CHEMICAL SAFETY REPORT

Substance Name: bis(2-ethylhexyl) phthalate

EC Number: 204-211-0

CAS Number: 117-81-7

Legal name of applicant(s): *Vinyloop Ferrara SPA*

Co-applicants :

Plastic Planet S.R L

Stena Recycling AB

Collectively referred to as "Soft PVC Recyclate Authorization Consortium" (in short : SPAC) for the purpose of this Application for Authorization

Submited by : *Vinyloop Ferrara SPA*

DECLARATION

We, the Soft PVC recyclate Authorisation Consortium, claim this report to be confidential. We hereby declare that, to the best of our knowledge as of today (9 August 2013), the information is not publicly available, and in accordance with the due measures of protection that we have implemented, a member of the public should not be able to obtain access to the information claimed confidential without our consent or that of the third party whose commercial interests are at stake. A non confidential version of the exposure scenarios discussed under section 9 has been provided separately.

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Part A

1. SUMMARY OF RISK MANAGEMENT MEASURES

DEHP is classified as Repr. Cat 1B according to Regulation 1272/2008. Applicants produce and market the substance exclusively as part of solid recyclate pellets/regrind containing DEHP (polymer matrix). These products are classified and labelled like mixtures according to Regulation 1272/2008 and Directive 676/548/EEC.
Risk management measures established to protect from any human health hazards at workplaces, where these recyclate containing DEHP are used, are described in detail in chapter 9 of this chemical safety report. No specific risk management measures are required due to exposure during service life when using articles manufactured from the solid mixtures containing DEHP. Please note that throughout this document the terms "soft PVC recyclate", "soft PVC recyclate containing DEHP" and "recycled soft PVC containing DEHP" shall be used interchangeably unless otherwise specified.

2. DECLARATION THAT RISK MANAGEMENT MEASURES ARE IMPLEMENTED

This application for authorisation does not include a manufacturing scenario. So, no risk management measures reported here relate to the applicants' own professional activities.

3. DECLARATION THAT RISK MANAGEMENT MEASURES ARE COMMUNICATED

We declare that all risk management measures described in the exposure scenarios in chapter 9 of this chemical safety report will be adequately communicated in the supply chain as soon as possible by the safety data sheets accompanying the recycled soft PVC containing DEHP.

This CSR is largely built by using relevant parts of the Authorisation CSR (chemical safety report) prepared by the authorisation task force for DEHP (ATF DEHP). The applicant acquired access for the use of relevant parts of the ATF's CSR, to ensure alignment and consistency across the applications for authorisation.

Part B

1. IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1. Name and other identifiers of the substance

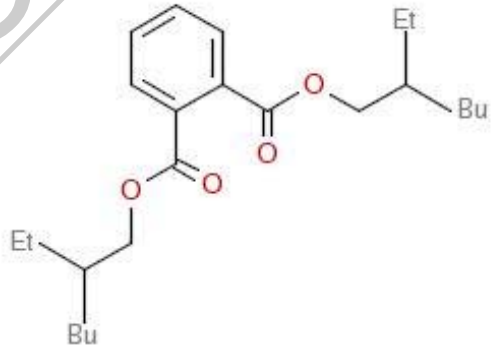
Bis(2-ethylhexyl) phthalate

The following public name is used: DEHP.

Table 1 Substance identity

EC number:	204-211-0
EC name:	bis(2-ethylhexyl) phthalate
CAS number (EC inventory):	117-81-7
IUPAC name:	bis(2-ethylhexyl) phthalate
Annex I index number:	607-317-00-9
Molecular formula:	C24H38O4
Molecular weight range:	390.5561

Structural formula:



1.2. Composition of the substance

Name: bis(2-ethylhexyl) phthalate

Description: DEHP contained in polymer matrix of recycled soft PVC

Table 2 Constituents

Constituent	Typical concentration	Concentration range	Remarks
bis(2-ethylhexyl) phthalate EC no.: 204-211-0	[redacted]	1 – 20%	DEHP in solid matrix of recycled soft PVC

As the submission substance is obtained from recycling soft PVC waste, it is exclusively handled within a polymer matrix. It enters the supply chain as a component of a solid soft PVC recyclate and is never dealt with as a free substance. Therefore, impurities or additives cannot be identified for DEHP from recycled soft PVC materials.

1.3. Physico-chemical properties

Table 3 Overview of physico-chemical properties

Property	Results	Value used for CSA / Discussion
Physical state at 20°C and 1013 hPa	Liquid Form:oily Colour: colourless Odour: no data	
Melting / freezing point	Melting point: from -55 to 50°C (from 218.15 to 223.15 K), at atmospheric pressure. Key values used in a weight of evidence approach have been selected in previous Risk Assessment Report, 2008.	Three values have been cited in various review articles and handbooks. Data selected for a weight of evidence approach: -50 °C and - 55°C (data from reference handbooks according to R7 guidance document). Data from supportive study: -47°C; Data from supportive study: -47°C (data cited in a review article without more details on method or origin, therefore considered as a supportive data but will not be inclueded in the weight of evidence approach.)
Boiling point	Boiling point: 374.15°C at 1022 mbar (ASTM E1782 standard: dynamic method under low pressure)	**Value used for CSA:** 647 K at 1013 hPa Five data are available for boiling point of DEHP. The key study, CCRA, 2008, is an experimental study according to ASTM E1782 standard. All other data are cited from reference handbooks and are consistent with the value of the key study. Therefore these data are considered as supportive studies. - Rippen, 2000: 385 °C at 1013 hPa - Verschueren, 384 at 1013 hPa - Patty, 1994: 230°C at ca 664 Pa (5 mmHg)

		- BUA, 1986: 230-233°C °C at 5 mbar , DIN 53 406, not distillable at 1030 mbar.
Relative density	Density: 0.98 g/cm³ at 20°C 1013 mbar. Weight of evidence approach on two data from reference handbooks: - 0.983 g/cm³, 20°C,(Rippen, 2000); - 0.9845 g/cm³, 20°C, 1013 hPa, according to DIN 51 757 (BUA, 1986).	**Value used for CSA:** 0.98 at 20°C Five data are available to estimate the density of DEHP. Two data selected for the weight of evidence. All other data are cited from handbooks or review articles which did not refered to test conditions or procedure. These data are consistent with th v lue of the weight of evidence and therefore are consider d a supportive studies. - Furtmann, 1996: 0.98 to 0.985 g/cm³ - Staples, 1997: 0.986 (specific gravity) - Spencer, 2001: 0.98 to (specific gravity)
Vapour pressure	Vapour pressure: very low pressure (below 10E-4 Pa) are difficult to measure therefore a weight of evidence is chosen in the case of DEHP . Based on measured, calculated and handbook values vapour pressure of DEHP is estimated between 1.8x10E-5 and 3.4x10E-5 Pa at room temperature (20 - 25°C)	The EU Risk Assessment Report 2008, validated by Swedish authoriti s used the value from Hüls AG (1997) for a ssing the environmental fate. This study has not been re-evaluated and considered as reliabl A literature average, and two recent studies re in ac ordance with this value. Therefore, the weight of e idence is based on the following data: _0.000034 Pa et 20°C (Hüls AG, 1997 in RAR, 2008) _0.000019 Pa 25°C, saturation chamber, (Clausen, 2002). _0.0000252 Pa at 25°C, where a QSAR relationship between water, octanol and air solubilities are correlated to the molar volume of phthalate esters (Cousins, 2000). _0.0000181 Pa at 20 °C, literature average in reference handbook (Rippen, 2000), A large range of values is observed and may be due to methodological difficulties to obtain precise measures of very low pressure and many values are overestimations due to interference from impurities (Rippen, 2000). Nonetheless, other data at ambient temperature are also recorded at ambient temperature (20, 25°C) supporting this range of very low vapour pressure for DEHP: _0.0000133322 Pa at 25°C, is an average from literature review (Staples, 1997) _0.00001 Pa at 20-25°C, another average value from selected data from a literature review (Tukker, 1998). _0.0000006 Pa at 20°C, ASTM E1782 (CCRA, 2008), _0.00086 (+/-0,00066) Pa at 25°C, US EPA Proposed

		rules, saturation method, 1980 (Howard, 1985), At least, RAR (2008) and Hoyer, (1958) give values of vapour pressure at higher and lower temperature (from 10 to 216°C). These data were validated in the risk assessment and some were extrapolated. In Hoyer 1958, the value is considered as unreliable because extrapolated outside the range of measured temperatures. However, these data are consistent and show an augmentation of vapour pressure with the temperature. Therefore these values were kept as supportive as it may give information on the substan e behaviour at higher temperatures.
Water solubility	0.003 mg/l @ 20°C	**Value used for CSA:** 0.003 mg/L at 20 °C A large range of values on the water solubility is available in the literature (0.003-1.3 mg/l at 20-25°C) Available studies clearly show considerable difficulties in estimating el ant water solubility. As reported by Cousin et al. 2003, and in OECD guideline standard method can be undertaken for compounds with solubilities greater than 1 mg/L. Below this limi problem occurs. In the cas of DEHP, a probable explanation of this is that DEHP e sily forms more or less colloidal dispersions in water due to flask shaking, which incr ase the amount DEHP in the water phase (Staples e al. 1997). The colloidal formation might be of rele ance in understanding laboratory studies in aquatic media. A colloidal water solubility of about 0.34 mg/l (Howard et al., 1985) is assumed in the Risk Assessment Report 2008. Thomsen et al. 2001, to avoid this problem, measured the unimeric solubility of DEHP via surface tension measurements. Although lower value was demonstrated (17 µg/L), this measure as it is an indirect method and may be affected by impurities present in the test sample, this result will not be considered for the assessment. The "true" water solubility value validated and used in the previous RAR, 2008 was the one proposed by Staples et al. 1997: water solubility = 0.003 mg/L at ambient temperature (20 -25°C). This value, considered as the key value, is today supported by 3 new studies: - Turner & Rawling 2000, estimated the DEHP water solubility in a experiment using slow stirring technique to avoid colloid formation(radiolabelled DEHP followed byLiquid Scintillation Countingafter16h equilibration periodon a lateral shaker). In addition, authors took particular caresto avoid contaminations and losses due to adsorption. The water solubility was thus estimated to be in a few

		µg/L range. - Ellington and Floyd, 1996, using SPARC calculation model estimated the water solubility of DEHP at 0.0026 mg/L. In 2009 the updated version 4.5 of the model gives a value at 0.00362 mg/L. - Cousins and Mackay, 2000 used the "three solubilities" approach to determine solubilities and partition coefficients of phthalate esters. In their model, authors have shown correlation between "apparent" solubilities in air, water and octanol, and the Le Bas molar volume as moleculardescr ptor (i.e. sum of atomic volumes with adjustement of the volume decrease arising from ring form tion). With this approach authors hightlighted he mea urement errors from water solubility data av ilable in he literature. Althought authors due to this variability, excluded data for substances of high molar volume (like DEHP) the water l bility c lculated with this model was 2.49 µg/L. Thi value is consistent other value previously alidat d in xperimatal and calculation model studies. Therefor , the ba ed on these studies, the true solubility of DEHP is considered to be at 0.003 mg/l at 20°C nd will be used in the current risk assessment.
Partition coefficient n-octanol/water (log value)	- n-Octanol - Water partition coefficient: Numerous data are reported in the literature. Due to the ability of DEHP to form a colloidal dispersion in w ter, results from stirring ethod OECD 107 are not considered as reliable (Leyder 1983). Only results bas d on s ow-stir method (similar to OECD 123) or HPL technique (OECD 117 or similar) are therefore assumed to be useful. Five measured values are available and considered as r li ble and are supported with 2 calculated values and 1 average value from literature review. Therefore a weight of evidence approach based on measured data and supported with estimated ones was applied. - Octanol-air partition coefficient: One calculated value is available. According to the model based on the 'three solubility approach' and the molar volume of phthalate esters, DEHP show an Log Koa 10.5 at 25°C. This calculated value is estimated to be reliable with accepatble restrictions as	**Value us d for CSA:** Log Kow (Pow): 7.5 at 20 °C A subset of the experimental and calculated data reported in the literature is presented below: **Method** / **Log Kow** / **Flag** / **Reference** Slow stir method (OECD 123 draft guideline) / 7.137 +/- 0,153 / WoE / Brooke et al. (1990) 7.453 +/- 0,061 / WoE / DeBruijn et al. (1989) 7.27 +/- 0,04 / WoE / Ellington and Floyd (1996) HPLC method (similar to OECD 117 guideline) / 7.94 / WoE / Howard et al. (1985) 7.8 +/- 1,4 / WoE / Klein et al. (1988) SPARC / 7.54 / SS / Ellington

<table>
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<td>the model give caculated values for vapour pressure, Log Kow and water solubility in accordance with recently measured ones.
- Particle-gas partition coefficient has been estimated from vapor pressure according to relationship reported by Naumova et al (2003) based on 1800 partition coefficients of polycyclic aromatic hydrocarbons considered as reasonably resemble phthalate esters. Log Kp = -0.6. This data is supported by value calculated by Cousins and Mackay, 2000 on basis of 'three solubilities approach' a model validated previously (Log Kp= -1.19) and which sustains the behaviour of DEHP to have more affinity to particles than for air.</td>
<td>
<table>
<tr><td>calculation model (2009 updated with v4.5)</td><td>(7.66)</td><td></td><td>and Floyd (1996)</td></tr>
<tr><td>QSAR model based on solubilities and Le Bas molar volumes</td><td>7.73</td><td>SS</td><td>Cousins and Mackay (2000)</td></tr>
<tr><td>Literature average validated in previous Risk Assessment Report (2008)</td><td>7.5</td><td>SS</td><td>Staples et l. (1997)</td></tr>
</table>
Consid ring he measured values the average value of DEHP partition coefficient is 7.52.

This value is supported by SPARC calculation model (considered as reliable in R7a guidance document) and t e model based on three solubilities developped by Cousins and Mackay (2000). Moreover, these values are in accordance with average value from measured data from literature (Staples et al. 1997). Value wich was recommended and used in the RAR (2008).
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<td>Viscosity</td>
<td>The same measur d value for dynamic viscosity at 20°C was cited in 3 r ferences without details on test procedure. As one of it (Hawley's dictionnary, 1981) s considered as a reliable document according to R7 guidance document, we consider this value as key parameter.</td>
<td>Value used for CSA: Viscosity at 20°C: 81 mPa · s (dynamic)

Dynamic viscosity of DEHP is recorded at 58 mPa.s at 25°C (Rippen 2000). We assume this reference as a reliable document and this data is therefore used a supportive value.</td>
</tr>
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Data wai ing

Information requirement: Surface tension

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the surface tension study (required in section 7.6) does not need to be conducted as the hydrosolubility is below 1mg/L at 20°C as adressed under section 4.8 water solubility.

Information requirement: Flash point

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the flash point study (required in section 4.11) does not need to be conducted as its value is estimated above 200 °C. Two data without enough detailled documentation are in accordance with this analysis.

Information requirement: Flammability

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the flammability study (required in section 4.13) does not need to be conducted as flammability of a liquid is addressed under 4.11 Flash point.

Based on experience in handling and use, the substance is not pyrophoric and is not flammable on c ntact with water.

One data is available from the literature.

Information requirement: Explosive properties

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the explosiveness study (required in section 4.14) does not need to be conduct d as no chemical groups associated with explosive properties is present in DEHP.

Information requirement: Self-ignition temperature

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the Autoflammability study (required in section 12) does not need to be conducted for liquids non flammable in air with no flash point up to 200 °C as addressed under section 4.11 Flash point. Three data without enough detailled documentation are consisten with waiving justification.

Information requirement: Oxidising p operties

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex VII, the explosiveness st dy (requ red in section 4.15) does not need to be conducted as no chemical groups associated with o idising properties is present in DEHP.

Information r quirement: Granulometry

R as n: other justification

Justification: In accordance with column 2 of REACH annex VII, the particle size distribution (required in sec ion 4.5) does not need to be conducted as the substance is marketed or used in a non solid or granular form (here the substance is liquid).

Information requirement: Stability in organic solvents and identity of relevant degradation products

Reason: other justification

Justification: In accordance with column 2 of REACH Regulation (EC) No 1907/2006, Annex IX, the stability in organic solvents study (required in section 4.17) does not need to be conducted as stability of the substance is not considered critical.

Information requirement: Dissociation constant

Reason: other justification

Justification: In accordance with REACH Regulation (EC) No 1907/2006, RIP 3.2.2, chapter R.7, the dissociation constant in water study (required in section 4.21) does not need to be conducted as there is no ionisable groups at environmentally relevant pH in the substance.

2. MANUFACTURE AND USES

Quantities

The applicants put on the market together approx. [redacted] of PVC recyclate. With an average measured DEHP content of [redacted], this represents approx [redacted] of DEHP. The modelling under the socio-economic assessment predicts higher quantities of DEHP in soft PVC recyclate at below [redacted] for the same amount of recyclate; the difference in these figures stems from the SEA taking an average figure across the EU and taking variations in the lifetime of different articles into account when predicting future concentrations in post-consumer wastes. Quantities of recyclate placed on the market might grow as discussed in the socio-economic assessment but at the most will double, giving an absolute maximum amount of DEHP contained in soft PVC recyclates at between [redacted].

2.1. Manufacture

Manufacturing process

Table 4 Manufacturing process

Related manufacture(s)	Description of manufacturing process
	Recycled soft PVC containing DEHP is mainly gained from post-consumer waste by separating soft PVC from other parts, followed by shredding or micronizing the soft PVC. Another manufacturing process includes di solution of soft PVC waste and purification by filtration followed by precipitation.

2.2. Identified uses

Table 5 Formulation

Identifiers	Use descriptors	Other information
F-1: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends	**Process category (PROC):** PROC 1: Use i closed process, no likelihood of expos re PROC 2: Use in closed, continuous process with occasional controlled exposure PR C 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of mixtures and articles (multistage and/or significant contact) PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 14: Production of mixtures or articles by tabletting, compression, extrusion, pelletisation PROC 15: Use as laboratory reagent **Product Category formulated:** PC 32: Polymer mixtures and compounds	Number of sites: 10-100 Substance supplied to that use: In a mixture

Identifiers	Use descriptors	Other information
	Technical function of the substance during formulation: Softeners	

Table 6 Uses at industrial sites

Identifiers	Use descriptors	Other information
IW-2: Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles	**Process category (PROC):** PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 6: Calendering operations PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or prepa ation (charging/discharging) from/to vessels/ arge containers at dedicated facilities PROC 14: Production of mixtures or ar icles y tabletting, compression, extru ion, p lletis tion PROC 21: Low energy manipulation of substances bound in materials and/or ar icles **Product Category used:** PC 32: Polymer mixt res nd compounds **Sector of end use:** SU 12: Manufacture of plastics products, including compounding and conversion **Technical function of the substance during formulation:** Sof eners	Number of sites: 10-100 Substance supplied to th t use: In a mix ure Subsequent servi e life relevant for that us : yes Link to the subsequent service li e: A 1: Professional handling of PVC products made from recycled soft PVC containing DEHP: Installation of building materials and similar activities) / inhalation exposure from volatile DEHP / professional PVC footwear) A-2: Exposure from consumer articles made from recycled soft PVC containing DEHP

Table 7 A ticle service life

Identi iers	Use descriptors	Other information
SL 1: Professional handl ng of PVC products made from recycled soft PVC containing DEHP: Installation of building materials and similar activities) / inhalation exposure from volatile DEHP / professional PVC	**Article category related to subsequent service life (AC):** AC 13: Plastic articles **Exposure related description of article:** Articles with intended or foreseeable skin contact, e.g. clothing or shoe ware Articles with foreseeable impact on indoor exposure due to large indoor surface, e.g. flooring Articles with foreseeable dispersion on use, e.g. tyres or roof-sheets	Article used by: workers Typical concentration of the substance in article: [redacted]

Identifiers	Use descriptors	Other information
footwear)	**Process category (PROC):** PROC 21: Low energy manipulation of substances bound in materials and/or articles **Technical function of the substance during formulation:** Softeners	
SL-2: Exposure from consumer articles made from recycled soft PVC containing DEHP	**Article category related to subsequent service life (AC):** AC 13: Plastic articles **Exposure related description of article:** Articles with intended or foreseeable skin contact, e.g. clothing or shoe ware Articles with foreseeable dispersion on use, e.g. tyres or roof-sheets Articles with foreseeable impact on indoor exposur due to large indoor surface, e.g. flooring **Technical function of the substance during formulation:** Softeners	Article used by: consumers Typical concentr tion of the substance in rticle [redacted]

Most common technical function of substance (what it oes):

Softeners

2.3. Uses advised against

Table 8 Uses at industrial sites

Identifiers	Use descriptors	Other information
IW-: Use as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles	**Product Category used:** PC 32: Polymer mixtures and compounds	Remarks: Restriction under Regulation 1907/2006: Not to be used in t ys and childcare articles

Table 9 Consumer uses

Identifiers	Use descriptors	Other information
C-: Use in substances and mixtures placed on the market for sale to the general public (see Restriction 30 Annex XVII REACH)		Remarks: See specific conditions in restriction 30 Annex XVII REACH
Use in food contact materials: Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food		Allowed uses and limits for DEHP stated in Annex I table 1of this Regulation

3. CLASSIFICATION AND LABELLING

3.1. Classification and labelling according to CLP / GHS

Name: Bis(2-ethylexyl) phthalate (DEHP)

Implementation: EU

State/form of the substance: liquid

Remarks: CLP classification calculated according to the European Regulation 1272/2008.

Classification

The substance is classified as follows:

Table 10 Classification and labelling according to CLP / GHS for physicochemical prope ties

Endpoint	Hazard category	Hazard statement	Reason for no las ification	CSR section*)
Explosives:			conclu ve but not sufficient for classificati n	6.1
Flammable gases:			onclusive bu not sufficient for lassificat on	6.2
Flammable aerosols:			con lusive but not sufficient for classification	6.2
Oxidising gases:			conclusive but not sufficient for classification	6.3
Gases under pressure:			conclusive but not sufficient for classification	
Flammable liquids:			conclusive but not sufficient for classification	6.2
Flammable solids:			conclusive but not sufficient for classification	6.2
Self-reactive substances and mixtures:			conclusive but not sufficient for classification	
Pyrophoric liquids			conclusive but not sufficient for classification	6.2
Pyrophoric lids:			conclusive but not sufficient for classification	6.2
Self-heati g su stances and mi tures:			conclusive but not sufficient for classification	
Subst nces and mixtures which in contact with water emit f ammable gases:			conclusive but not sufficient for classification	6.2
Oxidising liquids:			conclusive but not sufficient for classification	6.3
Oxidising solids:			conclusive but not sufficient for classification	6.3
Organic peroxides:			conclusive but not sufficient for classification	
Corrosive to metals:			data lacking	

*) Justification for (non) classification can be found in the CSR section indicated

Table 11 Classification and labelling according to CLP / GHS for health hazards

Endpoint	Hazard category	Hazard statement	Reason for no classification	CSR section*)
Acute toxicity - oral:			conclusive but not sufficient for classification	5.2.3
Acute toxicity - dermal:			conclusive but not sufficient for classification	5.2.3
Acute toxicity - inhalation:			conclusive but not sufficient for classification	5.2.3
Skin corrosion / irritation:			conclusive but not sufficient for classification	5.3.4 and 5.4.3
Serious damage / eye irritation:			conclusive but not sufficient for classification	5. .4
Respiration sensitization:			conclusive but not sufficient for classification	5.5.3
Skin sensitation:			conclusive but not s ffici nt for classification	5.5.3
Aspiration hazard:			conclus ve but not sufficient for classification	5.2.3
Reproductive Toxicity:	Repr. 1B Route of exposure: Oral	H360: May damage fertility or the unborn child		5.9.3
Reproductive Toxicity: Effects on or via lactation:			conclusive but not sufficient for classification	5.9.3
Germ cell mutagenicity:			conclusive but not sufficient for classification	5.7.3
Carcinogenicity:			conclusive but not sufficient for classification	5.8.3
Specific target organ toxicity - single:			conclusive but not sufficient for classification	5.2.3 and 5.3.4
Specific target organ toxicity - repeated:			conclusive but not sufficient for classification	5.6.3

*) Justification for (non) clas ification can be found in the CSR section indicated

Table 12 C assification and labelling according to CLP / GHS for environmental hazards

Endpoint	Hazard category	Hazard statement	Reason for no classification	CSR section*)
Ha ards to the aquatic environment (acute/short-term):			conclusive but not sufficient for classification	7.6
Hazards to the aquatic environment (long-term):			conclusive but not sufficient for classification	7.6
Hazardous to the ozone layer:			data lacking	7.6

*) Justification for (non) classification can be found in the CSR section indicated

Labelling

Signal word: Danger

Hazard pictogram:

GHS08: health hazard

Hazard statements:

H360: May damage fertility or the unborn child <state specific effect if known > <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>.

Precautionary statements:

P201: Obtain special instructions before use.
P281: Use personal protective equipment as required.
P308+P313: IF exposed or concerned: Get medical advice/attention

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3.2. Classification and labelling according to DSD / DPD

3.2.1. Classification and labelling in Annex I of Directive 67/548/EEC

Chemical name: Bis(2-ethylexyl) phthalate (DEHP)

Classification

The substance is classified as follows:

Table 13 Classification and labelling in Annex I of Directive 67/548/EEC for physicochemical properties

Endpoint	Classification	Reason for no classification	CSR section*)
Explosiveness:		conclusive but not sufficient for classification	6.1
Oxidising properties:		conclusive but not sufficient for classification	6.2
Flammability:		conclusive but not sufficient for classification	6.3
Thermal stability:		conclusive but not sufficient for classification	

*) Justification for (non) classification can be found in the CSR section indicated

Table 14 Classification and labelling in Annex I of Directive 67/548/EEC for health hazards

Endpoint	Classification	Reason for no classification	CSR section*)
Acute toxicity:		conclusive but not sufficient	5.2.3

		for classification	
Acute toxicity - irreversible damage after single exposure:		conclusive but not sufficient for classification	5.2.3
Repeated dose toxicity:		conclusive but not sufficient for classification	5.6.3
Irritation / Corrosion:		conclusive but not sufficient for classification	5.3.4 and 5.4.3
Sensitisation:		conclusive but not sufficient for classification	5.5.3
Carcinogenicity:		conclusive but not sufficient for classification	5 8 3
Mutagenicity - Genetic Toxicity:		conclusive but not suffi ient for classification	5 .3
Toxicity to reproduction - fertility:	Repr. Cat. 2; R60 May impair fertility.		5.9.3
Toxicity to reproduction - development:	Repr. Cat. 2; R61 May cause harm to the unborn child.		5.9.3
Toxicity to reproduction - breastfed babies:		data lacking	5.9.3

*) Justification for (non) classification can be found in the CSR s ction indi ated

Table 15 Classification and labelling in Annex I of Directive 67/548/EEC for the environment

Endpoint	Classification	Reason for no classification	CSR section*)
Environment:		conclusive but not sufficient for classification	7.6

*) Justification for (non) classi ication can be found in the CSR section indicated

Labelling

Indication of dange :

T - toxic

R-phrases:

R6 - May impa r fertility
R61 May cause harm to the unborn child

S phr ses:

S53 avoid exposure - obtain special instructions before use
S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

3.2.2. Self classification(s)

Not relevant

3.2.3. Other classification(s)

None

4. ENVIRONMENTAL FATE PROPERTIES

Not relevant for this application for authorisation.

5. HUMAN HEALTH HAZARD ASSESSMENT

General remarks:

The data summaries provided in this section are partly based on the elaborations of the EU Risk Assessment Report (ECB, 2008). This publication can be considered a general reference for the information in this section.

The primary data searches on health hazard data for DEHP were completed at the end of 2012, and the emerging literature has been monitored continuously and any relevant, new studies published up to the period of the 7 h June 2013 have been taken into account in this CSR.

5.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

According to the EU risk assessment, numerous studies on the toxicokinetics following ral administration of DEHP have been performed in experimental animals and a few studies on humans are avai able. Limited data are available concerning inhalation and dermal exposure. Studies using parenteral administration have also contributed to the understanding of the toxicokinetics.

5.1.1. Basic toxicokinetics

Studies have been performed to compare the toxicokinetic behav our of DEHP between humans, different strains of non-human primates, different strains of rats, mice, hamsters, gui ea pig , dogs, and miniature pigs. There are also studies that compare the toxicokinetics after different routes of expo ure.

The structures of the metabolites referred to in the text re given in Albro et al. (1983) and named according to the widely accepted nomenclature of Albro et al. (1983).

HOOC-C6H6-COO-CH2-CH-R'

|

R''

I 2-ethyl-3-carboxypropyl phthalate (R'=-CH2COOH; R''= -CH2CH3)

II 2-carboxyhexyl phthalate (R'=-[CH2]3CH3; R''= -COOH)

III 2-ethyl-4-carboxybutyl phthalate (R'=-[CH2]2COOH; R''= -CH2CH3)

IV 2-carboxymethylhexyl phthalate (R'=-[CH2]3CH3; R''= -CH2COOH)

V 2-ethyl-5-carboxypentyl phthalate (R'= -[CH2]3COOH; R''= -CH2CH3)

VI 2-ethyl-5-oxyhexyl phthalate (R'= -[CH2]2-CO-CH3; R''= -CH2CH3)

VII 2-(2-hydroxyethyl) hexyl phthalate (R'= -[CH2]3CH3; R''= -CH2CH2OH)

VIII 2-ethyl-4-hydroxyhexyl phthalate (R'= -CH2-CHOH-CH2CH3; R''= -CH2CH3)

IX 2-ethyl-5-hydroxyhexyl phthalate (R'= -[CH2]2]-CHOH-CH3; R''= -CH2CH3)

X 2-ethyl-6-hydroxyhexyl phthalate (R'= -[CH2]3CH2OH; R''= -CH2CH3)

XI 2-ethyl-pentyl phthalate (R'= -[CH2]3CH3; R''= -CH2CH3)

XII 2-ethyl-4-oxyhexyl phthalate (R'= -CH2-CO-CH2CH3; R''= -CH2CH3)

XIV 2-carboxymethyl-4-oxyhexyl phthalate (R'= -CH2-CO-CH2CH3; R' = H2COOH)

XV 2-ethyl-4-oxy-6-carboxyhexyl phthalate (R'= -CH2-CO-CH2COOH R''= -CH2CH3)

XVI 2-ethyl-4-hydroxy-6-carboxyhexyl phthalate (R'= -CH2-CHOH CH2COOH; R''= -CH2CH3)

XVII 2-(1-hydroxyethyl) hexyl phthalate (R'= -[CH2]3CH3; R''= -CHOH-CH3)

XVIII 2-carboxymethyl-4-hydroxyhexyl phthalate (R'= CH2-CHOH-CH2CH3; R''= -CH2COOH)

XIX 2-(1-hydroxyethyl) -5-hydroxyhexyl ph halate (R -[CH2]2-CHOH-CH3; R''= -CHOH-CH3)

XX 2-ethyl-4,6-dihydroxyhexyl phthalate (R'= -CH2-CHOH-CH2CH2OH; R''= -CH2CH3)

XXI 2-carboxymethyl-5-hydroxyhexyl phthal te (R'= -[CH2]2-CHOH-CH3; R''= -CH2COOH)

XXV 2-carboxymethyl-5-oxyhexyl phthalate (R'= -[CH2]2-CO-CH3; R''= -CH2COOH)

XXVI 2-(1-oxyethyl) hexyl phthalate R'= -[CH2]3CH3; R''= -CO-CH3)

5.1.1.1. Oral

The metabolism and e creti n of DEHP has been extensively studied in rats following oral administration. Other species in which the metabolism and excretion by the oral route have been studied include mice, guinea pigs, hamsters non human primates, and humans.

Humans

The primary objective of this GLP study was to determine the rate and extent of conversion of isotopically labell d DEHP into their primary and secondary metabolites in blood and urine following single oral dose administration at two different dose levels in healthy male and female subjects. The secondary objective was to collect and store biological samples for potential future chemical analysis (Douglas, 2010; Anderson et al., 2011).

This study was conducted in two parts. Part 1 was an open-label, single dose, pilot study in one male subject. Part 2 was an open-label, fixed sequence, single oral dose study in 20 healthy male and female subjects. During Part 2, all subjects participated in two study periods and there was a minimum of nine subjects of each gender.

In Part 1, one male subject entered and completed the study as planned. In Part 2, it was planned to study 20 healthy male and female subjects with a minimum of nine subjects of each gender. Twenty subjects (10 males and 10 females) entered and completed Part 2 of the study. Data for 10 subjects (Subjects 201 to 210) were included in the pharmacokinetic analysis and data for all 20 subjects were included in the safety data analysis.

Healthy male (Part 1), or male or female (Part 2) subjects of any ethnic origin, aged greater than 18 years, with a body mass index (BMI) between 19 and 32 kg/m^2 inclusive, and with a body weight >60 kg.

In Part 1, one subject received a single oral dose of 3.0 mg D^4-DEHP.

In Part 2, each subject received a single oral dose of 0.3 mg D^4-DEHP in Period 1, and 3.0 mg D^4-DEHP in Period 2.

All doses of D^4-DEHP were dissolved in the required volume of olive oil and added to a piece of toast, which he subject ate as part of a standard breakfast.

In Part 1, a single oral dose was administered in a single study period. In Part 2, single oral d ses were administered in two study periods with a minimum of 7 days between dose adminis rations There was approximately 1 year and 4 months between Parts 1 and 2 to allow for pharmacokine ic data analysis and protocol revisions to be performed which took into consideration advances in relevant scientifi knowledge.

Blood and urine samples were collected for the analysis of plasma and urinary concentra ions of the primary and secondary metabolites of D^4-DEHP (D^4-MEHP, D^4-5-oxo-MEHP, D^4-5-OH-ME P d D 5-carboxy-MEPP).

The pharmacokinetic parameters derived were: area under the plasma concentration time curve from time zero up to the last quantifiable concentration ($AUC_{0\text{-tlast}}$), maximum observed pla ma concentration (C_{max}), time of maximum observed plasma concentration (t_{max}), apparent terminal elimination half-life ($t_{1/2}$), amount of substance excreted in urine (Ae), percentage of the dose of D^4 DEHP xcr t d in urine based on individual metabolites measured (fe), renal clearance (CL_R), adjusted total amount and total percentage of the dose of D^4-DEHP excreted in urine based on all metabolites measured (Total Ae and Total fe, respectively), the combined secondary metabolites and each individual metabolite.

The pharmacokinetic parameters for all the metabolite are presented as the total analytes i.e. those generated from the analysis with enzyme hydrolysis.

The pharmacokinetic parameters of the primar metaboli e of D^4-DEHP, D^4-MEHP, are presented in Table 16:

Table 16 Pharmacokinetic Parame ers of the Metabolite D^4-MEHP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=5)	Females (N=5)	Overall (N=10)	Males (N=5)	Females (N=5)	Overall (N=10)
$AUC_{0\text{-tlast}}$ (nmol h/mL	0 089 (0 057, 0.121)	0.009 (0, 0.024)	0.049 (0, 0.121)	0.497 (0.167, 0.793)	1.12 (0.736, 1.61)	0.808 (0.167, 1.61)
C_{max} (nmol/mL)	0.029 (0.021, 0.042)	0.004 (0, 0.010)	0.017 (0, 0.042)	0.129 (0.056, 0.230)	0.348 (0.159, 0.627)	0.239 (0.056, 0.627)
t_{max}[a] (h)	2.50 (2.50, 4.00)	3.00, 4.00[b]	3.00[e] (2.50, 4.00)	4.00 (2.00, 8.02)	3.00 (2.50, 4.02)	3.50 (2.00, 8.02)
$t_{½}$ (h)	1.81, 3.07[b]	NC	1.81, 3.07[b]	4.02[c] (1.31, 9.41)	3.93[d] (2.45, 6.24)	3.97[e] (1.31, 9.41)

Arithmetic mean (min, max) data are presented
N = Number of subjects studied; NC = Not calculated;
[a] Median (min-max)
[b] N = 2 (individual values presented), [c] N = 3, [d] N = 4, [e] N = 7

Following single oral doses of D^4-DEHP at 0.3 mg and 3.0 mg, the primary metabolite, D^4-MEHP, steadily appeared in plasma. The systemic exposure to D^4-MEHP, based upon $AUC_{0\text{-tlast}}$ and C_{max}, appeared to increase in a supra-proportional manner over the dose range 0.3 mg to 3.0 mg, increasing by 17- and 14-fold, respectively, for the 10-fold increase in dose. The between-subject variability for $AUC_{0\text{-tlast}}$ and C_{max}, was high at both dose levels. Exposure to D^4-DEHP in male subjects was approximately 40% to 50% lower than in female subjects at the higher 3.0 mg dose level. D^4-MEHP was the most abundant of all the metabolites measured following oral administration of D^4-DEHP.

The urinary excretion of D^4-MEHP is presented in Table 17:

Table 17 Urinary Excretion of D^4-MEHP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=10)	Females (N=10)	Overall (N=20)	Males (N=10)	Females (N=10)	Overall (N=20)
$Ae_{0\text{-}48\ h}$ (µg)	17.7 (12.9, 24.0)	13.1 (8.51, 18.1)	15.4 (8.51, 24.0)	138 (91.6, 199)	115 (55.4, 176)	126 (55.4, 199)
$CL_{R\ 0\text{-}48\ h}$ (mL/min)	11.8[c] (9.63, 15.5)	26.8, 39.7[b]	17.9[d] (9.63, 39.7)	25. (6.82, 70.2)	7 38[e] (2.07, 9.66)	16.2[e] (2.07, 70.2)
$fe_{0\text{-}48\ h}$[a] (%)	7.75 (5.61, 10.5)	5.70 (3.71, 7.91)	6.72 (3.71, 10.5)	6.72 (4.46, 9.69)	5.58 (2.69, 8.56)	6.15 (2.69, 9.69)

Arithmetic mean (min, max) data are presented
N = Number of subjects studied
[a] Calculated using the actual maximum dose (mg)
i.e. 0.32 mg D^4-DEHP + 0.80 mg D^4-DINP and 2.87 mg D DEHP + 7.69 mg D^4-DINP
[b] N = 2 (individual values presented), [c] N = 5, [d] N = 7 [e] N = 10

The fraction of the dose excreted in urine s D^4-MEHP, up to 48 hours postdose at the 0.3 mg and 3.0 mg D^4-DEHP dose levels was low and similar in male and female subjects. The amount of D^4-MEHP excreted (Ae) increased in an approximate dose-proportional manner over the 0.3 mg and 3.0 mg doses. In general, the between-subject variability for fe (%) was low.

The pharmacokinetic parameters of the secondary metabolite of D^4-DEHP, D^4-5-oxo-MEHP, are presented in Table 18:

Table 18 Pharmacokinetic Parameters of the Metabolite D^4-5-oxo-MEHP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=5)	Females (N=5)	Overall (N=10)	Males (N=5)	Females (N=5)	Overall (N=10)
$AUC_{0\text{-tlast}}$ (nmol.h/mL)	0 (0, 0.001)	0 (0, 0)	0 (0, 0.001)	0.026 (0, 0.084)	0.037 (0, 0.111)	0.03 (0, 0.111)
C_{max} (nmol/mL)	0.001 (0, 0.003)	0 (0, 0)	0 (0, 0.003)	0.006 (0, 0.016)	0.008 (0, 0.020)	0.007 (0, 0.020)
t_{max}[a] (h)	4.00, 4.00[c]	NC	4.00, 4.00[c]	4.00[d] (4.00, 4.00)	4.02[d] (1.50, 6.00)	4.00[e] (1.50, 6.00)
$t_{½}$ (h)	NC	NC	NC	NC	4.75[b]	4.75[b]

Arithmetic mean (min, max) data are presented
N = Number of subjects studied; NC = Not calculated;
[a] Median (min-max)
[b] N= 1 (individual value presented), [c] N = 2 (individual values presented), [d] N = 3, [e] N = 6

The pharmacokinetic parameters of the secondary metabolite of D^4-DEHP, D^4-5-OH-MEHP, are presented in Table 19:

Table 19 Pharmacokinetic Parameters of the Metabolite D^4-5-OH-MEHP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=5)	Females (N=5)	Overall (N=10)	Males (N=5)	Females (N=5)	Overall (N=10)
$AUC_{0\text{-tlast}}$ (nmol.h/mL)	0.001 (0, 0.004)	0 (0, 0)	0 (0, 0.004)	0.266 (0, 1.12)	0.080 (0, 0.359)	0.173 (0, 1.12)
C_{max} (nmol/mL)	0.001 (0, 0.003)	0 (0, 0)	0 (0, 0.003)	0.032 (0, 0.117)	0.027 (0, 0.104)	0.013 (0, 0.117)
t_{max}[a] (h)	2.50, 3.00[b]	NC	2.50, 3.00[c]	3.50[c] (2.50, 4.00)	2.50[c] (1.50, 4.02)	2.75[d] (1.50, 4.02)
$t_{½}$ (h)	NC	NC	NC	NC	NC	NC

Arithmetic mean (min, max) data are presented
N = Number of subjects studied; NC = Not calculated;
[a] Median (min-max)
[b] N = 2 (individual values presented), [c] N = 4, [d] N = 8

The pharmacokinetic parameters of the secondary metabolite of D^4-DEHP, D^4-5-carboxy-MEPP, are presented in Table 20:

Table 20 Pharmacokinetic Parameters of the Metabolite D^4-5-Carboxy-MEPP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=5)	Females (N=5)	Overall (N=10)	Males (N=5)	Females (N=5)	Overall (N=10)
$AUC_{0\text{-tlast}}$ (nmol.h/mL)	0.028 (0, 0.074)	0.003 (0, 0.015)	0.016 (0, 0.074)	0.127 (0.006, 0.278)	0.135 (0.106, 0 213)	0 131 (006 0.278)
C_{max} (nmol/mL)	0.005 (0, 0.009)	0.001 (0, 0.006)	0.003 (0, 0.009)	0.021 (0.006, 0.035)	0.016 (0.009 0.025)	0.018 (0.006, 0.035)
t_{max}[a] (h)	2.75[c] (2.00, 3.00)	4.03[b]	3.00[d] (2.00, 4.03)	4.00 (4.00, 8.02)	4 00 (1.50, 6.00)	4.00 (1.50, 8.02)
$t_{½}$ (h)	NC	NC	NC	3.81	NC	3.81[b]

Arithmetic mean (min, max) data are presented
N = Number of subjects studied; NC = Not calculated;
[a] Median (min-max)
[b] N = 1 (individual value presented), [c] N = 4, [d] N = 5

Following single oral doses of 0.3 mg and 3.0 mg D^4 DEHP, the secondary metabolites D^4-5-oxo-MEHP, D^4-5-OH-MEHP and D^4-5-carboxy-MEPP, all steadily app ared in plasma. The between-subject variability for the secondary metabolites was high at both do levels. D^4-5-carboxy-MEPP and D^4-5-OH-MEHP were the most abundant metabolites and D^4-5-oxo-MEHP the l ast abundant. Exposure at the 3.0 mg D^4-DEHP dose level to D^4-5-oxo-MEHP and D^4-5-carboxy-MEPP w s similar in male and female subjects and was up to 3.7-fold higher in males compared to females for D^4-5-OH-MEHP.

The urinary excretion of the secondary metabolites of D^4-DEHP; D^4-5-oxo-MEHP, D^4-5-OH-MEHP and D^4-5-carboxy-MEPP is presented in Tables 21, 22 and 23:

Table 21 Urinary Excretion of the Metabolites D^4-5-oxo-MEHP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=10)	Females (N=10)	Overall (N=20)	Males (N=10)	Females (N=10)	Overall (N=20)
$Ae_{0\text{-}48\ h}$ (µg)	27.2 (21.8, 36.8)	31.2 (21.5, 41.7)	29.2 (21.5, 41.7)	227 (153, 371)	240 (170, 297)	34 (153 371)
$CL_{R\ 0\text{-}48\ h}$ (mL/min)	780, 1227[b]	NC	780, 1227[b]	329[c] (143, 526)	417[c] (149 800)	310[d] (143, 800)
$fe_{0\text{-}48\ h}$[a] (%)	11.3 (9.07, 15.3)	13.0 (8.95, 17.3)	12.2 (8.95, 17.3)	10.5 (7.09, 17 2)	11 1 (7.88, 13.8)	10.8 (7.09, 17.2)

Arithmetic mean (min, max) data are presented
N = Number of subjects studied; NC = Not calculated
[a] Calculated using the actual maximum dose (mg)
i.e. 0.32 mg D^4-DEHP + 0.80 mg D^4-DINP and 2.87 mg D^4-DEHP + 7.69 mg D^4-DINP
[b] N = 2 (individual values presented), [c] N = 3, [d] N = 6

Table 22 Urinary Excretion of the Metabolites D^4-5-OH-MEHP

	N minal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=10)	Fem les (N=10)	Overall (N=20)	Males (N=10)	Females (N=10)	Overall (N=20)
$Ae_{0\text{-}48\ h}$ (µg)	40.6 (22.6, 57.1)	36.0 (25.1, 4 .8)	38.3 (22.6, 57.1)	357 (243, 426)	342 (254, 406)	349 (243, 426)
$CL_{R\ 0\text{-}48\ h}$ (mL/min)	301, 912[b]	NC	301, 912[b]	189[c] (17.8, 284)	1607[c] (62.8, 3866)	898[d] (17.8, 3866)
$fe_{0\text{-}48\ h}$[a] (%)	6.8 (9.34, 23.6)	14.9 (10.4, 19.3)	15.8 (9.34, 23.6)	16.5 (11.2, 19.6)	15.8 (11.7, 18.7)	16.1 (11.2, 19.6)

Arithmetic me n (min-max) data are presented
N = Numb r of ubjects studied; NC = Not calculated
[a] Ca culated using the actual maximum dose (mg)
i . 0.3 mg D^4-DEHP + 0.80 mg D^4-DINP and 2.87 mg D^4-DEHP + 7.69 mg D^4-DINP
[b] N = 2 (individual values presented), [c] N = 4, [d] N = 8

Table 23 Urinary Excretion of the Metabolites D^4-5-Carboxy-MEPP

	Nominal Dose of D^4-DEHP					
	0.3 mg			3.0 mg		
Parameter	Males (N=10)	Females (N=10)	Overall (N=20)	Males (N=10)	Females (N=10)	Overall (N=20)
$Ae_{0\text{-}48\ h}$ (µg)	40.9 (28.4, 51.1)	37.2 (25.3, 48.7)	39.0 (25.3, 51.1)	318 (174, 493)	272 (160, 348)	295 (160, 493)
$CL_{R\ 0\text{-}48\ h}$ (mL/min)	116[c] (25.6, 276)	126[b]	118[d] (25.6, 276)	724[d] (47.0, 2981)	120[d] (51.8, 165)	422[e] (47.0, 2981)
$fe_{0\text{-}48\ h}$[a] (%)	16.1 (11.2, 20.2)	14.7 (10.0, 19.2)	15.4 (10.0, 20.2)	14.0 (7.64, 21.7)	12.0 (7.05, 15.3)	13 0 (7 05, 21.7)

Arithmetic mean (min-max) data are presented
N = Number of subjects studied
[a] Calculated using the actual maximum dose (mg)
i.e. 0.32 mg D^4-DEHP + 0.80 mg D^4-DINP and 2.87 mg D^4-DEHP + 7.69 mg D^4-DINP
[b] N = 1 (individual value presented), [c] N = 4, [d] N = 5, [e] N = 8

The fraction of D^4-DEHP excreted in the urine as the secondary metabolites up to 48 hours postdose, was similar in male and female subjects at both dose levels for each metabolite. The Ae of each econdary metabolite increased in an approximate dose-proportional manner across the 0.3 mg to 3.0 mg doses Th between-subject variability for fe (%) was generally low.

The overall fraction of D^4-DEHP excreted in the urine is presented in Table 24

Table 24 Summary of % molar elimination of D^4-DEHP metabolites in the urine over 48 hours post-dose (taken from Anderson et al., 2011)

	MEHP	5oxo-MEHP	5OH-MEHP	5cx-MEPP	Total
low dose	6.94	12.53	16.33	15.9	51.7
high dose	5.67	10.00	14.86	11.97	42.51
mean	6.31	11.27	15.5	13.93	47.11
standard errors of mean	0.309	0.426	0.50	0.558	1 345

Single oral doses of D^4-DEHP were considered to be safe and well tolerated by healthy male and female subjects when administered at dose levels of 0.3 mg and 3.0 mg D^4-DEHP. The incidence of adverse eve ts report d during the study was low, with a total of three adverse events reported by 2 subjects. All adverse events were moderate in severity and required treatment with concomitant medication. Only one adverse event of h adache was considered by the investigator to be related to the study investigational product however as this ccurred a the lower dose level only it is most probably unlikely to be truly substance-related. There were no serious or sev re adverse events reported during the study. There were no findings considered to be of clinical importance for th clinical laboratory evaluations (serum biochemistry, haematology, or urinalysis).

Conclusions:

- The primary (D^4-MEHP) and secondary (D^4-5-oxo-MEHP, D^4 5-OH MEHP and D^4-5-carboxy-MEPP) metabolites of D^4-DEHP were steadily formed following admini tration of single oral doses of 0.3 mg and 3.0 mg D^4-DEHP. All metabolites appeared in plasm at median t_{max} values ranging from 2.8 to 4.0 hours postdose.
- Systemic exposure ($AUC_{0\text{-tlast}}$) to D^4-MEHP ncre d n a supra-proportional manner of up to 17-fold for the 10-fold increase in dose. The dose proportionality for exposure to the secondary metabolites with increasing dose could not be assessed due to the low systemi exposure of these metabolites at the 0.3 mg dose level.
- The between-subject variability for systemic exposure (assessed for $AUC_{0\text{-tlast}}$ and C_{max}) to the primary and secondary metabolites of D^4 DEHP was high.
- A total of 6% to 7% of the D^4-DEHP dose was excreted in the urine as D^4-MEHP, and 11% to 16% as D^4-5-oxo-MEHP, D^4-5-OH-MEHP and D^4-5- arboxy-MEPP, up to 48 hours postdose.
- The overall fraction of the 0.3 mg and 3.0 mg doses of D^4-DEHP excreted as both the primary and secondary metabolites up t 48 hour postdose was 51.7% and 42.51% for the low and high dose, respectively.
- The renal excretion of the metabolites assessed for D^4-DEHP mainly occurred over the initial 24 hours following dosing.
- Systemic exposure and urinary excretion of the primary and secondary metabolites investigated for D^4-DEHP was generally similar for male and female subjects.
- There was no correlation evident between renal excretion (fe%) or systemic exposure ($AUC_{0\text{-tlast}}$ and C_{max}) and g nder, body weight or age.
- Single oral doses of D^4-DEHP were considered to be safe and well tolerated by healthy male and female subjects when administered at dose levels of 0.3 mg and 3.0 mg.

These data (urinary metabolites found after application of low dose) are used to calculate external doses from measured urinary concentrations. The methodology is described in detail in chapter 9.0.

The observed urinary excretion of DEHP metabolites in the Anderson et al. (2011) study is in close agreement with the results of another recent toxicokinetic study. Kessler et al. (2012) investigated metabolite profiles of DEHP in 4 human male volunteers, who ingested a single dose of approx. 0.645 mg/kg bw DEHP-D4. Total average amount of urinary excretion of the three metabolites MEHP-D4, 5OH-MEHP-D4 and 5 -oxo-MEHP-D4 was 29.1 and 31% after 22 and 46 h, respectively.

Kurata et al., (2012) looked at glucuronidation of the DEHP metabolites in the urine of human volunteers (10 males/10 females) after oral application of a single dose of 3 mg/kg bw. DEHP-D4 (and 9 mg/kg bw. di-iso-nonyl phthalate). MEHP-D4, 5OH-MEHP-D4 and 5 -oxo-MEHP-D4 were identified, the major part of it as glucuronides (69 to 86%). Urinary concentrations, but no cumulative excretion data are reported in this publication.

Further human studies:

Two healthy male volunteers (47 and 34 years old) received 30 mg DEHP (> 99% pure) as a single dose or 10 mg/day of DEHP for 4 days (Schmid & Schlatter, 1985). In the single dose study, urine was collected every six hours for 48 hours after dosage and metabolites were isolated and identified by GC/MS. Urinary excretion of DEHP occurred mostly within the first 24 hours and a urinary elimination half-life of about 12 hours was estimated. 11% and 15% of the administered dose was eliminated in the urine of the two volunteers, respectively. A total of 12 metabolites were detected with the major metabolites being identified and quantified as MEHP (6.4 and 12.7% of the detected metabolites, in the two volunteers, respectively) and metabolites I (1.9 and 2.1%), IV (3.7 and 1.8%), V (25.6 and 33.8%), VI (24.0 and 19.7%), VII (5.3 and 4.0%), and IX (33.0 and 25.9%). The amount of the remaining 5 metabolit s was less than 1%. About 35% of the metabolites were unconjugated in both volunteers. In the repeated do e study, urine was collected in 24 hour intervals until 48 hours after the last dose. Fifteen and 25% of the administe ed do e was eliminated in the urine of the two volunteers, respectively. Excretion of metabolites showed strong daily fluc uations. Based on the mean excretion of MEHP (9.6%), 5-oxo-MEHP (21.8%), 5-OH-MEHP (29.4 %), a d an assumed total urinary excretion of 25%, Koch et al. (2003a) have calculated conversion factors of 2.4% (MEHP), 5.5% (5-oxo-MEHP), and 7.4% (5-OH-MEHP) based on this study.

The time-course of DEHP metabolism and elimination in one human volunteer has be n investigated by Koch et al. (2004). A single oral dose of 48.1 mg deuterium-labelled DEHP (0.64 mg/kg b w.) was dministered to a male volunteer (the senior author of the paper, age 61, body weight 75 kg). DEHP was spiked into butter and administered on bread. By the use of deuterium-DEHP and the most modern technique (LC-LC/MS-MS), the results are very reliable even though they only represent one individual. The urinary excretion of MEHP, 5OH-MEHP, 5oxo-MEHP was monitored for 44 hours post-dosing and the serum levels was monitored for 8 h urs post-dosing. Peak concentrations in serum were found in the sample taken two hours after dosage, wit MEHP as the major metabolite. The half-time of all the three measured metabolites in serum was estimated to be l ss tha 2 hours. The excretion of DEHP metabolites in urine followed a multi-phase elimination pattern. After an absorption and distribution phase of 4 to 8 hours, the urine elimination pattern showed an initial half-time (8 to 16 hours p st-dosing) of about 2 hours for all three metabolites. The second phase, beginning 14 to 18 hours post-dosing, how d a half-time of about 5 hours for MEHP but 10 hours for the secondary metabolites. The study shows th t the secondary metabolites 5OH-MEHP and 5oxo-MEHP are the major metabolites of DEHP found in human urine at all time points following a single oral dose of DEHP and that the ratio between MEHP and the secondary metabolit s varies over time. Thus, the ratios of MEHP to 5OH-MEPH + 5oxo-MEHP varied over time from 1 to 4.9 during the firs phase (8-16 hours post dose) to 1 to 14.3 during the second phase (16 to 24 hours post dose). In the last samp e, taken 44 hours after the dose, the ratio was 1 to 74. This difference in elimination half-times has to be tak n into acc unt when DEHP ingestion are calculated based on either MEHP or the secondary metabolites. After 44 h 47% of the DEHP dose had been excreted in urine as the three measured metabolites. MEHP comprised 7.3% of the appl ed dose, 5OH-MEHP 24.7% and 5oxo-MEHP 14.9%. Thus, the ratio of excreted MEHP to 5OH-MEHP+5oxo-MEHP w s 1 to 5.4 in this individual, which appears lower than in the two recent studies on the general population (Koch et al., 2003b; Barr et al, 2003), where the ratio of MEHP to 5OH-MEHP +5oxo-MEHP were 1 to 8.7 and 1 to 14.1, respectively.

The metabolism of di(2-e hylhexyl)phthalate (DEHP) in humans was studied after three doses of 0.35 mg (4.7 µg/kg), 2.15 mg (28.7 µg/kg) and 48.5 mg (650 µg/kg) of D4-ring-labelled DEHP were administered orally to a male volunteer (Koch et al 2005).Two new metabolites, mono(2-ethyl-5-carboxypentyl)phthalate (5cx-MEPP) and mono[2-(carboxymethyl)he yl]phthalate (2cx-MMHP) were monitored for 44 h in urine and for 8 h in serum for the high-dose case, in addit on to the three metabolites previously analysed: mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono(2-e hyl-5-oxohexyl)phthalate (5oxo-MEHP) and mono(2-ethylhexyl)phthalate (MEHP). For the medium- and low-dose cas s, 24 h urine samples were analysed. Up to 12 h after the dose, 5OH-MEHP was the major urinary metabolit , after 12 h it was 5cx-MEPP, and after 24 h it was 2cx-MMHP. The elimination half-lives of 5cx-MEHP and 2cx-MMHP were between 15 and 24 h. 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, comprising 2cx-MMHP (1.6%), 5cx-MEPP (1.2%), 5OH-MEHP (0.6%) and 5oxo-MEHP (0.4%). In total about 75% of the administered DEHP dose was excreted in urine after two days. Therefore, in contrast to previous studies, most of the orally administered DEHP is systemically absorbed and excreted in urine. No dose dependency in metabolism and excretion was observed. The secondary metabolites of DEHP are superior biomonitoring markers compared to any other parameters, such as MEHP in urine or blood. 5OH-MEHP and 5oxo-MEHP in urine reflect short-term and 5cx-MEHP and 2cx-MMHP long-term exposure. All secondary metabolites are unsusceptible to contamination. Furthermore, there are strong hints that the secondary oxidised DEHP metabolites not DEHP or MEHP are the ultimate developmental toxicants.

Silva et al., 2003 report percentages of glucuronidation of four common phthalate monoesters, monoethyl (mEP), monobutyl (mBP), monobenzyl (mBzP), and mono-2-ethylhexyl phthalate (mEHP) in a subset of urine (mEP n=262, mBP n=283, mBzP n=328, mEHP n=119) and serum (mEP n=93, mBP n=149, mEHP n=141) samples from the general US population. The percentages of free and conjugated monoester excreted in urine differed for the various phthalates. For the more lipophilic monoesters (i.e., mBP, mBzP, and mEHP), the geometric mean of free monoester excretion ranged from 6 to 16%. The contrary was true for the most hydrophilic monoester, mEP, for which about 71% was excreted in urine as its free monoester. Furthermore, percentages of free and conjugated monoesters were similar for mEP, mBP and mEHP among serum and urine samples. Serum mBzP was largely below the method limit of detection. Interestingly, the serum mEP and mBP levels were less than 3% and 47%, respectively, of their urinary levels, whereas the level of mEHP was similar both in urine and serum.

The metabolism of DEHP in humans was investigated by identifying urinary oxidative metabolites of DEHP from individuals with urinary MEHP concentrations about 100 times higher than the median concentration in the gen ral US population (Silva et al., 2006). In addition to the previously identified DEHP metabolites MEHP, mono(2 ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-car oxypentyl) phthalate (MECPP), and mono(2-carboxymethylhexyl) phthalate (MCMHP), we also identified for the firs time in humans three additional oxidative metabolites, mono(2-ethyl-3-carboxypropyl) phthalate (MECPrP), mono(2-ethyl-4-carboxybutyl) phthalate (MECBP), and mono(2-(1-oxoethyl)hexyl) phthalate (MOEHP) based on their chromatographic behavior and mass spectrometric fragmentation patterns. Metabolites with two f nctional groups in the side alkyl chain were also tentatively identified as isomers of mono(2-hydroxyethyl-4- arboxybu yl) phthalate (MHECBP), mono(2-ethyl-4-oxo-5-carboxypentyl) phthalate (MEOCPP), and mono(2-ethyl-4-hydroxy-5-carboxypentyl) phthalate (MEHCPP). The presence of urinary DEHP metabolites in humans th have fewer than eight carbons in the alkyl chain was observed. These metabolites were previously identified in rodents. Although quantitative information is not available, these findings suggest that, despite potential differences among species, the oxidative metabolism of DEHP in humans and rodents results in similar urinary metaboli produc s.

Non-human primates

In a 65-week oral-dose toxicity study of DEHP in marmoset , which included a toxicokinetic study (Kurata, 2003), ring-labeled^{14}C-DEHP (99.6% purity) in corn oil was given to 3 groups f marmosets. The first group was treated at 3 months of age. The second group was treated at 18 mon hs of ge. The third group was treated for 65 weeks from 3 months of age with unlabeled DEHP and studied at 18 months of age. There were 3 animals of each sex in each treatment group. Treatments were by gavage at dos le ls of 100 or 2500 mg/kg bw. Blood samples were collected 1, 2, 4, 8, 12, 24, 48, 72, 120, and 168 hours after dosing Spontaneous urine and feces were collected for radioactivity determination. At least 2 weeks after the kinetic studies, animals were dosed again and tissues collected 2 hours later for determination of radioactivity. Radioactivi y determi ation was by liquid scintillation counting. The authors found the highest level of radiation in the kidneys afte a singl oral dose, and considered that high radioactivity levels in the prostate and seminal vesicles of some animals may have been due to urine contamination. Repeated dosing for 65 weeks did not appear to alter the d stribution of DEHP in 18-month-old animals. The authors called particular attention to the small amount of label distribu ed to the testis and postulated that differences in access of DEHP metabolites to the testis may explain a lack of testicular toxicity in marmosets compared to rodents, in which large amounts of MEHP are distributed to the testis fter DEHP treatment.

In a GLP study (Kurata, 2005) ^{1} C-labeled DEHP was orally administered to pregnant marmosets at a single dose of 100 mg/kg to ex min the transferability of the radioactivity to foetuses. The radioactivity (0.40 µg eq/g) of the foetal blood was comparable to that (0.41 µg eq/mL) in the plasma of the pregnant animals, and the radioactivity in the foetal kidney and liv r, 0 62 and 0.55 µg eq/g, respectively, was higher than that in the plasma of the pregnant animals at 24-hr post dose The whole-body autoradiography revealed high radioactive concentrations in the bladder urine and small intestinal ontents. As for the foetal testis, the radioactivity, 0.20 µg eq/g, was lower than that in the plasma of the pregnant animals, and no specific distribution was noted in this organ.

