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1st substance-group specific derivation of EU-wide health-based guidance values

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Chapter 1

Derivation of HBM health-based guidance values (HBM HBGVs): HBM $HBGV_{GenPop}$ for the general population & HBM $HBGV_{Worker}$ for workers

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Glossary

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
AFs	Assessment Factors
AFSSET	Former French Agency for Environmental and Occupational Health & Safety
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
BBP	Benzyl butyl phthalate
BE	Biomonitoring Equivalent
BGV	Biological Guidance Value
BLV	Biological Limit Value
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Confidence Limit
CDC	Center for Disease Control
CLP	Classification, Labelling and Packaging
CMD	Carcinogens and Mutagens Directive
DBP	Di-n-butyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate or di(2-ethylhexyl) phthalate
DFG	German Research Foundation
DIBP	Diisobutyl phthalate
DNEL	Derived No-Effect Level
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
GD	Gestational Day
HBM HBGV	HBM Health-Based Guidance Value
HBM	Human Biomonitoring
HBM4EU	European Biomonitoring Initiative
HED	Human Equivalent Dose
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
LC	Leydig Cell

MAK Commission	German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area
MCL	Mononuclear Cell Leukaemia
MEHP	Mono(2-ethylhexyl) phthalate
NHANES	National Health and Nutrition Examination Survey
NO(A)EL	No Observed (Adverse) Effect Level
OEL	Occupational Exposure Limit
PBPK/PBTK	Physiologically Based Pharmacokinetic/Toxicokinetic
PBT	Persistent Bioaccumulative and Toxic
PND	Post Natal Day
POD	Point Of Departure
PP	Peroxisome Proliferator
PPAR	Peroxisome Proliferator-activated Receptor
RAC	Risk Assessment Committee
RfD	Reference Dose
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCOEL	Scientific Committee on Occupational Exposure Limits
SEAC	Socio-Economic Analysis Committee
TDI	Tolerable Daily Intake

1 Introduction

1.1 Objectives

In task 5.2 of HBM4EU, HBM health-based guidance values (HBM HBGVs) are derived for HBM4EU priority substances.

The methodology applied to derive these values is based on the procedure described in the German Human Biomonitoring Commission's position papers, as well as on the guidance document elaborated by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) for the derivation of biological limit values for chemicals used in the workplace. These methodological approaches have been summarised and combined in a concept document previously released in the HBM4EU task 5.2 workframe (HBM4EU concept paper, 2017).

In the current report, a general population HBM HBGV (HBM $HBGV_{GenPop}$) and an occupational HBM HBGV (HBM $HBGV_{Worker}$) are derived for di(2-ethylhexyl) phthalate (DEHP) and discussed.

1.2 Methodological aspects of the HBM HBGVs derivation for DEHP

The current epidemiological knowledge based on human exposure to DEHP on whether and how it might cause human health effects is not sufficient to derive HBM HBGVs directly based on human data, be it for the general population (HBM $HBGV_{GenPop}$) or for the workers (HBM $HBGV_{Worker}$). Because of this, for deriving a HBM $HBGV_{GenPop}$, the German method for deriving HBM values for the general population based on an established tolerable intake level as the tolerable daily intake (TDI) is applied (Kommission Human-Biomonitoring, 2014; HBM4EU concept paper, 2017). The derivation of an occupational HBM $HBGV_{Worker}$ in this current report starts from an animal point of departure (POD) value identified from a key toxicity study, which then is translated into a biomonitoring equivalent as described by Hays et al. (2007).

As a first step in the underlying report, information on the general toxicological profile of DEHP is given and the requirements to be met for deriving HBM health-based guidance values from tolerable intake levels (as the TDI/ADI) or from a POD value are specified. Subsequently, HBM $HBGV_{GenPop}$ similar to HBM-I values are proposed, which could be seen as an update of the Opinion of the Human Biomonitoring Commission of the German Environment Agency (Kommission Human-Biomonitoring, 2007; 2005a). The proposal for a HBM $HBGV_{Worker}$ is based only partially on the ANSES collective expert appraisal on evaluation of DEHP biomarkers. Indeed, the ANSES Occupational Exposure Limit (OEL) Committee and the Working Group on Biomarkers, both active in the Occupational Safety and Health domain, preferred not to recommend any biological limit value (BLV) for DEHP, considering the many uncertainties underlying its calculation method (ANSES 2012a). Despite this, as it is key for the progress of HBM4EU to come up with HBM $HBGVs_{Worker}$, in this report an HBM $HBGV_{Worker}$ is derived using similar information as used by ANSES (ANSES 2012a) that was a merger of AFSSA and AFSSET. The biological reference value (BRV), which gives indication of the background burden of a reference population of working age and without recognisable specific exposure, as established in France in 2010 (AFSSET, 2010) is included in the current report and discussed in the context of more recent data.

1.3 General information & relevance of recommending HBM HBGVs for DEHP

Phthalates (phthalic acid diesters) are used as plasticizers or as solubilizers in many products of everyday life. They can be found in a variety of articles including electrical cables, hoses, flooring, wall coverings, coated textiles, footwear, sports equipment, toys, plastic sheets, food packaging, medical devices, as well as paints, varnishes or cosmetics (ECB, 2008). Due to toxicity classifications and use restrictions or bans, the last years have seen a significant change in the spectrum of phthalates used (European Council for Plasticizers and Intermediates, 2010; Ceresana market study, 2013). For instance, since February 2015 (Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) sunset date) phthalates listed in Annex XIV of the REACH regulation, currently diisobutyl phthalate (DIBP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and DEHP, may only be used or placed for usage on the EU market if an authorisation has been granted. Therefore DEHP, a general-purpose plasticizer used for more than 50 years in almost all soft PVC applications, has been replaced in the EU in various industrial processes by phthalates like diisononyl phthalates (DINP), and diisodecyl phthalates (DIDP), as well as by non-phthalate substitutes. Nevertheless, 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 Europe despite declining usage. For example, between 2011 and 2014, the tonnages of DEHP in imported articles placed on the EU market grew by close to 22%, as the authorisation requirements do not apply to imported articles and the volumes of articles imported have been increasing (ECHA Annex XV Restriction Report, 2016).

2 Identification of the substance

CAS Number: 117-81-7

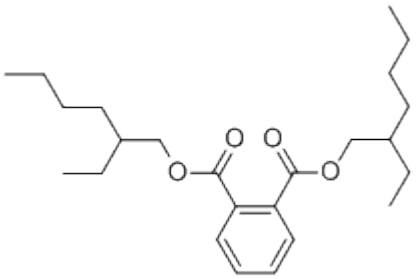
EINECS Number: 204-211-0

IUPAC Name: Bis(2-ethylhexyl) phthalate

Molecular weight: 390.6

Molecular formula: $C_{24}H_{38}O_4$

Structural formula:



source: CAS DataBase – ChemicalBook

3 Physicochemical properties

DEHP is a colorless liquid at room temperature. The vapour pressure is estimated to be $3.4 \cdot 10^{-5}$ Pa at 20°C. The Henry´s law constant for DEHP is 4.43 Pa · m^3/mol. The octanol-water partition coefficient, log Kow, is 7.5 (EU RAR, 2008).

4 Uses

The main use of DEHP is as a plasticizer in polymer products (in the EU this is more than 95% of the total use of DEHP), mainly in flexible PVC. Flexible PVC is used in many different articles, e.g. toys, building material such as flooring, cables, profiles and roofs, as well as in medical products such as blood bags and dialysis equipment. DEHP is also used in other polymer products and in non-polymer formulations and products. DEHP is known to migrate slowly from polymer products during their entire lifetime (EU RAR, 2008).

5 Classifications

Since its assessment in 2013, the International Agency for Research on Cancer (IARC) is considering DEHP as possibly carcinogenic to humans (Group 2B) due to sufficient evidence in experimental animals (IARC, 2013).

According to the Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008), DEHP may damage fertility and may damage the unborn child. It is therefore classified as reprotoxic 1B (H360FD). This substance was identified as a substance of very high concern (SVHC) because of its endocrine disrupting (ED) properties in the environment and for the human health. DEHP is thus included on the candidate list for authorisation and requires authorisation before it is used, according to Annex XIV of the REACH regulation. Some uses of this substance are also restricted under Annex XVII of REACH.

6 General toxicological profile

6.1 Toxicokinetics

Following oral administration, phthalates are generally rapidly absorbed from the gastrointestinal tract (probably in monoform). Phthalates can also be absorbed through the lungs, whereas absorption through the skin appears to be limited. For DEHP, the extent of oral absorption in rats is estimated to be around 60-70%. For humans, the Committee on Risk Assessment (RAC) considered at first (ECHA, 2012) that absorption of DEHP was 70% for adults and 100% in children. However, after revisiting the data, RAC concluded in its opinion of 8 March 2013 on the draft review report of ECHA concerning DINP and DIDP that humans appear to absorb DEHP at 100%. Human volunteer studies with DEHP demonstrate that the amount of radioactivity recovered in urine is dependent on the type and amount of metabolites that are measured in those studies. Measuring all metabolites most likely would result in near to 100% recovery of radioactivity in urine. An unknown amount of excretion via bile contributes further to the absorption estimate. Thus, absorption percentages of DEHP for humans are 100% oral absorption, 5% dermal absorption and 75% (adults) resp. 100% (child) inhalatory absorption (ECHA 2013a, b, c).

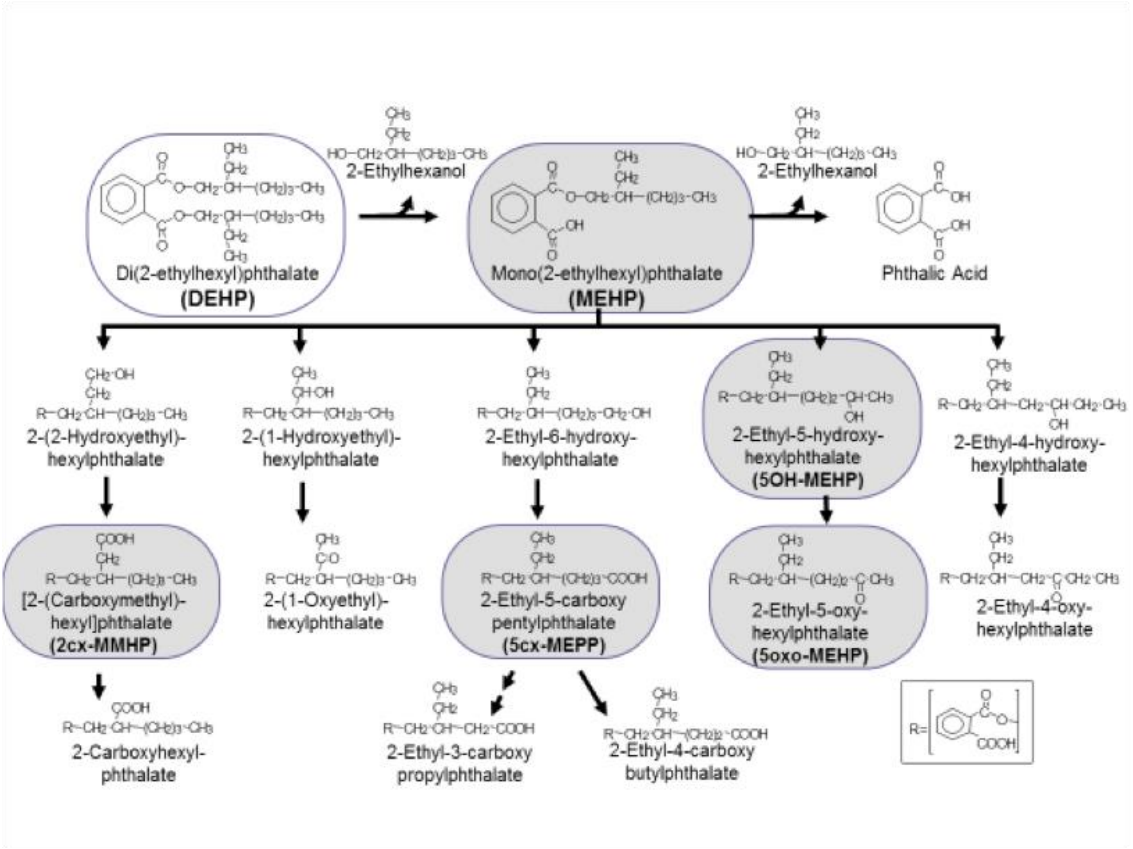
6.1.1 Animal studies

In rats, after absorption, DEHP is distributed mainly in the liver, kidneys, testes and blood (US-ATSDR, 2002) and there is no evidence of accumulation in the tissues. A comparative study of rats and marmosets showed similar distribution patterns in the two species (oral administration) whereas rats had higher tissue levels than marmosets (EU RAR, 2008). Thus, the difference in distribution between species is quantitative rather than qualitative.

The major step in the metabolism of DEHP is hydrolysis to mono(2-ethylhexyl) phthalate (MEHP) and to 2-ethylhexanol, which is common to all investigated species. The onset of hydrolysis takes already place in the oral cavity owing to the activity of saliva (Niino et al., 2001; 2003). Hydrolysing enzymes (hydrolases, unspecific lipases and/or esterases) are present in many tissues, especially so in the pancreas, the intestinal mucosa, in the liver, the lungs and in blood plasma. Further metabolism takes place in the liver. MEHP is a relatively major component in urine of monkeys, guinea pigs and mice but was in most cases not detected in rat urine. However, MEHP is present in plasma in all species tested. DEHP is excreted via the urine, mainly as MEHP-metabolites, but some excretion via bile also occurs in rodents. The half-life for DEHP and its metabolites was 3-5 days in the adipose tissue and 1-2 days in the liver. A comparative toxicokinetic study carried out in marmosets and rats, which were given (14C ring labelled) DEHP showed that excretion of the radiolabelled compounds was more rapid in rats than in marmosets (Rhodes et al., 1986; EU RAR 2008).

The oxidative metabolism of MEHP begins with hydroxylation of the ethylhexyl side chain in five different positions, forming primary and secondary alcohols, which are then oxidised into ketones or carboxylic acids (see figure 1).

Figure 1: Metabolism of DEHP (Koch et al., 2005). Major metabolites are highlighted.



6.1.2 Studies with human volunteers

In a human study with one male subject, isotope-marked DEHP was given orally in 3 different doses [0.35 mg (4.7 µg/kg), 2.15 mg (28.7 µg/kg), and 48.5 mg (650 µg/kg)] separated by at least a week. After 24 h, 67.0% (range: 65.8–70.5%) of the DEHP dose was excreted in urine, comprising 5OH-MEHP (23.3%), 5cx-MEPP (18.5%), 5oxo-MEHP (15.0%), MEHP (5.9%) and 2cx-MMHP (4.2%). An additional 3.8% of the DEHP dose was excreted on the second day (measured only for the highest dose), comprising 2cx-MMHP (1.6%), 5cx-MEPP (1.2%), 5OH-MEHP (0.6%), and 5oxo-MEHP (0.4%). In total 74.2% of the administered highest DEHP dose was excreted in urine after two days (Koch et al., 2005). No dose dependency in metabolism and excretion was observed.

Peak blood concentrations of the various DEHP metabolites were reached 2 to 4 hours after the start of ingestion. The half-lives of the oxidised metabolites (secondary metabolites) are between 2 and 5 hours with monophasic elimination kinetics in blood (Koch et al., 2004a and 2005). These compounds can be conjugated to glucuronic acid. All the metabolites identified in urine have biphasic elimination (Koch et al., 2005, see table 1).

In 2011, Anderson et al. published a study involving 20 volunteers (10 men and 10 women, all Caucasian) who ingested a dose (0.31 or 0.78 mg randomly) of DEHP. The dosed substance was deuterated di(2-ethylhexyl) phthalate (D(4)-DEHP). Urine samples were collected at intervals up to 48h post-dose. LC-MS/MS was used to measure metabolite concentrations. Excreted amounts were then calculated using urine volumes. Metabolite half-lives were estimated to be 4-8 h with more than 90% of metabolites excreted in the first 24 h of urine collection and the remainder in the 24-48 h period. The four metabolites of DEHP amounted to 47.1 ± 8.5% fractional excretion on a molar basis. The kinetic parameters are summarised in table 1:

Table 1: DEHP kinetic parameters (Anderson et al., 2011; Koch et al., 2004a, 2005)

Urinary metabolite	$T_{1/2(1)}$* (h)	$T_{1/2(2)}$* (h)	T_{max}* (h)	Molar excretion fraction (%) at 24h	Molar excretion fraction (%) at 48h
	(Koch et al., 2004a)	(Koch et al., 2005)		(Anderson et al., 2011)	
MEHP	2	5	2	6.2	6.3
5OH-MEHP	2	10	4	14.9	15.6
5oxo-MEHP	2	10	4	10.9	11.3
5cx-MEPP	3	12 to 15	4	13.2	13.9
2cx-MMHP	3	24	9 and 24	NR	NR

* $T_{1/2}$: half-life, $T_{1/2\ (1)}$: first phase, $T_{1/2\ (2)}$: second phase; T_{max}: time to obtain peak concentration

The authors (Anderson et al., 2011) state that the concentrations of the different metabolites are normally distributed and that the variability for the three secondary metabolites of DEHP is similar (RSD values of 20 - 25%). The exposure dose and gender do not significantly influence the excretion fractions. The metabolite concentrations ratio may, however, depend on the individual metabolic performance and also, on the age of the person concerned. Data on the age dependency of the ratio of the DEHP metabolites in urine have been condensed into table 2, as calculated from Becker et al. (2004).

Table 2: Ratios of DEHP metabolite concentrations in urine depending on age (Becker et al., 2004)

Study	Cohort	5OH-MEHP/MEHP		5-oxo-MEHP/MEHP		5OH-MEHP/5-oxo-MEHP	
		AM	RSD	AM	RSD	AM	RSD
Barr et al., 2003	Children and adults (N = 62)	8.2	80%	5.9	74%	1.4	22%
Becker et al., 2004 (GerES IV pilot study)	3 - 5 years (N = 55)	9.7	65%	7.5	68%	1.4	32%
	6 - 7 years (N = 50)	10.3	59%	8.0	60%	1.3	17%
	8 - 10 years (N = 39)	7.5	73%	5.8	74%	1.3	17%
	11 - 12 years (N = 58)	7.0	61%	5.3	59%	1.3	15%
	13 – 14 years (N = 48)	5.6	69%	4.3	63%	1.3	12%
	Total (3 - 14 years) (N = 254)	8.0	69%	6.2	70%	1.3	20%

Notes: AM = arithmetic mean, RSD = relative standard deviation

DEHP can cross the placenta barrier and distribute into foetal tissues. In addition, there are animal and human data showing that DEHP is transferred to mothers' milk.

Enke et al. (2013) showed that the metabolism in newborns differs distinctly from the one of the general population. In the newborns' urines, the carboxy-metabolites of the long chain phthalates (DEHP, DINP, DIDP) were by far the dominant metabolites with a relative share in the metabolite spectrum up to 6 times higher than in maternal urine.

Conclusion:

Basic toxicokinetic data in humans are available and the relation between intake and urinary excretion is known, however mostly after oral DEHP exposure. The few studies available concerning the inhalation route of exposure are not strictly toxicokinetic studies but case studies of patients or studies in the working environment (EU RAR 2008).

6.2 Toxicity

6.2.1 Human health effects

In humans, no acute toxicity has been observed following ingestion of DEHP (ANSES, 2012b).

The ECHA Restriction Report on four phthalates (DEHP, BBP, DBP, DIBP) published in 2016 states that "a number of epidemiological studies are available showing associations with developmental effects on male reproduction, such as congenital malformations of the male reproductive organs, reduced semen quality, reduced male reproductive hormone levels, and changes in pubertal timing[1]. Epidemiological studies are generally associated with considerable uncertainties and it is therefore difficult to draw exact conclusions on these studies. However, they contribute to the overall evidence that effects seen in rats from DEHP exposure are relevant in humans at exposure levels seen in the population" (ECHA Annex XV Restriction Report, 2016).

- Findings of Franken et al. (2017) demonstrate that in Western-European adolescents (418 14-15-year-old youngsters, recruited as a representative sample of residents of Flanders (Belgium)), asthma diagnosed by a doctor was significantly associated with the urinary sum of the three DEHP metabolites. This finding adds to the increasing literature on DEHP exposure and asthma (e.g. Wang et al., 2015). Also, a highly significant association of phthalate exposure with oxidative stress was observed in the study of Franken et al. for both HMW and LMW phthalates. This finding adds to the increasing evidence that phthalates activate oxidative stress and immune responses. The fact girls seem more responsive than boys may indicate that hormonal systems may be implied, however, the exact biological pathways are not yet well understood. The use of single spot urine samples and the cross-sectional nature of this study as well as life style associated co-exposures warrant cautious interpretation of the results.

[1] For example Aksglaede et al. (2009), Axelsson et al. (2015), Colon et al. (2000), Duty et al. (2004), Hauser et al. (2006), Jensen et al. (2015), Jørgensen et al. (2001), Jørgensen et al. (2002), Jørgensen et al. (2011), Lomenick et al. (2010), Main et al. (2006), Mendiola et al. (2011), Moral et al. (2011), Pan et al. (2006), Swan et al. (2005), Swan et al. (2015), and Wirth et al. (2008).

6.2.2 Animal studies

6.2.2.1 Acute toxicity

Acute toxicity studies indicate a low acute toxicity of DEHP. The oral LD50 is > 20 g/kg bw in rats and > 10 g/kg bw in mice (EU RAR, 2008). An inhalation LC50 of > 10.6 g/m^3 for 4 hours in rats has been reported. Although there are no adequate acute dermal toxicity data, a low acute dermal toxicity is assumed (EU RAR, 2008).

6.2.2.2 Irritation and sensitisation

The substance has no irritant and no sensitising effect (DFG, 2002).

6.2.2.3 Chronic toxicity

In animals, the organs affected by oral exposure to DEHP are mainly the liver and the kidneys (AFSSET, 2010).

In the liver, hepatomegaly (enlargement, elevated weight of the organ), peroxisome proliferation, replicative DNA synthesis, necrosis and liver tumours were observed (DFG, 2002). After experimental exposure of rats to concentrations of ca. 150 mg/kg bw/d, hepatocellular adenomas were seen. In rhesus monkeys subjected to i.v. administration of blood from PVC bags containing ca. 100 to 300 mg DEHP, abnormal histological findings in the liver and a reduced liver function were observed (Tickner et al., 2001). Renal damage may manifest in the form of histological changes in the kidney, formation of renal cysts and tubular atrophy (Tickner et al., 2001).

6.2.2.4 Reprotoxicity and developmental toxicity

The results of testing DEHP for reproductive toxicity in rodents demonstrate effects on reproduction and fertility in both sexes and developmental damage in the offspring. DEHP is classified as toxic to reproduction category 1B (H360FD) according to the CLP regulation.

