

Derivation of human biomonitoring (HBM) values on the basis of tolerable daily intake. Part III: HBM values for di(2-ethylhexyl)phthalate (DEHP)

Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency (Umweltbundesamt)

1 Introduction

Di(2-ethylhexyl)phthalate (DEHP) is one of the most commonly used phthalates (phthalic acid esters) [1], which are used as softeners in dispersions, paints, and varnishes, as emulsifiers, repellents and carrier fluids in biocides, in cosmetics and perfumes, and for many other common applications.

In animal experiments on chronic toxicity, renal, testicular and hepatic toxicity have been observed following intake of extremely low doses of DEHP. In addition to pronounced reproduction toxicity, DEHP has embryotoxic, fetotoxic and teratogenic effects on the offspring of exposed rodents. In vitro and in vivo studies have shown that DEHP can derange hormonal regulation, leading to estrogenic and anti-androgenic effects and the retardation of sexual maturity [2, 3]. As a result, phthalates are now also regarded in the environmental medicine literature as a priority substance group for human biomonitoring, since reliable analytic methods [4] are now available, and the current exposure level data for the general population [5, 6] indicates that individual exposure levels should be assessed toxicologically.

The present study describes how a method developed by the Human Biomonitoring Commission of the Federal Environment Agency (Umweltbundesamt) for the estimation of HBM values on the basis of tolerable daily intake [7] can be applied to di(2-ethylhexyl)phthalate (DEHP), in whose regard the Commission's recently published monograph on DEHP [8] will also be expressly referred to in the present paper.

In this method, after ascertaining whether the data and methodological requirements described below for the estimation of HBM values on the basis of tolerable/acceptable daily intake have been fulfilled [7], a recommended HBM I value is defined .

2 Data and methodological requirements for the estimation of HBM values

2.1 Tolerable/acceptable daily intake

Various organizations have estimated the tolerable daily intake of DEHP on the basis of Wolfe and Layton's [2] proposed 4.8 mg DEHP/kg/bw/day No Observed Adverse Effects Level (NOAEL), which is in turn based on a multigenerational study of testicular toxicity in Sprague-Dawley rats [2]. This study focused on reproduction toxicity that took the form of a reduced number of offspring, lower birth weights, male and female genital malformations, and infertility. This study is widely regarded as being more valid than studies such as Poon et al's classic animal toxicity study [9] which measured testicular toxicity (Sertoli cell vacuolization, atrophy of semen bearing tubuli) and hepatotoxicity (peroxisome proliferation) and defined a NOAEL of 3.7 mg/kg/bw/day.

The Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung (BfR), in accordance with the European Food Safety Authority (EFSA) has defined a tolerable daily intake of 0.05 mg/kg bw/day [10, 11] for DEHP on the basis of the Wolfe and Layton NOAEL [2]. At the request of the Federal Environment Agency, a tolerable resorbed dose was estimated (0.03 mg/kg/bw/day (intake equivalent: 0.05 mg/kg/bw/day)); this dose was also validated by a subsequent report [12]. The aforementioned Wolfe und Layton NOAEL of 4.8 mg/kg/bw/day [2] also formed the basis for the tolerable daily intake of 0.048 mg/kg/bw/day estimated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) [13, 14]; a total extrapolation factor of 100 [12, 13, 14] was used for each of these estimates. Tolerable DEHP intake defined by other organizations is listed in table 1.

Table 1 Tolerable daily intake of DEHP		
Term, Institution, Year [source]	Value (µg/kg/bw/day)	Underlying NOAEL (µg/kg/bw/day), year [source]
TDI ^{a)} (MPR ^{b)} NL-RIVM 2002 [27]	4	3,7; 1997 [9]
RfD ^{c)} US-EPA 1991 [28]	20	20; 1953 [29]
TDI ^{a)} WHO 2003 [30]	25	2,5; 2003 [30]
TDI ^{a)} EU-CSTEE 1998 [13]	37	3,7 ; 1997 [9] and ca. 3,5 LOAEL; 1998 [31]
TDI ^{a)} ECB/EU (RAR-DEHP) 2004 [32]	20 (0–3 month old infants; women of childbearing age) 25 (3-12 month old infants) 48 (all other population groups)	4,8; 2003 [2]
TDI ^{a)} Health Canada 1994 [33]	44	44 ; 1984 [34]
TRD ^{d)} D-UBA, 2003 [12]	50	2,9 (intake: 4.8); 2003 [2]
TDI ^{a)} BfR, 2005 [10] and EFSA, 2005 [11]	50	4,8; 2003 [2]
MRL ^{e)} US-ATSDR, 2002 [35]	60	5,8; 2000 [36]
<p><i>a) Tolerable Daily Intake</i> <i>b) Maximum Permissible Risk Level</i> <i>c) Reference Dose (for chronic exposure)</i> <i>d) Tolerable resorbed dose</i> <i>e) Minimal Risk Level (for chronic exposure duration)</i></p>		

In the present investigation, HBM values were estimated on the basis of the current European and German tolerable daily intake of 50 µg/mg/kg/bw/day, except for women of child bearing age for whom the amount is 20 µg/mg/kg/bw/day.