Kessler et al.(2004) compared blood levels of DEHP and MEHP in pregnant and non-pregnant Sprague-Dawley rats and rmosets in a Good Laboratory Practice (GLP) study. Sprague–Dawley rats and marmosets were treated orally with 30 or 500 mg DEHP/kg per day, nonpregnant animals on 7 (rats) and 29 (marmosets) consecutive days, pregnant animals on gestation days 14–19 (rats) and 96–124 (marmosets). In addition, rats received a single dose of 1000 mg DEHP/kg. Blood was collected up to 48 h after dosing. Concentrations of DEHP and MEHP in blood were determined by GC/MS. In rats, normalized areas under the concentration–time curves (AUCs) of DEHP were two orders of magnitude smaller than the normalized AUCs of the first metabolite MEHP. Metabolism of MEHP was saturable. Repeated DEHP treatment and pregnancy had only little influence on the normalized AUC of MEHP. In marmosets, most of MEHP concentration-time courses oscillated. Normalized AUCs of DEHP were at least one order of magnitude smaller than those of MEHP. In pregnant marmosets, normalized AUCs of MEHP were similar to those in nonpregnant animals with the exception that at 500 mg DEHP/ kg per day, the normalized AUCs determined on gestation days 103,

117, and 124 were distinctly smaller. The maximum concentrations of MEHP in blood of marmosets were up to 7.5 times and the normalized AUCs up to 16 times lower than in rats receiving the same daily oral DEHP dose per kilogram of body weight. From this toxicokinetic comparison, DEHP can be expected to be several times less effective in the offspring of marmosets than in that of rats if the blood burden by MEHP in dams can be regarded as a dose surrogate for the MEHP burden in their fetuses.

In a study of GLP-quality, male Cynomolgus monkeys (2 animals per group) received 100 or 500 mg/kg b. w. per day of unlabelled DEHP (99.8% pure) in corn oil by gavage for 21 days (Short et al., 1987). On day 22 each monkey received a single dose of (carbonyl-^{14}C) DEHP (radiochemical purity >97%) followed by three daily doses of unlabelled DEHP on days 23 to 25. Urine and faeces were collected at intervals on days 22 to 25 and then the animals were sacrificed. The percentages of (^{14}C) DEHP derived radioactivity in urine, faeces, and selected tissues (blood, liver, spleen, intestines, intestinal contents, fat, brain, kidneys, adrenals, testes, urinary bladder) were determined by liquid scintillation. Urine samples collected from 0-24 hours were analysed for metabolites of DEHP by normal and re ersed phase HPLC. (^{14}C) DEHP derived radioactivity was detected in some tissues (liver and intestines) at the 500 mg/kg dose level, but represented less than 0.2% of the dose administered. The plasma concentration curves (AUC) f r DEHP derived radioactivity for the first 48 hr was133 and 283 ug-hr/ml at 100 mg/kg b. w. and 387 and 545 ug-h /ml at 500 mg/kg b. w. For the dose of 100 mg/kg b. w., the two individual monkeys excreted 20 and 55% (20 and 55 mg) in the urine and 49 and 39% (49 and 39 mg) in the faeces. For the the 500 mg/kg b. w. dose, 4 and 13% (20 and 65 mg) were in the urine and 69 and 56% (345 and 280 mg) were in the faeces. This was measured within 96 h urs but the majority was excreted within the first 24 hours after dosing, with most of the remainder being excre ed during the next 24 hours.DEHP derived radioactivity in 0-24 hour urine samples was resolved into at least 15 me abolites and identified as MEHP, phthalic acid, metabolites I, III, IV, V, VI, IX, X, XII, XIII, XIV, and unident fied fra t ns. Major metabolites were MEHP, phthalic acid, metabolites V, IX, X, and probably XII, however, a great variability between the two individuals in both dose groups was observed. Polar components, including possibl glucuronides, made up only a small percentage of the urinary radioactivity. This study indicates a species difference n the metabolism of DEHP in rats and a nonhuman primate as two of the major metabolites identified in the u ine of rats (m tabolite I, the end-product of ß-oxidation of V; and metabolite VI, which is believed to be the proximate perox some stimulator in rodents) were minor metabolites in monkey urine.The recoveries of the amount of DEHP derived radioactivity in the urine indicated that absorption at 500 mg/kg b. w. is equivalent to that at 100 mg/kg b. w This suggests that a dependent reduction in the absorption of DEHP from the intestinal tract of Cynomolgus monkeys (f. Marmosets). However, the AUC is greater for 500 mg/kg b. w. than 100 mg/kg b. w. indicating that absorption is greater above 100 mg/kg b. w. than at 100 mg/kg b. w. [though one would expect proportionally higher values but AUC for a longer time than 48 hr should be conducted for 500 mg]. The difference in none recovered radioactivity in the urine at these two different dose groups may depend on dose, or a higher degree of an alternative excretion pathway (e. g. hepatobilary excretion) and/or a higher degree of retention in the body at 500 mg/kg.

A comparative species differences in the metabolism of DEHP was studied after administration of a single oral dose of 100 mg/kg b. w. (carbonyl-^{14}C) DEHP (radi chemical purity >97%) in corn oil by gavage to three male Cynomolgus monkeys, five male Fisher 344 rats and five groups of five male B6C3F1 mice at (Short et al., 1987; Astill et al., 1986). The study was using a method equ valent to guideline study and conducted according to GLP. Urine and faeces were collected at intervals of 12, 24, 48, 72 and 96 hours after dosing. Blood samples were taken from the femoral vein of monkeys at 2, 4, 8, 24 hours and just prior to sacrifice. All animals were killed around 96 hours after dosing for tissue collection (liver, stoma h, intestines, intestine contents, gall bladder wash and bile). Concentrations of radioactivity in urine, faeces and blood were dete mined at the specified intervals and concentrations of radioactivity in selected tissues and other biological samples we e determined by liquid scintillation at around 96 hours after dosing. Faeces samples collected from 0 48 hours were pooled for the monkeys, and urine samples collected from 0-24 hours were pooled for each species and wer analysed for metabolites of DEHP by HPLC. Urinary metabolites were isolated and the major metabolites w re analysed by GC/MS. All three species excreted 30-40% of the dose in the urine (rats 32.9%, mice 37.3% monk ys 28 2%), primarily during the first 12 hours for rats and mice and during the first 24 hours for monkeys. All three ecies excreted around 50% of the dose in the faeces (rats 51.4%, mice 52.0%, monkeys 49.0%), primarily during the first 24 hours for rats and mice and during the first 48 hours for monkeys. The rates and extent of urinary and faecal excretion varied widely among monkeys. DEHP was detectable in some tissues in all three species. The mean concentrations detected, with the exception of monkey liver and rat intestinal contents, were less than 1 µg/g. The highest concentrations were detected in liver, intestinal contents, and fat for monkeys, rats, and mice, respectively. Total recoveries of the radioactivity administered were 79 (68-91%), 87 (82-92%) and 90% (63-102%) for monkeys, rats and mice, respectively. Radioactivity in 0-24 hour urine samples were resolved into 13, 15, and 14 components in rats, mice, and monkeys, respectively. The components in urine were identified as MEHP (not detected in rat), phthalic acid, metabolites I, II (not detected in monkey), III (not detected in rat), IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. Major urinary components in rats were metabolites I, V, VI, and IX. Major urinary components in mice were MEHP, phthalic acid, metabolites I, VI, IX, and XIII, and in monkeys: MEHP, and metabolites V, IX, and X. In monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either absent or present in negligible quantities. Radioactivity in 0-48 hour monkey faecal extracts and

in 0-24 hour rat and mouse faecal extracts were resolved into 11, 10 and 10 components in rats, mice and monkeys, respectively. The faecal components were identified as DEHP, MEHP, phthalic acid, metabolites I-IV, VI, VII, IX, X, XII, XIII (not detected in monkey), and XIV (not detected in mouse). DEHP was a major faecal component in all three species and MEHP a major faecal component in rats and mice.Based on the amount of DEHP derived radioactivity recovered in the urine of Cynomolgus monkeys, rats and mice a similar degree of oral absorption of DEHP is indicated at a dose level of 100 mg/kg b. w.Two major species differences in the metabolism of DEHP in rat and mouse were observed as MEHP was a major component in mouse urine but was not detectable in rat urine; metabolite V was a major component in rat urine but a negligible component in mouse urine. Some overall similarities were observed in the metabolism of DEHP in monkeys and rats. In both species the MEHP formed by hydrolysis of DEHP was further metabolized via the ω-oxidation pathway, generating metabolites X, V, and I which collectively made up 34 and 44% of the radioactivity in the urine of monkeys and rats, respectively; and via the (w-1) -oxidation pathway, generating metabolites IX and VI which collectively made up 19 and 29% of the radioactivity in the urine of monkeys and rats, respectively. However, some overall differences in metabolism were also observed between the two species. MEHP was a relatively major component of monkey urine (11%) but was not detected in rat urine. Also in monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either bs nt or present in negligible quantities. Furthermore, MEHP was extensively converted to metabolite V in th monkey but, in contrast to the rat, further oxidation to metabolite I was negligible. Also metabolite I was a major componen in mouse urine. It appears therefore that ß-oxidative metabolism of DEHP is a major pathway in rodents b t not in monkeys.

The disposition of DEHP was studied in marmosets (Rhodes et al., 1983). Groups of three male marmosets received a single dose of (^{14}C-ring labelled) DEHP (radiochemical purity 97.5%) by the oral route (100 a d 2 000 mg/kg b. w.), intravenously (100 mg/kg b. w.), and intraperitoneally (1 000 mg/kg b. w.)(Rhodes et l., 1983; Rhodes et al., 1986). Urine and faeces were collected for seven days and the radioactive content det rmined. Tissue samples were removed 7 d after the administration.Following intravenous administration approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion) indicating a 2 to 1 r tio be ween the urinary and biliary (faecal) routes of excretion in the marmoset. Around 28% of the dose emained in th lungs with minimal levels in other tissues. A much smaller proportion of the dose was excreted following intraper oneal administration (10% in the urine and 4% in the faeces) in a similar 2 to 1 ratio. Around 85% of the dose r mained as unabsorbed ^{14}C in the peritoneal cavity with minimal amounts in the tissues (0.6%). Following oral adminis ation of 100 mg/kg b. w. 20-40% of the dose were excreted in urine and around 25% in faeces, and ollowing administration of 2 000 mg/kg b. w. around 4% and 84% were excreted in urine and faeces, respectively Minimal amounts remained in the tissues (< 0.1%). This indicates that oral absorption of DEHP by marmosets is d se-limited at 2000 mg/kg b. w. compared with 100 mg/kg b. w. Dose dependent reduction in the absorption of DEHP from the intestinal tract of the marmoset (according to the authors the amount absorbed is more equivalent to th t expected for a 150 to 200 mg/kg b. w. dose).

A comparative toxicokinetic study was carried out in marmosets (3 males and 3 females, 12-18 months) and Wistar derived albino rats (3 males and 3 females, Alderley ark Specific pathogen-free strain, 6-8 weeks). The animals were given (^{14}C ring labelled) DEHP (radi chemi al purity 97.9%) at doses of 2 000 mg/kg b. w. dailyby gavage for 14 days (Rhodes et al., 1986). Two sample of blood (0.5 ml) from each animal were taken during 0-8 hour period after dosing on day 1 and 14. The rats were ble via the t il vein and the marmosets via the femoral vein. Twenty-four hours after the final dose the animals were killed by inhalation of carbon dioxide/oxygen, and samples of blood (5 ml) were withdrawn from each animal via the vena cava. Excreta were collected for 24 hours after administration of the dose on days 6 and 13. Immedi tely after the blood samples were taken each animal was dissected and whole liver, kidneys and testes taken for radioch mical an lysis. Radioactivity was measured by liquid scintillation spectrometry. The radiolabelled compounds in urine and faeces were analysed by TLC to determine the distribution between DEHP and its metabolites.The uptak of radioactivity into the blood of rats was rapid and peaked after 2-3 hours (126 and 206 µg/g in males and females respectively) following administration on day 1, and after 6 hours (368 and 475 µg/g in males and females respe tive y) on day 14. On both days, blood levels did not decline significantly during the 8-hour sampling period, but 24 hours after dosing on day 14, the levels were 66 and 158 µg/g in males and females, respectively. Blood lev ls in marmosets were considerably lower. They peaked 1 hour after dosing (5 and 8 µg/g in males and females, espectively) t day 1 and after 1 and 3 hours after dosing in males and females, respectively, on day 14 (13 µg/g each) a d had n t declined significantly after 24 hours.After dosing on day 6, male rats excreted 83% (53% in urine and 30% in faece) and female rats excreted 63% of the dose (39% in urine, 24% in faeces). Male marmosets excreted 69 (1% in urine and 64% in faeces) and female marmosets excreted 80% of the dose (2% in urine and 75% in faeces) after the same exposure period. After dosing on day 13 male rats excreted 97% (56% in urine and 41% in faeces) and female rats excreted 96% of the dose (52% in urineand 43.6 in faeces), while male marmosets excreted 62% (1% in urine and 59% in faeces), and female marmosets excreted 75% of the dose (1% in urine and 71 in faeces). The discrepancy between the sum of urine and faeces and the total is due to cage washing. Two radiolabelled compounds were present in rat faeces (analysed by TLC), one being identified as DEHP (42% of the radioactivity from TLC), the other more polar compound (57% of the radioactivity) was not identified. In the faeces of marmosets, 98% of the recovered radioactivity was identified as DEHP. The levels of radioactivity in blood, expressed asmg equivalents of DEHP per g of blood in males and females, were 0.3 and 0.5% of the daily dose, respectively, in rats one hour after administration on day 1. The

corresponding levels for marmosets were 0.02 and 0.03%, respectively. The levels of radioactivity in blood 24 hours after administration on 14thday of exposure were 0.2 and 0.5%, respectively, for male and female rats. The corresponding values for marmosets were 0.03 and 0.06%, respectively. The very high faecal elimination and the low levels of radioactivity in urea, blood and tissues in marmosets compared with rats suggests, in agreement with the single dose study by Rhodes et al., (1983) that DEHP at 2000 mg/kg b. w. was poorly absorbed, whereas the urinary elimination data for rats indicate that at least half the dose was absorbed. The study also shows that repeated administration of DEHP in both rat and marmoset did not modify the proportion of dose excreted. The tissue levels in liver and in kidney were generally higher in female rats compared to male rats (liver 216 and 286 µg/g, kidney 115 and 176 µg/g in males and females, respectively). The mean residue level in testes was lower (36mg/g) than in other tissues. Tissue levels in marmosets were considerably lower than in rats (liver 29 and 47 µg/g, kidney 15 and 35 µg/g in males and females, respectively).

Rats

Sprague-Dawley

The toxicokinetic relationship between di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalat (MEHP), a major metabolite of DEHP, was investigated in Sprague-Dawley rats orally treated with a ing e dose of ^{14}C-DEHP (Koo, 2007). Urinary excretion of total ^{14}C-DEHP and of its metabolites was followed by liquid scintillation counting (LSC). Concentrations of DEHP and MEHP were determined 6, 24, and 48 h after treatment in rat serum and 6, 12, 24, and 48 h after treatment in urine by high-performance liquid chromatography (HPLC). After 2 h, peak concentrations of MEHP in both urine and serum were observed in animals treated with 40, 200 or 1000 mg DEHP/kg. HPLC showed that general toxicokinetic parameters, such as Tmax (h), Cmax (µg/ml), Ke (1 h), and AUC (µg-h/ml/) were greater for MEHP than DEHP in both urine and serum. In contrast, the half-lives (t1/2 [h]) of DEHP were greater than those of MEHP. The AUC ratios between DEHP and MEHP were relatively small r in se um than in urine, suggesting the important role of urinary DEHP data for exposure assessment of DEHP.

Sjöberg et al.(1985) studied the kinetics of DEHP and MEHP in immat re and mature Sprague-Dawley rats in two different studies. In one study (9-10 rats per group; 25, 40, or 60 day old n he day of dosing) were given a single dose of 1 000 mg/kg b. w. of DEHP (99% pure) in corn oil by gavage. Blood samples, 0.25 ml drawn from a jugular vein, were taken at 1, 3, 5, 7, 9, 12, 15, 24, and 30 hours after dos ng The area under the plasma concentration-time curve (AUC) and the elimination half-life was calculated. Dete table plasma concentrations of DEHP (>2 µg/ml) were found only in some of the animals 1-7 hours after dosing MEHP was detectable in all but five plasma samples (the 24- and 30-hour sample of two 60-day old rats, and the 30-h ur samp of one 60-day old rat). The maximal plasma concentration (Cmax) of MEHP generally appear d on hour after dosing, but in some 25-day old animals it was observed at 3-7 hours after dosing. No diff rences in Cmax were observed between the different age groups. Cmax ranged between 48 and 152 µg/ml, with a mean of 93 µg/ml. The mean AUC (0-30 hours) of MEHP of 25-day old rats (1213mg×hr/ml) was significantly higher than that of the 40- and 60-day old rats (611 and 555 mg×hr/ml, respectively). No significant differences in the mean plasma elimination half-life of MEHP were observed when comparing the different age groups. The mean pla ma elim nation half-lives of MEHP were 3.9, 3.1 and 2.8 hours, respectively for 25, 40 and 60 days old rats. The binding of MEHP to plasma proteins was 98% in all dose groups. In a second experiment by Sjöberg et al. (1985) the excretion of DEHP was studied in immature and mature Sprague-Dawley rats. Two groups of 6 rats which were 25 and 60 d ys old, respectively, on the day of dosing were given single doses of 1000 mg/kg b. w. of (carbonyl-^{14}C) DEHP (99% pure) in corn oil by gavage. The urine was collected daily for three days. The cumulative excretion of radi activity was 44 and 26% in 25- and 60-day old rats, respectively, within the first 72 hours after dosing. More than 85% of the rinary radioactivity appeared within the first 24 hours. No intact DEHP or MEHP was found in the urine wh n an lysed by TLC.

To examine the plasma concentration time profiles of MEHP and metabolites V, VI and IX after oral administration of DEHP, two separate experiments were performed by Sjöberg et al., (1986). In the first experiment, a suspension of DEHP (puri y not stated) in propylene glycol was given to five 35-day old male Sprague-Dawley rats in a dose of 2.7 mm l/kg . w. Blood samples drawn from one of the jugular veins 0.5, 1, 2, 3, 5, 7, 9, 12, 15, and 22 hours after dosing. In the second experiment, five rats were given daily doses of 2.7 mmol/kg b. w. of DEHP in propylene glycol for 7 days. After the final dose, blood samples were collected at the same time intervals as in the first experiment. The plasma concentrations of MEHP and the metabolites were determined by gas chromatography-electron impact mass spectrometry.The plasma concentrations and mean AUC's of each of the MEHP-derived metabolites were considerably lower than those of MEHP both after single and after repeated administration. The maximal plasma concentrations (MEHP, 0.55 and 0.56 µmol/ml; metabolite IX, 0.15 and 0.09 µmol/ml; metabolite VI, 0.06 and 0.07 µmol/ml; metabolite V, 0.06 and 0.09 µmol/ml after single and repeated doses, respectively) and mean AUC's (MEHP, 5.15 and 3.44 µmol/ml; metabolite IX, 0.84 and 0.46 µmol/ml; metabolite VI, 0.44 and 0.41 µmol/ml; metabolite V, 0.39 and 0.43 µmol/ml after single and repeated doses, respectively) did not differ significantly between animals given single or

repeated doses of DEHP. The mean elimination half-life of MEHP was significantly shorter in animals given repeated doses (1.8 hours) than in those given a single dose (3 hours).

The disposition kinetics of DEHP was studied in male Sprague-Dawley rats following single or multiple administration of DEHP by various routes (peroral by gavage: 2 000 mg/kg b. w.; intra-arterial: 100 mg/kg b. w.; intraperitoneal: 4 000 mg/kg b.w.) (Pollack et al., 1985).The animals were given a single dose of 2 000 mg/kg b. w. of DEHP (purity not stated) in corn oil by gastric intubation. Blood samples were drawn over a 30-hour period. Thereafter, repetitive doses of DEHP were administered to the same animals once daily for 7 days whereafter blood samples were collected over a 48-hour period. The concentrations of DEHP and MEHP in whole blood were determined by high performance liquid chromatography (HPLC). After a single oral dose, DEHP was absorbed relatively rapidly with a peak blood concentration of DEHP observed at approximately 3 hours. Systemic bioavailability of DEHP was low, approximately 13%. Blood concentrations of MEHP were much higher than those of the parent compound after oral administration. The blood concentrations of DEHP following repeated dosing were similar to those observed after a single dose Secondary increase in the concentration of DEHP in blood was observed following administration by all three rout s. Following a single intra-arterial injection a large apparent volume of distribution and a high rate of clearance was observed for DEHP. A marked route-dependency in the formation of MEHP from DEHP was observed. Pharmacokinetic calculations revealed that approximately 80% of an oral dose of DEHP undergoes m no-d - esterification, as compared to only about 1% of the dose following either intra-arterial or intrap ritoneal dministration. Multiple intraperitoneal injections resulted in an apparent decrease in the rate and/or extent of DEHP absorption from the peritoneal cavity, while no significant change in the peroral absorption of DEHP was o served. The difference in the MEHP to DEHP AUC ratio between peroral and intraperitoneal routes was still evident af er multiple dosing.

DEHP and MEHP were secreted into the milk of lactating Sprague-Dawley (CD) rats when given 3 oral doses of 2 000 mg/kg b. w. per day of DEHP in corn oil by gavage on days 15-17 of lactation (Dostal et al , 1987). Plasma collected 6 hours after the third dose contained virtually no DEHP but substantial amount of MEHP (76 µg/ml). Milk collected 6 hours after the third dose contained 216 µg/ml DEHP and 25 µg/ml MEHP A ve y eff cient extraction mechanism for DEHP was suggested because of a high milk/plasma ratio.

Male Sprague-Dawley rats (number not stated, 250-350 g) were given two dos s of 100 mg (7-^{14}C) DEHP (purity not stated) or (7-^{14}C) MEHP (purity not stated) in corn oil by gavage, 24 hours apart (Albro et al., 1983). Urine was collected from the time the first dose was given until 24 hours after the econd dose. Metabolites were isolated and analysed by HPLC and GC, and the profiles of radioactivity f he urinary metabolites were determined. Twenty metabolites were identified in the urine of rats. The metabolites identified in the urine of rats treated with either DEHP or MEHP were identical. No glucuronides or other conjugat s were detected.In a second experiment of the same study, (7-^{14}C) DEHP was given as a single dose to a rat (300 g) and the urine collected was freezed immediately. One week later the same rat was given a dose of (7-^{14}C) DEHP id ntical to that above and the urine was collected. Metabolites were isolated and analysed by HPLC and GC. The profiles of radioactivity of the urinary metabolites in the two different samples were qualitatively identic l indicat ng, according to the authors, that the presence of any of the metabolites found was not due to fu ther meta oli m by bacteria in the urine.According to the authors, previous studies of the metabolism in rats led to the suggestion that the enzymatic processes normally associated with w-, (w-1) -, a- and ß-oxidation of fatty acids could acc unt for the known metabolites of DEHP found in the urine. Several metabolites of DEHP have been identified in the pres nt study. Their formation requires that the initial hydroxylation process is less specific than fatty acid ω- and (ω-1) -oxidation are thought to be. Furthermore, it is necessary to postulate either that the aliphatic chain of MEHP can be oxidised at two sites simultaneously, or that oxidation products can be recycled for a second hydroxylation prior to excretion.

Adult male Sprague-Dawley rats (CD, 300-400 g; number not stated) were administered two doses of 200 µl (196 mg) (7-^{14}C) DEHP (>99% pure) in corn oil by gavage, 24 hours apart (Albro et al., 1973). The urine was collected for 48 hours after the first dose was given. Metabolites in the urine were analysed by TLC and gas chromatography (GC) and charact rised by infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy (MS). Five metabolites were ide tified n the urine. The metabolites identified correspond to phthalic acid and to metabolites I, V, VI, IX resulting rom ω and (ω-1) -oxidation of MEHP without attack on the aromatic ring. MEHP was not detected in the urine and phthalic a id amounted to less than 3% of the urinary metabolites. Conjugates were not detected. These results indicate, according to the authors, that DEHP is first hydrolysed to MEHP, which then undergoes w- and (w-1) -oxidation of the side chain. Alcohol intermediates may then be oxidized to the corresponding ketones. The metabolites found in the urine suggest that, in the rat, MEHP is metabolized like a fatty acid byw- and (w-1) -oxidation and then by ß-oxidation.

The elimination of DEHP was studied in rats and hamsters (Lake et al., 1984). A single dose of (carbonyl-^{14}C) DEHP (>99% pure) was administered to 5-week-old male Sprague-Dawley rats and male DSN strain Syrian hamsters at dose levels of 100 (5 rats, 3 hamsters) or 1000 mg/kg b. w. (5 rats, 5 hamsters) in corn oil by gastric intubation. Urine and faeces were collected over a period of 96 hours and then the animals were sacrificed. Radioactivity was measured in urine, faeces, and total gut contents by liquid scintillation spectrometry. Faecal metabolites were extracted and chromatographed on thin-layer plates. In both species the bulk of the radioactivity was excreted within 24 hours. At the

lower dose level, both species excreted more radioactivity in the urine (rat: 51%, hamster: 53%) than in the faeces (rat: 43%, hamster: 31%), whereas at the higher dose level, the major route of excretion was via the faeces (rat: 53%, hamster: 48%). In both species and at both dose levels, only negligible amounts of radioactivity were present at termination in either the liver, kidney, or total gut contents. Faecal radioactivity profiles were determined in 0-24 hour faeces samples. About 50% of the faecal radioactivity of rats at the higher dose level appeared to be the parent compound, the remainder comprised metabolites possibly including MEHP. In contrast, more than 95% of the faecal radioactivity of hamsters appeared to be the parent compound. Similar results were obtained with faecal extracts from rats and hamsters at the lower dose level.

Excretion and metabolism of DEHP were studied in a comparative study where DEHP (99.6% pure) was administered in the diet to adult male Sprague-Dawley rats (6 animals, 200-300 g), male beagle dogs (4 animals, approximately 1 year old, 7-10 kg), and male miniature pigs (5 animals, Hormel strain, between 4 month and 1 year, 10-25 kg) in doses of 50 mg/kg b. w. per day for 21-28 days before administration of a single dose of (carbonyl-^{14}C) DEHP (radioc emical purity >98%) (50 mg/kg b. w.) in corn oil by gavage (Ikeda et al, 1980). Administration of the DEHP containing di ts was continued until the animals were killed. Distribution and excretion of the radioactivity in urine, faeces, and various organs and tissues (liver, kidney, g. i. -tract with content, lungs, brain, fat and muscle) were analysed at various times by liquid scintillation. Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respectively; and after 4 days 37 and 53% (rats), 21 and 5% (dogs) and 79 and 26% (pigs), respectively. Elimination of radioactivity was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four rad oactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolized DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 all species and a small amount remained after 4 days. In other organs there was only a small amount of radioactivity present in all samples. Of the remaining organs the highest level (about 2% of the dose) was found in the livers from rats after 4 hours. Bile samples from dogs, and to a lesser extent from pigs, accounted for a significant mount f administered^{14}C dose. Less than 1% of the administered^{14}C dose was secreted in the bile from bile duct c nnu ated rats.

Metabolism and tissue distribution of mono-2-ethylhexyl phthalate (MEHP) ha been studied in male Sprague-Dawley rats (Chu et al., 1978). To study if MEHP was readily absorbed ora ly, th car tid arteries of 8 rats were cannulated and 4 days later 4 animals were given 69 mg (7-^{14}C) MEHP/kg (20mCi) in corn oil via stomach tube. Serial blood samples (0.2 ml) were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hou s after dosing and radioactivity was determined. The blood level was highest in the first sample, after 0.5 hours, an then rapidly decreased. About five hours after dosing there was a small increase in the bloodconcentration in all nimals whereafter the concentration slowly continued to decrease.Another four rats were given 35 mg (7-^{1}) MEHP/ g in 5% NaHCO3via the cannula. Serial blood samples were collected at 2, 4, 6, 8, 12, 14, 16, 18 and 20 minutes after the injection and analyzed for radioactivity. Immediately after the blood samples were taken the animals wer exs nguinated and tissues and organs were removed for determination of radioactivity. The rapid d crease was observed also in the rats administered i. v. The first blood sample showed that 53% of the radioactivity r main d in th blood. Approximately 1/3 and 1/5 of the radioactivity was retained in the blood 10 and 20 min after administration, respectively. Twenty minutes after an i. v. dose the liver, bladder and kidney were found to possess high radioactivity, but other tissues also had some radioactivity. One of the major deposition sites for radioactivity sho tly aft r i. v. injection was the liver.In a third experiment two groups of four rats were given 69 mg (20mCi) and 6.9 mg (2mCi) (7-^{14}C) MEHP/kg, respectively, in corn oil via stomach tube. The animals were exsaguinated after 24 hours and tissues and organs were removed for determination of radioactivity. Twenty-four hours afte an oral dose of 69 mg (7-^{14}C) MEHP virtually all radioactivity was removed from the body and only traces were present n the kidney, liver, heart, lung, intestine and muscle. No detectable amounts could be found in the tissues of rat 24 hours after an oral dose to 6.9 mg/kg.In a fourth study four rats were given a single oral dose of 69 mg (7-^{14}C) MEHP/kg (20mCi) in corn oil via stomach tube, and were kept individually in metabolism cages. Urine and faeces w re c llect d each day for 7 days for radioactivity measurements. Only the urine was examined for metabolites as this route of excr tion accounted for 81% of the dose. Excretion after 48 hours was insignificant. At least four metabolit were identified and they had previously been identified as DEHP metabolites.The bile ducts were annulated in four rats in a fifth study by the same author. These rats were given 3.5 or 35 mg (7-^{14}C) MEHP/kg in 5% NaHCO3 ia the superficial dorsal vein of the penis. Serial bile samples were taken hourly for 8 hours and assessed for radioactivity. Within 8 hours 52% (3.5 mg/kg) and 40% (35 mg/kg) of the dose was secreted, respectively, and secretion after this time was insignificant.Thus, the present study indicate that MEHP given orally to the rat undergoes ω- and (ω-1) -oxidation to yield the same metabolites as does DEHP, and suggest that MEHP is the intermediary product in the DEHP metabolism. More than 80% of the radioactivity of the orally administered (7-^{14}C) MEHP was excreted in urine within 24 hours. Up to 52% of the radioactivity entered the intestine from the bile whereas only 8% of the dose was excreted in faeces. This would indicate that resorption of radioactivity took place in the intestine. The rise in the radioactivity in the blood after the rapid decrease could be attributed to the reabsorption of biliary secreted material. The percent radioactivity secreted in the bile was lower, 40%, at the higher exposure level (35 mg/kg) compared to 52% at the lower exposure level (3.5 mg/kg).

Calafat et al.(2005) measured MEHP in maternal urine and amniotic fluid after gavage administration of DEHP [purity not specified] in corn oil to pregnant Sprague-Dawley rats on GD 8, 10, 15, 16, and 17. [The abstract indicates administration also on GD "5/7".] Doses were 0, 11, 33, 100, and 300 mg/kg bw (n = 2/dose group). Urine was collected approximately 6 hours after dosing and amniotic fluid was collected at necropsy on GD 18. MEHP was analyzed by HPLC-tandem MS after solid-phase extraction and enzymatic hydrolysis. There was no temporal trend in urinary MEHP levels over the collection period, and the 5 urine MEHP levels were combined for each animal. Creatinine-corrected and uncorrected urinary MEHP and uncorrected amniotic fluid MEHP were highly correlated with maternal DEHP dose (r values 0.964–0.998). [Data were presented only in graphic form. At the 300 mg/kg maternal DEHP dose level, urinary MEHP was estimated from a graph at 16.4 µg/L and amniotic fluid MEHP was estimated at 2.8 µg/L.] Maternal urinary MEHP was only 13.3% unconjugated while amniotic fluid MEHP was 88.2% unconjugated. The authors observed that the finding that MEHP was largely conjugated in urine did not agree with reports of other studies on urinary MEHP in rats. The authors also indicated that the lack of measurement of more oxidized MEHP metabolites may lead to an underestimation of exposure to DEHP and its biotransformation products.

Ono et al.(2004) evaluated the testicular distribution of DEHP in 8-week-old Sprague-Dawley rats. The r ts w re given a single gavage dose of DEHP 1000 mg/kg bw, radiolabeled either in the ring or the aliphatic side chains. Th animals were perfusion-fixed with paraformaldehyde and glutaraldehyde under anesthesia 6 or 24 hours after DEHP administration (n = 4 animals/time point). Testis, liver, and kidney were collected and processed for light and electron microscopic autoradiography. After ring-labeled DEHP was given, light microscopy showed pref rential distribution of grains to the basal portions of stage IX–I tubules at 6 hours. Grain counts were high in the kidney at 6 hours at the epithelial brush border and the abluminal cytoplasm of the proximal tubule. At 24 hours, grain counts in testis and kidney were much reduced, and hepatic grain counts were increased in a centrilobular distribu n in the liver. Electron microscopic autoradiography of Stage IX–I seminiferous tubules 6 hours after r nglabeled DEHP showed grains in Sertoli cell smooth endoplasmic reticulum and mitochondria. There were als grains at cell junctions involving neighboring Sertoli cells and Sertoli-germ cells. Fewer grains were seen in the S rtoli c ll Golgi apparatus and lysomes and in spermatocyte cytoplasm. By contrast, administration of side arm label d DEHP resulted in few grains in the seminiferous epithelium and 6 hours and no grains in any tissue examined at 24 hours. The authors concluded that phthalic acid is transported into tissue after DEHP administration nd is respon ible for the testicular toxicity of both DEHP and MEHP.

DEHP was orally administered to pregnant rats at a single dose of 100 mg/kg to examine the transferability of the radioactivity to the fetuses (Kurata, 200 4a; Kurata et al , 2012) In the pregnant animals, radioactivity was detected in the blood and plasma at a concentration of 1.88 and 2.74 µg eq /ml, respectively, at 24-hr postdose. In the fetuses at this time-point, however, radioactivity was found at 8 62 µg eq/g in kidney, at 8.20 eq/g in liver, at 5.63 µg eq/g in blood, and at 3.70 µg eq/g in testis. Thus, the concentrations of radioactivity in these tissues were higher than the plasma concentration in the pregnant animals. These resul s suggest that DEHP and/or its metabolite(s) absorbed into the circulating blood in a pregnant animal read ly migrat into the fetuses through the placenta barrier. Since concentrations of radioactivity in fetal tissues were higher han that n the plasma in the pregnant animals, the elimination rate of radioactivity from the fetuses is reasonably as umed to be slower than the rate from the pregnant animals. Thus, it is highly possible that the radioactiv ty that migrated into the fetal body through the placenta is accumulated in the fetal body.

The fetal kidney revealed a 3.1-fold higher concentration of radioactivity than the plasma in the pregnant animal. The localization of radioact vity at a high concentration in the kidney was also demonstrated by whole body autoradiography, sugge ting that he radioactivity migrated into the fetal blood via the placenta and was excreted into urine. Furthermore, the whole b dy autoradiograms of the fetuses demonstrated the highest concentration of radioactivity in the g strointestinal content, suggesting that the radioactivity in the blood was processed in the liver and excreted in the bile. The concentration of radioactivity in the testis was comparable with those in other tissues. Thus, no specific distri ution of radioactivity to the testis was observed. No significant transition of radioactivity to the brain, spinal ord, or eyeb ll was demonstrated

14C-labeled DEHP, was orally administered to juvenile rats (4 weeks old) at a single dose of 100 mg/kg to study hang in plasma concentration, distribution in the body, and excretion in urine and feces of the radioactivity (Kurata, 2004b). In addition, a structural profiling was performed for the metabolites in the plasma, urine, and feces. In the plasma concentrations of the radioactivity were 52.51, 34.85, and 16.97 µg eq./mL at 2-, 4-, and 8-hr post-dose, respectively. Radio-HPLC analysis revealed 8 radioactivity peaks, PM-1 to PM-8, including 6 unknown metabolites (unknown PA-PF), in the plasma. At 2-hr post-dose, MEHP was detected as the major radioactivity peak and accounted for 33.36% of the total radioactivity detected in the plasma. At this time-point, unchanged DEHP accounted for 1.37% of the total radioactivity detected in the plasma. At 4-hr post-dose, the major radioactivity peak was MEHP (24.98%), whereas unchanged DEHP was no longer detected. At 8-hr post-dose, the major radioactivity peaks were PM-5 (33.91%) and MEHP (14.71%), but unchanged DEHP was not detected. The radioactivity excreted in the urine and feces by 24-hr post-dose accounted for 58.04 and 28.54%, respectively, of the radioactivity dosed, indicating the predominance of urinary excretion as the route of excretion of the radioactivity associated with DEHP. In fact, the total excretion rate was 90.13%, when it was calculated including the radioactivity presumably adsorbed on the cage and

recovered in the cage washing. A total of 19 radioactivity peaks, UM-1 to UM-19, were detected in the urine, and all of them were unknown metabolites (unknown UA-US). Neither MEHP nor unchanged DEHP was detected. UM-13 (18.35%), UM-14 (18.33%), and UM-15 (17.84%) were detected as the major radioactivity peaks in the urine. From LC/MS/MS analysis, UM-13, UM-14, and UM-15 were elucidated as the carboxy-, hydroxy-, and oxo-derivatives of MEHP, respectively. Enzymatic hydrolysis of a urine sample containing these metabolites resulted in no significant changes in chromatographic elution pattern of the radioactive peaks attributable to these metabolites, suggesting that these metabolites were unconjugated forms. In the feces, a total of 17 radioactivity peaks, FM-1 to FM-17, were detected, and 15 out of the 17 peaks were unknown metabolites (unknown FA-FO). DEHP was the major radioactivity peak and accounted for 35.00% of the radioactivity dosed. In addition, MEHP (14.00%) and FM-3 (11.53%) were detected in the feces. Other radioactivity peaks accounted for less than 10% of the radioactivity in the sample. Among the tissues, the liver and kidney showed radioactivity at higher concentrations than the plasma at 8- and 24-hr post-dose. In contrast, the testis showed radioactivity at a lower concentration than the plasma at either one of the timepo nts. The whole-body autoradiography also suggested a low distribution of the radioactivity in the testis.

Wistar

The absorption, blood concentration and excretion of DEHP were determined in pregnant and non-pr gnan Wistar female rats following a single and a repeated oral administration at the dose levels of 200 mg/kg and 1000 mg/kg (Laignelet and Lhuguenot, 2000c,d). Blood samples were taken at defined time intervals aft r administrati n for quantification of total radioactivity. Urine and faeces were collected daily and DEHP and its metab lites were extracted and then identified by GC-MS and quantified by GC.In non-pregnant rats, [^{14}C]-DEHP was rapidly, extensively and dose-related absorbed following a single or repeated oral administration as mirrored y the blo d concentration curve profile and a rapid excretion in urine and faeces. A 5-day pre-treatment did not have any significant effect on the total absorption rate but increased slightly the half-life of elimination at the high dose lev l. Aft r a single or a repeated administration, [^{14}C]-DEHP was excreted very quickly in excreta and the recovery reached or exceeded 90% of the administrated dose. [^{14}C]-DEHP was excreted in majority as MEHP-deri d met bolit essentially in urine, as MEHP mainly in faeces and DEHP almost totally in faeces. Omega-1 oxidation was the ma n metabolic pathway (c.a. 60-70%) of the production of MEHP-derived metabolite. The repeated administration was characterised by a decrease of the DEHP excretion and a concomitant increase of the MEHP-derived metab lite but without alteration of the w/w-1 oxidation ratio. This effect was probably related to the metabolic activ tion which took place after a few days of treatment. In pregnant rats, [^{14}C]-DEHP was rapidly, exten ively and dose-related absorbed following a single or repeated oral administration as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. DEHP, MEHP and MEHP-derived metabolites wer found in blood. MEHP was the main circulating compound followed by DEHP. MEHP-derived metabolites we e pr ent t low concentration. A 5-day pre-treatment increased the total absorption rate and also increased the MEHP concentrations in blood to the detriment of DEHP. The distribution of [^{14}C]-DEHP in whole foetus was also rapid and exten ive and followed by a rapid clearance parallel to the blood concentration curve in dams. The radioacti ity conte ts in whole foetus were lower than the corresponding radioactivity concentrations in the blood of dams but did not reflect the actual concentrations in the blood of foetus. After a single or a repeated administration, [^{14}C]-DEHP was excreted very quickly in excreta and the recovery reached or exceeded 75 and 88% of the administrated dose respectiv ly. [^{14}C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, as MEHP mainly in fae es and DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway of the production of MEHP-derived metabolite.

The distribution and el mination of DEHP and MEHP after a single oral dose of 25 mmol DEHP /kg (corresponding to 9765 mg/kg b. w., purity not stat d) by gastric intubation were studied in male JCL: Wistar rats (number not stated, 200 g) (Oishi and Hi ga, 1982) Samples of blood and tissues were collected at 1, 3, 6, 24, 48 and 96 hours post-intubation, and analyzed by as-l q id chromatography and a electron capture detector. The concentration of DEHP and MEHP in blood and tis ues i creased to a maximum within 6-24 hours after dosing while the highest levels observed in the heart and lungs occurred within one hour. Both DEHP and MEHP were detected in brain and kidney, but the concentrations were very low. Only small amounts of MEHP were measured in the lung, and DEHP was detected in the spleen at very l w levels. The concentration of DEHP in fat increased gradually until 48 hours after dosing. The concentration of DEHP in liver declined with a half-life of 1 day while that in the epididymal fat declined more slowly with a half-life of 6.5 days. At 6 hours after administration, the highest ratio of MEHP/DEHP was recorded in testes (2.1). The ratio in blood was 1.1 while the ratio in other tissues was less than one. Biological half-lives of DEHP in different tissues ranged from 8 to 156 hours in the testicular tissue and epididymal fat, respectively and of MEHP from 23 to 68 hours in the blood and epididymal fat, respectively.

A study by Oishi (1990) reported on the distribution and elimination of DEHP after a single oral dose of DEHP (2 000 mg/kg b. w.) in male Wistar rats (35 days old). The blood was collected from the caudal vena cava under deep ether anestesia and then testes were removed at 1, 3, 6, 12 and 24 hours following DEHP administration. The concentration of MEHP in blood and in testis increased to a maximum 6 hours after administration of DEHP and then slowly decreased. For MEHP the biological half-lives in blood and testis were 7.4 and 8.0 hours, respectively, and the area under the concentration-time curve was 1497 and 436mg×h per ml or per g, respectively.

Young male Wistar rats (number not stated, 100-200 g) were treated with a single dose of (carbonyl-^{14}C) DEHP (purity not stated) at a dose level of 2 000 mg/kg b. w. in corn oil by gastric intubation following pre-treatment with DEHP (>99% pure) for 0, 6 or 13 days (Lake et al., 1975). At the end of 4 days, when no further radioactivity was detected in the excreta, the animals were sacrificed, and the organs and tissues were removed. The radioactivity in excreta, organs, and tissues were measured by liquid scintillation spectrometry. Following a single dose of DEHP, virtually all of the administered radioactivity was excreted in the urine (52%) and faeces (48%) within 4 days, and less than 0.1% of the radioactivity remaining in organs and tissues. Similar results were observed in rats pre-treated with DEHP for 6 or for 13 days (60% of the radioactivity was recovered in urine and 40% in faeces).

In several experiments by Tanaka et al., (1975), male Wistar rats (150-250g) were given single oral doses (500 mg/kg b. w.) or a single intravenous doses (50 mg/kg bw) of (carbonyl-^{14}C) DEHP (radiochemical purity >99%) to study distribution, metabolism and elimination. In the elimination studies there were two animals in each group, and in the distribution studies there were three animals in each. The peak blood level was observed about 6 hours after administration. The concentrations in liver and kidney reached a maximum in the first 2-6 hours. No significant retention was found in organs and tissues (brain, heart, lungs, liver, spleen, kidney, stomach, intestine, testicle, blood, muscle an adipose tissue). About 80% of the dose was excreted in the urine and faeces within 5-7 d ys fol owing both oral and intravenous administration. Excretion in the urine was generally slightly greater than that in the fae es. In experiments with rats in which the bile duct was cannulated, about 5% of the dose was recover d from th bile in 24 hours after oral administration, whereas about 24% was recovered after intravenous administration When urine and faecal extracts were analysed by thin-layer chromatography (TLC) after oral administration four major metabolites were detected in urine. Unchanged DEHP was excreted in the faeces, but DEHP or MEHP were not detected in the urine or bile. After intravenous administration about 75% of the dose was recovered from the l r after the first hour. The radioactivity of the liver declined rapidly by about 50% within the next 2 hours and nly 0.17% of the radioactivity remained on the 7thday. The intestine accumulated the next highest amount radi activity. The radioactivity increased as the radioactivity in the liver decreased. After intravenous application the acti ty in the liver and kidneys reach a maximum in the first 2-6 hours. Medium values were seen in the heart lung nd sple n. The peak blood level was observed about 6 hr after administration. The testicle and brain show d the low st values as in the case of intravenous application.

The distribution, accumulation, and excretion of DEHP were studied in several experiments with Wistar rats (Daniel and Bratt, 1974). Following a single oral dose of (carbonyl ^{14}C) DEHP (2.9 mg/kg b. w.; purity not stated), rats (5 adult males) excreted 42% and 57% of the administered radio ctivi y in the urine and faeces, respectively, within 7 days. Rats (5 adult males) fed a diet containing 1 000 ppm of DEHP 7 days prior to dosing with (carbonyl-^{14}C) DEHP excreted 57% and 38% in the urine and faeces, respectively, withi 4 d ys.In studies with biliary-cannulated rats, administered 2.6 mg/kg DEHP by intubation, around 10% was excr ted in the bile.In rats (24 females) fed a diet containing 1 000 or 5 000 ppm of (carbonyl-^{14}C) DEHP for 35 nd 49 d ys, respectively, the amount of radioactivity in liver and abdominal fat rapidly attained a steady-state concentr tion without evidence of accumulation. When returned to a normal diet, the radioactivity in the liver declined with a half life of 1-2 days and in fat with a half-life of 3-5 days. DEHP was extensively metabolized with 14 metabolites, including MEHP, present in urine (analysed by TLC, MS and NMR). DEHP was not detected in the urin . The pri cipal metabolites detected correspond to phthalic acid and metabolites IV or V, VI and IX i. e. the acid, alcohol and ketone resulting fromw- and (w-1) -oxidation of MEHP. The hexobarbital sleeping time was reduced 39 and 43% in male and females, respectively, when given five daily oral doses of DEHP. When DEHP was administered intravenuosly the hexobarbital sleeping time increased by approximately 40% in male rats compared with the corresponding controls.Rats were injected 600 mg^{14}C-DEHP/kg as an emulsion prepared by subjecting to ultrasonication thr ugh the femoral vein. After 2, 24, 72 and 96 hours the animals were killed and the lungs, liver, sple n, blood and portions of abdominal fat were removed for radiochemical analysis. Radioactivity disappeared rapid y from the blood and about 60-70% was recovered in the liver and lung within 2 hours of dosing. After 4 days 44% was recovered from the urine, 29% from the faeces. About 1% was recovered in fat.

Lhuguenot et al.(1985) studied the metabolism of DEHP and MEHP in rats following multiple dosing. Adult male Wistar rats (180-220 g, 3 rats per group and per chemical) were administered (7-^{14}C) DEHP (>98% pure) or (^{14}C) MEHP (position of label not stated; highest available purity) by gastric intubation in corn oil at doses of 50 or 500 mg/kg b. w. for three consecutive days. Urine was collected for 4 days, at 24-hour intervals, metabolites were extracted, analysed by GC and detected by MS. After exposure to DEHP approximately 50 and 60% and after exposure to MEHP approximately 70 and 80% of the total daily doses were recovered in the urine at the low and high dose levels, respectively. No water-soluble conjugates were detected in the urine following administration of DEHP or MEHP. After a single dose of either compound, the main metabolites excreted were I, V, VI and IX. At the lower dose level, no or minor changes in urinary metabolite profiles were seen with time; after multiple dosing at the higher dose level, increases in ω-β-oxidation products (metabolites I and V) and decreases in (ω-1) -oxidation products (metabolites VI and IX) were seen.

Fischer 344

Male Fischer 344 rats (12 animals per group) received a total of 10 daily doses of 1.8, 18, or 180 mg/kg b. w. of radiolabelled DEHP (purity not stated) in cottonseed oil by gavage. All rats received the same amount of radioactivity (1.8 mg/kg b. w. (^{14}C) DEHP, position of label not stated) with different amounts of non-radioactive DEHP diluent (Albro et al., 1982). Urine and faeces were collected daily. Three rats from each group were sacrificed 1, 3, 10, or 12 days after receiving their first dose of DEHP. Various tissue samples and faeces were radioassayed using a tissue oxidizer; urine was radioassayed directly in liquid scintillation fluid. The profiles of the radioactive metabolites in urine were determined by HPLC. Unhydrolyzed DEHP was measured by Radio-TLC. The percentage of ^{14}C retained in the liver tended to decrease with exposure time and also with increasing dose. There was no evidence for accumulation of DEHP in the liver. Essentially the same observations applied to the testes except that testes had lower concentrations than the liver. After about 4 days, excretion (cumulative excretion of ^{14}C as a percentage of the cumulative dose) became quite independent of the dose. Up to a dose of 180 mg/kg per day there was no indication of beginning to saturate the overall elimination mechanism.In a separate experiment, rats and mice were given single oral doses of DEHP in cottonseed oil by gavage at doses ranging from 1.8 to 1 000 mg/kg bw. The animals were sacrificed 6 hours later and the livers were assayed for intact DEHP as described above. In Fisher rats, as the dose increased, a threshold was reached, at about 450 mg/kg bw, above which there was a steady increase in the amount of unhydrolysed DEHP reaching the liver. According to the authors, intact DEHP will reach the liver of rats whenever its concentration exceeds 0.43% in the diet. In contrast, an absorption threshold could not be determined in either CD-1 or C3B6F1mice for doses up to 1000 mg/kg b. w. According to the authors, this may reflect the higher level of DEHP hydrolase in the intestines of mice than in rats. Preliminary experiments with both Sprague-Dawley (CD) and Fischer 344 rat revealed that the maximum amount of DEHP that could be given as a single oral dose without significant excretion of unabsorbed DEHP in the faeces was 200 mg/kg bw. According to the authors, pharmacokinetic studies using ^{14}C-labelled DEHP at doses above 200 mg/kg bw would rapidly become dominated by unabsorbed DEHP and, according to the authors, could not be directly compared to rats of elimination at lower doses.

Male Fischer 344 rats (100-150 g bw, 12 animals per group) were fed diets containing 1 000, 6 000, or 12 000 ppm (estimated to correspond to 85, 550 and 1 000 mg/kg b. w. per day, respectively) of non-radiolabelled DEHP (99.8% pure) (Short et al., 1987; Astill et al., 1986). These groups were divided into three subgroups each consisting of 4 rats which received diets for 0, 6 or 20 days, followed by a diet containing a similar level of (carbonyl-^{14}C) DEHP (radiochemical purity >97%) for 24 hours. Urine and faeces were collected at 24-hour intervals from 24-96 hours after administration of radiolabelled DEHP and then the animals were sacrificed. Four animals from each dose group were sacrificed on each of days 5, 11 and 25 and terminal blood samples were collected. Liver, lung, spleen, intestines, fat, brain, kidney, adrenals, testes and urinary bladder were removed. Urine samples collected from 0-48 hours were pooled for each of the three dose levels and three prior exposure regimens and analysed by normal and reverse phase HPLC for metabolites of DEHP. Urinary metabolites were isolated and the major metabolites were analysed and identified by GC-MS. Faeces samples were similarly pooled and analysed by normal HPLC for the metabolites of DEHP. Radioactivity was measured by liquid scintillation.At all dose levels and exposure times radioactivity was excreted primarily via the urinary route and primarily during the first 24 hours. The percentage of the dose excreted in urine increased with dose, from 53% at 1 000 ppm to 62-66% at 6 000 ppm and 66-69% at 12 000 ppm. The faecal excretion occurred primarily during the second 24 hours. The percentage of the dose excreted in faeces decreased with dose, from 35-38% at 1 000 ppm to 26-30% at 6 000 ppm and 24-28% at 12 000 ppm. At all dose levels, prior exposure to DEHP did not affect the extent or rate of urinary or faecal excretion. Less than 1% of the administered dose remained in tissues 4 days after treatment. The radioactivity in the urine samples was resolved into 14 components and identified as phthalic acid, metabolites I, II, III, IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. The major urinary metabolites, I and V, were followed by metabolite IX and phthalic acid; the remainders were all present in only minor quantities. DEHP and MEHP were not detected in the urine. The radioactivity in the faeces sample was partially resolved into 15 components and tentatively identified as DEHP, MEHP, phthalic acid, metabolites I-V as a pool, metabolites VI, VII, IX, X, XII, XIII, and XIV. The major faecal metabolites were MEHP, metabolites I-V, VI and IX.The urinary, and to a lesser extent the faecal, metabolite excretion patterns changed with dose and prior exposure to DEHP. The major changes in metabolism occurred between the 1 000 and 6 000 ppm dose levels and between 0 and 6 days prior exposure to DEHP. Minor changes were observed with increase in dose to 12 000 ppm. Urinary elimination of metabolites I and V were measured as a function of dose and duration of treatment. The output of metabolite 1 was relatively constant at all dietary levels on day 0, while the output of metabolite V increased with dietary level on day 0. Following prior exposure to DEHP at the 1 000 ppm dose level the urinary excretion of metabolite I doubled compared with no prior exposure, while the the urinary excretion of metabolite V remained relatively constant. Following prior exposure to DEHP at the 6 000 or 12 000 ppm dose level the urinary excretion of metabolite I increased three or four times, respectively, compared with no prior exposure. The urinary excretion of metabolite V decreased two or three times, respectively, following prior exposure to DEHP at the 6 000 or 12 000 ppm dose level compared with no prior exposure. According to the authors the increase in urinary levels of metabolite V at 6000 ppm and higher while metabolite I remained relatively constant, indicated that at high exposure levels the initial dose of DEHP exceeded the rats ability convert metabolite V to metabolite I. However, the capacity forb–oxidation appeared to increase with repeated exposure to DEHP since urinary levels of metabolite V decreased with subsequent doses of DEHP while those of metabolite I increased.The tissue distribution was examined in rats sacrificed 112-116 hours after receiving the

radiolabelled DEHP. A major source of radioactivity was found in the intestinal contents. Additional sources of radioactivity included the liver, fat, kidney and adrenals. A comparison of the tissue levels of radioactivity after 0 and 20 days of pretreatment, indicated that pretreatment with DEHP for 20 days did not significantly alter the tissue distribution of ^{14}C-DEHP derived radioactivity.

To study peroxisome proliferation Fisher 344 rats (5 males / group) were fed diets containing 100, 1000, 6000, 12000, 25000 ppm DEHP, corresponding to 11, 105, 667, 1223 and 2100 mg DEHP/kg bw/d (Short et al., 1987). After an overnight fast the rats were sacrificed and their livers were examined for parameters indicative of peroxisome proliferation. Liver weight expressed as percent of body weight was significantly increased in rats that received 667 mg/kg bw/d and above. Palmitoyl-CoA oxidation and lauric acid 11- and 12-hydroxylation weight was significantly increased in rats that received 667 and 105 mg/kg bw/d, respectively, and above. Since an increased peroxisomal score was observed at dose levels that also produced significant changes in the biochemical parameters, these observations appear to be correlated.

Fischer 344 rats, CD mice, Syrian golden hamsters, and Hartley albino guinea pigs were given two doses of (carbonyl ^{14}C) DEHP (purity not stated) in cottonseed oil by stomach tube at 24 hours intervals (Albro et al., 1982). The maximum single dose of DEHP was 180 mg/kg b. w for rats and guinea pigs, 360 mg/kg b. w for mi e, and 20 mg/kg b. w for hamsters. Urine was collected for a total of 48 hours following the first dose. The urinary metabolites were analysed by HPLC and GC-MS. Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. No components were detected in urine from hamsters or guinea pigs that were not also present in urine from rats a d mice. In the hamsters the main metabolites were: di methyl phthalate (DMP), metabolite I, V, VI and IX. T e ain m tabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating metabolite. MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three speci s excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the speci sted

Parmar et al.(1985) studied the effects on rat pups from dams (strain ot stated) given DEHP through the lactation period. Pups from 10 litters were pooled and seven pups were randomly assign d to each mother. Five mothers were given 2 000 mg/kg b. w. of DEHP (vehicle not stated) daily by oral gavage f om day 1 of birth up to day 21, and five mothers served as control group and was given saline. DEHP was detec ed in the livers of pups from treated mothers indicating that DEHP can be transferred through the milk.

The metabolism of 2-ethylhexanol (2-EH), a metabolite of DEHP was studied in two adult male rats administered (1-^{14}C) ethylhexanol (purity not stated) in cottonseed il by gavage (Albro, 1975). Carbon dioxide (from expired air), urine, and faeces were collected at hourly intervals for 28 hours after administration. Metabolites in the urine were identified by GC and MS. 2-EH was efficiently absorbed and radioactivity from 2-EH was rapidly excreted in respiratory carbondioxide (6-7%), faeces (-9%), and urine (80-82%), with essentially complete elimination by 28 hours after administration. Other metab lites identified were 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid and 2-ethyl-1,6-hexandioic ac d. Only about 3% of 2-EH was excreted unchanged. Thus, these data indicate that the carbon chain of 2-EH is ultima ely metabolized through oxidation pathways (w- and (w-1) -oxidation with subsequent ß-oxidation) to acetate and carbon dioxide.

Conclusion on oral ab orption n rats

The two recent nd highly r liable toxicokinetic studies of Laignelet and Lhuguenot, (2000c,d) with pregnant and non—pregnant Wistar r ts are the most reliable data available to estimate the exposure in experimental rats after long-term expo ur

In the fo lowing table the results from these studies are summarised. It becomes clear from these data that absorption is higher after repeated exposure compared to single application. Metabolites found in urine alone summed up to 73.5% low amounts found in the high dose group of pregnant rats were probably due to a low recovery rate), without accounting for residues in the carcasses and amounts excreted via the bile. A nearly complete oral absorption can be assu d in rats after repeated dosing.

Based on these data the oral absorption in rats with repeated dosing regimens is estimated in a conservative way at 75%.

Table 25 Data used to estimate the oral bioavailability in rats

Strain	Age or body weight	Route/Dose, mg ^{14}C-DEHP/kg b.w./day	Recovery of the radioactivity (% of the dose)				Total recovery (%)	% of the dose as DEHP in faeces	% of the dose excreted in bile	Oral bioavailability (%) (Total recovery - % of the dose excreted as DEHP in faeces)	Reference
			Urine	Faeces	Carcass ± cage washing	Air					
Wistar	10-12 weeks	Gavage/200 (10 days)									Laignelet and Lhuguenot, 2000d
		after 1d	39.8	10.5			50.3	12% after 1d,		Nc	
		after 10d	68.7	23.2			101	0.7% after 10d		100 after 10d	
		Gavage/1000 (10 days)			Nd	Nd			Nd		
		after 1d	39.9	25.2			65.1	33% after 1d		Nc	
		after 10d	73.4	27.8			101	7% after 10d		93 after 10 d	
Wistar (pregnant rats)	10-12 weeks	Gavage/200 (from GD 6 to GD15)									Laignelet and Lhuguenot, 2000c
		after 1d	62.7	11.4			74.1	30% after 1d		Nc	
		after 10d	73.5	22.2			95.5	2% after 10d		93 after 10d	
		Gavage/1000 (from GD 6 to GD15)			Nd	Nd			Nd		
		after 1d	36.4	38.1			74.5	55% after 1d		Nc	
		after 10d	54.0	33.6			87.6	13% after 10d		(75 after 10d)	

Nd = Not Determined

Nc = not calculated due to missing data or total recovery <90% of the dose

Mice

The absorption, blood concentration and excretion of DEHP were determined in pregnant and non-pregnant CD1 female mice following a single and a repeated oral administration at the dose levels of 200 mg/kg and 1000 mg/kg (Laignelet, 2000). Blood samples were taken at defined time intervals after administration for quantification of total radioactivity. Urine and faeces were collected daily and DEHP and its metabolites were extracted and then identified by GC-MS and quantified by GC.In non-pregnant mice, [^{14}C]-DEHP was rapidly and extensively absorbed following a single or repeated oral administration as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. The absorption was not dose-related. It was significantly in excess of the 5-fold difference in dose levels after a single administration and significantly below after a repeated administration. At low dose, a 5-day pre-treatment increased slightly the absorption rate, but at high dose, it induced a decrease of the absorption. After a single or a repeated administration, [^{14}C]-DEHP was excreted very quickly in urine and faeces and the total recovery reached 60 or 69% whatever the dose level respectively. [^{14}C]-DEHP was excreted in majority as MEHP-derived metabolite essentially in urine, a MEHP equally in urine and faeces after a single administration and mainly in urine after a repeated administrati n and as DEHP almost totally in faeces. Omega-1 oxidation was the main metabolic pathway of e produc ion of MEHP-derived metabolite. However, repeated administration of DEHP induced a metabolic a tivation and a displacement of the oxidation pathway in favour of the ω-oxidation. A large part of MEHP-deriv d metabolites were excreted as glucuro-conjugates in urine but this proportion decreased after a repeated administration of high dose. A low proportion of MEHP was excreted as glucuro-conjugate.In pregnant mice, [C] DEHP was rapidly, extensively and dose-related absorbed following a single or repeated oral administrat on as mirrored by the blood concentration curve profile and a rapid excretion in urine and faeces. A pre-trea ment with a low dose did not have any effect on the total absorption rate but increased the MEHP concent ations in blood to the detriment of DEHP. However, a pre-treatment with a high dose induced an incr ase of he Cmax and paradoxically a decrease of the AUC. This effect could be related to an extensive initial ab orption of non-metabolised DEHP and an increased of the excretion rate due to metabolic activation and/or ent ro-hepatic excretion. Only DEHP and MEHP were found in blood, no MEHP-derived metabolites were detec d. MEHP was the main circulating compound. The distribution of [^{14}C]-DEHP in whole foetus w s also rapid and extensive and followed by a rapid clearance parallel to the blood concentration curve in dams The radioactivity contents in whole foetus were lower than the corresponding radioactivity concentrati ns in the blood of dams but did not reflect the actual concentrations in the blood of foetus. After a single or a r peated administration, [^{14}C]-DEHP was excreted very quickly in excreta and the recovery reached or exceeded 60% of the administrated dose. [^{14}C]-DEHP was excreted in majority as MEHP-derived metabo ite e sentially in urine, as MEHP mainly in faeces and DEHP almost totally in faeces. Omega-1 oxid tion was he main metabolic pathway of the production of MEHP-derived metabolite. However, repeated dministr tion of DEHP induced a metabolic activation and a displacement of the oxidation pathway in fa ur of the ω-oxidation. A large part of MEHP and MEHP-metabolites were excreted as g ucuro-conjugates in urine but this proportion seemed to decrease after a repeated administration.