DEHP produces impairment of the structure and function of the testicles. Reductions in testicle weight, sometimes massive atrophy of the seminal channels and effects on spermatogenesis have been observed. Initial effects in rats have been described to consist in a vacuolisation of Sertoli cells (13 weeks study, no observed adverse effect level (NOAEL): 3.7 mg/kg bw/d) (Poon et al., 1997). Sertoli cells seem to be a critical target site for producing testicular atrophy and effects on the germinal cells (Poon et al., 1997; Arcadi et al., 1998; Li et al., 1998). Animals in the development phase have shown a higher sensitivity than mature ones capable of reproduction (Gray and Butterworth, 1980; Sjöberg et al., 1985, 1986; Wolfe and Layton, 2003). Exposure to doses showing only minor effects in adult animals will result in irreversible damage to the testicles if experienced during the prenatal or lactation phases. Testicle damage with atrophy of the seminal canals is associated with an inhibition of spermatogenesis that may end with aspermatogenesis. These effects are attributable to an anti-androgenic mode of action (EU RAR, 2008). The anti-androgenic effects in male rats have further been demonstrated by Gray et al. (2000), Parks et al. (2000) and Wichert Grande (2006).

In order to determine a TDI for DEHP exposure, studies with evidence of reprotoxicity, which is considered as the critical effect for DEHP, were assessed. The most protective NOAEL refers to testicular effects and was determined by Wolfe and Layton (2003) in Sprague-Dawley rats (n=17 males and females) in a study over three generations. Dietary concentrations of DEHP were 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm of DEHP, corresponding to 0.1, 0.47, 1.4, 4.8, 14, 46 and 359 mg/kg bw/d in F2 animals. From this study where the testicular effects were much more pronounced in the F1 and F2 generations than in the F0 generation, a NOAEL for testicular effects

and developmental toxicity of 4.8 mg/kg bw/d can be derived. This study plays a central role in the EU Risk Assessment Report (EU RAR, 2004) on DEHP and its quality characteristics were described in detail by Blystone et al. (2010).

More recent literature has been evaluated by the Danish Environmental Protection Agency in the context of the Annex XV Restriction Report (2016). According to this report, there is no reason to deviate from the NOAEL of 4.8 mg/kg bw/d which forms the basis for the TDI value derivation.

The study overview of the Annex XV Restriction Report is divided into studies evaluated as critical for NOAEL selection and those ranked as not critical. The text describing the study design of single studies as well as the evaluation is mainly taken over:

- Andrade et al. (2006) performed a crucial study on in utero and lactational exposure of Wistar rats to DEHP at low and high doses by gavage. Pregnant Wistar rats were gavaged from gestational day (GD) 6 to post natal day (PND) 21 with 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135 and 405 mg DEHP/kg bw/d (n=11 to 16 litters per dose). The authors describe effects on daily sperm production from 15 mg/kg bw/day and a low, but increased incidence of cryptorchidism at 5 mg/kg bw/day (one animal per dose group 5, one per dose group 135, and one per dose group 405 mg/kg bw/d of DEHP had undescended testes). Effects on hormone levels were seen at low doses, but did not exhibit dose-response relationships. Despite the low number of affected animals, a NOAEL of 1.215 mg/kg bw/d based on cryptorchidism was concluded, as cryptorchidism is less common in Wistar rats compared to other rat strains. The weights of testes and epididymides were not affected in any treatment group, whereas the weight of seminal vesicle plus coagulating glands was significantly reduced at the highest dose group. Ventral prostate weight was also reduced at this dose, although not statistically significant. The Annex XV Restriction Report however is concluding not to take the results into consideration due to the observation of cryptorchidism in only few animals.
- Christiansen et al. (2010) found in another relevant study a reduced anogenital distance and increased nipple retention in male rats, perinatally (GD 7 to PND 16) exposed by gavage to 10 mg DEHP/kg bw/d and above, with a NOAEL of 3 mg/kg bw/d. Part 1 of the study included 16 mated dams in the control group and 8 mated dams per group in six exposure groups receiving either 10, 30, 100, 300, 600 or 900 mg/kg bw/d of DEHP. Part 2 of the study included 16 mated dams in the control group, 16 mated dams receiving 3 mg/kg bw/d of DEHP, and 8 mated dams per group receiving either 10, 30, or 100 mg/kg bw/d of DEHP. A number of reproductive endpoints were investigated postnatally and at PND 16. In a combined evaluation of the two parts of the study, the anogenital distance was significantly decreased and the nipple retention significantly increased at 10 mg/kg bw/d of DEHP with a NOAEL of 3 mg/kg bw/d. At the same dose (10 mg/kg bw/d) and above, decreased weights of ventral prostate and levator ani/bulbocavernosus muscle were observed, though these effects did not show clear dose-response relationships. Additionally, mild dysgenesis of external genitals was observed at all doses and also in one of the male control rats. When the two study parts were combined the incidences of mild dysgenesis were significantly increased at all dose levels except 30 mg/kg bw/d ($p = 0.075$ for litter incidences). There is thus not a clear dose-response relationship in the percentage of affected males. Although DEHP appears to affect external genitals at the lowest dose level, the effect on reduced anogenital distance at 10 mg/kg bw/d may be considered a more robust LOAEL for DEHP. Indeed, the authors concluded that the results are consistent with the NOAEL of 4.8 mg/kg bw/d (ECHA Annex XV Restriction Report, 2016) used for TDI derivation.

The following further studies all included doses of at least 10 mg/kg bw/day and were therefore not taken into consideration for a NOAEL determination:

- Howdeshell et al. (2008) found that DEHP decreased fetal testosterone production in rats at doses from 300 mg/kg bw/d (NOAEL 100 mg/kg bw/d). In this study, pregnant Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/d of DEHP from GD 8 to 18 by gavage in corn oil (n=5 to 8 dams per group). Testicular testosterone production *ex vivo* was assessed by incubation of testes of 18 days old foetuses for 3 hours and testosterone measurement in the media. Because of the dose amount, the results are not relevant for an update of the TDI.
- Hannas et al. (2011) compared the exposure of SD and Wistar rats to 0, 100, 300, 500, 625, 750 or 875 mg DEHP/kg bw/d from GD 14 to 18. Testicular testosterone production ex vivo was assessed by incubation of testes of 18 days old foetuses for 3 hours and testosterone measurement in the media. A decrease in testosterone production was seen at 300 mg/kg bw/d and above with a NOAEL of 100 mg/kg bw/d.
- A study by Jones et al. (2014) found permanently reduced mRNA levels of the gene Hsd3b in adult testes following in utero exposure to 10 mg/kg bw/d of DEHP. Hsd3b codes for the steroidogenic enzyme 3beta hydroxysteroid dehydrogenase (3βHSD), and reduced levels of this protein were seen in Leydig cells (LCs) of these DEHP exposed adult animals. This points out permanent effects of foetal exposure to low doses of DEHP.
- For the sake of completeness: Tanaka et al. (2003), Tanaka et al. (2005), Gray et al. (2009), Wilson et al. (2007), Howdeshell et al. (2007), Shirota et al. (2005), Borch et al. (2005), Tomonari et al. (2006), Cammack et al. (2003), Wilson et al. (2004), Liu et al. (2008), Noriega et al. (2009).

The following recent studies examined doses at or below 10 mg/kg bw/d, but are not considered critical:

- A study by Zhang et al. (2014) examined the ovarian effects of a very low dose of DEHP (40 ug/kg bw/d by gavage in DMSO and water from GD 1 to 19). Reduced primordial follicle number and increased secondary follicle number was observed in F1 offspring at PND 21. Also, the second-generation females (F2) had reduced numbers of primordial follicles and increased numbers of secondary follicles at PND 21, indicating accelerated follicle recruitment in two generations. These findings were associated with changes in gene methylation and expression of several genes in F1 ovaries. These findings may indicate an effect at a lower dose than the NOAEL of 4.8 mg/kg bw/d selected for the TDI determination, but only one dose group is included and the number of dams/litters per endpoint is not clearly presented.
- Pocar et al. (2017) showed that gestational and lactational exposure to DEHP at environmental doses promotes transgenerational effects on reproductive health in female offspring, as adults, over three generations in the mouse. Gestating F0 mouse dams were exposed to 0, 0.05, and 5 mg/kg/d DEHP in the diet from GD 0.5 until the end of lactation. The incidence of adult-onset disease in reproductive function was recorded in F1, F2 and F3 female offspring. In adult F1 females, DEHP exposure induced reproductive adverse effects with: i) altered ovarian follicular dynamics with reduced primordial follicular reserve and a larger growing pre-antral follicle population, suggesting accelerated follicular recruitment; ii) reduced oocyte quality and embryonic developmental competence; iii) dysregulation of the expression profile of a panel of selected ovarian and pre-implantation embryonic genes. F2 and F3 female offspring displayed the same altered reproductive morphological phenotype and gene expression profiles as F1, thus showing transgenerational transmission of reproductive adverse effects along the female lineage. These findings indicate that in mice exposure to DEHP at doses relevant to human exposure during gonadal sex determination significantly perturbs the reproductive indices of female adult offspring and subsequent generations.

- In a number of in-vitro tests (e.g. Harris, 1997), DEHP has also shown a weak oestrogenic effect. Under *in-vivo* conditions, an influence of DEHP (2 g/kg) on the oestrus cycle of adult female rats has been described by Lovekamp-Swan and Davis (2003).

In 2017, the European Commission adopted the decision on identification of DEHP as SVHC, due to its endocrine disrupting properties[2]. The Support document to the Opinion of the MSC for identification of DEHP as SVHC indicates that "when available information from toxicological and ecotoxicological studies are combined, DEHP can be considered an endocrine disruptor (ED) for both the environment and for human health as it fulfils the WHO/IPCS definition of an ED, and the recommendations from the European Commission's EDs Expert Advisory Group for a substance to be identified as an ED" (ECHA, 2014). Also, "DEHP should be identified as a SVHC in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is a substance with endocrine disrupting properties for which there is scientific evidence of probable serious effects on human health and the environment which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57. DEHP has been shown to adversely affect the endocrine system of mammals primarily through *in vivo* findings on reduced fetal testosterone. These findings are further substantiated by mechanistic findings, also *in vivo*, of down-regulation of genes in the steroidogenic biosynthesis pathway. The spectrum of adverse effects observed in rats includes increased nipple retention, decreased anogenital distance, genital malformations, reduced number of spermatocytes and testicular changes including multinucleated gonocytes, tubular atrophy and LC hyperplasia."

Determination of an occupational limit or guidance value, be it an external one or a biomonitoring limit or guidance value requires a key study in which the exposure protocol would be appropriate with an occupational exposure scenario (e.g. in terms of window of exposure, exposure duration, route of exposure). Therefore, the multigenerational oral study on rats conducted by Wolfe et al., 2003 used as the key study for the TDI derivation was not chosen for the OEL calculation, as its protocol of exposure was not appropriate for occupational exposure (continuous exposure starting at the juvenile stage of the F1 and F2 rat generations, in which the more significant testicular effects were observed).

The chosen key study for the derivation of an HBM $HBGV_{worker}$ is a chronic oral (diet) toxicity study in rats involving male and female groups, performed by David et al. (2000) (AFSSET, 2010). Dose levels tested were 0; 100; 500; 2500 and 12,500 ppm for 104 weeks. These doses correspond to 0; 5.8; 28.9; 146.6 and 789.0 mg/kg bw/d for males and 0; 7.3; 36.1; 181.7 and 938.5 mg/kg bw/d for females. There was no significant change in the weight of the animals at the end of the study regardless of the exposure dose. Bilateral aspermatogenesis was directly observed in groups exposed to 500, 2500 and 12,500 ppm, defining a NOAEL at 100 ppm, or 5.8 mg/kg bw/d, with a significant difference compared to the study's control group.

6.2.2.5 Genotoxicity and mutagenicity

In the majority of tests for genotoxicity and mutagenicity, DEHP and the metabolites MEHP and 2-ethylhexanol proved to be negative. An exception was seen regarding the results of tests which also considered the effects of non-genotoxic chemicals such as spindle poisons, tumour promotors, or peroxisome proliferators. Thus, in a variety of cell cultures and by *in-vivo* testing, cell-transforming properties, an induction of aneuploidy and an induction of cell proliferation by DEHP could be demonstrated, in part also for its metabolites (Kommission Human-Biomonitoring, 2005a). Based on all the results, both negative and positive, DEHP and its major metabolites are considered to be non-

[2] https://echa.europa.eu/documents/10162/812c42cd-0304-74d8-ff84-2853b4c83e62

mutagenic substances. The data available on genotoxicity do not suggest a classification of DEHP according to the criteria for classification and labelling of dangerous substances (Annex IV to Commission Directive 93/21/EEC of 27 April 1993 adapting to technical progress for the 18th time, Council Directive 67/548/EEC) (EU RAR 2008).

6.2.2.6 Carcinogenicity

According to an assessment by the IARC in 2013, there is sufficient evidence in experimental animals for the carcinogenicity of DEHP, while no data were available to the Working Group on carcinogenic effects in humans. For this reason, DEHP has been considered as „possibly carcinogenic to humans“, Group 2B (IARC, 2013).

The Senate Commission for the investigation of health hazards of chemical compounds in the work area (MAK Commission) of the German Research Association (Deutsche Forschungsgemeinschaft – DFG) has classified DEHP regarding its carcinogenicity as coming under Category 4, i.e. as a carcinogenic substance for which genotoxic effects do not play a role, or only a minor one and for which no essential contribution towards a reduction of the cancer risk for humans by adherence to the MAK value can be expected (DFG, 2002).

EU RAR (2008) states that the results of the studies clearly show DEHP to be carcinogenic in rats and mice (a statistically significant increase in the incidence of liver tumours with a dose-response relationship in rats and mice of both sexes, and an increase in the incidence of LC tumours and Mononuclear Cell Leukaemia (MCL) in male rats). However, there is a plausible mechanism for the peroxisome proliferators (PPs)-induced hepatocarcinogenicity in rodents (activation of peroxisome proliferator-activated receptor α (PPARα)) and there is evidence showing that humans are less sensitive to the hepatotoxic effects of PPs by the suggested mechanism. Therefore, the relevance for humans of the liver tumours in rodents induced by DEHP, a weak PPs, is regarded to be negligible. Also, the relevance of the DEHP-induced MCL in F344 rats is questionable. On the other hand, the induction of LC tumours in rats exposed for DEHP should be regarded as relevant to humans and, therefore, a careful evaluation of the orignal data of Berger (1995) is necessary before concluding the possible carcinogenic risk of DEHP. Based on the overall evaluation of the available data, no classification for human carcinogenicity has been proposed.

7 Exposure

As the phthalate plasticizers are not chemically bound to PVC, they can leach or migrate into foodstuff and other materials, or evaporate into indoor air and atmosphere. The occurrence of phthalates in consumer products can result in human exposure through direct contact and use, indirectly through leaching into other products, or general environmental contamination. Humans are possibly exposed through ingestion, inhalation, and dermal exposure during their whole lifetime, including intrauterine development (Heudorf et al., 2007).

The food chain has been identified as the main source of exposure in adults. In addition, house dust and the so-called mouthing behaviour was described as an important path for the uptake of DEHP in children. However, because of considerable uncertainties in estimating the exposure, a systematic and structured study on the basis of national data has been performed to describe the exposure in the German population (Heinemeyer et al., 2012, see table 3). In particular for the food consumption, data were available from the National Food Consumption Survey. With the ESKIMO study also, there were current data for school children and adolescents.

Martine et al. (2013) investigated the occurrence of six phthalates in drinking water (both tap and bottled), in common foodstuff and in ambient air (both indoor and outdoor) in the urban centre of Paris in order to document the everyday exposure of humans via digestive and respiratory tracts. The estimations of the DEHP daily intakes were 1.05; 1,454 and 3.13 ng/kg bw/day through water, food and air respectively (total of 1.458 µg/kg bw/day).

Biodegradation of DEHP, which under aerobic and humid conditions in the environment is a rapid process ($t_{1/2}$ = 2 – 4 weeks) (Wams, 1987), will be clearly delayed under indoor conditions where it is bound to dust. House dust will also contain abrased particles from DEHP-containing objects.

Given the uncertainties in the exposure estimates for articles, food etc., the ECHA Risk Assessment Committee RAC (2012) considers the data from biomonitoring studies to be important for a risk assessment. This could be done by deducing the external exposure to DEHP from its metabolite levels in urine, and relating it to an accepted TDI value respectively to other acceptable exposure levels or by comparing urine levels of selected metabolites directly with a HBM health-based guidance value. An overview of relevant European biomonitoring studies with urine sampling years from 2001 on and with published intake estimates (µg/kg bw/day) of DEHP is given in table B18 of the annexes of the Annex XV Restriction Report.

Table 3: Absolute shares of different exposure paths for DEHP [µg/kg bw/d] for children, adolescents and adults (Heinemeyer et al., 2012)

	Source	„Realistic“ estimation [µg/kg bw/d]			Conservative estimation	Uncertainty
		children	adolescents	adults	single events	
Oral (incl. mouthing)	food	6.5-15.1	6-25	10.1-21.3	30	low
	toys	0.6-7.0	x	x	10	low
	other consumer products	0.3-3.8	x	x	6	low
	house dust	2.3-4.7	x	x	8	medium
Inhalative	airborne particulate matter	0.08-0.11	0.1	0.05-0.08	0.3	low
	gaseous, here: car interior	0.2	0.2	0.1	0.2	medium
Dermal	textiles	0.4	0.4	0.3	0.7	high
	cosmetics	0.006-2.4	0.006-2.4	0.006-2.4	5	high
	products made of plastics, here shoes	4.6-10.3	3.4-9.2	2.4-6.7	20-38	high
	Total exposure	15-44	10-37	13-31	–	

7.1 General population exposure

One of the more recent studies is a study among the Austrian population aged 6-15 and 18-81 years. 14 urinary phthalate metabolites of 10 parent phthalates were analysed by HPLC-MS/MS in order to assess phthalate exposure. In the total study population, ranges of urinary DEHP metabolite concentrations were n.d.-20 µg/L (median n.d.) for mono-(2-ethylhexyl) phthalate (MEHP), n.d.-80 µg/L (median 2.6 µg/L) for mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), n.d.-57 µg/L (median 1.9 µg/L) for mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), n.d.-219 µg/L (median 11 µg/L) for mono-(5-carboxy-2-ethylpentyl) phthalate (5cx-MEPP). Generally, children exhibited higher levels of exposure. Individual daily intakes were estimated based on urinary creatinine and urinary volume excretion and were then compared to acceptable exposure levels, leading to the identification of exceedances of mainly the TDI, especially among children (Hartmann et al., 2015).

Newer and therefore not listed in the table is the publication of Franken et al. (2017) describing and evaluating the urinary levels of DEHP metabolites of adolescents investigated during the time periods 2008–2009 (FLEHS II) and 2013 (FLEHS III). An important finding is that the geometric mean (GM) of the sum of determined DEHP metabolites was reduced by 69% over the 5 years time span between the two studies.

Also relevant is the time trend analysis of Koch et al. (2017). By using the German Environmental Specimen Bank, which continuously collects 24-h urine samples since the early 1980s in Germany,

Koch et al. (2017) analysed 300 urine samples from the years 2007 to 2015 for 21 phthalate metabolites (representing exposure to 11 parent phthalates). The data were then combined with the data of two previous retrospective measurement campaigns (1988 to 2003 and 2002 to 2008). The combined dataset comprises 1162 24-h urine data of samples spanning the years 1988 to 2015. With this detailed set of human biomonitoring data, the time course of phthalate exposure in Germany over a time frame of 27 years could be described. For the metabolites of DEHP, a roughly ten-fold decline in median metabolite levels from their peak levels in the late 1980s/early 1990s compared to most recent levels from 2015 could be observed. In a considerable number of samples collected before 2002 health based guidance values (BE and HBM-I) have been exceeded for DEHP but also in recent samples some individual exceedances still can be observed (BE value DEHP 1.0%).

A comparison across Europe was intended with the DEMOCOPHES project where spot urine samples from mother-child pairs in 16 Member States and Switzerland were collected and analysed. The sampling period was from September 2011 until February 2012 and the majority of the samples collected were morning samples (99.2% in children and 98.8% in mothers). Children probed were 6-11 years old. The median age of the mothers was 39 years with a 25th percentile of 35 years and a 75th percentile of 42 years (FPS 2013). The results of the DEMOCOPHES survey regarding DEHP are summarised in table 4.

Table 4: Results of the DEMOCOPHES survey regarding urinary DEHP metabolites (FPS, 2013)

	Children		Mothers	
Biomarker	Mean	P90	Mean	P90
Urinary DEHP metabolites (µg/L) (Sum of MEHP + 5OH-MEHP + 5oxo-MEHP)	47.6	141.0	29.2	93.0

According to Den Hond et al. (2015), the highest geometric mean level of DEHP metabolites in urine of children was found in Slovakia and the lowest level in Luxembourg. In general, urinary DEHP metabolites levels tended to be higher in Eastern Europe compared to Western Europe. Comparison of exposure to DEHP with average gross domestic product per capita shows that exposure to restricted phthalates is negatively correlated with affluence (WHO, 2015). This correlation might be due to a consumer preference towards cheaper imported plastic materials in countries with lower gross domestic product per capita.

A comparison of German DEMOCOPHES data for children (6-11 years) in 2011/12 with the weighted average of creatinine corrected intake estimates for the age groups 5-6, 7-8, and 9-11 years from 2001-2002 revealed a decline of DEHP (metabolite) levels of about 50% over this 10 years. Concerning adults in Germany it was found, that there is a decline of 40-50% in DEHP (metabolite) levels from 2001/2003 to 2011. DEMOCOPHES data intake estimates from Denmark for children compared with data from 2007 (children aged 6-10 years) indicated that DEHP levels in urine decreased about 50-70% in the respective four years. For adults a decline in exposure of 30% was assumed (FPS 2013).

7.2 Occupational exposure

Occupational exposure to DEHP, mainly through inhalation but also via the dermal route, occurs in the production of DEHP, industrial use of DEHP as an additive, and at industrial end-use of semi-manufactured products and end-products containing DEHP (EU RAR, 2008).

Occupational data on biological monitoring of DEHP exposure are currently scarce. Only the study by Dirven et al. in the Netherlands reports both concentrations of urinary metabolites of DEHP and atmospheric concentrations of DEHP (individual samples) in several sectors of the PVC industry (Dirven et al., 1993b). Other studies in the workplaces (Gaudin et al. in France and Hines et al. in the United States) only report the urinary concentrations of biomarkers (Gaudin et al., 2008 and 2011; Hines et al., 2009). As in the environmental field, workers primarily excrete the secondary metabolites, of which the most abundant is 5cx-MEPP (Preuss et al., 2005). The table 5 below only lists biomonitoring studies that used secondary oxidative metabolites in addition to MEHP, such as 5cx-MEPP, to assess DEHP exposure.