Conclusion: A generally accepted tolerable/acceptable daily intake value is available at the national and international level.

2.2 Exposure levels for the general population and basic toxicokinetic data

In recent years researchers (predominantly in Germany) have investigated renally excreted DEHP metabolites in various population groups (see section 2.3). The German studies have investigated both adults and children (see table 2). Virtually all of the studies detected the DEHP metabolites 5oxo-MEHP and 5OH-MEHP in urine, with median levels ranging from 20-40 µg/l for the former and 30-50 µg/l for the latter. Peak renal excretion was 544 µg/l for 5oxo-MEHP and 818 µg/l for 5OH-MEHP.

Author [source]	Age groups (years)	Sample size	5oxo-MEHP (µg/l) Median (P 95)	5OH-MEHP (µg/l) Median (P 95)
Koch et al. [37] Koch et al. [6]	7 – 64	85	36.5 (156)	46.8 (224)
Koch et al. [38]	20-59	19	19.6 (36.7)	32.1 (64.0)
	2-6	36	33.8 (71.0)	49.1 (107.0)
Becker et al. [39]	3-14	254	41.4 (138)	52.1 (185)

P 95 = 95th percentile

Mammals rapidly convert DEHP into numerous metabolites, beginning with oral hydrolysis of DEHP into MEHP and 2-ethylhexanol via salivary enzymes. The latter compound is completely broken down via a series of intermediate steps.

Oxidative MEHP metabolism begins with hydroxylation at five locations along the ethylhexyl side chain, resulting in the formation of primary and secondary alcohols which are then oxidized into ketones or carboxylic acids.

Several studies have analyzed 12 metabolites as well as their interrelationships and concentrations following both oral and intravenous intake of 30 mg of DEHP. The main DEHP metabolites are MEHP, 5oxo-MEHP and 5OH-MEHP, the latter of which accounts for 50-66 percent of DEHP metabolites in urine [15, 16, 17].

A HBM study using isotope marked DEHP found that of the 47 percent of the applied dose detected in urine, 7 percent was MEHP, 15 percent was 5oxo-MEHP and 25 percent was 5OH-MEHP. Thus 40 percent of the applied DEHP dose was excreted as 5oxo-MEHP and 5OH-MEHP [16].

As with rats, such assessments must allow for a base amount of biliary excretion.

The aforementioned DEHP metabolites in urine have a half-life of approximately 12 hours. Secondary DEHP metabolite concentration in urine is only slightly higher than MEHP concentration; these metabolites also have longer half lives in urine than MEHP.

Conclusion: Basic human toxicokinetic data is available, and the ratio between intake and urinary excretion is known.

2.3 Assay method

MEHP and other secondary metabolites in urine are assayed using gas chromatography-mass spectrometry [15] and high pressure liquid chromatography-mass spectrometry [4, 18]. However, only secondary DEHP metabolites in urine and blood can be assayed accurately since during the pre-assay phase or under ambient conditions MEHP is readily formed from DEHP through various hydrolytic processes. This does not occur with secondary metabolites.

Conclusion: An established assay methodology is available for a readily accessible human biological matrix.

3 Derivation of a HBM value for DEHP

HBM values for DEHP metabolites in urine are estimated on the basis of the *composite sum* of the metabolites 5oxo-MEHP and 5OH-MEHP. 40 percent of the applied (oral) DEHP dose is excreted renally as 5oxo-MEHP and 5OH-MEHP [16, 17].

Inasmuch as concentration data is generally based on mass, the metabolite values must undergo a conversion on the basis of the following molecular weights: DEHP: 390, 5-oxo-MEHP: 292 g/mol; and 5-OH-MEHP: 294 g/mol. This process can be simplified by using a molecular weight of 293 g/mol for the target metabolite.