Male C57BR mice (10-12 g, three groups of one control animal and 8 exposed) were given a single oral dose of 6.72 mg (carbonyl- C) DEHP (radiochemical purity >98%) by gavage The animals were killed after 1, 2, 4, 8, 24 hr and 3, 5, 7 days, respectively, and were examined by whole-body autoradiography (Gaunt and Butterworth, 1982). Following abso ption, the radioactivity was widely distributed in organs and tissues without evidence of tissue storage The ontents of stomach and small intestine showed marked evidence of radioactivity in all mice during the first 24 h, but only a slight reaction of one animal was recorded on day 3. Radioactivity was present in the aecal ontents at 1 hr, increased to a maximum at 2 hr and persisted for 1 day, but was found only in one animal on day 3. No radioactivity was detected in the colon contents or faeces after 1 hr the activity reach a maxim m at 2 and 4 hrs in colon contents and faeces, respectively. The decline in radioactivity was similar to that in oth r parts of the gastro-intestinal tract. In the bladder there was a high level of activity between 1-24 hr and some activity in 2 of 3 mice on day 3. In the kidney activity in the parenchyma was similar to that in many tissues of the same animal, but it was more concentrated in the renal pelvis and papillae. Radioactivity in the testis was obvious only in one animal (killed after 4 hr) and was similar to the general tissue levels. In other tissues the level of radioactivity varied considerably between animals even at the same examination interval.

The distribution and tissue retention of DEHP following intravenous and oral administration was studied in mice with whole body autoradiography (Lindgren et al., 1982). In one experiment two male C57BL mice received 10 µCi of (2-ethylhexyl-1-^{14}C) DEHP (chemically and radiochemical purity >99%) intravenously, corresponding to 9.6 mg/kg b. w. of DEHP. Another 2 male mice were given 10 µCi (carbonyl-^{14}C) DEHP intravenously, corresponding to 3.6 mg/kg b. w of DEHP. The distribution was similar following administration of either substance. Four hours after intravenous injection, a very high activity was observed in the gall bladder, intestinal contents and urinary bladder. A high uptake was also seen in the liver, kidney, and brown fat. Some activity was

observed in the white fat, myocardium, and muscles. The level in blood, bone, cartilage, testes, and nervous system was very low. Twenty-four hours after administration the activity in the gall bladder, intestinal contents and urinary bladder was still very high. The concentration in brown fat was high, but the activity in the liver and kidney was lower than after 4 hours.

In a second experiment of the same study (Lindgren et al., 1982), the effects of pre-treatment on the distribution of (^{14}C) DEHP were studied in male mice by whole body autoradiography. Four mice were given DEHP (10 mg/kg) by oral intubation once daily for 5 consecutive days, 2 mice were given daily intraperitoneal injections of phenobarbital sodium (75 mg/kg/d) in physiological saline for 3 consecutive days, and another 2 mice were intraperitoneally treated with 3-methylcholantrene (30 mg/kg/d) for 4 consecutive days. Twenty-four hours after the last administration the animals received 10mCi DEHP in 20ml ethanol (corresponding to 9.6 mg DEHP/kg for 2-ethylhexyl-1-^{14}C and 3.6 mg DEHP/kg forcarbonyl-^{14}C). The mice were killed 24 hours after injection with (^{14}C) DEHP. Following pretreatment of male mice with either DEHP, phenobarbital sodium or 3-methylcholantrene, the distribution 24 hours after the injection of either (carbonyl-^{14}C) DEHP or (2-ethylhe yl-1-^{14}C) DEHP was similar to that in the non-pre-treated animals, except that the concentration in he brown fat was higher in all the pre-treated animals as compared to non-pre-treated animals.

In a third experiment of the same study, Lindgren et al. (1982), six pregnant mice were each given 10mCi (^{14}C) DEHP in soy bean oil by oral intubation. The mice were killed after 4 and 24 hours. Mice at ges ation day 8 were given 7.7 mg DEHP/kg for 2-ethylhexyl-1-^{14}C and 2.9 mg DEHP/kg forcarbonyl-^{14}C, and mice at day 16 of gestation received 4.8 mg DEHP/kg for2-ethylhexyl-1-^{14}C and 1.8 mg DEHP/kg r arbonyl-^{14}C. Whole body autoradiography was performed as described previously. After the pregnant mice had been killed the uteris were removed with their embryos, except for a few fetuses from mice in the lat stage of pregnancy which were removed surgically from the uterus. As for the uteris, the maternal livers and kidneys were also removed for autoradiography. At early gestation marked uptake was seen in the yolk ac. There was a high concentration in the gut of the embryo 4 hours after administration of (carbonyl-^{14}C) DEHP on g station day 8. On gestation day 9, 24 hours after administration of (2-ethylhexyl-1-^{14}C) DEHP pronounced ctivity was observed in the neuroepithelium of the embryos. A high concentration was also seen in th uterine fluid. Except for the gut and the neuroepithelium, uptake in the embryo was low. In the mice at late gestation, a very high accumulation was seen in the yolk sac after oral administration of either (2 ethy hexyl-1-^{14}C) DEHP or (carbonyl-^{14}C) DEHP. The distribution in the fetuses was very similar after administra ion of the two^{14}C-labelled DEHP compounds. Four hours after administration on gestation day 16 there wa high activity in the renal pelvis, urinary bladder and intestinal contents. Some activity was seen in th sk l ton nd liver. On gestation day 17 there was little activity left in the fetuses, although a rather high activity was observed in the renal pelvis, urinary bladder and intestinal contents.

The distribution and retention of DEHP was stud ed in the NMRI mouse brain and liver (Eriksson and Darnerud, 1985). (7-^{14}C) DEHP (0.7 mg/kg b. w.; purity not stated) was administered by gavage to young mice (3-20 days old). One and 7 days after treatment the mount of radioactivity in the liver and brain was measured. The amount of radioactivity in the brain was low, esp cially in 10- and 20 days-old mice, and retention of radioactivity in the brain was minimal. The amount of r dioactivity in the liver was about 10 times that in the brain. After 24 hours the amount of radioactivity found in the livers ranged from about 27 to 2% in the order 3-, 10- and 20-day-old mice, showing sign ficant de reases in all ages after 7 days.

Male CD mice, guine pigs rats, and hamsters were given two doses of (carbonyl-^{14}C) DEHP (360 mg/kg b. w. for mice) by tom ch tube at 24 hours intervals to compare the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982). The study is presented in the section on oral administration to Fischer 344 rats. Rats predomina ely excreted metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids req ire fr m three to six oxidative steps for their formation. No components were detected in urine from hamst s or guinea pigs that were not also present in urine from rats and mice. In the hamsters the main metabolit s were: di-methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating metabolite.MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the species tested.

Studies to compare the toxicokinetic behaviour of DEHP between male B6C3F1 mice, rats and Cynomolgous monkeys has been performed (Short et al., 1987; Astill et al., 1986) and is presented in the section on oral administration to non-human primates.All three species excreted 30-40% of the dose in the urine (rats 32.9%, mice 37.3%, monkeys 28.2%), primarily during the first 12 hours for rats and mice and during the first 24 hours for monkeys. All three species excreted around 50% of the dose in the faeces (rats 51.4%, mice 52.0%, monkeys 49.0%), primarily during the first 24 hours for rats and mice and during the first 48 hours for monkeys. The rates

and extent of urinary and faecal excretion varied widely among monkeys. DEHP was detectable in some tissues in all three species. The mean concentrations detected, with the exception of monkey liver and rat intestinal contents, were less than 1 µg/g. The highest concentrations were detected in liver, intestinal contents, and fat for monkeys, rats, and mice, respectively. Total recoveries of the radioactivity administered were 79 (68-91%), 87 (82-92%) and 90% (63-102%) for monkeys, rats and mice, respectively. Radioactivity in 0-24 hour urine samples were resolved into 13, 15, and 14 components in rats, mice, and monkeys, respectively. The components in urine were identified as MEHP (not detected in rat), phthalic acid, metabolites I, II (not detected in monkey), III (not detected in rat), IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified fractions. Major urinary components in rats were metabolites I, V, VI, and IX. Major urinary components in mice were MEHP, phthalic acid, metabolites I, VI, IX, and XIII, and in monkeys: MEHP, and metabolites V, IX, and X. In monkeys 15-26% of the radioactivity excreted may represent glucuronic acid conjugates whereas in rat glucuronides are either absent or present in negligible quantities. Radioactivity in 0-48 hour monkey faecal extracts and in 0-2 hour rat and mouse faecal extracts were resolved into 11, 10 and 10 components in rats, mice and monk ys, respectively. The faecal components were identified as DEHP, MEHP, phthalic acid, metabolites I-IV VI VII IX, X, XII, XIII (not detected in monkey), and XIV (not detected in mouse). DEHP was a major f ecal component in all three species and MEHP a major faecal component in rats and mice.

In mice (strain and number not stated) given a single oral dose of 400 mg/kg b. w. of (^{14}C) MEHP (radiochemically pure, position of label not stated) in corn oil, the major metabolites (id ntified by GC/MS) in urine were recovered in the form of glucuronides (Egestad and Sjöberg, 1992). Three new metabolites were isolated and characterised as conjugates of ß-glucose. Thus glucosidation has been shown to e an alternative conjugation pathway, although less important.

A single oral dose of 400 mg/kg b. w. of (carbonyl-^{14}C) MEHP in corn oil wa given to 11 male mice (strain not stated) and male guinea pigs (Dunkin Hartley, number of animals not st ted) (Egestad et al., 1996). The radiolabelled DEHP was diluted with unlabelled DEHP to give a pecific a tivity of 2.7 and 6.1mCi/mmol for the guinea pigs and mice, respectively. Urine was collected over 4 hours. Following extraction, individual metabolites were purified and separated using a combination of ion-exchange chromatography and reversed-phase HPLC. Analysis of intact conjugates, as well as nonconjugat d metabolites, was performed by GC/MS. Enzymatic methods were used for further characterisati n. The study confirmed glucuronidation as the major conjugation pathway for MEHP in the investigated species The recovery of^{14}C was 83-103% and 74-78% in guinea pigs and mice, respectively. In guinea pigs MEHP glu uronide were the dominating metabolite whereas in the mice it was an even distribution of the gl uro ides of MEHP and its metabolites. In mice approximately 3% of the administered dose was found in the urin as ß-glucose conjugates. The ß-glucose conjugates were not observed in the guinea pigs.

Guinea pig

Male Hartely guinea pigs, rats, mice, and hamsters were given two doses of (carbonyl-^{14}C) DEHP by stomach tube at 24 hours intervals to compa e the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982). The study is p esented in the section on oral administration to Fischer 344 rats.Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), these diacids require from three to six oxidative steps for their formation. No components were detected in urine from hamsters or guinea pigs that were n t also present in urine from rats and mice. In the hamsters the main metabolites were: dimethyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolites in the mouse were: DMP, MEHP, metaboli s I, VI, IX, while in the guinea pigs MEHP was the dominating metabolite. MEHP accounted for 5% of the met bolit s in hamsters, and 19 and 70%, respectively, in mice and guinea pigs. Rats did not excrete conj gat s of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjuga es other than glucuronides were detected in any of the species tested.

A single oral dose of (carbonyl-^{14}C) MEHP was given to male guinea pigs (Dunkin Hartely: number of animals not stated) and mice to compare the toxicokinetic behaviour of MEHP (Egestad et al., 1996). The study is presented in the section on oral administration to mice. The study confirmed glucuronidation as the major conjugation pathway for MEHP in the investigated species. The recovery of^{14}C was 83-103% and 74-78% in guinea pigs and mice, respectively. In guinea pigs MEHP glucuronide were the dominating metabolite whereas in the mice it was an even distribution of the glucuronides of MEHP and its metabolites. In mice approximately 3% of the administered dose was found in the urine as ß-glucose conjugates. The ß-glucose conjugates were not observed in the guinea pigs.

Hamster

The elimination of DEHP was studied in rats and hamsters (Lake et al., 1984). A single dose of (carbonyl-^{14}C) DEHP (>99% pure) was administered to 5-week-old male Sprague-Dawley rats and male DSN strain Syrian hamsters at dose levels of 100 (5 rats, 3 hamsters) or 1000 mg/kg b. w. (5 rats, 5 hamsters) in corn oil by gastric intubation. The study is presented in the section on oral administration to Sprague-Dawley rats. Urine and faeces were collected over a period of 96 hours and then the animals were sacrificed. Radioactivity was measured in urine, faeces, and total gut contents by liquid scintillation spectrometry. Faecal metabolites were extracted and chromatographed on thin-layer plates. In both species the bulk of the radioactivity was excreted within 24 hours. At the lower dose level, both species excreted more radioactivity in the urine (rat: 51%, hamster: 53%) than in the faeces (rat: 43%, hamster: 31%), whereas at the higher dose level, the major route of excretion was via the faeces (rat: 53%, hamster: 48%). In both species and at both dose levels, only negligible amounts of radioactivity were present at termination in either the liver, kidney, or total gut contents. Faecal radioactivity profiles we e determined in 0-24 hour faeces samples. About 50% of the faecal radioactivity of rats at the higher dose level appeared to be the parent compound, the remainder comprised metabolites possibly including MEHP. In contrast, more than 95% of the faecal radioactivity of hamsters appeared to be the parent compound. S mil r results were obtained with faecal extracts from rats and hamsters at the lower dose level.

Male guinea pigs, rats, mice, and Syrian golden hamsters were given two doses of (carbony ^{14}C) DEHP by stomach tube at 24 hours intervals to compare the toxicokinetic behaviour of DEHP between the species (Albro et al., 1982). The study is presented in the section on oral administration to Fischer 344 rats. Rats excreted predominately metabolites having carboxyl groups on the side chain (metabolites I-V), the e diacids require from three to six oxidative steps for their formation. No components were detected in urine f om hamsters or guinea pigs that were not also present in urine from rats and mice. In the hamsters th main metabolites were: di methyl phthalate (DMP), metabolite I, V, VI and IX. The main metabolit s in the mouse were: DMP, MEHP, metabolites I, VI, IX, while in the guinea pigs MEHP was the dominating m tabolit . MEHP accounted for 5% of the metabolites in hamsters, and 19 and 70%, respectively, in mice and guin a pigs. Rats did not excrete conjugates of DEHP metabolites. In contrast, each of the other three species excreted glucuronide conjugates. No conjugates other than glucuronides were detected in any of the speci s tested.

Dog

A study to compare the toxicokinetic behaviour of DEHP between dog, rats and miniature pigs has been performed by Ikeda et al., 1980. The study is pr sent d in he section on oral administration to Sprague-Dawley rats.Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respecti ely; and aft r 4 days 37 and 53% (rats), 21 and 75% (dogs), and 79 and 26% (pigs), respectively. Elimination o radioactivity was rapid in rats, slightly prolonged in dogs and least rapid in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four radioactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolized DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 in all species and a small amount remained after 4 days. Bile samples from dogs, and to a lesser extent from pigs, accounted for a signific nt amount of administered^{14}C dose. Less than 1% of the administered^{14}C dose was secreted in the bile from bile duct cannulated rats.

Pigs

The kin tics of DEHP and its metabolite mono(2-ethylhexyl) phthalate (MEHP) was studied in the young male pig an om ivor model-species for research in reproductive toxicology (Ljungvall, 2004). Eight pigs were given 100 mg DEHP/kg bodyweight by oral gavage. The concentrations of DEHP and MEHP were then measured in the plasma and tissues of the pigs at different time points after administration. There was no consistent rise above cont mination levels of concentrations of DEHP in the plasma of the pigs. However, the metabolite MEHP reached the systemic blood circulation. The half-life of MEHP in the systemic blood circulation was calculated to b 6.3 h. Absorption from the intestine was biphasic in six of the eight pigs and the mono-exponential elimination-phase started 16 h after the after the administration of DEHP. To conclude, MEHP consistently reaches the systemic circulation in the pig when DEHP is administered orally. The kinetic pattern of the parent substance on the other hand is more difficult to characterise.

A study to compare the toxicokinetic behaviour of DEHP between miniature pigs, rat and dog has been performed by Ikeda et al., 1980. The study is presented in the section on oral administration to Sprague-Dawley rats. Excretion of radioactivity in urine and faeces during the first 24 hours was 27 and 57% (rats), 12 and 56% (dogs), and 37 and 0.1% (pigs), respectively; and after 4 days 37 and 53% (rats), 21 and 75% (dogs), and 79 and 26% (pigs), respectively. Elimination of radioactivity was rapid in rats, slightly prolonged in dogs and least rapid

in pigs; excretion in all three species was virtually complete in 4 days. TLC revealed four radioactive metabolites in rat urine, three in dog urine and five in pig urine. Only a trace of unmetabolized DEHP was found in the urine of rats, dogs, or pigs. A substantial amount of radioactivity was present in the gastro-intestinal tract at day 1 in all species and a small amount remained after 4 days. Bile samples from dogs, and to a lesser extent from pigs, accounted for a significant amount of administered^{14}C dose. Less than 1% of the administered^{14}C dose was secreted in the bile from bile duct cannulated rats.

The distribution and retention of DEHP and DBP orally administered in feed to piglets has been studied (Jarosová et al., 1999). Six piglets (33-50 kg) of which 4 received DEHP (5 g per day and head (ca. 125 mg/kg b. w. /day) for 14 days and 2 served as controls were used. After 14 days, 2 treated and the 2 controls were sacrificed. The remaining two treated-piglets were then maintained on a DEHP-free diet, and one each sacrificed on day 14 and 28, respectively, post dosing. DEHP and MEHP were determined by HPLC analysis. Whole blood and urine samples were collected before sacrifice and on from treated animals on day 7 of treatment The concentration of DEHP was measured in the whole (wet weight) and/or fat extracted tissues/organs of mus le renal fat, subcutaneous fat, kidneys, lungs, brain, heart, and liver. MEHP was only determined in the whole liver, whole blood and urine samples.Body and tissue/organ weights were not affected by administration of DEHP.The highest levels of DEHP were in the subcutaneous (treated cf. control: ca 19 cf.0.42 mg/kg f t) and ren l fat (ca. 25 cf. 0.37 mg/kg fat), muscle (ca 25 cf. 2.4 mg/kg fat), heart (ca 12 cf. <0.2 mg/kg fat) and lu gs (ca 13 cf. 0.25 mg/kg fat). The amount of DEHP in kidney (ca 2 cf. <0.2 mg/kg fat) was low. MEHP, ut not DEHP, level was increased in the liver, whole blood and urine: individuals values greatly varied but an increase up to, for example, of 20-1000-fold in blood occurred. DEHP was not increased in the brain In the 14 day post dosing (recovery) animal, the level of DEHP was decreased by around 50% in subcutaneous and renal fat, muscle, heart and lungs. MEHP returned to control levels in liver, whole blood and urine. By 28 days, DEHP was reduced to control levels in all tissues/organs except in renal fat and the lungs. Although only one animal per recovery time interval was used, these data indicate that in piglets that DEHP is r tained for a c nsiderable time post dosing.The authors further investigated the reason for the presenc of DEH in the organs (not blood) of the control animals. Analysis of the piglet feed showed that around 0.4 mg DEHP and 0.5 DBP per Kg commercial feed was present. Based on a 40 kg pig eating 2 kg of feed a day, he d ily intake of DEHP is around 0.02 mg/kg b. w. Comparing the daily intake (0.8 mg DEHP) with the residue of DEHP in whole muscle of control pigs (0.10 mg/kg), a biotransfer factor (BTF: concn. in meat (mg kg) /daily intake of DEHP (mg/d)) of 0.125 d/kg is derived.

Broiler hens

The distribution and retention of DEHP and DBP orally administered in feed to broiler hens has been studied (Jarosová et al., 1999). Eighteen br iler hens (750 g) per treated and control group were used. DEHP (100 mg per day and head (ca. 135 mg/kg b. w. /day) was administered for 14 days. Six hens each per treated and control group were sacrificed at 14 days, and on ost treatment days 14 and 28. DEHP and MEHP were determined by HPLC analysis. Liver and blood sample were obtained by heart puncture to determine DEHP and MEHP. Muscle (pooled sample of breast and thigh samples), skin (thoracic area), and mesenterial fat were analysed for DEHP. Post DEHP dosing samples of blood, muscle and skin were pooled from 6 individuals.The highest levels of DEHP were in th mesente ial fat (treated cf. control: ca 31cf.0.33 mg/kg fat), skin (ca 26cf.3.8 mg/kg fat) and muscle (ca 26cf.2 5 mg/kg fat). DEHP (ca 6.3cf.0.47 mg/kg fat) and MEHP (ca 0.15cf. <0.01 mg/kg whole tissue) were dete ted in the liver. In whole blood, levels of MEHP varied but indicated an increase of more than 7-fold. In the 4 day post dosing (recovery) animal, the level of DEHP decreased by more than 50% in muscle, skin adipose tissue and liver. However, by comparison with the all the control groups and the treated group, DEHP is apparently retained in the muscle, skin and adipose tissue around 30%). MEHP levels in the liver and blood were reduced to control levels by post recovery day 14.The authors further investigated the reason for the presence of DEHP in the organs (not blood) of the control animals. Analysis of the hens feed showed that around 1.0 mg DEHP and 2.0 DBP per Kg commercial feed was present.

5.1.1.2. Inhalation

There are few studies available concerning the inhalation route of exposure. Studies available for humans are not strictly toxicokinetic studies but case studies of patients and in the working environment, which are reported therafter. There is only one toxicokinetic study with rats exposed to a DEHP aerosol.

Rats

In a study performed by General Motors (1982), adult male Sprague-Dawley rats (200 g) were exposed, either once or repeatedly, by inhalation to a DEHP aerosol (98% pure).In the single exposure study, six rats were exposed to 129±26 mg/m^{3}of (carboxyl-^{14}C) DEHP for 6 hours (head-only chamber; radiochemical purity of the labelled compound 95%). Accurate particle size determination was not technically possibly to conduct; however, the particle size was estimated to be 0.4 - 0.5 µm. Three animals were sacrificed immediately after exposure and the remaining three after the follow-up time. The follow-up time for collection of urine and faeces samples was 72 hours before the animals were sacrificed. Samples were collected at 12- and 24-hour intervals for urine and faeces, respectively, and assayed for radioactivity. Blood samples (0.1 ml) were taken from the jugular vein 1, 3, 6, hr during exposure and 1, 3, 6, 12, 18, 24 and 36 hours post-exposure. Tissues (lung, liver, kidney, fat, adrenal, heart, spleen, thymus, testes, and brain) were removed post mortem and assayed for radioactivity. Radioactivity was determined by liquid scintillation spectrometry. Metabolites were determined by HPLC in urine samples collected between 0-12, 12-24, 24-36 hours. Data were calculated as µmole equivalents DEHP.During single dose inhalation exposure (^{14}C) DEHP was absorbed rapidly as indicated by the m unt of radioactivity in the blood. Following exposure, the decrease in radioactivity in blood was log non-linear. Immediately after exposure, approximately 1 mg (based µmole equivalents DEHP) or 75% of the body urden was recovered in the carcass and skin. 10% was recovered in the lung and ca. 2% in all the ther tissues excluding the brain where no radioactivity was detected. Radioactivity associated with the lu g probably represents that fraction of aerosol particles deposited in the larger airways. This fracti n could b absorbed through the gastrointestinal tract following clearance from the pulmonary spaces by muc ciliary ladder mechanism. It is probable that a large portion of DEHP is ingested. After 72-hou , pproximately 1.5 mg (3.94 µmole equivalents DEHP) was recovered mainly in the urine and faeces. Combined urine (52%) and faeces (40%) accounted for greater then 90% of total recovered radioactivity. Ar und 6% of t e original body burden radioactivity was determined in tissues (low amount of radioactivity in the lung and liver, and trace amount in kidney), carcass, and skin. Based on this information the retention of DEHP c n be derived by comparison with the calculated inhaled dose. Assuming a minute volume of 0.2 l/min and 100% inhalation of the aerosol the inhaled dose is around 36 mg/kg b. w. /day or 7.2 mg/rat/day for a 6 hour per day inhalation exposure. Retention is thus around 21% (1.5/7.2) for DEHP with an estimated particle size of 0.4 - 0.5 µm.The rate of excretion of radioactivity in the faeces was approximately first order kinetics d ring 72-hour. The elimination half-life was ca. 22 hours and the elimination rate constant (Ke) 0.032 hr-1. In urine, excretion was biphasic. The initial rapid phase was ca. 10 hours (Ke = 0.069 hr-1) and was sustained for 30 hours. The slower phase half-life was 22 hours.At least three peaks were identified by HPLC in rine. Phthalic acid (3-5% total radioactivity) was identified. The remaining radioactivity was con ined to tw other peaks, however, the metabolites were not identified: DEHP was not detected in the urine Under the experimental conditions used in this study, around 1.5% of a nominal aerosol concentration of 100 mg/m^{3}was absorbed by rat following a 6 hour exposure period. Both pulmonary and gastrointestinal tr ct absorpt on are expected to contribute to the total body burden level of detected^{14}C-DEHP derived radioactivity

For comparison, General Moto s (1982) lso studied the disposition in male Sprague-Dawley rats (200 g) (number not stated) after a sing peroral (gavage) dose of (carboxyl-^{14}C) DEHP (25 mmoles/kg bw (10 mg/kg bw): 25 mmoles/ml, 9.7mCi/ml) administered in corn oil with the results of the single exposure inhalation study (see above). The dos was selected to approximate DEHP absorbed during inhalation (2 mg/animal). Faeces were collected over 24 hours interval and urine over 12 hours interval. The animals were killed after 72 hours Tissues (lung, liver, kidney, fat, adrenal, heart, spleen, thymus, testes, and brain) were removed post mortem and assayed for radioactivity Radioactivity was determined by liquid scintillation spectrometry. A more rapid excretion of^{1} C- in he urine and increased body burden clearance (half-life»12 hours) was observed following oral administra ion compared with inhalation exposure. The percentage cumulative recovery of radioactivity in urine (54%) and faeces (43%) after 72 hours was not significantly different as compared with inhalation expo ur (52 and 40%, respectively, in urine and faeces). Within 72 hours the recovery was 3.94 and 3.82mmole equival nts DEHP (ca 1.5 mg) following single oral and inhalation administration, respectively. To assess the rel v nce of this comparison route-specific metabolism, biliary excretion and reabsorption, and the contribution of GI- ract absorption during and following inhalation exposure should be considered compared with unabsorbed rally administered DEHP. Urinary excretion following single oral exposure was also biphasic with half-lifes of about 10 and 22 hours, respectively. The excretion in urine and decline in body burden were more rapid following oral administration, especially initially.In the repeated exposure study General Motors (1982), 16 rats were pretreated with 100 mg/m3unlabelled DEHP for 2 weeks (6 hours per day, 5 days per week in an exposure chamber, mass median aerodynamic diameter of aerosol particles: 0.6 µm) except that the last exposure was to (carboxyl-^{14}C) DEHP (head-only chamber).Following repeated inhalation exposure, around 90% of the radioactivity was excreted in the urine (50%) and faeces (40%), and 8% recovered in the carcass and skin. Excretion of radioactivity in urine was apparently first order with a half-life of about 25 hours. Urinary excretion seems to be initially slower compared with single inhalation exposure (see above) but was parallel to the curve from single exposure after less than 24 hours.The study indicates that following repeated inhalation exposure

long-term retention does not occur and that the excretion profile is not modified by prolonged inhalation exposure (2-weeks) compared with single exposure. Hence, disposition characteristics following repeated exposure were similar to single dose exposure.

5.1.1.3. Dermal

Data are reported in section "Dermal absorption".

5.1.1.4. Other routes

Exposure of humans to DEHP through medical treatment practices such as dialysis, respiration therapy blood transfusions, or parenteral nutrition where the source of DEHP is the plastic materials used in the med cal treatment devices or storage bags may also occur. The data are summarized in sections 7.10.5 of he IUCLID dossier and 5.10.2 of the CSR.

Non-human primates

The disposition of DEHP was studied in marmosets (Rhodes et al., 1983). Groups of thr m le marmosets received a single dose of (^{14}C-ring labelled) DEHP (radiochemical purity 97 5%) by the intravenous (100 mg/kg b. w.), intraperitoneal (1 000 mg/kg b. w.) and oral (100 and 2 000 mg/kg b. w.) outes Urine and faeces were collected for seven days and the radioactive content determined. Following in ravenous administration approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion for 7 days) indicating a 2 to 1 ratio between the urinary and biliary (faecal) outes of excretion in the marmoset. Around 28% of the dose remained in the lungs 7 days after adminis ration o 14C-DEHP, with minimal levels in other tissues. A much smaller proportion of the dose was excreted foll wing intraperitoneal administration (10% in the urine and 4% in the faeces) in a similar 2 to1 ratio Around 85% of the dose remained as unabsorbed DEHP in the peritoneal cavity with minimal amounts in he tissues (0.6%). The results for oral administration are presented above.

The Urinary excretion and metabolites of DEHP were studied in two African Green monkeys following a bolus infusion with ^{14}C-DEHP (carbonyl-^{14}C-DEHP-en iched plasma containing 96% DEHP and 4% MEHP) (Albro et al., 1981; Peck and Albro, 1982). To closely simulat the manner in which man is exposed to DEHP when receiving blood products, a^{14}C-DEHP impregnated PVC plastic strip was immersed in 20-ml aliquot of plasma and stored for up to 5 months at 4°C Se ial plasma, urine and stool samples were obtained and measured. Urine metabolites were isolated and identified by GC/MS. Plasma^{14}C concentration rapidly decreased: less than 5% by 30 min post infusion. C14-MEHP increa ed rapidly followed by^{14}C-MEHP oxidation products. The levels of MEHP was greater than the oxidation pr ducts. MEHP is apparently quite stable since^{14}C-MEHP in plasma increased to a plateau within7 minutes and then remained at a constant level for more than 30 minutes even though the total^{14}C-plasma levels decreased in the plasma. The cumulative^{14}C excretion in urine of three monkeys was 50% nd greater than 70% by 4 and 24 hours, respectively.Urine samples from two monkeys were collected for a 5-hou post-in usion period. Approximately 80% of the urinary metabolites were excreted in the form of glucuronide conjugates. According to the authors the glucuronide frequency is analogous to what has been reported for hum n leukemia patients but in clear contrast to rats where rat urinary metabolites are excreted unconjuga ed. Seven metabolites (MEHP, V, VI, VII, VIII, IX, X, XI) were identified (GC/MS) with MEHP (29%) and meta olite IX (38%) being the major metabolites. The remaining metabolites amounted to 7% or less.

The eff cts of DEHP on hepatic function and histology were evaluated in the rhesus monkey undergoing chronic transfusion (Jacobson et al., 1977). These studies demonstrates that intravenous administration of solubilised DEHP results in detectable concentrations of DEHP in biopsy material for up to 14 months following transfusion. The initial liver biopsies (100-400 mg) of all the exposed animals contained significant amount of DEHP. Plasma or PRP (platelet-rich-plasma) stored at 22°C yielded higher levels of DEHP in the livers of the transfused animals than plasma or PRP stored at 4°C. The 5 month follow up samples contained concentrations similar to that initially measured. Since the post-transfusion samples were in the lower range of sensitivity one of the animals were killed 14 month post-transfusion and the level of DEHP in different organs was analysed. Significant levels were found in the liver (0.7%), testis (0.4%), heart (0.8%) and fat (2.0%). The residual organ level excluding fat was less than 1% of the dose administered. One animal had tuberculosis and was killed 5 month post-transfusion. In this animal significant levels were found in the spleen (10%), lung (15%), fat (20%) and liver (1.7%). Based on organ weight (excluding fat) and DEHP content, the residual DEHP was 5 percent of the dose administered, according to the authors.

Rats

The disposition kinetics of DEHP was studied in male Sprague-Dawley rats following single or multiple administration of DEHP by various routes (intra-arterial: 100 mg/kg b. w.; intraperitoneal: 4 000 mg/kg b. w.; peroral: 2 000 mg/kg b. w.) (Pollack et al., 1985, the study is described in section oral; rats).

The disposition of DEHP and four of its metabolites was studied in Sprague-Dawley rats (40-days-old, 8 in each group) given single infusions of a DEHP emulsion in doses of 5, 50 or 500 mg DEHP/kg bw (Sjöberg et al., 1985). Plasma concentrations of DEHP, MEHP and metabolites V, VI and IX were followed for 24-h after the start of infusion. The kinetics of the primary metabolite MEHP was studied separately. The concentrations of DEHP in plasma were at all times higher than those of MEHP which were much higher than the concentrations of the other metabolites investigated. In animals given 500 mg/kg bw the area under the plasma concentration time curves (AUCs) of the other investigated metabolites were at most 15% of that of MEHP. Parallel decreases in the plasma concentrations of DEHP, MEHP and metabolites V, VI and IX indicated that the elimin tion f DEHP was the rate limiting step in the disposition of the metabolites. This was partly supported y the observation that the clearance of MEHP was higher than that of DEHP. Nonlinear increases n the AUCs of DEHP and MEHP indicated saturation in the formation as well as the elimination of MEHP

Transplacental transfer of DEHP has been observed following intraperitoneal administration of (arboxy-^{14}C) DEHP on gestational day 5 or 10 in SD rats (Singh et al., 1975). The dams were killed at 24 hours interval starting on days 8 and 11 until day 20 of gestation. Radioactivity was detected in foetal i su s, amniotic fluid and placenta at all time points. The radioactivity peaked at 48 hours and declined rapidly thereafter. The concentration was less than that of maternal blood and less than 1% of the administered dose.

Mice

The distribution of (carbonyl-^{14}C) DEHP was studied in male CD-1 mice following intravenous injection by tail vein of 1 ml DEHP-enriched plasma (2.293 µg/ml) (Waddell et al 1977). After 1, 3, 9, 24, and 168 hours, the mice were killed and subjected to whole-body autoradi graphy. The radioactivity was rapidly accumulated in the kidney and liver, with high concentrations in urine, bile, and intestinal contents. There was no evidence of retention in any tissues in the body. From the amount f radi activity seen in the sequence of time intervals, it seems clear that the material is rapidly and com letely el minated by the kidney and the liver. The secretion by the liver, via the bile into the intestine appears to e the major route. No enterohepatic circulation of DEHP was indicated since there was a persistently high concentration in the intestinal lumen.

5.1.1.5. In vitro studies

The enzymatic hydrolysis of DEHP by d fferent lipases was studied in tissues from the Sprague-Dawley rat (CD-strain), guinea pig, hamster and mic (Albro and Thomas, 1973). DEHP was hydrolysed to MEHP by lipases from a variety of rat tissues with pancreas, liver, and intestinal mucosa containing the bulk of the DEHP hydrolase activity. here was no difference in DEHP hydrolase activity in terms of units per mg of protein between intestinal h mogenates from young adult (200g) and old (450g) rats, but there was higher activity from male than fr m female r t (1.7 and 0.45 units/g protein, respectively), and from fed than from fasted rats (1.7 and 0.74 units/g pr t in respectively). Variation among species was not extreme, although different mouse strains sh wed onsiderable differences (e. g. 1.2 and 4.0 units/g protein for CD-1 and C57B1/6f, respectively). Out of 15 t ssue mixtures, only the liver alkaline lipase preparation was able to further hydrolyse MEHP to phthalic acid, at a rate of 2% at which it hydrolyzed DEHP.

The activities of liver, lung and kidney of rats of various age group and that of placenta in hydrolyzing di(2-ethylh xyl) phthalate to mono(2-ethylhexyl) phthalate have been measured (Gollamudi et al., 1985). Male and female Sprague-Dawley rats of 45 days of age, neonatal rats within 12 hours of parturition, and fetuses and placenta on day 19 of gestation were used. The liver was most active in all age groups; however, the lung and the kidney also had considerable activity. The tissues of the fetuses and the neonate had significant activity. The Km values of the enzyme were 4, 1.25 and 5.9 mM, respectively, in the neonatal, adult and old livers (Gollamudi et al., 1983).

The absorption of DEHP and MEHP was studied using an everted gut-sac preparation form the male Sprague-Dawley (300-400 g) rat small intestine (White et al., 1980). MEHP was significantly less lipophillic than DEHP, and was absorbed by the everted gut sac in a significantly greater quantity than DEHP. Esterases within the mucosal epithelium hydrolysed DEHP quantitatively to MEHP.

Lake et al.(1977) studied the hydrolysis of DEHP in hepatic and intestinal mixtures from various species. Hepatic postmitochondrial supernatant mixtures from Sprague-Dawley rat, olive baboon, and albino ferret were able to hydrolyse DEHP to MEHP as well as were intestinal mucosal cell mixtures from rat, baboon, ferret, and man. According to the authors, these results show a species similarity in the metabolism of DEHP between man, rodent, nonrodent, and nonhuman primate species. Furthermore, the results suggest that orally ingested DEHP would most probably be absorbed from the gut of the rat, baboon, ferret, and man primarily as the corresponding monoester.

Gray et al.(1982) studied the hydrolysis of DEHP in intestinal mixtures from young Sprague-Dawley rats (28-42 days) and Dunkin-Hartely hamsters. During a 16-h incubation, DEHP was hydrolysed to MEHP which was significantly different between rats (18.9±2.1%) and hamsters (4.1±1.0%).

The hydrolysis of DEHP was studied in hepatic post-mitochondrial supernatant fractions and in intest nal mucosal cell whole homogenates obtained from untreated Sprague-Dawley rats and Syrian ham ters (L ke et al., 1984). Rat hepatic DEHP hydrolase activity was more than twice as active as the enzyme present in ham ter liver. Rat intestinal DEHP hydrolase activity was thrice as active as the hamster intestinal a tivity. Ac ording to the authors, these differences may explain the different hepatic response of rats and hamsters t DEHP.

Two groups of 6 Sprague-Dawley rats which were 25 days old and two groups of 6 Sprague-Dawley rats which were 60 days old were used to studyin vitrometabolism (Sjöberg et al., 1985). On gr up of ach age were pre-treated with DEHP (gavage, 1000 mg/kg bw for 14 days) and the other group of eac age were pre-treated with phenobarbital (intraperitoneal injection, 100 mg/kg bw for 3 days). After the animals were killed liver microsomes were prepared and concentrations of mono-(2-ethyl-5-hydroxyhexyl) phthalate was determined. The conversion of MEHP to mono-(2-ethyl-5-hydroxyhexyl) phthalate in liv r mi rosomes from untreated 25- and 60-day-old rats was 0.37±0.07 and 0.39±0.08 nmol/mg protein/m n, respec ively. The rate of (ω-1) hydroxylation in liver microsomes from rats pretreated with DEHP was 0.3 ±0.05 nmol/mg protein/min. Liver microsomes pretreated with phenobarbital showed a two fold in rease in the conversion rate 0.73±0.15 nmol/mg protein/min.

In another experiment the protein binding of MEHP in pl sma from 25, 40 and 60-day-old Sprague-Dawley rats at 25mg/ml was determined using the equilibrium dialysis technique (Sjöberg et al., 1985). The binding of MEHP to plasma proteins was 97.6±1.5, 98.0±0 2 and 97 5±0.4% in the 25, 40 and 60-day age group, respectively. The plasma protein binding was constant in the concentration interval 5-150mg/ml.

The hydrolysis rates of DEHP were me sured in susp nsions of contents of the Wistar rat stomach, small intestine or caecum (Rowland et al., 19 7). After 16 hours, 1.0% of DEHP was hydrolysed by stomach, 22.1% by small intestine, and 6.9% by ca um contents. The hydrolysis product was identified as MEHP.

In studies reported by Lhuguenot et al. (985), the metabolism of MEHP was studied in isolated and cultured rat (Wistar derived) hepatocytes. A concent ations of 50 or 500 µM (^{14}C) MEHP (position of label not stated, highest available purity) the substance was extensively metabolized. No water-soluble conjugates were detected. At the low concentration, recoveries of radioactivity of 71-79% were obtained. The amount of unchanged MEHP remaining in the m dium increased slightly from day 1 to day 3. Metabolites I and V are final products of MEHP metabolism, no furth r metabolism was detected in this study. Metabolite X was transformed to metabolite V, which was f rther transformed to metabolite I, and metabolite IX was transformed to metabolite VI. A small increase in m tabolit I and a decrease in metabolite VI were seen from day 1 to day 3. At the high concentra ion, coveries of radioactivity ranged from 74 to 83%. The amount of unchanged MEHP decreased from 149 µM af er 1 day to 93 µM after 3 days. A fivefold increase in metabolite I and a smaller increase in meta olite V along with time-dependent decreases in metabolites VI and IX were observed.

Ito et al.(2005) evaluated enzyme activities in tissues from rats, mice, and marmosets to assess possible specifies differences in the biotransformation of DEHP. CD-1 mice and Sprague-Dawley rats were 11 weeks old and Common marmosets were 18 months old when liver, kidney, lung, and small intestine were harvested. Tissues were stored at −85ºC until used. Tissue homogenates or microsomal fractions were assayed for lipase activity based on hydrolysis of DEHP to MEHP. UDP-glucuronyl transferase by measuring glucuronidation of MEHP, naphthol, and bisphenol A. Alcohol dehydrogenase was measured using 2-phenoxyethanol and 2-ethylhexanol as substrates, and aldehyde dehydrogenase was measured using 2- phenylpropionaldehyde and 2-ethylhexanal as substrates. Lipase activity was highest in liver, small intestine, and kidney in mice. The lowest lipase activity was found in marmosets. Marmoset hepatic lipase activity was 4–5% that of mouse activity, and small intestine lipase activity in marmosets was <1% of mouse small intestine activity. Rat lipase activities in these organs were intermediate between mouse and marmoset. Lipase activities were comparably low in rat and mouse lung and were undetectable in marmoset lung. UDP-glucuronyl transferase was detectable only in liver in the 3 species.

Although activity was greater in mouse than marmoset, the difference between species was not as great as for lipase. Alcohol and aldehyde dehydrogenases were higher in marmoset than in rodents; however, the authors concluded that the possible increased ability of marmosets over rodents to convert MEHP to its ω-oxidation products was unlikely to be important given the small amount of MEHP that would be expected to be generated in marmosets from oral or intravenous exposures.

The following information is taken into account for any hazard / risk assessment:

DEHP is rapidly absorbed from the GIT following oral administration. The liver, kidney, testes and blood are the main sites of distribution following orally administered DEHP. DEHP is oxidised into a large number of metabolites with 2-EH, MEHP and oxidative metabolites of MEHP and being the main constituents. There is no evidence of accumulation in animal tissues. DEHP and metabolites are excreted in the urine and faeces. Th extent of absorption in rats, non-human primates and humans is around 50% after single dose application for doses up to about 200 mg/kg bw. At higher doses it appears that absorption in non-human primates is do e-limited in contrast to rodents. A recent study showed that the overall fraction of the 0.3 mg and 3.0 mg dos s of D4-DEHP excreted as both the primary and secondary metabolites up to 48 hours postdose was 51 7% nd 42.5%, respectively.

Absorption in rats after repeated application was consistently higher compared to single applic tion. Urinary excretion was up to 73.5% in reliable studies and absorption in was estimated to be 75% As abso ption was comparable between rats and humans after single dose administration, the same absorption rate was also used for humans for long-term oral exposure.

The oral absorption characteristics of human sub-populations e.g. age- and health depe dent factors, are not known and no convincing experimental data are available to account for th t As the estimated absorption rate of 75% in rats is considered to be conservative (i.e. lower bound) and r al bsorption s probably nearly complete in rats and humans, , for children it is considered appropriate to assume the same rate of75% value for oral absorption.

75% absorption is also assumed for inhalation exposure for rats and humans.

Value used for CSA: no bioaccumulation potential

Summary of exposure route dependent ystemic bioavailability in humans and experimental animals

Species and exposure rou e	systemic bioavailability (%)
Oral	
Rats	75
Humans - adults	75
Humans - infants/children	100
Inhalation	
Adults	75
Infants/child en	100

5.1 2 Dermal absorption

Human

No rel able study is available.

Rats

Dermal absorption distribution, and excretion were studied in rats by Elsisi et al. (1989). Hair from a skin area (1.3 cm in diameter) on the backs of male F344 rats (number not stated) was clipped, [^{14}C]-DEHP (> 96% radiochemically pure, uniformly labelled on the ring) was applied at a single dose of 30-40 mg/kg bw (5-8 mg/cm2) in ethanol and after evaporation the area of application was covered with a perforated cap (non-occluded). Rats were kept in metabolic cages and urine and faeces were collected every 24 hours for 7 days. On the 7th day the rats were sacrificed and samples from various organs and tissues (brain, spinal cord, lung, liver, spleen, intestine, kidney, testis, fat, muscle, and skin) were collected. The skin area of application was also removed and analysed. The radioactivity was determined using liquid scintillation spectrometry. Cumulative

excretion in the urine and faeces was around 4.5%. The amount of radiolabel remaining in the body 7 days after dosing was less than 2% of the applied dose. Retention in the different organs and tissues examined was low ≥ 0.3%: muscles showed the highest amount 1.17%. Most of the unabsorbed dose (86%) remained at the skin area of application after 7 days. Dermal absorption (considered as the cumulative amount detected in excreta and tissues, excluding the dosed skin (a lower bound)) is calculated to be 6.5%.

The dermal absorption of [^{14}C]-DEHP was evaluated in male F344 rats (Melnick et al., 1987). A single dose of 30 mg/kg bw (6 mg assuming 200 g/rat: 4.5 mg/cm2) of [14C]-DEHP (purity and position of label not stated), dissolved in ethanol, was applied to a circular area of 1.3 cm diameter (1.326 cm2) in the middle of the back of three rats. After the ethanol had evaporated, a perforated plastic cap was glued on the skin over the site of application. Urine and faeces were collected every 24 hours for 5 days and radioactivity was determined by liquid scintillation spectrometry. Five days after dosing, recovery of 14C in the urine and faeces was around 5% with 3%, respectively. Body organs and the skin in the area of DEHP application site were also collected and analysed. The amount of radiolabel retained in the body 5 days after dosing was less than 2% of the applied dose. The highest amount was recovered in the muscle (1.2%). About 95% of the applied dose was rec v red from the application site and the plastic caps, which were used to cover the application site. These esult indicate that DEHP is not well absorbed through the skin of rats. Dermal absorption (consid red as th cumulative amount detected in excreta and tissues, excluding the dosed skin (a lower bound)) s calculated to be 9%.

The absorption of [14C]-DEHP contained in a PVC plastic film (40% w/w (25.5mg/ m2): 0 5 mm thick) in male Fischer 344 rats was studied (Deisinger et al., 1998). Sheets of PVC film (15 cm2) were applied to the shaved backs of eight rats in two separate experiments (4 rats/experiment). In a short-term study ("study II"), the PVC was removed after 24 hours and the animals killed. In a longer term study ("study I") after removal of the PVC film at 24 hours the animals were rewrapped with aluminium foil and ba dag at the exposure site and sacrificed after a further 6 days. In both studies, [14C]-label was determined in the fo lowing: urine and faeces up to sacrifice; cage washes; washed and rinsed residue from the exposu e site be ore sacrifice; entire clipped area including the dermal exposure site was exercised after washing nd ri sing; the remainder of the body (carcass). The PVC films were also analysed for remaining [14C]-DEHP after the 24-hour exposure period. In "study I" (longer term study), the aluminium and bandage used fr m 2 hours to 7 days were analysed. The migration of (14C) DEHP from the film was 261 and 505.6 µg du ing 2 hours (0.725 and 1.4 µg/cm2/hour). Based on the materials mass balance information on the combined amount of [14C]-DEHP for urine, faeces, cage washes and carcass, and residual amount in the skin at the applic tion ite, the authors calculate that the percutaneous absorption rate (J) is around 0.24 µg/cm2/hr in both studies.

Guinea pig

Dermal absorption of a single dos adio labelled DEHP was determined in 5 female hairless guinea pigs [Crl:LAF/HA(hr/hr)BR, 20-30 weeks old] (Ng et al., 1992). [Carbonyl-14C]-DEHP (53 µg, 13.2 µg/cm2, > 98% pure) was dissolved in 50 µl ac tone and applied topically on 4 cm2 of the washed upper dorsal area. The exposed area was then covered by a protective non-occlusive pad to prevent ingestion of the compound. Animals were kept individually i glass metabolism cages. 24h after dosing the pad was removed and the dosing site cleansed with soap nd water to remove unabsorbed material. Urine and faeces were collected at 6 and 12 hours on the first day and hen at da ly intervals for 7 days post-treatment for radioassay. Seven days post-administration the site of application was stripped with tape 10 times. Radioactivity content on the tape was determined by liq id scintillation spectrometry. To correct the dermal absorption for incomplete excretion (e.g. body retention), the excretion rate of an intramuscular dose, in 5 animals, was compared with the excretion rate of a topica dos . Three percent (7% after correction) of the dermally administered dose was absorbed in vivo and excreted in 24 hours. Around 60% of the topical dose was not excreted in the faeces and urine after 7 days. The pe cent of [14C]-label recovered was 31% from skin surface wash (performed at 24 hours), 53% (21% before co rected) cumulative urine and faeces excretion up to 7 days, and 11.3% was tape strip recovery (perfo med at 7 days). 13% and 5% were recovered in the protective skin pad and body tissues (liver, fat, muscle, skin), respectively. The total recovery was 95% (76% before corrected). To determine the amount of DEHP that might have been have volatilized during the penetration process, DEHP was applied on a piece of skin that was kept in a petri dish at 33ºC for 7 days. Analysis of the radioactivity content revealed that 10% of the dosed material was lost. Thus volatilisation can only in part account for the loss of DEHP from the application site. Dermal absorption (considered as the cumulative amount detected in excreta and tissues, excluding the dosed skin (a lower bound)) is calculated, without consideration of correction, to be 26%.

The skin reservoir and bioavailability of dermally administered [14C]-DEHP in hairless guinea pigs was determined (Chu et al., 1996). Different amounts of [14C]-DEHP were applied to the washed dorsal region of 4 female Hartley hairless guinea pigs in four different experiments for different times and dosages: 119 µg/cm2 for 24 hours; 107 µg/cm2 for 48 hours; 442 µg/cm2 for 7 days; and, 529 µg/cm2 for 14 days. Radioactivity was

measured in skin sections by autoradiography or liquid scintillation to determine the amount of radioactivity. The authors conclude that the results indicate that the amount of DEHP remaining in the skin after washing will eventually enter the systemic circulation and should be considered as part of the total dose absorbed, and that the hair follicle may play a role in percutaneous penetration. Dermal absorption (considered as the cumulative amount detected in excreta and tissues, including skin dose) is calculated to be 9.7-18.9% from the four different experiments.

In vitro

Absorption, permeability constant and percutaneous absorption rate of [14C]-DEHP was determined in the epidermis of nonviable human skin (autopsy sample) and rats (AL/pk, Wistarderived) with a glass diffusion cell (Scott et al., 1987). 0.5 ml (14C)-labelled undiluted DEHP was applied to the skin mixtures. Apparently th skin discs were 7 cm2, therefore, 70 mg/cm2 [14C]-DEHP was applied. 50% v/v aqueous ethanol was used as the receptor fluid and 50 µl samples collected at regular intervals. Diffusion cells were maintained at 30º . The results are presented in the following Table.

Table 26 Absorption rate data of DEHP. Adapted form Errata to Scot et al. (1987)

	Human*	Rat*
Permeability constant (Kp: 10-5 cm/hr)	0.57	2.28
Percutaneous permeability rate (J: µg/cm 22/hr)	5.59	22 37
Lag time (hr)	3.1	3 9

* Epidermis, 50% aqueous ethanol

Absorption, permeability constant and percutaneous abs rption rate of [14C]-DEHP was determined in the stratum corneum of human skin (autopsy sample) and full th ckness rat skin Fischer-334 with a Franz-types diffusion cell (Barber et al., 1992). 0.3 ml [14C]-labelled undiluted DEHP was applied to 1.02 or 0.636 cm2 of skin (288-576 mg/cm2). The receptor solution was based on Dulbecco's phosphate-buffered (pH 7.1) isotonic saline. Diffusion cells were maintained at 37 or 30 C. Th esults are presented in the following Table.

Table 27 Absorption rate data of DEHP. Inf rmation are adapted

	Human*	Rat**
Permeability constant (Kp: 10-5 cm/hr)	0.0105	0.0431
Percutaneous permeability rate (J: µg/cm22/hr)	0.1	0.42

* Stratum corneum

** Full thick ess skin. Buffer

The perme bility constant of [14C]-DEHP in the epidermis and dermis of male Sprague-Dawley rats was compared using buffer or aqueous ethanol with a diffusion cell (Pelling et al., 1998). Absorption was determined using b th phosphate buffer saline and 50% aqueous ethanol as receptor medium. [14C]-DEHP was applied in 50 µl acetone (78.6 µg; 78.6 µg/cm2 assuming application to 1 cm2). Diffusion cells were maintained at 31.5ºC. The re ults are presented in the following Table. The authors comment that the rate and extent of DEHP bs rption through the epidermis was greatly increased (40- and 80-fold, respectively) using 50% aqueous ethanol as receptor fluid compared with buffer. Also in comparison with Scott et al. (1987) it is noted that the Kp for the aqueous ethanol system is much higher (16-fold) in this study.

Table 28 Absorption rate data of DEHP. Information are adapted

Rat		
Buffer	50% aq. Ethanol	
Permeability constant (Kp: 10-5 cm/hr)		
Epidermis	1.3	94.6
Dermis	4.76	9.83
Percutaneous permeability rate (J: µg/cm 2/hr)*		
Epidermis	0.02	0.786

* Calculated based on data detailed in the study. The area of the applied (14C)DEHP was assum d to b 1 cm2

The absorption of (14C) DEHP was determined in full thickness viable and nonviable guinea pig skin with a diffusion cell (Ng et al., 1992). The receptor solution was based on Hepes-buffered Hanks' balanc d salt solution, containing gentamicin (50 mg/l) and 4% bovine serum albumin. [14C]-DEHP wa applied in 10 µl acetone (53.2, 228 and 468 µg/cm2). Diffusion cells were maintained at 37ºC The mount of [14C] recovered in receptor fluid, on skin disc and in skin wash after 24 hours was determined In vitro experiments were also carried out using perfusate containing an esterase inhibitor, phenylmethyl ulfonyl fluoride (174 mg/l). Metabolites were identified by GC/MS. Total recovery was between 78-90% The results are presented in the following Table.

The authors comment that for a dose of 53.2 µg/cm2 the in vitro absorption of 47% (receptor fluid + skin disc) after 24 hours is comparable with the corrected in vivo cumulati e excretion (urine + faeces) of 53% after 7 days.

To determine the amount of DEHP that may have v latised during the penetration process, DEHP was applied on a piece of skin that was kept in a petri dish at 33ºC for 7 days. Analysis of the radioactivity content revealed the 10% of the dosed material was lost. Thus v l tilisation is apparently low and can only in part account for the dose of DEHP from the application site.

DEHP was metabolised to a monoester MEHP. In the presence of the esterase inhibitor, the dose that permeated into the receptor fluid decreased from 3 36% in the control to 2.67% in 24 hours. The proportion of MEHP was also reduced from 2.36 to 1.23% in the in ibitor treated group.

Table 29 Percent radiolabel recovered for DEHP at 24 hr post application. Information are adapted

	Dose (µg/cm2)			
	53.2	53.2*	228	468
Receptor fluid	6.1	5.0	2.4	2.5
Skin disc	41	40	37	36
Receptor fluid + skin disc	47	45	40	39
Skin was	38	41	50	40
Total recovery	85	86	90	78
Estimate Per Ab rate (J: µg/cm2/h)#	0.13	0.11	0.23	0.49

* Non-viable skin.

Calculated based on % in receptor fluid and 24 hours. Information on the rate of excretion is not detailed in the study other than for at 24 hours.

The absorption of (14C) DEHP was determined in the perfused porcine skin flap (Wester et al., 1998). Isolated skin flaps were perfused in a non-recirculating chamber. 10 cm2 was dosed with (14C) DEHP (18.5 µg/cm^2). After 8 hours, 0.14% was recovered in the perfusate, 14.5% was recovered in skin strips (taped 12 times), 3.8%

in the remaining skin and 71% in skin surface wash, a total material balance recovery of 94%. Based on the combined amount in the perfusate and skin strips (14.6%) the percutaneuos absorption rate is 0.34 µg/cm2/hour.

The following information is taken into account for any hazard / risk assessment:

Based on in vivo studies in animals the cumulative bioavailability of DEHP is 20%. Based on the in vivo data, and application of a across-species correction factor of 4 a dermal absorption value of 5% is considered reasonable for potential human percutaneous absorption. A dermal bioavailability of 5% is also used for exposure to DEHP contained in PVC products because the migration coefficient of DEHP from the product has also to be considered. Each stage is, therefore, clearly defined. Hence, 5% is used for both exposure to free DEHP and DEHP contained in plastic products.

Value used for CSA: Absorption rate (%): 5

Summary of exposure route dependent systemic bioavailability

Human exposure route	Human systemic bioavailability (%)
Dermal (free DEHP and in products)	
Adults	5
Infants/children	5

5.2. Acute toxicity

5.2.1. Non-human information

5.2.1.1. Acute toxicity: oral

The results of experimental studies are summarised in the following table:

Table 30 Overview of experimental studies on acute toxicity after oral administration

Method	Results	Remarks	Reference
rat (Fischer 344) male/female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD0: > 20000 mg/kg bw (male/female)	1 (reliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982a)
rat (Fischer 344) female oral: gavage equivalent or similar to OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure)	LD0: > 5000 mg/kg bw (female)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Berman E, Schlicht M, Moser VC (1995)
rat (Sprague-Dawley) male oral: gavage	LD0: > 2000 mg/kg bw (male)	2 (reliable with restrictions)	Chu I, Secours VE, Marino IA,

equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)		supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Villeneuve DC and Valli VE (1981)
mouse (B6C3F1) male/female oral: gavage equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD0: > 20000 mg/kg bw (male/female)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982a)

5.2.1.2. Acute toxicity: inhalation

The results of experimental studies are summarised in the following table:

Table 31 Overview of experimental studies on acute toxicity after inha ation exposure

Method	**Results**	**Remarks**	**Reference**
rat (Sprague-Dawley) male/female inhalation (nose only) equivalent or similar to OECD Guideline 403 (Acute Inhalation Toxicity)	LC0 : > 10620 mg/m³ air (nominal) (male/f male)	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Greenough RJ (1981a)

5.2.1.3. Acute toxicity: dermal

The results of exp rimental studies are summarised in the following table:

Tabl 32 Overview of experimental studies on acute toxicity after dermal administration

M thod	**Results**	**Remarks**	**Reference**
rabbit Food and Drug Administration's cuff test	LD50: ca. 20 mL/kg bw (= 19800 mg/kg bw)	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Shaffer CB, Carpenter CP (1945)

5.2.2. Human information

No data available.

5.2.3. Summary and discussion of acute toxicity

Acute oral toxicity

In rats:

Acute oral toxicity of DEHP in the rat has been estimated as a prerequisite to a carcinogenicity study by NTP (1982). Doses from 800 to 20,000 mg/kg of DEHP (99.5% pure) were administered in a single dose by gavage to groups consisting of 5 males and 5 females. The vehicle was corn oil. No deaths were observed during a 14 day observation period, giving an LD_0 in excess of 20,000 mg/kg. No individual animal data are given.

Berman (1995) evaluated the acute oral toxicity of DEHP (>99% pure) in rats according to a protoc l similar to the OECD N°425 guideline. Groups of 7-15 female Fischer 344 rats were given a single ra dose of DEHP at doses of 0, 150, 500, 1500, 5000 mg/kg and were then observed daily for 7 days. No mortality t the highest dose tested (5000 mg/kg). The oral LD_0 of DEHP is higher than 5000 mg/kg in female Fischer 344 rats.

Groups of six male Sprague Dawley rats were administered single oral doses of DEHP 2000 mg/kg in 2% gum acacia and were observed clinically for seven days (Chu et al., 1981). No mortality a d clinical signs were observed. Body weight and food consumption were not affected by treatment. The liver weight increased in the treated group. Hepatic microsomal aniline oxidase activity was not altered y treatment. The acute oral LD0 is higher than 2000 mg/kg in rats.

In mice:

Acute oral toxicity of DEHP in mice has also been estimat d as prer qu site to a carcinogenicity study by NTP (1982). Doses from 800 to 20,000 mg/kg of DEHP (99 5% pure) w re administered as a single dose by gavage to groups consisting of 5 males and 5 females. The vehicle wa corn oil. No deaths were observed during a 14-day observation period, thereby giving an LD_0 value high r tha 20,000 mg/kg. No individual animal data are given in the report.

Acute inhalation toxicity

In a study performed according to GLP principle groups of 5 male and 5 female rats were exposed for 4 hours to clean air (control group) or DEHP (purity not specified) in concentrations of either 3.39, 6.82, or 10.62 mg/litre (3,390, 6,280, or 10,620 mg/m3) (Greenough, 1981). The highest dose was considered the technical limit of aerosol generation for the test material. The control group and the lowest dose group were exposed on the same day. The mid-dose gr up and the highest dose group were exposed on different days. The exposure was nose-only. The rats were observed for linical signs throughout the exposure period and for the first 4 hours after dosing. During the subsequent 14-day observation period the rats were inspected twice daily. Body weights were measured before exposure and with regular intervals during the observation period. A detailed macroscopic examination was performed on all animals at sacrifice at the end of the observation period. No animals died during or aft r the exposure. All treated animals showed a slightly unkempt appearance for 1-2 days after exposure, tho e in he highest dose group had a yellowish staining on their fur. This group also had a reduced body weight g in on the second day after exposure, which subsequently returned to the normal pattern. In all groups, da k red foci and patches were observed in the lungs at post mortem inspection. These findings were mor frequent in the treated animals. In conclusion, the LC_0 of DEHP via inhalation was in this study found to be in exce s of 10,620 mg/m3for 4 hours.