Table 5: Urinary concentrations of 5cx-MEPP in various sectors of industrial activity (AFSSET, 2010; ANSES, 2012a)

5cx-MEPP (urinary) at the end of the work shift		
Sector of activity	**Biomarker concentration** Median - maximum value in µg/L and [µg/g of creatinine]	**References**
Footwear	124.7 [91.6] - NR	Dirven et al., 1993b
Cables	48.4 [35.6] - NR	
DEHP manufacture	Not informed	Hines et al., 2009
PVC film	283.0 [142.0] - 2030 [625]	
Automotive filters	Not informed	
PVC compounding	391.0 [200.0] - 1080 [444]	
Tubing	51.4 [31.0] - 497 [53]	
Footwear	132.0 [69.7] - 3520 [1180]	
DEHP manufacture	18.8 [14.3] - 219 [122]	Gaudin et al., 2011
Plastisol coatings	103.7 [63.0] - 961 [533]	
PVC granules 1	166.4 [105.1] - 1320 [372]	
PVC granules 2	57.6 [26.9] - 488 [579]	
Moulding polymers	34.3 [27.7] - 529 [177]	
Wall coverings	134.6 [78.6] - 1410 [600]	

8 HBM parameters

8.1 Choice of biomarkers

8.1.1 For general population HBM measures

Given DEHP's short half-life in blood (estimated at 30 minutes), it is difficult to use this biomarker for biological monitoring.

Use of the DEHP metabolites 2-ethylhexanoic acid, 2-ethyl-3-hydroxyhexanoic acid, and 2-ethyl-3-oxohexanoic acid as biomarkers for DEHP exposure is not suitable due to their lack of specificity. Indeed, ethylhexanol and its oxidation products become released from numerous other chemical compounds such as di(2-ethylhexyl) adipate (another plasticiser), or tri(2-ethylhexyl) phosphate (a widely used flame retardant). In addition, ethylhexanol by itself is used in numerous applications like wood preservatives, as a solvent in paint manufacture etc.

The specific DEHP biomarkers identified in the scientific literature are the following (abbreviations given in brackets):

- Mono(2-ethylhexyl) phthalate, urine (MEHP)
- Mono(2-ethyl-5-hydroxyhexyl) phthalate, urine (5OH-MEHP)
- Mono(2-ethyl-5-oxo-hexyl) phthalate, urine (5oxo-MEHP)
- Mono(5-carboxy-2-ethylpentyl) phthalate, urine (5cx-MEPP)
- Mono[(2-carboxymethyl) hexyl] phthalate, urine (2cx-MMHP)

Measuring MEHP alone can lead to overestimation (contamination) or an underestimation (low excretion) of exposure. This is the reason why the measurement of this metabolite has a greater inter-individual variability (RSD of 31% for 24h and 48h urine collection) than the three secondary metabolites, 5OH-MEHP, 5cx-MEPP and 5oxo-MEHP (RSD of 19 to 27% for 24h urine collection and 20% to 25% for 48h urine collection) (Anderson et al., 2011). Therefore, MEHP was not selected for the biological monitoring.

Also for toxicological reasons it is not recommended to measure DEHP or MEHP. Regnier et al. (2004) conducted in-vitro studies regarding the embryotoxicity of DEHP, MEHP and a number of oxidative metabolites in embryonal rats. They examined effects on growth, development and morphology and found the oxidised metabolites, in particular 5OH-MEHP to be the ultimate species responsible for reprotoxicity. If referred to morphological changes in the embryo, 5OH-MEHP proved to be 100 times more toxic than MEHP. For the parameters examined, all oxidative secondary metabolites were clearly more toxic than DEHP, MEHP, 2-EH or 2-EHA. The study of Stroheker et al. (2005) confirmed these results. The two secondary metabolites, 5OH-MEHP and 5oxo-MEHP, rather than DEHP or MEHP, exhibited anti-androgenic activity in an *in vitro* transcriptional assay.

Additionally, compared to MEHP, the secondary metabolites of DEHP are excreted in urine in much larger concentrations and the half-life periods for their excretion are obviously longer.

With a half-life of 24 hours, urinary 2cx-MMHP seems relevant as a biological indicator of exposure. However, very few data on it are available. Urinary 2cx-MMHP was therefore not selected as a biological indicator of exposure to DEHP.

As suitable biomarkers the following are left: 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP. For reasons of preciseness, 2 metabolites should be considered for HBM HBGV derivation for the general population, and two alternatives are given to perform the comparisons with the respective analytical

results of biomonitoring programs. For note, individually urinary DEHP biomarkers were considered in the fourth American National Health and Nutrition Examination Survey (NHANES), which are: MEHP, 5OH-MEHP, 5cx-MEPP and 5oxo-MEHP (CDC, 2017).

8.1.2 For occupational HBM measures

No studies in workplaces using DEHP or MEHP in blood were identified; neither were studies measuring the urinary 2cx-MMHP biomarker for workers (AFSSET, 2010).

Urinary MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP are documented in some studies in workplaces. These biomarkers are specific to DEHP exposure, have half-lives enabling samples to be taken at the end of the work shift and thus can be selected for the biological monitoring of occupational exposure to DEHP (ANSES, 2012a).

Given the greater inter-individual variability in the measurement of MEHP, as indicated above, this biomarker of exposure was not selected.

5cx-MEPP was selected as biomarker of occupational exposure for DEHP, according to a French field study, establishing a relationship between atmospheric DEHP concentration and urinary 5OH-MEHP, 5oxo-MEHP or 5cx-MEPP: a median atmospheric DEHP concentration of 0.04 mg/m^3 (0.002 - 1.13) was measured and a correlation factor of 0.41 between atmospheric DEHP concentration and urinary 5cx-MEPP was determined (Gaudin et al., unpublished data, ANSES, 2012a).

8.2 Analytical methods

Analytical methods used for MEHP and other, secondary metabolites in urine include both gas chromatography-mass spectrometry (Dirven et al., 1993a) and high-performance liquid chromatography-mass spectrometry (Blount et al., 2000a; Koch et al., 2003a; Koch et al., 2003b; Preuss et al., 2005; Anderson et al., 2011; Kasper-Sonnenberg et al., 2012; Kasper-Sonnenberg et al., 2014; Koch et al., 2017).

However, it has to be emphasised that only the secondary DEHP metabolites in urine (and in blood) can be measured reliably since MEHP is readily formed from DEHP already during the pre-analytic phase or under certain ambient conditions, as a result of various hydrolytic processes. This does not apply to the secondary metabolites.

Conclusion:

Established analytical procedures are available for an easily accessible human biological matrix.

9 Derivation of HBM HBGVs for DEHP

9.1 Summary

The existing database on biomonitoring data on DEHP does not allow for the derivation of HBM health-based guidance values based on a relationship between human internal concentrations (parent compound or biomarker(s)) and health effects. According to the ECHA Annex XV Restriction Report (2016), epidemiological studies are generally associated with considerable uncertainties and it is therefore difficult to draw exact conclusions on these studies. However, they contribute to the overall evidence that effects seen in rats from DEHP exposure are relevant in humans at exposure levels seen in the population.

Thus, a TDI value of 0.05 mg DEHP/kg bw/d, based on a NOAEL identified from an animal study is used as a basis for the HBM HBGVs for the general population.

Regarding the derivation of the HBM $HBGV_{Worker}$, calculating the urinary 5cx-MEPP concentration at the French recommended OEL of 0.8 mg/m^3 DEHP based on a NOAEL of 5.8 mg/kg bw/d identified in the study from David et al., 2000 is not possible, because no solid evidence of a relationship between atmospheric concentration of DEHP and urinary concentration of the biomarker 5cx-MEPP is available (ANSES, 2012a). Therefore, the derivation method for the HBM $HBGV_{Worker}$ is based on calculation of the urinary 5cx-MEPP biomarker equivalent at the NOAEL of 5.8 mg/kg bw/d identified in the study from David et al., 2000.

Factsheets summarising the data which are relevant to perform the derivation of HBM HBGVs, as described here below, are provided in the section 13 of this report: section 13.1 summarises the information used for the general population HBM HBGV derivation, as section 13.2 summarises the information used for the workers HBM HBGV derivation.

9.2 Derivation of HBM HBGVs for the general population based on the TDI

9.2.1 TDI/ADI values

Based on a NOAEL of 4.8 mg DEHP/kg bw/d determined by Wolfe and Layton (2003) in a multi-generation study in Sprague-Dawley rats, a TDI value of 0.05 mg DEHP/kg bw/d has been established by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung – BfR, 2005) in accordance with the European Food Safety Authority (EFSA, 2005). The key study focused especially on reproductive toxicity resulting in a reduced number of offspring, lower birth weights, genital malformation in male and female offspring, and infertility inter alia. The NOAEL of 4.8 mg/kg bw/day refers to testicular effects (germ cell depletion, reduced testis weight) in male offspring. The effects can be attributed to an anti-androgenic mode of action. This study was also used as a starting point for risk characterisation in the European Chemicals Bureau Risk Assessment Report (2004, 2008), by ECHAs RAC (ECHA 2012, 2013c), and in the Annex XV Restriction Report prepared by ECHA and Denmark (2016). RAC considers the NOAEL of 4.8 mg/kg bw/day to be “conservative”, given the low incidences of the reported effects at the LOAEL. In deriving an oral Derived No-Effect Level (DNEL) for DEHP, which is defined as the level of a substance above which a human should not be exposed, RAC concluded that assessment factors (AFs) needed to be applied for intra- and interspecies differences. For intraspecies differences, a factor of 10 (default) was applied, as well as a factor of 2.5 for interspecies differences and an allometric scaling factor of 4. Other AFs (e.g. for different duration/exposure time) were not regarded necessary. The internal DNEL of 0.034 mg/kg bw/day was calculated after application of these mentioned AFs and a correction factor of 0.7 for the oral absorption fraction in rats to the NOAEL of 4.8 mg/kg bw/day (ECHA, 2016).

Conclusion:

Generally accepted TDI/ADI values are available at the European level (EFSA, 2005). However, after revisiting the data, RAC concluded in its opinion of 2013 (ECHA, 2013b) that humans appear to absorb DEHP at 100 % instead of the assumed 70 %. Because the extent of oral absorption in rats is estimated to be around 60-70%, a correction of the NOAEL to 3.36 mg/kg bw/d had to be performed and might also be considered when revising the TDI value.

The proposal for restriction notes that aside from the study by Wolfe and Layton (2003) also the studies by Christiansen et al. (2010) and Andrade et al. (2006) might be critical for the selection of the starting point for DNEL derivation, but there are comprehensible reasons for staying with the TDI (see above 6.2.2.4).

9.2.2 HBM HBGVs$_{GenPop}$ for the excretion of DEHP metabolites in urine referring to the sum of the urinary metabolites 5-oxo-MEHP and 5-OH-MEHP

It is assumed that the general population is virtually continuously exposed to DEHP and a steady state of the metabolites is reached. A share of 26.9 % of the DEHP dose absorbed by the oral route is excreted in 48 h in urine in the form of the two metabolites 5-oxo-MEHP and 5-OH-MEHP (Anderson et al., 2011), giving the fractional urinary excretion (F_{ue}) value. The 48 h values for excretion are preferred for determining the F_{ue} instead of the 24 h values because within this time frame a nearly complete excretion is covered.

As a rule, concentrations are stated based on mass. This is why the values have to be converted according to molecular weights (MWs). The MWs are 390.56 g/mol for DEHP, 292.33 g/mol for 5-oxo-MEHP, and 294.34 g/mol for 5-OH-MEHP. For conversion to weight proportions, the ratio between the MWs of the metabolites and DEHP must be taken into account. For reasons of simplification, a mean MW of 293.34 g/mol for the metabolites can be used, thus the MW ratio is 0.75.

For the derivation of the HBM HBGVs$_{GenPop}$, it is assumed in a first step that an adult's daily intake of DEHP corresponds to the TDI value (50 µg/kg bw/d).

Ideally, the HBM guidance value should refer to a complete 24-hour urine sample, stating the quantity of a substance in µg/day. For reasons of practicability, the excretion is referred to a body weight-related urine excretion of 30 mL/kg bw/d for children and 20 mL/kg bw/d for adults (Wissenschaftliche Tabellen Geigy, 1977).

The calculated value corresponds to the German HBM-I value, because it is derived from a TDI value, i.e. no risk of adverse health effects from concentrations (at and) below this value, according to current assessment.

HBM HBGV$_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5\text{-}OH\text{-}MEHP)}$, adults

$$= [\text{TDI} \cdot \frac{\text{averaged molecular weight of metabolites}}{\text{molecular weight of DEHP}} \cdot F_{ue}] \text{ / amount of urine}$$

$$= 50\ \mu g/kg\ bw/d \cdot \frac{293.34}{390.56} \cdot 0.269\ /0.02\ L/kg/day$$

= 50 · 0.751 · 0.269 / 0.02 = 505.1 µg/L, rounded to 500 µg/L

HBM HBGV$_{GenPop\ (5\text{-}oxo\text{-}MEHP\ and\ 5\text{-}OH\text{-}MEHP)}$, children

$$= 50\ \mu g/kg\ bw/d \cdot \frac{293.34}{390.56} \cdot 0.269\ /0.03\ L/kg/day$$

= 50 · 0.751 · 0.269 / 0.03 = 336.73 µg/L, rounded to 340 µg/L

9.2.3 HBM HBGVs for the excretion of DEHP metabolites in urine referring to the sum of the urinary metabolites 5cx-MEPP and 5-OH-MEHP

A share of 29,5 % of the DEHP dose absorbed by the oral route is excreted in 48 h in urine in form of these two metabolites (Anderson et al., 2011).

The MWs are: 308.33 g/mol for 5cx-MEPP and 294.34 g/mol for 5-OH-MEHP. Thus, the averaged MW is: 301.34 g/mol.

HBM HBGV$_{GenPop\ (5cx\text{-}MEPP\ and\ 5\text{-}OH\text{-}MEHP)}$, adults

$= 50 \cdot 0.7716 \cdot 0.295 \,/\, 0.02 = 569.06\ \mu g/L$, rounded to $570\ \mu g/L$

HBM HBGV$_{GenPop\ (5cx\text{-}MEPP\ and\ 5\text{-}OH\text{-}MEHP)}$, children

$= 50 \cdot 0.7716 \cdot 0.295 \,/\, 0.03 = 379.37\ \mu g/L$, rounded to $380\ \mu g/L$

Table 6: Derivation of HBM HBGV$_{GenPop}$ for the sum of two DEHP metabolites

Metabolites, Population	TDI (µg/kg bw/d)	Urine volume[a] (L/kg bw/d)	HBM HBGV$_{GenPop}$ (rounded) (µg/L urine)[b]
5-oxo-MEHP and 5-OH-MEHP, children (6 to 13 years of age)	50	0.03	340
5-oxo-MEHP and 5-OH-MEHP, adults incl. women of child-bearing age	50	0.02	500
5cx-MEPP and 5-OH-MEHP, children (6 to 13 years of age)	50	0.03	380
5cx-MEPP and 5-OH-MEHP, adults incl. women of child-bearing age	50	0.02	570
[a] Urine volume has to be recalculated during the course of HBM4EU, for this reason the HBM HBGVs$_{GenPop}$ should be regarded as preliminary **[b] Calculation method: (TDI x F_{ue} x ratio of MWs) / urine volume**			

Deriving a HBM HBGV for the subgroup of children under 6 years of age is not appropriate, considering the lack of relevant toxikokinetic data and the knowledge gaps regarding DEHP exposure.

For reasons of preciseness, HBM HBGVs derived on the basis of two biomarkers are preferred. Nevertheless values for single biomarkers of exposure are given for information and for allowing comparison to values measured during various studies

Table 7: HBM $HBGV_{GenPop}$ calculation with single biomarker of exposure

Metabolite Population	Biomarker MW	Ratio of Biomarker MW over DEHP MW	F_{ue} (at 48 h) from Anderson et al., 2011	TDI (µg/kg bw/d)	Urine volume (L/kg bw/d)	HBM $HBGV_{GenPop}$ (µg/L urine)[a]
5-OH-MEHP **Adults**	294.34	0.754	0.156	50	0.02	294.1
5-oxo-MEHP **Adults**	292.33	0.748	0.113	50	0.02	211.3
5cx-MEPP **Adults**	308.33	0.789	0.139	50	0.02	274.2
[a] Calculation method: (TDI x F_{ue} x ratio of MWs) / urine volume						

9.3 Derivation of HBM $HBGV_{worker}$ based on an animal POD

9.3.1 Choice of a POD value from a key study

As previously mentioned, a POD value of 5.8 mg/kg bw/d (NOAEL) for a critical effect of DEHP corresponding to the onset of aspermatogenesis was determined in the study conducted by David et al., 2000. This study was performed in adult rats and can therefore potentially be transposed to workers. Moreover, this was a long-term study (2 years in rats), which virtually corresponds to lifetime exposure in accordance with an occupational exposure scenario (AFSSET, 2010).

The critical effect chosen, aspermatogenesis, is consistent in transposition from animals to humans (AFSSET, 2010).

9.3.2 Calculation of HBM $HBGV_{Worker}$

The MAK Commission concluded on DEHP that it was not possible to extrapolate the results of oral studies in rodents to inhalation exposure in humans (Hartwig, 2016). ANSES confirms that extrapolation of dose-response data from one exposure route to another is accompanied by many uncertainties, the main limitation beeing the pharmacokinetic differences between the oral and inhalation route (Hartwig, 2016), leading to an inaccurate dosimetry to normalise the internal dose.

Thus, in the current state of knowledge, route-to-route extrapolation for DEHP is not recommended for the derivation of an occupational health reference value.

9.3.2.1 Interspecies extrapolation

Extrapolation of the NOAEL of 5.8 mg/kg bw/d to humans taking into account the same route of exposure (oral) and thereby determining a human equivalent dose (HED) from the dose determined in rats is performed by:

Application of an allometric adjustment, according to the U.S. EPA recommendations (U.S. EPA, 2006):

Oral adjusted NOAEL = animal dose x (animal weight/human bodyweight)$^{1/4}$

A mean animal weight of 322 g was determined in the key study of David et al., 2000 (mean of all animal weights at the end of the study, no observed significant change in the weight of animals regardless of the exposure dose). The human bodyweight was set at 70 kg. This leads to an oral NOAEL of 1.5 mg/kg bw/d adjusted to humans regarding the toxicokinetic interspecies differences.

b) Application of an assessment factor of 2.5 to the oral adjusted NOAEL of 1.5 mg/kg bw/d, accounting for the interspecies toxicodynamic differences. This leads to an oral HED of 0.6 mg/kg bw/d.

9.3.2.2 Biomonitoring equivalent calculation

The selected urinary biomarker for occupational biomonitoring is 5cx-MEPP, as mentioned in section 8.1.2.

The estimated concentration of the urinary 5cx-MEPP consistent with an exposure to the calculated HED of 0.6 mg/kg bw/d is obtained with a urinary mass balance approach, either on an urinary bodyweight-related excretion rate basis (as performed for the HBM $HBGV_{GenPop}$ in the sections 9.2.2 and 9.2.3), or on a creatinine-adjusted basis.

A share of 13.2 % (F_{ue}) of the DEHP dose absorbed by the oral route is excreted in 24h in urine in form of the metabolite 5cx-MEPP, as indicated from Anderson et al., 2011.

a) Bodyweight-related urine excretion rate adjustment (0.02 L/kg bw/d for adults):

Urinary 5cx-MEPP equivalent concentration = [HED x F_{ue} x ratio of molecular weights of 5cx-MEPP over DEHP] / adult bodyweight-related urine excretion = [0.6 x 0.132 x (308.33/390.6)] / 0.02 = 3.12 mg/L

b) Bodyweight-normalised creatinine excretion rate adjustement (0.02 g/kg bw/d for a 70 kg male person, as determined from Aylward et al., 2009):

Urinary 5cx-MEPP equivalent concentration = [HED x F_{ue} x ratio of molecular weights of 5cx-MEPP over DEHP] / creatinine excretion rate normalised to bodyweight = [0.6 x 0.132 x (308.33/390.6)] / 0.02 = 3.12 mg/g creatinine

9.3.2.3 Calculation of HBM $HBGV_{Worker}$ value

An assessment factor accounting for intraspecies differences (AF_H) is applied to the urinary 5cx-MEPP equivalent concentration. This AF_H is set to 5 when workers are the targeted population for which the HBM value is derived, which leads to:

Urinary 5cx-MEPP concentration measured at the end of the workshift:

HBM $HBGV_{Worker}$ = urinary 5cx-MEPP equivalent concentration / AF_H = 0.62 mg/g creatinine (or 0.62 mg/L = 620 µg/L)

10 Level of confidence attributed to the derived HBM HBGVs

As mentioned in the HBM4EU concept document to derive HBM HBGVs, it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM HBGV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to revise later on values with estimated 'lower' level of confidence.

However, it should be specified that the levels of confidence proposed in this document are not determined according to an established recognised methodology, but rather rely on expert judgment regarding the reliability of the data and the calculation method used to derive the HBM values.

The levels of confidence attributed to the HBM HBGV for the general population are set to '**medium'**. Please, refer to the n°2 note of the factsheet presented in section 13.1 for explanation on this level of confidence appreciation.

The level of confidence attributed to the HBM $HBGV_{worker}$ is set to '**low'**. Please, refer to the n°2 note of the factsheet presented in section 13.2 for explanation on this level of confidence appreciation.

11 Reference values

11.1 For the general population (RV_{95})

To be added during the course of HBM4EU.

11.2 For the occupationally exposed population (BRV)

The BRV is a statistical description of the background exposure to a chemical substance in a reference population of persons who are not occupationally exposed to the substance. Unlike the RV_{95}, the BRV is derived from persons of working age. Definition of the BRV is similar to the BAR (Biologische Arbeitsstoff-Referenzwerte), as established by the MAK Commission, for occupational health purposes (Angerer et al., 2011).

The American NHANES studies with cohorts of over 1500 people (aged 20 years and over) are reference studies. The ANSES Working Group on Biomarkers proposed in 2012 a BRV of 200 µg/g of creatinine for 5cx-MEPP (ANSES, 2012a), which is a rounded value of the 95th percentile of the distribution of urinary concentrations of 5cx-MEPP equal to 214 µg/g of creatinine measured from the NHANES urine samples collected in 2007-2008 (CDC, 2011).

More recent results were published in 2017 from the CDC regarding the NHANES samples collection (CDC, 2017). They indicate that urine samples collected in 2011-2012 are giving a value for the 95th percentile of the distribution of urinary concentrations of 5cx-MEPP equal to 53.7 µg/g of creatinine this time (CDC, 2017). A creatinine non-corrected value of 61.8 µg/L is also given (CDC, 2017). Starting from the assumption that the characteristics of the US population are close to those of the EU population, a rounded concentration of 54 µg/g of creatinine (or 62 µg/L) for 5cx-MEPP is thus proposed in this current report as a BRV.

12 Summary and outlook

For recording and evaluating exposure to DEHP in the general population it is recommended to determine the sum of the DEHP metabolites 5-oxo-MEHP and 5-OH-MEHP or alternatively 5cx-MEPP and 5-OH-MEHP in morning urine (µg/L) and to compare the analytical results with the HBM HBGVs for the general population described here above (see also section 9.2).

