responder-Analyse. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 50: 547-556
109. Becker K, Pick-Fuß H, Conrad A, Zigelski C, Kolossa-Gehring M, Göen T, Seidel A (2009) Kinder-Umwelt-Survey (KUS) 2003-2006. Human-Biomonitoring-Untersuchungen auf Phthalat- und

Phenanthrenmetabolite sowie Bisphenol A. Umwelt & Gesundheit 04/2009. Umweltbundesamt, Berlin http://www.umweltbundesamt.de/uba-info-medien/3822.html
110. Schulz C, Babisch W, Becker K, Dürkop J, Roßkamp E, Seiwert M, Steiner M, Szewzyk R, Ullrich D, Englert N, Seifert B, Eis D (2004) Kinder-Umwelt-Survey – das Umweltmodul im KiGGS. Teil 1: Konzeption und Untersuchungsprogramm. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 47: 1066-1072
111. Wittassek M, Wiesmuller GA, Koch HM, Eckard R, Dobler L, Muller J, Angerer J, Schlüter C (2007) Internal phthalate exposure over the last two decades - a retrospective human biomonitoring study. Int J Hyg Environ Health 210: 319-333
112. Göen T et al., Internal phthalate exposure of young adults in Germany over the last decade – the follow up of a retrospective human biomonitoring study Publikation in Vorbereitung.
113. Kommission „Human-Biomonitoring" des Umweltbundesamtes (1996) Konzept der Referenz und Human-Biomonitoring-Werte (HBM) in der Umweltmedizin. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 39: 221-224
114. Poulsen OM, Holst E, Christensen JM (1997) Calculation and application of confidence and tolerance intervals for biological reference values. Technical Report. Pure Appl Chem 69: 1601-1611
115. Angerer J, Ewers U, Wilhelm M (2007) Human biomonitoring: state of the art. Int J Hyg Environ Health 210: 201-228
116. Angerer J, Goen T, Lehnert G (1998) Mindestanforderungen an die Qualität von umweltmedizinisch-toxikologischen Analysen. Umweltmed Forsch Prax 3: 307-312
117. Kommission „Human-Biomonitoring" des Umweltbundesamtes (2005) Normierung von Stoffgehalten im Urin - Kreatinin. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 48: 616-618
118. Stellungnahme des Bundesinstitutes für Risikobewertung (BfR): 'Übergang von Phthalaten aus Twist off-Deckeln in Lebensmittel - Gesundheitliche Bewertung Nr. 042/2005' (2005) Berlin, Germany.
119. Stellungnahme des Bundesinstitutes für Risikobewertung (BfR): 'Weichmacher gehören nicht ins Speiseöl!' (2005) Berlin, Germany.
120. Stellungnahme des Bundesinstitutes für Risikobewertung (BfR): 'Di-isobutylphthalat in Papier und Kartons für dem Kontakt mit Lebensmitteln' (2007) Berlin, Germany.
121. Wormuth M, Demou E, Scheringer M, Hungerbuhler K (2007) Assessments of direct human exposure: the approach of EU risk assessments compared to scenario-based risk assessment. Risk Anal 27: 979-990
122. Becker K, Conrad A, Lusansky C, Schulz C, Seiwert M, Hünken A, Kolossa-Gehring M (2008) German Environmental Survey for Children (GerES IV): Metabolites of DEHP, DnBP, DiBP, BBzP, and DiNP in urine of German children. 'Joint Annual Conference of ISEE/ISEA'. 12. Oct. 2008. Pasadena
123. Stellungnahme der Kommission Human-Biomonitoring des Umweltbundesamtes (2007) Ableitung von Human-Biomonitoring-(HBM-) Werten auf der Basis tolerabler Aufnahmemengen–Teil III: HBM-Werte für Di(2-ethylhexyl)phthalat (DEHP). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 50: 255-259
124. Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LE, Jr. (2000) The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 58: 339-349
125. Mylchreest E, Cattley RC, Foster PM (1998) Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? Toxicol Sci 43: 47-60
126. Borch J, Axelstad M, Vinggaard AM, Dalgaard M (2006) Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis. Toxicol Letters 163: 183-190
127. Gray LE, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58: 350-365
128. Tyl RW, Myers CB, Marr MC, Fail PA, Seely JC, Brine DR, Barter RA, Butala JH (2004) Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. Reprod Toxicol 18: 241-264
129. Hotchkiss AK, Parks-Saldutti LG, Ostby JS, Lambright C, Furr J, Vandenbergh JG, Gray LE, Jr. (2004) A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. Biol Reprod 71: 1852-1861
130. Foster PM, Thomas LV, Cook MW, Gangolli SD (1980) Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. Toxicol Appl Pharmacol 54: 392-398
131. European Commission Environment (2005) Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Richtlinie zur Beschränkungen des Inverkehrbringens und der Verwendung gewisser gefährlicher Stoffe und Zubereitungen) http://ec.europa.eu/environment/chemicals/dansub/home_en.htm,
132. European Parliament and Council (2009) Directive 2009/2/EC amending, for the purpose of its adaptation to technical progress, for the 31st time, Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:011:0006:0082:EN:PDF
133. European Parliament and Council (2001) Directive 2001/59/EC adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:225:0001:0333:EN:PDF

134. Centers for Disease Control and Prevention; National Center for Environmental Health; Division of Laboratory Sciences. Atlanta, GA. (2009) Fourth National Report on Human Exposure to Environmental Chemicals. http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf.
135. Fromme H, Bolte G, Koch HM, Angerer J, Boehmer S, Drexler H, Mayer R, Liebl B (2007) Occurrence and daily variation of phthalate metabolites in the urine of an adult population. International Journal of Hygiene and Environmental Health 210: 21-33
136. Silva MJ, Reidy JA, Preau JL, Jr., Needham LL, Calafat AM (2006) Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. Environ Health Perspect 114: 1158-1161
137. Seiwert M (2010) personal communication