Acute dermal toxicity

The acute dermal toxicity of DEHP has not been investigated in a study of guideline quality. In an older study, rabbits were exposed dermally for 24 hours with doses up to 20 ml/kg (Shaffer et al., 1945). That amount killed 2 of 6 rabbits. The LD_{50} by skin absorption is ca. 20 ml/kg (19800 mg/kg bw). However, details of the experimental design and original results are not presented in the report.

The following information is taken into account for any hazard / risk assessment:

In experimental animals, DEHP exhibits low acute oral, dermal and inhalation toxicity.

Value used for CSA:

discriminating dose (oral): 20000 mg/kg bw

LD50 (dermal): 19800 mg/kg bw

discriminating conc. (inhalation): 10620 mg/m³ air

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classification is warranted for the acute toxicity.

5.3. Irritation

5.3.1. Skin

5.3.1.1. Non-human information

The results of experimental studies on skin irritation are summarised in the following table:

Table 33 Overview of experimental studies on skin irritation

Method	Results	Remarks	Reference
rabbit (Little White Russian) Coverage: occlusive (clipped (10x10cm)) OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	Slightly irritating (not classified) Erythema score: 1 of max. 4 (animal #1) (Time point: 2 48, 72h) (fully reversible within: 8d) 1.33 of max. 4 (animal #2) (Time point: 24, 48, 72h) (fully reversible wi hin: 8d) 1 of max. 4 (animal #3) (Time po nt: 24, 48, 72h) (fully rever ible ith : 8d) Edema score 0 of max. 4 animal #) (Time point: 24, 48, 72h) 0.3 of max. 4 (animal #2) (Time point: 24, 8, 72h) fully reversible within: 48h) 0.33 of max. 4 (animal #3) (Time point: 24, 48, 72h) (fully reversible within: 48h)	1 (reliable without re trict on) key s udy exp rimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Mürmann P (1987a)
rabbit (New Zealand White) Coverag : occ usive (clipped and 2 of 4 abraded) EPA OPP 81-5 (Acute Derm l Irritation)	Slightly irritating (not classified) Erythema score: 0.33 of max. 4 (mean) (Time point: 24, 72h) (fully reversible within: 72 h) (Non-Abraded) 0.58 of max. 4 (mean) (Time point: 24, 72h) (fully reversible within: 72h) (Abraded) Edema score: 0.25 of max. 4 (mean) (Time point: 24, 72h) (fully reversible within: 72h) (Non-abraded) 0.5 of max. 4 (mean) (Time point: 24, 72h) (fully reversible within: 72h) (Abraded)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Greenough RJ (1981b)

5.3.1.2. Human information

No data available.

5.3.2. Eye

5.3.2.1. Non-human information

The results of experimental studies on eye irritation are summarised in the following table:

Table 34 Overview of experimental studies on eye irritation

Method	Results	Remarks	Reference
rabbit (Little White Russian) OECD Guideline 405 (Acute Eye Irritation / Corrosion)	Slightly irritating (not classified) Cornea score: 0 of max. 4 (animals # 1, 2 and 3) (Time point: 24, 48, 72h) Iris score: 0 of max. 2 (animals # 1, 2 and 3) (Time point: 24, 48, 72h) Conjunctivae score: 0 of max. 3 (animals # 1, 2 and 3) (Tim point: 24, 48, 72h) Chemosis score: 0 of max. 4 (animals # 1, 2 3) (Time point: 24, 48 72h)	1 (reliable without restriction) key study experimental result **Te t material (EC name) bis(2 ethylhexyl) phth late**	Mürmann P (1987b)
rabbit (New Zealand White) EPA OPP 81-4 (Acute Eye Irritation)	not irritatin Cornea score: 0 of max. 4 (mean) (Time point: 24, 48, 72h) Iris score: 0 f max. 2 (mean) (Time point: 24, 48, 72h) Conjunctivae score: 0.14 of max. 3 (mean) (Time point: 24, 48, 72h) Chemosis score: 0.14 of max. 4 (mean) (Time point: 24, 48, 72h)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Greenough RJ (1981c)

5.3.2.2. Human information

No data available.

5.3.3. Respiratory tract

5.3.3.1. Non-human information

No data available.

5.3.3.2. Human information

No data available.

5.3.4. Summary and discussion of irritation

Skin irritation

The acute dermal irritative and corrosive properties of DEHP have been investigated in a study confo mi g with OECD guideline 404 (Mürmann, 1987a). The hair was clipped from the dorsal and lateral part of the trunk of three male Little White Russian rabbits. Undiluted DEHP (> 99% pure) (0.5 ml) was applied to a kin a ea of 6 cm2 and covered with a patch. After 4 hours, the patch was removed and the skin gently rinsed with warm water. The skin reactions were evaluated after 1, 24, 48, and 72 hours, and after 6 and 8 days, u ing he grading system of the OECD guideline. At the 1-hour observation, all rabbits showed very slight eryth ma, and one rabbit also a very slight oedema. At 24 hours, the reaction of one rabbit had progressed to a well-defin d erythema. At 48 hours, all rabbits had very slight erythema which at 72 hours was accompanied by a d y app arance of the skin. At 6 days, the skin surface appeared scaly, and at 8 days the skin was free from reac ion. It was concluded that DEHP was slightly irritating to the skin. Mean scores calculated for times 24, 48 and 72h of animals 1, 2 and 3 were 1, 1.33 and 1 for erythema and 0, 0.33 and 0.33 for oedema, respectiv ly

In a skin irritation study performed according to GLP principles and confo ming with the U. S. Food and Drug Administration (FDA) recommended method, 3 male and 3 femal New Ze land White rabbits were used (Greenough, 1981b). The skin of the back was clipped free of hair and 2 of the 4 patch test areas were abraded. One square inch chromatography paper patches were wett d with the undiluted test substance (DEHP), or with 10% aqueous sodium lauryl sulphate (as positive control substance) Patches were applied to both intact and abraded skin and left in position for 24 hours, thereafter t e skin was cleansed. The test sites were scored immediately (24-hour reading) and 48 hours later (72 our r ading). Reactions were evaluated according to the FDA recommended scoring system. DEHP cau d mild t moderate reactions at 24 hours. At the 72-hour reading, no treated sites showed any response to reatment. Mean scores calculated for times 24 and 72h of the 6 treated animals (non-abraded) were 0.33 and 0 25 for erythema and for oedema, respectively. After abrasion of the skin, these scores were 0.58 and 0.5 for eryth ma and oedema, respectively.

Eye irritation

In a study performed in accord nce with OECD guideline 405, eye irritation of DEHP (>99% pure) was studied (Mürmann, 1987b). DEHP in a olume o 0.1 ml was installed in the right eye of three male Little White Russian rabbits. The left eye served as control. The animals were evaluated after 1, 24, 48, and 72 hours, and after 6 days of application, using the grading system of the OECD guideline. At 1 hour, the conjunctivae of all three rabbits showed mild redne s and one rabbit showed mild discharge. No conjunctival reactions were observed at the later observation times. All observations for chemosis, corneal opacity and lesions of the iris were negative. Mean irritation sco e calculated for times 24, 48 and 72h of the animals 1, 2, 3 were 0, 0, 0 for cornea, 0, 0, 0 for iris, 0, 0, 0 for conjunctiv and 0, 0, 0 for chemosis, respecitvely.

In a tudy erfo med according to GLP principles and conforming with the U. S. Food and Drug Administration (FDA) re ommended method, DEHP in a volume of 0.1 ml was introduced into the right eye of 3 male and 3 f male New Zealand White rabbits (Greenough, 1981c). The eyes were examined at 1, 24, 48, and 72 hours, and at 7 days, and the reactions were scored according to the FDA recommended scoring system. No reactions were ound n the cornea or iris at any point of time. The conjunctivae of 5 eyes exhibited mild redness at 1 hour, while one eye showed very mild redness. At 24 hours, mild redness persisted in 3 eyes, while the remainder had no redness. No redness was observed at 72 hours or 7 days.

Mean irritation scores calculated for times 24, 48 and 72h of the 6 treated animals were 0 for cornea, 0 for iris, 0.14 for conjunctiva and 0.14 for chemosis.

Respiratory tract irritation

No studies specifically addressing this issue have been found.

In a study of acute toxicity by inhalation, groups of rats were exposed to DEHP in concentrations of 3.39, 6.82 or 10.62 mg/litre for 4 hours (Greenough, 1981). The respiratory tract was subjected to detailed macroscopic

examination in all animals and revealed dark red foci and patches in the lungs. These foci and patches were observed more frequently in the treated animals (present in 19 out of 30 rats exposed to DEHP) than in the controls (present in 2 out of 10 rats exposed to clean air). The lung-to-body weight ratios of all treated groups were similar to the ratios obtained in the control group.

The following information is taken into account for any hazard / risk assessment:

DEHP induced minimal skin and eye irritation in animals and did not induce skin irritation in human volunteers. Data are insufficient to determine the respiratory irritant potential of DEHP.

Value used for CSA:

Skin irritation / corrosion: slightly irritating

Eye irritation: slightly irritating

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classification is warranted for skin, eye and respi atory irritation.

5.4. Corrosivity

According to the skin and eye irritation assays, DEHP is not corrosive.

5.5. Sensitisation

5.5.1. Skin

5.5.1.1. Non-human information

The results of experimental studies on skin sens isation summarised in the following table:

Table 35 Overview of experimental studies n skin sensitisation

Method	Resul s	Remarks	Reference
guinea pig (Dunkin-Hartley) female Guinea pig maxim ation tes Induction: intradermal nd epicutaneous Challenge epicutaneous, oc lusive equival nt or similar to OECD Guid line 406 (Skin Sensitisation)	not ensitising No. with positive reactions: 1st reading: 0 out of 20 (test group); 24 h after chall.; dose: 50% 1st reading: 0 out of 10 (negative control); 24 h after chall.; dose: 50%	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Greenough RJ (1981d)

Data waiving

Assay: LLNA

Reason: study scientifically unjustified

Justification: A valid guinea-pig maximization assay is available

5.5.1.2. Human information

No data available.

5.5.2. Respiratory system

5.5.2.1. Non-human information

The results of experimental studies on respiratory sensitisation are summarised in the following table:

Table 36 Overview of experimental studies on respiratory sensitisation respiratory sensitisation

Method	Results	Remarks	Reference
Species: mouse (B6C3F1) female Induction: dermal Challenge: dermal (on the ears) topical application (and challenge) of test substances to mice followed by measurements of serum IgE. auricular lymph nodes harvesting for measurement of IL-4 and IL-13 proteins and their corresponding mRNAs. liver weight increase monitoring (for evaluation of skin absorption).	not sensitising All animals survived. No significant differences in body weight was observed. Liver weights of DEHP-treated mice were significantly elevated with respect to control. Phthalate treatment had no effect on IL-4 or IL-13 mRNA levels.	1 (reliable without restriction) key study experimental result **Test material (EC name) bis(2-ethylhexyl) phthalate**	Butala JH David RM, Gans G (2004)

5.5.2.2. Human information

This part has been quoted from the review article of Kimber and Dearman in 2009 (An assessment of the ability of phthalates to influence immune and allergic responses).

Occupational exposure to high concentrations of phthalate fumes, such as those inhaled by individuals performing hot-wire cutting of PVC film for example, has been linked to asthma and other respiratory symptoms (Polakoff et al., 1975; Andrasch et al., 1976; Falk and Portnoy, 1976;Brooks and Vandervort; 1977; Eisen et al., 1985;Markowitz, 1989; Nielsen et al., 1989). A variety of respiratory symptoms such as cough, work-related shortness of breath, wheezing and rhinitis, as well as a decline in FEV_1, were found to be increased in exposed workers compared with non-exposed reference groups.

It should be noted however, that the majority of these studies were not adjusted for confounders. The role of exposure of adults at home or at work to PVC containing materials has been investigated also (Norback et al., 2000; Tuomainen et al., 2004; Jaakkola et al., 2006).

A study of Swedish hospital workers revealed a higher risk of asthma symptoms in those individuals that occupied buildings showing signs of dampness-related degradation of DEHP in PVC flooring (Norback et al., 2000) In an intervention study it was shown that removal of damaged PVC flooring, and a consequential reduction of indoor pollutants such as 2-ethyl-hexanol, was associated with a significant reduction in the prevalence of respiratory, nasal and conjunctival symptoms (Tuomainenet al., 2004).

In a case controlled population-based study a correlation was found between the presence of plastic wall coverings at work and an increased risk of asthma (Jaakkola et al., 2006). .

There has also been a number of epidemiological studies in which the association of exposure to phthalates and the exacerbation of respiratory symptoms, such as bronchial obstruction or wheeze, or the development of atopic diseases (rhinitis or eczema), in children has been investigated (Oie et al., 1999; Jaakkola et al., 1999; 2000; 2004; Bornehag et al., 2004; Kolarik et al., 2008).

In some of these studies, the relationship between PVC floorings or wall material and the development of asthma, allergy or related respiratory outcomes were measured (Oie et al., 1999; Jaakkola et al., 1999; 2000; 2004). In general, following adjustment for various confounders, risks of the various respiratory outcomes measured were reported to be increased in the presence of the plastic materials in the home environment. Two independent studies were conducted in Sweden (Bornehag et al., 2004) and in Bulgaria (Kolarik et al., 2008) in which the association between the phthalate content of house dust and allergic symptoms in children has been investigated. In both studies there was reporteda statistically significant association between the concentration in house dust of DEHP and allergic symptoms (asthma or wheezing) in children. Despite the presence of higher levels of BBP in the Bulgarian homes compared with the Swedish homes, it was only in the Swedish study that an association with BBP in house dust and allergic symptoms (eczema and rhinitis) was found (Bornehag et al., 2004; Kolarik et al., 2008). It should be noted that both studies demonstrated an association, rather than a c usal link, between DEHP exposure and symptoms, and, importantly, measurements of phthalate concentrations were made after the development of symptoms. That is, the degree of correlation between the levels of the e materials during the relevant period of disease development and the period after which a diagnosis was made (w en the measurements were performed) is unknown.

One important issue is that in most epidemiological studies it is not possible to exclude th possibility that products other than phthalates, which are also released from PVC materials following pyrolysi or dampness-related degradation, may contribute to the apparent association between phthalate conc ntrations nd allergic symptoms. Indeed, a recent systematic review of the role of exposure to phthalates and the risk of development of allergies and asthma concluded that although there was some evidence to sugge t that pht alate exposure may be associated with increased risk, the lack of objective exposure information limited he interpretation of these epidemiologic data (Jaakkola and Knight, 2008). A slightly earlier review (Mendell, 2007) also noted that although there were strong associations between indoor residential chemical missions (such as formaldehyde and phthalates) and respiratory health or allergy, causality was not demon trated nd that these risk factors may only be correlative indicators for truly causal exposures.

5.5.3. Summary and discussion of sensitisation

Skin sensitisation

One Magnusson-Kligman guinea pig maximiza on te t h been performed, in which female albino Dunkin-Hartley guinea pigs were used (Greenough, 1981d) The maximisation test comprised two procedures. The induction procedure consisted of an intradermal njec ion of the test material into the skin of the shoulder region followed by a topical application 7 day later. Th intradermal injection (actually 3 injections) consisted of 0.1 ml Freund's adjuvant alone, 0.1 ml 10% DEHP in paraffin oil, and 0.05 ml 10% DEHP in paraffin oil emulsified with 0.05 ml Freund's adjuvant The control group received 2 injections of Freund's adjuvant only. For the topical application, a 2.2 cm pa ch of filt r paper was wetted with 50% DEHP in paraffin oil and applied for 48 hours to the pre-treated area. The control group was not subjected to topical application. The challenge procedure which consist d of a topical application was carried out 14 days after the completion of the induction period. In prelimina y experiments, a solution of 50% DEHP was determined to be non-irritant; higher concentrations were not teste . A 2 x 2 cm patch wetted with 50% DEHP was applied to a challenge site (on the right flank) of all animals for 24 hours. The degree of response was assessed 24 hours after removal of the challenge patch and rated. Any animal showing erythema at the challenge site was considered to have shown a positive response DEHP was unequivocally not sensitising in the guinea pig maximization test; there were no positive re ponses.

The foll wing information is taken into account for any hazard / risk assessment:

DEHP ha not been found to induce skin sensitisation in animals.

Value used for CSA: not sensitizing

Respiratory sensitisation

Di(2-ethylhexyl) phthalate (DEHP) was tested using for respiratory sensitization in B6C3F1 mice (Butala et al., 2004). This test involves topical application and challenge of DEHP to mice followed by measurements of serum IgE. In addition auricular lymph nodes were harvested for measurement of IL-4 and IL-13 proteins and their corresponding messenger RNAs. Liver weight increase, a measure of peroxisomal proliferation, was monitored to assure that internal dosing had been achieved. ELISA and RNAse protection assays demonstrated that DEHP treatment did not significantly affect IgE, IL-4 or IL-13 levels. Similarly IL-4 and IL-13 mRNA

levels were not elevated. In contrast, all of these were significantly elevated by trimellitic anhydride (TMA), a respiratory sensitizer used as the positive control in this assay. Liver weights were significantly elevated by DEHP, providing evidence of sufficient percutaneous absorption to induce physiological responses. Under these experimental conditions, DEHP is not considered as a respiratory sensitizer.

The PVC flooring material is an important source for phthalates, but several other sources contribute in indoor environment. PVC products indoors (different surface materials) have been associated with airway effects in epidemiological studies (Jaakkola et al. 2006) but only in one study has the concentrations of di(2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBP) been measured (Bornehag et al., 2004). In that study DEHP was associated with asthma and BBP with rhinitis in children at the highest exposure quartile (Bornehag et al., 2004). Long-term exposure to DEHP (Larsen et al 2007) and metabolite, mono-2-ethylhexyl phthalate (Hansen et al. 2007), together with a model allergen did not show promoting effects on the development of the allerg n specific IgE antibodies. Phthalates are not skin sensitizers for humans and there is no evidence of respiratory sensitization. Based on the lack of mechanistic support and taking into account the low exposure level of phthalates by inhalation, the EU Scientific Committee on Health and Environmental Risks (SCHER, 2007) does not find consistent scientific evidence which indicate that phthalates should be high concern hemi als in indoor air.

The following information is taken into account for any hazard / risk assessment:

DEHP is not considered as a respiratory sensitizer

Value used for CSA: not sensitizing

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classifica ion is warranted for skin and respiratory sensitization.

5.6. Repeated dose toxicity

5.6.1. Non-human information

5.6.1.1. Repeated dose toxicity: oral

The results of experimental studies are summarised in the following table:

Table 37 Overview of experimental studies on repeated dose toxicity after oral administration

Method	Results	Remarks	Reference
rat (Fischer 344) ale/female chronic (ral: f ed) 100 500 2500, 125000 ppm (nominal in diet) 0, 5.8 28.9, 146.6 or 789.0 mg/kg/day, respectively, for males, d 0, 7.3, 36.1, 181.7 or 938.5 mg/kg/day, respectively, for females (actual ingested) Exposure: 104 wk (7/wk) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL: 500 ppm (male/female) (NOAEL = 28.9 mg/kg bw/day [males] and 36.1 mg/kg/day [females] Liver: ↑ weight (males) and peroxisome proliferation at 500 ppm; kidney: ↑ weight from 2,500 ppm; ↑ mineralization of the renal papilla (males), tubule cell pigment (both sexes), and chronic progressive nephropathy (males) at 12,500 ppm; pituitary: ↑ castration cells (30/60 males) ar 12,500 ppm; testes: ↓ weight, ↑	1 (reliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	David RM, Moore MR, Finney DC (2000a) David RM, Moore MR, Finney DC (2001)

	incidence and severity of bilateral aspermatogenesis; ↓ incidence of interstitial cell neoplasms; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 12,500 ppm; changes in the kidneys, testes, and pituitary were not reversible upon cessation of exposure)		
rat (Fischer 344) male subacute (oral: feed) 0, 100, 1000, 6000, 12000, 25000 ppm (nominal in diet) 0, 11, 105, 667, 1223, and 2100 mg/ kg/d (average daily doses) Exposure: 21 days (ad libitum) Assessment of the peroxisome proliferation potential	NOAEL: 11 mg/kg bw/day (actual dose received) (male) (increase in palmitoyl Co A activity at 105 mg/kg)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Short RD, Robinson EC Lington AW Chi AE (1987)
rat (Alderley Park) male/female subacute (oral: gavage) 2000 mg/kg (actual ingested) Exposure: 14 days (once daily) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	LOAEL: 2000 mg/kg bw/day (nominal) (male/female) (↓bwg (males). Liver: ↑absw ↑relw, ↑pp, ↑pSER mitoch changed. Kidney: ↑weight (females), ↑pp. Testes: ↓weight, atrophy. ↓CHO (males) ↓TG (males))	2 (r liable with restri tions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Rhodes C, Orton TC, Pratt IS, Batten PL, Bratt H, Fackson SJ, Elcombe CR (1986) ICI (1982)
rat (Sprague-Dawley) male subacute (oral: gavage) 0, 25, 100, 250, 1000 mg/kg (actual ingested) Exposure: 14 days (daily) Assessment of the peroxisom proliferation potential	OAEL: 25 mg/kg bw/day (a tual dose received) (male) (liver: ↑relw from 100 mg/kg/day, ↑p.enz.act. from 25 mg/kg/day)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Lake BG, Gray TJB, Foster JR, Stubberfield CR, Gangolli SD (1984)
rat (Fi her 344) male subacute oral: f ed) 0, 0.02 0.05, 0.1, 0.5, 1.0, 2.5 % (n inal in diet) Exposure: 28 days (ad libitum) Evaluation of the peroxisome proliferation potential and testicular toxicity	NOAEL (induction of palmitoyl-CoA oxidation): 51.7 mg/kg bw/day (actual dose received) (male) NOAEL (testicular atrophy): 1093 mg/kg bw/day (actual dose received) (male) LOAEL: 200 ppm (LOAEL = 24 mg/kg/day ↓bw at 2.5%. Liver: ↑absw from 0.5%, ↑relw from 0.02%, ↑p.enz.act. from 0.1%. Testes: ↓absw and relw and atrophy at 2.5%)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Worrell NR (1990)

rat (Sprague-Dawley) male/female subchronic (oral: feed) 0, 5, 50, 500, 5000 ppm (nominal in diet) 0.4, 3.7, 37.6, 375.2 mg/kg/d (males); 0.4, 4.2, 42.2, 419.3 mg/kg/d (females) (calculated based on daily food consumption) Exposure: 90 d (13W) (ad libitum) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 50 ppm (male/female) (NOAEL = 3.7 mg/kg bw Liver: enlarged (both sexes), ↑absw and relw, hypertrophy, ↑nb peroxisomes at 5,000 ppm. Kidney: ↑relw at 5,000 ppm (both sexes). Thyroid: histological changes at 5,000 ppm. Testes: mild to moderate Sertoli cell vacuolation from 500 ppm (7/10); ↓ absw and relw testicular weight, atrophy, and complete loss of spermattogenesis at 5,000 ppm (9/10)) NOAEL (Kidney toxicity): 37.6 mg/kg bw/day (nominal) (male)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Poon R, Lecavalier P, Mueller R, Valli VE, Procter BB and Chu I (1997)
rat (Wistar) male subacute (oral: feed) 0, 60, 200, 600, 2000 mg/kg (nominal in diet) 0, 5, 18, 52, 182, 549 mg/kg (actual ingested) Exposure: 14 or 28 days (ad libitum) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	NOAEL: 5 mg/kg bw/day (actual dose received) (male) (liver: dose-related ↑absw from 182 mg/kg bw following 2 or 4 weeks of treatme t, ↑n peroxisomes from 18 mg/kg bw, ↑p.enz.act. f om 5 mg/kg bw)	2 (reliable with restric ions) supp rting study expe imental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Jansen EHJM, van den Ham WA, Dormans JAMA, van Apeldoorn ME, van Leeuwen (1992) Jansen EHJM, Van Den Ham WA, De Fluiter P, Laan CA and Van Leeuwen FXR (1993)
rat (Wistar) male subacute (oral: feed) 0 or 2 % (nominal in diet) Exposure: 3, 10, 21 days (ad libitum) 21D in diet	conc. level:: 2 % in diet (male) (Body weight decrease Increase of liver weight Increase of the peroxysome proliferation)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Mann AH, Price SC, Mitchell FE, Grasso P, Hinton RH and Bridges JW (1985)
rat (Wista) male subch onic (oral: gavage) 0 125, 250, 500, 1000 mg/kg bw (actual ingested) Exposure: 9 wk (daily) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	NOAEL: 1000 mg/kg bw/day (nominal) (male)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Dalgaard M, Ostergaard G, Lam HR, Hansen EV and Ladefoged O (2000a)
rat (Wistar) male/female subacute (oral: gavage) 2500 mg/kg/d (actual ingested)	no NOAEL identified: dose level:: 2500 mg/kg bw/day (actual dose received)	2 (reliable with restrictions) supporting study	Mangham BA, Foster JR, Lake BG (1981)

Exposure: 3 weeks (ad libitum) Assessment of the liver and testicular effects	(↓bwg (males). Liver: ↑relw, no histological findings, ↑nb peroxisomes, ↑pSER)	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	
rat (Fischer 344) male/female chronic (oral: feed) 0, 6000, 12000 ppm (nominal in diet) Exposure: 104-105 Weeks of exposure (ad libitum) OECD Guideline 451 (Carcinogenicity Studies)	LOAEL: 6000 ppm (male/female) (No NOAEL determined)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982b) Kluwe WM, Haseman JK Douglas JF (1982a) Kluwe WM (1983a)
rat (Fischer 344) male chronic (oral: feed) 1.2% (nominal in diet) Exposure: 1, 2, 4, 8, 18, 39, 77, 151, 365 days (ad libitum) rats fed with DEHP-diet for max 365 days	conc. level:: 12000 ppm (male) (Pulse-labelling technique: labelling index of hepatocyte nuclei significantly increased only at 2 days. Pump infusion technique: significant increase in h patic nuclear labelling at 8 days)	2 (reliable with restriction supp rting study exp rimental r sult **Test ma erial (EC nam): bis(2-ethylhexyl) phthalate**	Marsman DS, Cattley RS, Conway JG and Popp JA (1988)
rat (Sprague-Dawley) male subacute (oral: feed) 1, 25, 100, 400, 1,600 or 6,400 ppm (nominal in diet (28 days)) 0, 1, 5, 25, 125 or 625 ppm (nominal in diet (3 and 6months)) Exposure: 28 days, 3 and 6 monrths (ad libitum) equivalent or similar t OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity n Rodent)		2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Chu, I., Secours, V.E., Marino, I.A. (1981)
rat (Fischer 344) female subacut (oral gavage) 50 150, 500 or ,500 mg/kg bw/day (actu l ingested) Exp sure 14D (daily) Fonc ional Observation Battery and motor activity measurements after repeated exposure	NOAEL: 1500 mg/kg bw/day (actual dose received) (female)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Moser VC, Cheek BM, MacPhail RC (1995) Moser VC, MacPhail RC, Gennings C (2003)
rat (Sprague-Dawley) male/female subchronic (oral: feed) 0, 0.2, 1.0, 2.0 % (nominal in diet) 0, 143, 737 or 1,440 mg/kg/day in males and 0, 154, 797, or 1,414	LOAEL: 0.2 % (male/female) (LOAEL = 143-154 mg/kg bw ↓bw from 1.0%. liver: ↑absw and relw from 0.2%, no histological findings. testes:	2 (reliable with restrictions) supporting study experimental result **Test material (EC**	Gray TJB, Butterworth KR, Gaunt LE (1977)

mg/kg/day in females (actual ingested) Exposure: 17 Weeks (ad libitum) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	↓absw and ↑relw from 1.0%, atrophy)	**name): bis(2-ethylhexyl) phthalate**	
rat (Wistar) male chronic (oral: feed) 0, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0 % (nominal in diet) 0, 8, 22, 42, 88, 500, 900 mg/kg/day (estimated intake) Exposure: 16 days (ad libitum) Evaluation of the liver effects	NOAEL: 42 mg/kg bw/day (actual dose received) (male) (increase in liver relative weight)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Fukuhara M, Takabatake E (1977)
rat (Sprague-Dawley) male subacute (oral: gavage) 0, 10, 100, 1,000 or 2,000 mg/kg/d (actual ingested) Exposure: 3, 6, 12, or 24 months (ad libitum) Dvaluation of the DEHP toxicity in pups, wealing and adult rats	NOAEL: 10 mg/kg bw/day (nominal) (male) (Mortality: all pups in three youngest age groups at 2,000 mg/kg/day groups. ↓bw in two highest dose groups. Liver: ↑absw and relw from 100 mg/kg/day ↑pp, ↑p.enz.act. ↓TG, ↓CHO)	2 (reliable with restrictions) supporting study exp rim n al result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Dostal LA, Jenkins WL, Schwetz BA (1987)
rat (Fischer 344) female subacute (oral: gavage) 0, 50, 150, 500, 1500 mg/kg (actual ingested) Exposure: 14 days (daily) equivalent or similar to OECD Guideline 407 (Repeated Dose 28 Day Oral Toxicity in Rodents)	NOAEL: 50 mg/kg bw/day (actual dose received) (f male) (Hepatocellular ytomega y at 150 mg/kg bw)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Berman E, Schlicht M, Moser VC and MacPhail RC (1995)
rat (Sprague-Dawley) male chronic (oral: feed) 0, 0.02, 0 2, 2% (nominal in diet) 0, 70, 700mg/kg (actual ingested) Exposure: 102 weeks (ad libitum) 102-week exposure in diet in rat	LOAEL: 7 mg/kg bw/day (actual dose received) (male) (↓bw from 70 mg/kg bw. Liver: ↑pp and nb mitoch from 70 mg/kg bw, ↑p.enz.act. from 7 mg/kg bw, no tumours. Testes: atrophy and inhibition of spermatogenesis from 7 mg/kg bw)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ganning AE, Brunk U, Edlund C, Elhammer Å and Dallner G (1987) Ganning AE, Olsson MJ, Brunk U and Dallner G (1990)
rat (Wistar) male subacute (oral: gavage) 0, 1000, 5000, 10000 mg/kg bw (actual ingested) Exposure: 4 wk (daily) equivalent or similar to OECD	NOAEL: 1000 mg/kg bw/day (nominal) (male) LOAEL: 5000 mg/kg bw/day (nominal) (male)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-**	Dalgaard M, Ostergaard G, Lam HR, Hansen EV and Ladefoged O (2000b)

Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)		**ethylhexyl) phthalate**	
rat (Alderley-Park) male/female subacute (oral: feed) 0, 50, 200, 1000 mg/kg (nominal in diet) Exposure: 28 days (ad libitum) equivalent or similar to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	LOAEL: 50 mg/kg diet (male/female) (↓bw at 1,000 mg/kg/day for 9 months. liver: ↑w from 50 mg/kg/day, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed (males).)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Mitchell FE, Price SC, Hinton RH, Grasso P and Bridges JW (1985) Hinton RH (1982)
rat (Fischer 344) male/female subacute (oral: feed) 0, 0.01, 0.1, 0.6, 1.2, 2.5 % (nominal in diet) Exposure: 21 days (ad libitum) Assessment of the peroxisome proliferation potential	no NOAEL identified: LOAEL: 0.01 % in diet (male/female) (corresponding to 11 mg/kg per day in males and 12 mg/kg per day in females)	2 (reliable with restrictions) supporting study experimental resul **Test material (EC name): bis(2-ethylhexyl) phthalate**	Barber ED Astill BD, M ran EJ, Schneider BF, Gr y TJB, Lake BG, Evans JG (1987) CMA (1984)
rat (Fischer 344) male/female subchronic (oral: feed) 0, 1600, 3100, 6300, 12500, 25000 ppm (nominal in diet) Exposure: 13 weeks of treatment (ad libitum) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 6300 ppm (male/female) (NOAEL = 320 mg/kg bw ↓bwg at 25,000 ppm. Testes: atrophy from 12,50 ppm)	2 (rel able with restri tions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982c)
mouse (B6C3F1) male/female chronic (oral: feed) 100, 500, 1500, 6000 ppm (males: 19.2, 98.5, 292.2, and 1266.1 mg/kg/d; females: 23.8, 116 , 354.2, and 1458.2 mg/kg/d) (nominal in diet) Exposure 104 wk (7/wk) OECD Guideline 453 (Combined Chroni Toxicity / Carcinogenicity St dies)	NOAEL: 100 ppm (male/female) (NOAEL = 19.2 mg/kg [males] and 23.8 mg/kg [females]) Liver: peroxisome proliferation and ↑ weight (males) from 500 ppm; ↑ weight, adenomas and carcinomas (both sexes) from 1,500 ppm; kidney: ↓ weight (especially males) and chronic progressive nephropathy (both sexes) from 1,500 ppm; testes: ↓ weight, ↑ incidence and severity of bilateral hypospermia from 1,500 ppm; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 1,500 ppm; ↓ survival (males); changes in liver, kidneys, and testes were at least partially reversible following recovery period)	1 (reliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	David RM, Moore MR, Finney DC (2000b) David RM, Moore MR, Finney DC (2001)

mouse (B6C3F1) male/female subchronic (oral: feed) 0, 800, 1600, 3100, 6300, 12500 ppm (nominal in diet) Exposure: 13 weeks of treatment equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 800 ppm (male/female) (NOAEL = 100 mg/kg bw ↓bwg from 3,100 ppm (males) and from 1600 ppm (females))	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982c)
monkey (Marmoset) male/female subacute (oral: gavage) 2000 mg/kg (actual ingested) Exposure: 14 days (once daily) OECD Guideline 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents)	LOAEL: 2000 mg/kg bw/day (actual dose received) (male/female) (decrease relative kidney weights in females, increase in hepatic catalase activity in males.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Rhodes C Orton TC, Prat IS, Bat en PL Bratt H, Fa kson SJ, Elcombe CR (1986)
hamster, Syrian (DSN) male subacute (oral: gavage) 0, 25, 100, 250, 1000 mg/kg (actual ingested) Exposure: 14 days (daily) 14 by gavage	NOAEL: 250 mg/kg bw/day (actual dose received) (male) (Liver weight and activitie of PCoA and CAT)	2 (reliable with re trict ons) supp rting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Lake BG, Gray TJB, Foster JR, Stubberfield CR, Gangolli SD (1984)
mouse (B6C3F1) male/female chronic (oral: feed) 0, 3000, 6000 ppm (nominal in diet) Exposure: 104-105 Weeks of exposure (ad libitum) OECD Guideline 451 (Carcinogenicity S udies)	NOAEL: 30 0 ppm male/fem le) (The LOAEL f r carcinogenicity has been det mined at 3000 ppm.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982b) Kluwe WM, Haseman JK, Douglas JF (1982b) Kluwe WM (1983a)
mouse (Sv/129, PPARalpha-null or wild type) male chr nic (o al: feed) 1200 ppm (nominal in diet) ca 2400 mg/kg bw (calculated) Exposure: 24 weeks (ad libitum) equivalent or similar to OECD Guideline 452 (Chronic Toxicity Studies)	LOAEL: 12000 ppm (male) (mortallity, body weight, relative organ weight, peroxysome proliferation)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ward JM, Peters JM, Perella CM and Gonzalez FJ (1998)
monkey (Macaca fascicularis) male subacute (oral: gavage) 0, 100, 500 mg/kg (nominal in diet)	NOAEL: 500 mg/kg bw/day (nominal) (male) (no liver effects)	2 (reliable with restrictions) supporting study experimental result	Short RD, Robinson EC, Lington AW, Chin AE (1987)

Exposure: 21 days (daily) 21D in diet. Peroxysome proliferation study.		**Test material (EC name): bis(2-ethylhexyl) phthalate**	
monkey (Macaca fascicularis) male subacute (oral: gavage) 0, 500 mg/kg bw (actual ingested) Exposure: 14 consecutive days (daily) Screening study gavage 14 D in non-rodent	NOAEL: 500 mg/kg bw/day (actual dose received) (male) (No effects)	2 (reliable with restrictions) supporting study **Test material (EC name): bis(2-ethylhexyl) phthalate**	Pugh Jr G, Isenberg JS, Kamendulis LM (2000)
mouse (B6C3F1) male subchronic (oral: feed) 0, 6000, 12000 ppm (nominal in diet equivalent to 0, 1000, 2000 mg/kg/d) Exposure: 2, 8, 24, 40 weeks (ad libitum) 40Weeks exposure in diet in mouse	LOAEL: 1000 mg/kg bw/day (actual dose received) (male) (Hepatocyte labelling index: significantly elevated at 2000 mg/kg bw (24,40 weeks). Thymidine kinase activity in liver: at 2000 mg/kg bw , increase at 2 weeks, decrease at 8 weeks; at 1000 mg/kg bw , increase at 2 and 40 weeks)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylh xyl) phthalate**	Ward JM Hagiwara A, And rson LM (1988)
primate (Marmoset) male/female chronic (oral: gavage) 0, 100, 500, 2500 mg/kg (actual ingested) Exposure: 65 Weeks (once daily) equivalent or similar to OECD Guideline 452 (Chronic Toxicity Studies)	NOAEL: 2500 mg/kg bw/day (actual dose received) (male/female) (N toxicologically relev nt effect)	1 (eliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kurata Y (2003b)

5.6.1.2. Repeated dos toxicity: inhalation

The results of expe imental s udies are summarised in the following table:

Table 38 Overview of experimental studies on repeated dose toxicity after inhalation exposure

M thod	**Results**	**Remarks**	**Reference**
rat (Wis ar) male/female subacute (inhalation: aerosol) (nose/head only) 1.0, 0.05 and 0.01 mg/l (nominal conc.) 230, 11 and 2.3 mg/kg for males and 360, 18 and 3.6 mg/kg (estimated daily intake) Vehicle: unchanged (no vehicle) Exposure: 6h (daily)	NOAEC systemic and respiratory tract irritation: 50 mg/m³ air (male/female) (At 1000 mg/m3: Increase in relative lung weights accompanied by foam cell proliferation and thickening of the alveolar septi, absolute liver weights (females) and relative liver weights (both sexes) slightly but significantly increased but	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Klimisch HJ, Gamer AO, Hellwig J (1992) Klimisch HJ (1990) Klimisch HJ (1988)

OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	there were no corresponding histological findings)		
rat (Wistar) male subacute (inhalation) (whole body) 5 and 25 mg/m3 (nominal conc.) Vehicle: unchanged (no vehicle) Exposure: 6 hours/day (5days/week) Effect on the testes of prepubertal rats after 4 and 8 wk inhalation (6 h per day)	LOAEC: 5 mg/m³ air (nominal) (male) (increase in testosterone and in weight of seminal vesicles)	2 (reliable with restrictions) supporting study experimental result Test material (EC name): bis(2-ethylhexyl) phthalate	Kurahashi N, Kondo T, Omura M (2005)

5.6.1.3. Repeated dose toxicity: dermal

No reliable data is available.

5.6.1.4. Repeated dose toxicity: other routes

The results of studies on repeated dose toxicity (other routes) are summaris d in the following table:

Table 39 Overview of studies on repeated dose toxicity (oth routes)

Method	**Results**	**Remarks**	**Reference**
rat male/female subacute (intravenous) 0, 30.8, 91.7, 164.8 mg/kg Vehicle: Bovine Serum Albumin (BSA) 4% in physiol. salilne Exposure: 18 d (1 injection da ly) Repeated intraveinous treatment in neonatal rats	LOAEL: 30.8 mg/kg bw/day (male/female) (B dy weight gains and average eight gain/d ignificantly and dose-ependently decreased from d to 21. Absolute and relative liv r weights significantly increased in a dose-related m nner.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Greener Y, Gillies B, Wienckowski D (1987)
rat (Sprague-Dawl y) male subacute (intravenous) 0, 5, 50, 500 mg/kg Vehicle: i emulsion (20% DEHP, frac ioned egg yolk phosphatides (1.2%) glycerol (2.2%) and distilled wat r Expo ure: 6 d (1 infusion daily via the implanted canula (3 hours/1ml/h)) Repeated intraveinous treatment in rats with specific investigations in the liver and the testes.	NOAEL: 50 mg/kg bw/day (male) (Some altered Sertoli cells at 500 mg/kg bw/d)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Sjöberg P, Lindquist NG, Montin G (1985)

5.6.2. Human information

A case report suggests that inhalation of DEHP may induce toxic damage of the lungs in the preterm infant. In preterm infants, artificially ventilated with PVC respiratory tubes, unusual lung disorders resembling those observed in hyaline membrane disease were observed during the fourth week of life in two infants (Roth et al., 1988). In a third infant, who died two weeks after birth, DEHP was detected in the lung tissue but not in the liver tissue. The estimated inhalative exposure in the three infants ranged between 1µg/hour – 4,200µhour. DEHP, but not MEHP, could be demonstrated in urine samples. The authors assumed that these findings were causally related to the exposure to high doses of DEHP released from the PVC tubes.

A morbidity study was carried out on a group of 97 men and 4 women employed in a German plant producing DEHP (Thiess et al., 1978b). The average exposure period was 12 years (4 months to 35 years). Background levels were generally low (0.001-0.004 ppm≈0.016-0.064 mg/m3) with higher levels up to 0.01 ppm (0.16 mg/m3) in the vicinity of the chemical reactor. Blood lipids, serum activities of liver enzymes, and routin haematological tests were normal, and no excess of any pathological condition was found. All 58 children fathered by the exposed men were normal. Due to the low exposure levels and the lack of a referent gro p, this study is considered inadequate with respect to the risk assessment.

A mortality study of 221 workers exposed to DEHP in the plant was also conducted. Eight deat s occurred in the cohort compared with expected values of 15.9 and 17.0 for city and country, respectively (Thiess t al., 1978c). This study is considered inadequate with respect to the risk assessment due to sm ll cohort size, short follow-up, and low exposure levels.

Three epidemiological studies on neurological symptoms in workers exposed to phthala e esters, including DEHP, by inhalation are available. However, due to several limitations including lack of an appropriate referent group, small size of the exposed population, inadequate documentation, nd mixed exposure to other substances than DEHP, these studies are considered inadequate with respect o risk ass ssment.

A morbidity study was conducted in the USSR of 147 workers t a PVC-processing plant (Milkow Milkov et al., 1973). The workers were exposed to a mixture of phthalates, including DEHP as a minor constituent. Tricresyl phosphate (a neurotoxin) was a component of the incombustible mat rials produced in 10-20% of machines assigned to various workers. The total phthalate air once rations recorded varied between 1.7 and 66 mg/m3. No referent group was included in the study. Frequent ompl ints of ill-effects were made by those exposed to phthalates. A high incidence of pain in the upp d low r extremities was reported in 57% of those employed for 6-10 years and 82% of those employed for more than 10 years.

Polyneuropathy was evident in 47 workers (32% ; the incidence increased with length of employment. Another 22 workers (15%) were said to have functional di orders (not specified) of the nervous system. Vestibular abnormalities were evident in 63 workers (78%) of 81 workers specifically examined.

In a cross-sectional study, sym toms and signs of polyneuropathy were reported in 12 out of 23 workers at a plant for phthalate production in Italy (Gilioli et al., 1978). The workers were exposed to a mixture of phthalates, including DEHP, but also, to a lesser degree, to the corresponding alcohols and to phthalic anhydride. Total phthalate air concen rations recorded varied between 1 and 60 mg/m3. No referent group was included in the study. The authors oncluded that no definite conclusion could be drawn from the study because of the limited number of workers examined. The study is reported in Italian with an abstract in English.

In a study involving a Swedish PVC-processing factory, 54 male workers were examined for peripheral nervous system symptom and clinical signs. The workers were exposed mainly to DEHP, di-isodecyl phthalate, and butylbenzyl phthalate (Nielsen et al., 1985). They were divided into three groups of approximately equal size and with mean phthalate exposures of 0.1, 0.2, or 0.7 mg/m3. Some workers displayed various peripheral nervous system symptoms and signs, but these were not related to the level of exposure. None of the workers reported symptoms indicating work-related obstructive lung disease. Neither did conventional lung function test results show ny association with exposure levels. Some biochemical parameters (haemoglobin, alpha-1-antitrypsin, and immunoglobulin A) showed exposure-related associations.

Conclusion: the available studies in humans are inadequate for risk assessment.

5.6.3. Summary and discussion of repeated dose toxicity

Discussion

This part has been adapted from the EU Risk Assessment 2008 (p 343 -363)

Studies in animals

A few inhalation studies in experimental animals are available and the only study available following dermal exposure to DEHP is inadequate for risk assessment. A large number of studies have investigated the toxicity of DEHP following repeated oral administration to experimental animals, mainly rats. The most pronounced effects included, effects on the liver (hepatomegaly, peroxisome proliferation and replicative DNA synthesis), testes (tubular atrophy), effects on the kidneys and cardiopulmonary tissues and hypolipidemic effects (decreased plasma levels of cholesterol and triglyceride). Other, less pronounced, effects have also been observed, e. g. reduced body weight and body weight gain, and alterations in clinico-chemical parameters.

Short-term repeated dose studies (up to 28-days exposure)

Inhalation

Rats

Wistar rats (10 males and females per group in the main dose group; 2 males and females per group in satellite group I, 15 males and 2-5 females per group in satellite group II; an equal number of con rats in each group; 9 weeks old at the beginning of exposure) were exposed in head-nose inhalation syst ms to DEHP (99.7% pure) aerosols of respirable particle size (mass median aerodynamic diameter < 1.2 ± 2.9-9.5 µm) or air (controls) (Klimisch, 1988, 1990; Klimisch et al., 1992). Exposure duration was 6 hour per day, 5 days per week for 4 weeks at 0, 0.01, 0.05, or 1.0 mg/litre (0, 10, 50, or 1,000 mg/m3). The animal of the main dose group were sacrificed at the end of the exposure period. Before sacrifice, mal rats from satellite group II had a recovery period of 2 or 6 weeks after termination of exposure. Livers of anim ls from satellite groups I and II were examined by light and electron microscopy. No animals di d during th study. Clinical examination and blood chemistry parameters did not reveal treatment-related effects. Body weights of treated rats and controls were similar. In the highest dose group, a significant increase in r lative lung weights was seen in male rats. This was accompanied by foam cell proliferation and thickening of the alveolar septi. Absolute liver weights (females) and relative liver weights (both sexes) were slightly but ignificantly increased but there were no corresponding histological findings. All these effects were reve sible with n the post-exposure observation period. No testicular toxicity was detected histologically. Electron microscopical examination of liver samples from all three concentration groups and controls at th end of exposure and after the post-exposure period did not reveal clear ultrastructural changes in hepatocytes that could be attributed to the exposure or to peroxisome proliferation. The NOAEL in this study is 50 mg/m3.

The effects of inhalation of DEHP on tes es of prepubertal rats was researched (Kurahashi et al., 2005). The results showed that inhalation of DEHP by 4-wk-old male Wistar rats at doses of 5 or 25 mg/m3, 6 h per day, for 4 and 8 wk significantly increased the concentration of plasma testosterone and weight of seminal vesicles. However, the concentration of luteinizing hormone (LH), follicular stimulating hormone (FSH) and the expression of mRNAs of androgen biosynthesis enzyme, cytochrome P450 cholesterol side-chain-cleavage enzyme (P450scc), 3 beta-hydroxysteroid dehydrogenase (3beta-HSD), cytochrome P450 17 alpha-hydroxylase/17, 20 lyase (CYP17 and aromatase (CYP19) did not change. Rats with precocious testes did not increase in any of he DEHP groups. It has also been found that the estimated effective dose in this study was less than those eported in previous studies, which uses oral dosing.

Peroral

Rats

Male Wistar rats (4 rats per dose group, 6 control rats) were fed a diet containing 0 or 2% DEHP (> 99.5% pure) for 3, 10, or 21 days (Mann et al., 1985). Body weights were significantly decreased after 10 and 21 days of treatment. Relative liver weights were increased in all dosed rats; electron microscopic examination showed significantly increased peroxisome proliferation, changes in mitochondria, and proliferation of the smooth endoplasmatic reticulum already after 3 days of treatment. The activities of peroxisomal enzymes (PCoA, α-GD, and catalase) were significantly increased.

In an other study comparable to a guideline study and performed according to GLP principles, Alderley Park rats (10 animals/sex) were given 2,000 mg/kg bw/day of DEHP (99.7% pure) by gavage in corn oil for 14 days (Rhodes et al., 1986). The control group was given the vehicle. The body weight gain was significantly reduced in males but not in females. Slight signs of systemic toxicity were observed in treated rats of both sexes. Plasma

cholesterol and triglyceride levels were significantly reduced in male rats but not in females. Absolute and relative liver weights (both sexes) and kidney weights (females) were significantly increased. Testis weights were significantly reduced in 3 male rats and testicular tubular atrophy was observed in 4 male rats. Brain weights were unaffected. Electron microscopy of livers revealed marked peroxisome proliferation (an 8-fold increase in males and 5-6-fold increase in females). Both number and size of peroxisomes were increased. Disorganisation of rough endoplasmic reticulum, mild proliferation of smooth endoplasmic reticulum, and increased number of lysosomes and mitochondria were observed in a number of rats. A significantly increased activity in the peroxisomal enzymes α-GD and PCoA and an increased activity in catalase (males only, not significant) was observed. Peroxisome proliferation was also observed in the proximal tubule of the kidney (2-fold increase in both sexes).

In a study performed according to GLP principles, Fischer 344 rats (5 animals/sex/group) were fed 0, 0.01, 0 1, 0.6, 1.2, or 2.5% DEHP (99.9% pure; corresponding to 0, 11, 105, 667 1,224, or 2,101 mg/kg bw in males and 0 12, 109, 643, 1,197, or 1,892 mg/kg bw/day in females) in the diet for 21 days (Barber et al., 1987). In the highest dose group, rats of both sexes lost weight during the first week of the study and body weights were significantly reduced compared to controls from day 3 of the study. Animals given 1.2% gained less weight than the controls during the first three days of treatment, although the differences were not sign f cant. Food intake was significantly decreased in the highest dose group (both sexes) and in females from the 1.2% dose-group. Absolute and relative liver weights were significantly increased from 0.6%. Histological examina ion showed a reduction in cytoplasmic basophilia in livers from male rats given 0.6% and more and in f male rats given 1.2% and more. Relative kidney weights were significantly increased in the highest dose group; n histological abnormalities were, however, observed. Testis weights were significantly reduced in male rats in the highest dose group and moderate to severe testicular atrophy were noted. In male rats, serum triglyceride levels were significantly increased at 0.01% but significantly reduced from 0.6%. In fem le rats serum triglycerides were significantly increased from 1.2%. There was no dose-related redu tion in serum holesterol levels. Electron microscopy revealed a dose-related increase in numbers of peroxisomes from 0.1% in males and from 0.6% in females. At dietary levels of 0.6% and above, the size range of the p roxisomes was also increased and there were changes in peroxisomal morphology. PCoA showed a dose related increase at dietary levels of 0.6% and above. LAH-11 and LAH-12 were increased in males from 0 1% and more and in females from 1.2% and more. No NOAEL can be derived from this study. The LOAEL is 0.01% in the diet (corresponding to 11 mg/kg per day in males and 12 mg/kg per day in females).

In an other study realised by Fukuhara (1977), EHP was mixed into diet and given to Wistar male rats for 16 days. A significant and dose-dependent increase in the relative liver weight (RLW) was observed in the rats fed DEHP at dietary levels greater than 0.1%. The RLW increased progressively with the duration of the treatment, reaching its maximum in two weeks. B ochemica analysis of the principal hepatic components has shown that the increase in RLW induced by DEHP was due to an increase in the total amounts of protein, water, lipid, and nucleic acids, The increase in p otein was most marked and was due mainly to the increase in non-collagen protein. The total amounts of g ycogen a d collagen did not change. However, most of these components were found to be unchanged or reduced in the r contents per liver weight, except that of protein. The increase in RLW is caused equally by th increase in cell number and in the cell volume, the nuclear DNA content being unaffected. The content of cytochrome P-450 in microsomes increased in the rats of both sex fed a 0.5% DEHP-diet for 16 days. However, th activities per mg of microsomal protein or per g of liver of aminopyrine N-demethylase and aniline hyd oxylase decreased in male rats but increased in female rats. Total activities of these enzymes increased markedly in both sex of animals. Glucose 6-phosphatase, acid phosphatase and cytochrome c oxidase were reduced significantly in their activities per liver weight but glucose 6-phosphate dehydrogenase was uncha ged The significance of the liver enlargement induced by DEHP is discussed in relation to the physiological response of liver and to possible pathological changes of liver.

In a study performed according to GLP principles, DEHP (99.9% pure) was fed to groups of 5 male Fischer 344 rat a dietary levels of 0.02, 0.05, 0.1, 0.5, 1.0, or 2.5% (24, 52, 115, 559, 1,093, or 2,496 mg/kg bw/day) for 28 days (BIBRA, 1990). The control group (10 male rats) received basic diet. A significant reduction in body weight and a reduced food intake was observed after 7 days of treatment in high-dose rats and was persistent throughout the study. Absolute liver weights were significantly increased from 0.5% while relative liver weights were significantly increased at all dose levels. Hepatic PCoA was significantly increased from 0.1% in the diet. Testis weights were significantly reduced at 2.5% in the diet; marked testicular atrophy was observed histologically. This study identifies no NOAEL; the LOAEL corresponds to the lowest dose administered, that is 0.02% in the diet (24 mg/kg/day).

Male Sprague-Dawley rats (5 animals per group) were given 25, 100, 250 or 1,000 mg/kg bw/day of DEHP (> 99% pure) by gavage in corn oil for 14 days (Lake et al., 1984). Control rats (5 animals) received the vehicle. Relative liver weights were significantly and dose-dependently increased in the three highest dose groups. A

marked dose-dependent increase in the activities of PCoA and CAT was also observed.

The Mangham study (1981) was conducted on the effect of oral administration of either DEHP or dialkyl 79 phthalate (DA79P) at a dose level of 2500 mg/kg/day for 7 and 21 days in young male and female Wistar albino rats. Both DEHP and DA79P increased liver site in both sexes and reduced the relative weight of testes in male rats. Liver enlargement was accompanied by alterations in several marker enzyme activities. Both DEHP and DA79P depressed mitochondrial succinate dehydrogenase in male but not in female animals. While certain parameters of hepatic xenobiotic metabolism were elevated in female rats receiving either DEHP or DA79P, a marked inhibition of xenobiotic metabolism was observed in male rats treated with DA79P. Parallel morphological investigations revealed histological evidence of liver damage in male rats given DA79P and ultrastructural investigation revealed changes in the structure of the nuclei, mitochondria, and endoplasmic reticulum. These effects were largely absent from female animals. DEHP produced no hepatic histological changes in either sex but ultrastructural studies indicated proliferation of the smooth endoplasmic reticulum, an increase in the numbers of microbodies (peroxisomes), and mitochondrial changes. Treatment of rats with ither DEHP or DA79P resulted in hepatic changes, although the effects were not necessarily common to both agents. Male animals appeared to be more susceptible than female animals. Finally, both agents caused tes icular atrophy as indicated by decreased testicular weight and atrophy of seminiferous tubules in male rats tr ated for 21 days.

Male Fischer 344 rats (5 animals per group) were fed 0, 100, 1,000, 6,000, 12,000 or 25,000 ppm (0, 11, 105, 667, 1,223, or 2,100 mg/kg bw/day) of DEHP (99.8% pure) in the diet for 21 day (Short et l., 1987). The relative liver weight and PCoA oxidation in liver homogenates were significantly increased from 6,000 ppm. LAH-11 and LAH-12 hydroxylation were significantly increased from 1,000 ppm. Per xisome proliferation was examined by electron microscopy and evaluated as moderate to very mark d in the three highest dose groups.

In a 28-day study, male weanling Sprague-Dawley rats (10 animals per gr up) w re fed diets containing 0, 25, 100, 400, 1,600 or 6,400 ppm of MEHP (Chu et al., 1981). Major organs w re excised and weighed (including testes). At 6 400 ppm a significant growth retardation was not d. Liver and heart weight were significantly increased from 1,600 ppm. Histological examination revealed, however no treatment-related abnormalities.

In a study designed to reveal a NOAEL for peroxisome proliferation, male Wistar rats (6 animals per group) were fed 0, 60, 200, 600, 2,000 or 6,000 mg/kg of DEHP (98.4% pure) (0, 5, 18, 52, 182, or 549 mg/kg bw/day) in the diet for 2 or 4 weeks (Jansen, 1992). Per xisome proliferation was evaluated by morphometric analysis (light and electron microscopy) and by meas m nt of the activity of peroxisomal associated enzymes (PCoA, ECoA, catalase, CAT, LAH-11, and LAH 12). There were no significant differences in body weights between control animals and treated groups. The liver weights of animals in the two highest dose groups were significantly increased in a dose-related manner c mpared to the control group following 2 or 4 weeks of treatment. The morphometric analy is re ealed a significant increase in volume density and number of peroxisomes in animals given 200 mg/kg DEHP or more in the diet for two weeks. For all enzymes, a dose-response relationship was observed. The NOAEL for induction of LAH-11, LAH-12, and ECoA was 200 mg/kg, for PCoA 600 mg/kg, and for cat lase 2 000 mg/kg. The activity of CAT (the most sensitive parameter in the study) was significantly increased in all treated groups. An overall NOAEL for peroxisome proliferation was established as 60 mg/kg DEHP (5 mg/kg bw/day) in the diet. The results for CAT were not taken into account in the establishment of the NOAEL as this enzyme is not specific for peroxisome proliferation.

In a study comparable to guideline study, male Sprague-Dawley rats were given 10, 100, 1,000 or 2,000 mg/kg bw/day of DEHP (99% pure) in corn oil by gavage for 5 days beginning at an age of 6 (1-week-old), 14-16 (2-week- ld) 21 (3 week-old), 42 (6-week-old) or 86 (12-week old) days (Dostal et al., 1987b). The control group wa given he vehicle. After two doses of 2,000 mg/kg bw/day virtually all pups in the three youngest age groups died wh reas 6- and 12-week-old rats showed significantly decreased body weights with no fatalities. Five daily doses of 1,000 mg/kg bw/day caused significant decreases in body weight gain in 1-, 2-, and 3-week-old rats. Absolute and relative liver weights were significantly increased at 100 mg/kg bw/day in all age-groups except in 1-week-old rats and in all age groups at higher dose levels. Absolute kidney weight was reduced in some cases h reas relative kidney weight was increased at doses of 1,000 mg/kg bw/day or more in 3-week-old rats or older rats. Morphological examinations revealed increased peroxisome proliferation in neonatal as well as adult rats. The activities of PCoA and CAT were increased in a dose-dependent manner in all age groups. The activities of these enzymes were similar in control rats of all ages. Plasma cholesterol concentrations were higher in suckling control rats (1- and 2-week-old) than in weanling (3-week-old) and adult controls. In DEHP-treated rats, plasma cholesterol concentrations were significantly reduced in weanling and adult rats given doses of 1,000 mg/kg bw/day or more. In suckling rats plasma cholesterol levels were increased at 1,000 mg/kg/bw/day. Plasma triglyceride levels in the control group were similar at all ages whereas significant decreases in plasma triglycerides were observed in weanling and adult rats; in suckling rats only small decreases (not significant) occurred.

The neurobehavioural effects were tested in rats by a functional observational battery (FOB) and motor activity measurements before exposure, at specified times after a single dose exposure, and during and after a 14-day repeated dose exposure (Moser et al., 1995, 2003). Female Fischer 344 rats (number not given) were administered 150, 500, 1,500 or 5,000 mg/kg bw/day of DEHP (> 99% pure) (single dose study), or 50, 150, 500 or 1,500 mg/kg bw/day of DEHP (repeated exposure, 14 days) in corn oil by gavage. The FOB included following measures: autonomic, activity, excitability, neuromuscular, sensorimotor, and physiological measures. Motor activity was measured in a maze. The FOB was performed on each rat just prior to the first dose. Thereafter, the FOB followed by motor activity assessments was conducted at 4 and 24 hours after exposure (single dose study), and on day 4 and 9 (before the daily dose) and 24 hours after the last dose. No lethality occurred. A single administration of the highest dose produced pronounced signs of general debilitation in two rats 24 hours after dosing. No changes in body weight were observed in either study. No functional domain was overall affected in either study.

Hamsters

Male DSN Syrian hamsters (5 animals per group) were given 25, 100, 250 or 1,000 mg/kg bw/day of D HP (> 99% pure) by gavage in corn oil for 14 days (Lake et al.,1984b). Control hamsters (5 animals) received the vehicle. The relative liver weight was significantly increased in high-dose animals. No signif cant increase in the activities of PCoA and CAT was observed. Administration of similar doses of DEHP o rats res lted in a significant and dose-dependent (from 100 mg/kg bw/day) increase in liver weight as well as marked and dose-dependently increased activities of PCoA and CAT.

Monkeys

In a study comparable to a guideline study and performed according to GLP principles, marmosets (5 animals of each sex) were given 2,000 mg/kg bw/day of DEHP (99.7% pure) by ga age n corn oil for 14 days (Rhodes et al., 1986). The control group was given the vehicle. Body weight gains wer unaffected. No effects were observed on liver and testis weights whereas the relative kidney weights we e significantly reduced in females. Gross and microscopic examination of the liver, kidney, testes, nd pi uita y showed no changes. Electron microscopy revealed only a slight increase in peroxisomes. Plasma cholesterol and triglyceride levels were similar in treated and control animals. A significant increase n hepatic catalase activity was seen in males. In a comparative study in rats (Rhodes et al., 1986), hepa omeg ly, marked peroxisome proliferation, and an increase in the activity of peroxisomal, mitochondrial and smoot end plasmatic reticulum (microsomal) enzymes concerned with fatty acid metabolism were obs ved f ll wing a similar dosage regimen.

Male cynomolgus monkeys (one animal per gr up) were given 0, 100 or 500 mg/kg bw/day of DEHP (99.8% pure) by gavage in corn oil for 21 days Short et l., 1987). There were no treatment related changes in relative liver weight, PCoA oxidation, CAT, or AH-11 andLAH-12 hydroxylation. In addition, no treatment related effects were observed at light and electron mi roscopic examination of the livers. In contrast to these results, relative liver weight and PCoA oxidation and LAH-11 and LAH-12 hydroxylation were significantly increased in male rats given doses from 6 000 ppm (667 mg/kg bw/day) or from 1,000 ppm (105 mg/kg bw/day), respectively, in the diet for 21 days Peroxisome proliferation was moderate to very marked from 6,000 ppm.