For evaluating DEHP exposure in the occupational field, measurement of the biomarker of exposure 5cx-MEPP at the end of the workshift should be performed and compared to the suggested HBM HBGV for the workers described here above (see also section 9.3).

The derivation in this current document of HBM HBGVs for the general population as well as for workers is based on the conversion of external doses (i.e. a TDI for the general population and a HED for the workers) to their estimated equivalent internal doses. The TDI and the HED have in common that they are calculated from PODs derived from reproductive toxicity (observed in rats' male offspring in the key study used in the TDI calculation and in adult male rats in the key study selected for the HED calculation). In establishing these HBGVs based on reprotoxicity we acknowledged an endocrine mode of action. However according to current policy (as for example in the REACH context), substances identified as having endocrine disruptive properties should be considered not to have a threshold, except where it can be demonstrated that a threshold exists (EU Commission, 2014). In this case, for DEHP, the existence of a threshold can be assumed based on available data, even if not formely demonstrated. Thus, this leads to uncertainties regarding the appropriateness of the derived HBGVs. Efforts for deriving HBGVs for non-threshold substances should be made by the task 5.2 partners during the course of the HBM4EU project.

Also, several recent studies showing adverse effects of phthalate exposure on the immune system (allergy, asthma and eczema) could indicate that reproductive toxicity may not be the most sensitive endpoint for the effects of DEHP. Those studies are briefly described in the Restriction Report of four phthalates (ECHA, 2016): "Studies in mice and rats showed that DEHP could enhance the sensitisation to allergens (adjuvant effect), and this was suggested as an underlying risk factor in the increase in severity of asthma (Guo et al. 2012; You et al. 2014). Increased serum IgE responses were seen after 52 days exposure of adult mice to very low doses of DEHP (30 µg/kg bw/day) (Guo et al. 2012). Tonk et al. (2012), examined developmental and immunological effects of 1 to 1000 mg/kg bw/day of DEHP in juvenile and adult male rats, and found effects on immune parameters in juvenile males beginning from around 1 mg/kg bw/day, i.e. at lower doses than the doses affecting reproductive organ weights". Thus, there is a need for further robust data regarding adverse effects of phthalates on the immune system, which could be a task undertaken by the partners from WP13 on "Establishing exposure-health relationships".

Another lead to reduce uncertainties towards the HBM HBGVs hereby derived ("medium" for the HBM $HBVG_{GenPop}$ and "low" for the HBM $HBGV_{Worker}$, see section 10) would be to have a validated PBPK or TK model for DEHP enabling the extrapolation of the selected animal POD value to the corresponding human POD value, thereby avoiding the default factor value of 10 as AF for the interspecies differences applied to the animal PODs to obtain either the TDI or the HED. Thus, A DEHP PBPK or TK model validated by WP12 working on toxicokinetic modeling could allow reducing the uncertainty factors applied for the derivation of the HBGVs.

Most studies on the toxicity of phthalates have focused on single phthalates, but it is also important to consider the effects of phthalate mixtures because humans are generally exposed to several phthalates. In a next step, a HBM HBGVs should be derived which refer to those phthalates and their metabolites which show anti-androgenic effects. The feasibility of deriving HBGVs for combined phthalates exposure should be explored in 2018 by the task 5.2 partners, according to the Annual Work Plan 2018.

13 Factsheets

13.1 Factsheet on the HBM HBGV for the general population

Rapporteur: UBA **Reviewer: ANSES**			
Compound	**DEHP** bis (2-ethylhexyl) phthalate		**Factsheet** **General population**
Parameter	**Note**	**Value / descriptor**	**Comments**
HBM HBGV$_{GenPop}$ and Status			
Target population: general population			
HBM HBGVs$_{GenPop}$	1	**Σ [5-oxo-MEHP and 5-OH-MEHP] in the urine:** **Children (6 - 13 y): 340 µg/L** **Adults: 500 µg/L** **Σ [5cx -MEPP and 5-OH-MEHP] in the urine:** **Children (6 - 13 y): 380 µg/L** **Adults: 570 µg/L**	As information, creatinine adjusted values: **Σ [5-oxo-MEHP and 5-OH-MEHP] in the urine:** **Adults: 500 µg/g creat** **Σ [5cx -MEPP and 5-OH-MEHP] in the urine:** **Adults: 570 µg/g creat**
Level of confidence	2	**Medium**	See note for explanation
HBM HBGV$_{GenPop}$ status	3	**Provisional**	See note for explanation
HBM HBGV$_{GenPop}$ year of issue	4		
General Information			
CLP-INDEX-Nr.	5	607-317-00-9	
EC-Nr.	6	204-211-0	
CAS-Nr.	7	117-81-7	
Harmonised CLP classification	8	category 1B reproductive toxicant	
Molar mass	9	390.6 g/mol	
Biomarker(s)			
Identification	10	urinary 5-oxo-MEHP and 5-OH-MEHP or urinary 5cx-MEPP and 5-OH-MEHP	
Molar mass of biomarker(s)	11	5-oxo-MEHP: 292.33 g/mol 5-OH-MEHP: 294.34 g/mol 5cx-MEPP: 308.33 g/mol	
Toxicokinetic data			
Key study for TK data, authors, year	12	A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate	Anderson et al., 2011 Food Chem Toxicol. 49(9):2022-9
Type of study	13	Human:	

		20 volunteers (10 female + 10 male), all Caucasian	
Route of exposure	14	Oral single dose: 0.31 or 0.78 mg randomly	
Half-life of biomarker(s) (second phase)	15	5-oxo-MEHP: 10h 5-OH-MEHP: 10h 5cx-MEPP: 12-15h	From Koch et al, 2005
Factor for metabolic conversion (F_{ue})	16	F_{ue} = 0.269 (urinary 5-oxo-MEHP + 5-OH-MEHP) F_{ue} = 0.295 (urinary 5cx-MEPP + 5-OH-MEHP)	From Anderson et al., 2011 Fractional urinary excretion ratios of biomarkers relative to the oral dose of DEHP taken up, at 48h: 5-oxo-MEHP: 11,3% 5-OH-MEHP: 15,6% 5cx-MEPP: 13,9%
Derivation method and calculation			
Derivation method	17	**Based on a defined tolerable intake (TDI)**	
Defined tolerable intake	18	**TDI value:** **0.05 mg/kg bw/d**	**TDI from EFSA 2005 based on:** Multigenerational reproductive assessment by continuous breeding when administered to Sprague-Dawley rats in the diet Wolfe and Layton, 2003 (chronic, oral study) **Critical endpoint: developmental impairment** NOAEL = 4.8 mg/kg/d AFs = 100 See note for more details
Biomonitoring equivalent	19	**Based on urinary mass balance** **Σ [5-oxo-MEHP and 5-OH-MEHP]** **Children, adults: 10.1 µg/kg bw/d** **Based on urinary mass balance** **Σ [5cx-MEPP and 5-OH-MEHP]** **Children, adults: 11.4 µg/kg bw/d**	= TDI x F_{ue} x ratio of molecular weights (mean biomarkers MW over DEHP MW) F_{ue} for Σ [5-oxo-MEHP and 5-OH-MEHP] in 48-hour urine
HBM HBGVs$_{GenPop}$ and RV95 value			
Calculated HBM HBGVs$_{GenPop}$	20	**Σ [5-oxo-MEHP and 5-OH-MEHP] in the urine (rounded value):** **Children (6 to 13 y): 340 µg/L** **Adults: 500 µg/L** **Σ [5cx-MEPP and 5-OH-MEHP] in the urine (rounded value):**	Calculated with the following urine volumes: Children (6 to 13 years): 0.03 L/kg bw/d Adults: 0.02 L/kg bw/d

		Children (6 to 13 y): 380 µg/L **Adults: 570 µg/L**	
Reference value (RV_{95})	21	To be added during the course of HBM4EU	See note for the definition
Additional Comments			

Explanation of notes:

1) **HBM $HBGV_{GenPop}$**: numerical value of the **HBM $HBGV_{GenPop}$** in µg/L or mg/L

2) **Level of confidence of the HBM $HBGVs_{GenPop}$** reflects the reliability of the proposed HBM HBGV. The level of confidence takes into account the various uncertainties underlying its derivation. An attribution of a global level of confidence is suggested for the proposed DEHP HBM $HBGV_{GenPop}$, considering the assessment of the various uncertainties:

   - regarding the nature and quality of the DEHP toxicological data: the database on DEHP is based on a large number of both human and animal studies → medium/high

   - regarding the critical endpoint and mode of action: the confidence in the evidence of developmental effect (testicular developmental impairment) is high, confirmed by other independent toxicological animal studies (subchronic and chronic). Nevertheless, no evidence for these effects in humans is available from the scientific literature → medium/high

   - regarding the selected key study for the TDI derivation: the study used for the TDI derivation is rated 1 on the Klimisch classification, which indicates a reliable study, carried out according to the OECD internationally accepted testing guideline. However, the best case for derivating an HBM HBGV would have been availability of human data → medium

   - regarding the extrapolations of the POD value for the TDI calculation: the NOAEL determined from the high-quality key study is being extrapolated across species (AF_A = 10) and according to intra individual variability (AF_H) → medium (using a validated PBPK model or TK model for extrapolating from animal to human could increase the level of confidence)

   - regarding the calculation of the HBM HBGV: the determination of the factor for metabolic conversion (F_{ue}) is based on a study with 20 volunteers (male and female volunteers) (see note 12) → high

Altogether the global level of confidence for the DEHP HBM $HBGV_{GenPop}$ is set to 'medium'.

3) **HBM $HBGV_{GenPop}$:** the status value is fixed to 'provisionnal', thereby reflecting that the HBM $HBGV_{GenPop}$ could be revised later on in the project. Uncertainties underlying the TK data for example could be reduced by means of a validated PBPK model for DEHP.

4) **HBM $HBGV_{GenPop}$ year of issue:** year of agreement on the HBM $HBGV_{GenPop}$ in the HBM4EU workframe.

5) **CLP Number:** according to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Implementing the globally harmonised system of chemical classification or GHS. http://guidance.echa.europa.eu/docs/guidance_document/clp_introductory_en.pdf

6) **EC Number:** under European Inventory of Existing Commercial chemical Substances (EINECS), ELINCS (European List of Notified Chemical Substances) in support of Directive 92/32/EEC, the 7th amendment to Directive 67/548/EEC, NLP (No-Longer Polymers). See: ESIS: European chemical Substances Information System: http://esis.jrc.ec.europa.eu/index.php?PGM=dat

7) **CAS Number:** collection of disclosed chemical compound information by Chemical Abstracts Service. Almost all molecule databases can be searched by CAS Registry Number.

8) **Harmonised CLP classification:** CLP classification including CMR and other health relevant effects. In the case that classification is not harmonised, this should be stated. For selfclassifications by industry the ECHA- CLP inventory can be searched at: http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

9) **Molar mass**: mass of DEHP per amount of this substance expressed in g/mol

10) **Biomarker(s) identification:** Given DEHP's short half-life in blood, it is difficult to use this biomarker for biological monitoring. Compared to MEHP the secondary metabolites of DEHP are excreted in urine in much larger concentrations and the half-life periods for their excretion are obviously longer. With a half-life of 24 hours, urinary 2cx-MMHP seems relevant as a biological indicator of exposure. However, very few data on it are available. Urinary 2cx-MMHP was therefore not selected

as a biological indicator of exposure to DEHP. As suitable biomarkers the following are left: 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP. For reasons of preciseness, 2 metabolites should be considered for HBM guidance value derivation for the general population.

11) **Molar mass of the biomarker(s)**: mass of the selected biomarkers (expressed in g/mol).

12) **Study for TK data, Authors, Year:** Anderson et al. (2011) published a study involving 20 volunteers (10 men and 10 women) who ingested a dose (0.31 or 0.78 mg randomly) of radiolabeled DEHP. Over a 48-hour period, urine was collected at several intervals. The molar excretion fractions of urinary metabolites were determined at 24 and 48 hours.

13) **Type of study:** human study (10 men and 10 women).

14) **Route of exposure:** oral single dose of radiolabeled DEHP (0.31 or 0.78 mg randomly).

15) **Half-life of biomarker:** estimated elimination half-lives for the DEHP metabolites 5-oxo-MEHP, 5-OH-MEHP, and 5cx-MEPP, determined for the second elimination phase in the high dose experiment from Koch et al, 2005.

16) **Factor for metabolic conversion (F_{ue}):** a share of 26,9 % of the DEHP dose administered is excreted as 5-oxo-MEHP and 5-OH-MEHP at 48h, and a share of 29,5% of the DEHP dose administered is excreted as 5cx-MEPP and 5-OH-MEHP, as measured by Anderson et al., 2011.

17) **Derivation method:** a study on humans allowing for a dose-relationship between internal concentration of a DEHP biomarker and health effect was not available. Therefore, a derivation method based on a defined tolerable intake is used (see concept document).

18) **Defined tolerable intake:** the TDI value of 0.05 mg/kg bw/d, based on a NOAEL of 4.8 mg DEHP/kg bw/d determined by Wolfe and Layton (2003) in a multigeneration study in Sprague-Dawley rats was retained, in accordance with the value of EFSA (2005). The critical endpoint is the testicular developmental impairment. An assessment factor of 10 was applied to the NOAEL accounting for interspecies differences (AF_A) as well as an assessment factor of 10 accounting for human variability (AF_H).

19) **Biomonitoring equivalent:** it is assumed in a first step that an adult's daily intake of DEHP corresponds to the TDI value (50 µg/kg bw/d), i.e. this adult's intake would amount to 50 µg DEHP per kg body weight per day. A share of 26,9% of the dose absorbed is excreted in the form of the two metabolites, 5-oxo-MEHP and 5-OH-MEHP. For conversion to weight proportions (µg/L), the ratio between the molecular weights of DEHP and its metabolites must be taken into account (the MW of DEHP is 390,56 and the mean MW of both 5-oxo-MEHP and 5-OH-MEHP is 293,34, thus the MW ratio is 0.75).

    For adults, children: Σ [5-oxo-MEHP & 5-OH-MEHP] = 50 µg/kg bw/d x 0.75 x 0.269 ($F_{ue(48h)}$) = 10.1 µg/kg bw/d

    The same calculation is performed for the sum of 5cx-MEPP and 5-OH-MEHP ($F_{ue(48h)}$ = 0.295; ratio of MW = 0.771):

    For adults, children: Σ [5cx-MEPP & 5-OH-MEHP] = 50 µg/kg bw/d x 0.771 x 0.295 = 11.4 µg/kg bw/d

20) **HBM $HBGV_{GenPop}$:** Ideally, the HBM $HBGV_{GenPop}$ should refer to a complete 24-hour urine sample, stating the quantity of a substance in µg/day. For reasons of simplification, in accordance with the approach described in the concept document, the excretion is referred to a body weight-related urine excretion of 0.03 L/kg bw/d for children and 0.02 L/kg bw/d for the adults.

    HBM $HBGV_{GenPop}$ for (Σ 5-oxo-MEHP + 5-OH-MEHP) in the urine:

    For children (6 to 13 y): 10.1 / 0.03 = 337 µg/L, rounded 340 µg

    For adults: 10.1 / 0.02 = 505 µg/L

    HBM $HBGV_{GenPop}$ for (Σ 5cx-MEPP + 5-OH-MEHP) in the urine:

    For children (6 to 13 y): 11.4/ 0.03 = 380 µg/L

    For adults: 11.4 / 0.02 = 570 µg/L

21) **Reference value:** indication of the background burden, established if possible from measurements among a suitable reference population (population without recognisable specific exposure).

13.2 Factsheet on the HBM $HBGV_{Worker}$ value derivation

Rapporteur: ANSES **Reviewer: UBA**			
Compound	**DEHP** bis (2-ethylhexyl) phthalate		**Factsheet** **Occupational field**
Parameter	**Note**	**Value / descriptor**	**Comments**
HBM $HBGV_{Worker}$ and Status			
Target population: workers			
HBM $HBGV_{Worker}$	1	**[5cx-MEPP] in the urine at the end of the workshift:** **0.62 mg/g of creatinine** **(0.62 mg/L of urine)**	
Level of confidence	2	**Low**	See note for explanation
HBM $HBGV_{Worker}$ status	3	**Provisionnal**	See note for explanation
HBM $HBGV_{Worker}$ year of issue	4		
General Information			
CLP-INDEX-Nr.	5	607-317-00-9	
EC-Nr.	6	204-211-0	
CAS-Nr.	7	117-81-7	
Harmonised CLP classification	8	category 1B reproductive toxicant	
Molar mass	9	390.6 g/mol	
Biomarker(s)			
Identification	10	urinary 5cx-MEPP	
Molar mass of biomarker(s)	11	308.33 g/mol	
Toxicokinetic data			
Study for TK data, authors, year	12	A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate	Anderson et al., 2011 Food Chem Toxicol. 49(9):2022-9
Type of study	13	Human: 20 volunteers (10 female + 10 male), all Caucasian	
Route of exposure	14	Oral single dose: 0.31 or 0.78 mg randomly	
Half-life of biomarker(s)	15	12-15 h	
Factor for metabolic conversion (F_{ue})	16	F_{ue} = 0.132 Fractional urinary excretion ratio of biomarker relative to the oral dose of DEHP taken up, at 24h: 5cx-MEPP: 13.2%	From Anderson et al., 2011 The fractional urinary excretion ratio is taken here at 24h
Derivation method and calculation			
Derivation method	17	From a Point of departure (POD)	

Key study, Author(s), Year	18	Chronic toxicity of di(2-ethylhexyl) phthalate in rats. David et al., 2000	Key study (Wolfe and Layton, 2003) for derivation of the TDI from EFSA (2005) not appropriate for occupational scenario of exposure (see note)
Species	19	Rat	
Route/type of study	20	Oral, chronic	
Exposure duration	21	2 years (104 weeks)	
Critical endpoint	22	**Bilateral aspermatogenesis, reprotoxicity**	
POD value	23	**oral NOAEL: 5.8 mg/kg bw/d**	
Interspecies adjustment	24	Allometric adjustement: 1.5 mg/kg bw/d HED (oral - human): 0.6 mg/kg bw/d	Toxicokinetic part (allometric adjustement): oral HED = animal dose x (animal weight/human bodyweight)$^{1/4}$ Toxicodynamic part: AF_A = 2.5
Biomonitoring equivalent	25	**Urinary 5cx-MEPP concentration for a daily HED:** **3.12 mg/g creatinine** With creatinine excretion rate normalised to bw: 0.02 g/kg bw/d	Based on urinary mass balance approach: Urinary 5cx-MEPP concentration = [HED x F_{ue} x ratio of molecular weights of 5cx-MEPP over DEHP] / creatinine excretion rate
HBM HBGV$_{Worker}$			
HBM HBGV$_{Worker}$	26	**urinary [5cx-MEPP]:** **0.62 mg/g of creatinine** with creatinine excretion rate normalised to bw: 0.02 g/kg bw/d 620 µg/L with bodyweight-	**Uncertainty factor related to the intraspecies differences:** **AF_H = 5 (workers)**
Biological Reference Value	27	**urinary [5cx-MEPP]:** **54 µg/g of creatinine** **(62 µg/L)**	95th percentile of distribution of urinary [5cx-MEPP] NHANES study (2011 - 2012), US general population > 20 y: 53.7 µg/g of creatinine (1704 samples) 61.8 µg/L (1705 samples)
Additional Comments			

Explanation of notes:

1) **HBM HBGV$_{Worker}$**: numerical value of the HBM HBGV in µg/L or mg/L

2) **Level of confidence of the HBM HBGV(s)$_{Worker}$:** reflects the reliability of the proposed HBM HBGV$_{Worker}$. The level of confidence takes into account the various uncertainties underlying its derivation. An attribution of a global level of confidence is suggested for the proposed DEHP HBM HBGVs$_{Worker}$, considering the assessment of the various uncertainties:

- regarding the nature and quality of the DEHP toxicological data: the database on DEHP is based on a large number of both human and animal studies) → medium/high

- regarding the critical endpoint and mode of action: the confidence in the evidence of developmental effect (testicular developmental impairment) is high, confirmed by other independent toxicological animal studies (subchronic and chronic). Nevertheless, no evidence for these effects in humans is available from the scientific literature → medium/high

- regarding the selected key study for identification of the NOAEL: the study has been realised on adult rats during 2 years, which corresponds almost to an entire-life exposure in accordance with an occupational exposure scenario. However, the best case for derivating an HBM HBGV$_{Worker}$ would have been availability of human data. The second best case would have been an inhalation animal study → low

- regarding the extrapolations of the POD value (animal oral NOAEL) to the biomonitoring equivalent: allometric adjustment accounting for the toxicokinetic differences between rats and humans and uncertainty factor accounting for the toxicodynamic interspecies differences (AF_A = 2,5) were applied by default. Occupational exposure is not continuous but follows the working time schedule (usually 8 h/day and 5 d/week), thus derivating an HBM HBGV$_{Worker}$ on the basis of a continuous POD is certainly leading to an overestimation of the value. As a perspective, using a validated PBPK model for extrapolating from animal to human or for the extrapolation from continuous to discontinuous exposure could increase the level of confidence of the calculated HBM HBGV$_{Worker}$ → low

- regarding the calculation of the HBM HBGV$_{Worker}$: the kinetic parameters of DEHP and of the metabolites (e.g. the factor for metabolic conversion (F_{ue})) were measured for oral absorption of a single dose of DEHP on 20 volunteers (male and female) (see note 12), which is considered as a solid study. However, the molar excretion fraction of 5cx-MEPP was not determined for exposure by inhalation → medium/low

Altogether the global level of confidence for the DEHP HBM HBGV$_{Worker}$ is set to 'low'.

3) **HBM HBGV$_{Worker}$ status:** the status value is fixed to 'provisionnal', thereby reflecting that the HBM HBGV$_{Worker}$ could be revised later on in the project if for example uncertainties related to the TK data can be reduced (i.e. use of a validated DEHP PBPK model). A higher level of confidence attributed to the HBM HBGV$_{Worker}$ would lead to a 'confirmed' value status.

4) **HBM HBGV$_{Worker}$ year of issue:** year of agreement on the HBM HBGV$_{Worker}$ in the HBM4EU workframe.

5) **CLP Number:** according to the CLP Regulation (EC) No 1272/2008 of substances and mixtures. See: http://guidance.echa.europa.eu/docs/guidance_document/clp_introductory_en.pdf

6) **EC Number:** under European Inventory of Existing Commercial chemical Substances (EINECS), ELINCS (European List of Notified Chemical Substances) in support of Directive 92/32/EEC, the 7th amendment to Directive 67/548/EEC, NLP (No-Longer Polymers). See: http://esis.jrc.ec.europa.eu/index.php?PGM=dat

7) **CAS Number:** collection of disclosed chemical compound information by Chemical Abstracts Service.