Derivation of the HBM value is based on the assumption that an adult's daily intake of DEHP will be constituted by his actual (and tolerable) daily intake of 50 µg/kg/bw/day. 40 percent of the applied dose is excreted renally as the metabolites 5oxo-MEHP and 5OH-MEHP (1 mol DEHP ~ 0.4 mol = 5-oxo-MEHP + 5-OH-MEHP). The ratio between the molar mass of DEHP and its metabolites must be factored into the weight percentage conversion (µg/l). Inasmuch as the molecular weight of DEHP is 390 and the mean molecular weight of both 5oxo-DEHP and 5-OH-DEHP is 293, the molecular weight ratio is 0.75.

$$\text{Sum of 5oxo- and 5OH-MEHP in urine} = \text{TDI} \times \frac{\text{Molecular weights of the metabolites}}{\text{Molecular weight of DEHP}} \times 0.4$$

Composite sum of the molecular weights of the metabolites 5oxo-MEHP and 5OH-MEHP in 24 hour urine = 50 µg/kg/bw/day x 293/390 x 0.4 = 15 µg/kg/bw/day.

Thus of the 50 µg/kg/bw/day of DEHP (tolerable daily intake) that is putatively ingested by the average adult, 15 µg x kg/bw is excreted renally over a 24 hour period as the metabolites 5oxo-MEHP and 5OH-MEHP. This calculation method

provides the HBM I value for the average adult but yields a different value for population subgroups such as women whose tolerable daily intake differs from that of the average adult.

The HBM value thus calculated comprises a HBM I value that was derived from a No Observed Adverse Effects Level (NOAEL) [19].

Ideally estimated HBM I values should be based on 24 hour urine expressed as µg/day. For reasons of simplicity, in accordance with the procedure described in part II of the present report [7] the metabolite calculation described above was based on 30 ml/kg/bw/day for adults and 20 ml/kg/bw/day of urine for all other population subgroups [20], in proportion to body weight (see table 3). Inasmuch as the ratio between renal elimination of DEHP metabolites and creatinine is unknown, the calculation of standard values for renal elimination of creatinine was dispensed with in the present study (also see [21, 22]).

Table 3			
Derived HBM I values for the sum of the metabolites 5oxo-MEHP and 5OH-MEHP			
Population group	Tolerable daily intake (µg/kg/bw/day)	Urine volume (l/kg/bw/day)	HBM I value (ug/l) of morning urine*
Children aged 6-13	50	0.030	500
Women of childbearing age	20	0.020	300
All other population groups	50	0.020	750
* Calculation method: Tolerable daily intake x concentration in urine (f= 0.4) x the ratio of molecular weight (0.75) to urine volume (ml/kg/bw).			

Inasmuch as the physiology of children and adults differs, and it is not known whether metabolic ratios in adults and in children under the age of six are the same, the Commission decided to forego estimation of a HBM value for this population group.

A comparison of these HBM I values and the current reference values for the DEHP metabolites 5oxo-MEHP and 5OH-MEHP estimated by the Commission [8] can be found in table 4.

Table 4 Comparison of the HBM I values and the sum of the current reference values for the DEHP metabolites 5oxo-MEHP and 5OH-MEHP		
Population group	HBM I value (ug/l)	Reference value (µg/l) [8]
Children aged 6-13	500	5oxo-MEHP: 150 5OH-MEHP: 220
Women of childbearing age	300	
Males 14 years of age and older, and all other population groups	750	<u>Σ: 370</u>

4 Summary and perspective

We recommend that DEHP exposure be measured and evaluated on the basis of (a) the HBM I values described in the present report; and (b) the composite total of the DEHP metabolites 5oxo-MEHP and 5OH-MEHP in morning urine (µg/l).

Recent studies of phthalate exposure in pregnant women [23, 24] show that 2 to 36 month old male infants apparently exhibit reduced anogenital distance on exposure to DEHP doses that are substantially lower than the US Environmental Protection Agency's reference dose of 20 µg/kg/bw. However the results of these studies have not yet been applied to risk assessments for human reproduction [25]. If new findings modify the risk-assessment of DEHP and the consequent tolerable daily intake values, the HBM I values will also have to be adjusted using the calculation method described in the present report.

One recent study [26] has shown that population groups such as nursery school children and their parents and teachers are exposed to endocrine-active phthalates such as DnBP, DiBP, BBzP and DiNP [26]. This factor should be taken into account in phthalate exposure assessments for the population as a whole. However, a standard HBM I value for the aggregate total of all endocrine-active phthalates

cannot be defined at present owing to the diverse effects involved and the fact that the relevant mechanisms of action have yet to be described.

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