The effects of DEHP as a peroxisome proliferator were evaluated in young adult male cynomolgus monkeys after 14 days of trea ment, wi h emphasis on detecting hepatic and other effects seen in rats and mice after treatment with high d ses of phthalates (Pugh et al, 2000). Monkeys weighing 2-2.9 kg were exposed to 500 mg DEHP/kg/day (dissolved in 0.5% methylcellulose) administrated in a constant volume of 10 ml/kg once a day for 14 consecutiv days using an adult/pediatric nasogastric tube. All animals were observed twice a day for mortality, morb dity, and toxicological or other clinical signs, including behavioural changes, appetite, and exc eta. Blood samples were collected during the second and fourth week of the pre-test period and prior to necrop y. Monkeys were sacrificed on the day following the last dose. A gross necropsy was performed including a thorough visual examination of all organs and body tissues. Organ weights were obtained for the liver, kidney, and testes/epididymis, adrenals, brain, heart, lung, spleen, and thyroid/parathyroid, and organ to body weight ratios were calculated. Sections of liver, kidney, and testes, were fixed and embedded for subsequent histopathological evaluation, assessment of replicative DNA synthesis by immunohistochemical detection, evaluation of GJIC and for assessment of peroxisomal activity. There were no overt changes in the general health or behaviour of the monkeys following14 days of dosing. Treatment with DEHP had no effect on body weights, food consumption, or relative weights of any organs assessed. There were no changes in haematological parameters, serum chemistry or in the urine analysis. No inflammation or necrosis was seen in any of the tissues examined. The test substance did not produce any toxicologically important changes in the monkeys (Pugh et al., 2000).

Other Routes

A 3-day-old neonatal rat model was used to assess DEHP toxicity following intravenous administration (Greener et al., 1987). Neonates (12 rats per group, 2 to 4 days old) were injected 30.8, 91.7, or 164.8 mg/kg bw of DEHP (purity not specified) in 4% bovine serum albumin (BSA) solution for 18 consecutive days. Control neonates were injected a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1 to 3 hours later. After sacrifice, a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum, and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were detected in the tissues with the exception of local lesions at the injection site (subacute dermatitis), also noted in half of the BSA and saline control rats.

In the Sjoberg study (1985), six intravenous infusions of DEHP (0, 5, 50 or 500 mg/kg bw) were given to 25-day- or 40-day-old rats. In Epon-embedded testicular materials from animals given the highest dose, (dilated cisternae of endoplasmic reticulum) were observed. No age-related testicular effects were obser ed.

Subchronic toxicity studies (>28-days exposure <chronic exposure)

Oral

Rats

Fischer 344 rats (10 animals/sex/group, five- to six-week old) were given 0, 1 600, 3,100, 6,300,12,500 or 25,000 ppm of DEHP (> 99.5% pure) in the diet for 13 weeks to determine he high a d low doses for a following chronic study (NTP, 1982). One male rat fed 6,300 ppm died. Depression of mean body weight gain of male and female rats fed 25,000 ppm was 29% and55%, respectively relativ to con rols. Testicular atrophy was observed in all males fed 25,000 ppm but was less pronounced in males fed 12,500 ppm (1,250 mg/kg/day). No other compound-related histopathological findings were observed

In a study performed according to OECD guideline 408 and GLP prin ipl s, young male (105-130 g) and female (93-111 g) Sprague-Dawley rats (10 animals/sex/group) were admi istered 0, 5, 50, 500 or 5,000 ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw in males and 0.4, 4.2, 42.2 or 419.3 mg/kg bw/day in females) of DEHP (99.6% pure) in the diet for 13 weeks after a one-week acclimatisa ion period (Poon et al., 1997). No clinical signs of toxicity were observed. Body weight gain and food con umption were not affected. There were slight but significant decreases in red blood cell counts and seru h moglobin, albumin, and potassium levels in male rats fed the 5,000 ppm diet. A reduction in the h lesterol concentration was observed in female rats of the same dose group. In the 5,000 ppm dose group, the liv r w s enlarged in 10 male and in 5 female rats and absolute and relative liver weights and relative kidney weights were significantly increased in rats of both sexes. Microscopic examination revealed minimal to mild hepatocellular hypertrophy in the liver from all rats of both sexes, minimal focal necrosis in one male and two females. Electron microscopy of liver samples revealed an increased number of peroxisomes in rats f both se es. Significantly decreased absolute and relative testicular weight and mild to moderate seminiferous tu ule at ophy and mild to moderate Sertoli cell vacuolation in male rats (9/10) were also found at 5,000 ppm. In the thyroid, mild histological changes consisting of reduced follicle size and colloid density wer detected in eight animals. In the 500 ppm dose group, the liver of one male rat was enlarged and minimal Sertoli cell vacu lation was observed in 7 male rats. The NOAEL for the testicular effects (based on Sertoli cell vacuol tion in male rats) was considered to be 50 ppm DEHP in the diet (3.7 mg/kg bw/day). A NOAEL for the effects on the kidney can be considered to be 500 ppm (37.6 mg/kg bw in males).

In two separate tudies with exposure duration of 9 weeks or 4 weeks, male Wistar rats were dosed with DEHP by gavage and exposed to drinking water with or without acetone (0.5% wt/v in the 9-week study, 1% wt/v in the 4-we k tudy) (Dalgaard et al, 2000). 2-ethylhexanol is a metabolite of DEHP, which role is not fully elucidated. Both th phthalic acid and the aliphatic (ethylhexyl) part of DEHP may be toxic to the testis. The possible c ntribution of the aliphatic part to the testis toxicity can be examined by administering acetone concomitantly with DEHP.

In the 9-week study the doses of DEHP were 0, 125, 500 or 1,000 mg/kg bw with soya oil as a vehicle in a dose volume of 2 ml/kg bw. In the 4-week study the doses of DEHP were increased to 1,000, 5,000 and 10,000 mg/kg bw. In each study 80 male rats were used; 10 animals per group, weighing approximately 160g. In the 4-week study additionally 80 female rats, approximately 10 weeks of age, were used to investigate male fertility. The rats were housed two per cage in wire cages with a 12-hour reversed day/night cycle. They were given a standard diet and they were observed twice a day for clinical signs of toxicity. During both studies bodyweight was measured once a week. Food and water consumption per cage was registered on a weekly basis. The rats were tested in a Functional Observational Battery (FOB). On the day before sacrifice, rats were anaesthetised and blood was collected for clinical biochemical analyses. During the last week of the 4-week study all male rats

from each dose group were mated with undosed females. On day 15 after mating or at the end of the allocated mating period, the female rats were sacrificed, and the uteri and ovaries removed. The uterus was opened and the number of implantations and dead or retarded foetuses was counted. All males from each group underwent a thorough autopsy. Liver, kidneys, adrenals, heart, spleen, testes, epididymis, seminal vesicles, and brain were excised and weighed. In the 9-week study the histopathology was only performed in the control group and in the group receiving the highest dose level of DEHP. Histopathological findings of the testes were graded into normal, slight/moderate atrophy, or severe atrophy. In the 4-week study immunohistochemical investigations were performed on testis vimentin.

In the 9-week study no animal died and no other clinical abnormalities were observed. No effect on body weight, food and water consumption was observed. No histopathological changes were observed in any of he investigated organs of he control and animals exposed to 1,000 mg/kg bw DEHP. The only effect of DEHP was the statistically significant increase in relative liver weight in the dose group exposed to 500 and 1,000 mg DEHP/kg bw with or without acetone in the drinking water.

In the 4-week study a statistically significant reduction in body weight and food and water consumption was observed in the middle and high dose groups. This reduction was getting more pronounced during th study and was dose-dependent for DEHP. Six animals died due to emaciation, two animals in the g oup dosed wi h 10,000 mg/kg bw DEHP and four animals dosed with the same amount of DEHP combined with 1% ac tone in the drinking water. No differences were found between groups in the FOB test. Hindlimp grip strength was statistically significantly reduced in the groups receiving acetone compared to th gr ups not receiving acetone. Forelimb grip strength was statistically significantly reduced with increasing doses f DEHP. There was a statistically significant reduction in the level of cholesterol in animals dos d with 125mg, 500 or 1,000 mg DEHP/kg bw and in animals exposed to 250 mg DEHP/kg bw in combination with 0 5% acetone.

The number of males without recognised mating increased in a dose-relat d m nner although the effect was only statistically significant in the group receiving 10,000 mg DEHP/kg bw plus 1%acetone (4/6). The number of pregnant females decreased with increasing DEHP dose levels (9/10→1/8) The most pronounced effect of DEHP was a statistically significant increase in relative liver weight, which was observed in treated animals. In the middle and high dose groups body weight was stati tically significantly reduced, while the relative weights of kidney and brain were statistically significantly increa ed. Testes weight was statistically significantly reduced in the groups exposed to 5,000 and 10,000 mg DEHP/kg bw with or without acetone.

The weight of epididymals and seminal vesicles were tat tically significantly reduced at the highest dose level. DEHP had no effect on the weight of splee The t stis of two rats dosed with 5,000 mg/kg bw showed severe atrophied tubules with massive loss of spermatids and spermatocytes sloughed into the lumen. In most tubules the majority of the germinal epithelium was lined by spermatogonia and Sertoli cells or by Sertoli cells alone. 5 animals that received 5,000 mg DEHP/kg bw + acetone had some microscopic features of severe atrophy of the testes and occasionally thicken ng of the basal lamina of the tubule. Slight to moderate atrophy was seen in two animals: one dosed with 10,000 mg DEHP/kg bw and one dosed with 5,000 mg DEHP/kg bw + 1% acetone. All other surviving animals dosed wi h 10,000 mg DEHP/kg bw with or without acetone in the drinking water showed severe seminif us tubular atrophy. A slight diffuse Leydig's cell hyperplasia was observed in testes with severe atrophy

Mice

B6C3F1 mic (10 nimals/sex/group) were given 0, 800, 1,600, 3,100, 6,300 or 12,500 ppm of DEHP (> 99.5% pure) in the di t for 13 weeks (NTP, 1982). Seven out of 10 mice in the highest dose group died; these deaths were, how ver, accidental. Two female mice in the 3,100 ppm group and one female in each of the control, 6,3 0 and 12,500 ppm groups died. A mean bodyweight gain depression of 10% or more was observed in males fed 3,1 0 ppm and more and in all female dose groups except for those fed 1,600 ppm. No other compound-related effects were observed.

Monk ys

In a 13-week oral study performed according to GLP principles, marmosets (4/sex/group) were daily administered 0 (corn oil), 100, 500 or 2,500 mg/kg DEHP (purity not specified) in corn oil (Kurata et al., 1995). Males were dosed from 13 to 14 months of age and females from 12 to15 months of age. The body weight gain was significantly suppressed in males administered 2,500 mg/kg. There was a significant decrease of the absolute weight of the spleen in dosed males and also a similar trend for the relative weight; this was thought to be of little toxicological significance as no histopathological changes were found. Other organ weights, including liver, testes, and pancreas, were not different from the control weights. In the DEHP dosed groups there was a significant rise in the total and free cholesterol and phospholipid levels in administration week 4. In week13, only the total cholesterol value in the 500 mg/kg males was different from the control value. It could not be

concluded that the effect was caused by the administration of DEHP. A clear rise in blood testosterone and oestradiol concentrations in all groups, including controls, were concluded to be hormonal changes accompanying sexual maturity occurring at the age of about12 months. In the 500 and 2,500 mg/kg group males, a significant increase in the average hepatic peroxisome area was observed, but there was no difference from the control group in terms of the number of peroxisomes per cell or the area density with respect to the cell area of the peroxisomes. No difference from the control group was seen concerning hepatic peroxisome enzyme activities in the DEHP dosed groups. A rising trend (not statistically significant) in the hepatic microsome protein content and an accompanying rising trend in the cytochrome P-450 content per unit liver weight were observed in all dosed males and in mid- and high-dose females. The effects on various organs of long-term oral administration of DEHP have also been studied in marmosets (Kurata et al., 1996; 1998). Marmosets (12-15 months old; 4 animals per sex and group) were given daily doses of 0, 100, 500 or 2,500 mg/kg bw of DEHP (purity not specified) by gavage in corn oil for 13 weeks. The control group was given the vehicle. A significant suppression of body weight gain was observed in high-dose males. Dose-related decreases in spleen weight were observed in all dosed males. Light and electron microscopic examination revealed no substance-related abnormality in the liver in any dosage group. A slight but significant increase in the mean hepatic perox some volume was observed in mid- and high-dose males, but the number of peroxisomes and their volume den ity was not different from those in the control group. No substance-related change of peroxisome rel ted enzyme activity (catalase, CAT, and PCoA) was observed in any dosage group. No effects on the teste were se n.

Chronic Toxicity studies (more than 10% of the test animals lifespan)

Inhalation

No relevant data are available.

Oral

Rats

In a studyg comparable to a guideline study, Sprague-Dawley ra s (15 animals/sex/group) were given diets containing 0, 0.2, 1.0 or 2.0% DEHP (purity specified as conforming with British Standards Institution) (0, 143, 737 or 1,440 mg/kg/day in males and 0, 154, 797, or 1,414 mg/kg bw/day in females) for 17 weeks (Gray et al., 1977). Rats in the highest dose-group showed clinical sign of toxicity (loss of fur) from week 1 through week 17 of treatment. The bodyweights of rats in the two highest d se groups were reduced compared to controls, significantly from day 2 in both sexes given 2% DEHP and from day 6 or day 83 in males or females, respectively, given 1% DEHP. The body w ights of low-dose rats were lower (not significant) than in controls. Food intake was significantly reduced only in ra s giv n 2% DEHP. Both absolute and relative liver weights were significantly increased in all dose groups when compared to controls. The absolute weights of most other organs (including testes) in rats of the tw highe t dose groups 2were lower than those of controls while the relative weights were increased Histological examination revealed severe seminiferous tubular atrophy and cessation of spermatogenesis, which cou d be related to the dietary level of DEHP. These changes were evident as early as week 2. No histologic l changes attributable to DEHP treatment were observed in the livers. No NOAEL can be identified from this study; the LOAEL corresponds to the lowest dose level, 0.2% in the diet (143 mg/kg/day in males and 145 mg/kg/day in females).

In 3- and 6-month fe ding studies, weanling Sprague-Dawley rats (20 animals/sex/group) were fed diets containing 0 1 5 25, 125 or 625 ppm of MEHP (Chu et al., 1981). After 3 months, 10 animals of each sex from each group w re n cr psied. The remaining animals were maintained on the same diet and sacrificed after six month . Relati organ weights were not altered in the 3-month period, but the liver weights of high-dose females w re significantly increased in the 6-month period. Treatment-related lesions were found in the liver (midzonal and periportal eosinophilic cytoplasmic inclusions, and vacuolations with isolated binucleated and necrotic hepatocytes), heart (mild enlargement of myocardial nuclei and segmental deregistration of myocardial st iati ns), and adrenals (vacuolation of the zona fasciculata).

In a study comparable to a guideline study and performed according to GLP principles, groups of male and female Alderley Park rats (20 animals/sex per treatment group, 30 animals/sex in the control group) were fed diets containing sufficient DEHP (> 99.7% pure) to ensure intakes of 0, 50, 200 or 1,000 mg/kg bw/day (Hinton, 1982; Mitchell et al., 1985a). Four rats from each treatment group and six controls and were sacrificed 3, 7, 14, 28 days, and 9 months after beginning of feeding. No clinical signs of toxicity were observed during the study. Food consumption of the treated rats was either similar to or greater than that of controls. Body weight was significantly reduced only in rats given 1,000 mg/kg bw/day for 9 months. Liver weights were increased in male rats fed 50 and 200 mg/kg bw/day for 14 days or more and in all high dose rats at all time intervals. Examination of liver sections by light microscopy showed periportal accumulation of fat and mild centrilobular loss of glycogen, both effects were dose-dependent. Electron microscopy revealed that the number of peroxisomes was

significantly increased at 50 mg/kg bw/day after 14 days in males and after 9 months in females. Higher doses caused increased number of peroxisomes after 3 days in males and after 14 days in females, the increase was dose-dependent in both sexes. Dose-dependent alterations to the endoplasmatic reticulum (ER) were also observed. Smooth endoplasmatic reticulum proliferation was significantly increased at 50 mg/kg bw/day after 7 days in males and after 14 days in females. Changes in rough endoplasmatic reticulum were observed at 200 mg/kg bw/day after 3 days in males and after 28 days in females. The density of the mitochondrial matrix was increased in male rats although there was no dose dependence. Biochemical studies revealed a marked induction of the peroxisomal enzymes PCoA and α-GD; the induction was dose and time-dependent. The effects in female rats increased more slowly than in male rats but were equivalent by 28 days. The ER-associated enzymes cytochrome P-450 and LAH were dose-, but not time-dependently increased with maximal activity observed at 3 days of treatment. No effect on testis weight was observed. Alterations of the kidneys (lysosomes in the cells of the proximal tubule were enlarged) were observed at 200 and 1,000 mg/kg bw/day. In thyroids of rats fed 1,0 0 mg/kg bw/day for 9 months showed also alterations (basophilic deposits in the colloid and enlargement f the lysosomes).

Male Fischer 344 rats (5-10 animals per group) were fed 1.2% DEHP (purity not specified) in the diet for 1, 2, 4, 8, 18, 39, 77, 151 or 365 days (Conway et al., 1989). Livers were examined biochemically. Catalase activity was increased (25%) after 8 days and remained at this level up to 365 days. Glutathione peroxidase activity showed a 50% and 80% decrease after 8 or 365 days of treatment, respectively. Lipofuscin, which was cont ined within lysosomes, was increased 3-fold after 39 days and remained at this level up to 365 days of reatment. The activities of the lysosomal enzymes α-fucosidase, ß-galactosidase and N-acetylglu osaminid se were increased 50-100% for 39-365 days of treatment.

In a more recent long-term study, comparable to a guideline study and conducted according to the principles of GLP (David et al., 2000, 2001), F-344 rats were administered DEHP at dietary concentrations of 0, 100, 500, 2,500 or 12,500 ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day, respectively, for males, and 0, 7.3, 36.1, 181.7 or 938.5 mg/kg/day, respectively, for females), 70 males and females/group, for at least 104 weeks. An additional group was administered 12,500 ppm DEHP for 78 weeks, followed by a r covery period of 26 weeks. There were no treatment-related effects at 100 and 500 ppm. At 2,500 ppm the mean serum albumin concentration and mean liver weights were significantly increased. At We k 79 and at study termination also absolute and relative kidney weights were increased in both sexes at 2,500 ppm At the highest dose level, there was a decreased survival, increased incidence of clinical abnormalities, nd d creased body weight gain in both sexes. A diffuse hepatomegaly and histopathological hepatic ch nges were demonstrated as were effects on the kidneys, including increased absolute and relative kidney w ights (both sexes), increased incidence and severity of mineralisation of the renal papilla in males, incr ased incidence and/or severity of tubule cell pigment in both sexes, and increased severity of chronic progress e nephropathy in the males. In the males, also absolute and relative testis weights were signifi ntly decreased at 12,500 ppm, with associated increased incidence of bilateral aspermatogenesis and decreased incidence of interstitial cell neoplasms. In the pituitary, an increased number of castration cells were observed in 30/60 males compared to 1/60 of the control males. There was no indication in rats killed at study t rmina ion that DEHP-related changes in the kidney, testis, and pituitary were reversible upon cessati of DEHP-exposure. The NOAEL for systemic non-neoplastic effects, including the effects on the kidney is considered to be 500 ppm DEHP in the diet (corresponding to 28.9 mg/kg bw/day in the males and 36.1 mg/ g/day in he females) based on increased absolute and relative kidney weight in both sexes at the next higher dos level (LOAEL: 2,500 ppm corresponding to 146.6 mg/kg bw/day in the males and 181,7 mg/kg bw/day in the females).

In a two years- tudy, Fischer 344 rats (50 animals/sex/group) were given 0, 6,000 or 12,000 ppm of DEHP (> 99 5% pur) in the diet. (NTP, 1982) Mean daily ingestions of DEHP were calculated to 322 and 674 mg/kg bw/d y for low- and high-dose male rats, respectively, and to 394 and 774 mg/kg bw/day for low- and high-dose female rats, respectively. The survival rate was unaffected. At the end of the study, mean body weights of dosed m le ats and high-dose female rats were marginally to moderately lower than those of the corresponding contro s. Food consumption was slightly reduced in rats of either sex. In high-dose males, the incidence of hyp rtrophy of the anterior pituitary was significantly increased (45%); and in the testis, degeneration of the seminiferous tubules occurred in 90% of the animals.

Male Sprague-Dawley rats (a total of 520 animals) were fed 0, 0.02, 0.2 or 2% DEHP (0, 7, 70 or 700 mg/kg bw/day; > 99% pure) in the diet for 102 weeks (Ganning et al., 1987, 1990). The body weights were significantly reduced in the highest dose group reaching 20% lower values after 25 weeks of treatment. Significantly reduced body weights were also observed in the mid dose group but to a lesser extent (around 10%). Electron microscopy revealed characteristic changes in hepatocytes. After one week of treatment with 2% DEHP, peroxisome proliferation (varying size) was observed and was persistent throughout the study. The number of mitochondria was increased whereas no changes were observed in rough and smooth endoplasmatic reticulum. After feeding

with 0.2% DEHP for 16 months, an increased number of peroxisomes and mitochondria and well-developed endoplasmic reticulum were observed. The number of peroxisomes and mitochondria was not significantly increased after feeding of 0.02%DEHP when compared with the control group. The activity of PCoA was significantly increased in all dose-groups with a doubling of the activity in the lowest dose group after 2 years of treatment. This long-term treatment with DEHP had complex effects on catalase activity with decreasing activity during the initial phase, at 10 weeks the activity exceeded that of the control, but returned to normal at the end of the study. The activity of urate oxidase decreased throughout the study. No hyperplastic nodules or primary liver carcinoma or other tumours were observed. In all dose-groups, DEHP exerted a pronounced effect on the function of the testes after prolonged treatment, consisting of inhibition of spermatogenesis and general tubular atrophy. No NOAEL can be derived from this study; the LOAEL is 0.02% in the diet (7 mg/kg bw/day), the lowest dose administered. In a following study investigating the reversibility of the observed effects, rats w re fed a diet containing 2% DEHP for 1 year and then the basal diet for 3 weeks. PCoA and catalase activities decreased after cessation of treatment and reached control levels after 2 weeks.

Mice

In a study comparable to a guideline study and performed according the principles of GLP (David et al., 000, 2001) B6C3F1 mice (70-85 of each sex/dose group) were administered DEHP daily in the di t at concentrations of 0, 100, 500, 1,500 and 6,000 ppm (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg/day, respe tively, fo males, and 0, 23.8, 116.8, 354.2 or 1,458.2 mg/kg/day, respectively, for females), for 104 weeks. One dditional group (55 males and females/group) were administered 6,000 ppm DEHP for 78 weeks, fol w d by a 26-week recovery period. At 1,500 ppm, there was a significant decrease in kidney weight in males and an increased incidence and/or severity of chronic progressive nephropathy in both sexes. The testicular weigh was also significantly decreased, with an increased incidence and severity of bilateral hypospermia and an associated increased incidence of immature/abnormal sperm forms and hypospermia in the epididymis At the highest dose level, there was a statistically significant decrease in survival in males, treatment related clinical signs and a significantly reduced body weight gain for both males and females In both males and females, the kidney weight indices were significantly decreased at study termination. In the recov ry group, some treatment-related findings were reversible or did not progress after cessation of DEHP exposure such as effects in the liver, including induction of neoplasms. In contrast to the liver, the effe ts of DEHP on the kidney and testis were not reversible following cessation of exposure. The NOAEL in this study is considered to be 500 ppm (due to effects on the kidney at the next higher dose level) corresponding to 98.5 mg/kg in males and 116.8 mg/kg in females.

In a study performed according to GLP principle B6C3F1 mice (50 animals/sex/group) were given 0, 3,000, or 6,000 ppm of DEHP (> 99.5% pure) in the diet for 103 weeks (NTP, 1982). Mean daily ingestion of DEHP was calculated to 672 and 1,325 mg/kg bw for low- and high-dose males, respectively, and to 799 and 1,821 mg/kg bw for low- and high-dose females, respectively. The survival rate was unaffected in male mice but several female mice in the lower dose group died fter 75-90 weeks of treatment. These deaths were not attributed to DEHP administration. Mean b dy weigh s of female mice were marginally to moderately lower than those of the corresponding controls at the end of the tudy. A significantly higher incidence of chronic inflammation of the kidney was observed in high-dose m les. Bilateral seminiferous tubular degeneration and testicular atrophy were observed in 14% of the male mice in the high dose group. This lesion was also found in one control male mouse and in two low-dos males. Non-neoplastic lesions were not observed in female mice.

Monkeys

In a study rea ised ac ording to guidelines and GLP (Kurata 2003), DEHP was administered by oral gavage at doses f 0 100 500, and 2500 mg/kg for 65 weeks to juvenile common marmosets (about 3 months old) of both sex s, and ts toxicity was assessed. An extensive and intensive investigation focused on testicular morphology, and f nction was realised. Treatment-related change in the body weight was not evident. Treatment-related changes were not observed in general except for adaptive liver changes. During the treatment period, all males experienced a surge in testosterone, and the testosterone levels in all treated groups were similar to that of the control group. For the testis, electron microscopic examination was additionally applied, however, this revealed n treatment-related abnormalities. Histochemical examination after 3beta-hydroxysteroid dehydrogenase (3beta-HSD) staining did not reveal any alteration in steroid synthesis. Consumption of peroxide scavenger like GSH, GST, or GSH-Px in the testis was not noticed, which suggests that the peroxysomal enzyme may not be operating in this organ. For functional examination, sperm count was conducted to show no treatment related effect in numerical changes. The liver weight and its body weight ratio were not affected. P450 content tended to increase with dose-dependent manner in general, and that was considered to be adaptive change to the DEHP-exposure. However, with regard to specific CYP, CYP3A and 2E these are related to testosterone-6-beta-hydroxylation and lauric acid gamma-1 hydroxylation, respectively, and CYP4A related to lauric acid hydroxylation, no marked increases were noticed. That showed induction of non-PP dependent oxidation and proved absorption of the test substance. In the toxicological study, despite the high dose of 2500 mg/kg/day and

adoptive liver change proving absorption, no testicular change was morphologically or functionally noticed in the extensive examinations. In conclusion, when DEHP was administered orally to juvenile marmoset at dosage levels of 100, 500, and 2500 mg/kg/day for 65 weeks, the testicular effect that is well known in rodents were not observed despite extensive examination.

The following information is taken into account for any hazard / risk assessment:

Repeat dose effects of DEHP have been evaluated in a number of animal species by several routes of exposure. The most pronounced findings were effects on the liver (hepatomegaly, peroxisome proliferation), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). For liver and kidney toxicity (increases in serum albumin, absolute and/or relative liver and kidney weights and hepatic peroxisome proliferation) a LOAEL was established from a well-conducted 104-week rat dietary study at 146.6 mg/kg bw/d. The NOAEL was 28.9 mg/kg bw/d. Studies of DEHP in monkeys failed to elicit the liver, kidney or testicular effects seen in rodents.

Value used for CSA (route: oral):

NOAEL: 29 mg/kg bw/day

Target organs: urogenital: kidneys

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF HE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classification is warr nted f r specific target organ toxicity-repeated exposure.

5.7. Mutagenicity

5.7.1. Non-human information

5.7.1.1. In vitro data

The results of experimental studies are ummaris d in the following table:

Table 40 Overview of exp rimental in vitro genotoxicity studies

Method	Results	Remarks	Reference
bacterial reverse m tation as ay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537 TA 98 and TA 100 (met. act : with nd w thout) Doses 0, 100, 333, 1000, 3333, 10000 µg/plate equivalent or similar to OECD G ideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Zeiger, E., Haworth, S., Mortelmans, K (1985) Zeiger E, Haworth S, Speck W (1982)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA97, TA98, TA100, TA1535 (met. act.: with and without)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA97, TA98, TA100,	2 (reliable with restrictions) **key study** experimental result **Test material (EC**	Zeiger E, Haworth S (1985) Ashby, J., Paton, D. (1985a)

Doses: 0, 100, 333, 1000, 3333, 10000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	TA1535(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	**name): bis(2-ethylhexyl) phthalate**	
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA1535, TA1537, TA1538, TA98 and TA100 (met. act.: with and without) Doses: 0, 0.02, 0.06, 0.20, 0.66, 2.00 mL of urine from 6 sprague-Dawley rats treated daily with corn oil (negative control) or 2000 mg/kg DEHP for 15 day, or singly with 600 mg/kg 8-hydroxyquinoline (positive control) equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA1535, TA1537, TA1538, TA98 and TA100(all strains/cell types tested); met. act.: with and without; cytotoxicity: no (but tested up to a maximal volume of urine compatible with the test)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	DiVincenzo GD, Hamilton ML, Mueller KR (1985)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 102 (met. act.: with and without) Doses: 0, 1, 2.5, 5.0, 10.0, 20.0 µmol/plate Bacterial gene mutation assay on TA102	Evaluation of results: negative Test results: negative for S. typhim rium TA 102(all strains/cell types tested); met act.: with and without	2 (eliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Schmezer P, Pool BL, Klein RG, Komitowski D, Schmahl D (1988)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, oth r: TA 1535, TA 1537, TA1538 TA 98 and TA 100 (met. act.: with and without) Doses: 0.1, 0 5, 2 5, 5.0, 10.0 µl/plate equival nt or similar to OECD Guidel ne 471 (Bacterial Reverse M t tion Assay)	Evaluation of results: egative Test results: negative for S. typhimurium, other: TA 1535, TA 1537, TA1538, TA 98 and TA 100(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kirby PE, Pizzarello RF, Lawlor TE (1983)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 102 (met. act.: with and without) Doses: up to 5000µg/plate Bacterial gene mutation assay on TA 102	Evaluation of results: negative Test results: negative for S. typhimurium TA 102(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Jung R, Engelhart G, Herbolt B (1992)

	(5000µg/plate)		
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA 1535, TA 1537, TA1538, TA 98 and TA 100 (met. act.: with and without) Doses: 0, 0.25, 0.5, 1.0, 2.0 mg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA 1535, TA 1537, TA1538, TA 98 and TA 100(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result **Test material (IUPAC name): mono (2-ethylhexyl) phthalate**	Ruddick, J.A., Villeneuve, D.C., Chu, I. (1981)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA98, TA100,TA1535, TA1537, TA1538 (met. act.: with and without) Doses: 50, 100, 500, 1,000, 5,000 µg/plate, equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA98, TA100,TA1535, TA1537, TA1538(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentration	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylh xyl) phthalate**	Rexroat MA, P obst GS (1985) Ashby, J., Paton, D. (1985a)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA97, TA98, TA100, TA102 (met. act.: with and without) Doses: 0, 320, 1,000, 3,200, 10,000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: neg tive for S typhimurium, other: TA97, A98, TA100, TA102(all s rains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Baker RSU, Bonin AM (1985) Ashby, J., Paton, D. (1985a)
bacterial reverse m tation as ay (e.g. Ames t st) (gene mutation) S. typhimurium, ther TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, a mix of 7000-strains TA1537, TA98 (met. act.: with) Doses 0.5-1000 µg/ml Ames-Test using a commercial test system AMAX, Mutagenicity Assay by Xenometrix	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, a mix of 7000-strains, TA1537, TA98(all strains/cell types tested); met. act.: with; cytotoxicity: > 1000 µg/ml	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Gee, P., Sommers, C.H., Melick, A.S. (1998)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA98, TA100 (met. act.: with and without)	Evaluation of results: negative Test results: negative for S. typhimurium,	2 (reliable with restrictions) supporting study experimental result	Yoshikawa K, Tanaka A, Yamaha T (1983)

Doses: 50, 100, 200, 500, 1,000, 2,000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	other: TA98, TA100(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes (2000 µg/plate)	**Test material (EC name): bis(2-ethylhexyl) phthalate**	
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA102 and TA2638 (met. act.: with and without) E. coli, other: WP2 uvrA/pKM101 and WP2/pKM101 (met. act.: with and without) Doses: 0, 313, 625, 1250, 2500, 5000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay) equivalent or similar to OECD Guideline 472 (Genetic Toxicology: Escherichia coli, Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for S. typhimurium, other: TA102 and TA2638(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations negative for E. coli, other: WP2 uvrA/pKM101 and WP2/pKM101(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Watanabe K, Sakamoto K and Sasaki T (1998)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium, other: TA97, TA98, TA100, TA102 (met. act.: with and without) Doses: 0, 100, 200, 500, 1,000, 2,000, 5,000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Revers Mutation Assay)	Evaluation of results: negative Test results: neg tive for S typhimurium, other: TA97, A98, TA100, TA102(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Matsushima T, Muramatsu M, Haresaku M (1985) Ashby, J., Paton, D. (1985a)
mammalian cell ge e mutati n assay (gene mutation) mouse lymphoma L5178Y cells (met ac : with and without) Doses: 125, 250 500, 750, 1000, 2000, 3000, 5000 nl/ml OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Te t)	Evaluation of results: negative Test results: negative for mouse lymphoma L5178Y cells(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Myhr B, Bowers L, Caspary WJ (1985) Ashby, J., Paton, D. (1985a)
mammalian cell gene mutation assay (gene mutation) human lymphoblastoid cells (TK6) (met. act.: with and without) Doses: 0, 200, 250, 400, 600, 750, 800, 1,000 µg/ml	Evaluation of results: negative Test results: negative for human lymphoblastoid cells (TK6)(all strains/cell types tested); met.	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-**	Crespi CL, Ryan CG, Seixas GM (1985) Ashby, J., Paton, D. (1985a)

equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations	**ethylhexyl) phthalate**	
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Doses: with metabolic activation: 1.0, 2.5, 5.0, 7.5, 10, 20, 40, 80 µg/ml without metabolic activation: 10, 20, 30, 40, 50, 100, 200, 400 µg/ml equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Evaluation of results: positive with metabolic activation negative without metabolic activation Test results: negative for mouse lymphoma L5178Y cells(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes (>= 30µg/ml)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Oberly TJ, Bewsey BJ, Probst GS (1985) Ashby, J., Paton, D. (1985a)
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells and L5178Y clone 372+/+ (met. act.: with and without) Doses: 0, 78, 392, 1,962, 9,810 µg/ml equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	Evaluation of results: negative Test results: negative for mouse lymphoma L5178Y cells(all str ins/cell types tested); me act. with and without; cytotoxicity: no, ut tested up to imit c nc ntrations	2 (rel ble with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Styles JA, Clay P, Cross MF (1985) Ashby, J., Paton, D. (1985a)
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Doses: DEHP from 0.016 to 21.0 µl/ml (non-activated cul ures from 0.089 to 2 µl/ml (ac ivated cultur s) equival nt or similar to OECD Guid line 476 (In vitro Mammalian Cell Gene Mutation Te t)	Evaluation of results: negat ve Test results: negative for mouse lymphoma L5178Y cells(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kirby PE, Pizzarello RF, Lawlor TE (1983)
mammalian cell gene mutation assay (gene mutation) mouse embryo cell Balb/c-3T3 (met. act.: with) Doses: 0, 79, 250, 791, 2,000, 7,910 nl/ml (77-7,752 µg/ml)	Evaluation of results: ambiguous with metabolic activation Test results: ambiguous (2-fold increase in the appearance of Oua R mutants at	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-**	Matthews EJ, DelBalzo T, Rundell JO (1985) Ashby, J., Paton, D. (1985a)

Ouabaine resistance in Balb/c-3T3 cells	250 nl/ml, but statistically significant) for mouse embryo cell Balb/c-3T3; met. act.: with; cytotoxicity: yes (>= 2000 nl/ml)	**ethylhexyl) phthalate**	
in vitro gene mutation assay in fungi (gene mutation) yeast, other: Schizosaccharomyces pombe P1 (met. act.: with and without) Doses: 369, 738, 1,467, 2,935, ,5870 µg/ml Forward mutation assay in S. pombe	Evaluation of results: ambiguous Test results: ambiguous for yeast, other: Schizosaccharomyces pombe P1(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Loprieno N, Boncristiani G, Forster R (1985) Ashby, J., Paton, D. (1985a)
in vitro gene mutation assay in fungi (gene mutation) Saccharomyces cerevisiae (met. act.: with and without) Doses: 1,541, 3,081, 6,163, 12,325 nl/ml (1,510-12,080 µg/ml) equivalent or similar to OECD Guideline 480 (Genetic Toxicology: Saccharomyces cerevisiae, Gene Mutation Assay)	Evaluation of results: ambiguous Test results: ambiguous for Saccharomyces cerevisiae(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting t dy experimental r sult **Test material (EC n me) bis(2-ethylhexyl) phth late**	Mehta RD, von Bor tel RC (1985) Ashby, J., Paton, D. (1985a)
in vitro gene mutation assay in fungi (gene mutation) Saccharomyces cerevisiae (met. act.: with and without) Doses: 0, 40, 200, 1,000, 5,000 µg/ml equivalent or similar to OECD Guideline 480 (Genetic Toxicology: Saccharomyces cerevisiae, Gene Mutation A say)	Evaluation of results: negative Test results: posi ive (only at 5000 µg/ml) for Saccharomyces cerevisiae(all strains/cell types tested); met. act.: with and without; ytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Arni P (1985) Ashby, J., Paton, D. (1985a)
in vitro gene mutation ssay in fungi (gene mutat n) Saccharomyce cerevisiae (met. ac : with and w thout) Doses 200, 500, 1000, 2000, 3000 5000 µg/ml equivalent or similar to OECD Guideline 480 (Genetic Toxicology: Saccharomyces cerevisiae, Gene Mutation Assay)	Evaluation of results: negative Test results: negative for Saccharomyces cerevisiae(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parry JM, Eckardt F (1985) Ashby, J., Paton, D. (1985a)
in vitro mammalian chromosome aberration test (chromosome aberration) Chinese hamster Ovary (CHO) (met. act.: with and without)	Evaluation of results: negative Test results: negative for Chinese hamster	2 (reliable with restrictions) **key study** experimental result	Gulati DK, Witt K, Anderson B (1989) Gulati DK, Sabharwal PS,

Doses: 0, 5, 16, 50, 600, 1600, 2000, 3000, 4000, 5000 µg/ml equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Ovary (CHO)(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	**Test material (EC name): bis(2-ethylhexyl) phthalate**	Shelby MD (1985)
in vitro mammalian cell micronucleus test (chromosome aberration) Chinese hamster lung fibroblasts (V79) (met. act.: with and without) Doses: 0.1, 0.25, 1, 2.5, 10, 25, 100, 250, 1000 µg/ml in vitro micronucleus assay	Evaluation of results: negative Test results: negative for Chinese hamster lung fibroblasts (V79); met. act.: without; cytotoxicity: yes (> 10 µg/ml) negative for Chinese hamster lung fibroblasts (V79); met. act.: with; cytotoxicity: no, but tested up to limit concentrations	1 (reliable without restriction) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	von der Hude W, Kalweit S, Engelhardt G, McKiernan S et al (2000)
in vitro mammalian chromosome aberration test (chromosome aberration) primary culture, other: Chinese hamster primary liver cells (CH1-L) (met. act.: without) Doses: 0, 5, 12.5, 25, 50 µg/ml Effects on mitosis and the mitotic spindle in Chinese hamster primary liver cells (CH1-L) in culture	Evaluation of results: positive without metabolic activation Test results: positive (increases in hyperploidy cells (chromosome number > 22) but negative for hypoploidy and polyploidy cells) for primary culture, other: Chinese hamster primary liver cells; met. act.: without; cytotoxicity: yes (50 µg/ml)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parry EM (1985) Ashby, J., Paton, D. (1985b)
in vitro mammalian chromosome aberration test (chromosome aberration) hepatocytes: rat liver cells RL4 (met. act.: not applicable) Doses: 0, 250, 500, 1,000 µg/ml equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Evaluation of results: negative Test results: negative for hepatocytes: rat liver cells RL4; met. act.: not applicable; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Priston RAJ, Dean BJ (1985) Ashby, J., Paton, D. (1985a) Shell (1983)
in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: Chinese hamster lung fibroblasts (CHL) (met. act.: with and without) Doses: 1375, 2750, 4130 µg/ml equivalent or similar to OECD Guideline 473 (In vitro	Evaluation of results: negative Test results: negative for mammalian cell line, other: Chinese hamster lung fibroblasts (CHL); met. act.: with; cytotoxicity: no (Cytotoxic concentrations were determined in a preliminary study)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ishidate Jr M, Sofuni T (1985) Ashby, J., Paton, D. (1985a)

Mammalian Chromosome Aberration Test)			
in vitro mammalian chromosome aberration test (chromosome aberration) primary culture, other: Chinese hamster primary liver cells (CH1-L) Doses: 0, 5, 12.5, 25, 50 µg/ml equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Evaluation of results: positive without metabolic activation Test results: positive (increases in hyperploidy cells (chromosome number > 22) but negative for hypoploidy and polyploidy cells) for primary culture, other: Chinese hamster primary liver cells(all strains/cell types tested); met. act.: without; cytotoxicity: yes (50 µg/ml)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parry JM, Danford N, Parry EM (1984) Ashby, J., Paton, D. (1985c)
in vitro mammalian cell micronucleus test (chromosome aberration) hepatocytes: rat (met. act.: not applicable) Doses: 0.039, 0.39, 3.9, 39, 390, 3900 mg/l (nominal 1E-07 to 1E-02 M) in vitro mammalian cell micronucleus assay on rat hepatocytes	Evaluation of results: negative Test results: negative for hepatocytes: Isolated rat hepatocyte; met. act.: not applicable; cytotoxicity: no, but tested up to limit concentration	2 (reliable with restrictions) supporting st dy experimental result **T st m terial (EC nam): bis(2-ethylhexyl) phthalate**	Müller-Tegethoff K, Kasper P, Müller L (1995)
sister chromatid exchange assay in mammalian cells (DNA damage and/or repair) Chinese hamster Ovary (CHO) (met. act.: with and without) Doses: 0, 5, 16, 50, 160, 500, 1600, 2000, 3000, 4000, 5000 µg/ml equivalent or simil r to OECD Guideline 479 (Gen tic Toxicology In Vitro Si te Chromatid Exchang Assay in Mammalian C lls)	Evaluation r ults: negative Tes results: negative for Chinese hamster Ovary (CHO); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Gulati DK, Witt K, Anderson B (1989) Gulati DK, Sabharwal PS, Shelby MD (1985) Ashby, J., Paton, D. (1985a)
sist r chromatid exchange assay in mammalian cells (DNA damage and/or repair) Chin se hamster Ovary (CHO) (met. act.: with and without) Doses: 3.9, 19.5, 39, 195, 390, 1170, 2340, 3900 µg/ml equivalent or similar to OECD Guideline 479 (Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells)	Evaluation of results: negative Test results: negative for Chinese hamster Ovary (CHO); met. act.: with and without; cytotoxicity: yes (determined in a preliminary assay)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Douglas GR, Blakey DH, Liu-Lee VW (1985) Ashby, J., Paton, D. (1985a) Douglas GR, Hugenholtz AP, Blakey DH (1986)

sister chromatid exchange assay in mammalian cells (DNA damage and/or repair) mammalian cell line, other: rat liver cells RL4 (met. act.: not applicable) Doses: 0, 125, 250, 500, 1,000 µg/ml equivalent or similar to OECD Guideline 479 (Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells)	Evaluation of results: negative Test results: negative for mammalian cell line, other: rat liver cells RL4; met. act.: not applicable; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Priston RAJ, Dean BJ (1985) Ashby, J., Paton, D. (1985a)
yeast cytogenetic assay (chromosome aberration) yeast, other: S. cerevisiae RS112 (met. act.: with and without) Doses: 0, 3000, 10000, 30000, 100000, 200000 µg/ml equivalent or similar to OECD Guideline 481 (Genetic Toxicology: Saccharomyces cerevisiae, Mitotic Recombination Assay)	Evaluation of results: negative Test results: negative for yeast, other: S. cerevisiae RS112(all strains/cell types tested); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name) bis(2 ethylhexyl) phth late**	Carls N, Schiestl RH (1994)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) hepatocytes: rat (met. act.: not applicable) Doses: 0.19, 0.39, 1.95, 3.9, 1 .5, 39, 195, 390, 1,950, 3,900 µg/ml equivalent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	Evaluation of resu ts: negative Test results neg tive for h patocytes:; met. et.: not applicable; cytotoxicity: no, but tested up to limit concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Probst GS, Hill LE (1985) Ashby, J., Paton, D. (1985a)
DNA adenoviru SA7 transf mation (DNA damage and/or repair) Syrian hamster embryo cells Doses: 0, 0.2, 0.3, 0.6, 1.3, 2.6 mM (78-1016 µg/ml) DNA adenovirus SA7 transformation	Evaluation of results: ambiguous Test results: ambiguous for Syrian hamster embryo cells; met. act.: without	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Hatch GG, Anderson TM (1985) Ashby, J., Paton, D. (1985a)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair)	Evaluation of results: negative Test results:	2 (reliable with restrictions) supporting study	Williams GM, Tong C, Brat SV (1985)

hepatocytes: rat (met. act.: not applicable) Doses: 0.1, 1, 10, 100, 1,000, 10,000 µg/ml equivalent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	negative for hepatocytes:(all strains/cell types tested); cytotoxicity: no, but tested up to limit concentrations	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ashby, J., Paton, D. (1985a)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) mammalian cell line, other: rat hepatocytes (met. act.: without) Doses: 391, 1172, 3907 µg/ml Hepatocyte/DNA single strand break assay	Evaluation of results: negative Test results: negative (for single-strand breaks) for mammalian cell line, other: hepatocytes(all strains/cell types tested); met. act.: without; cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis 2 ethylhexyl) phthalate**	Bradley MO (1985) Ashby, J , P ton, D. (1985a)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) hepatocytes: rat (met. act.: not applicable) Doses: 10e-5 to 10e-2 M equivalent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	Evaluation of results: negative Test results: negative for hepatocy e :; met. act.: not applicable cyto oxicity: yes (0.01 M l/L)	2 (re iable with restri tions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kornbrust DJ, Barfknecht TR, Ingram P (1984a)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/ r repair) hepatocytes: rat and human (met. act.: with ut) Dos s: 0.1, 1, 10 mM (39-3,900 µg/ml) equiv lent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	Evaluation of results: negative Test results: negative for hepatocytes: rat and human(all strains/cell types tested); cytotoxicity: no, but tested up to precipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Butterworth BE, Bermudez E, Smith-Oliver T (1984)
in vitro mammalian cell transformation assay (genome mutation) mammalian cell line, other:	Evaluation of results: negative Test results:	2 (reliable with restrictions) supporting study	Sanchez JH, Abernethy DJ, Boreiko CJ (1987)

C3H/10T1/2 (met. act.: without) Doses: 0.0, 5.0, 10, 50, 75, 100 µM (39µg/ml) in vitro mammalian cell transformation assay in C3H/10T1/2 cells	negative for mammalian cell line, other: C3H/10T1/2; met. act.: with and without; cytotoxicity: yes (>= 75 µM)	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	
in vitro mammalian cell transformation assay (genome mutation) mammalian cell line, other: C3H/10T1/2 (met. act.: with and without) Doses: without metabolic activation: 10, 20, 40 µg/ml with metabolic activation: 250, 500, 1,000 µg/ml in vitro mammalian cell transformation assay on C3H/10Tl Clone 8 cells	Evaluation of results: negative Test results: negative for mammalian cell line, other: C3H/10T1/2; met. act.: with and without; cytotoxicity: yes (determined in a preliminary cytotoxicity assay)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Lawrence N, McGregor DB (1985) Ashby, J Paton, D. (1985)
(genome mutation) mammalian cell line, other: Syrian hamster embryo cells (met. act.: with and without) Doses: 0, 3, 10, 30, 100µM in vitro mammalian cell transformation assay in Syrian hamster embryo cells	Evaluation of results: ambiguous without metabolic activation positive with metab lic a tivation Test results: ambiguous for ma m lian cell line, oth : Syrian hamster embryo cells met act.: without; cytotoxicity: no positive fo mammalian cell line, other Syrian hamster embryo cells met. act.: with; ytotoxicity: no	2 (re iable with restri tions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tsutsui T, Watanabe E, Barrett JC (1993)
in vitro mammalian cell transformation assay (genome mutation) mammalian c ll line, other: Syrian hamster embryo cells (m t. act without) Doses: 0.8, 4, 20, 100, 133, 300 µg/ml Cell ransformation assay in Syrian hamster embryo cells	Evaluation of results: positive Test results: positive for mammalian cell line, other: Syrian hamster embryo cells; met. act.: without; cytotoxicity: yes (>= 100 µg/ml)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Sanner T, Rivedal E (1985) Ashby, J., Paton, D. (1985a)
in vitro mammalian cell transformation assay (genome mutation) mammalian cell line, other: Syrian hamster embryo cells (met. act.: without)	Evaluation of results: positive Test results: positive for mammalian cell line, other: Syrian hamster embryo cells; met. act.: without;	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-**	Jones CA, Huberman E, Callaham MF, Tu A, Halloween W, Pallota S, Sivak (1988)

Doses: 13-4000µg/ml Cell transformation assay in Syrian hamster embryo cells	cytotoxicity: yes (variable according to the laboratories)	**ethylhexyl) phthalate**	
in vitro mammalian cell transformation assay (genome mutation) mammalian cell line, other: Syrian hamster embryo cells (met. act.: without) Doses: 0.01, 0.1, 1.0, 10, 100 µg/ml Cell transformation assay in Syrian hamster embryo cells	Evaluation of results: positive Test results: positive for mammalian cell line, other: Syrian hamster embryo cells; met. act.: without	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Barrett JC, Lamb PW (1985) Ashby, J., Paton, D. (1985a)
in vitro mammalian cell transformation assay (genome mutation) mouse embryo cell Balb/c-3T3 (met. act.: with and without) Doses: Without RLC: 0, 3.91, 7.81, 15.6, 31.3 and 62.5 nl/ml With RLC: 0, 10, 25 and 50 µl/ml	Evaluation of results: negative Test results: negative for mouse embryo cell Balb/c-3T3; met. act.: with and without; cytotoxicity: yes (>= 16.0 nl/ml without RLC, >=20.0 µl/ml with RLC)	2 (reliable with restrictions) supporting st dy experimental result **T st m terial (EC nam): bis(2-ethylhexyl) phthalate**	Mat hews EJ, DelBalzo T, Rundell JO (1985) Ashby, J., Paton, D. (1985a)
(genome mutation) primary culture, other: Syrian hamster embryos (SHE) (met. act.: without) Doses: 0-77 µM in vitro mammalian cell transformation assay in SHE c lls	Evaluation of results: positive without met bolic activation Test results: posi ive for pr mary culture, other: Syrian hamster embryos (SHE) cells; met. act.: without; cytot xicity: no, but tested up to pr cipitating concentrations	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Mikalsen SO, Holen I, Sanner T (1990)

5.7.1.2. In vivo data

The results of exp rimental studies are summarised in the following table:

Table 41 O erview of experimental in vivo genotoxicity studies

Meth d	**Results**	**Remarks**	**Reference**
transgenic animal mutagenicity assay (gene mutation) rat (Sprague-Dawley gpt delta rats carrying about ten tandem copies of the transgene lambda EG10 per haploid genome) female oral: feed	Evaluation of results: negative Test results: Genotoxicity: negative (female); toxicity: yes (increased liver weight)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kanki K, Nishikawa A, Masumura KI, Umemura T et al. (2005)

12000 ppm (nominal in diet) 187 mg/rat/day (actual ingested) gpt delta transgenic rats mutation assay			
transgenic animal mutagenicity assay (gene mutation) mouse (lacZ-plasmid transgenic) male/female oral: gavage 2333 mg/kg (actual ingested) lacZ plasmid-based transgenic mouse mutation assay	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: no effects (increase in the mutant frequency in liver, but not in kidney or spleen)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Boerrigter METI (2004)
chromosome aberration assay (chromosome aberration) rat (Fischer 344) male oral: gavage 0.5, 1.7, 5.0 ml/kg (nominal conc.) equivalent or similar to OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects	2 (reliable with restrictions) **key study** experimental result **T st m terial (EC nam): bis(2-ethylhexyl) phthalate**	Putman DL, Moore WA, Schechtman LM (1983)
micronucleus assay (chromosome aberration) mouse (B6C3F1) male intraperitoneal 600, 3000, 6000 mg/kg Micronucleus assay on periphe al blood erythrocytes	Evaluation of results: negative Test results: Genotoxicity negative (male); toxi ity: yes (decreased body weight gain at 6000 mg/kg)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Douglas GR, Hugenholtz AP, Blakey DH (1986)
micronucleus assay (chromo ome aberration) rat (Fisher F344 o SD) male oral: gavage 0, 1 00 2000 mg/kg (nominal conc.) Peripheral blood micronucleus assay in young rats	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects (at the highest dose tested (2000mg/kg))	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Suzuki H, Ikeda N, Kobayashi K, Terashima Y et al. (2005)
micronucleus assay (chromosome aberration) mouse (CD-1) male intraperitoneal 0, 500, 1000, 2000 mg/kg (nominal conc.)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: yes (mortality (1/6) at 2000 mg/kg)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl)**	Morita T, Asano N, Awogi T (1997) Morita T (1997)

OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)		**phthalate**	
micronucleus assay (chromosome aberration) rat (Fisher F344 or SD) male oral: gavage 0, 1000, 2000 mg/kg (nominal conc.) Micronucleus assay in rat hepatocytes	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects (at the highest dose tested (2000mg/kg))	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Suzuki H, Ikeda N, Kobayashi K, Terashima Y et al. (2005)
Drosophila SLRL test (chromosome aberration) Drosophila melanogaster (Canton-S) male injection 0, 20 ppm (nominal in diet) equivalent or similar to OECD Guideline 477 (Genetic Toxicology: Sex-linked Recessive Lethal Test in Drosophila melanogaster)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: no effects	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylh xyl) phthalate**	Yoon JS Mason JM, Valencia R (1985)
Drosophila SLRL test (chromosome aberration) Drosophila melanogaster (Canton-S) male oral: feed 0, 18600ppm (nominal in diet) equivalent or similar to OECD Guideline 477 (Genetic Toxicology: Sex-linked Recessive Lethal Test in Drosophila melanogaster)	Evaluation of results: negative Test results Genotoxici y: n gative (ma e/female toxicity: yes (19% mor ality)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Zimmering S, Mason JM, Valencia R (1989)
unscheduled DNA ynthesis (DNA d mage and/or repair) rat Fischer 344) male/female oral: g vage 150, 500 mg/kg (nominal conc. (by gavage)) 12000 ppm (nominal in diet) equivalent or similar to OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo)	Evaluation of results: negative Test results: Genotoxicity: negative (male/female); toxicity: yes (at 1000 mg/kg the hepatocytes exhibited altered morphology and were badly clumped togethe)	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Butterworth BE, Bermudez E, Smith-Oliver T, Earle L (1984)
DNA synthesis stimulation (DNA damage and/or repair)	Evaluation of results: negative Test results:	2 (reliable with restrictions)	Hagiwara A, Tamano S, Ogiso

rat (Fischer 344) male oral: feed 1.2% (nominal in diet (equivalent to 600mg/kg/d)) DNA synthesis stimulation in urinary bladder epithelium	Genotoxicity: negative (urinary bladder epithelium labelling index unaffected) (male); toxicity: yes (decrease in body weight)	supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	T, Asakawa E, Fukushima S (1990)
in vivo- in vitro replicative DNA synthesis (DNA damage and/or repair) rat (Fischer 344) male oral: gavage 0, 1000, 2000 mg/kg (nominal conc.) in vivo- in vitro replicative DNA synthesis synthesis in hepatocytes was measured at 24, 48 and 72 hours after administration	Evaluation of results: positive Test results: Genotoxicity: positive (male)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Uno Y, Takasawa H, Miyagawa M (1994)
unscheduled DNA synthesis (DNA damage and/or repair) rat (Sprague-Dawley) male by gavage and in diet 5000 mg/kg (nominal conc. (by gavage)) 1000 ppm (2%) (nominal in diet (followed by 5000mg/kg by gavage)) equivalent or similar to OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver C lls in vivo)	Evaluation of results: negative Test results: Genotoxicity: negat ve (m le); toxicity: yes (peroxis me proliferation)	2 (rel able with restr ctions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kornbrust DJ, Barfknecht TR, Ingram P (1984b)
single strand break evaluati n (DNA damage and/or repair) rat (Fischer 344) mal oral feed 2% (900mg/kg) (nominal in diet)	Evaluation of results: positive Test results: Genotoxicity: positive (5-fold increase in single strand breaks in tumour-bearing rats; no increase in other rats) (male); toxicity: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tamura H, Iida T, Watanabe T, Suga T (1991)
unscheduled DNA synthesis (DNA damage and/or repair) mouse (B6C3F1) male by gavage and in diet 0, 10, 100, 500 mg/kg (nominal conc. (by gavage)) 6000 ppm (nominal in diet)	Evaluation of results: negative Test results: Genotoxicity: negative (male); toxicity: yes (hepatomegaly on day 28 of the feeding study)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl)**	Smith-Oliver T, Butterworth BE (1987)

equivalent or similar to OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo)		**phthalate**	
DNA damage evaluation in liver and kidney after long-term administration (DNA damage and/or repair) rat (Fischer 344) male oral: feed 0 and 1.2% (nominal in diet) 8-hydroxydeoxyguanosine (8-OH-dG) formation in liver and kidney DNA of rats	Evaluation of results: positive Test results: Genotoxicity: positive (male); toxicity: yes (increased liver weight)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Takagi A, Sai K, Umenura T (1990)

5.7.2. Human information

No data available.

5.7.3. Summary and discussion of mutagenicity

Discussion

In vitro studies

Gene mutation

Bacteria

DEHP and MEHP have been tested for mutageni potential in a number of Ames-type assays. Unfortunately, several of these studies have been marred by in deq ate reporting, for instance the omission of details of the types of tester strains used and phthalat concentr tions; many have only been published in abstractform.

In studies comparable to a guideline study DEHP was tested in *S. Typhimurium* (TA98, TA100, TA1535 and TA1537 or TA97, TA98, TA100 and TA1535 or TA97, TA98, TA100, TA102) with quantities of 100-10,000 µg DEHP/plate (purity > 99%), wi h and wi hout metabolic activation with S9-mix from rat and/or hamster (Zeiger et al., 1982, 1985a, 1985b; Baker and B nin, 1985). DEHP exhibited no mutagenicity in any of the tester strains neither with nor without metabolic activation.

In a study compara le to a gu deline study, DEHP was tested in *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) a concentrations from 0.1-10 µl DEHP/plate (purity: 99.9%) (98-9,800 µg/plate) with and without met b lic activation with Aroclor-induced rat liver S9 (Kirby et al., 1983). No significant mutagenic activity was observed

In a tudy omp rable to a guideline study, DEHP was tested in*S. typhimurium*(TA97, TA98, TA100, TA102) with quantities of 100-5,000 µg DEHP/plate (purity > 99%), with and without metabolic activation with S9-mix from r and hamster (Matsushima et al., 1985). DEHP exhibited no mutagenicity in any of the tester strains neither w th nor without metabolic activation.

In a study comparable to a guideline study, DEHP was tested for the induction of bacterial mutation using five hi idine auxotrophs of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) according to the method of Ames (Rexroat and probst, 1985). The test was conducted at concentrations of 50-5000 µg/plate with and without metabolic activation using an S9 fraction prepared from the livers of Aroclor-1254-induced rats. N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2NF) and 9-aminoacridine (9AmAc) served as the positive controls for the non-activated test, while 2-aminoanthracene (2AA) served as the positive control for the activated test. Either with or without metabolic activation no revertants were induced by treatment with DEHP.

DEHP and MEHP were tested in *Salmonella typhimurium* (TA98, TA100) and*Escherichia coli*trp- (*uvr+, uvr-*) at concentrations from 50 to 2,000 µg/plate with and without metabolic activation with rat S9-mix (Yoshikawa et

al., 1983). DEHP and MEHP did not show any mutagenic activity but some lethality towards the *S. typhimurium* strains.

DEHP (0.5-1000 µg/ml) was tested in the Ames test on *Salmonella typhimurium* TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, and a mix of 7000-strains, TA1537, TA98 with metabolic activation (Gee et al., 1998). Each of the *Salmonella typhimurium* strains TA7001-7006 carry a target missense mutation in biosynthetic operon that reverts to phototrophy by base-substitution events unique to each strain. TA7001-3 detect point substitutions at A: T and TA7004-6 at G: C. TA98 and TA1537 detect frameshift mutations. DEHP exhibited no mutagenicity in any of the tester strains with metabolic activation.

The potential of Diethylhexylphthalate DEHP to induce reverse mutation in *Salmonella typhimurium* was evaluated in strain TA 102 (Jung et al., 1992). DEHP was tested in two independent experiments in three different laboratories, with and without a metabolic activation system. Bacteria cultures were exposed DEHP t five dose-levels (three plates/dose-level) up to 5000µg/plate in DMSO or ethanol. After 48 to 72 hours of incubation at 37°C, the revertant colonies were scored. DEHP did not induce any noteworthy increase in the number of revertants, both with and without S9 mix. DEHP did not show any mutagenic activity in the acterial reverse mutation test with *Salmonella typhimurium*.

In a study comparable to a guideline study, MEHP was tested in *S. typhimurium* (TA 1535, TA 1537, TA1538, TA 98 and TA 100) with quantities of 250-2,000 µg MEHP/plate (purity > 99%), with nd witho t metabolic activation with S9-mix from rat (Ruddick et al., 1981). MEHP exhibited no mutagenicity i any of the tester strains neither with nor without metabolic activation.

DEHP was tested at concentrations of 0, 313, 625, 1250, 2500, 5000 µg/plate on *Salmonella typhimurium* strains: TA102 and TA2638 and *Escherichia Coli* WP2 uvrA/pKM101 and WP2/pKM101 with and without metabolic activation (Watanabe et al., 1998). DEHP exhibited no mutag nicity in any of the tester strains neither with nor without metabolic activation.