8) **Harmonised CLP classification:** CLP classification including CMR and other health relevant effects. In the case that classification is not harmonised, this should be stated. For selfclassifications by industry the ECHA- CLP inventory can be searched at: http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

9) **Molar mass**: mass of DEHP per mole, expressed in g/mol

10) **Biomarker identification:** urinary mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) was chosen as the most relevant biomarker of exposure among the biomarkers identified in the scientific literature (urinary MEHP, urinary 5OH-MEHP, urinary 5oxo-MEHP, urinary 5cx-MEPP, urinary 2cx-MMHP and systemic DEHP, systemic MEHP). Blood biomarkers and urinary 2cx-MMHP were not selected because no study at the workplace reported DEHP and MEHP measurements in blood resp. 2cx-MMHP in urine. Urinary MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP are documented in some studies in workplaces. These biomarkers, specific to exposure to DEHP, have half-lives enabling samples to be taken at the end of the work shift. These biological indicators of exposure to DEHP can be selected for the biological monitoring of occupational exposure. However, urine samples collected for measuring MEHP require special measures to be taken at the time of collection to prevent transformation of DEHP to MEHP (leading to an overestimation of concentrations). Measuring MEHP alone can lead to an underestimation (low excretion) or overestimation (contamination) of exposure. This is the reason why the measurement of this metabolite has a greater inter-individual

variability (30%) than the three secondary metabolites, 5OH-MEHP, 5cx-MEPP and 5oxo-MEHP (20 to 25%) (Anderson et al., 2011). This biomarker was not selected for the biological monitoring of occupational exposure. 5OH-MEHP and 5cx-MEPP account for the highest urinary fractions of the three secondary metabolites. One study (Dirven et al., 1993b) establishes a relationship between 5OH-MEHP, 5oxo-MEHP or 5cx-MEPP with atmospheric concentrations of DEHP.

5cx-MEPP was finally selected according to the correlation (r=0,41) reported between atmospheric DEHP concentration and urinary 5cx-MEPP concentration (Gaudin et al., unpublished data).

11) **Molar mass of the biomarker**: mass of the selected biomarker (5cx-MEPP) per mole, expressed in g/mol.

12) **Study for TK data, Authors, Year:** Anderson et al. (2011) published a study involving 20 volunteers (10 men and 10 women) who ingested a dose (0.31 or 0.78 mg randomly) of radiolabeled DEHP. Over a 48-hour period, urine was collected at several intervals. The molar excretion fractions of urinary metabolites were determined at 24 and 48 hours.

13) **Type of study:** human study (10 men and 10 women, all Caucasian).

14) **Route of exposure:** oral single dose of radiolabeled DEHP (0.31 or 0.78 mg randomly).

15) **Half-life of biomarker:** cx-MEPP has a half-life of 12 to 15 h, determined from the second phase of elimination of measured urinary metabolites.

16) **Factor for metabolic conversion (F_{ue}):** molar excretion fraction (expressed in %) at 24 h of the urinary 5cx-MEPP measured from Anderson et al., 2011.

17) **Derivation method:** using the OEL of 8 mg/m^3 as atmospheric exposure concentration to derive an HBM $HBGV_{Worker}$ (which would correspond to the urinary 5cx-MEPP concentration at this concentration of exposure) is not possible because no solid evidence of a relationship between atmospheric concentration of DEHP and urinary concentration of the biomarker 5cx-MEPP is available. Thus, starting from the critical effect (POD) is necessary, which corresponds to reproductive toxicity as assessed while derivation from the TDI value of DEHP (EFSA 2005) was retained by ANSES in 2012).

18) **Key study, Authors, Year:** the multigenerational oral study on rats conducted by Wolfe et al., 2003, which has been used for the TDI value derivation, could not serve as a basis for the HBM $HBGV_{Worker}$, due to the exposure scenario not appropriate with an occupational exposure scenario. Therefore, the chronic oral toxicity study in rats (male and female groups) from David et al., 2000 used to identify the POD value for the 8h-OEL value derivation has been selected.

19) **Species**: F344 male and female rats.

20) **Route/type of study**: oral chronic study. Doses of DEHP corresponding to 0 ; 100 ; 500 ; 2500 and 12500 ppm were administrated (corresponding to DEHP doses of 0 ; 5.8 ; 28.9 ; 146.6 and 789.0 mg/kg bw/d for males and 0 ; 7.3 ; 36.1 ; 181.7 and 938.5 mg/kg bw/d for females).

21) **Duration of exposure**: exposure through oral doses given during 104 weeks.

22) **Critical endpoint:** the bilateral aspermatogenesis is selected as first adverse effect observed.

23) **Point of departure (POD) value:** a NOAEL of 5.8 mg/kg bw/d for a critical effect corresponding to the onset of aspermatogenesis was determined in this study. The lowest dose (LOAEL) leading to the significant effect of bilateral aspermatogenesis is 28.9 mg/kg bw/d.

24) **Interspecies extrapolation**: the interspecies extrapolation from rats to humans includes: a) an allometric adjustment (toxicokinetic part of the animal to human extrapolation) and b) application of an assessment factor taking into account the toxicodynamic differences.

a) The NOAEL determined in rats is adjusted to humans by an allometric adjustment, according to the U.S. EPA (2006) recommendations: oral HED = animal dose x (animal weight/human bodyweight)$^{1/4}$

The mean animal weight of 322 g was determined from the key study of David et al., 2000 (mean of all animal weights at the end of the study, no observed significant change in the weight of animals regardless of the exposure dose). The human bodyweight was set at 70 kg, which leads to a NOAEL of 1.5 mg/kg bw/d adjusted to humans.

b) The oral NOAEL adjusted to humans is divided by an assessment factor of 2.5 accounting for the interspecies toxicodynamic differences, leading to an **oral Human Equivalent Dose (HED) of 0.6 mg/kg bw/d**.

25) **Biomonitoring equivalent**: under steady-state conditions, the estimated concentration of the urinary biomarker 5cx-MEPP consistent with an exposure to the HED of 0.6 mg/kg bw is obtained with an urinary mass balance approach:

- With a bodyweight-related urine excretion of 0.02 L/kg bw/d for adults:

Urinary 5cx-MEPP equivalent concentration = [HED x F_{ue} x ratio of molecular weights of 5cx-MEPP over DEHP] / adult bodyweight-related urine excretion = [0.6 x 0.132 x (308.33/390.6)] / 0.02 **= 3.12 mg/L**

- With the creatinine excretion rate normalised to the bodyweight and estimated to 0.02 g/kg bw/d:

Urinary 5cx-MEPP equivalent concentration = [HED x F_{ue} x ratio of molecular weights of 5cx-MEPP over DEHP] / creatinine excretion rate normalised to bodyweight = [0.6 x 0.132 x (308.33/390.6)] / 0.02 **= 3.12 mg/g creatinine**

26) **HBM HBGV$_{Worker}$**: an assessment factor accounting for intraspecies differences (AF_H) is applied to the urinary 5cx-MEPP equivalent concentration. This AF_H is set to 5 when workers are the targeted population for which the HBM HBGV is derived, which leads to:

HBM $HBGV_{Worker}$ = urinary 5cx-MEPP equivalent concentration / AF_H = 3.12 / 5 = **0.62 mg/g creatinine (or 620 µg/L)**

27) **Biological Reference Value**: indication of the background burden established from measurements among a suitable reference population of working age (population without recognisable specific exposure). The indicated value corresponds to the 95th percentile of the distribution of the urinary 5cx-MEPP concentration determined from the American NHANES study with samples collected in 2011-2012 (CDC, 2017). Analysis of urinary 5cx-MEPP was performed on 1704 samples coming from US volunteers over 20 years of age. Starting from the assumption that the characteristics of the US population are close to those of the EU population, the concentration of 54 $\mu g.g^{-1}$ of creatinine (62 µg/L) for 5cx-MEPP can be proposed as the biological reference value.

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Chapter 2

Hexamoll® DINCH® (1, 2-cyclohexane dicarboxylic acid diisononyl ester) - Derivation of HBM health-based guidance values (HBM HBGVs): HBM $HBGV_{GenPop\ (adults)}$ & HBM $HBGV_{GenPop\ (children)}$

Chapter 2: Table of contents

Glossary

AGD	Anogenital Distance
AGI	Anogenital Index
ANSES	French Agency for Food, Environmental and Occupational Health and Safety
BfR	German Institute for Risk Assessment
CHDA	1,2-cyclohexane dicarboxylic acid
CPSC	U.S. Consumer Product Safety Commission
cx-MINCH	cyclohexane-1,2-dicarboxylic acid mono-carboxyisooctyl ester
DEHP	di-2-ethylhexyl phthalate
DINCH	1,2-cyclohexane dicarboxylic acid diisononyl ester
DBP	Di-n-butyl phthalate
EFSA	European Food Safety Authority
EU	Europe
FLEC	Field and Laboratory Emission Cell
Fue	Urinary Excretion Factor
HBGV	Health-based Guidance Value
HBM	Human Biomonitoring
HBM4EU	European Biomonitoring Initiative
GGT	Gamma-glutamyl transferase
IV	Intravenous
IVF	In Vitro Fertilization
IVLV	Industry Association for Food Technology and Packaging
LOAEL	Lowest Observed Adverse Effect Level
LoQ	Limit of Quantification
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHxCH	cyclohexane-1,2-dicarboxylic acid mono carboxyhexyl ester
MoA	Mode of Action
NOAEL	No Observed Adverse Effect Level
NICNAS	Australian National Industrial Chemicals Notification and Assessment Scheme
OECD	Organisation for Economic Co-operation and Development
OH-MINCH	cyclohexane-1,2-dicarboxylic acid mono-hydroxyisononyl ester
oxo-MINCH	cyclohexane-1,2-dicarboxylic acid mono-oxoisononyl ester
PND	Postnatal Day
POD	Point Of Departure
RfD	Reference Dose
RIVM	Netherlands National Institute for Public Health and the Environment
RMOA	Risk Management Option
sc	subcutaneous
SCENIHR	Scientific Committee on Emerging and Newly-Identified Health Risks

SHBG	Sex Hormone-Binding Globulin
SVF	Stromal Vascular Fraction
T3	Triiodothyronine
T4	Thyroxine
TDI	Tolerable Daily Intake
TSH	Thyroid Stimulating Hormone
UBA	German Environment Agency

1 Introduction

1.1 Objectives

In task 5.2 of HBM4EU, health-based HBM guidance values (HBGVs) are derived for HBM4EU priority substances.
The methodology applied to derive these values is based on the procedure described in the German Human Biomonitoring Commission's position papers, as well as on the guidance document elaborated by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) for the derivation of biological limit values for chemicals used in the workplace. These methodological approaches have been summarised and combined in a concept document previously released in the HBM4EU task 5.2 workframe (HBM4EU concept paper, 2017).

In the current report, general population HBGVs ($HBGVs_{GenPop}$) are derived for the sum of the DINCH metabolites cyclohexane-1,2-dicarboxylic acid mono-hydroxyisononyl ester (OH-MINCH), and cyclohexane-1,2-dicarboxylic acid mono-carboxyisooctyl ester (cx-MINCH) in the urine of adults and children.

Derivation of an HBGV for workers ($HBGV_{Worker}$) is not presented in this current report. To our knowledge, studies exploring the internal concentration of DINCH in workers via biomonitoring measurements are not published at this time. However, efforts will be made during the course of the HBM4EU project to derive a specific value for workers, starting from an animal study compatible with an occupational scenario of exposure, as indicated in the HBM4EU concept paper.

1.2 Methodological aspects of the HBGVs derivation for DINCH metabolites

The current epidemiological knowledge on whether and how human exposure to DINCH might cause human health effects is not sufficient to derive $HBGVs_{GenPop}$ directly based on human data. Because of this, for deriving $HBGVs_{GenPop}$, the German method for deriving health-based HBM guidance values for the general population based on an established tolerable intake level as the tolerable daily intake (TDI) is applied (HBM4EU concept paper, 2017; German HBM Commission, 2014). The following proposal is also based on the Analysis of the most appropriate Risk Management Option (RMOA) elaborated by ANSES (2016). The scientists who prepared an expertise as basis for the work of the German Human Biomonitoring Commission as well as ANSES were allowed by the company BASF to review studies including the rough data submitted for registration of DINCH (studies marked with an asterix in the reference list).

As a first step in the underlying report, information on the general toxicological profile of DINCH is given, and the requirements to be met for deriving health-based HBM guidance values from tolerable intake levels (as the TDI) are specified. Subsequently, $HBGVs_{GenPop}$ similar to HBM-I values are proposed, which could be seen as an update of the Opinion of the Human Biomonitoring Commission of the German Environment Agency (2014).

1.3 General information and relevance of recommending HBGVs for DINCH metabolites

1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamoll® DINCH®) [CAS Nº 166412-78-8] is a plasticizer used in the production of plastic articles. It was developed as an alternative to the harmful plasticizer di-2-ethylhexyl phthalate (DEHP) (Wadey, 2003; BASF, 2012). The abbreviation DINCH is derived from the chemical name diisononyl cyclohexane-1, 2-dicarboxylate and will be used as a short form of Hexamoll® DINCH® herein. As compared to DEHP, DINCH has, according to the current state of knowledge, less harmful toxicological properties and, in contrast to DEHP, has not

shown any reproductive toxicity in toxicological *in vivo* studies. DINCH is considered as suitable for health sensitive applications such as use in toys (Janssen and Bremmer, 2009) or medical devices (SCENIHR, 2008). After a positive opinion by the European Food Safety Authority (EFSA) in 2006, DINCH was approved for the production of plastic materials for food packaging in the context of the Regulation (EU) No 1935/2004 on materials and articles intended to come into contact with food. DINCH has also been listed for applications with food contact materials as FCM substance nº 775 (Regulation (EU) No 10/2011, Annex I), whereby the overall migration limit of 60 mg/kg of food (corresponding to 10 mg /dm^2 of plastic surface area) applies by default to the group nº 32 where DINCH belongs to.

When DINCH was introduced into the market in 2002, the production capacity by BASF, Ludwigshafen amounted to 25,000 tonnes per year. Up to mid-2007, it was quadrupled to 100,000 tonnes per year (Badina, 2008; Nagorka et al., 2011). In another step, the production capacity was doubled again to 200,000 tonnes per year in 2014 (BASF, 2014).

2 Identification of the substance

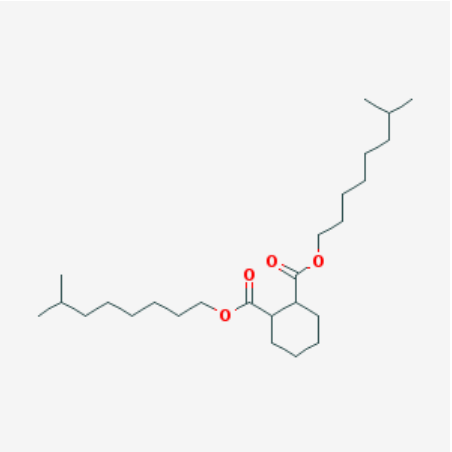
CAS Number: 166412-78-8

IUPAC Name: Diisononyl cyclohexane-1,2-dicarboxylate

Molecular weight: 424.67 g/mol

Molecular formula: $C_{26}H_{48}O_4$

Structural formula:



Source: Pubchem Open Chemistry Database

3 Physicochemical properties

At room temperature, DINCH is a colourless liquid with a barely perceptible odour. The molar mass is 424.7 g/mol (BASF, 2011). For other physicochemical properties see table 1.

Table 1: Physicochemical data for DINCH (BASF 1999a and 2000a)

State	Liquid
Relative density	0.95 g/cm^3 at 20 °C
Boiling point	394 °C (extrapolation); decomposition at ca. 351 °C
Vapour pressure	2.2 × 10^{-7} hPa at 20 °C
Partition coefficient log K_{OW}	10.0 (Calculated with K_{ow}Win, V1.51, measured > 6 with HPLC method)
Water solubility	< 0.02 mg/L at 25 °C

4 Use and exposure

DINCH is used as an alternative non-phthalate plasticizer in the production of shoes and handbags (synthetic leather), in textile coating (e.g. raincoats), but also in the production of household products such as tablecloths, shower curtains, wallpapers, floor coverings, adhesives and office supplies (e.g. plastic films or rubbers) (Wadey, 2003). DINCH has also been used in more sensitive areas such as toys and medical devices, e.g. blood bags (Bhaskaran et al., 2011; Nussbaumer et al., 2010). In addition, and as following from the listing under Regulation (EU) No 10/2011 (see above), DINCH is also used in plastic materials for food packaging. The “Industrievereinigung für Lebensmitteltechnologie und Verpackung e. V.” (IVLV, 2011) (Industry Association for Food Technology and Packaging) has reported DINCH to be used as a PVC plasticizer (up to 40 %) and as an additive in polystyrene, according to the approval of the manufacturer (EFSA, 2006).

Migration tests into mineral water and fruit juice/lemonade from bottle closures containing a PVC sealing layer with 37 % DINCH showed migration in the range of 10–30 µg/kg after storage periods of up to 25 days (at 28°C) (EFSA, 2006). From a polystyrene sample containing 3 % DINCH, the migration in 50 % ethanol was 53 µg/kg. In 10 % ethanol and olive oil, the migration was < 31 µg/kg resp. < 37 µg/kg (EFSA, 2006). Tests with PVC wrapping film (DINCH content of 10–17.8 %) on the migration into food and food simulants showed values of up to 29 ± 2 mg/dm^2 for sunflower oil and high fat cheese, thus exceeding the overall migration limit of 10 mg/dm^2. Low fat cheese showed a low migration level of 2.4 mg/dm^2.

Studies on DINCH levels in house dust were published by Nagorka et al. (2011). In the 2001 - 2006 period, the authors tested the house dust from about 900 German households with children between 4 and 14 years of age. The samples from vacuum cleaner bags were sieved and the 63 µm fraction was analysed. The limit of quantification (LoQ) for DINCH was 0.31 mg/kg dust. During the pilot phase in the 2001 - 2002 period, DINCH could not be detected in any of the 274 house dust samples tested. This result had been expected since it was not until 2002 that DINCH was introduced into the market. In the main phase of the study, i.e. during the 2003–2006 period (593 samples), DINCH was predominantly used in medical products and toys (Nagorka et al., 2011). During this period, as much as 20 % of the samples were already found to exceed the LoQ. The median was below the LoQ, that means < 0.31 mg/kg dust, the 95th percentile was 2.5 mg/kg dust and the maximum was 112 mg/kg dust (arithmetic mean: 1.2 mg/kg dust, values < LoQ were set 2/3 LoQ). In April 2009, 36 samples (only 7 of these from households with children) were analysed using the same method (Nagorka et al., 2011). In these tests, DINCH could be detected in almost all house dust samples. The concentrations measured were clearly above those found in the 2003–2006 period. The median was 2.2 mg/kg dust, the 95th percentile was 76 mg/kg dust and the maximum was 105 mg/kg dust (arithmetic mean: 12 mg/kg dust).

Fromme et al. (2016) measured 2011/2012 DINCH levels in indoor air and dust samples from 63 daycare centres in Germany. DINCH was present in indoor air with a median value of 108 ng/m^3. In dust, a median value of 302 mg/kg was found for DINCH. In relation to previous studies, the concentration of DINCH in dust has an increasing time trend.

Analysis of Larsson et al. (2017) showed that preschool buildings built after the year 2000 had higher levels of DINCH in dust than older buildings, whereas the banned phthalate di-n-butyl phthalate (DBP) was found in higher concentrations in older buildings.

In a study by Schossler et al. (2011), the DINCH concentration was measured in an emission chamber [Field and Laboratory Emission Cell (FLEC), Chematec] in the gaseous phase under controlled conditions after introduction of a soft PVC sample (DINCH concentration 17 %). At a

temperature of 23 °C and 50 % relative humidity, the DINCH concentration in the air was measured over a period of 5 months. After 50 days, a steady-state concentration of 0.42 ± 0.02 µg/m^3 was reached. For a sample surface of 0.018 m^2, a chamber volume V of 3.5 × 10^{-5} m^3 and an air exchange rate of n = 514/h, the authors calculated an area-specific emission rate of 0.41 µg DINCH per hour and per m^2 for the PVC material tested. Based on the data on the vapour pressure (see table 1) and on the octanol-air partition coefficient (log K_{oa} = − 6.88 at 25 °C and 1013 mbar), DINCH concentrations of < 0.5 µg/m^3 have to be expected in the gaseous phase (Schossler et al., 2011). This estimate has been confirmed by the measurements.

5 General toxicological profile

Presentations of the relevant toxicological studies and their evaluations are included in an EFSA report (EFSA, 2006), in the documentation of the Australian chemicals authority (NICNAS, 2012), and in the publication by Bhat et al., 2014. The use of DINCH in medical devices was assessed by a scientific panel of experts of the EU Commission (SCENIHR, 2008). An assessment of DINCH regarding its use in toys has been performed by the Dutch National Institute for Public Health and the Environment (RIVM, 2009). In 2004, DINCH was also assessed by the German Institute for Risk Assessment (BfR) and included in the recommendations I (as a plasticizer for PVC), XXVII (conveyor-belts), and XXIX (plastic hoses for beverages) (BfR, 2004). Another assessment by BfR was carried out in 2011.

5.1 Toxicokinetics

5.1.1 Studies in rats

Studies on the toxicokinetics according to the OECD Guideline No. 417 (BASF 2003, 2005a) and on the metabolism (BASF, 2005b) with ^{14}C-labelled DINCH have been performed in male and female rats. In general, no sex-specific differences were found in these studies.

After oral administration by gavage, DINCH is rapidly absorbed from the gastrointestinal tract. However, the absorption rate was found to be subject to saturation with increasing doses. After a high oral dose of 1000 mg/kg bw, the bioavailability was only 5–6 %; however, after a low dose of 20 mg/kg bw, it was 40–49 % (BASF, 2003a, 2005a). The highest concentrations in plasma were found about 1 h after oral administration. The distribution in the organs was found to vary; the highest concentrations of radioactivity were found in the gastrointestinal tract, the adrenal glands, and liver, the lowest levels, in the brain, in muscle tissues, and in the bones (BASF, 2003, 2005a). Radioactivity is eliminated from plasma in a biphasic manner. It is rapidly and almost completely excreted in the faeces and urine, however, not in the breathing air. The toxicokinetic data have not provided any indication of DINCH accumulation.

The main metabolite detected in urine after oral administration was 1,2-cyclohexane dicarboxylic acid (CHDA, 0.6–4 % of the radioactivity administered). Via the faeces mainly the unchanged parent substance, DINCH, is excreted (24–76 % of the radioactivity administered), but also 1,2-cyclohexane dicarboxylic acid monoisononyl ester (MINCH, 3–5 % of the radioactivity administered). In the bile, the main metabolite identified was the MINCH glucuronide, but also MINCH by itself and its degradation products. Studies with repeated oral administration did not provide any indication of an increased degradation of the test substance by enzyme induction (BASF, 2005b). Silva et al. (2011 and 2012) analysed the urine of female rats 24 h prior and 24 or 48 h after oral or subcutaneous (sc) administration of 500 mg/kg bw DINCH. Also in this study, the main metabolite detected in urine is CHDA. In addition, MINCH and 14 oxidized metabolites were identified.