DEHP (purity: > 99%) was tested in *Salmonella typhimurium* strain TA102 a strain sensitive to mutations arising as a cause of oxidative DNA damage, in concentrations fr m 1. o 20.0 µmol/plate (391-7,812 µg/plate) in the presence of enzymes proposed to be responsible for the metab lic activation of DEHP (Schmezer et al., 1988). No mutagenic response was observed. Similarly, MEHP (purity not stated) was not mutagenic in *S. typhimurium* (TA100, TA102) when tested in concentr tions from 0.16 to 1.25 µmol/plate (45-348 µg/plate) with and without metabolic activation with S9 (rat)

The mutagenicity of urine from rats treated ith DEHP was examined (DiVincenzo et al., 1985). Male Sprague-Dawley rats were administered a daily dose of 2 000 mg/kg of DEHP in corn oil by gavage for 15 days. Urine was collected and pooled and tested un iluted by a direct plating procedure (0.02, 0.06, 0.20, 0.66, 2.0 ml urine per plate) in *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) with and without metabolic activation with rat S9-mix. There was no vidence that mutagenic substances were excreted in the urine following administration of DEHP.

Yeast

DEHP (purity: not tated) was tested in *Saccharomyces cerevisiae* for point mutations in strains XV185-14C and RM52 using two different cel culture conditions at concentrations from 1,541 to 12,325 nl/ml (1,510-12,080 µg/ml) with and without metabolic activation with rat S9 (Mehta and van Borstel, 1985). DEHP induced point mutations his+with S9 in strain XV185-14C cultured in buffer pH 7.0 being significant only in the lowest and the highest concentration.

Wh n cult red in YEPD medium pH 6.3, DEHP induced point mutations his+in strain RM52 (-S9), arg+/ is+(+/-S9), trp+(+S9) in strain XV185-14C. Mutation frequencies were 2-fold increased but not dose-dependent.

DEHP was tested in the *ade* forward-mutation system in *Schizosaccharomyces pombe* P1 in concentrations from 369 to 5,870 µg/ml with and without metabolic activation with S9 (rat) (Loprieno et al., 1985). According to the authors, 3-fold increases in mutant frequency were obtained over 3 consecutive doses in the assay without metabolic activation, but in a second experiment this finding was not confirmed. The results were therefore regarded as equivocal.

DEHP was tested in a gene mutation test in the yeast *Sccharomyces cerevisiae* at doses of 0, 40, 200, 1000, 5000 µg/ml (Arni, 1985). DEHP induced mitotic gene conversion with and without activation at the concentration of 5000 µg/ml.

DEHP was tested for mitotic gene conversion, point mutation and mitotic segregation using the yeast *Saccharomyces cerevisiae* strain D7 at doses of 200, 500, 1000, 2000, 3000, 5000 µg/ml (Parry and Eckardt,

1985). No genotoxic effect has been observed with and without metabolic activation.

Mammalian cells

DEHP was tested in an *in vitro* gene mutation assay using mammalian cells cultures both in the absence and presence of metabolic activation (S9 mix), according to a protocol similar to the OECD n° 476 Guideline (Kirby et al., 1983). Mouse lymphoma L5178Y cells cultured in vitro were exposed to DEHP (99.9 %) at concentrations from 0.016 to 0.21 µg/ml (non-activated cultures) and from 0.089 to 1.2 µg/ml (activated cultures), in ethanol. Appropriate positive controls were used. After a 48 rest period, cells were then incubated mutagenicity evaluation with trifluorothymidine during 10-12 days. None of the cultures treated with DEHP at any dose-level, exhibited mutation frequencies that were significantly greater (two-fold greater than background) than that of the corresponding ethanol solvent control. The positive control chemicals, on the other hand, demonstrated significant increases in mutation frequencies for both S9 activated and non-activated cultures. DEHP was not mutagenic in the L5178Y TK+/- mammalian mutagenicity assay.

DEHP was tested for the ability to induce mammalian cell mutation in the L5178Y TK+/- mous lymphoma cell forward-mutation assay with and without metabolic activation with rat S9 at concentrations f om 10 to 400 µg/ml (-S9) or 1.0-80 µg/ml (+S9) (Oberly et al., 1985). A dose dependent decrease in valu s for total survival was obtained in the absence of S9 ranging from 5 to 73% and a 2-fold or greater increase in m tation frequency was seen at 2 dose levels, however, survival was only 12 and 5% at these dose levels. In the presence of S9, a dose-related decrease in total survival was observed ranging from 5 to 90% and a 2-fold or greater increase in mutant frequency was seen at three dose levels. According to the authors, DEHP was mutag nic in the assay with metabolic activation.

DEHP was tested in an *in vitro* gene mutation assay using mammalian cell cultures both in the absence and presence of metabolic activation (S9 mix), according to the a protoc l similar to th OECD n° 476 Guideline (Myhr et al., 1985). Mouse lymphoma L5178Y cellscultured*in vi o*were exposed to DEHP in ethanol for 4 hours at concentrations between 125 and 5000 nl/ml in the presenc and abs nce of metabolic activation. Appropriate positive controls were used and showed a stati tical incre se in mutant colonies. After a 48h rest period, cells were then incubated for 11-12 days for mutagenicity evaluation with trifluorothymidine 3µg/ml. The evaluation of DEHP should be based on soluble concentrations less than 25 nl/ml, and it was assumed from the current data that such treatments would be relativ ly n n-toxic and not mutagenic. Under these experimental conditions, DEHP did not induce any increase in mutan colonies and is not considered as mutagenic.

DEHP was tested for mutagenicity in mouse lymphoma L5178Y and L5178Y clone 372+/+ cells at concentrations of 0, 78, 392, 1,962, 9,810µg/m with and without metabolic activation (Styles et al., 1985). DEHP did not induce any increase in mutant colonies and is not considered as mutagenic.

DEHP was tested for gene mutatio in a human lymphoblasts TK6 and AHH-1 assay at concentrations of 0, 200, 250, 400, 600, 750, 800, 1,000 µg/ml with and without metabolic activation (Crespi et al., 1985). DEHP was found negative for gene-locus mutations

DEHP (purity: 98%) was tested in mouse embryo cells Balb/c-3T3 for gene mutations at concentrations of 0, 79, 250, 791, 2,000, 7,910 nl/ml (77 -7,752 µg/ml) with metabolic activation (Matthews et al., 1985). DEHP induced greater than a 2-fol increase in the appearance of Oua R mutants at one intermediate dose. However, this activity was not stati tically s gnificant; and, therefore, its mutagenic activity in this study was classified as questionable

DNA damages

Mammalian cell

DEHP purity 99%) was tested for the capacity to enhance the adenovirus (SA7) transformation of Syrian hamster embryo cells (SHE) at concentrations from 0, 0.2, 0.3, 0.6, 1.3 and 2.6 mM (78-1,016 µg/ml) (Hatch and Ander on, 1985). No enhancement of virus transformation was observed in a first experiment. A second expe iment detected activity at the two highest concentrations that was independent of cytotoxicity for significance.

The potential of DEHP to induce sister-chromatid exchanges in Chinese hamster ovary cells was evaluated with and without a metabolic activation system according a protocol similar to the OECD 479 (Gulati et al., 1985, 1989). Using the standard protocol, without delay in the cell harvest time, all results were negative. However, in the absence of S9, extending the harvest time to 40 hours to compensate for DEHP-induced cell cycle delay permitted the detection of a significant dose-related increase in SCE. The frequency of SCE/cell increased with increasing concentrations of DEHP over a range of 20-100µg/ml. The magnitude of the increase being between 30% to 40% above the solvent control (or about three induced SCE/cell). Although the effect was small, they were reproducible, dose-related, and statistically significant. The autors considered that the observed effects

were not DEHP dependant.

DEHP was tested for genotoxicity in a sister chromatic exchange assay on Chinese hamster ovarian cells at doses of 3.9, 19.5, 39, 195, 390, 1170, 2340, 3900 µg/ml (Douglas et al., 1985, 1986). No genotoxicity has been observed.

DEHP was tested for genotoxicity in a sister chromatic exchange assay on rat liver cells RL4 at doses of 0, 125, 250, 500, 1,000 µg/ml (Priston and Dean, 1985). No genotoxicity has been observed.

DEHP was tested at concentrations of 391, 1172, 3907 µg/ml for single strand breaks in rat primary hepatocytes (Bradley, 1985). No genotoxic activity was observed.

The ability of DEHP to induce DNA damage or repair was examined in rat and human hepatocytes in vitro (Butterworth et al., 1984). Unscheduled DNA synthesis was measured by incorporation of [^{3}H]thymidine into primary hepatocyte cultures immediately isolated from hepatocyte cultures incubated directly with DEHP. DNA damage was measured by alkaline elution of cellular DNA from the same cultures. In vitro conditions were 0.1 1.0 and 10.0 mM DEHP in the cultures for 18 h. Primary cultures of human hepatocytes were prepared from freshly discarded surgical material and exposed to the same concentration of DEHP. Concentrations up to 0.5 mM mono(2-ethylhexyl) phthalate, a principal metabolite of DEHP, were also examined in the human hepatocyte assay. No chemically induced DNA damage or repair was observed in vitro in rat or human hepatocytes under any of the conditions employed. However, an increase in the percentage of cells in S-phase in the animals given DEHP was observed. These data indicate that DEHP does not inhibit direct genotoxic activity in the animals even with a treatment regimen which eventually produced tumors in a long term bioassay, and that both rat and human hepatocytes are similar in their lack of a genotoxic response to DEHP exposure in culture.

DEHP was tested for induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes at concentrations from 0.01 to 10 mM DEHP (purity: 99.8%) (Kornbrust et al., 198). Incubation of DEHP with rat hepatocytes did not produce any evidence of unscheduled DNA synthesis, as assessed by the autoradiographic determination of [^{3}H]thymidine incorporated during the incubation.

DEHP was tested for the induction of unscheduled DNA synthesis in cultured hepatocytes (Probst and Hill, 1985). Primary cultures of adult rat hepatocytes were incubated for 20 h with 8 concentrations (0.19, 0.39, 1.95, 3.9, 19.5, 39, 195, 390, 1,950, 3,900 µg/ml). N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 2-acetylaminofluorene (2AAF) were also tested and served as the positive controls. Unscheduled DNA synthesis was measured by autoradiography, and the study was replicated using 2 independent hepatocyte mixtures. A concentration-dependent induction of DNA repair synthesis was observed in hepatocytes exposed to the carcinogens MNNG and 2AAF. No induction of DNA-repair synthesis was observed in any of the cultures treated with DEHP.

DEHP was tested for the induction of unscheduled DNA synthesis in cultured hepatocytes (Williams et al., 1985). Primary cultures of adult rat hepatocytes were incubated for 18-20 h with 6 concentrations (0.1, 1, 10, 100, 1,000, 10,000 µg/ml). Benzo(a) pyrene was also tested and served as the positive controls. Unscheduled DNA synthesis was measured by autoradiography. A concentration-dependent induction of DNA-repair synthesis was observed in hepatocytes exposed to benzo(a) pyrene. No induction of DNA-repair synthesis was observed in any of the cultures treated with DEHP.

Chromosomal effects

Yeast

DEHP was tested at doses of 0, 3000, 10000, 30000, 100000, 200000 µg/ml for mitotic recombination in *Saccharomyces cerevisiae* (Carls and Schiestl, 1994). No genotoxic effect has been observed.

Mammalian cells

DEHP was tested in an *in vitro* micronucleus test on Chinese hamster V79 cells in compliance with the Principles of Good Laboratory Practice (von der Hude et al., 2000). Cells were treated with and without metabolic activation at doses of 0.1, 0.25, 1, 2.5, 10, 25, 100, 250, 1000 µg/ml. In the absence of S9 the cells were incubated for 24 h, and for 3 h in the presence of S9. Thereafter cells were washed, fixed and Giemsa stained. Appropriate negative and positive control substances were tested. DEHP was tested in 3 laboratories. The common result was that DEHP did not increase the incidence of micronuclei, neither in the presence nor in the absence of metabolic activation. DEHP was not considered to be genotoxic in the Chinese hamster V79*in-vitro*micronucleus assay.

The potential of DEHP to induce structural chromosome aberrations in Chinese hamster ovary cells was evaluated with and without a metabolic activation system according a protocol similar to the OECD 473 (Gulati et al., 1985, 1989). Cells were treated with 0, 5, 16, 50, 600, 1600, 2000, 3000, 4000, 5000 µg DEHP/ml for

about 10-11 h and colcemid was added 2-3 hr prior to tell harvest by mitotic shake-off. The chromosome number was recorded for each cell and chromosome or chromatid type aberrations were classified into three categories: simple (breaks, fragments, double minutes), complex (interchanges, rearrangements), and other (pulverized, more than ten aberrations/cell). DEHP did not induce any increase in the number of cells with structural chromosome aberration, both with and without S9 mix.

DEHP (purity: not stated) was tested in a primary liver cell line (CH1-L) from Chinese hamster at concentrations up to 50 µl/ml (49 mg/ml) without metabolic activation (Parry et al., 1984). DEHP produced increases in hyperploidy cells (chromosome number > 22) but was negative for hypoploidy and polyploidy cells.

DEHP (purity > 99%) was tested in primary liver cells (CH1-L) from Chinese hamster at concentrations from 5 to 50 mg/ml (Parry, 1985). The mitotic index was reduced at the top dose, the AT/M ratio was reduced in a dose related manner, and the chromosome cluster group and the abnormal division stage was increased by treatmen According to the author, these observations indicate a positive spindle effect. There was no effect on chromosome dislocation, multipolar spindles, or chromosome lagging and bridge formation.

DEHP was tested in a cytogenetic assay in Chinese hamster lung fibroblasts (CHL) (Ishidate and S funi 1985). The cells were treated for 24 and 48 h with 1375, 2750, 4130 µg DEHP/ml with and withou metaboli activation. DEHP did not show any cytotoxicity at even the maximum dose used here and this finding may relate to the insolubility of this chemical. DEHP showed negative results on structural a d numer cal chromosome aberrations.

DEHP was tested in micronucleus assay on primary cultures of rat hepatocyt s (Mü ler-Tegethoff et al., 1995). The cells were treated for 48 h with 0.039, 0.39, 3.9, 39, 390, 3900 µg DEHP/ml DEHP had no influence on the incidence of micronuclei. No cytotoxicity was noted in tested concentration range.

In vivo studies

Gene mutation:

Male and female *lacZ*-plasmid based transgenic mice wer treated at 4 months of age with 6 doses of 2333 mg DEHP per kg over a two-week period (Boerrigter, 2004). Con rol an mals were treated with the vehicle only (35% propyl glycol). The mutant frequency in liver kidn y and spleen DNA was determined as the proportion of retrieved mutant and wild-type *lacZ* plasmids expressed in Escherichia Coli C host cells employing a positive selection system for mutant plasmids. Exposur to DEHP significantly increased the mutant frequency in liver, but not in kidney or spleen, of both female and m le mice. The results indicate that some peroxisome proliferators display an organ-specific mutagenicity in *lacZ* plasmid-based transgenic mice consistent with historical observations of organ- and compound-specific carcinogenicity.

In vivo mutagenicity and mutation spectr of DEHP was investigated in guanine phosphoribosyltransferase (gpt) delta transgenic rats (Kanki et l., 2005) After 13-wk treatment, in the DEHP-treated rats (12000 ppm in diet), marked hepatomegaly with cen rilobular hypertrophy of hepatocytes occurred, although GST-P staining was consistently negative. (MFs) in th liver DNA were 188.0 x 10e-6 and 56.5 x 10e-6, approximately 35-fold and 10-fold higher, respectiv ly, than that of non-treatment control rats (5.5 x 10e-6). There were no increases in mutant frequencies n the DEHP-treated rats as compared to the non-treatment control value. These data provided support fo the conc usion that DEHP exerts its influence via a nongenotoxic promotional pathway.

DNA damag

To evaluate th r lationship between hydrogen peroxide generation and subsequent DNA damage caused by peroxisom pro iferation, DNA damage and changes in peroxisomal ß-oxidation activity in rat liver were examined (Tamura et al., 1991). In male F-344 rats (4 rats/group) fed 2% DEHP in the diet for 52 or 78 weeks, hepato arcinomas or neoplastic nodules were seen in 1/4 or 2/4 rats, respectively. The hepatic DNA from tumour-b aring rats showed a 5-fold increase in single strand DNA breaks whereas no increase was observed in non tumour-bearing rats. According to the authors, the results show that although prolonged treatment with peroxisome proliferators induces markedly peroxisomal ß-oxidation activity, the active oxygen species from peroxisomal ß-oxidation are not enough to give rise to significant DNA damage.

To elucidate the relationship between hepatic peroxisome proliferation and oxidative DNA damage induced by hepatocarcinogenic peroxisome proliferators, DEHP were fed at doses of 1.2% to male F-344 rats for up to 1 year (Takagi et al., 1990). Evidence of hepatic peroxisome proliferation and 8-hydroxydeoxyguanosine (8-OH-dG) formation in liver and kidney DNA were assessed at 1, 2, 3, 6, 9 and 12 months. Peroxisomal (beta-oxidation enzyme activities were increased 3- to 8-fold and catalase was elevated to 1.4- to 2.2-fold the control level by DEHP from months 1 to 12 of the treatment. 8-OH-dG levels in liver DNA of DEHP-fed rats were increased approximately 2-fold after 1 month, the tendency for elevation also being observed in the liner DNA at 2, 3, 9 and 12 months. The results thus clearly demonstrate that persistent peroxisome proliferation in the liver

leads to continued specific oxidative DNA damage.

The ability of DEHP to induce DNA damage or repair was examined in rat hepatocytes in vivo (Butterworth et al., 1984). Unscheduled DNA synthesis was measured by incorporation of [^{3}H]thymidine into primary hepatocyte cultures immediately isolated from treated animals. DNA damage was measured by alkaline elution of cellular DNA from the same cultures. In vivo-in vitro treatment regimens were: (i) female rats, 12 000 p. p. m. DEHP in the diet for 30 days; (ii) female rats, 12 000 p. p. m. in the diet for 30 days, followed by 500 mg/kg DEHP by gavage 2 h before sacrifice; (iii) male rats, 500 mg/kg DEHP by gavage 2, 12, 24, or 48 h before sacrifice; and (iv) male rats, 150 mg/kg/day by gavage for 14 days. No chemically induced DNA damage or repair was observed in vivo in rat hepatocytes under any of the conditions employed. However, an increase in the percentage of cells in S-phase in the animals given DEHP was observed. These data indicate that DEHP does not exhibit, direct genotoxic activity in the animals even with a treatment regimen which eventually produced tumors in a long term bioassay, and that both rat and human hepatocytes are similar in their lack of a genotoxic response to DEHP exposure in culture.

DEHP was tested for mutagenic potential in an UDS assay in vivo. Sprague Dawley rats were tre ted da ly by gavage during 4 or 8 weeks at 5000mg/kg or were fed in diet at 2% during 4 or 8 weeks followed by a single gavage of 5000mg/kg (Kornbrust et al., 1984). In vivo administration of 5 g DEHP/kg b dy weight by gavage did not elicit DNA repair in hepatocytes, isolated 2, 15, or 24 h following the administration of DEHP. When rats were treated with DEHP at a dosed-feed level of 2% for 4 or 8 wk and administered dose of 5 g DEHP/kg body weight by gavage 15 h prior to isolation of hepatocytes, DNA repair was st n t dete ted. DNA repair was not induced in hepatocytes isolated from treated rats.

Genotoxicity as DNA repair or unscheduled DNA synthesis (UDS) and c ll replication as the percentage of cells undergoing scheduled DNA synthesis (SDS or S phase) were determined in ouse hepatocytes in vivo in response to DEHP (Smith-Oliver and Butterworth, 1987). UDS and SDS were determined bv autoradiographic quantitation of [^{3}H]-thymidine incorporation in primary hepatocy e cultures isolated from B6C3F1 male mice treated in vivo. No DNA repair was observed in cultures from mice reated with up to 500 mg/kg DEHP 12, 24 or 48 h previously or from animals treated up to 28 days with 6000 ppm DEHP in the diet. At 24 h following treatment with 500 mg/kg DEHP, 3.1% of the hepatocytes were in S phase compared to control values of 0.27%. Administration of DEHP in the diet at 6000 ppm produc d 9.2% of the cells in S phase at day 7 with the value returning to control levels by day 14. On day 28 of the feeding study the liver to body weight ratios had almost doubled in the group treated with DEHP comp red to controls. No increase in the liver-specific enzyme alanine aminotransferase was seen in the serum following treatment with 500 mg/kg DEHP, indicating that the hyperplasia was due to mitogenic stimulation r ther than regenerative hyperplasia in response to cytotoxicity.

8-OH-dG levels in liver DNA of DEHP fed rats were increased approximately 2-fold after 1 month, the tendency for elevation also being observed i the l ner DNA at 2, 3, 9 and 12 months. The results thus clearly demonstrate that persistent peroxisome prol feration in the liver leads to continued specific oxidative DNA damage.

Chromosomal effects

Drosophila

DEHP was tested f r mutagenicity in the sex-linked recessive lethal (SLRL) mutation assay after a single injection of 0 or 20 ppm to D osophila melanogaster larvae (Yoon et al., 1985). Adult males emerging from the treatment (1 600 ppm in diet) were mated at approximately 24 hr of age with two successive harems of three to five Basc fem les to tablish two single-day broods. The percentage of lethals was 3% compared to 5% in the control group. No genotoxic effect has been observed.

DEHP was tested for mutagenicity in the sex-linked recessive lethal (SLRL) mutation assay after being fed t *Dros phila melanogaster* larvae (Zimmering et al., 1989). Adult males emerging from the treatment (18600 ppm in di t) were mated at approximately 24 hr of age with two successive harems of three to five Basc females to establish two single-day broods. Males were then discarded, and the conventional SLRL assay carried out. The percentage of lethals was 7% compared to 11% in the control group. No genotoxic effect has been observed.

Mammals

In a study performed according to GLP principles, DEHP, MEHP and 2-EH were tested for their ability to induce chromosomal damage in male Fischer rats after oral administration (Putman et al., 1983). Five rats per group were given by gavage in corn oil 0.5, 1.7, 5.0 ml/kg/day of DEHP (purity: 99.9%), 0.01, 0.05, 0.14 ml/kg/day of MEHP (purity: 94.7%), or 0.02, 0.07, 0.21 ml/kg/day of 2-EH (purity: 99.7%) for 5 consecutive days. A positive control group was included. No significant increase in chromatid and chromosome breaks or structural rearrangements were noted and the mitotic index was also unaffected.

DEHP was tested in a Mammalian Erythrocyte Micronucleus Test, according to the OECD n° 474 Guideline and

in compliance with the Principles of Good Laboratory Practice (Morita et al., 1997). Groups of 6 males CD-1 mouse were treated twice intraperitoneally with DEHP (purity >98%) at doses of 0, 500, 1000, 2000 mg/kg. One group of 6 males received the vehicle under the same experimental conditions, and acted as control group. One group of 6 males received the positive control substance (mitomycin C 0.5 mg/kg) once by intraperitoneal route. For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 1000 polychromatic erythrocytes. Mitomycin C induced a highly significant increase in the frequency of MPE, indicating the sensitivity of the test system under our experimental conditions. At the high dose (2000mg/kg), the mortality was 1/6. DEHP did not induce any noteworthy increase in the number of micronucleated with structural chromosome aberration, both with and without S9 mix, in any experiment or at any harvest time. In conclusion, DEHP did not induce an increase in micronucleus mouse bone marrow when tested at concentrations up to 2000 mg/kg.

DEHP was tested for mutagenicity in an*in vivo*micronucleus test on mouse. Groups of 15 mice were injected intraperitoneously DEHP at concentrations of 600, 3000 and 6000 mg/kg and peripherical blood was xam ed for micronucleus immediately after the last injection, two and four we'eks after the last injection (Doug a et al., 1986). The positive control showed a significantly elevated number of micronuclei on the day following the last treatment but levels returned to control values by the second week. No increase in micronu lei was de ected at any of the doses or times following DEHP exposure, nor with the solvent or untouched control

Simultaneous liver and peripheral blood micronucleus assays in young rats was performed with DEHP. DEHP was administered by gavage at dose levels of 0, 1000 and 2000 mg/kg bw to gro s f 4 rat (Suzuki et al., 2005). Positive control animals received DEN at 40 mg/kg (liver micronucleus assay) or CP at 10 mg/kg (peripheral blood micronucleus assay). DEHP was negative for micronucl us induction in both liver and peripheral blood.

Other *in vitro* and *in vivo* studies related to mutagenicity and/or carcinogenicity

DEHP (purity not stated) was tested in*Saccharomyces cerevisiae*fo gene conversion in strain D7-144 using two different cell culture conditions at concentrations from 1,541 to 12,325 nl/ml (1,510-12,080 µg/ml) with and without metabolic activation with rat S9 (Mehta and van Borstel, 1985). When cultured in YEPD medium pH 6.3, DEHP induced gene conversion with and without metab lic activation but not when cultured in buffer pH 7.0. According to the authors, overall test results wer pos ive.

DEHP was examined for activity in the C3H/10T½ murine fibroblast cell transformation system in concentrations from 0 to 100 µM DEHP (purity: 99.8%) (0 39 µg/ml) (Sanchez et al., 1987). DEHP did not produce cell transformation, initiate tran forma ion in cultures treated with a tumour promoter or promote transformation in cultures pre-treated with a chemical carcinogen.

DEHP was evaluated in the Syrian h mst r embryo (SHE) cell transformation assay in three different laboratories using the same bas c experimental protocol with minor modifications at concentrations from 13 to 4,000 µg/ml (Jones et al., 1988 In one l boratory, DEHP induced a high level of transformation in two assays but gave only one transformed colony at a single dose (> 1,000 µg/ml) in a third assay. In a second laboratory, transformation was observed generally at concentrations above 1,000 µg/ml. In a third laboratory, a low number of transformed colonies were observed in two assays. It was concluded by the authors that DEHP was positive for induction of morphologic l transformation in this assay.

DEHP (purit 99%) induced morphological cell transformations when evaluated in the Syrian hamster embryo (SHE) cell transform tion assay at concentrations from 0.8 to 300 µg/ml (Sanner and Rivedal, 1985).

The following information is taken into account for any hazard / risk assessment:

The pos ible genotoxic effect of DEHP has been thoroughly investigated in several different short-term tests. Most of the studies are performed according to GLP principles and are comparable to guideline studies.

The re ults have been negative in the majority of the in vitro and in vivo studies on DEHP for detection of gene muta ion, DNA damage, and chromosomal effects. The more conclusive positive results were obtained on cell transformation, induction of aneuploidy and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic substances such as tumour promoters and/or peroxisome proliferators. Taken together all the results, both negative and positive, DEHP is considered to be non-genotoxic

Value used for CSA: Genetic toxicity: negative

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classification is warranted for germ cell mutagen.

5.8. Carcinogenicity

5.8.1. Non-human information

5.8.1.1. Carcinogenicity: oral

The results of experimental studies are summarised in the following table:

Table 42 Overview of experimental studies on carcinogenicity after oral administration

Method	Results	Remarks	Reference
Carcinogenicity studies			
rat (Fischer 344) male/female oral: feed 100, 500, 2500, 12500 ppm (nominal in diet) 5.8, 28.9, 146.6, 780 mg/kg/d (actual ingested (males)) 7.3, 36.1, 181.7, 938.5 mg/kg/d (actual ingested (females)) Exposure: 104 wk A set of 10 animals per sex from Groups 1, 4, and 5 were sacrificed after week 78 for histopathology. (ad libitum) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (carcinogenicity): 500 ppm (nominal) (male/female) (= 28.9 mg/kg bw/d for males and 36.1 mg/kg bw/d for females ↑ incidence of hepatocellular adenoma and carcinomas from 2500 ppm ↑ incidence of mononuc ar cell leukaemia (males)) Neoplastic effects: yes	1 (reliable without restriction) **key study** exp rimental re ult **Test material (EC name): bis(2-ethylhexyl) phthalate**	D vid RM, Moore MR, Finney DC and Guest D (2000a) David RM, Moore MR, Cifone MA, Finney DC and Guest D (1999)
rat (Fischer 344) male/female oral: feed 0, 6000, 12000 ppm (nominal in diet) 322, 674 mg kg bw/d (actual ingested (mal s)) 394, 774 mg/kg bw/d (actual ingested (females)) Exposur : 104-105 Weeks of xposure (ad libitum) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	LOAEL (carcinogenicity): 6000 ppm (nominal) (male/female) (No NOAEL determined. ↑ incidence of hepatocellular carcinomas and neoplastic nodules ↓ incidence of pituitary, thyroid C-cell, and testicular interstitial cell tumours in males) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982c) Kluwe WM, Haseman JK, Douglas JF (1982c) Kluwe WM (1983b)
rat (Fischer 344) male oral: feed 2% (nominal in diet (equivalent to 1000 mg/kg/d))	conc. level: (carcinogenicity): 20000 mg/kg diet (male) (liver tumours in 6/10 dosed rats and in 0/8 controls) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result	Rao MS, Usuda N, Subbarao V, Reddy JK (1987)

Exposure: 95weeks (ad libitum) Male F-344 rats were fed a diet containing 2% di-(2-ethylhexyl)phthalate (DEHP) for 95 weeks		**Test material (EC name): bis(2-ethylhexyl) phthalate**	
rat (Fischer 344) male oral: feed 20000 ppm (nominal in diet) ca. 1000 mg/kg bw/d (actual ingested) Exposure: 95 weeks (ad libitum) Male F344 rats (14 treated, 10 controls) were treated with DEHP in diet during 108 weeks	conc. level: (carcinogenicity): 20000 ppm (nominal) (male) (hepatocellular carcinomas and neoplastic nodules in 11/14 dosed rats and in 1/10 controls) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Rao MS, Yeldandi AV, Subbarao V (1990)
rat (Sprague-Dawley) male oral: feed 0, 600, 1897, 6000 ppm (nominal in diet) 0, 30, 95, 300 mg/kg (actual ingested) Exposure: life-time (ad libitum) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	NOAEL (carcinogenicity): 95 mg/kg bw/day (actual dose received) (male) (increase incidence of neoplasias at 300 mg/kg) Neoplastic effects: yes	2 (reliable with restrictions) supporting study exp rim n al result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Voss C, Zerban H, Bannasch P and Berger MR (2005) Berger MR (1995)
mouse (B6C3F1) male/female oral: feed 100, 500, 1500, 6000 ppm (nominal in diet (equivalent to (males: 19.2, 98.5, 292.2, and 1266.1 mg/kg/d females: 23.8, 116.8 354.2, and 1458.2 mg/kg/d))) 19.2, 98.5, 292.2, and 1266.1 mg/kg/d (ac u l ingested (males)) 23.8, 116.8, 354 2, and 1458.2 mg/kg/d (ctua ingested (females)) Exp sure: 104 wk A set of 0–15 animals per group was terminated during Week 79. (7 days wk) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (carc nogenicity): 00 ppm (ominal) (male/female) (= 98.5 mg/kg bw/d for males and 116.8 m /kg bw/d for females ↑ incidence of hepatocellular adenoma and carcinomas) Neoplastic effects: yes	1 (reliable without restriction) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	David RM, Moore MR, Finney DC and Guest D (2000b) David RM, Moore MR, Cifone MA, Finney DC and Guest D (1999)
mouse (B6C3F1) male/female oral: feed 0, 3000, 6000 ppm (nominal in diet)	LOAEL (carcinogenicity): 3000 ppm (nominal) (male/female) (No NOAEL identified.	2 (reliable with restrictions) supporting study experimental result	NTP (1982c) Kluwe WM, Haseman JK, Douglas JF

672 and 1,325 mg/kg bw/day (actual ingested (males)) 799 and 1,821 mg/kg bw/day (actual ingested (females)) Exposure: 104-105 Weeks of exposure (ad libitum) equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	↑ incidence of hepatocellular carcinomas) Neoplastic effects: yes	**Test material (EC name): bis(2-ethylhexyl) phthalate**	(1982b) Kluwe WM (1983b)
Initiation/promotion studies			
rat (Fischer 344) male oral: feed 12000 ppm (nominal in diet (equivalent to 550mg/kg/d)) Exposure: 24W (ad libitum) Initiating and promoting test, 24 weeks exposure to DEHP in the diet, Phenobarbital (PB) was used as positive promoting chemical and N-2-fluorenylacetamide (FFA) as positive initiating chemical.	conc. level: (Initiating and promoting test): 12000 ppm (nominal) (male) (No initiating, promoting, or sequential syncarcinogenic effect was reported) no NOAEL identified : Neoplastic effects: no effects	2 (reliable with restrictions) supporting study experimental resul **Test material (EC name): bis(2-ethylhexyl) phthalate**	Williams GM, Maruy ma H, Tanaka T (1987)
rat (Sprague-Dawley) female oral: gavage 10, 100, 200, 500mg/kg (actual ingested) Exposure: 11 Weeks (3 times per week) Promoting test, DEHP by gavage in olive oil, 3 times a week, for 11 weeks, di-ethylnitrosamine (DEN) was used for initiation	NOAEL (promo ing a tivity): 100 mg/kg bw/day actual dose received) (femal) (A weak pr mo ing effect was noted (a 2-fold ↑ in the number a d ar a of ATPase-eficient f ci) Ne plastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Oesterle D, Deml E (1988)
rat (Fischer 344) female oral: feed 12000 ppm (analytical conc.) ca 600 mg/kg bw (actual ingested) Expo re: 3 or 6 months (ad libitum) Pr moting test, DEHP in diet for 3 or 6 months, di-ethylnitrosamine (DEN) was used for initiation and phenobarbital (PB) as positive promoting control	conc. level: (Promoting effect): 12000 ppm (analytical) (female) (No promoting effect was reported) Neoplastic effects: no effects	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Popp, J.A., Garvey, L.H., Hamm,Jr., T.E. (1985)
rat (Fischer 344) male oral: feed 12000 ppm (nominal in diet) ca. 600mg/kg/d (actual ingested)	conc. level: (Promoting activity in kidney): 12000 ppm (nominal) (male) (Positive promoting activity (↑ in incidence of renal cell adenomas and	2 (reliable with restrictions) supporting study experimental result	Kurokawa Y, Takamura N, Matushima Y (1988)

Exposure: 24weeks (ad libitum) Promoting test, DEHP in diet for 24 weeks, N-ethyl-N-hydroxyethylnitrosamine (EHEN) was used for initiation	adenocarcinomas and the number of tumours per kidney, in rats given DEHP after N-ethyl-N-hydroxyethylnitrosamine (EHEN)) Neoplastic effects: yes	**Test material (EC name): bis(2-ethylhexyl) phthalate**	
rat (Sprague-Dawley) male/female oral: gavage 50, 200, 500, 1000, 2000 mg/kg (actual ingested) Exposure: 7-11Weeks (3 times a week) Promoting test, DEHP by gavage in olive oil, 3 times a week, for 7-11 weeks; diethylnitrosamine (DEN) was used for initiation	(Promoting activity in liver): (male/female) (A weak promoting effect was noted in some treated groups (increase in the number and area of ATPase deficient foci and GGTase-positive foci in the lower dose groups but were decreased in the higher dose groups)) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Gerbracht U, Einig C, Oesterle D Deml E, Schlatterer B and Eigenbrodt E (1990)
rat (Fischer 344) male oral: feed 0.3, 0.6, 1.2% (nominal in diet) ca. 250, 300, 600 mg/kg bw (actual ingested) Exposure: During experimental weeks 5-8 and weeks 12-20 (ad libitum) Promoting test (uracil-accelerated transitional cell proliferation model), DEHP in the diet during experimental weeks 5-8 and week 12-20, N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) was used for initiation	NOAEL (Promoting activity in urinary bladder): > 12000 mg/kg diet (male) (No promoting activity) Neoplastic effects: no eff cts	2 (eliabl with restri ions) supporting study expe imental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Hagiwara A, Tamano S, Ogiso T (1990)
rat (Fischer 344) female oral: gavage 10000 mg/kg (ac ual ingested) Expo ure Single administration Ini iating test, DEHP at 6, 12, or 24 hours ingle oral dose ; 2-acetylaminofluorene (AAF) was used as positive promoting chemical and diethylnitrosamine (DEN) as positive initiating chemical.	dose level: (initiating test in liver): 10000 mg/kg bw/day (actual dose received) (female) (No tumour initiating activity of DEHP observed. Number and volume of preneoplastic foci was increased in the positive control group)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Garvey LK, Swenberg JA, Hamm Jr TE (1987)
rat (Fischer 344) male oral: feed 3000 ppm (nominal in diet) Exposure: 6 weeks of DEHP treatment (ad libitum)	conc. level: (Promoting activity in liver): 3000 mg/kg diet (No promoting effect was reported)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-**	Ito N, Tsuda H, Tatematsu M (1988)

Promoting test, DEHP in the diet for 6 weeks, diethylnitrosamine (DEN) was used for initiation. All rats were partially hepatectomised to maximise any interaction between proliferation and the effect of the tested compound.		**ethylhexyl) phthalate**	
rat (Fischer 344) female oral: feed 12000 ppm (analytical conc.) ca. 600 mg/kg bw/d (actual ingested) Exposure: 12 weeks (ad libitum) Initiating test, DEHP 12 weeks exposure in the diet; Phenobarbital (PB) was used as promoting chemical and diethyl-nitrosamine (DEN) as positive initiating chemical.	conc. level: (initiating test in liver): 12000 ppm (analytical) (female) (No tumour initiating activity of DEHP observed. Number and volume of preneoplastic foci was increased in the positive control group)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Garvey LK, Swenberg JA Hamm Jr TE (1987)
mouse (C3H) male/female oral: feed 12000 ppm (nominal in diet) ca. 2400 mg/kg bw/d (actual ingested) Exposure: 24weeks (ad libitum) Promoting test, DEHP in diet for 26 weeks, N-nitrosodiethylamine (NDEA) was used for initiation.	conc. level: (Promoting test): 12000 ppm (nominal) (male/female) (Positive promoting activity (↑ in incidence of liver tumours in mice given NDEA and DEHP compared wit mic exposed to NDEA alone) Neoplasti effects: yes	2 (relia le with restr ctions) supp rting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Weghorst CM, Devor DE, Henneman JR (1994)
mouse (B6C3F1) male oral: feed 3000 ppm (nominal in diet (equivalent to 1200mg/kg)) Exposure: 28, 84 and 168 days (ad libitum) Promoting te t, DEHP in diet for 28, 84 or 168 days di-ethylnitrosamine (DEN) was used for initiation.	con level: (promotion activity): 3000 mg/kg diet (male) (Positive promoting activity after only 28 days of exposure (significant and time-dependent ↑ in incidence of focal hepatocellular proliferative lesions; significant ↑ in incidence of liver tumours at 168 days) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ward JM, Ohshima M, Lynch P and Riggs C (1984)
mouse (B6C3F1) male/female oral: feed 6000 ppm (nominal in diet (equivalent to 1200 mg/kg/d)) Exposure: 72weeks (ad libitum) Promoting test, DEHP in diet for up to 78 weeks of age, transplacental initiation: N-nitrosoethylurea (NEU), ip to pregnant C57BL/6NCr mice. DNA synthesis: BrdU, ip, for	conc. level: (Promoting test): 6000 ppm (nominal) (male/female) (Positive promoting activity (↑ in incidence of focal hepatocellular proliferative lesions (FHPL) including hyperplastic foci, hepato-cellular adenomas and carcinomas). Authors Concl.: tumour promotion in liver may be a	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ward JM, Konishi N, Diwan BA (1990)

determination of the DNA synthesis in kidneys tubular cells and in the hepatocytes	consequence of ↑ DNA synthesis in initiated or focus cells rather than in nonproliferative hepatocytes.)		
mouse (B6C3F1) male oral: feed 6000 ppm (nominal in diet (equivalent to 1200mg/kg/d)) Exposure: 29 weeks (ad libitum) Promoting test, DEHP in the diet for 29 weeks, di-ethylnitrosoamine (DEN) was used for initiation	conc. level: (Promoting activity in liver): 6000 mg/kg diet (male) (Positive promoting activity (↑ in incidence of focal hepatocellular proliferative lesions(FHPL) and number of FHPL per area); 4 mice given DEN and DEHP had hepatocellular adenomas) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Hagiwara A, Diwan BA, Ward JM (1986)
mouse (B6C3F1) male/female oral: gavage 25000 or 50000 mg/kg (actual ingested) Exposure: Single administration Initiating test, single dose of DEHP by gavage. Phenobarbital (PB) was used as promoting chemical Promoting test, DEHP in diet for 2, 4, or 6 months, di-ethylnitrosoamine (DEN) was used for initiation	dose level: (Initiation): 50000 mg/kg bw/day (actual dose received) (male/female) (No initiating activity was reported.) Neoplastic effects: yes	2 (reliable with restrictions) suppor ing study experimental result **Test materi l (EC name): bis(2-ethylhexyl) phthalate**	Ward JM, Rice JM, Creasia D (1983)
mouse (B6C3F1) male/female oral: feed 3000, 6000, 12000 ppm (nominal in diet) ca. 600, 1200 or 2400mg/kg/d (actual ingested) Exposure: 2, 4, 6 months (ad libitum) Initiating te t single dose of DEHP by gavage. Phenoba bital (PB) was used a promo ing chemical Pr moting test, DEHP in diet for 2, 4, or 6 months, di-ethylnitrosoamine (DEN) was used for initiation	LOAEL (P omotion): 3000 ppm (analytic l) male/fem le) (Positive promoting activity (numerous foci and neoplasms were seen in mice given DEHP after DEN)) Neoplastic effects: yes	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ward JM, Rice JM, Creasia D (1983)

5.8.1.2. Carcinogenicity: inhalation

No data available.

5.8.1.3. Carcinogenicity: dermal

No data available.

5.8.1.4. Carcinogenicity: other routes

The results of experimental studies are summarised in the following table:

Table 43 Overview of experimental studies on carcinogenicity (other routes)

Method	Results	Remarks	Reference
mouse (B6C3F1) male/female (intraperitoneal) 5 and 10 µmoles Exposure: Neonates are treated via intraperitoneal injection (IP) with test chemical dissolved in DMSO on Days 8 and 15. One third of the total dose is delivered on day 9 and two thirds are delivered on day 15 (in 10 and 20 ul total volume respectively). Rat neonates are treated via IP injection with DEHP on Days 8 and 15 and sacrificed after 1 year for tumor observation	dose level: (carcinogenicity): 10 µmoles (male/female) (no increase in the incidence of liver, lung or other tumors) Neoplastic effects: no effects	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	McClain RM (2001)
hamster, Syrian (albino) male/female (intraperitoneal) 3000 mg/kg Exposure: life-time (grp1: once weekly grp2: once every 2 weeks grp3: once every 4 weeks) Life-time, ip injections of DEHP every week, every 2 weeks, or every 4 weeks	dose level: (carcinogenicity): 3000 mg/kg bw/day (male/female) (No differences in the tumour incidence in the treated groups compared with the control group) Neoplastic effects no effects	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Schmezer P, Pool BL, Klein RD (1988)

5.8.2. Human information

5.8.2.1. Studies specific for exposure to DEHP

Occupational exposure

Thiess *et al* (1978) evaluated, the mortality of 221 workers in a di(2-ethylhexyl) phthalate production plant in Germany was followed between 1940 and 1976. Most subjects (135/221) were hired after 1965 and the process was completely enclosed in 1966. No information on level of exposure was provided. Information on vital status for foreigners [number not stated] was obtained for only 55% of them, but appeared to be complete for the remaining cohort. Reference rates were obtained from local populations (the city of Ludwigshafen, the Rheinhessen-Pfalz land) and national rates. Altogether, eight deaths occurred during the follow-up period versus 15.9 expected using local rates [standardized mortality ratio, 0.50; 95% confidence interval, 0.22–0.99] and 17.0 expected using national rates. One death from pancreatic cancer (0.13 expected) and one from bladder papilloma (0.01 expected) occurred among workers with a long exposure time (≥ 20 years). No further report on a longer follow-up for this cohort was available to the IARC Working Group (Thiess et al., 1978). [The IARC Working Group (Monograph no. 101) noted that the majority of the cohort members were employed after exposure levels had been considerably reduced, and that the methods for this study were poorly described.]

Case–control study

Lopez-Carrillo *et al.* (2010) conducted a case–control study in northern Mexico to evaluate the association between urinary levels of nine phthalate metabolites and breast cancer. They interviewed 233 women with breast cancer and 221 age-matched controls from 2007 to 2008, and collected sociodemographic and reproductive characteristics and first morning void urine samples before any treatment. Exposure assessment was based on the measurement of biomarkers: no data on personal habits involving exposure to phthalates were available. Phthalate metabolites, detected in at least 82% of all women, were measured in urine samples by isotope dilution/high-performance liquid chromatography coupled to tandem mass spectrometry. After adjusting for risk factors and other phthalates, increased odds ratios for breast cancer were associated with urinary concentrations of four DEHP metabolites: MEHP, MEHHP, MEOHP, and MECPP; however, this increased risk was only statistically significant for MECPP, with a dose–response trend ($P = 0.047$). A nonsignificant negative association was observed for MEOHP. With regard to other phthalate metabolites, urinary concentrations of DEP and monoethyl phthalate (MEP) metabolites were positively associated with breast cancer (odds ratio of highest versus lowest tertile, 2.20; 95%CI: 1.33–3.63; P for trend < 0.01). In contrast, significant neg ti e associations were found for monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP) and mono(3-carboxylpropyl) phthalate (MCPP) metabolites. The odds ratios for the sum of all nine metabolites (incl ding five non-DEHP phthalate metabolites), for the 2nd and 3rd versus the 1st tertile were 0.94 (95%CI: 0 57– 56) and 1.41 (95%CI: 0.86–2.31; P for trend = 0.114). [The IARC Working Group (Monograph 101) considered that this study had an appropriate design, although the timing of exposure assessment was a con ern. Biological samples to measure DEHP metabolites were obtained after diagnosis among cases, befor any treatment; metabolites were measured in the urine, and it is not known whether disease stat uld have affected metabolite levels. A limitation of this study was the lack of consistency in effect between the four DEHP metabolites measured and the lack of a dose–response for all metabolites Further, it is unclear which metabolite is the best biomarker for exposure to DEHP.]

5.8.2.2. Occupational exposure to phthalate plasticizers

Cohort study

Hagmar *et al.* (1990) reported on the mortality of 2031 Swedish w rkers at a PVC-processing factory that produced flooring, film and pipes from PVC. DEHP w s the major p asticizer used in all of these products. A significant excess of total cancer morbidity (standard incid nce ratio, 1.28; 95%CI: 1.01–1.61; 75 cases) and respiratory cancer morbidity (SIR, 2.13; 95%CI: 1.27–3 46; 17 cases) was observed among the PVC-processing workers, but no statistically significant associa on was found with cumulative exposure to plasticizers. [The IARC Working Group noted that only 6% of the cohort was exposed only to plasticizers.]. Respiratory cancer risk was increased in individuals who w re exp sed o both asbestos and plasticizers but not to vinyl chloride (SIR, 10.70; 95%CI: 2.20–31.20; three ases) and in workers not exposed to any of the three agents. [The limitations of this study were the small number o workers and exposed deaths/cases of site-specific cancers, potential confounding by tobac o smoking or other risk factors and crude exposure estimates.]

Case–control studies

Three population-based case–control studies — two in Sweden and one in Denmark — evaluated the association between occupational exposure to PVC plastics or products and testicular cancer. A small Swedish study found an increased risk for testicula cancer among men exposed to PVC (OR, 6.6; 95%CI: 1.4–32; seven exposed cases and tw exposed ontrols) (Hardell *et al.*, 1997). [The IARC Working Group noted that the results were only from liv ng su jects, which could introduce bias if the risk factor were associated with poor prognosis.] In a larger D nish s udy, no increased risk for testicular cancer was observed for men ever exposed to mainly PVC (OR, 0.7; 95%CI: 0.5–1.2) or plastics in general (OR, 1.0; 95%CI: 0.8–1.2) (Hansen, 1999). [However, this study had limited statistical power because of small numbers of exposed cases; the prevalence of exposure to PVC for 1 year or more among controls was only 0.5%.] In the second Swedish study (Hardell *et al.*, 2004; We tberg *et al.*, 2005) of 791 men with germ-cell testicular cancer and 791 matched controls, ever exposure to PVC plastics was associated with an increased risk for testicular cancer (OR, 1.35; 95%CI: 1.06–1.71); a non-significant increased risk was reported for exposure to soft (containing plasticizer) plastics (OR, 1.48; 95%CI: 0.94–2.34; 54 cases and 37 controls) but not to rigid plastics (OR, 1.06; 95%CI: 0.55–2.01; 23 cases and 26 controls). The risk was elevated among workers with a 10-year latency (OR, 1.45; 95%CI: 1.06–1.98). However, odds ratios for exposure decreased with increasing exposure for all four measures of exposure (duration, maximum intensity, median intensity over the subject work history and cumulative median intensity). [The questions on exposure were focused on PVC in general and not on exposure to specific substances, which could decrease the possibility of detecting an effect due to phthalates.]

Selenskas *et al.* (1995) conducted a nested case–control study of pancreatic cancer among a cohort of workers employed at a plastics manufacturing and research and development plant in New Jersey, USA (Dell & Teta,

1995). Individuals with potential exposure to phthalates worked in either the vinyl- and polyethylene-processing department, or the fibres and fabrics department. Vinyl processing involved the compounding and calendering (a fabrication step) of PVC polymers and copolymers that are produced at other loca tions. Potential exposure to DEHP, specifically mentioned as being used in this plant, occurred in the production of flexible plastics. A significantly increased risk for pancreatic cancer was observed only in the vinyl- and polyethylene-processing workers (relative risk, 7.15; 95%CI: 1.28–40.1; five exposed cases who had worked for more than 16 years). No trend of increasing risk with increasing duration or latency was observed. [However, there were only nine cases and 40 controls in the combined production areas, so that the number of cases and controls in each exposure stratum (duration or latency) was small.] Most of the cases (eight of nine) and controls (34/40) in the vinyl- and polyethylene-processing areas worked in the vinyl- and polyethylene-processing sub-de partment in that area. Among these workers, an elevated risk for pancreatic cancer was observed among those exposed for more han 18 years (relative risk, 8.98; 95%CI: 0.90–89.8). All of the cases of pancreatic cancer that occurred in the vinyl- and polyethylene-processing department worked in the building where both vinyl and polyethylene wer processed and none of the cases occurred among workers in the building where only polyethylene w s processed. [The IARC Working Group (Monograph no. 101) noted that the limitations of this study nclude the small numbers of exposed cases, the categorization of exposure that was not specific to DEHP, the ck of quantitative exposure measures and potential confounding from exposure to occupational ag nts, toba co smoking or other risk factors.]

The relationship between multiple myeloma and exposure to phthalates (and other occupa nal agents) was evaluated in a population- based case–control study among Danish men (Heineman *et al.*, 1992). Exposure to phthalates was associated with elevated but non-significant odds ratios f r multiple myeloma, with a higher risk estimate for probable exposure (OR, 2.0; 95% CI: 0.9–4.4; 11 cases and 21 controls) than possible exposure (OR, 1.3; 95% CI: 0.9–2.0; 34 cases and 94 controls). Risk estimates for prob bl exposure increased with increasing duration of exposure when latency was not considered (OR for probable exposure greater than 5 years, 2.5; 95% CI: 0.9–7.0; *P* for trend = 0.02). When time s nce fi st exp sure was lagged for 10 years, risks increased with duration of exposure for possible but not probable exp ure. Exposure to vinyl chloride was also associated with an increased risk for multiple myeloma and the risk estimate increased with increasing duration of exposure. Stratified analysis was conducted to separa e the effects of exposure to phthalates from exposure to vinyl chloride. Increased risk estimates were observed for: xposure to phthalates but never to vinyl chloride (OR for 5 or more years, 2.0; 95% CI: 0.1–27; one case) exposure to vinyl chlo- ride but never to phthalates (OR for 5 or more years, 2.6; 95% CI: 0.3–19 2; two cases); and exposure to both (OR, 5.2; 95% CI: 1.0–29.5; four cases). However, the numbers of exposed cases in each stratum were small. In logistic regression analyses that controlled for exposure to vinyl hloride, engine exhaust and gasoline, risk estimates for exposure to phthalates no longer increa ed with duration of exposure. [If duration of exposure were a poor surrogate for cumulative exposure, this w uld lead to a misclassification of exposure and thus make it hard to detect an exposure–response r lationship.]

5.8.3. Summary and discussion of carcinogenicity

Discussion

Studies in humans

The human dat is inadequate for risk assessment.

Studies in animals

Long-te m carcinogenicity studies in rats and mice have been published. The studies are comparable to guideline studies and performed according to GLP.

Oral

Rat

In a study compliant to OECD guideline 453, F-344 rats (70/sex/group) received DEHP in the diet at doses of 0, 100, 500, 2500, or 12500 ppm (M/F: 0/0, 5.8/7.3, 28.9/36.1, 146.6/181.7, or 789/938.5 mg/kg bw/d) for 104 weeks(David *et al*., 1999, 2000). In an additional recovery group, rats (55/sex/group) were administered 12500 ppm DEHP for 78 weeks, followed by a 26-week recovery period. Increases in hepatocellular adenomas and mononuclear cell leukaemia (MCL) in males at 2500 ppm and above and hepatocellular carcinomas in males and females at 12500 ppm, were observed. However, the incidence of hepatocellular adenomas/carcinomas was decreased in recovery animals at 12500 ppm (2-week recovery period), compared with the same dose group at

the end of the dosing period. Peroxisome proliferation was induced from 2500 ppm. Effects on the liver, kidney and testis induced at 2500 ppm and above are described in Section "repeated dose toxicity". The LOAEL for tumour induction (hepatocellular neoplasms and MCL in male rats) was 2500 ppm (147 mg/kg b. w. per day for males). The NOAEL was 500 ppm (28.9 mg/kg bw/d, males).

In a study performed following a protocol comparable to OECD guideline 451, groups of 50 male and 50 female Fischer 344 rats, five to six weeks of age, were fed diets containing 6000 or 12 000 mg/kg diet (ppm) di(2-ethylhexyl) phthalate (> 99% pure) for 103 weeks(NTP, 1982; Kluwe et al., 1982 and Kluwe et al., 1983). All surviving rats were killed at 104–105 weeks. Other groups of 50 males and 50 females served as controls. There was a dose-related decrease in body weight gain in both sexes but no effect on survival. More than 60% of the animals survived to the end of the study. High-dose male rats had significant increases (p= 0.01, Fisher's exact test) in the combined incidence of hepatocellular carcinomas and neoplastic nodules (control, 3/50; low-dose 6/49; high-dose, 12/49). The Cochran–Armitage test also indicated a significant trend (p= 0.007). [The IARC Working Group (2000) noted that the term neoplastic nodule is now generally assumed to represent hepatocellular adenomas.] The incidence of hepatocellular carcinomas alone or neoplastic nodules alone was not significantly increased. In female rats, the incidence of hepatocellular carcinomas was increa ed in high dose rats (8/50; p= 0.003, Fisher's exact test) compared with controls (0/50) and that of neoplast c nodules was also increased in high-dose females (5/50; p< 0.028) compared with controls (0/50). The incidence of hepatocellular carcinomas and neoplastic nodules combined was also increased in low-dose (6/49; p= 0 012) and high-dose (13/50; p< 0.001) females compared with controls (0/50). The LOAEL for tumour inducti n in rat was 6/000 ppm DEHP in the diet (320 mg/kg/day for male rats).

DEHP was administered in the diet at 0, 600, 1897, and 6000 mg/kg to male Sprague-Dawley rats beginning at an age of 90–110 days and continuing for the remaining lifetime of the animal (up to 159 weeks) (Voss et al., 2005). DEHP dose levels were 0, 30, 95, and 300 mg/kg bw/d. Significa tly increa ed incidence of hepatocellular adenomas and carcinomas were observed at the highest dose The percentage of benign Leydig cell tumors in the highest dose group was almost twice as high as the percentage in the control group (28.3% versus 16.4%). There was a significant dose-related trend in inc denc of hepatic neoplasms and Leydig cell tumours. Leydig cell tumours have not been reported in previous s udies in Sprague-Dawley rats, most likely due to late appearances outside the normal observation rang s of arcinogenicity studies.

Male F-344 rats were fed a diet containing 2% di-(2-e hylhexyl)phthalate (DEHP) for 95 weeks (Rao et al., 1987). Liver nodules and/or hepatocellular car inomas (HCC) developed in 6/10 rats fed DEHP and none were found in controls (P less than 0.005 by chi 2 test) All the nodules and HCC were negative for gamma-glutamyl transpeptidase. In the non-tumorous portions of live the hepatocytes contained an increased number of peroxisomes and extensive accumulation of lipof scin. By immunocytochemical analysis, the liver peroxisomes in rats treated chronically with DEHP h d visually detectable decrease in the H2O2-degrading catalase and increase in H2O2-producing fatty acyl-CoA oxidase. These results show that higher dietary level of DEHP, which causes substantially gre ter degre of peroxisome proliferation than the 1.2% dietary level used in the National Toxicology Program bioassay (1982, Publication no. NTP-80-37, Tech. Report Series No. 217), can induce liver tumors in male rats.

F-344 male rats were given a diet containing 2% DEHP ad libitum for 108 wk (Rao et al., 1990). At necropsy livers were quantita ively ana yzed for total tumor incidence and the number of lesions per liver after slicing the entire organ at 1- to 2 mm intervals. Neoplastic nodules and/or hepatocellular carcinomas were observed in 11 of 14 rats (78.5%). When evaluated according to the size, 57, 16, and 36% rats contained nodules ranging from 1 to 3, 3 to 5 and greater than 5 mm in size, respectively. The number of nodules per liver ranged from zero to four. Thes resu ts indicate that DEHP induces tumors in a large number of animals at 2% dose levels.

Mice

In a study compliant to OECD guideline 453, B6C3F1 mice (70/sex/group) received DEHP in the diet at concentrations of 0, 100, 500, 1500, or 6000 ppm (M/F: 0/0, 19.2/23.8, 98.5/116.8, 292.2/354.2, or 1266 1/1458.2 mg/kg/d) for 104 weeks(David*et al*., 1999, 2000). In an additional recovery group, mice were dosed with 6000 ppm of DEHP for 78 weeks, followed by a 26-week recovery period. Significantly increased incidences of hepatocellular adenomas and carcinomas were observed at 1500 ppm and 6000 ppm in male mice. In these two high dose groups, induction of peroxisome proliferation but not hepatocellular proliferation was more pronounced in both sexes. In the 6000 ppm recovery group, the incidence of hepatocellular adenomas, but not carcinomas, was less than in the 6000 ppm group. Non-tumour endpoints are described in Section "repeated dose toxicity". The LOAEL for tumour induction (hepatocellular neoplasms in male mice) in this study was 1500 ppm (292 mg/kg bw/d). The NOAEL was 500 ppm (98 mg/kg bw/d).

In a study performed following a protocol comparable to OECD guideline 451, groups of 50 male and 50 female B6C3F1mice, six weeks of age, were fed diets containing 3000 or 6000 mg/kg diet (ppm) di(2-ethylhexyl)

phthalate (> 99% pure) for 103 weeks weeks(NTP, 1982; Kluwe et al., 1982 and Kluwe et al., 1983). All surviving mice were killed at 104–105 weeks. There was a clear dose-related decrease in body weight gain in females. Survival at the end of the study was more than 60% in males and more than 50% in females. High-dose males had a slightly decreased body weight gain. In male mice, significant increases in the incidence of hepatocellular carcinomas were observed (control, 9/50; low-dose, 14/48; high-dose, 19/50; p= 0.022, Fisher's exact test). The Cochran-Armitage test also indicated a significant trend (p= 0.018). The incidence of hepatocellular adenomas and carcinomas combined was also increased in males (control, 14/50; low-dose, 25/48, p= 0.013; highdose, 29/50, p= 0.002, Fisher's exact test). In females, significant increases in the incidence of hepatocellular carcinomas were seen (control, 0/50; low-dose, 7/50, p= 0.006; high-dose, 17/50, p< 0.001, Fisher's exact test) and of hepatocellular adenoma and carcinoma combined (control, 1/50; low-dose, 12/50; high-dose, 18/50, p< 0.001, trend and Fisher's exact tests). The LOAEL for tumour induction in mice was 3 000 ppm DEHP in the diet (670 mg/kg/day for male mice).

Inhalation

No reliable studies have been reported.

Dermal

No studies have been reported.

Studies on tumour initiating and/or promoting activity

Since DEHP is considered to be a non-genotoxic substance, it has been sugg sted th t the carcinogenic effect is exerted during the promotion phase of hepatocarcinogenicity. DEHP has therefore been tested in several initiation/promotion experiments in rats and mice where the end-point has een the number and/or volume of altered liver cell foci.

In conclusion, DEHP have no tumour initiating activity (Garvey e al., 1987 Williams et al., 1987; Ward et al., 1983), a positive promoting activity in mice liver (Ward et al, 983, 1984; Schuller and Ward, 1984; Hagiwara et al., 1986; Ward et al, 1990; Weghorst et al, 1994), a weak or no p omoting activity in rat liver (Popp et al., 1985; Williams et al, 1987; Oesterle et al., 1988; Ito et al., 1988; Gerbracht et al., 1990) and a promoting activity in rat kidneys (Kurokawa et al., 1988).

Mecanism of carcinogenicity and relevance to human

Hepatocarcinogenicity

Concerning the hepatocarcinogenicity of DEHP, prev ously, two different modes of action have been suggested for DEHP and other Peroxisome Prolif rators (PPs) (see also section 5.10.3):

-induction of peroxisome prolif ration leading to oxidative stress and generation of electrophilic free radicals and/or

-increased hepatocyte proliferatio / suppression of hepatocellular apoptosis which could lead to fixation of a previously existing DNA damage; enhancing the conversion rate of initiated cells to tumor cells; as well as increasing the susceptiblity of hepatocytes to replication and a subsequent neoplastic transformation.

None of these mechanistic pr mises provides a wholly satisfactory explanation of the mechanism of cancer induction ca sed by DEHP. However, in view of the available evidence, a mechanism due to oxidative stress seems to be th least likely to play a major role (Cattley et al., 1998; Youssef and Badr, 1998; IARC, 1995).