5.1.2 Studies with human volunteers

Koch et al. (2013) examined the metabolism of DINCH in humans. The metabolites and the elimination kinetics were determined by means of HPLC - MS/MS with labelled internal standards. Three male volunteers were administered 50 mg DINCH by the oral route (0.552–0.606 mg/kg body weight). Over a period of 48 h, all single urine samples were collected in separate containers and stored deep-frozen until analysis. Within a period of 24 h, circa 13 % of the oral dose were eliminated via the urine in form of DINCH metabolites, oxidized in the aliphatic side chain (cyclohexane-1,2-dicarboxylic acid mono-hydroxyisononyl ester (OH-MINCH), cyclohexane-1,2-dicarboxylic acid mono-oxoisononyl ester (oxo-MINCH), and cyclohexane-1,2-dicarboxylic acid mono-carboxyisooctyl ester (cx-MINCH)); merely 1.7 % of the dose absorbed were eliminated on the 2nd day in form of these

three metabolites. Altogether, 39 % of the dose administered could be recovered as metabolites in the urine within 48 h. Cyclohexane-1, 2-dicarboxylic acid (CHDA) accounted for 23.7 % of the dose administered. Nevertheless, this substance is unsuitable as a biomarker for human biomonitoring due to its lacking specificity for DINCH. Biomarkers of exposure to DINCH, identified as diagnostically specific and sufficiently sensitive include the secondary oxidation products of the alkyl side chain of the monoester MINCH: primarily OH-MINCH, followed by oxo-MINCH, and cx-MINCH. The excretion of the DINCH metabolites in urine as a % of the oral DINCH dose administered has been summarised in table 2.

Table 2: Excretion of DINCH metabolites in urine, in % of the oral DINCH dose administered in humans

	CHDA	MINCH	OH-MINCH	oxo-MINCH	cx-MINCH
0-24 h	22.20	0.65	9.55	1.85	1.67
24-48 h	1.46	0.07	1.18	0.18	0.36
0-48 h	23.7 (19.98–26.54)	0.72 (0.31–1.26)	10.73 (7.70–12.91)	2.03* (1.52–2.56)	2.03 (1.75–2.29)

Table reprinted from Koch et al. (2013), *based upon a new reference standard, oxo-MINCH could be updated to 2.6 % of the applied dose (Schütze et al., 2017)

In a follow-up study of the same working group, Schütze et al. (2017) further investigated the extensive oxidative metabolism of DINCH after oral dosage of 50 mg to three male volunteers (0.552–0.606 mg/ kg bw). New metabolites were tentatively identified and quantified via fragmentation analogies and new standard substances. In addition to the five urinary DINCH metabolites previously reported, two groups of extensively oxidized metabolites were identified, characterised (a) by multiple side chain oxidation and breakdown and (b) by hydroxylation at the cyclohexane ring. In total five newly identified carboxylated breakdown metabolites represented in sum 5.12 ± 0.49 % of the applied dose. MCHxCH (cyclohexane-1,2-dicarboxylic acid mono carboxyhexyl ester) was identified as a major metabolite (2.71 ± 0.34 %) and thus represents the second most important specific metabolite of DINCH after OH-MINCH (10.7 ± 2.1 %). It might be used in future human biomonitoring studies as an additional valuable and specific biomarker to assess DINCH exposure. Less than 1 % was excreted as ring-hydroxylated metabolites (four metabolites identified). The DINCH metabolism in humans is shown in figure 1.

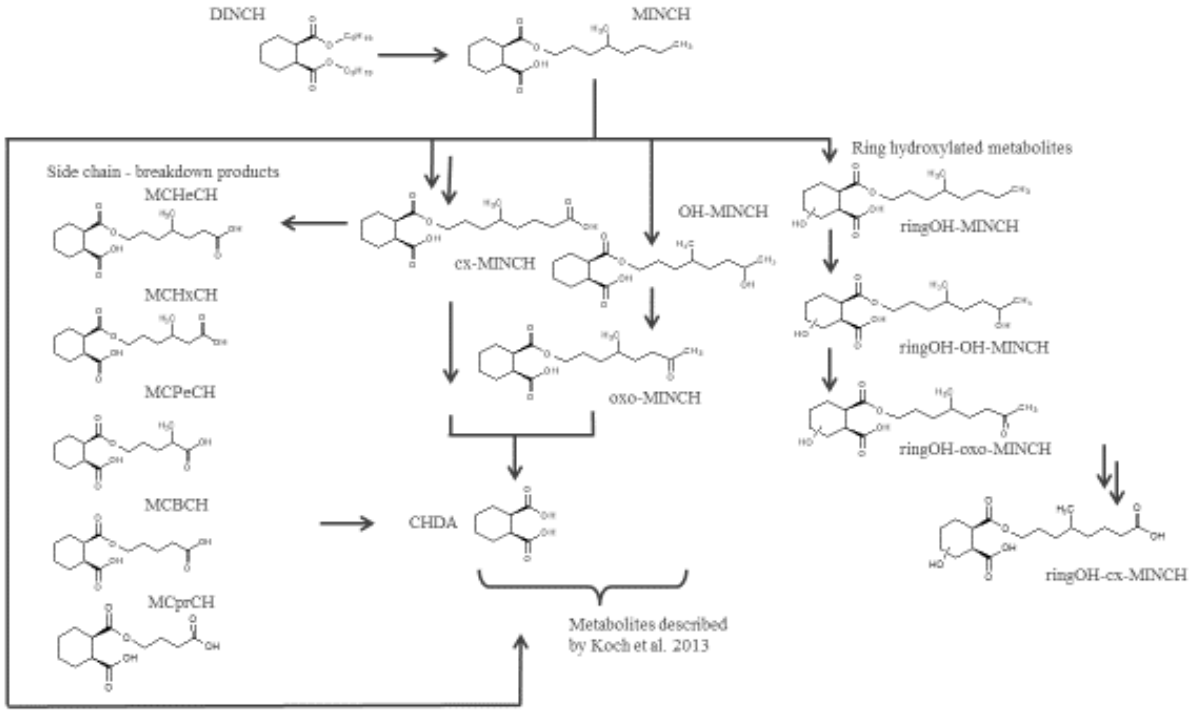


Figure 1: The metabolic breakdown of DINCH as proposed for humans based on a human volunteer study (Schütze et al., 2017; modified)

5.2 Effects on humans

Mínguez-Alarcón et al. (2016) investigated the associations between urinary concentrations of metabolites of DINCH and markers of ovarian response among women undergoing *in vitro* fertilization (IVF) treatments. They could show that there are suggestive negative associations between urinary MINCH concentrations and peak estradiol levels and the number of total oocyte yields. This association was stronger in older women (> 37 years old) compared with younger women (< 37 years old). This is of concern because it is more likely that women over 37 years of age will undergo IVF.

5.3 Effects shown in animal studies

5.3.1 Acute Toxicity

DINCH has shown a low acute toxicity after oral or dermal administration. A study on acute oral toxicity of DINCH (BASF, 1999b) found a LD_{50} of > 5000 mg/kg bw in male and female rats. In dermal toxicity tests (BASF, 1999c), no systemic effects were observed in male and female rats after administration of 2000 mg/kg bw. No studies were found on exposure via inhalation.

5.3.2 Skin and mucosal irritation

DINCH has a slight irritant effect on the skin and no irritant effect on the eyes of rabbits. No effects were found that would have to result in a classification as irritant (BASF, 2004a and 1999d)

5.3.3 Sensitization

The skin-sensitizing potential of DINCH was examined in a guinea pig maximization test (BASF, 1999e). None of the 10 experimental animals showed any sensitizing effect.

5.3.4 Toxicity after repeated administration

5.3.4.1 Oral administration

The effects of DINCH after repeated oral administration were examined in rats in subacute, subchronic and chronic studies performed according to the OECD guidelines.

In a **subacute feeding study** (BASF, 2000b) (OECD 407, 28 days exposure, 14 days recovery, 5 Wistar rats per sex and dose), the highest dose with female rats (1674 mg/kg bw/d) resulted in an increase in the activity of gamma-glutamyl transferase (GGT) and a decrease in bilirubin levels in the serum. These effects were interpreted by the authors as results of adaptive metabolic processes. In male rats of the highest dose group (1585 mg/kg bw/d), degenerated transitional epithelial cells of the urinary tract were found in the urine sediment. All effects seen were reversible. Microscopic examination of liver did not show any signs of cell hypertrophy or any accumulation of liver peroxisomes (NICNAS, 2012). The authors of the study derived a NOAEL of 3000 ppm, corresponding to 342 mg/kg bw/d for female rats, and 318 mg/kg bw/d for male rats. Thyroid weight and thyroid hormone levels were not measured (ANSES, 2016).

No effects on mortality, clinical signs or feed/water consumption were found in a **subchronic feeding study** with 20 six-weeks-old Wistar rats per sex and dose (OECD 408 compliant, 13 weeks; 0, 1500, 4500, 15000 ppm (males: 0,107, 326, 1103 mg/kg bw/d; females: 0, 128, 389, 1312 mg/kg bw/d)) (BASF, 2002a). Minimal to slight hypertrophy/hyperplasia of thyroid gland follicular epithelia was observed for male and female rats in all dose groups including the control group. The incidence of this effect was clearly dose-dependent in males: control (2/20), low dose group (14/20), mid dose group (11/20), high dose group (16/20). A similar dose-dependency was observed in female rats: control (1/20), low dose (1/20), mid dose (3/20), and high dose group (15/20) (NICNAS, 2012). At the highest dose a significant increase of relative weight of liver in both sexes and of thyroid in males was reported in addition to hypertrophy of the thyroid follicles. The relative weight of liver was also increased in females of the mid dose. A significant increase of serum GGT activity and thyroid stimulating hormone (TSH) levels was observed at the highest dose in females. These effects seemed to suggest a mode of action via an enzymatic induction process according to the authors. Nevertheless, TSH response in males and thyroxine (T4)/triiodothyronine (T3) response in both sexes were less consistent (ANSES, 2016; Bhat et al., 2014).

An increase of the testes relative weight was noted at all doses (with no dose-response relationship) but without histological changes in testes or reproductive tract (NICNAS, 2012).

Blood and degenerated transitional epithelium cells were observed in urine of male rats from 4500 ppm on. These effects (from day 86 on no longer statistically significant) were interpreted by the authors as substance-related. Additionally, increased relative kidney weights were observed in male rats from 107 mg/kg bw/d on. However, based on comparison with historical controls, the authors concluded these effects to be toxicologically irrelevant. As to the female rats, the increased relative kidney weight in the highest dose group (1312 mg/kg bw/day) was discussed as whether or not to be an adverse effect. Histopathology did not reveal any treatment-related effects (German HBM Commission, 2014). The NOAEL was set by the authors of the study at 389 mg/kg bw/d for females and 107 mg/kg bw/d for males and refers to kidney effects.

In a **study on cell proliferation over 1, 4, or 13 weeks** (BASF, 2005c), the treated animals (10 Wistar rats per sex and dose) neither developed clinical manifestations nor showed effects on their body weight and feed intake after oral administration of 0, 40, 200 or 1000 mg/kg bw/d of DINCH. While increased cell proliferation was observed at all dose levels, the pattern of response was both organ- and sex-dependent.

Cell proliferation induced by DINCH was observed in the liver and thyroid gland, and to a lesser extent in kidneys (males only). Liver and thyroid cell proliferative effects were apparent in all dose groups of both sexes, but mainly after 1 and 4 weeks of treatment. By week 13, the cell proliferation response had subsided. However, there was evidence of follicular cell hypertrophy of the thyroid, mainly in the mid and high dose groups of both sexes, which progressively increased towards 13 weeks of treatment.

In the kidneys, a significant increase in the proliferation of tubular cells was observed in male rats at doses of 200 mg/kg bw/d and above, after 1 and 4 weeks of treatment. At 13 weeks, the only significant increased labelling was seen in the outer stripe of the medulla but only in the low dose group. The lack of a clear dose-response relationship for the 13-week kidney findings suggests that their toxicological significance is questionable (NICNAS, 2012).

5.3.4.2 Intravenous administration

To assess possible risks of the use of DINCH in blood bags, David et al. (2015) investigated the toxicity of DINCH in rats after intravenous (IV) administration. A series of studies was performed by slow IV injection or IV infusion of DINCH suspended in Intralipid® 20% (20% intravenous fat emulsion). Rats were injected once, followed by 14 days of recovery; injected daily for 5 days followed by 5 days of recovery, or infused for 29 days (4 h/d) followed by 14 days of recovery. Dose levels were 0, 62, 125, and 250–300 mg/kg bw/d. These dose levels represent the limits of suspension and far exceed any anticipated exposures from migration out of plasticized blood bags. Animals were observed for signs of toxicity; body weight and feed consumption were measured; blood collected for clinical chemistry and haematology; and tissues collected and processed for histopathology. Urine was collected during the 4-week study to quantify urinary metabolites of DINCH. The results of the studies indicate that no substance-related toxicity occurred: no effects on behaviour, no effects on organ weight, no effect on serum chemistry including thyroid hormones; and no effect on major organs, especially no testicular toxicity, and no indication for peroxisome proliferation in the liver. The only effects seen were petechia and granulomas. However, the results of metabolite analyses demonstrate that DINCH was bioavailable.

5.3.5 Carcinogenicity

In a combined chronic toxicity/carcinogenicity study (Organisation for Economic Co-operation and Development (OECD) 453 compliant) male and female Wistar rats (50 per sex and dose plus 10 per sex and dose) were exposed via feed to DINCH doses of 0, 40, 200 or 1000 mg/kg bw/d (BASF, 2005d). In the satellite groups, urine analyses and examinations on haematology and clinical chemistry were carried out (sampling after 3, 6 and 12 months). The rats of the satellite groups were killed after 12 months, those of the main groups, after 24 months of exposure and subjected to histopathological examination.

There was neither an increase in malignant neoplasia observed, nor any treatment-related effects on the body weight, the feed and the water consumption, or the mortality rate. Concerning the haematology, mean corpuscular volume (MCV) was slightly but significantly reduced in all treated males and similarly mean corpuscular hemoglobin (MCH) was a little but statistically significant decreased in both low and high dose groups after 6 months. Low and high dose male rats showed decreased MCV and MCH values after 12 months also. Slightly, but statistically

significant increased red blood cell counts were found in mid dose and high dose males after 12 months. Finally, high dose females exhibited higher platelet counts after 12 months. As in previous studies, an increased alkaline phosphatase activity in the serum of high dose male rats after 12 months of DINCH exposure was detected. This was possibly indicative of mild and adaptive impairment of liver function. GGT activity was increased substantially in high dose females and bilirubin levels in serum of both sexes were decreased after administration of the high dose. These effects were interpreted by the authors as results of an adaptive metabolic response. Also, the increase in relative liver weights in female rats observed after the high dose was interpreted by the authors in this sense.

In the urine sediment of male rats of the high dose group, degenerated cells of the transitional epithelium and granular urinary casts and/or casts with degenerated epithelial cells were found. Such casts were also found in the urine sediment of male animals after 200 mg/kg bw/d. These findings of urine analysis were restricted to the sampling performed after 3 months. Examinations carried out later, i.e. after 6 and 12 months, did not show such results. The findings of urine analysis were interpreted by the authors of the study as an adaptive effect because of their temporary occurrence and a lack of corresponding histopathological renal findings. The results of urine analysis could possibly also be associated with the increase in relative kidney weights found in male rats of the satellite groups after 12 months of exposure (+ 8.0 % and 10.4 % after 200 resp. 1000 mg/kg bw/d; only significant after the medium dose). After 12 months of exposure, the absolute kidney weight of the male rats in the satellite group at the medium dose of 200 mg/kg bw/d showed a significant increase by 20 % ($p < 0.01$). After 24 months of exposure, the absolute kidney weight showed an increase by 4.5 % at the same dose, the effect was weakly significant ($p < 0.05$). The absolute kidney weight in male rats was significantly elevated also at the high dose (1000 mg/kg bw/d). Nevertheless, no dose-effect relationship was seen. Altogether, however, these effects were not classified as adverse effects because histopathological examinations of the kidneys had resulted in normal findings.

Thyroid weight was increased in both sexes after the high dose and in male rats also after the medium dose. The thyroid weight increase was associated with follicular cell hyperplasia and the presence of follicular adenomas. In the following table 3, the incidence of thyroid gland adenoma in rats exposed to DINCH during 2 years is reported (U.S. CPSC, 2010).

Table 3: Incidence of thyroid gland adenoma in rats exposed to DINCH during 2 years (U.S. CPSC, 2010)

Dose (mg/kg bw/d)	Males	Females
0	3/50	1/50
40	5/50	3/50
200	11/50*	3/50
1000	14/50**	9/50**

The observed effect of DINCH on the thyroid was discussed by the authors as a secondary effect after induction of the microsomal liver enzymes (see also chapter 6 below): The induction of liver enzymes leads to an increased formation of T4 glucuronide, which is eliminated via the bile. The increased elimination of T4 is followed by a decrease in the T4 serum level and subsequent compensation by means of TSH production which may induce hypertrophy and hyperplasia and eventually, also tumours in the thyroid.

It should however be noted that the thyroid hormone levels were not measured in this study (ANSES, 2016).

Information on changes in organ weights can be taken from the following table 4.

Table 4: Changes in the weight of kidney, liver, thyroid, and uterus observed in the OECD 453 compliant study (NICNAS, 2012; Bhat et al., 2014)

		Males			Females		
Dose (mg/kg bw/d)		40	200	1000	40	200	1000
Rats exposed during 1 year							
Kidney	Absolute weight	+3.1%	**+20.3%****	**+14.2%****			
	Relative weight	-3.8%	**+8. %***	+10.4%			
Liver	Absolute weight	+5.2%	**+15.9%***	+11.1%	+6.0%	+6.0%	**+14.0%****
	Relative weight				+11.5%	**+11.7%****	**+22.2%****
Thyroid	Absolute weight				**-18.5%***	-2.2%	+2.7%
Rats exposed during 2 years							
Kidney	Absolute weight	-1.9%	**+4.5%***	**+3.1%***			
Liver	Absolute weight	+0.8%	**+6.7%***	**+68%***	-1.0%	+6.7%	**+13.8%****
	Relative weight	-2.1%	**+4.5%***	+1.3%	-2.5%	+4.9%	**+14.6%****
Thyroid	Absolute weight	-1.8%	**+68.9%****	**+52.4%****	+6.3%	+13.9%	**+70.4%****
	Relative weight	0.0%	**+71.4%***	**+42.9%****	0.0%	+11.1%	**+55.6%****
Uterus	Absolute weight				-29.2%	**-70.1%***	**-77.5%****

* p <0.05; ** p < 0.01

The decrease in absolute uterus weights was attributed to the lower incidence of uterine adenocarcinomas compared to controls (14/50, 4/34, 3/35, and 2/50 in the control, low-. mid-, and high-dose respectively).

After 2 years of treatment, a significant increase of fibroadenomas was observed in the mammary glands of females treated with the mid and the highest dose. According to the authors, the mammary fibroadenomas are in the range of the historical control, the incidence of mammary fibroadenomas in the concurrent control (2%) being very low compared to historical control data (6-16.1%). The relevance of this finding remains unclear despite the fact that this common benign and spontaneously occurring tumor type is unique to rats. Since in the 1-year interim sacrifice group after treatment with DINCH no findings at all in the mammary gland were reported, any signs of an early induction of fibroadenomas or precursor lesions were missing. Thus, evidence indicated that these mammary fibroadenomas appeared to be only incidental, age-related lesions and should not be considered (Bhat et al., 2014).

5.3.6 Effects on reproduction and development

5.3.6.1 Reproductive toxicity

The effects of DINCH on reproduction were examined in a two-generation study in rats (BASF, 2003b).

Groups of 25 male and 25 female Wistar rats each were administered 0, 100, 300 or 1000 mg of DINCH/kg bw/d. At least 74 days after the beginning of the treatment, the animals (F0 generation) of the same dose group were mated to produce the F1A generation. The F1B generation was produced by another, i.e. subsequent mating of the F0 animals. 25 male and 25 female F1A rats each of the same dose group were mated to produce the F2 generation; the mating took place ≥ 73 days after weaning of the young F1A animals. The study was completed after weaning of the F2 offspring. F0 and F1 rats were subjected to continuous exposure. The F0 animals were killed after weaning of the young F1B animals and examined macroscopically and histopathologically; the same procedure was applied to the F1A parent animals after weaning of the F2 generation.

Reprotoxic effects: DINCH doses of up to 1000 mg/kg bw/d did not have any influence on the oestrous cycle prior to mating of the F0 and F1A females, the mating behaviour, fertility (gestation, parturition, and lactation), semen parameters of the F0 and F1A males found at necropsy (sperm morphology and motility in all dose groups; sperm count in the cauda epididymidis as well as testicles

only in the control and at the highest dose), the weight of sexual organs and their histopathological findings.

Developmental toxicity: Doses of up to 1000 mg/kg bw/d did not result in any effects on the F1 and F2 generations with regard to viability, development of body weight from days 0 to 21 post- partum, and sexual maturation of the F1A parent animals. Likewise, no clinical manifestations or pathological changes were found at necropsy. Altogether, no treatment-related effects were observed in the F1 and F2 generations.

Systemic toxicity: The treatment-related effects on the parent animals in the F0 and F1 generation are presented in table 5.

The histopathological effects on the kidneys and thyroid and the related elevated organ weights were interpreted by the authors of this study as a substance-induced toxicity. All other effects including the induction of liver enzymes were classified as adaptive effects. The evaluation of the effects on the thyroid observed in the F1 generation as adverse is in contrast to the evaluation of the outcome of the subchronic (BASF, 2002a, cf. chapter 5.3.4.1) and chronic feeding study (BASF, 2005d, cf. chapter 5.3.5) by the respective authors. The above studies reported similar effects, however, the authors considered them as secondary to the induction of liver enzymes and/or as toxicologically irrelevant.

According to ANSES (2016) additional studies are missing to consider this mechanism of enzyme induction as the mode of action of DINCH.

In the two-generation study, the NOAEL for developmental toxic and reprotoxic effects was set at 1000 mg/kg bw/d. For the systemic toxicity in the F0 generation, the authors of the study determined a NOAEL of 1000 mg/kg bw/d, and for the systemic toxicity in the F1 generation, a NOAEL of 100 mg/kg bw/d (LOAEL 300 mg/kg bw/d, cf. table 5).

The two-generation study was subjected to re-evaluation both by EFSA (2006) and Bhat et al. (2014) (cf. chapter 6).