A third an a m re feasible mechanistic basis for hepatocarcinogenicity through activation of Peroxisome Proliferator activated Receptor alpha (PPARα) has been accepted by most of the experts in this field. Activation of PPARαis also required for the induction of peroxisome proliferation, cell proliferation, and most probably also of se eral other aspects of the multifaceted effects brought about by the PPs (Peters et al., 1997, Ward et al., 1998, Cattley et al., 1998). The role of PPARαin the toxicity of DEHP has been investigated, recently, in a subchronic study in PPARα-null and wild-type male Sv/129 mice (Ward et al., 1998). Whereas the wild-type mouse fed DEHP exhibited typical lesions in the liver (such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly), kidney, and testes, no signs of liver toxicity was detected in the PPARα-null mice. On the other hand, evidence of lesions in kidneys and testes were found also in the PPARα-null mice, indicating a PPARα-independent pathway for induction of toxicity in these organs. It has been demonstrated in another study with knockout mice fed the potent peroxisome proliferator Wy-14,643 (Peters et al., 1997) that PPARαis required for the hepatocarcinogenicity of this substance. However, there is still no clear evidence showing that the carcinogenicity of DEHP in rodent is mediated through activation of PPARα.

Species differences are evident regarding the response to the different effects of the PPs on the liver. Rats and mice are very sensitive, Syrian hamsters appear to exhibit an intermediate response, whereas guinea pigs and

monkeys appear to be relatively insensitive. The potential human response to PPs has been examined in liver biopsies obtained from patients treated with hypolipidemic drugs with no evidence of peroxisome proliferation. The low sensitivity of human liver to the effects of PPs could be explained by the low level of PPARαfound in human liver (1-10% of the level found in rat and mouse liver) and genetic variations that render the human PPARαless active as compared to PPARαexpressed in rodent liver (Palmer et al., 1998; Tugwood et. al, 1996; Woodyatt et al., 1999). The potential carcinogenic risk of hypolipidemic therapy with fibrates, potent PPs, has been evaluated in two limited clinical trials with no evidence for carcinogenesis obtained. No relevant data are available on humans exposed to DEHP.

It has been suggested that the hepatocarcinogenic effects of PPs, such as DEHP, in experimental animals are rodent-specific and irrelevant for human. This position is held by a number of experts and is a defensible conclusion based on the available mechanistic data. However, the following arguments still indicate that a certain human cancer risk cannot, with certainty, be excluded:

1. The arguments for rodent-specificity of the liver tumours and the irrelevance of the experimental da a fo humans are based on the overall evidence available for all the PPs together. The weight of eviden e ava able for each of the PPs, for example DEHP, is weaker.

2. The available data indicate a quantitative but not a qualitative, species variation in the expre sion of PPARα. Humans express PPARαin liver, albeit in levels lower than those found in rodents (Tugwood et a 1996; Palmer et al., 1998). Therefor, a certain human cancer risk may still exist for some of the highly p tent peroxisome proliferators. Also inter-individual differences in expression of human PPARα hav been de onstrated (Tugwood et al., 1996). This evidence supports the conclusion reported by Vanden H uvel (1999) “Therefore, although PPs may pose little risk to the population as a whole, the potenti l human carcinogenicity of these chemicals cannot be summarily ignored. ”

3. DEHP has shown positive activity in several cell transformation assays a d this effect is correlated with inhibition of gap junctional intercellular communication. It may be argued t at these effects on cell transformation and intercellular communication by DEHP point at a different mechanism of carcinogenicity independent of PPARα (Dybing and Sanner, 1997; Mikalsen and S nner, 1993; Tsutsui et al., 1993).

4. An association between non-peroxisomal effects of PP and the carcinogenic process could exist. Possible changes in non-peroxisomal parameters (such as mito hondrial effects; regulation of cytochrome P-452, hormonal disturbances; and effects on cellular biology and ion homeostasis) in experimental animals and the relevance of such effects to humans have not bee well studied (Youssef and Badr, 1998; Eagon et al., 1996).

Most recently, a Working Group of the International Agency for Research on Cancer”(IARC) have concluded that the mechanism by which DEHP in reases the incidence of liver tumours in rodents (activation of PPAR-á) is not relevant to humans. Therefore, nd b sed on the overall evaluation of the available data, the DEHP-induced liver tumours in rats and mice will not be considered in the present Risk Assessment Report on DEHP.

Leydig cell (LC) tumours in rats

An increase in the incidence of testicular interstitial cell tumours (LC tumours) was observed in rats exposed to DEHP in a long-term study (Voss et al., 20005). In this study, 2,170 male Sprague-Dawley rats were exposed, lifelong, for the three non-genotoxic liver carcinogens DEHP, phenobarbital-sodium (PHB), and carbon tetrachloride (CCl4) t assess their hepatocarcinogenic effects alone or in combination. DEHP at dose levels of 30, 95 and 300 mg/kg, in the diet, did not cause liver tumours but induce LC tumours.

In a two-g ner ion reproduction toxicity range-finding study in Wistar rats (Schilling et al., 1999), DEHP (approximately 0 110, 339 and 1,060 mg/kg/d) was administered, in the diet, to groups of 10 males and 10 femal s sexually immature animals (F0 parental generation). Males and females from the same dose group were mated 70 days after the start of treatment. The females were allowed to litter and rear their pups (F1generation pups) until day 21 post partum. All male and female F1generation pups with the exception of one male and one femal pup/litter (each first surviving pup/sex) were sacrificed on day 21 post partum.

The selected pups (F1generation pups) were reared for at least 10 weeks to become the F1generation parental animals. The male animals of the F1generation parental animals were killed after the mating period. In the 3-month-old F1males, a slight (grade 2) diffuse LC hyperplasia were observed in all six high dose animals. The authors considered the lesion to be treatment-related.

This DEHP-related effect is critical as the prenatal exposure period was relatively short, the onset of the lesions is early and the development of LC hyperplasia to LC tumours is possible if the 3-month-old animals had been allowed to age. This report supports the results of Voss et al., (2005) showing induction of LC tumours in Sprague-Dawley rats exposed for DEHP.

The relevance for humans of rodent LC tumours has recently been evaluated in an international workshop (summarised in Clegg ED, Cook JC, Chapin RE, Foster PM and Daston GP (1997) Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproductive Toxicology;11(1), 107-121) as well as in a published review (Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM and Foster PMD (1999) Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Crit. Rev. Toxicol.29, 169-261). It was concluded that the pathways for regulation of the Hypothalamo-Pituitary-Testis (HPT) -axis in rats and humans are similar and hence, compounds that induce LCTs in rats by disruption of the HPT-axis pose a risk to human health with exception of two classes of compounds GnRH and dopamine agonists. Since it has been demonstrated that DEHP and other phthalates has a direct effect on the foetal testes the two latter mechanisms are not relevant for phthalates, and the induction of LC tumours in rats exposed for pthalates should be regarded as relevant to humans taking into consideration the species differences in sensitivity (Jones et al., 1993; Mylchreest et al., 1999; Foster, 1999).

MCL

The observed increase in the incidence of MCL in F344 rats is within the range of NTP's historic l cont ol data. However, the concurrent study control groups remains most appropriate for comparisons, and the hi torical control data, if considered, must be from the test laboratory itself. Therefore, the increas in t e incidence of MCL in male rats (Davis et al., 2000) may be DEHP-related, as the incidence were significantly increased compared to the study control and in addition to the historical control data from the same laboratory.

Additionally, it should be noted that increases in the incidence of MCL in F344 rats exposed to other phthalates, for example, diisononyl phthalate, diallyl phthalate, and butylbenzyl phthalate have b en reported.

Whereas Ward and Reynolds (1983, Large granular lymphocyte leukemia, a heterogeneous lymphocyte leukemia in F-344 rats. Am. J. Pathol.111, 1-10) consider MCL in F344 rats as hav ng similar pathology to an uncommon human tumour (large granular lymphocytic leukemia) and representing a unique model for study of natural tumour immunity, other experts regard MCL as F344 rats-specific, with little relevance for humans (Caldwell DJ (1999). Review of mononuclear cell leukemia (MNCL) in F 344 rat bioassays and its significance to human cancer risk: a case study using alkyl phthalates Regul. T xicol. Pharmacol.30(1), 45-53). Based on the available data the relevance for humans of the DEHP-induced MCL in F344 rats is not clear.

The following information is taken into account for any haz rd / risk assessment:

No relevant studies in humans on the carcinogenicity f DEHP is available. The results show that DEHP is carcinogenic in rats and mice. A statistically ignificant increase in the incidence of hepatocellular tumours with a dose-response relationship was observed in rat and mice of both sexes and a significant dose-related increase in the incidence of Leydig cell tumours was observed in male rats. It was also noted that low doses did not cause hepatocellular tumours, which sugg sts a threshold for this effect.

However, there is a plausible mechanism for the PPs-induced hepatocarcinogenicity in rodents (activation of 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.

Leydig cell tumours have been reported in only one study in Sprague–Dawley rats at doses that have been used in two other studies (u ing F 344 rats). It has been argued that the differing results are the consequence of the high spontan ous incidence of Leydig cell tumors in Fischer 344 rats compared to Sprague–Dawley rats.

In a 104-week rat study, an increased incidence of mononuclear cell leukaemia (MCL) was also noted. The rel vance f MCL is unknown, but it was only seen in one of two rat studies and in neither of the two mouse studi s Moreover, this tumour type is well known to occur spontaneously with high incidence in the F344 rat strain us d in the study.

The LOAEL and the NOAEL for tumour induction in rats (both liver tumours and MCL) were established as 2500 ppm (146.6 mg/kg bw/d for males) and 500 ppm (28.9 mg/kg bw/d for males) respectively (David et al., 1999, 2000). In mice, the LOAEL and the NOAEL for induction of liver tumour were 1500 ppm (292 mg/kg bw/d for males) and 500 ppm (98 mg/kg bw/d for males) respectively (David et al., 1999, 2000).

Recently, the International Agency for Research on Cancer (IARC) re-evaluated the carcinogenicity of DEHP. Based on mechanistic information the Agency concluded that activation of peroxisome proliferator-activated receptor α represents an important mechanism in rodents, but that "multiple molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to the induction of cancer in rats and mice." The Agency concluded on a classification in Group 2B (possibly carcinogenic to humans). (Rusyn and Corton, 2012).

It should be emphasised that no convincing data are available pointing to involvement of genotoxic modes of action. Furthermore, humans do not appear susceptible to peroxisomal proliferation (PP) as exemplified by clinical studies of populations exposed for long periods to hypolipidemic drugs (rodent hepatocarcinogens and strong rodent PPs). Liver tumours and the modes of action (including alternative modes of action as discussed by IARC) by which they form are likely not relevant to humans; however, a data gap for demonstrating the lack of cell proliferative events (key event in the rodent MOA) in humans was acknowledged.

In conclusion, these new data and evaluation by the IARC Working Group don't warrant a modification of the actual non-classification of DEHP as carcinogenic according to the criteria of Regulation (EC) N° 1272-2008 (CLP) and will not impact the risk assessment performed under Regulation (EC) N° 1907-2006 (REACH), as DNELs for DEHP have been derived from the reproductive toxicity data, which is the most sensitive end-point.

Value used for CSA (route: oral):

NOAEL: 29 mg/kg bw/day

Justification for classification or non classification

According to the criteria edicted in REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008, no classification is warranted for carcinogenicity.

5.9. Toxicity for reproduction

5.9.1. Effects on fertility

5.9.1.1. Non-human information

The results of experimental studies are summarised in the following table:

Table 44 Overview of experimental studies on fe tility

Method	Results	Remarks	Reference
Multig neration reproductive toxicity studies			
rat (Sprague-Dawley) male/female multigeneration study oral: feed 1.5 (2 groups), 10, 30, 100, 300, 1000, 7500, 10000 (2 group) ppm (nominal in diet) 0.12, 0.78 2.4 .9, 23, 77, 592, and 775 mg/k /day actual ingested (F0 animals)) 0.09 0.48 1.4, 4.9, 14, 48, 391, and 543 mg/kg/day (actual ingested (F1 animals)) 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg/day (actual ingested (F2 animals)) Exposure: Premating exposure period (males): 81 +/- 10 d Premating exposure period (females): 81 +/- 10 d	NOAEL (Effects not related to reproductive toxicity in adult animals) (F0, F1 and F2): 300 ppm (nominal) (male/female) ((equivalent to approximately 23 mg DEHP/kg bw/day in the F0 animals, and 14 mg DEHP/kg bw/day in the F1 and F2 animals) based on reductions in bodyweights noted in both sexes at 7,500 (F1, F2 animals) and 10,000 ppm (F0, F1 animals), absolute and/or relative organ weight changes noted at 1000 ppm and above (increased liver: 1,000 ppm and above; increased kidneys: 1,000 ppm and above; increased adrenals: 10,000 ppm; increased pituitary: 10,000 ppm), and microscopic pathological findings noted at 1,000 ppm and above (liver	1 (reliable without restriction) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Wolfe GW and Layton KA (2004) Peckham JC (2003)

Duration of test: 3 generations in F3 (F3c) (no data) equivalent or similar to OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	hypertrophy: 1,000 ppm and above; cortex vacuolisation of the adrenals: 7,500 ppm and above; dilation of the tubules and mineralization in the kidneys occasionally associated with chronic pyelonephritis: 1,000 ppm and above). Microscopic pathological findings in the adrenal glands were also indicated in F1 animals at 1,000 ppm (no further data)) NOAEL (Reproductive toxicity) (F0, F1 and F2): 1000 ppm (nominal) (male/female) ((equivalent to approximately 77 mg DEHP/kg bw/day in the F0 animals, and 48 and 46 mg DEHP/kg bw/day in the F1 and F2 animals respectively) and was based on impaired fertility and litter paramet s noted at 7,500 ppm and above, and decreased var ous sperm end-points noted at 7500 (F1-, F2-, F3 m les) and 10,000 ppm (F0-, F males)) NOAEL (Developmental toxicity) (F1 and F2): 100 ppm (nominal) (male) equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and approximately 5 mg DEHP/kg bw/day in the F and F2 animals) and was based on decreased absolute and/or relative testis weights noted at 7,500 (F1, F2 and F3 males) and 10,000 ppm (F0 and F1 males), macroscopic pathological findings (small or aplastic testes) noted at 300 (3/45 non-mating F1 males, 1/21 non-mating F2 males), 1,000 (3/25 non-mating F2 males), 7,500 (7/10 mating F1 males, 10/30 non-mating F1 males, 9/10 mating F2 males, 11/20 non-mating F2 males) and 10,000 ppm (2 or 3 of 10 F0 males, 10/10 mating F1 males, 21/21 non-mating F1 males), and microscopic pathological findings (testis seminiferous tubular atrophy) noted at 300 (1/10 F1 males),		

	7,500 (all F1 and F2 males) and 10,000 ppm (all F1 males, 2 or 3 of 10 F0 males))		
rat (Wistar) male/female two-generation study oral: feed 0, 1000, 3000 or 9000 ppm (analytical conc.) ca. 0, 110, 339, 1060 mg/kg bw/day (actual ingested) Exposure: through a premating period of at least 73 days, through mating, gestation, parturition and lactation to weaning (day 21 post partum) of the offspring (F1 generation.) (ad libitum) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	NOAEL (Reproductive performance and fertility) (P0 and F1): 3000 ppm (analytical) (male/female) LOAEL (Prenatal and developmental toxicity) (F1 and F2): 1000 ppm (analytical) (male/female) (Focal tubular atrophy)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Schilling, K, Gembardt, C, and Hellwig, J. (2001a)
rat (Wistar) male/female two-generation study oral: feed 1000, 3000 or 9000 ppm (nominal conc.) 110, 339, 1060 mg/kg bw/day (actual ingested) Exposure: Exposure on P female/male, 90 days before mating during weaning of F2 litter. (Daily) OECD Guideline 416 (Two-Generation Reproduction Toxici y Study)	NOAEL (Fertility) (P): 1060 mg/kg bw/day (male/female) (↑ relative liver weight in F0 females from 1,000 ppm and in F0 males from 3,00 ppm (negative hist path logy); ↓ food consumption, body weight, nd b dy w ight gain and ↑ postimplantation loss in f males a 9,000 ppm;) NOAEL (ertility) (F1): 339 mg/kg bw/day (male/female) (F1 parental animals : ↓ food consumption, body weight, and mortality in both sexes initially at 9,000 ppm and ↓ body weight gain in females; ↓ fertility, ↓ testicular and epididymal weight and size, atrophy of the testes, Leydig cell hyperplasia, interstitial oedema, and altered spermatogenesis and aspermia at 9,000 ppm; dose related decrease of prostate weight from 1,000 ppm;) NOAEL (Development) (F1 and F2): 110 mg/kg bw/day (female) (F1 pups :↓ number of delivered and live born pups and ↓ viability index neonatally at 9,000 ppm; loss of spermatocytes at 3,000 ppm (2/10) and 9,000 ppm (7/9); ↑ presence of	1 (reliable without restri tion) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Schilling, K, Deckardt, K, Gembardt, C, and Hildebrand, B. (1999a)

	areolas/nipple anlagen; retarded preputial separation and vaginal opening at 9,000 ppm. F2 pups: ↑ number of still born pups from 3,000 ppm, ↓ number of delivered pups and mean number of pups/dam at 9,000 ppm.)		
Single generation reproductive toxicity studies			
rat (Wistar) male/female fertility and testicular toxicity inhalation: aerosol (nose/head only) 1.0, 0.05 and 0.01 mg/l (nominal conc.) 230, 11 and 2.3 mg/kg for males and 360, 18 and 3.6 mg/kg for females (estimated daily intake) Exposure: 6h (daily for 28days) OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	NOAEC (Fertility) (P): ≥1 mg/L air (nominal) (male) (no effects on male fertility, no testicular toxicity)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bi 2 ethylhexyl) phthalate**	Klimisch HJ, Gamer AO Hellwig J (992) BASF (1990)
rat (Fischer 344) male Male fertility and testicular toxicity oral: feed 320, 1,250, 5,000 or 20,000 ppm (nominal in diet) 18, 69, 284 and 1,156 mg/kg bw/d (actual ingested) Exposure: 60 days (ad libitum) Sexually mature male F344 rats received DEHP in he diet for 60 days and were mated with undosed females at e posure day 61 to 66.	NOAEL (P): 125 ppm (nominal) (mal) ((= 69 mg/kg bw/d) Dos dependent ↓ in total ody, test , epididy is, nd prostate weights fr m 5,000 ppm ↓mean litt r size at 20,000 ppm correlated with d generative testicular changes, ↓ testicular zinc content, epididymal sperm density and motiliy, ↑ number abnormal sperm cells)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Agarwal DK, Eustis S, Lamb IV JC et al. (1986a)
mouse (CD-1) male/female on generation study oral: fe d 300 p m (nominal in diet) 44 7 mg/kg bw/d (actual ingested) Exposure: 5 weeks of age of the F0 generation to birth of the F1 generation. (ad libitum) Evaluation of the reproductive effects of DEHP in a cross-mating method of mice. The single-dose method was used in a cross-mating method since the multi-doses	NOAEL (P): >= 300 ppm (nominal) (male/female) based on: test mat.	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tanaka T (2003)

method was too complicated to analyze the results.			
mouse (CD-1) oral: feed 0.03% (nominal conc.) 53–57 mg/kg bw/day during the preconception period, ~43 mg/kg bw/day during mating, 46–49 mg/kg bw/day during gestation, and 154–171 mg/kg bw/day during lactation (actual ingested) Exposure: From 5 weeks of age of the F0 generation to 9 weeks of age of the F1 generation (In the diet (therefore daily)) Evaluation of the reproductive and neurobehavioural effects of DEHP in a cross-mating method of mice.	NOAEL (maternal and developmental toxicity): 0.03 %	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tanaka, T. (2005)
mouse (CD-1) male/female two-generation study oral: feed 0.01, 0.025 or 0.05% (nominal in diet) 0, 18.89, 47.70 and 94.90 mg/kg/d (actual ingested (calculated according to daily intake)) Exposure: gestation day 0 to 17 (ad libitum) equivalent or similar to OECD Guideline 416 (Two-Generati n Reproduction Toxicity Study)	NOAEL (P): >= 95 mg/kg bw/day (female) (No effect) NOAEL (F1): 48 mg/kg bw/day (male/female)(↑ prenatal mortality for F1-litters at 0.05% ↓ number of viable pups n onata ly at 0.05%) NOAEL (F2): >= 95 mg/kg bw/day (mal /female) (No ffect)	1 (rel ble without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Price CJ, Tyl, RW, Marr MC (1988a)
mouse (CD-1) male female fertility oral: feed 0.01, 0 1, 0.3% (nominal in diet) 0, 14, 141 and 25 mg/kg/d (actual inge ted (Doses were converted to mg/kg w/day assuming mice c ns me 5.1 g/day and weigh 36 g, regar less of treatment group)) Exposure: 7 days (premating) and then 98 days (cohabitation) (ad libitum) Reproduction and fertility were assessed. The mice were exposed to the chemical for a 7-day pre-mating period and during the a 98-day	NOAEL (P): 14 mg/kg bw/day (female) based on: test mat. (dose-dependent ↓ in the number of litters and proportion of pups born alive from 0.1% (0.1%: 14/19 fertile, 0.3%: 0/18); ↑ absolute and relative liver weight (both sexes) and ↓ reproductive organ weights and atrophy of seminiferous tubules at 0.3%; no effect on bw crossover mating trial: treated males and control females: 4/20 fertile; control males and treated females: 0/16 fertile)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Lamb JC, Chapin RE, Teague J (1987)

cohabitation period. Cross mating tests was realised to evaluate the influence of the sex on reproductive functions.			
mouse (ICR) male/female Male fertility subcutaneous 0, 1, 2, 5, 10, 15, 20, 40, 60, 80 and 100 ml/kg (nominal conc.) Exposure: on days 1, 5, and 10 Evaluation of the reproductive performance after subcutaneous administration of DEHP	no NOAEL identified (P): (male/female) (There is a conspicuous reduction in the incidence of pregnancies in DEHP-treated mice, and it exhibits a dose-dependent trend. At a dose level greater than 10 ml/kg the pregnancy rate is so low the animals may be considered almost sterile)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Agarwal, D.K., Lawrence, W.H., and Turner, J.E. (1989)

Toxicity to reproduction: other studies

The results of experimental studies are summarised in the following table:

Table 45 Overview of experimental studies on the toxicity to rep duction (other studies)

Method	Results	Remarks	Reference
Testicular toxicity in y ung and adult animals			
rat (Sprague-Dawley) male Testicular toxicity oral: gavage 10, 100, 1000 and 2000mg/kg bw/d (actual ingested (Neonatal and adult rats (1, 2, 3, 6, and 12 weeks o age))) 100, 200, 500 and 1000 mg/kg bw/d (actual ingested (Suckling rats)) Exposure: 5 days (d ily) Evaluation of the male fertility	NOAEL (testicular toxicity) (Neonat and du rats (1, 2, 3, 6 and 12 weeks of age)): 100 mg/kg bw/day (male) (↓ bsolute and relative testis w ights at 1,000 mg/kg bw/day in 1, 2, 3,and 6-week ol rats; ↓ Sertoli cell nuclei in 1-week-old rats and loss of spermatocytes in 2- and 3-week old rats; ↓ testis weight also in 6-and 12-week old rats at 2,000 mg/kg bw/day; fatalities in suckling rats at 2,000 mg/kg) NOAEL (Fertility) (Suckling rats): 200 mg/kg bw/day (male) (testis: ↓ number of Sertoli cells in adult rats at 500 and 1,000 mg/kg bw, no effect on fertility after mating to untreated females)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Dostal LA, Chapin RE, Stefanski SA et al. (1988)
rat (Sprague-Dawley) male Testicular toxicity oral: gavage 2800 mg/kg (actual ingested) Exposure: 10 days (daily)	dose level:: 2800 mg/kg bw/day (male) (↓ abs. wghts of testes, seminal vesicles, and prostate, severe testicular atrophy in 4-week-old rats. No effect in 15-week-old rats.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC**	Gray, T.J.B, Gangolli, S.D. (1986)

Study the age-dependent effects on male reproductive organs in 4, 10, or 15 weeks-old rats		**name): bis(2-ethylhexyl) phthalate**	
rat (Sprague-Dawley) male Testicular toxicity oral: gavage 1000 mg/kg Exposure: 14days (daily) equivalent or similar to OECD Guideline 417 (Toxicokinetics)	dose level:: 1000 mg/kg bw/day (male) (severe testicular damage in the 25-day-old rats, whereas the older animals were unaffected)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Sjöberg, P., Bondesson, U., Kjellen, L. (1985)
rat (Sprague-Dawley) male Testicular toxicity oral: gavage 1,000 and 1,700 mg/kg (nominal in diet) Exposure: 14 days (daily) Study the age-dependent testis toxicity of DEHP in rats at 25, 40 and 60 days of age	dose level:: 1000 — 1700 mg/kg bw/day (male) (decreased testicular weight in 25- and 40-day-old rats given 1,700 mg/kg bw. Severe testicular damage for the 25- and 40-day-old rats at both dose levels. No changes in the 60-day-old rats.)	2 (reliable with restrictions) supporting study experimen 1r ult **Test material (EC name): bis(2-ethylhexyl) phthalate**	Sjöberg P., Lindqvist, N.G., Plö n, L. (1986)
rat male Testicular toxicity oral: gavage 250, 500, 1000 and 2000 mg/kg body weight Exposure: 15 days (daily)	LOAEL (P): 250 mg/kg bw/day (male) based on: test mat. (↓ testes wght a 2000 mg/kg, ↓ sperm c unt >= 250 mg/kg, ↑ GGT, LDH activities >=500 mg/kg)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parmar D, Srivastava SP, Seth PK (1986)
rat (Wistar) male Testicular toxicity oral: gavage 0, 50, 100, 250 or 500 mg/kg (actual ingested) Exp sure 30 d ys (daily) Inve tigation of the activities of the testicul r enzymes associated with spermatogenesis	LOAEL: 50 mg/kg bw/day (male) (effects on absolute and relative testis weight, and reduced testicular enzyme activities)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parmar, D., Srivastava, S.P., Srivastava, S.P. (1995)
rat (Sprague-Dawley) male Testicular toxicity oral: feed 0, 5, 50, 500, 5000 ppm (nominal in diet) ca. 0.4, 3.7, 37.6, 375.2 mg/kg bw/d (actual ingested)	NOAEL (P): 50 ppm (nominal) (male/female) (High incidence of minimal to mild Sertoli cell vacuolation at 500 ppm)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Poon R, Lecavalier P, Mueller R. (1997)

Exposure: 90 days (ad libitum) equivalent or similar to OECD 408			
rat (Fischer 344) male/female Testicular toxicity oral: feed 0, 1,600, 3,100, 6,300, 12,500 or 25,000 ppm (nominal in diet) 0, 80, 160, 320, 630 or 1,250 mg/kg/day (analytical conc.) Exposure: 13 weeks (ad libitum) equivalent or similar to OECD 408	NOAEL (P): 6300 ppm (analytical) (male/female) ((= 320 mg/kg bw/d) ↓bwg at 25,000 ppm testis atrophy from 12,500 ppm)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982c)
rat (Sprague-Dawley) male/female Testicular toxicity oral: feed 0, 0.2, 1.0, 2.0 % (nominal in diet) 0, 143, 737 or 1,440 mg/kg/day in males and 0, 154, 797, or 1,414 mg/kg/day in females (actual ingested) Exposure: 17 Weeks (ad libitum) equivalent or similar to OECD 408	LOAEL (P): 0.2 % in diet (male) based on: test mat. (↓bw from 1.0% liver: ↑absw and relw from 0.2%, no histological findings testes: ↓absw and ↑relw from 1.0%, atrophy. LOAEL: 143 mg/kg/day) no NOAEL identified :	2 (reliable with restrictions) supporting s udy experimental result **Test material (EC name): bi (2-ethylhexyl) phthalate**	Gr y TJB, Butterworth KR, Gaunt LE (1977)
rat (Fischer 344) male/female Testicular toxicity 0, 100, 500, 2,500 or 12,500 ppm (nominal in diet) 0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day (actual inges ed) Exposure: at least 04 weeks (ad libitum) OECD 453	NOAEL P): 500 p m (nominal) (male) ((= 28.9 mg/kg bw/day) pituitary: ↑ astration ells (30/60 males) at 12500 ppm; testis: ↓ weight, ↑ incidence and se erity of bilateral hypospermia at 12500 ppm; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 12500 ppm; changes in the testis and pituitary were not reversible upon cessation of exposure)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	David RM, Moore MR, Finney DC and Guest D (2000c) David RM, Moore MR, Finney DC (2001)
rat Sprague-Dawley) male Testicular toxicity ral: feed 0 0.02, 0.2, 2% (nominal in diet) 0, 7, 70, 700mg/kg (actual ingested) 102-week exposure in diet in rat	LOAEL (P): 0.02 % in diet (male) (testes: atrophy and inhibition of spermatogenesis from 0.02% (7 mg/kg bw/day))	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ganning AE, Brunk U, Edlund C (1987) Ganning AE, Olsson MJ, Brunk U (1990)
rat (Fischer 344) male/female Testicular toxicity oral: feed	LOAEL (P): 322 mg/kg bw/day (male/female) (testes: seminiferous tubulardegeneration at 6,000	2 (reliable with restrictions) supporting study	NTP (1982c) Kluwe WM, Haseman JK,

6,000 or 12,000 ppm (nominal in diet) 322 or 674 mg/kg/day for males (actual ingested) Exposure: 103 weeks (ad libitum) OECD 451	(5%) and at 12,000 ppm (90%))	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Douglas JF (1982d) Kluwe WM (1983a)
mouse (CD-1) male/female Testicular toxicity oral: feed 3000 ppm (nominal in diet) ca. 150 mg/kg bw (actual ingested) Exposure: 98days (ad libitum) equivalent or similar to OECD 408	conc. level: (P): 3000 ppm (nominal) (male/female) (Reduction in epididymal and testicular weights, sperm motility, and sperm concentration and an increased number of abnormal sperm cells were observed at the only dose tested.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Morrissey RE, Lamb IV JC, Schwetz BA et al (1988) Mo rissey RE, Schw tz BA, Lamb JC et al. (1988)
mouse (B6C3F1) male Testicular toxicity oral: feed 100, 500, 1500, 6000 ppm (nominal in diet (equivalent to 19.2, 98.5, 292.2 and 1266.1 mg/kg/d)) Exposure: 104 wk A set of 10–15 animals per group was terminated during Week 79. (7/wk) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (P): 500 ppm (nominal) (male) ((= 98.5mg/kg bw/d) testis: from 1,500 ppm ↓ weight, ↑ incidence and severity of bilateral hypospermia; epididymis: from 1,500 ppm ↑ immature or abno mal sperm forms and hyposperm a; changes in tes es pa tially reversible)	1 (reliable wi hout restriction) suppo ing study expe imental result **Test material (EC n me): bis(2-ethylhexyl) phthalate**	David RM, Moore MR, Finney DC (2000c) David RM (1999) Moore MR (1997)
mouse (B6C3F1) male Testicular toxicity oral: feed 3000, 6000 ppm (nominal in diet) 672 or 1,325 mg/kg/day (males) and 799, or 1 821 mg/kg/day (females) (actual ingested) Exposu e: 104-105 Weeks of exposur (ad libitum) equiv lent or similar to OECD 451	NOAEL (P): 672 mg/kg bw/day (male) (testes: seminiferous tubular degeneration and testicular atrophy at 6,000 ppm)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	NTP (1982c) Kluwe WM, Haseman JK, Douglas JF (1982b) Kluwe WM (1983b)
monkey (Marmoset) male Testicular toxicity oral: gavage 100, 500 and 2500 mg/kg (actual ingested) Exposure: 13 wk (91 consecutive days) (daily)	NOAEL (P): >= 2500 mg/kg bw/day (actual dose received) (male) (no effect on testis)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl)**	Kurata Y, Kidachi F, Yokoyama M (1998)

equivalent or similar to OECD Guideline 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents)		**phthalate**	
monkey (Marmoset) male/female Ovarian and testicular toxicity oral: gavage 0, 100, 500, 2500 mg/kg (actual ingested) Exposure: 65 Weeks (once daily) Repeated dose toxicity study in juvenile male and females Marmosets with special examination of the reproductive organs and liver activity.	NOAEL (P): >= 2500 mg/kg bw/day (actual dose received) (male) based on: test mat. NOAEL (P): 100 mg/kg bw/day (actual dose received) (female) based on: test mat. (increased ovarian and uterine weights and elevated blood estradiol level were observed in higher dosage groups)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tomonari Y, Kurata Y, David RM, Gans G, Kawasuso T and Katoh M (2006) Kurata Y (2003c)
rat (Wistar) male Testicular toxicity inhalation (whole body) 5, 25 mg/m3 (nominal conc.) Exposure: 4 or 8 weeks (6 h per day, 5 d per week) Study of the effects of inhalation of DEHP on testes of pre-pubertal rats	LOAEC: 5 mg/m³ air (male) (↑ plasma testosterone and weight of seminal vesicles. No histological change in the testis)	2 (reliable i h restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Kurahashi N (2005)
Testicul r toxicity in neonatal rats			
rat (Sprague-Dawley) male Neonatal testicular toxicity oral: gavage 20, 100, 200, or 500 mg/kg (ac ual ingested) Exposure single administration impact on both Ser oli cells and gonocytes of a single dose o DEHP administered in vivo to 3-day old rat pups	NOAEL (P) 20 mg/kg bw/day (a tual dose received) (male)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Li LH, Jester WF, Laslett AL et al. (2000)
rat Sprague-Dawley) male Neona al testicular toxicity ral: gavage 300, 600 and 1000 mg/kg bw/day (analytical conc.) Exposure: 21 days (daily) Evaluation of Reproductive Development following Oral Exposure to DEHP in Male Neonatal Rats	dose level: (neonatal rats): >= 300 mg/kg bw/day (male) (Testicular changes, consisting of a partial depletion of the germinal epithelium and/or decrease in diameter of seminiferous tubules)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Cammack JN, White RD, Gordon D, Gass J et al. (2003)
rat (Sprague-Dawley) male	NOAEL (neonatal rats): 60	2 (reliable with	Cammack JN,

Neonatal testicular toxicity intravenous 0, 60, 300, or 600 mg/kg/day (analytical conc.) Exposure: 21 days (daily) Evaluation of Reproductive Development Following Intravenous and Oral Exposure to DEHP in Male Neonatal Rats	mg/kg bw/day (actual dose received) (male) (Testicular toxicity at 300 and 600 mg/kg bw/d)	restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	White RD, Gordon D, Gass J et al. (2003)
Effects on the development of the male reproductive tract			
rat (Long-Evans) male Male reproductive tract development oral: gavage 10, 500, or 750 mg/kg (actual ingested) Exposure: from postnatal day 21 to 49 (daily) The potential of phthalate exposure to advance or delay the timing of puberty	no NOAEL identified:	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bi (2-ethylhexyl) phthal te**	Ge RS (2007)
rat (Sprague-Dawley and Long-Evans) male Male reproductive tract development oral: gavage 0, 10, 100, 300, and 900 mg/kg/day (exp1) (actual ingested (in LE and SD rats)) 0, 100, 300, and 900 mg/kg/day (exp2) (actual ingested (only in SD rats)) Exposure: from PND 22 to PND 28 (exp1). From PND 3 until PND 37 (exp2) (dail) Effect of DEHP treatment during pub rty on pub rty, testosterone production and male reproductive tract d velopment	NOAEL: 10 mg kg bw/day (male) (Any hang in reproductive end poin s in both stra ns) OAEL: 100 mg/kg bw/day (male) (Subtle changes in some androgen-dependent organ weights at 100 mg/kg. Testis histology altered at 300 and 900 mg/kg in both strains. Puberty delayed in LE rats at >= 300 mg/kg and SD rats at 900 mg/kg)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Noriega N, Howdeshell KL, Furr J. (2009)
Ovarian toxicity			
rat (Sprague-Dawley) female Ovarian toxicity oral: gavage 2000mg/kg (actual ingested) Exposure: over two to three cycles (time-to-effect study) 8 days (pathogenicity study) (daily)	no NOAEL identified :	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Davis, B.J., Maronpot, R.R., Heindel, J.J. (1994)

Regularly cycling Sprague-Dawley rats were dosed daily with DEHP by gavage for 1-12 days. Ovarian morphology and levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and progesterone were determined.			
rat (Sprague-Dawley) female Ovarian toxicity oral: gavage 0, 1500mg/kg (actual ingested) Exposure: 10 consecutive days (daily) Effects of in vivo administered DEHP on in vitro ovarian steroid profiles in immature and cycling female rats	LOAEL (P): 1500 mg/kg bw/day (female) based on: test mat. (Alterations of the oestrus cycle and concentration changes of testosterone and oestradiol in rats in dioestrus.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Laskey, J.W Berman, E. (1993)
in vitro study Ovarian toxicity primary granulosa cells (Fischer 344rat) male in medium 25, 50, 100, 200 µM (nominal conc.) Exposure: 48h (once) in vitro effects of a 48 hours exposure period to MEHP on primary rat granulosa cells.	: (dose-dependent effects of MEHP on the levels of aromatase RNA)	2 (relia le with restr ctions) supp rting study experimental result **Test material (EC name): mono(2-ethylhexyl) phthalate**	Lovekamp and Davis (2001)
Effect on the development of the female reproductive tract			
rat (Wistar-Imamichi) female Female reproductive tract development inhalation 5, and 25 mg/m3 (nominal conc.) Exposure for 6 h/day (5 contiguous days/ eek) Evaluation of the effects of inhaled di(2-e hylhexyl)phthalate (DEHP) on the onset of puberty and on postpubertal reproductive functions in prepubertal female rats	LOAEL: 5 mg/m³ air (female) (advanced puberty onset and alter postpubertal reproductive functions)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Ma M, Kondo T, Ban S,Umemura T et al. (2006)
Mechanistic studies			
rat (Fischer 344) male Testicular toxicity oral: gavage	: (male) (Organ weights of testis, epididymis, prostate, and seminal vesicles significantly and dose-	2 (reliable with restrictions) supporting study	Agarwal DK, Eustis S, Lamb IV JC et al. (1986b)

330, 1000, 3000 mg/kg (actual ingested) Exposure: 13 weeks (ad libitum) Rats were maintained one week on synthetic diets containing 2 ppm (low), 20 ppm (normal) or 200 ppm (high) zinc, then gavaged for 13 days with DEHP	dependently reduced in rats on low-zinc diet)	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	
rat (Wistar) male Testicular toxicity oral: gavage 2000mg/kg (actual ingested) Exposure: 15 days (daily) The involvement of testosterone in the testicular atrophy caused by DEHP was examined by co-administration of testosterone subcutaneously along with DEHP to adult male Wistar rats for 15 days	dose level:: 2000 mg DEHP/kg bw/d + 1 mg testosterone/kg bw (male) (Co-administration of testosterone seemed to normalise the testes weights, sperm count and the activity of testicular enzymes)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Parmar, D., Srivastava S.P., Singh, G B. (987)
rat and mouse (Sprague-Dawley and Swiss) male Testicular toxicity subcutaneous and intraperitoneal 1, 5, 10, 20, 50, 100mg/kg (nominal conc.) Exposure: 5, 10, 20days (daily) Changes in testes and sex accessory weight as well as gonadal zinc n mice and rats via subcutaneou and intraperitoneal administration	: (The results indicated that the male rat is more sensi ive to DEHP- or MEHP-induced effects on male gonad than the male mou e. sc r ip injected DEHP or MEHP caused g nadal in depletion)	2 (rel able with rest ictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Curto, K.A., Thomas, J.A. (1982)
mouse (Jcl:ICR and Crj:CD-1(ICR) male Testicular toxicity oral: feed 0.1 0.2, 0 4 or 0.8% (nominal in die) 150, 300 600, 1,200 mg/kg bw/day Expo ure: 14 days (ad libitum) T eatment in diet during 14days in two strains of mice. Evaluation of zinc content and enzyme activity in testes.	NOAEL (CD1 mice): 150 mg/kg bw/day (male) (↓ testicular weights >= 0.2%, ↓ testicular zinc content at 0.4 and 0.8%, ↓ testicular LDH-X activities at 0.2%) NOAEL (ICR mice): 300 mg/kg bw/day (male) (↓ testicular zinc content at 0.4 and 0.8%, ↓ testicular LDH-X activities at 0.8%)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Oishi S (1993)
rat (Wistar) male Testicular toxicity oral: gavage	dose level:: 2000 mg/kg bw/day (Co-administration of adenosylcobalamin, but not methylcobalamin, prevented the DEHP-induced testicular	2 (reliable with restrictions) supporting study experimental result	Oishi S (1994)

2000mg/kg (actual ingested) Exposure: 7 days (daily) Study of the influence of the vitamin B12 derivative adenosylcobalamin on testicular toxicity	effects)	**Test material (EC name): bis(2-ethylhexyl) phthalate**	

5.9.1.2. Human information

The exposure-related observations in humans are summarized as followed:

Male fertility

A Swedish epidemiologic study by Modigh and colleagues (2002) assessed the association between o cup tional exposure to DEHP and male fertility as determined by evaluating the time to pregnancy in 227 couples and their 397 pregnancies where male partner was working in a plant producing polyvinyl chloride (PVC) plastics Exposure assessment was based on air measurements at work place and questionnaire info mation on work tasks and locations. Time to pregnancy was compared between three exposure categories of no exposure, low (<0.1 mg/m3) and high (>0.1 mg/m3). There was no association between exposure and time to pregnancy.

Gonadal hormones and semen quality

Phthalate monoesters including MEHP, the initial metabolite of DEHP, and MBP are known testicular toxicant in rodents. The balance of gonadotropin and gonadal hormones is an imp rtant indicator of male fertility.

Main and colleagues (2006) studied 62 cryptorchid boys and 68 healthy boys from a prospective cohort of Danish and Finnish boys. As biomarkers of exposure, they analysed breast milk amples collected 1-3 months postnatally for phthalate monoesters including MMP, MEP, MBP, MBzP, MEHP, and MINP. Serum samples were analysed for gonadotropins, sex-hormone binding globulin (SHBG) estosterone, and inhibin B. No association was found between phthalate monoesters and cryptorchidism. MEP and MBP were positively, but weakly correlated with SHBG (Spearman correlation c efficient [r]=0.323, p=0.002 and r=0.272, p=0.01 respectively). MMP, MBBEP, and MBP were correl ted w th LH: free testosterone ratio and MINP with LH (r=0.243, p=0.019). MBP was negatively correlated wi h free testosterone (r=-0.22, p=0.033). These findings suggest some phthalates may have adverse effe s hum n Leydig cell development and function, which may be related to incomplete virilization in infant boys exposed to phthalates.

Pan et al.(2006) reported the effect of o cupation l exposures to high levels of the phthalate esters, DBP and DEHP on the balance of gonadotropin and gonad l hormones including the circulating concentration and/or balance of free testosterone (fT) luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2). They compared blood and urine concentrations of 74 male workers in a factory producing unfoamed polyvinyl chloride flooring and 63 men f om a construction company matched for age and smoking status. The exposed workers had significantly levated urinary concentrations of MBP (644.3 vs. 129.6 µg/g creatinine, p <0.001) and MEHP (565.7 vs. 5.7 µg/g creatinine, p<0.001). The fT concentration was significantly lower (8.4 vs. 9.7 µg/g creatin ne. P=0.019) in the exposed workers compared with the unexposed. Among the exposed, fT had a negative corre ation with MBP (r=-0.25, p=0.03) and MEHP (r=-0.19, p=0.095). In the regression analysis fT decreased significan ly with increasing total phthalate ester score. However, a cross-sectional study of 295 men attending an andrology clinic in Massachusetts found no association between phthalate levels in urine and serum le els of testosterone (Duty 2005), although in this instance phthalate exposure would have been notably low r than for the PVC workers.

Duty t al.(2003a, 2003b, 2004, 2005) and Hauser et al. (2006) conducted a series of studies in male partners of subfertile couples recruited at an infertility clinic (US). They estimated associations between blood and urinary bioma kers of exposure to phthalates and various measures of semen quality and morphology. Sperm concentration, motility and motion parameters were measured using computing aided sperm analysis. Sperm DNA damage was measured using neutral comet assay. In an analysis of 168 males (Duty et al. 2003b), there was an exposure-response relation between MBP levels and sperm motility and concentration. Monobutyl benzyl phthalate (MBBP) levels were inversely associated with sperm concentration. Hauser et al.(2006) studied 463 male partners of subfertile couples (including the 168 men in the previous study) who presented semen analysis at the infertility clinic. They compared urine concentrations of phthalates esters between 76 men with compromised sperm concentrations (<20 million/mL), 221 men with compromised sperm motility (<50% motile) and 114 with compromised morphology (<4% normal) with 210 subjects whose sperm concentration, motility and morphology was normal (above the three cut points). There was a dose-response relation between MBP and low sperm concentration (adjusted odds ratios per quartile: 1.00; 3.1; 2.5; 3.3, P for trend = 0.04) and

suggestive evidence for a dose-response relation between MBzP and low sperm concentration (adjusted odds ratios per quartile: 1.00; 1.1; 1.1; 1.9, P for trend = 0.13). No association was found between monoethyl phthalate, monomethyl phthalate and the DEHP metabolites and the three semen parameters.

In an analysis of 220 males, straight-line velocity (VSL), curvilinear velocity (VCL) and linearity (VCL/VCL) of sperm motion were inversely associated with levels of MBP, MBzP, and MEHP (Duty et al. 2004). The association between urinary concentration of phthalate metabolites and sperm DNA damage was reported in two analyses with partly same study subjects (Duty et al. 2005, Hauser et al. 2006). Various measures of sperm DNA damage were measured, including comet extent and tail distributed moment. The studied metabolites were MMP, MEP, MBzP, MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate, and mono(2-ethyl-5-oxohexyl) phthalate. There was an association between MEP and DNA damage. MEHP, a metabolite of DEHP, was associated with DNA damage after adjustment for the oxidative DEHP metabolites mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate. There is an indication of altered sperm motility and sperm DNA damage (as measured in chromosomal breaks) after exposure to DEHP and several other phthalates.

Huang et al. (2011) investigated sperm concentration, motility, morphology, and chromatin DNA integr ty in 45 male workers employed in two PVC pellet manufacturing plants in Taiwan.DEHP exposure was me sured by breathing-zone DEHP personal sampling and analysis covering the full work shift of workers Workers were divided in two subgroups with 23 low-DEHP-exposed subjects (average exposure con entration 7.9 µg/m^3) and 22 high-DEHP-exposed subject (average exposure concentration 56.3 µg/m^3) with comp rable demographic and reprodutive characteristics. After adjusting for coffee drinking, cigarette smoking a d age ersonal air concentrations of DEHP showed positive associations with sperm DNA denaturatio induction (beta = 0.038) and DNA fragmentation index (beta = 0.140) and negative associations wi h sperm mo ility (beta = -0.227).

Measures of testosterone (total, calculated free testosterone and the free andr gen index-FAI) were inversely correlated with the urinary concentrations of four DEHP metabolie in a s udy wi h 425 fertile US men (Mendiola et al., 2010).After adjustment to covariates like age, body mass i dex, smoking status, ethnicity, urinary creatinine concentration and time of sample collection here was no longer an association between urinary DEHP metabolite concentrations and total testoste one le els. But FAI was significantly inverse associated with the urinary concentration of the DEHP metabolites MEHP, 5OH-MEHP and 5oxo-MEHP. SHBG was positively associated with the urinary concen ra ion of MEHP, but not with other DEHP metabolites. According to the authors these data show only weak a socia ions between hormonal alterations and environmental exposure to DEHP. No associations were bserved between urinary DEHP metabolites and FSH, LH, inhibin B and estradiol.

These findings were widely confirmed when a s cond US population, n=425 male partners in an infertile couple, was investigated (Mendiola et al., 2012 .The obs ved associations between phthalate metabolites and reproductive hormones were robu nd i sensitive to the characteristics of the subpopulation. If the pooled population was analysed the investigators observed an inverse association between FAI and free testosterone and urinary concentrations of MEHP, 5OH-MEHP and 5oxo-MEHP. 5OH-MEHP and 5oxo-MEHP were positively associated with SHBG, and MEHP was nversely associated with E2.

Lin et al. (2011) inv stigated 155 maternal and infant pairs from Taiwan to evaluate the association between maternal phthalate xposure (nalysis of MEHP, 5OH-MEHP and 5oxo-MEHP for DEHP) and cord sex steroid hormones (free testo terone fT and estradiol). For male newborns no significant correlation was found between cord steroid h rmone le ls and DEHP metabolites.In female newborns, the maternal urinary levels of MEHP and 5OH-MEHP wer negatively correlated with fT and fT/estradiol with Pearson correlation coefficients ranging b twee -0.27 and -0.32 ($p<0.05$). This correlation persisted even after adjustment for gestational age.

Pubertal development

Two studies have investigated associations between pubertal development and phthalate exposure (Colon et al. 2000, Rais-Bahrami et al. 2004). The relation between serum phthalate concentrations and premature breast development was studied in a case-control study of 41 patients from the San Juan City Hospital Pediatric Endocrinology Division and 35 controls from the general pediatric care who did not have signs of premature sexual development (Colon et al. 2000). Higher serum levels of DMP, DEP, DBP, and DEHP plus its metabolite MEHP were measured in cases than controls. The average concentration of DEHP was 450 ppb in cases and 70 ppb in controls, the difference being statistically significant. This was not seen with other phthalates studied. There appears to be a correlation between DEHP exposure and breast development in young females. However, the quality of the data is uncertain due to laboratory and/or diagnostic procedures performed (CERHR 2005).

Rais-Bahrami et al. 2004 reported a 14-16 years follow-up study to DEHP toxicity noted in adolescents after a high DEHP exposure as neonates during extracorporeal membrane oxygenation (ECMO) support. The onset of puberty and sexual maturity was evaluated in 19 adolescents (13 males and 6 females). The results showed that

there were no significant adverse effects on their physical growth and pubertal maturity. Thyroid, liver, renal and male and female gonadal functions tested were within normal range for age and sex distribution. It was suggested that the acute and short-term exposure to DEHP by the intravenous route, and a lack of conversion of DEHP to MEHP may be protective against its long term adverse effects (Rais-Bahrami et al. 2004). A limitation of the study is the low number of individuals studied and the evaluation period of maximal 16 years.

In a 20 year follow up study Hack et al. 2002 compared young adults with a normal birth weight (mean 3279 gram, n=233) to very low birth weight (mean 1179 gram, n=242) individuals, assumed to have had a high DEHP exposure. The very low birth weight individuals showed educational disadvantages persisting into early adulthood. There were no differences observed concerning male fertility.

Endometriosis

Two case-control studies have investigated the relations between biomarkers of DEHP exposure and the risk of endometriosis. A case-control study of Cobellis and colleagues (2003) provided first evidence of an associa ion between plasma and peritoneal fluid levels of DEHP and the risk of endometriosis. The 24 cases were patients who underwent diagnostic laparoscopy for ovarian cysts or chronic pelvic pain and dysmeno rhoea and who had a histological confirmation of endometriosis. The 35 controls were healthy age matched individuals without infertility or reproductive diseases. The cases had a higher plasma concentration of DEHP (median 0.57 µg/ml, interquartile range 0.06-1.23) than the controls (0.18 µg/ml 0-0.44, P=0.0047), but the plasma MEHP and peritoneal DEHP and MEHP concentrations were similar. However, certain limitations in hese studies include possible exposure due to medical procedures, information on the selection of controls, evalu tion of confounding factors, and small sample size (CERHR Expert Panel 2005).

Reddy and colleagues (2006a) conducted a case-control study with 49 infertile women with endometriosis and two control groups. The first control group (I) included 38 age-matched wom n wi hout endometriosis but with infertility related to tubal defects, fibroids, polycystic ovaries, idi pathic infertility and pelvic inflammatory disease diagnosed by laparoscopy. The second control group (II) comprised 21 age-matched fertile women undergoing laparoscopic sterilisation. The endometriosis cases h d a signi icantly higher concentration of DBP (mean 0.44 µg/ml, SD 0.41), BBP (0.66, 0.61), di-n-octyl phthalate (DOP)_ (3.32, 2.17) and DEHP (2.44, 2.17) compared with both the first (DBP 0.08, 0.14; BBP 0.12 0.20; DOP 0; DEHP 0.50, 0.80) and second control group (DBP 0.15, 0.21; BBP 0.11, 0.22; DOP 0; DEHP 0. 5, 0.68). These studies indicate a correlation between the phthalate ester concentrations and the severity of endome riosis for all compounds.

Conclusion

There are various epidemiological stud es exami ing he reproductive effects of DEHP. These studies largely examine the relationship between urine levels of the DEHP metabolites and various effect parameters. Although some of these studies indicated ad rse effects o DEHP on reproductive functions and the developing organism, associations in these studies we e weak, contradictory, or confounded by exposure to other substances. In agreement with the EU Risk Assessment herefore the risk assessment is based on experimental data.

5.9.2. Developmental toxicity

5.9.2.1. Non human inf mation

The results of xperimental studies are summarised in the following table:

Table 46 Overview of experimental studies on developmental toxicity

Meth d	Results	Remarks	Reference
Standard developmental toxicity/teratogenicity studies			
rat (Wistar) inhalation: aerosol (nose/head only) 0, 0.01, 0.05, 0.3 mg/l (nominal conc.) Exposure: 6 hours (daily)	NOAEC (maternal toxicity): 0.3 mg/L air (nominal) NOAEC (fetotoxicity): 0.3 mg/L air (nominal)	1 (reliable without restriction) **key study** experimental result **Test material (EC**	Merkle J, Klimisch HJ, Jäckh R (1988) BASF (1986)

OECD Guideline 414 (Prenatal Developmental Toxicity Study)		**name): bis(2-ethylhexyl) phthalate**	
rat (F 344)) oral: feed 0.5, 1.0, 1.5 or 2% (nominal in diet) 357, 666, 856 and 1,055 mg/kg bw/day (actual ingested) Exposure: (ad libitum) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 357 mg/kg bw/day (actual dose received) (↓ maternal food intake from 0.5%; ↓ maternal bw gain, ↑ absolute and relative liver weights) NOAEL (embryotoxicity): 357 mg/kg bw/day (actual dose received) (↓ mean foetal bw from 0.5%; ↓ foetal bw/litter from 1.0%, ↑ number and percentage of resorptions, nonlive and affected implants/litter at 2%) NOAEL (teratogenicity): >= 1055 mg/kg bw/day (actual dose received)	1 (reliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tyl RW, Price CJ, Marr MC (1988)
rat (Wistar) oral: gavage 0, 40, 200, 1000 mg/kg bw/d (actual ingested) Exposure: day 6 through day 15 of gestation (daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	LOAEL (maternal toxicity): 1000 mg/kg bw/day (The maternal toxicity was only moderate.) LOAEL (teratogenicity): 1000 mg/kg bw/day (The number of foetuses bearing external (13.4% paar litter) soft tissue (75.3%) a d skeletal malformations (67.4%) was istinctly ncreased.) NOAEL (maternal toxicity): 200 mg/kg bw/day NOAEL (developmental toxicity): 200 mg/kg bw/day	2 (reliable w th restr ctions) **key study** experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Hellwig, J., Freudenberger, H., and Jäckh, R. (1997)
rat (Wistar) oral: gavage MEHP : 0, 2 5, 4 0 or 900 mg/kg bw/day (actua ingested) MEHP : 0, 50, 100 or 200 mg/kg bw/d y (actual ingested) Exp sure On the 6th day of gestation to 15th day of gestation. (Daily: between 9-10 o'clock each morning) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 50 mg/kg bw/day NOAEL (teratogenicity): 225 mg/kg bw/day LOAEL (teratogenicity): 450 mg/kg bw/day (This dose affected the mean litter weight of the live pups.)	2 (reliable with restrictions) supporting study experimental result **Test material (IUPAC name): mono-2-ethylhexylphthalate (MEHP)**	Ruddick, J.A, Villeneuve, D.C, Chu, I, Nestmann, E, and Miles, D. (1981)
mouse (CD-1) oral: feed	NOAEL (maternal toxicity): 91 mg/kg bw/day (actual dose received) (↓ maternal body	1 (reliable without restriction) key study	Tyl RW, Price CJ, Marr MC (1988)

0.025, 0.05, 0.10, 0.15 % DEHP (250, 500, 1000, and 1500 ppm, respectively) (nominal in diet) 44, 91, 190.6 or 292.5 mg/kg bw/day (actual ingested) Exposure: (ad libitum) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	weight gain from 0.10% (mainly due to ↓ uterine weight, ↓ foetal body weight and number of live foetuses per litter)) NOAEL (embryotoxicity): 44 mg/kg bw/day (actual dose received) (↑ number and percent of resorptions, late foetal deaths, dead and malformed foetuses, and percent malformed foetuses/litter from 0.05% (open eyes, exophtalmia, exencephaly, short, constricted or no tail); visceral malformations and skeletal defects (fused and branched ribs, mis-alignement, and fused thoracic vertebral centra))	experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	
mouse (CD-1) oral: gavage 0, 40, 200, 1000 mg/kg/d (actual ingested) Exposure: day 6 through 15 of gestation (1/d) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (embryotoxicity): 40 mg/kg bw/day (↑ Skeletal, visceral, and external variations and malform tions ↑ Prenatal mortality at higher doses) NOAEL (mat rnal oxicity): 200 mg/kg bw/d y (↑ Liver weight, ↓weigh ga n at 1000 mg/kg)	2 (rel ble with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Reader SCJ (1996)
mouse (ICR) oral: feed 0.05, 0.1, 0.2, 0.4 and 1.0% (nominal in diet) 70, 190, 400, 830 and 2,200 mg/kg bw (actual ingested) Exposure: (d libitum) equivalent or imilar to OECD Guideline 414 Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 400 mg kg bw/day (decrease in body weight gain from 0 %) NOAEL (developmental toxicity): 70 mg/kg bw/day (increase of fetal resorption and external malformations from 0.1%)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Shiota, K., Nishimura, H (1982)
mouse (CD-1) oral: feed 0, 0.017, 0.035, 0.070 or 0.140 % (nominal in diet) 35, 73, 134 or 269 mg/kg/bw (actual ingested (Based on the average food intake for mice during gestation was 175 g/kg/day)) Exposure: from day 0 to day 17 of gestation (continuously)	NOAEL (maternal toxicity): 134 mg/kg bw/day (increased relative liver weight, decreased weight gain) LOAEL (teratogenicity): 35 mg/kg bw/day (↑ Litters with resorptions, ↑ Fetuses with malformations, ↓ Fetal weight)	1 (reliable without restriction) supporting study experimental result **Test material (IUPAC name): mono(2-ethylhexyl) phthalate (MEHP)**	National Toxicology Program (NTP) (1991)

equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)			
Other developmental toxicity studies			
rat (Sprague-Dawley) oral: feed 1.5 (2 groups), 10, 30, 100, 300, 1000, 7500, 10000 (2 groups) ppm (nominal in diet) 0.12, 0.78, 2.4, 7.9, 23, 77, 592, and 775 mg/kg/day (actual ingested (F0 animals)) 0.09, 0.48, 1.4, 4.9, 14, 48, 391, and 543 mg/kg/day (actual ingested (F1 animals)) 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg/day (actual ingested (F2 animals)) Exposure: 3 generations in F3 (F3c) (Daily) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)	NOAEL (generations F0, F1 and F2) (maternal toxicity): 300 ppm (nominal), equivalent to approximately 23 mg DEHP/kg bw/day in the F0 animals, and 14 mg DEHP/kg bw/day in the F1 and F2 animals (Reductions in bodyweights noted in both sexes at 7,500 (F1, F2 animals) and 10,000 ppm (F0, F1 animals), absolute and/or relative organ weight changes noted at 1000 ppm and above (increased liver: 1,000 ppm and above; increased kidneys: 1,000 ppm and above; increased adrenals: 10,000 ppm; increased pituitary: 10,000 ppm), and microscopic pathological findings no d at 1,000 ppm and ab ve (liver hypertrophy: 1,000 ppm and above; cortex vacu isation of the adrenals: 7,500 ppm and above; d ati n of t e tubules and mineralization in the kidneys ccasionally ssociated with chronic pyelonephritis: 1,000 ppm and above). Microscopic pa hological findings in the a renal glands were also indicated in F1 animals at 1,000 ppm (no further data)) NOAEL (generations F1 and F2) (developmental toxicity): 100 ppm (nominal), equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and approximately 4.8 mg DEHP/kg bw/day in the F1 and F2 animals (Decreased absolute and/or relative testis weights noted at 7,500 (F1, F2 and F3 males) and 10,000 ppm (F0 and F1 males), macroscopic pathological findings (small or aplastic testes) noted at 300 (3/45 non-mating F1 males, 1/21 non-mating F2 males), 1,000 (3/25 non-mating F2	1 (reliable without restriction) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Wolfe GW and Layton KA (2004) Peckham JC (2003) Blystone CR, Kissling GE, Bishop JB, Chapin RE, Wolfe GW and Foster PMD (2010)

	males), 7,500 (7/10 mating F1 males, 10/30 non-mating F1 males, 9/10 mating F2 males, 11/20 non-mating F2 males) and 10,000 ppm (2 or 3 of 10 F0 males, 10/10 mating F1 males, 21/21 non-mating F1 males), and microscopic pathological findings (testis seminiferous tubular atrophy) noted at 300 (1/10 F1 males), 7,500 (all F1 and F2 males) and 10,000 ppm (all F1 males, 2 or 3 of 10 F0 males))		
rat (Wistar) male/female Testicular toxicity oral: gavage 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg/day (actual ingested) Exposure: Females F0 was exposed from day 6 of gestation to day 21 of lactation. (Daily) Evaluation of the possible long-term effects of developmental DEHP exposure on male reproductive tract structure and function, including the investigation of reproductive organ weights, testicular function, hormonal status, sexual behavior and fertility	NOAEL (F1): 1.215 mg/kg bw/day (male) LOAEL (reduction of sperm production) (F1): 15 mg/kg bw/day (male) LOAEL (reproductive tract abnormalities) (F1): 5 mg/kg bw/day (male)	1 (reliable without restriction) supporting study experimen l r ult **Test material (EC name): bis(2-ethylhexyl) phthalate**	Andrad A M, Grande, SW, Ta sness, CE, Grote, Golombiewski, A, Sterner-Koc (2006b)
rat (Long-Evans) male Testicular toxicity oral: gavage 100 mg/kg/day (actual ingested) Evaluation of the impact on steroidogen sis in male t after DEHP-treatment in tero (GD 12-21).	dos lev l: : 100 mg/kg bw/day (female) (decreas. se um concentration of testosterone in offspring at 21 and 35 days of age)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Akingbemi BT (2001)
rat (Sprague-Dawley) oral: gav ge 0, 37 , 750, 1500, 3000 (nominal in diet (mg/kg/day)) Exposure: From gestation day 3 to postnatal day 21. (Daily) Exposition from day 3 through post natal day (day 21). Group of 5-8 rats. Study of male reproductive system development. 3 doses and control group.	LOAEL (male reproductive system development) (developmental toxicity): 375 mg/kg bw/day (Two effects were statistically significant at this dose: reductions in anterior prostate weight and permanent nipple retention.)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Moore, RW., Rudy, TA., Lin, TM., Ko, K. and Peterson, RE. (2001)

rat (Sprague-Dawley) oral: gavage 0, 11, 33, 100, 300 mg/kg/day (actual ingested) Exposure: From day 8 of gestation to day 17 of lactation. (Daily) Dams were exposed during pregnancy and lactation to relatively low levels of DEHP by gavage, followed by a thorough examination of a sufficient number of adult offspring to be able to detect low incidence of males with the Phthalate Syndrome	no NOAEL identified : LOAEL (developmental toxicity): 11 mg/kg bw/day (A significant percentage of F1 males displayed one or more Phthalate Syndrome lesions at 11 mg/kg/day DEHP and above)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Gray, L.E.-Jr, barlow, N.J., Howdeshell, K.L., Ostby, J.S., Furr, (2009)
rat (Sprague-Dawley) oral: gavage 0, 100, 300, 600, and 900 mg/kg/day; (actual ingested) Exposure: on GD 8–18 (daily) Effects of DEHP on male fetal testosterone production after in utero exposure	NOAEL (maternal toxicity): >= 900 mg/kg bw/day (No effect on maternal body weight gain, litter size, resorptions, and fetal mortality) NOAEL (developmental toxicity): 100 mg/kg bw/day (↓ testicular testosterone production at >= 300 mg/kg bw)	2 (reliable with restrictions) suppo ting study experim ntal result **Te t m terial (EC nam): bis(2-ethylhexyl) phthalate**	Howdeshell KL, Wilson VS, Furr J, Lambright CR et al. (2008)
rat (Sprague-Dawley) oral: gavage 500, 1000 mg/kg (actual ingested (Exp 1)) 125, 250, 500mg/kg (actual ingested (Exp 2)) Exposure: (daily) Observation of the histopathological changes of testicul r development by intra-uterine exposu e to DEHP	NOAEL (developmen al toxicity) 125 g/kg bw/day OAEL (d velopmental toxicity): 250 mg/kg bw/day (degeneration of germ cells in G 6 fetuses and localized p oliferation or hyperplasia of interstitial cells in G18 and 20 fetuses from 250 mg/kg bw)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Shirota M, Saito Y, Imai K, Horiuchi S et al. (2005)
rat (Sprague-Daw ey) oral: gavage from 58 to 1250 mg/kg/day (actual ingested) Expo ure: (daily) Th effects of fetal exposure to a wide range of DEHP doses were examined on fetal, neonatal, and adult testosterone production. Pregnant rats were administered DEHP from Gestational Day (GD) 14 to the day of parturition (Postnatal Day 0).	no NOAEL identified :	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Culty M (2008)
rat (Wistar)	NOAEL (maternal toxicity):	1 (reliable without	Grande, SW,

oral: gavage 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg bw/day (actual ingested) Exposure: From day 6 of gestation to day 21 of lactation. (Daily) Evaluation of the possible reproductive effects of low (human relevant) and high doses of DEHP on female offspring rats exposed in utero and during lactation.	135 mg/kg bw/day (significant increase in liver and kidney weights at 405 mg/kg bw/d) NOAEL (developmental toxicity): 5 mg/kg bw/day (delays in the time of pubertal onset in female offspring at >= 15 mg/kg/d)	restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Andrade, AJM, Talsness, CE, Grote, K, and Chahoud, I. (2006)
rat (Wistar) oral: gavage 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg/day (actual ingested) Exposure: From day 6 of gestation to day 21 of lactation. (Daily) Evaluation of the sexual dvelopment of male offspring from birth until puberty including the investigation of androgen sensitive endpoints, sexual developmental landmarks and testicular histology	NOAEL (maternal toxicity): 135 mg/kg bw/day (significant increase in liver and kidney weights at 405 mg/kg bw/d) LOAEL (developmental toxicity): 5 mg/kg bw/day (delay in age at preputial separation at >= 15 mg/kg/day, histopathological changes of testis at >= 135 mg/kg/day, marginal tes is weight increase on PND 22 at >=5 mg/kg/day educed anogenital distanc and nipple retention at 405 mg/kg/day)	1 (reliable without restriction) supporting study experimental resul **Test materi l (EC name): bis(2-ethylhexyl) phtha ate**	Andrade, AJM, Grande SW Talsness, CE, Gr te, K, Golombiewski, A (2006a)
rat (Wistar) oral: gavage 0.015, 0.045, 0.135, 0.405, 1.215 mg/kg/day (actual ingested) 5, 15, 45, 135, 405 mg/kg/day (actual ingested) Exposure: From day 6 of gestation to day 21 of lactation (Daily) Evaluation of the pos ible effects of low and high DEHP doses on female reproductive fun tion later in life (adulthoo) fol owing in utero and lac ational DEHP exposure	LOAEL (developmental toxicity): 135 mg/kg bw/day significan increase in t rtiary fo licles undergoing atre ia at 405 mg/kg bw.)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Grande, SW, Andrade, AJM, Talsness, CE, Grote, K, Golombiewski, A (2007)
mouse (CD-1) oral: feed 00.01, 0.1, 0.3% (nominal in diet) 14, 141, and 425 mg/kg bw/day, (actual ingested) Exposure: (ad libitum) Reproduction and fertility were assessed The mice were exposed to the	NOAEL (maternal toxicity): 425 mg/kg bw/day (↑ absolute and relative liver weight (both sexes) and ↓ reproductive organ weights and atrophy of seminiferous tubules at 0.3%; no effect on bw) NOAEL (developmental toxicity): 14 mg/kg bw/day (dose-dependent ↓ in the number of litters and proportion of pups born alive	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Lamb JC, Chapin RE, Teague J (1987) Kimmel C (1997)

chemical for a 7-day pre-mating period and during the a 98-day cohabitation period. Cross mating tests was realised to evaluate the influence of the sex on reproductive functions.	from 0.1% (0.1%: 14/19 fertile, 0.3%: 0/18))		
rat (Sprague-Dawley) oral: gavage 0, 00.08, 0.4, 2, 10µmol//ml (nominal conc. (MEHP (PART 1))) 0.002, 0.01, 0.04, 0.2, 1, 5µmol/ml (nominal conc. (metabolites I, V, VI, IX (PART 2))) 0, 0.04, 0.2, 1.0, 5.0 µmol/ml (nominal conc. (2-ethylhexanol, 2-ethylhexanoic acid (PART 3))) Exposure: (once) Influence of DEHP/MEHP metabolites upon in vitro growth and development of GD9 rat embryos	(embryotoxicity): (Potential embryotoxicity: metabolites IX >I>VI>V>MEHP>DEHP = 2-ethyl hexanol > 2-ethyl hexanoic acid.)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Bowden HC (2001) Laignelet L, Lhuguenot JC (2000e)
mouse (CD-1) male/female Developmental neurotoxicity oral: feed 0, 100, 300, 900ppm (nominal in diet) 0, 17, 47, and 140 mg/kg bw/day during gestation, 0, 60, 172, and 493 mg/kg bw/day during lactation, and 0, 16–19, 48–56, and 145–171 mg/kg bw/day from weaning to 9 weeks of age in F1 offspring (a tual ingested) Exposure: from 5 weeks of age for the Fo generation t 9 weeks of age for the F1 generation (ad libitum) Examination of the n urobehavioral toxicity in mic exposed to DEHP	NOAEL (P): >= 140 — 493 (female) NOAEL (F1): 47 — 1 2 mg/kg bw/day (mal /female) based on: test m t (de reased survival uring l c tion period)	2 (reliable with r trictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Tanaka T (2002)

5 9 2 2. Human information

The exposure-related observations in humans are summarised as followed:

Hypospadias and cryptorchism.