Table 5: Systemic effects of DINCH in the two-generation study in rats (BASF, 2003b), effects classified as adverse by the authors are in bold type

Dose (mg/kg bw/d)	Female F0 animals	Male F0 animals	Female F1A animals	Male F1A animals
1000	γ-glutamyl transferase ↑	-	γ-glutamyl transferase ↑	-
	Total bilirubin ↓	-	Total bilirubin ↓	Total bilirubin ↓
	Absolute and relative liver weight ↑	Absolute and relative liver weight ↑	Absolute and/or relative liver weight ↑	Absolute and/or relative liver weight ↑
	Absolute and relative kidney weight ↑	Absolute and relative kidney weight ↑	Absolute and/or relative kidney weight ↑	**Absolute and/or relative kidney weight ↑**
			Absolute and relative thyroid weight ↑	**Vacuolisation of the renal tubular epithelium (in 25/25)**
			Hypertrophy and/or Hyperplasia of the thyroid	
			Change of the thyroid colloid	
300	γ-glutamyl transferase ↑	-	γ-glutamyl transferase ↑	-
	Total bilirubin ↓	-	Total bilirubin ↓	Total bilirubin ↓
	Absolute and relative liver weight ↑	Absolute and relative liver weight ↑	Absolute and/or relative liver weight ↑	Absolute and/or relative liver weight ↑
	Absolute and relative kidney weight ↑	Absolute and relative kidney weight ↑	Absolute and/or relative kidney weight ↑	**Absolute and/or relative kidney weight ↑**
			Hypertrophy and/or Hyperplasia of the thyroid	**Vacuolisation of the renal tubular epithelium (in 9/25)**
			Change of the thyroid colloid	
100	No effects	No effects	Relative kidney weight ↑	Relative kidney weight ↑

5.3.6.2 Developmental toxicity

Studies relevant for the assessment of the effects of DINCH on the embryonic and foetal development were performed in rats and rabbits according to OECD Guideline No. 414. Maternal or developmental toxic effects were reported for doses of ≥ 1000 mg/kg bw/d.

In experiments with Wistar rats (BASF, 2002b), groups of 25 animals were administered DINCH doses of 0 (solvent control olive oil), 200, 600 or 1200 mg/kg bw/d from day 6 until day 19 post-coitum by gavage. The volume administered was 5 mL/kg bw each. Caesarean section was performed on day 20 post-coitum. Also at the highest dose, no maternal toxic effects on feed consumption, the development of body weight, or weight of the uterus were observed and likewise, no clinical manifestations or pathological changes were found at necropsy. Furthermore, no effects were reported regarding the conception rate, or the corpus luteum count, the implantation and resorption rate, the number of live foetuses, or the foetal weight, and the distribution of male and female foetuses. No treatment-related external, visceral or skeletal malformations of the foetuses were found.

In this study, the NOAEL for maternal toxic and prenatal developmental toxic effects was established to be1200 mg/kg bw/d.

In a feeding study with Himalayan rabbits (BASF, 2004b), groups of 25 artificially inseminated rabbits were exposed to 0, 102, 311 or 1028 mg DINCH/kg bw/d on days 6 to 29 after insemination. On day 29, the mothers were killed and subjected to necropsy. Implantations were found in 19 to 24 animals per group. Evaluation was performed similar to the study in rats and as a result, no maternal or developmental toxic effects were found up to and including 1028 mg/kg bw/d. Based on these results, the NOAEL was set as 1028 mg/kg bw/d.

Pre- and postnatal developmental toxic effects in Wistar rats were examined in a gavage study that served as a dose-finding study for the subsequent two-generation study and was partly based on OECD Guidelines No. 414 and 415 (BASF, 2002c). Ten pregnant rats per group were treated with 0, 750 or 1000 mg DINCH/kg bw/d from day 3 post coitum to day 20 post-partum. The solvent control contained olive oil. The mothers (F0) were killed and subjected to necropsy after weaning (day 21 post-partum) of the offspring (F1). The feed intake and weight development of the treated F0 animals corresponded to those of the controls, no clinical manifestations were observed. Likewise, gestation, parturition and lactation remained unaffected by the treatment. Necropsy of the F0 animals did not result in any abnormal findings. All male F1 animals and three female F1 animals per litter were selected for further examinations on day 21, non-selected offspring was discarded. The study was completed 100 to 105 days post-partum by necropsy of the F1 rats. Anogenital distance (AGD) and anogenital index (AGI = AGD divided by kg bw) were measured at day 1 after birth, and sexual maturation was also determined (for males testes descent, balanopreputional separation, penis appearance, and sperm quality, and for females vaginal opening). Clinical examinations, sexual maturation, organ weights, gross and histopathological findings, and sperm motility all showed no indications of substance-related adverse effects in F1 progeny. The AGD ($p < 0.05$) and AGI ($p < 0.01$) were significantly decreased in the male high dose group (1000 mg/kg bw/d), respectively AGD 7% and AGI 8% below the control group. In the female high dose group the AGI was significantly reduced by 8% ($p < 0.05$).

The analysis of these observations is not unanimous. According to the SCENIHR report (2008) and NICNAS (2012), the limited (7-8% change compared to controls) although significant alterations in the AGD and AGI are not considered of biological significance as other corresponding parameters (like testes descent, preputial separation, vaginal opening, testes weight and histology, and sperm parameters) were not affected. Moreover, in females the AGI was decreased to the same extent, contradicting the AGI to be an effect of impaired androgen-dependent development. However, Silva et al. (2013) reported that the apparent effect was likely exaggerated due to the slight increase in body weight among the respective female pups. Thus, the significant change in AGI would be likely driven by the very slight increase in female pup body weights combined with the very slight but not significant decrease in AGD. Moreover, according Silva et al. (2013), the reported reduction in female AGI does not reduce the validity of the male AGD findings since in 2005 Piepenbrink et al. reported that the AGD was affected in neonatal female rats exposed in utero to DEHP by gavage for the last 16 days of gestation. But this is not always the case since in a study by Gray (2000) the AGD was decreased by 29% in male pups compared to controls and there was no effect seen in females, when animals were exposed during a full 2-generation study. Nevertheless, no reproductive effects were reported in the 2-generation study performed with DINCH. Therefore, despite an effect on the AGD the reproductive function seemed not to be consequently affected (ANSES, 2016).

Furr et al. (2014) have described the results of a foetal phthalate screening test examining the influence on the foetal testosterone production and the gene expression of the steroidogenesis. Pregnant Sprague-Dawley rats were administered the respective phthalate and also DINCH (gavage, in maize-germ oil, dose: 750 mg/kg bw/d) from day 14 to day 18 of gestation, i.e. within the critical

time window for the sexual differentiation of the male animals. On day 18 of gestation, the foetuses were removed and their testicles used for ex vivo determination of the testicular testosterone production and for the determination of testicular gene expression patterns via RT-PCR array. DINCH did not exert effect on the testis testosterone production.

In another study by Nardelli et al. (2017) timed-pregnant Sprague-Dawley rats were gavaged with vehicle or with DINCH or DEHP i.a. (30 or 300 mg/kg bw/d) from gestational day 8 until postnatal day (PND) 21. The offspring were examined for effects on developmental and endocrine markers until PND 46. While DINCH had no effects on many of the endpoints associated with DEHP exposure, it did significantly increase the incidence of hemorrhagic testes in exposed offspring. The increase in hemorrhagic testes occurred already at 30 mg/kg bw/d. The authors of this study emphasize the need for a deeper exploration of the possible endocrine disruptive properties of DINCH.

5.4 Toxicity in *in vitro* and *in silico* studies

An *in vitro* study on the effects of DINCH on rat primary stromal vascular fraction (SVF) of adipose tissue was performed by Campioli et al. (2015). The authors concluded that DINCH and its metabolite CHDA do not affect SVF differentiation directly, but MINCH showed to be a potent PPAR-α agonist and a metabolic disruptor.

Nardelli et al. (2015) used the immortalised TM4 Sertoli cell line in order to screen different DEHP substitutes for toxicogenomic effects. The exposure to DINCH resulted in an altered expression of a large number of genes involved in major signal transduction pathways including ERK/MAPK and Rho signalling, which is why the authors suggested that DINCH is biologically active.

Cell survival, proliferation, steroidogenesis and mitochondrial integrity after treatment with DINCH was investigated by Boisvert et al. (2016) using the mouse MA-10 Leydig and C18-4 spermatogonial cell lines. A biphasic effect could be observed on steroid production in MA-10 and fetal Leydig cells.

In another recent *in vitro* study Eljezi et al. (2017) examined cytotoxic effects of DEHP-alternative plasticizers on a L929 cell line which is androgen receptor positive. MINCH was found to cause a decrease in cell viability and to reduce the cell proliferation significantly at 0.1 mg/mL. In this study DINCH is considered as potentially toxic in the standard EN 10993-5.

A structural binding study on DEHT, TOTM, and DINCH was conducted by Sheikh et al. (2016) to help in predicting potential endocrine disrupting risks of the three alternate plasticizers against sex hormone-binding globulin (SHBG). Induced Fit Docking of the three alternate plasticizer compounds indicated that each of the three compounds fitted well into the steroid binding pocket of SHBG. About 82–91 % of the SHBG interacting residues for the bound ligand, DHT, overlapped with SHBG interacting residues for the three alternate plasticizers. The Dock score and Glide score were highest in DHT and progressively decreased for DINCH, TOTM, and DEHT. However, the three alternate plasticizers showed high binding affinity with SHBG that was higher than that for the native bound ligand, DHT, indicating more tight interactions with the SHBG. Thus, there is according to the authors, on a preliminary basis, a high risk of these alternate plasticizers i.e. DEHT, TOTM, and DINCH binding to the SHBG in the circulation and potentially displacing the endogenous testosterone and estradiol leading to potential disruption of the androgen-estrogen homoeostasis in the body.

5.5 Genotoxicity

DINCH was found to be non-genotoxic, both in *in-vitro* studies and *in vivo*. The studies were performed according to OECD guidelines, the individual results are presented below:

DINCH was non-mutagenic in the bacterial mutation assay with Salmonella typhimurium TA1535, TA100, TA1537, TA98 and Escherichia coli WP2uvrA both with and without metabolic activation (BASF, 2000c). The solvent used was acetone. Testing was performed with concentrations from 20 µg/plate to 5 mg/plate.

DINCH did not show any clastogenic or aneugenic effects in the chromosome aberration assay with V79 cells of the Chinese hamster in three independent experiments with different periods of exposure and culture (BASF, 2000d). The solvent used was acetone. Testing included concentrations of up to 1 mg/mL.

DINCH was also found non-mutagenic in an HPRT gene mutation test with CHO cells (BASF, 2001a). Testing included concentrations up to 5 mg/mL (solvent acetone) in 2 independent test series performed both with and without addition of a metabolizing system.

In a micronucleus assay in mice, DINCH did not show any clastogenic or aneugenic effects (BASF, 2001b). In a first test, 6 male NMRI mice were injected intraperitoneally 0 (solvent olive oil), 500, 1000 or 2000 mg/kg bw of DINCH per dose. The positive control was treated with cyclophosphamide. The animals were killed 24 h after administration, the bone marrow was prepared and the micronuclei in the polychromatic erythrocytes were determined. No increase of the micronucleus rate was measured in any of the treatment groups, however, a clear increase was found in the positive control. Likewise, no mutagenic effect was observed in a second test with a dose of intraperitoneally 2000 mg/kg bw and a survival time of 48 h.

6 Evaluation

Different panels and authors who had reviewed the data base of DINCH for risk assessment focused on different key studies and endpoints (see table 6).

6.1 European Food Safety Authority

EFSA (2006) derived a TDI of 1 mg/kg bw/d based on nephrotoxic effects. On the one hand haematuria and degenerated cells of the transitional epithelium in the urine of male (and female rats[3]) observed in the subchronic feeding study (BASF, 2002a) are considered as relevant effects (NOAEL 107 mg/kg bw/d for males). On the other hand effects on the kidneys found in the two-generation study in rats are classified as relevant (BASF, 2003b), namely the vacuolisation of the tubular epithelium observed in male F1 rats at the dose of 300 mg/kg bw/d and above. The NOAEL of this study was 100 mg/kg bw/d. Using an assessment factor of 100 (for inter- and intraspecies differences) a TDI of 1 mg/kg bw/d was derived from this NOAEL.

The DINCH-induced hypertrophy/hyperplasia of the thyroid found in the subchronic and two-generation study as well as the increased incidence of thyroid adenomas in the chronic study (BASF, 2005d) were not considered to be a suitable parameter for TDI derivation due to a higher sensitivity of rats as compared to humans.

6.2 National Industrial Chemicals Notification and Assessment Scheme (NICNAS)

NICNAS (2012) considered effects on kidney, namely elevated kidney weights in male rats as observed in the chronic feeding study (BASF, 2005d) to be the relevant effect for risk assessment. In contrast to the study authors, NICNAS didn't interpret the elevated kidney weight as an adaptive process because of lacking evidence, such as the induction of the corresponding enzymes in the kidneys. The NOAEL for this endpoint was 40 mg/kg bw/d. By using an assessment factor of 100, a TDI of 0.4 mg/kg bw/d was derived[4].

Reasoning in detail (essentially literally):

Kidney effects

Treatment-related increases in kidney weights were predominantly observed in male rats in the 90-day and 2-year repeated dose studies, in the cell proliferation study, and in the 2-generation study. While these increases correlate with the observation of male-only kidney cortical cell proliferation in the S-phase response study (although this finding was of questionable significance), increased kidney weights were also observed in female rats in the 90-day and 2-generation studies. No data was available regarding the reversibility of kidney weight changes.

The treatment-related vacuolisation of the tubular epithelia of male F1 animals in the two-generation study is considered a relevant effect of treatment with DINCH, although what it may indicate is uncertain (NOAEL: 100 mg/kg bw/d).

Degenerated epithelial cells were found in the urine of male rats in the 28- and 90-day studies. The notifier's toxicology laboratory reports that these effects are a transient effect in younger animals of the strain used, and have been observed following treatment with several structurally unrelated

[3] Evaluation of the original study (BASF, 2002a) by the German Fraunhofer Gesellschaft (FhG) on behalf of UBA did not indicate any occurrence of degenerated cells of the transitional epithelium in the urine of **female rats**.

[4] There are a number of weak points regarding the selection of this study as a pivotal study: 1) No abnormal renal findings were made in the histopathological examinations; 2) There was no increase in the relative kidney weight after 24 months in any of the treatment groups; and 3) A significant increase ($p < 0.05$) in the relative kidney weight in male rats was only found after 12 months of exposure to 200 mg/kg bw/d.

chemicals. This claim is supported by the fact that these effects were not observed in the 2-year study or in animals over 20 weeks of age in the 2-generation study. However, in both the 28-day and the 90-day studies, these findings were reported as treatment-related adverse effects, and no data has subsequently made available to support this claim. In the 90-day study, mid- and high-dose animals also showed increased blood cells in urine at one measurement interval (NOAEL: 107 mg/kg bw/d for males).

Liver effects and enzyme induction

Treatment of test animals with DINCH was found to result in increases in liver weights in the 90-day and 2-year repeated dose studies, in the cell proliferation study, and in the 2-generation study. Other signs of liver effects in these studies included elevations of serum γ-glutamyltransferase activity and decreased serum bilirubin concentrations. No histopathological evidence of liver toxicity was observed. This spectrum of treatment-related effects is known to result from hepatic enzyme induction. To show that the notified chemical was able to induce liver enzymes, two special studies were carried out. These studies showed that (1) DINCH is an inducer of both phase I and phase II enzymes in the liver (BASF, 2005e), and (2) that treatment of rats with DINCH induces cell proliferation in the liver that accounts for the increased organ weights observed (BASF, 2005c). Therefore, any observed effects that are thought to result from liver enzyme induction are interpreted to be adaptive metabolic changes, and not pathological changes.

Thyroid effects

The 2-year combined chronic toxicity/carcinogenicity study with DINCH revealed effects on the thyroid as the most significant adverse effect. The key findings were increased absolute and relative thyroid weight, altered thyroid colloid, and an increased incidence of thyroid follicular cell adenomas at 24 months. Thyroid follicular cell proliferation and changes in TSH levels were also observed at comparable dose levels in the 90-day rat study, in a 13 weeks cell proliferation study, and also in female rats in the 2-generation reproduction toxicity study.

The notifier has argued that these thyroid effects are not significant for human health risk assessment because of differences between rats and humans in thyroid hormone handling and sensitivity to thyroid-disturbing mechanisms. This conclusion is reasonable, and consistent with EFSA (2006) and IARC (Rice et al., 1999) opinion on the significance of thyroid follicular cell tumours induced by chemicals which alter thyroid hormone metabolism and which demonstrate a lack of genotoxic potential. It is reasonably well established that in the rat, thyroid follicular-cell tumours are commonly associated with imbalances in TSH levels resulting in sustained stimulation of the thyroid gland by TSH feed-back stimulation of the hypothalamic-pituitary-thyroid axis, leading to secondary hyperplastic or neoplastic changes with thyroid adenoma or carcinoma formation. The human thyroid gland is much less susceptible to this pathological phenomenon than rodents (Capen, 1997). Even in patients with markedly altered changes in thyroid function and elevated TSH levels, there is little if any increase in the incidence of thyroid cancer (Capen, 1997; Curran and DeGroot, 1991). The increased sensitivity of the rodent thyroid gland to perturbations by drugs and chemicals is related to the shorter plasma half-life of thyroxine (T4) in rodents (12-24 h) when compared to humans (5-9 days), due to the considerable differences in the transport proteins for thyroid hormones between species (Capen, 1997). In humans, serum T4 is bound primarily to thyroxine-binding globulin, a protein that is not present in rodents.

The proposal that thyroid effects of the notified chemical in rats are associated with an indirect mechanism was supported by the performance of special mechanistic studies. These demonstrated that, at relevant dose rates in rats, hepatic metabolic pathways involved in T4 conjugation are strongly induced, and that T3, T4 and FSH levels are perturbed in a manner consistent with an indirectly acting enzyme inducer (phenobarbital) (BASF, 2005f). The effects observed were not comparable to

those associated with a direct inhibitor of iodine incorporation into thyroid hormones (propylthiouracil). The effects of DINCH on thyroid hormone metabolism are also not unlike those of polyhalogenated biphenyls, which increase the glucuronidation of T4 and increase TSH, and thyroid uptake of iodine in rats, but less so in mice (Capen, 1997; Craft, 2002). What is not known (although it appears to be unlikely for the notified chemical), is whether hydroxylated metabolites can have a direct effect on thyroid hormone receptor-activated gene expression, as has been suggested for PCB metabolites (Kimura-Kuroda, 2007).

A dose-dependent increase in the incidence of altered thyroid follicular colloid (described as “flaky”) was observed in female animals in the 2-year study (at 12 months), in male rats after 13 weeks in the cell proliferation study, and in female F1 rats in the 2-generation study (<1 year at terminal sacrifice). This effect is neither assumed to be an effect of aging, nor is it known to be caused by liver enzyme induction. Therefore, as the nature and/or pathogenesis of the effect are not known, it cannot be considered as non-adverse. Altered colloid was observed at 300 mg/kg bw/day (F1 females) in the 2-generation study (NOAEL: 100 mg/kg bw/d).

Lack of proliferative effects on peroxisomes

No peroxisome proliferative effects related to activation of the PPARα were observed for DINCH (c.f. phthalate esters like DINP). No effects were observed on cyanide-insensitive palmitoyl CoA oxidase in the 90-day study, and no peroxisome accumulation was observed in any of the repeated dose oral toxicity studies. Also unlike DINP, DINCH caused no increase in the incidence of hepatic or pancreatic acinar cell tumours, nor did it appear to cause testicular degeneration (NICNAS, 2007).

6.3 Bhat et al.

In the opinion of Bhat et al. (2014), sufficient data are available fulfilling the U.S. EPA criteria for evidence of a potential for thyroid impairment via a hormone-mediated MOA with a threshold (EPA, 1998). Therefore, the authors have considered the two-generation study in Wistar rats to be the pivotal study for deriving a RfD. In this study, the adult F1 generation showed a higher incidence of thyroid and renal effects, as compared to the rats of the chronic study (see chapter 5.3.5, OECD 453 compliant). Based on human equivalent doses, benchmark modelling was conducted, which resulted in a $BMDL_{10}$ of 21 mg/kg bw/d for the endpoint of thyroid hypertrophy/hyperplasia seen in adult F1 rats also exposed in utero. Taking into account an assessment factor of 10 for intraspecies differences and an assessment factor of 3 due to the lack of a second chronic study in another animal species, a RfD of 0.7 mg/kg/d was derived. An interspecies extrapolation factor was not applied since rodents are more sensitive than humans with respect to the proposed indirect MOA for thyroid gland lesions.

BMD modelling was also performed for the endpoint renal tubular vacuolization (males) resulting in a $BMDL_{10}$ of 41 mg/kg bw/d. Overall, the lowest human equivalent $BMDL_{10}$ value and thus POD for the RfD was considered to be the human equivalent $BMDL_{10}$ of 21 mg/kg bw/d for thyroid follicular hypertrophy/hyperplasia in adult female F1 rats from the two-generation study (BASF, 2003b), and the log-logistic model was the best fitting model.

6.4 Other assessments

The Scientific Committee on Emerging and Newly-Identified Health Risks derived a NOAEL of 107 mg/kg bw/d based on the renal effects observed in the subchronic feeding study in rats (SCENIHR, 2008; BASF, 2002a). A TDI was not stated.

In a report of the U.S. Consumer Product Safety Commission, a NOAEL of 40 mg/kg bw/d has been stated by the authors based on the chronic feeding study in rats, with reference to the thyroid effects (U.S. CPSC, 2010; BASF, 2005d). A TDI was not derived.

The National Institute for Public Health and the Environment (RIVM, the Netherlands) used for risk assessment with regard to the exposure from toys, a NOAEL of 100 mg/kg bw/d based on the two-generation study (Janssen and Bremmer, 2009; BASF, 2003b), thus being in accordance with the assessment by EFSA (2006).

Table 6: Overview of NOAELs and TDIs/reference doses derived for DINCH

Evaluating panel	Crucial effects	Crucial study	NOAEL (mg/kg bw/d)	TDI/RfD
EFSA	Nephrotoxicity: Haematuria, degenerated cells of the transitional epithelium in the urine, vacuolation of tubular epithelium	Subchronic study and two-generation study	100	TDI: 1 mg/kg bw/d
NICNAS	Elevated kidney weights	Chronic study	40	TDI: 0.4 mg/kg bw/d
Bhat et al.	Hypertrophy and hyperplasia of the thyroid in the F1 generation	Two-generation study	$BMDL_{10}$: 21 (human equivalent dose)	RfD: 0.7 mg/kg bw/d
SCENIHR	Nephrotoxicity: Haematuria, degenerated cells of the transitional epithelium in the urine	Subchronic study	107	Not derived
RIVM	Nephrotoxicity: Vacuolation of tubular epithelium	Two-generation study	100	Not derived
U.S. CPSC	Thyroid effects: Adenomas	Chronic study	40	Not derived

6.5 Overall assessment

The existing data base for DINCH does not allow for the derivation of health-based guidance values from human data. But there is a substantial data set on toxicity to laboratory animals to serve as the basis for hazard identification of DINCH. Target organs detected in repeated dose toxicity tests, and studies on carcinogenicity and reproductive toxicity are kidney, thyroid, liver, and mammary glands.

Nephrotoxic effects are considered relevant by the majority. Depending on the study used and the endpoint chosen the TDI is between 0.4 and 1 mg/kg bw/d. The chronic study has a number of weak points regarding the selection of this study as a pivotal study: 1) No abnormal renal findings were made in the histopathological examinations; 2) There was no increase in the relative kidney weight after 24 months in any of the treatment groups; and 3) A significant increase ($p < 0.05$) in the relative kidney weight in male rats was only found after 12 months of exposure to 200 mg/kg bw/d. This is the reason why the German Human Biomonitoring Commission based the HBM value derivation on the TDI of 1 mg/kg bw/d, derived by EFSA on the basis of the two-generation study.

The effects observed on the thyroid gland are discussed controversially. According to the authors of most of the studies, the REACH registrants, as well as institutions like EFSA and NICNAS, the effects observed on the thyroid gland are secondary (compensatory) effects only associated with liver enzyme induction and therefore of limited relevance to humans. But nevertheless, these effects would well correlate with Gamma-GT levels and increased liver weights, and with humans the thyroid-liver axis also exists (Malik and Hodgson, 2002). According to ANSES (2016), it should be pointed out that lot of other mechanisms than induction of liver enzymes can explain a hyperthyroidism, and an increase of TSH level which have not been examined. U.S. CPSC (2010) perceives thyroid effects observed in rodents as relevant for humans and quotes the U.S. EPA point of view who considers that rodent noncancer thyroid effects resulting from disruption of the thyroid-pituitary axis are presumed to pose a noncancer health hazard to humans and that rodent cancer effects resulting from this mechanism may pose a cancer health hazard to humans, and the effects on thyroid observed in this study may indeed be relevant to humans.

Also, other scientists argue that induction of Phase II enzymes is a relevant mode of action (MoA). Concurrent elevated TSH levels and increased thyroid organ weights are regarded as clear adverse effects and should be used a basis for setting a POD. Therefore, Bhat et al. (2014) conducted a benchmark modelling for the endpoint of thyroid hypertrophy/hyperplasia seen in the two-generation study which resulted in a $BMDL_{10}$ of 21 mg/kg bw/d (human equivalent dose). Taking into account an assessment factor of 10 for intraspecies differences and an assessment factor of 3 due to the lack of a second chronic study in another animal species, a RfD of 0.7 mg/kg/d was derived.

2009 it has been shown that a possible link exists between hypothyroidism and hepatocellular carcinoma in humans (Hassan et al., 2009). However, it should be noted that the question of relevance of effects on thyroid observed in rodents is not specific to the DINCH itself but is a general question. This topic has to be touched upon at the European level, as asked by France in the ECHA Endocrine Disruptor expert group in November 2014 (ANSES, 2016).

Based on all the information available, the increase of fibroadenomas in mammary glands and foci in liver are not considered to be of substantial relevance given that mammary fibroadenomas in female rats are common benign and spontaneously occurring tumours unique to rats and no clear dose-response relationship for the increase of foci in the female liver exists, resp. only a slight increase in numbers of male liver foci, but only in the mid- and high-dose satellite groups (12 months).

Concerning the effects of DINCH on the reproduction, the various studies available indicate that DINCH has only minor concerns. The effect on the AGD observed in the development study in male

and female pups can be considered as weak and the effect observed for males is well below the decrease observed following an in utero exposure to DEHP for instance (Gray, 2000). It is nevertheless indicating an anti-androgenic activity which is however not related to any histologic changes or to changes in testis weight, or sperm parameters. The absence of anti-androgenic activity seems also to be confirmed by the absence of effects seen in the study by Furr et al. (2014). The study results of Nardelli (2017) are heterogeneous and need further clarification and classification. Not any possible related adverse effects were identified in the two-generation study available.

However, there may be a remaining concern for newborns exposed through PVC feeding tubing since newborns and premature children are considered as sensitive populations and therefore at risk, taking into account that the cognitive skills are closely linked to a proper thyroid functioning and since the mode of action of the substance remains unknown, despite some assumptions (ANSES, 2016).

Taken together all the information presented, there are good reasons to derive the health-based HBM guidance values on the TDI derived by EFSA. On the other hand, the TDI/RfD value derivation by other institutions remains a contentious issue. Therefore, with reference to the uncertainties related to the mode of action and the critical effect chosen a low level of confidence is attributed to the proposed values (see chapter 9). If a newly elaborated or updated TDI value is published in the future by a recognised body on the basis of new toxicological evidences or reasoning towards the choice of the endpoint, the revision of the hereby derived HBM HBGVs will be considered.

7 HBM parameters and analysis

Biomarkers identified as valid for a diagnosis of exposure to DINCH include oxidation products of the alkyl side chain of the monoester, MINCH:

- OH-MINCH, cyclohexane-1,2-dicarboxylic acid mono-hydroxyisononyl ester,
- cx-MINCH, cyclohexane-1,2-dicarboxylic acid mono-carboxyisooctyl ester, and
- oxo-MINCH, cyclohexane-1,2-dicarboxylic acid mono-oxoisononyl ester (Koch et al., 2013).

Because oxo-MINCH has until now been predominantly described in semi-quantitative terms, and only recently, an analytical standard has been developed synthetically, the focus has so far mainly been on the analysis of OH-MINCH and cx-MINCH. Consequentially, those 2 metabolites should be taken into consideration for a HBM HBGV derivation. For reasons of preciseness, it is proposed to include both metabolites in the HBM HBGV derivation for the general population (German HBM Commission, 2014). The different elimination half-life periods are as follows: OH-MINCH: 10 h, cx-MINCH: 16.5 h (derived graphically, $t_{1/2}$ after 12-48 h) resp. OH-MINCH: 9.5 h, cx-MINCH: 18 h (calculated values, $t_{1/2}$ after 12-48 h) (Koch et al., 2013).

Analysis is performed by means of HPLC-MS/MS. The preparation of samples includes the addition of the radiolabelled internal standards for the DINCH metabolites OH-MINCH and cx-MINCH, and the cleavage of possible conjugates of these two metabolites by means of beta-glucuronidase. The LoQ values for the oxidised metabolites are close to 0.05 µg/L. The relative standard deviations of the concentrations of both metabolites for the measurements in series and the day-to-day measurements are below 10 % (Schütze et al., 2012).

Results of human biomonitoring of DINCH metabolites

In the context of a research project commissioned by the German Environment Agency, the new human biomonitoring analysis method for DINCH and its metabolites was applied to analyse urine specimens of the human environmental specimen bank (ESB Hum) (Schütze et al., 2014). It was intended to determine, for the first time, body burdens in a sub-cohort of the German general population and to identify a possible time course of these values over the period the specimens had been collected (1995-2012). Altogether, 300 24-h urine specimens were analysed in the context of the study. These had been collected in 1999, 2003, 2006, 2009 and 2012. For each year, 60 samples were tested, among these, 30 samples each from female and male test persons. The samples mainly originated from students of the University of Münster aged between 20 and 30 years.

The test results revealed OH-MINCH to be the dominating DINCH metabolite, which could be detected in 29.7 % of all samples. Other metabolites include cx-MINCH (24 %), oxo-MINCH (22 %) and MINCH (1.7 %).

Based on the DINCH metabolites analysed, a clear correlation was found with the year when the samples had been collected: up to and including 2003, not any DINCH metabolites could be found in the samples. As from 2006 up to 2012, an increase was found both in the percentage of urine samples tested positive for DINCH metabolites, and in the concentration of metabolites in the individual urine samples. In 2012, DINCH metabolites could be detected and quantified in almost all samples tested (98.3 %). Also, the highest metabolite levels detected were measured in this last year of the sample series. The geometric mean of OH-MINCH increased significantly from concentrations below the LoQ in 2006 to 0.40 µg/L in 2012. Similar trends were also observed for the metabolites, cx-MINCH and oxo-MINCH. Also, the concentration of cx-MINCH increased from a value below the LoQ in 2006 to 0.18 µg/L in 2012, that of oxo-MINCH from a value below the LoQ in 2006 to 0.25 µg/L

in 2012. For the analyte MINCH of which only a small share is eliminated, the detection rates and concentrations determined were too low to identify a significant temporal trend.

For the samples of the year 2012, the resulting 95th percentile for OH-MINCH was 2.09 µg/L, and the resulting maximum value 236 µg/L. For cx-MINCH, the resulting 95th percentile was 0.86 µg/L, and the resulting maximum value, 98.4 µg/L. A further examination of the time trend up to now is currently under way and may also allow a first estimation of possible sex differences.

In 2016 and 2017 additional results on human exposure to DINCH have been published. The urinary levels of DINCH metabolites have been determined in Norway (adult study population, 2013/14) (Giovanoulis et al., 2016), in Germany (children, 2011/12) (Fromme et al., 2016), and in Portugal (children, 2014/15) (Correia-Sa et al., 2017). In the Portugal study the range of children age was pre- and post-pubertal which means effects may be different due to hormonal status. The exposure of children seems to be 5 times higher than the one of adults. Due to the increasing presence of DINCH in plastic products, levels found are higher than those reported before 2012.

8 Derivation of health-based HBM guidance values

Health-based HBM guidance values are derived by leaning on the procedure described by the German HBM Commission in 2007 and 2014, see also Apel et al., 2017:

HBM HBGV$_{GenPop}$ = [TDI × (averaged molecular weight metabolites/molecular weight DINCH) × Fue]/amount of urine [L/kg bw/d]

- Determination of the ratio of averaged molar masses of the metabolites, OH-MINCH and cx-MINCH, to the molar mass of DINCH: [(OH-MINCH + cx-MINCH): 2]/ DINCH = [(314 + 328): 2]/424.7 = 0.75
- Determination of the metabolic conversion factor (fractional urinary excretion ratio of biomarkers relative to the oral dose of DINCH taken up). The 48 h values for excretion are preferred for determining the F_{ue} instead of the 24 h values because within this time frame a nearly complete excretion is covered. According to the results of the toxicokinetic study from Koch et al. (2013), 10.73 % of orally ingested DINCH are eliminated within 48 h in the urine in the form of OH-MINCH, and 2.03 %, in the form of cx-MINCH, i.e. altogether 12.76 %. Thus, the urinary excretion factor (Fue) is 0.1276. A shortcoming to be kept in mind however is, that this Fue was deduced from a study with 3 males only.
- Amount of urine for children: 0.03 L/kg bw/day; for adults: 0.02 L/kg bw/d.

HBM HBGV$_{GenPop\ (OH\text{-}MINCH\ +\ cx\text{-}MINCH)\ children}$: [1000 × 0.75 × 0.1276]/0.03 = 3190 µg/L;
rounded value 3 mg/L

HBM HBGV$_{GenPop\ (OH\text{-}MINCH\ +\ cx\text{-}MINCH)\ adults}$: [1000 × 0.75 × 0.1276]/0.02 = 4785 µg/L;
rounded value 4.5 mg/L

9 Level of confidence attributed to the derived HBM HBGVs

As mentioned in the HBM4EU concept paper to derive HBGVs, it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBGV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to revise later on HBGVs with estimated 'low' or 'medium' level of confidence. However, it should be specified that the levels of confidence proposed in this document are not determined according to an established recognised methodology, but rather rely on expert judgment regarding the reliability of the data and the calculation method used to derive the HBGVs. The levels of confidence attributed to the $HBGV_{Gen\ Pop(adult)}$ and $HBGV_{GenPop\ (children)}$ are both set to '**low'** (see note n°2 of the factsheet, section 11).

10 Summary and outlook

The exposure of the general population to DINCH should be determined by measuring the sum of the DINCH metabolites OH-MINCH and cx-MINCH in morning urine (µg/L), and should be evaluated by comparing the analytical results with the respective HBM HBGVs for adults (4.5 mg/L) and children (3 mg/L). The derivation of the HBM HBGVs for the general population is based on the conversion of an external dose (TDI) (EFSA, 2006) to its estimated equivalent internal dose. The TDI in this connection refers to nephrotoxicity.

Further effects observed on the thyroid gland are discussed controversially and were not considered for setting a POD, but would have led to a value of similar order of magnitude.

However, there may be a remaining concern for newborns exposed through PVC feeding tubing since newborns and premature children are considered as sensitive populations and therefore at risk, taking into account that the cognitive skills are closely linked to a proper thyroid functioning and since the mode of action of the substance remains unknown, despite some assumptions (ANSES, 2016).

Concerning the effects of DINCH on the reproduction, the various studies available indicate that DINCH has only minor concerns. The effect on the AGD observed in the development study in male and female pups can be considered as weak and the effect observed for males is well below the decrease observed following an in utero exposure to DEHP for instance (Gray, 2000). It is nevertheless indicating an anti-androgenic activity which is however not related to any histologic changes or to changes in testis weight, or sperm parameters.

With reference to the uncertainties related to the mode of action and the critical effect chosen a low level of confidence is attributed to the proposed values. Thus, there is a need for further robust data regarding the mode of action of DINCH which could be tackled by the partners from WP13 on “Establishing exposure-health relationships”.

An incentive to reduce uncertainties towards the HBM HBGVs hereby derived (with a low level of confidence) would be to have a validated PBPK or TK model for DINCH enabling the extrapolation of the selected animal POD value to the corresponding human POD value, thereby avoiding the default value of 10 as AF for the interspecies differences applied to the animal PODs to obtain the TDI. Thus, a PBPK or TK model for DINCH validated by WP12 working on toxicokinetic modeling could allow reducing the uncertainty factors applied for the derivation of the HBGVs.

Also, further research efforts are necessary to ensure that metabolism and sensitivity are similar in adults and children.

11 Factsheet on the HBM HBGVs for the general population

Rapporteur: UBA **Reviewer: ANSES**			
Compound	**DINCH** Diisononylcyclohexane-1,2-dicarboxylate		**Factsheet** **General population**
Parameter	**Note**	**Value / descriptor**	**Comments**
$HBGV_{GenPop}$ and status			
Target population: general population			
$HBGV_{GenPop}$	1	**Σ [OH-MINCH and cx-MINCH] in urine:** **Children: 3 mg/L** **Adults: 4.5 mg/L**	
Level of confidence	2	**low**	
$HBGV_{GenPop}$ status	3	provisional	
$HBGV_{GenPop}$ year of issue	4	2017	
General Information			
CLP-INDEX-Nr.	5	-	
EC-Nr.	6	431-890-2	
CAS-Nr.	7	166412-78-8	
Harmonised CLP classification	8	-	
Molar mass	9	424,7 g/mol	
Biomarker(s)			
Identification	10	urinary OH-MINCH and cx-MINCH	specific oxidized metabolites
Molar mass of biomarker(s)	11	OH-MINCH: 314 g/mol cx-MINCH: 328 g/mol	
Toxicokinetic data			
Key study for TK data, authors, year	12	Metabolism of the plasticizer and phthalate substitute diisononyl cyclohexane-1,2-dicarboxylate (DINCH®)in humans after single oral doses	Koch et al., 2013
Type of study	13	Human study: 3 male volunteers (age 26-38, bw 28-90 kg)	
Route of exposure	14	Single oral dose: 50 mg	
Half-life of biomarker(s)	15	Derived graphically ($t_{1/2}$ after 12-48h): OH-MINCH: 10 h cx-MINCH: 16,5 h Calculated values ($t_{1/2}$ after 12-48h): OH-MINCH: 9,5 h cx-MINCH: 18 h	Koch et al., 2013
Factor for metabolic conversion (F_{ue})	16	F_{ue}: 0,1276 (urinary OH-MINCH + cx-MINCH Fractional urinary excretion ratios of biomarkers relative to the oral dose of DINCH taken up, at 48h: OH-MINCH: 10.73% cx-MINCH: 2.03% Total: 12.76%	48 h and not 24 h: Important for deriving F_{ue} is that the time choosen encompasses the time frame of a quantitatvely relevant excretion, further assumption: steady state
Derivation method and calculation			
Derivation method	17	**Based on a defined tolerable intake (TDI)**	

Defined tolerable intake	18	TDI value: 1.0 mg/kg bw/d	**TDI from EFSA, 2006; based on:** Two-generation reproduction toxicity study in Wistar rats BASF (2003b) Critical endpoint: nephrotoxicity NOAEL: 100 mg/kg bw/d AF: 100 See note for more details
$HBGV_{GenPop}$			
Calculated $HBGV_{GenPop}$	19	**For the Σ [OH-MINCH and cx-MINCH]** Children: (1 × 0.75 × 0.1276)/0.03 = 3 mg/L (rounded value) Adults: (1 × 0.75 × 0.1276)/0.02 = 4.5 mg/L (rounded value)	Body weight-related urine excretion (L/kg bw/d) Children: 0.03 L/kg bw/d Adults: 0.02 L/kg bw/d
Additional Comments			

Explanation of notes:

1) **HBM $HBGV_{GenPop}$**: numerical value of the HBM $HBGV_{GenPop}$ value in mg/L

2) **Level of confidence of the HBM $HBGV_{GenPop}$:** reflects the reliability of the proposed HBM HBGV. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM HBGV (e.g. reliability of the key study used to derivate the TDI value, uncertainties related to the calculation of the TDI, toxicokinetic data on the substance of interest, on the extrapolations and calculation of the final HBM HBGV).

   Attribution of a global level of confidence for the proposed DINCH HBM $HBGV_{GenPop}$ is suggested, considering:

   - the nature and quality of the DINCH toxicological data: the toxicological database on DINCH is well supported, however based almost exclusively on animal studies. → medium

   - the critical endpoint and mode of action: the evidence of the DINCH nephrotoxicity is based on two animal studies (sub chronic and chronic). Nevertheless, no evidence for these effects in humans is available from the scientific literature. → low/medium

   - the selected key study for the TDI derivation: the key study used for the TDI derivation is an unpublished industrial study from BASF. Also, the best case for derivating HBM HBGVs would have been availability of human data. → low

   - the extrapolations of the POD value for the TDI calculation: the NOAEL determined from the key study is being extrapolated across species (AF_A = 10) and according to intra individual variability (AF_H). → medium (using a validated PBPK model or TK model for extrapolating from animal to human could increase the level of confidence.)

   - data gaps to compare adults and children: data are missing to ensure that metabolism and sensitivity are similar in adults and children. → low

   - the calculation of the HBM HBGVs, the determination of the factor for metabolic conversion (F_{ue}) is based on a study with only 3 male volunteers (see note 12) → low

   Altogether the global level of confidence for the DINCH HBM HBGVs in the general population is set to **'low'**.

3) **HBM $HBGV_{GenPop}$ status:** the status value is fixed to 'provisional', thereby reflecting that this value could be revised later on in the project, if uncertainties underlying the data could be reduced. For example, the uncertainties of the TK data could be reduced by means of a validated PBPK model for DINCH. A higher level of confidence attributed to the value would lead to a 'confirmed' value status.

4) **HBM $HBGV_{GenPop}$ year of issue:** year of agreement on the HBM $HBGV_{GenPop}$ in the HBM4EU workframe.

5) **CLP Number:** according to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Implementing the globally harmonized system of chemical classification or GHS. http://guidance.echa.europa.eu/docs/guidance_document/clp_introductory_en.pdf

6) **EC Number:** under European Inventory of Existing Commercial chemical Substances (EINECS), ELINCS (European List of Notified Chemical Substances) in support of Directive 92/32/EEC, the 7th amendment to Directive 67/548/EEC, NLP (No-Longer Polymers). See: ESIS: European chemical Substances Information System: http://esis.jrc.ec.europa.eu/index.php?PGM=dat

7) **CAS Number:** collection of disclosed chemical compound information by Chemical Abstracts Service. Almost all molecule databases can be searched by CAS Registry Number.

8) **Harmonised CLP classification:** CLP classification including CMR and other health relevant effects. In the case that classification is not harmonised, this should be stated. For selfclassifications by industry the ECHA- CLP inventory can be searched at: http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database

9) **Molar mass**: mass of DINCH per amount of this substance expressed in g/mol

10) **Biomarker(s) identification:** Biomarkers identified as valid for a diagnosis of exposure to DINCH include oxidation products of the alkyl side chain of the monoester, MINCH: Cyclohexane-1,2-dicarboxylic acid mono-hydroxyisononyl ester (OH-MINCH), cyclohexane-1,2-dicarboxylic acid mono-carboxyisooctyl ester (cx-MINCH) and cyclohexane-1,2-dicarboxylic acid mono-oxoisononyl ester (oxo-MINCH) (Koch et al., 2013). Because oxo-MINCH has until now been predominantly described in semi-quantitative terms, and only recently, an analytical standard has been developed synthetically, the focus has so far mainly been on the analysis of OH-MINCH and cx-MINCH. Consequentially, those 2 metabolites should be taken into consideration for a HBM HBGV derivation.

11) **Molar mass of the biomarker(s)**: mass of the selected biomarkers (expressed in g/mol).

12) **Study for TK data, Authors, Year:** Koch et al. (2013) examined the metabolism of DINCH in humans. The metabolites and the elimination kinetics were determined by means of chromatographic-mass spectrometric methods. Three male test persons were administered 50 mg DINCH by the oral route. Over a period of 48 h, all single urine samples were collected in separate containers and stored deep-frozen until analysis.

13) **Type of study:** human oral study

14) **Route of exposure:** DINCH oral single administration of 50 mg.

15) **Half-life of biomarker:** estimated elimination half-lives for the DINCH metabolites

16) **Factor for metabolic conversion (F_{ue}):** Fractional urinary excretion ratios of biomarkers relative to the oral dose of DINCH taken up, at 48h: OH-MINCH: 10.73%; cx-MINCH: 2.03%; total: 12.76%. 48 h are preferred instead of 24 h because it is important for deriving F_{ue} that the time choosen encompasses the time frame of a quantitatvely relevant excretion, further assumption: steady state

17) **Derivation method:** The existing data base on DINCH did not allow for the derivation of health-based guidance values from human data, based on internal concentration and health effects relationship. Therefore, a derivation method based on a defined tolerable intake is used (see concept document).

18) **Defined tolerable intake:** The DINCH TDI derivation by EFSA is based on nephrotoxic effects. In the two-generation reproduction toxicity study in Wistar rats with DINCH continuous dietary administration (BASF, 2003b), vacuolation of the tubular epithelium was observed in male F1 rats at the medium dose of 300 mg/kg bw/day and above. The NOAEL was 100 mg/kg bw/day. Based on the renal findings, EFSA established a NOAEL of 100 mg/kg bw/day. Using an uncertainty factor of 100, a TDI of 1 mg/kg bw/d was derived from this NOAEL.

19) **HBM HBGVs:** Ideally, the HBM HBGVs should refer to a complete 24-hour urine sample, stating the quantity of a substance in µg/day. For reasons of simplification, in accordance with the approach described in the concept document,

the excretion is referred to a body weight-related urine excretion of 30 mL/kg bw/d for children and 20 mL/kg bw/d for the remaining subgroups of the population.

Children: HBM HBGV$_{GenPop}$ = 3 mg/L
Adults: HBM HBGV$_{GenPop}$ = 4.5 mg/L

The resulting values are recommended as HBM HBGV$_{GenPop}$ for the sum of the concentrations of OH-MINCH and cx-MINCH in urine.

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