Van Tongeren and colleagues (2002) developed a job-exposure matrix (JEM) to assess exposure to potential endocrine disrupting agents, including phthalates. Vrijheid and colleagues (2003) applied this JEM in a study of 3471 hypospadias cases identified from the National Congenital Anomaly System of England and Wales in 1980-1996, which included a total of 35962 cases of congenital anomalies. The authors compared the prenatal exposures of hypospadias cases with exposures of all the cases. The risk of hypospadias was not related to estimated maternal occupational exposure to phthalates. For 1992-96 there was an increased risk of hypospadias related to probable exposure, mainly among hairdressers, with an adjusted odds ratio of 1.52 (1.05-2.20) without

social class adjustment, and 1.26 (0.81-1.97) after such adjustment. The JEM was also applied in a Dutch nested case-control study of 56 cases of hypospadias and 78 cases of cryptorchism and 313 controls selected from a cohort of 8,698 male newborns. No association was found between estimated occupational exposure to potential endocrine disrupting agents and these outcomes (Pierik et al. 2004). In a study on contamination of breast milk with phthalates no association was found between breast milk phthalate monoester levels and cryptorchidism, but other potential anti-androgenic metabolites were not measured (Main et al. 2006).

Birth weight and gestational age

Latini and colleagues (2003a) measured serum DEHP and MEHP concentrations in the cord blood of 84 consecutive newborns. Detectable cord blood pthtalates concentrations were found in almost 90 % of these individuals. In this single study the mean gestational age was significantly lower among newborns with detectable cord blood MEHP compared with those without (38.2 vs. 39.4 weeks). Also the mean birth weight was lower (3,150 vs. 3475 g) although the difference was not statistically significant. In logistic regressi n analysis adjusting for potential confounders, the absence of MEHP was a significant determinant of ge tati nal age. This study suggests a possible effect of DEHP on pregnancy outcome.

Decreased anogenital distance

According to a recent review (Scott, 2009), the effects of phthalate exposure on perina al testost rone production (assessed as AGD) in humans has been investigated in several studies, the results of which are conflicting. The first cross-sectional study (in the United States) examined 85 boys at 2–36 month of ge and found a negative correlation between AGD (corrected for body weight) and the level of certain phtha te metabolites, including monobutyl phthalate (MBP) and monoethyl phthalate (MEP), found in maternal urine during pregnancy (Swan 2005). A recent expansion of this study to include a total of 106 boys has c nf rmed the negative correlation between AGD and maternal (urinary) levels of phthalates, including MEP MBP, monoethyl hexyl phthalate (MEHP) and the further MEHP metabolites, monoethyl hydroxyhexyl phth late and monoethyl oxohexyl phthalate (Swan 2008). Both of these studies also demonstrated tha AGD c rrelated to penile volume/length (Swan 2005, Swan 2008) and the incidence of cryptorchidism (Swan 2005), similar to rat studies (Welsh 2008). However, the data were considered insufficient as solid evidence f r an effect and need further elaborations with larger studies, but do add to the concern for male reproductive effects (Kaiser 2005, Sharpe 2005).

Another study of 73 pregnant Mexican women in a ho pital based cohort investigated the association between exposure to MEHP, monobenzyl phthalate, MEP and MBP during pregnancy and AGD in male newborns (Bustamente-Montes 2008). This study found a s atistically significant association between MEP exposure and reduced AGD and also between monobenzyl phthal te exposure and reduced penis length and width. These studies are consistent with the possibili y that per natal phthalate exposure can inhibit testosterone production in the male fetus (during the masculinizati n programming window), resulting in reduced AGD and penile volume/length, as well as inhibi ing normal te tis descent; such effects are in broad, but not total, agreement with studies in rats. However, one point of disagreement is with regard to diethyl phthalate and its metabolite MEP.

In contrast to the above-mentioned stud es, a smaller (prospective) study in Taiwan, involving 33 boys, found no relationship between lev ls of MBP or MEHP, measured in AF or maternal urine, during pregnancy, and AGD of the male offspring (Huang 2009).

This finding fits with two in vitro studies that have investigated the effects of MBP or MEHP on human fetal testis testost rone production. In the first study, second-trimester human fetal testis explants were cultured with MBP in short term culture, but there was no effect on basal or hCG-stimulated testosterone production (Hallmark 2007). In the second study, exposure of first-trimester human fetal testis explants to MEHP in the pre ence o absence of LH/hCG found no effect on testosterone production or on steroidogenic enzyme expr ssion (Lambrot 2009). The latter study did, however, demonstrate that MEHP has negative effects on germ cells, as it did in fetal rat testis explants, which makes the lack of effect on testosterone production more c nvincing.

An increase in SHBG would result in less free testosterone because more would be able to bind to SHBG, and this would result in an increase in LH because of reduced negative feedback, thus explaining the increased LH-free testosterone ratio observed without the need to invoke direct inhibition of steroidogenesis by the phthalates. Phthalates are known to act on the liver, which is also the source of SHBG, but it is unknown whether phthalate exposure affects SHBG production.

Conclusion

There are various epidemiological studies examining the reproductive effects of DEHP. These studies largely examine the relationship between urine levels of the DEHP metabolites and various effect parameters. Although some of these studies indicated adverse effects of DEHP on reproductive functions and the developing organism,

associations in these studies were weak, contradictory, or confounded by exposure to other substances. In agreement with the EU Risk Assessment therefore the risk assessment is based on experimental data.

5.9.3. Summary and discussion of reproductive toxicity

Discussion

About 90 studies were reviewed in the evaluation of the reproductive toxicity of DEHP. Collectively, these studies were undertaken predominantly in rodents and build on the original observation that DEHP produced testicular atrophy in a subchronic toxicity study. The literature contains many redundant studies, usually at high doses (e.g., 2 g/kg, usually in rats), all of which show similar effects on the testes. A number of more specifi studies in the rat have attempted to investigate the mode of action of DEHP using *in vivo* and *in vitro* rotocols.

Multigeneration reproductive toxicity studies

Rats

Oral

Wolfe and Layton (2004) studied the multigenerational reproductive toxicity of DEHP in Spragu -Dawley rats. The methodology used in this study to a large extent complied with OECD Guid line 416. The number of animals in each test and control group was 17 males and 17 females only (the Guid line recommends a sufficient number of animals in each test and control group to yield preferably not les than 20 pregnant females at or near parturition). However, it is considered that enough pregnancies were produced in the study to assure a meaningful evaluation. Therefore the failure to achieve the desired number pregnant animals does not invalidate the study. The F0 animals were administered the test article during a 6 week pre-mating period (according to the Guideline a dosing continued for at least 10 weeks before he mating period is required). This, however, is not considered to be a serious deviation since the s udy was conducted on three generations instead of two, and males of two generations (F1 and F2 animals) were d sed d ring complete spermatogenic cycle. Thus the study has provided satisfactory information c ncerning effects on spermatogenesis.

The 10,000-ppm animals only completed the F1 generation and were terminated due to the inability to produce any F2 generation animals. This, however, is not consid red t be a serious deviation since the number and choice of original dose levels (10,000-ppm group not in uded) is considered to be satisfactory for the purpose of the study.

In excess of Guideline requirements, crossover cohabitation was performed on the control and selected F1/F2 animals. This is considered to be scientifically ad antageous as it may provide additional data on effects on fertility. Except for females dur ng the lacta i n period body weight and food consumption of parent animals was measured at limited time point approximately every second week (the Guideline recommends weekly measurements at a minimum). However the limited number of measurements made in this study is considered to be satisfactory for the purpose of the study.

At the time of term nation brain and spleen were not weighed in adults or in pups. Nor where thymus weighed in pups. However, the deviation from the Guideline do not affect the scientific validity of this study. A complete necropsy was perform d on all surviving control animals and 10 treated animals from each dose group for each sex (according to th Guideline full histopathology should be performed for all high dose and control animals selected for m ting, and organs demonstrating treatment-related changes should also be examined in the low-and mid dose group). However, tissue samples from a number of 10 animals/sex/group are in this study considered eno gh to assure a meaningful histopathological examination. The deviations and/or omissions from the Guidel ne do not affect the scientific validity of this study.

The study is performed according to GLP (although statement not yet signed), and considered acceptable.

DEHP (purity 99.8%) was administered in the diet at concentrations of 1.5 (Control 1 and 2), 10, 30, 100, 300, 1,000, 7,500 and 10,000-ppm to groups of 17 male and 17 female Sprague-Dawley Crl: CD®BR rats (source: Charles River Laboratories, Portage, Michigan). The control dose level was set at 1.5-ppm, as this was the amount of DEHP found in the control feed. The 10,000-ppm group and their corresponding control group (Control 2) were added to the study structure after the initiation of the original seven dose groups and followed the same study design. Mating pairs were allowed to produce three litters (a, b, c) each.

Animals in the F0 generation began exposure as adults (5 weeks of age) and were bred to produce the F1 generation (F1a, 1b, 1c), the F1 adults (selected from F1c weanlings) were bred to produce the F2 generation (F2a, 2b, 2c), and the F2 adults (selected from F2c weanlings) were bred to produce the F3 generation (F3a, 3b,

3c). The animals were administered the test article during the pre-mating period (6 or 10 weeks), and also during the mating-, gestation- and lactation periods for breeding of the F1, F2 and F3 litters/pups until the day of necropsy (approximately 2 weeks after the last weaning). The F1, F2 and F3 animals received diets containing DEHP after weaning (day 21 post partum) with the same concentration of DEHP as their parents received until necropsy. Additional non-mating males (up to three per litter) were selected from the F1c, and F2c litters, and were maintained following similar procedures as those for mating males, except they were not cohabited with females. The 10,000-ppm animals only completed the F1 generation and were terminated due to the inability to produce any F2 generation animals. A one-week cross over cohabitation was performed on the control and 10,000-ppm F1 animals (up to 17 animals/sex/group), and on the control and 7,500-ppm F2 animals (up to 17 animals/sex/group) in order to determine the affected sex. F1 and F2 animals were then paired with naive animals and received control feed during the cohabitation. Upon separation, the F1 and F2 animals received dosed feed.

Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, reproductive performance, anogenital distance, pup survival, sexual development, oestrous cyclicity, sperm endpoints, gross pathology, organ weights, and limited/selected histopathology. B sed on measured feed consumption, mg/kg daily doses were calculated to be 0.12, 0.78, 2.4, 7.9, 23 77, 592 nd 775 mg/kg bw/day in the F0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391 and 543 mg/kg bw/day in the F1 animals; and 0.1, 0.47, 1.4, 4.8, 14, 46 and 359 mg/kg bw/day in the F2 animals.

Parental data(general condition and behaviour, bodyweight, food intake)

The incidence of intercurrent deaths amongst treated F0 animals (17 animal /sex/group) was 1, 1, 0, 0, 1, 1, and 0 in the 10, 30, 100, 300, 1,000, 7,500 and 10,000-ppm groups. The respective numbers for the F1 generation (17 animals/sex/group) were 0, 1, 1, 1, 1, 2, and 0 in the 10, 30, 100, 300, 1 000 ,500 and 10,000-ppm groups. The incidence of intercurrent deaths amongst treated F2 animals (17 animals/s x/gr up) was 1, 2, 0, 0, 1, and 1 in the 10, 30, 100, 300, 1,000 and 7,500 groups. The incidence of intercurrent dea hs amongst animals in the control groups was 0-2. Clinical signs were generally comparable among all group in all generations and were not treatment-related in incidence or severity (stated by the au hor). Comm nt: No data were available to confirm this statement.

Statistically significant reductions in terminal body weight were noted in adult animals at 10,000 (F0 males: 6%; F1 mating males: 16%; F1 non-mating males: 21% F1 f males: 19%) (There were no F2 animals at 10,000-ppm) and at 7,500-ppm (F1 non-mating males: 0%; F2 m ting males: 14%; F2 non-mating males: 14%; F2 females: 8-18% during Week 1-6). Statistically significant reductions in dam body weights were also noted at delivery (9-11%) and during lactation (11-20%) in F0 females at 10,000-ppm.

Parental feed consumption was generally comparable in all groups in all generations on a g/animal/day basis, but was statistically significant incr ased at 7,500 and 10,000-ppm on a g/kg bw/day basis, except during lactation where dam feed consumption was statistically significant decreased in F0 animals at 7,500 (17%) and 10,000-ppm (11%, PND 4-7).

Reproductive toxicity: F1-, F2-and F3-Mating Trial

Pregnancy indices were decr ased at 7,500 and 10,000-ppm. None of the F1 mating pairs produced offspring at 10,000-ppm (this finding was correlated with no sperm or spermatids noted in these animals), and at 7,500-ppm statistically sig ificant decreases in the pregnancy indices were noted for the F2 mating pairs. The total number of males per l tter as d creased at 10,000-ppm in the F1a litter (26%) and at 7,500-ppm across all F1 litters combined (F1a F1b+F1c) (approximately 20%). The total number of F1a pups per litter was decreased at 7,500-ppm (22% and t 10,000-ppm (21%). The total number of pups per litter across all F1 (F1a+F1b+F1c) litters combined (18%) was also decreased at 7,500-ppm. There was also an increase in the number of cumulative days to deliv r the F1a litter for F0 animals at 10,000-ppm.

At 10, 00-ppm, male and female pup weights, unadjusted and/or adjusted for litter size, were decreased in the F1a F1b and F1c litters (7-12%). At 7,500-ppm male and female pup weights, unadjusted and adjusted for litter size, were decreased in the F2c litter (14%) and combined F2a, 2b, 2c litters (10%).

Male anogenital distance (AGD) was decreased at 10,000-ppm in the F1a, F1b, and F1c pups (8-15%) and at 7,500-ppm in the F1a and F1b pups (6.6-8%), in the F2a and F2c pups (13- 17%) and in the F3a pups (13%). No changes were noted in the female AGD throughout all the mating trials. Retained nipples were observed in the F3c male pups (11%) at 7,500-ppm. Testes descent, vaginal opening, and preputial separation were delayed at 10,000-ppm in the F1c pups, and at 7,500-ppm in the F1c, F2c and F3c pups.

The relative length of time spent in estrous stages was statistically significant increased for the F0 females at 10, 300, 1,000 and 7,500-ppm. However, no changes were revealed in the number of females with regular cycles,

cycle length, number of cycles and in number of cycling females across the dose groups as compared to the control.

Reproductive toxicity: F1- and F2 Crossover-Mating Trial

At 7,500 and 10,000-ppm, when treated males were crossed with nulliparous naive females, there were decreased numbers of implantation sites (54% at 7,500-ppm, 98% at 10,000-ppm), and decreased indices of mating, pregnancy (8/17 versus 15/17 at 7,500-ppm; 0/17 versus 11/17 at 10,000-ppm), and fertility (8/14 versus 15/17 at 7,500-ppm; 0/17 versus 11/17 at 10,000-ppm).

At 7,500 and 10,000-ppm, when treated females were crossed with naive males, there was a decrease in AGD in the male pups (11.5% at 7,500-ppm; 17% at 10,000-ppm). Also at 7,500-ppm, male, female, and combined pup weights were decreased, both when unadjusted and adjusted for litter size (8-16%).

Sperm end-points

At terminal necropsies, various sperm end-points were found to be decreased at 7,500-ppm in th F1, F2 and F3 males and at 10,000-ppm in the F0 and F1 males. Epididymal sperm density was decreased at 7,500-ppm in the F2 (64%) and F3 males (94%), and at 10,000-ppm in the F1 males (99.6%). Comment: A a result of echnical difficulties the epididymal sperm data for the 10,000-ppm F0 males was not obtained. Total sp rmatid/cauda was decreased at 7,500-ppm in the F1 (61%), F2 (73%) and F3 males (95%), and at 10,000-ppm in th F1 males (99.8%). Total spermatid/testis was decreased at 7500-ppm in the F1 (69%), F2 (74%) and F3 males (79%), and at 10,000-ppm in the F0 males (31%). At 10,000-ppm no spermatids were present n the testes of F1 males. Spermatid/mg testes was decreased at 7,500-ppm in the F1 (56%), F2 (57%) and F3 males (67%). Decrease in the motile percentage was noted in the F2 males (25%) at 7,500-ppm. De rease of 12.8% in track speed was revealed along with a 15.6% decrease in the lateral amplitude in the F0 male at 10 000-ppm. Abnormal sperm morphology was seen in the F2 males at 100, 300, 1,000 and 7,500 ppm (stated by the author). Comment: No further data were available.

The results above show statistically significant organ weight ch nges noted in adult animals in the liver, kidney, and male accessory sex organs at the 7,500 and 10,000-ppm doses, and in the liver at 10 (males only), 300 (females only) and 1,000-ppm. At 10,000-ppm organ w ight changes were also observed in adrenal glands (males only), pituitary (males only), uterus and ovari s.

Statistically significant organ weight changes w re also noted in F3 animals. A dose-related increase in the absolute and relative liver weights were noted in he 1,000-ppm (21% and 17%, respectively) and 7,500-ppm (51% and 63%, respectively) males. The relativ liv r weight was also increased (36%) in the 7,500-ppm females. Absolute and relative right tes is weight were decreased in the 7,500-ppm males (48% and 45% respectively). Decreases in absolute dor olateral prostate weight (41%) and relative epididymis weights (35%) were also noted for the 7,500-ppm males.

Comment: no individual anima data on ross observations were available.

All other gross findings seen at necropsy were considered not dose related and incidental. Aplastic testes, epididymis and seminal vesicles, and small testes and epididymis were noted in 1-3 non-mating males at 300-ppm. At 1,000-ppm small pro tates were noted in 3 or 4 non-mating males. In comparison of the incidence of these findings to Th rImmun s (the laboratory in question) historical control data, the incidence of the findings in the semin l esicle and prostate is similar while the incidence for male testis and epididymis is increased (stated by the auth r)

Comment No d ta were available to confirm this statement (historical control data were not included in the draf).

Histopathology

In the estes, minimal to marked atrophy of the seminiferous tubules characterized by loss of germ cells and the presence of Sertoli cell-only tubules, as well as occasional failure of sperm release, were noted at 10,000-ppm in the F0 and F1 males, and at 7,500-ppm in the F1 and F2 males. Minimal atrophy of seminiferous tubules was also observed in F1 males at 100-ppm (1/10) and at 300-ppm (1/10). The changes noted in the testes were correlated with “small testis” observed grossly in the most severe cases of F0 males, and were found in all 7,500 and 10,000-ppm F1 males. In F2 males atrophy of the seminiferous tubules, presents in 10/10 males at 7500-ppm, was correlated to the gross observation of atrophy, and there was failure of sperm release in 1/10 males. Comment: No data were reported for the 1,000-ppm group. Secondary changes were present in the corresponding epididymis including sloughed epithelial cells/residual bodies (3/10 F0 males at 10,000-ppm; 6/10 F1 males at 7500-ppm) and aspermia (1/10 F0 males at 10,000-ppm; 4/10 F1 males at 7,500-ppm; 9/10 F1 males at 10,000-ppm). Secondary changes (including aspermia, oligospermia, residual bodies/sloughed epithelial cells)

were also present in the corresponding epididymis of F2 males at 7,500-ppm (number of animals not specified).

Minimal to mild hepatocellular hypertrophy was noted at 10,000-ppm in the F0- (males: 9/10; females: 10/10) and F1 animals (males: 6/10; females 9/10), at 7,500-ppm in the F0- (males: 10/10; females 9/10), F1- (males: 10/10; females: 10/10) and F2 animals (males: 10/10; females: 10/10), and at 1,000-ppm in the F1- (males: 5/10) and F2 animals (number of animals not specified).

Dilatation of the tubules and mineralization occasionally associated with chronic pyelonephritis was observed at 10,000-ppm in the F1 animals (males: 5/10; females: 3/10), at 7,500-ppm in the F1- (males: 3/10; females: 5/10) and F2 animals (males: 4/10; females. 5/10) and at 1,000-ppm in the F1 animals (females 1/10)

Cortex vacuolisation of the adrenals was noted at 10,000-ppm in the F0- (males 6/10 versus 1/10 in the controls) and F1 animals (males 5/10 versus 1/10 in the controls), and at 7,500-ppm in the F1 animals (males 4/10 ver us 2/10 in the controls).

Comment: Findings in the adrenal glands (not specified) were also noted in the F1 animals at 1,000-ppm (tated by the author). No further data were available.

Results of the Pathology Working Group's (PWG's) reexamination

Sertoli cell vacuolation was observed in the control group as well as in the 1,000-ppm nd 7,500 ppm F1 males. It was not observed in the 10,000-ppm animals with diffuse seminiferous tubule atrophy. In the 7,500-ppm males, Sertoli cell vacuolation was observed in seminiferous tubules without atrophy This vacuolation was similar to that observed in the control group males

Comment: The vacuolation of Sertoli cells observed resulted from distortion during fixation and processing of the tissues according to the PWG. This distortion could have obscured any m nimal oxic effects that may be present.

Conclusion

The no-observed adverse effect level (NOAEL) for testicular toxi ity in his study was 100-ppm (equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animal and approximately 5 mg DEHP/kg bw/day in the F1 and F2 animals) and was based on decreased absolute and/ r relative testis weights noted at 7,500 (F1, F2 and F3 males) and 10,000-ppm (F0 and F1 males), macroscopic pathological findings (small or aplastic testes) noted at 300 (3/45 non-mating F1 males, 1/21 non-mati g F2 mal s), 1,000 (3/25 non-mating F2 males), 7,500 (7/10 mating F1 males, 10/30 non-mating F1 males, 9/ 0 mating F2 males, 11/20 non-mating F2 males) and 10,000-ppm (2 or 3 of 10 F0 males, 10/10 mating F1 m les, 21/21 non-mating F1 males), and microscopic pathological findings (testis seminiferous tubular atrophy) not d at 300 (1/10 F1 males), 7,500 (all F1 and F2 males) and 10,000-ppm (all F1 males, 2 or 3 of 10 F0 males)

Microscopic and/or macroscop c pathological findings and organ weight changes (absolute and/or relative) were also noted in the epididymis, seminal vesicles and prostate. Thus, macroscopically small and/or aplastic epididymides were noted at 300 (2/45 non-mating F1 males, 1/21 non-mating F2 male), 1,000 (3/25 non-mating F2 males), 7,500 (1 or 2 of 10 mating F1 males, 9/10 mating F2 males, 7/20 non-mating F2 males) and 10,000-ppm (21/21 non-ma ing F1 males). Small seminal vesicles were noted at 300 (1/45 non-mating F1 males) and 7500-ppm (1/10 ma ing F1 males), and small prostate was noted at 1,000 (3 or 4 of 43 F1 non-mating males), 7,500 (1/10 F0 mating male , 1/10 F1 mating males, 1/30 non-mating F1 males) and 10,000-ppm (1 or 2 of 21 non-mating F1 males). Microscopic pathological changes in the epididymis including sloughed epithelial cells/residual bodies and aspermia/oligospermia were found in F0 and F1 males at 7,500 and 10,000-ppm. Organ weight changes were noted in the epididymis (F1 and F2 males at 7,500-ppm; F0 and F1 males at 10,000-ppm), seminal vesicles (F2 males at 7,500-ppm; F1 males at 10,000-ppm) and prostate (F1 males at 7,500 and 10,000-ppm). t 7,500-ppm changes in epididymis and prostate weights were also noted in F3 males.

The low observed adverse effect level (LOAEL) for testicular toxicity was set at 300-ppm (equivalent to approximately 23 mg DEHP/kg bw/day in the F0 animals and 14 mg DEHP/kg bw/day in the F1 and F2 a imals). At this dose level macroscopic pathological findings in testes (aplastic and/or small) were noted in animals of both generations (F1 and F2), and microscopic pathological findings in testes (seminiferous tubular atrophy) were noted in 1/10 F1 males.

Further on, macroscopic pathological findings in male accessory sex organs other than testes (mentioned above) were also present at this dose level and at higher doses. Atrophy of seminiferous tubules in testis was also observed at 100-ppm. However, this effect on testis at 100-ppm was only noted in one animal in one generation (F1) and in the absence of any accompanying findings. At 300-ppm additional parameters and several generations of animals were affected. Effects on male accessory sex organs other than testis could also be taken into consideration at this dose level. Therefore the LOAEL was set at 300-ppm.

The NOAEL for fertility toxicity in this study was 1,000-ppm (equivalent to approximately 77 mg DEHP/kg bw/day in the F0 animals, and 48 and 46 mg DEHP/kg bw/day in the F1 and F2 animals respectively) and was based on impaired fertility and litter parameters noted at 7,500-ppm and above, and decreased various sperm end-points noted at 7500 (F1-, F2-, F3 males) and 10,000-ppm (F0-, F1 males). None of the F1 mating pairs produced offspring at 10,000-ppm (this finding was correlated with no spermatids present in the testes of F1 males at 10,000-ppm). At 7,500-ppm statistically significant decreases in the pregnancy indices were noted for the F2 mating pairs (8/17 vs. 17/17). The total number of males per litter was decreased at 10,000-ppm in the F1a litter (26%) and at 7,500-ppm across all F1 litters combined (F1a+F1b+F1c) (approximately 20%). The total number of F1a pups per litter was decreased at 7,500-ppm (22%) and at 10,000-ppm (21%). The total number of pups per litter across all F1 (F1a+F1b+F1c) litters combined (18%) was also decreased at 7,500-ppm. There was also an increase in the number of cumulative days to deliver the F1a litter for F0 animals at 10,000-ppm.

The NOAEL for developmental toxicity in this study was 100-ppm (equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and approximately 5 mg DEHP/kg bw/day in the F1 and F2 animals) and w s bas d on the fact that the testicular effects were much more severe in the F1 and F2 generations than in F0 indic ting the developmental phases as sensitive to the testicular toxicity of DEHP.

The NOAEL for effects not related to reproductive toxicity in adult animals was 300-ppm (equivalent t approximately 23 mg DEHP/kg bw/day in the F0 animals, and 14 mg DEHP/kg bw/day in the F1 and F2 animals) and was based on reductions in bodyweights noted in both sexes at 7,500 (F1, F2 animals) and 10,000-ppm (F0, F1 animals), absolute and/or relative organ weight changes noted at 10 ppm and above (increased liver: 1,000-ppm and above; increased kidneys: 1,000-ppm and above; increased adrenals: 10,000-ppm; increased pituitary: 10,000-ppm), and microscopic pathological findings noted at 1,000 ppm and above (liver hypertrophy: 1,000-ppm and above; cortex vacuolisation of the adrenals: 7 500-ppm and above; dilation of the tubules and mineralization in the kidneys occasionally associated with chroni pyelonephritis: 1,000-ppm and above). Microscopic pathological findings in the adrenal glands were also i dicated in F1 animals at 1,000-ppm (no further data).

In conclusion, a NOAEL of 4.8 mg/kg/day is obtained for testicul r toxi ity and developmental (testicular) toxicity. The NOAEL for fertility is 46 mg/kg/day.

Results from a 2-generation reproduction toxicity study in Wistar rats indicate effects on reproductive performance, several organs, survival (overall, 8 of 50 adult high dose females died or were killed for humane reasons), as well as on development (**Schilling t al., 200 a**). The study was performed according to current guidelines and in conformity with GLP. Wi t r rat (25 rats/sex and generation) were exposed to dietary levels of 0, 1,000, 3,000 or 9,000-ppm DEHP corresp nding to approximately 0, 113, 340 or 1,088 mg/kg bw and day). The F0 animals were exposed as from the age of 37 days, for at least 73 days before mating, and until weaning. F1 pups were raised and ated o produce a F2 generation. Selected F2 male and female animals (10 of each sex) performed a functional observation battery, motor activity, and a water maze test at 21 days of age.

Histopathology of the testis was perform d with light microscopy after Bouins fixation, paraplast embedding, and Haematoxylin and Eosin staining Evaluation of the testis showed focal tubular atrophy to be the most frequent finding. In the F0 animals, the frequency was 0/25, 1/25, 3/25, and 6/25 in the control, low, mid, and high dose groups, r spectively. The number of affected tubules/testis, as well as the presence of diffuse tubular atrophy, was increas d in the high dose group.

In the F1 adult males, the frequency of focal tubular atrophy was 3/25, 7/25, 4/25, and 14/25 in the control, low, mid, and high do e groups, respectively. Although fewer animals were affected in the mid than in the low dose group, the ffec s in the mid dose animals were more pronounced than in the low dose animals. Thus, the number o affected tubules/testis was increased in the two highest dose groups. In addition, diffuse tubular atrophy was observed in the high dose group (3/25). Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. A reduced or absent sperm-/spermatid counts together with perm abnormalities was observed in the high dose groups in 2 and 1 animal(s) (of 25) in the F0 and F1 adult male , respectively.

A reduced testis weight (absolute and relative) was observed in the high dose F2 pups.

Other findings: F0

Observations on the F0 parental females were mortality (2/25), a decreased food consumption (25%), reduced body weights, body weight loss during lactation (14%), and a retarded body weight gain (25%) in the high dose group. Effects on organ weights and/or histopathology were observed among both females and males. Besides effects on the testis (see above), there was also an affect on the ovaries in the high dose group (reduced number of growing follicles and of corpora lutea, 15%* and 25%**, respectively). Effects on reproductive performance were evident in the high dose group, as illustrated by a reduced fertility index among both females and males,

and an increased postimplantation loss.

Other findings: F1 pups

Observations on the F1 pups in the high dose group included reduced number of live (viability index) and total number of pups, increased number of stillborn pups, increased pup mortality, reduced body weights (31%) and body weight gains (36%) until weaning (day 21 post partum).

Feminisation of male pups was indicated by a reduced anogenital distance (14%), a reduced anogenital index (8%), and an increased frequency of areolas/nipple anlagen in male pups.

The timing of sexual maturation was delayed in both females (vaginal opening) and males (preputial separation). The weight of the thymus and spleen were reduced.

Some of these effects were also significant in the mid dose group (e. g., the viability index, the anoge ital index and the weights of thymus and spleen), and although not statistically significant in most cases, there appear to be a trend also including small effects in the low dose group.

Other findings: F1 parental animals

In the high dose group, there was an increased mortality/sacrifices among dams (6/25) and malformed external genital organs in males (2/25). Food consumption, body weights, and body weight gains were reduced in both males and females. The effects on reproductive performance and organ weights/histopathology were almost identical to those in the F0 generation.

In the lower dose groups, there were besides the effects on the testis (see above) also an increased number of stillborn pups in the mid dose group.

Other findings: F2 pups

In the high dose group, the observations in the F2 pups were almos identical to those in the F1 pups, but included a reduced weight of the testis.

In the mid dose group, the effects in F2 pups seemed more severe than in the F1 pups. There were an increased number of stillborn pups, a decreased live birth index and viability index, lower body weights (6%) and body weight gains (7%), a reduced anogenital distance/index (9% nd 8%, respectively), an increased presence of aerola/nipple anlagen affecting 49% of the mal , and a d creased thymus weight in males. Although not statistically significant, there appears to be a trend also including small effects on the thymus and the testis (see also above) in the low dose group.

Timing of sexual maturation was not st died in the F2 generation.

In the high dose group, a functional observation battery performed on selected animals at day 21 post partum revealed reduced values for gr p strength in males, and reduced values for landing foot-splay in both males and females. The body weights of th se animals were reduced (27-38%), but it is not clear whether the reduced body weight could account for the functional effects. No effects were observed on the water maze test or on motor activity. No effects were observed in the lower exposure groups.

Evaluation of imm nological data

The present study indicates that DEHP induces atrophy of spleen and thymus. There was a significant decrease in spleen weight at all doses in both male and female F1-pups and a significant decrease in thymus weight in the mid and h gh d se groups in F1 males. In the F1-females a significant reduction of the thymus was only ob erved at the highest dose level, however, a non-significant but clear dose dependent trend was observed also for th low and mid dose groups. In the F2-pups, splenic weight was significantly reduced in the high dose group with 30 nd 34% in males and females, respectively. The effect on the thymus weight in the F2-pups is similar to that in the F1-pups. A significant reduction in the mid and high dose groups of the F2-males, and for the F2-femal s a non-significant but dose-dependent reduction in the mid and high dose groups.

In the highest dose group the reduced spleen and thymus weights were observed in parallel with a significant reduction in male and female F1 and F2 pup body weights. Thus, it is possible that in the highest dose group the effect on spleen and thymus weights could be associated with the reduced body weight. However, the effect on the spleen observed in the low-dose group of both male and female F1 pups and on the thymus weight in the mid dose group of male F1 and F2 pups was not accompanied by a reduced body weight. Therefore, without further testing of the immunotoxicity of DEHP a direct immunotoxic effect of DEHP cannot be excluded. Thus, for the effect on the spleen a LOAEL of 1,000-ppm can be concluded from this study.

Evaluation of testicular data

In this study, significant and severe effects on testicular histology, sperm morphology, fertility, and sexual development of the offspring have been observed in the high dose group of both generations. Several of these effects are also clearly apparent in the mid dose group, e. g., a reduced testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring (as indicated by the presence of aerola/nipple anlagen in the males). Some of these effects are also occurring in the low dose group (e. g., focal tubular atrophy), although few tubuli are affected per testis. However, based on the clear dose-response, we conclude that there is an adverse effect on the testis also in the low dose group (113 mg/kg and day), which thus constitutes the LOAEL of the study.

Overall evaluation

It should be observed that although there has been some focus on the testicular effects, the testis have only been studied by standard methods (Bouins fixation, paraplast embedding, and Haematoxylin and Eosin staining) and no measurements of, e. g. hormone concentrations have been conducted. Still, there were effects on numerous parameters relating to reproductive success in the low dose animals (testicular tubular atrophy, relative weight of testis in F2, pup production, pup viability, anogenital index, pup weight gain, and time of sexual maturation of males and females), effects that were not statistically significant in this dose group but at higher ones. Although some of these effects are small in the low dose group, the relevance is supported by known mechanisms of action and clear dose-responses involving the three dose groups.

Wistar rats have been used in the present study. Although it is not the first time Wistar rats have been used in studies on the toxicity of DEHP, the fact that none of the previous studies giving low LOAELs have used Wistar rats, gives some concern relating to the sensitivity of this strain as compared to, e. g. the more commonly used Sprague-Dawley rat.

The low dose in this study (113 mg/kg and day) is considered a LOAEL and hence, no NOAEL can be deduced from this study.

Results from a 2-generation range finding study in Wistar rats indicate effects on fertility and developmental toxicity (**Schilling et al., 1999a**). The study was performed according to current guidelines and in conformity with GLP. Wistar rats (F0 generation = 10 rats/sex) were exposed to dietary levels of 0, 1,000, 3,000 or 9,000-ppm DEHP (corresponding to approximately 0, 110, 339 or 1,060 mg/kg bw/day). F1 pups were raised and mated to produce a F2 generation, which was sacrificed two days after birth. The mean relative liver weight was significantly increased in F0 parental males at 3 000 and 9 000-ppm (at the higher dose level also the absolute liver weight). No treatment related histopathological changes were, however, noted. There was a reduced total number of delivered F1 pups and the viability index was reduced on post partum day 0 and 4 at 9,000-ppm. In F1 male pups a treatment related loss of spermatocytes was found at 3,000 and 9,000-ppm (2/10 and 7/9, respectively). At the highest dose level, the presence of areolas/nipple anlagen was significantly increased and the male sexual maturation (based on preputial separation) was significantly retarded. A reduced anogenital distance was observed in F2 male pups at 9,000-ppm (not investigated in F1 pups).

Mortality occurred in F1 parental males (3/9) at 9,000-ppm in the premating phase, initially also reduced food consumption and reduced mean body weights were noted. At this dose level, the fertility was also reduced in the males (fertility/mating index 83%). The absolute and relative testicular weight and the absolute epididymidal weight were significantly decreased at 9,000-ppm. The prostate weight showed a dose-related decrease from 1,000-ppm. The testes and the epididymides were reduced in size in three out of six animals at 9,000-ppm.

Histopathology revealed focal or diffuse atrophy of spermatogenesis of the testes and diffuse Leydig cell hyperplasia in all males, interstitial oedema in the testes in three out of six animals, and debris of an altered spermatogenesis in the epididymides in five out of five animals. Also aspermia (2/5), missing seminal vesicle (1/6) and areolas/nipple anlagen (1/6) were noted. There was a dose-related increase of stillborn pups from 3,000-ppm and a decrease of delivered F2 pups, statistically significant at 9,000-ppm.

The effects found in F1 parental males indicate that DEHP exerts a specific action on male genital organs such as the testicle and the epididymis, when males are exposed during early development. This is strengthened by the fact that female gonads were unaffected. However, concerning testicular effects in developing male pups only one testicle per litter was studied histopathologically in F1 pups and none of the F2 pups. F1 pups were culled at day 21 and neither undescended testes nor hypospadia were investigated. Neither is there any information on effects on Sertoli cells in F1 parental male rats in this range finding study.

Single generation reproductive toxicity studies

Rat

Inhalation

In a 4-week inhalation study conducted according to OECD guideline 412 and the principles of GLP, male Wistar rats (10 rats per group) were exposed 5 days/week, 6 hours/day to 0, 0.01, 0.05 or 1 mg DEHP/litre (0, 10, 50 or 1,000 mg DEHP/m3) (99.7% pure) as liquid aerosol (**Klimisch et al., 1992**). The males were mated to untreated females. No effects on male fertility were observed 2 and 6 weeks after the end of exposure and no testicular toxicity was detected histologically.

Oral

In a study comparable to a guideline study, **Agarwal et al. (1986a)** administered DEHP (> 99% pure) to groups of 24 sexually mature male F344 rats (age about 15 weeks) in the diet at 0, 320, 1,250, 5,000 or 20,000-ppm (equivalent to doses of 0, 18, 69, 284 and 1,156 mg/kg bw) per day for 60 days. The males were mated with undosed females at exposure days 61 to 66. A dose-dependent reduction in total body, testis, epididymis, and prostate weights was observed at 5,000 and 20,000-ppm. The only functional reproductive consequence of exposure of male rats to DEHP was a significantly reduced mean litter size at 20,000-ppm (1,156 mg kg bw/day). This effect was directly correlated with degenerative changes in the testes, along with decrea ed testicular zinc content, significant reduction in epididymal sperm density and motility, and increa ed oc urrence of morphologically abnormal sperm. There was a trend towards decreased (not statistically ignificant) testosterone and increased luteinising hormone (LH) and follicle stimulating hormone (FSH) in serum t 5,000 and 20,000-ppm. The incidence of pregnancy, mean litter weight on day 1, frequency f stillbirt and neonatal death, and mean litter growth up to 7 days of age were unaffected. A NOAEL of 69-mg/kg bw/day is derived.

Mouse

Oral

Tanaka (2003) examined the effects of prenatal DEHP exposure on sex rati in mice. It appears that the study was conducted to address concerns about DEHP effects on sex. Starting at 5 weeks of age, 20 male and female CD-1 mice/sex/group were fed diets containing 0 or 0.03% DEHP (purity > 97.0%). At 9 weeks of age, each female was mated for 5 days with a male from the same or opposite reatment group (i. e., cross-mating). There were 4 treatment groups consisting of 10 mice/sex: control femal s × c ntrol males, control females × treated males, treated females × control males, and treated females × treated males. Females continued to receive the DEHP-containing or control diets during the mating peri d and throughout gestation. The study authors estimated that intake of DEHP was 49 mg/kg bw/day in mal s and ~55–58 mg/kg bw/day in females during the preconception period. Intakes by females were timated t ~45 mg/kg bw/day during the mating period and ~50 mg/kg bw/day during the gestation period. Fema s were allowed to litter, and endpoints examined on day of birth were litter size, litter weight, individual offspring weight, and sex ratio. Statistical analyses included ANOVA or Kruskal-Wallis test follow d by Bon erroni multiple comparisons to assess food intake, litter size, and litter and body weights. Chisquared test was used to evaluate sex ratio based on offspring, and the Steel test was used to assess sex ratio bas d on litter. A a result of pregnancy failures or abortions, there were 8–10 litters delivered in each treatment group. Compared to the group consisting of control males and females, mean body weights of male offspring were increased in all groups containing a treated female and/or male parent. No significant effects were noted for lit size, litter weight, total or average sex ratio, or female offspring weights. The study authors concluded that the concentrations of DEHP used in this study did not produce adverse effects on sex ratios.

Tanaka et al (2005) gave DEHP (>97% purity) to CD-1 mice in the diet from 5 weeks of age in the F0 genera ion to 9 weeks of age in the F1 generation. A single dietary dose level of 0.03% was used, with control animals re eiving untreated basal feed (n = 20/sex/treatment group). At 9 weeks of age, 10 DEHP treated fema es were paired with DEHP-treated males, 10 DEHP-treated females were paired with control males, 10 control females were paired with DEHP-treated males, and 10 control females were paired with control males. The f males' diet was available to males during the 5-day cohabitation phase. Females reared their own unadjusted litters, which were weaned at 4 weeks of age. One female and male from each litter were retained and f d their dam's diet until 9 weeks of age. All F1 offspring underwent neurobehavioral testing during the lactation period, including surface righting and negative geotaxis on PND 4 and 7, cliff avoidance on PND 7, swimming behavior on PND 4 and 14, and olfactory orientation on PND 14. Exploratory behavior was assessed in 1 male and 1 female from each litter at 3 weeks of age. Post-weaning tests included multiple-T water maze at 7 weeks of age and exploratory behavior at 8 weeks of age. Statistical analyses were performed using ANOVA or Kruskal-Wallis test followed by Bonferroni multiple comparison tests. Proportions were evaluated using chi-squared or Fisher test. [It is not stated whether litter was considered in the analysis of the preweaning neurobehavioral tests.] Based on measured feed consumption, mean DEHP intake by treated males [rounded by CERHR] was 46 mg/kg bw/day. Treated females received 53–57 mg/kg bw/day during the preconception period, ~43 mg/kg bw/day during mating, 46–49 mg/kg bw/day during gestation, and 154–171 mg/kg bw/day during lactation.

DEHP had no effect on feed consumption or dam body weight. As repeated in Section 4.2.3, there were no significant treatment effects on the number of pregnant females, number of litters, number of offspring, average litter size or weight, or offspring sex ratio. Offspring body weight during the lactation period was similar between groups except for an 8% decrease in body weight on PND 14 in female offspring when the parents both had received DEHP. The author did not consider this isolated alteration to be treatment related. Swimming ability was accelerated in PND 4 female offspring when the dam received DEHP. The number of movements in the test of exploratory behavior was decreased in male offspring the parents of which both received DEHP. There were isolated differences in T-maze performance by sex, trial, and treatment group that were not considered to represent treatment-related alterations in maze-learning. None of the other behavioral tests revealed effects of DEHP treatment. The author concluded that "few adverse effects on several behavioral parameters were produced at the dose level of DEHP in the present study."

Price et al. (1988a) conducted a study in CD-1 mice of the postnatal effects of prenatal exposure to DEHP. Th design is similar to Tyl et al. except that developmental toxicity was evaluated postnatally rather than at the nd of pregnancy. Pregnant mice, 28–29 per group, were fed a diet containing 0, 0.01, 0.025, or 0.05% DEHP, resulting in mean doses of 0, 19, 48, or 95 mg/kg bw/day (estimated by authors), respectively, from gd 0 to 17. Dams were evaluated for maternal toxicity and their offspring were evaluated for viability, growth, and development. The F1 pups were then mated within dose groups and their F2 offspring were ev luated for viability and growth through pnd 4. There were no observed adverse effects on the (F0) females during pregnancy. The only indication of a maternal DEHP effect was a trend for decreasing body weight gain on pnd 4 and 7 that was not significant in a pairwise comparison with controls. For F1 litter the perc nt prenatal mortality was significantly increased at the 95 mg/kg bw/day dose with a concomitant decrease in live litter size at pnd 1. On pnd 4, the percentage of viable pups was also decreased in litters exp sed to 95 mg/kg bw/day. No other effects of DEHP upon growth, viability, age at acquisition of landmarks, or pontaneous locomotor activity were seen in any dose groups on pnd 14, 21, or 50. The NOAEL for dev lopme tal toxicity was 48 mg/kg bw/day and the maternal NOAEL was 95 mg/kg bw/day. No adverse effects were consi tently observed upon the reproductive performance of the F1 generation or the F2 offspring through pnd 4.

CD-1 Swiss mice, 11 weeks old at the start of exposure, were used for continuous breeding phase and cross-over mating studies **(Lamb et al., 1987)**. There were 20 breeding pairs in each treated dose group, and 40 pairs in the control group. DEHP was mixed with feed to levels f 0, 0 01, 0.1, and 0.3% (w/w); this yielded calculated doses of 0, 14, 141, and 425 mg/kg bw/day. Following 7-day premating period, the mice were housed as breeding pairs for 98 days. Litters were remove imm di ely after birth. Endpoints in-life were clinical signs, parental body weight and food consumption fertility (numbers of pairs producing a litter/total number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex ratio, pup body weights within 24 hours of birth, nd water onsumption. There was clear indication that DEHP affected fertility when administered in the di t. A 425 mg/kg bw/day no breeding pairs delivered a litter. At 141 mg/kg bw/day, fertility was significan ly reduced as evidenced by fewer litters, fewer pups/litter, and fewer pups born alive. A cross-over mating study was conducted between the 425 mg/kg bw/day treatment group and the controls. Fertility was significantly redu ed in the groups of treated males and control females and the groups of treated females and con ol males. The treated females produced no litters and only 4/20 treated males sired a litter. Only the control and high-dose groups were necropsied. High-dose males had reduced testicular and epididymal weights and histo ogic evidence of seminiferous tubular destruction accompanied by major changes in epididymal sperm number, morphology, and motility. In addition, the males had decreased prostate weight, reduced serum te tosterone, and elevated LH and FSH. There were no histopathologic effects in the reproductive tracts of high os females, but the weight of the reproductive tract was lower than controls (probably because the animal wer not pregnant). The high-dose group was infertile, the mid-dose group was affected, and the low dose group was unaffected. Thus, the NOAEL was at a calculated dose of ~14 mg/kg bw/day. The LOAEL was at 141 mg/kg bw/day, based on reductions in litter size and in proportions of pairs having litters.

Subc taneous

Agarwal et al., (1989) dosed adult male ICR mice in groups of 25 (control) or 10-13 (treated) were treated subcutaneously with undiluted DEHP at volumes of 1, 2, 5, 10, 15, 120, 40, 60, 80, and 100 mL/kg, on days 1, 5, 10, a regimen used in the previous study (Agarwal et al., 1985). Doses in g/kg were not provided; in the previous report, a dose of 10 mL/kg was equivalent to 9.86 g/kg. The animals were mated from day 21-28, and then sacrificed. Other animals were sacrificed on day 21; one testis and epididymis from 10 mice was fixed in formalin and evaluated histologically. The other testis was used for biochemical measures. The disposition of the animals was not clear from the Methods description in the prescribed study. The data were analyzed by ANOVA and then compared pairwise by t-test; this is an incorrect comparison, as it produces false positives. The authors noted that doses of 20 mL/kg or more were incompletely absorbed, and the animals had fluid filled pouches

containing some DEHP and some apparent lymph. Testis weights (unclear when during the study these were collected) were reduced at and above 20 mL/kg. There were various biochemical changes (whose meaning is difficult to determine) at 10 mL/kg and higher. Testicular histology was affected at and above 10 mL/kg, with inflammation being most common at 10 mL/kg, and tubular changes appearing at 40 mL/kg (which might be calculated to be an administered dose of 39.4 g/kg). The testicular pathology (description of the changes and the doses at which they began to appear) was not tabulated, and the text description was cursory. The effect on fertility was limited to determining how many females per group were pregnant; this was 76% in the controls, and as the dose increased, the proportions of pregnant females were: 50, 25, 33, 42, 25, 8, 20, 0, 8, and 0%. The authors did not perform statistical analyses on these data, and it can be seen that even at the lowest dose (which can be calculated to be 0.99 g/kg) there may be a decrease in percent pregnant females, and the next highest dose(2 mL/kg, wherein 25% of the females were pregnant), gave the fourth lowest pregnancy value, the same as that found with 15 mL/kg. Pregnancy and histology were evaluated in this study, which is informative. Howe er, the methods used for histologic evaluation were suboptimal; the time between treatment and mating and necropsy meant that there might have been some degree of recovery from any DEHP effect; the concentration and volume of the dosing solution meant that residual neat DEHP was in a depot at the site of inj ction, complicating the kinetics in vivo; the numbers of animals for the mating trials (10) were relatively small, and the measures of fertility (the % of females getting pregnant) are gross; the apparently most s nsi ive period of male development was not evaluated.

The following information is taken into account for any hazard / risk assessment:

There are various epidemiological studies examining the reproductive effects of DEHP. These studies largely examine the relationship between urine levels of DEHP metabolites and various measurements. Although some of these studies indicated adverse effects of DEHP on reproductive functio s and the developing organism, associations in these studies were weak, contradictory, or confounded by exp sure to other substances. In agreement with the EU Risk Assessment therefore the risk assessment is b sed on experimental data.

There are many experimental animal studies, largely oral, using rats or mic . The most sensitive effects, perturbations in testicular structure and function, have been consi tently bserved in several reproductive toxicity studies in rats and mice by both oral and parenteral routes of exposure. In vivo and in vitro assays have demonstrated that the Sertoli cell is the most sensitive targe of toxicity, causing subsequent germ cell depletion. Rats appear to be the more sensitive species than mice for t sticular effects.

The NOAEL for impairment of fertility in a mu igen ti n study (Wolfe and Layton (2004) was 1,000-ppm (equivalent to approximately 77 mg DEHP/kg bw/day in the F0 animals, and 48 and 46 mg DEHP/kg bw/day in the F1 and F2 animals respectively) and was bas d on impaired fertility and litter parameters noted at 7,500-ppm and above, and decreased various sperm end-poin s noted at 7500 (F1-, F2-, F3 males) and 10,000-ppm (F0-, F1 males).

The consistent finding of testicular effec s in rats and mice is in contrast to studies in marmosets. No treatment-related changes in testicular his ology or more gross parameters were observed at the highest dose used, 2500 mg/kg bw/d.

Value used for CSA (route: oral): NOAEL: 46 mg/kg bw/day

Developmen al t xicity

Nume ous stud es have shown that DEHP is embryotoxic in rats at doses close to maternally toxic dose levels. In mi e, seve al studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those prod cing observable evidence of toxicity to the dams.

Standard developmental toxicity/teratogenicity studies

Inhalation

Twenty-five pregnant Wistar rats per dose group were used to study the teratogenicity of DEHP by inhalation as liquid aerosol at dose levels of 0, 0.01, 0.05 or 0.3 mg/litre (0, 10, 50 or 300 mg/m3) (**Merkle et al., 1988, BASF, 1986**). The particle MMAD was < 1.2 ± 7.3, ± 16.8 and ± 5.8 µm for the low, middle and high dose group, respectively. The study was performed according to OECD Guideline 414 and GLP principles. The dams were exposed by head-nose exposure for 6 hours per day from gestation day 6 through 15 (the period of male sexual differentiation between days 16-19 is not included in this study). Twenty rats per group were sacrificed on day 20 of pregnancy and five rats per group were allowed to litter. The offspring was raised and observed for postnatal signs of toxicity. In a range-finding study, "exposure-related" peroxisome proliferation was observed in dams from 200 to 1,000 mg/m3. The number of live foetuses/dam was slightly, but statistically significantly

decreased in the 50 mg/m3 group and the percentage of resorptions/dam was elevated. These effects, however, were not seen at the next dose level. That effects were only seen in the middle dose group may reflect the large standard deviation of the particle MMAD. The effects reported were not regarded as exposure related, since no dose dependency was observed. The number of corpora lutea, uterine weights, body weights, living and death implants, early and late resorptions, dead foetuses, pre- and post-implantation losses were unchanged compared to controls. The validity of this study is questioned, as the systemic dose was not determined. By comparison with another inhalation dose study there may be problems with delivering the expected dose of DEHP. Hence, this study is considered inadequate for use in risk characterisation.

Oral

Rats

In a study comparable to a guideline study and performed according to GLP principles, dietary levels of 0, 0.5, 1.0, 1.5 or 2% of DEHP (no information on mg/kg bw/day is given) were given to groups of F344/CrlBr ra (34-25) throughout gestation (days 0-20) (**Tyl et al., 1988**). The rats were sacrificed on day 20. Food intake was significantly decreased at all dose levels. Reduced maternal body weight gain and increased absolu e and relative liver weights were observed at a dietary level of 1.0%. Reduced foetal body weights per litt r were ob erved at the same dietary level. There were no treatment-related differences in the number of corpora lu ea or implantation sites per dam, nor in the percent pre-implantation loss. At a dietary level of 2% the number and percent of resorptions, non-live and affected implants per litter were significantly increased and the number of live foetuses per litter was significantly decreased. Mean foetal body weight was s gnificant y reduced at all dose levels. The number and percentage of malformed foetuses per litter was not significantly different from control. The NOAEL for maternal and developmental toxicity was 0.5% DEHP (approx mately 357 mg/kg bw/day).

In a study performed according to OECD Guideline 414 and GLP principles, DEHP was tested for its prenatal toxicity in Wistar rats (**Hellwig et al., 1997**). DEHP (99.8% pure) was adm nistered as an oily solution to 9-10 pregnant female rats/group by stomach tube at doses of 40, 200 or 1 000 mg kg bw on day 6 through 15 of gestation. On day 20 of pregnancy, all females were sacrificed a d ass ss d by gross pathology. Maternal toxicity at 1,000 mg/kg bw was reported: Slightly reduced maternal food consumption was noted. Reduced uterus weight was assessed as to be associated with the igh mbryolethality. The corrected body weight gain did not show any differences of biological relevance Stati ically increased relative kidney and liver weights was observed. Developmental toxicity at 1,000 mg/kg bw: Severe developmental effects were observed: statistically significantly increased implantation loss (bo 40%). There also was a statistically significant lower number of live foetuses/dam, decreased foetal body weights, a drastically increased incidence of of external, soft tissue, and skeletal malformed foetuses litter (in otal approximately 70% of the foetuses/litter), predominantly of the tail, brain, urinary tract, gonads, ver ebral column, and sternum. There also were an increased percentage of foetuses/litter with soft tissue and k letal variations and skeletal retardations. The NOAEL for maternal and developmental toxicity was 200-mg/kg bw/day.

Pregnant female Wistar rats were exposed to **MEHP** by gavage daily on the day 6 to the day 15 of gestation, at the dose of 0, 225, 450 or 900 mg/kg bw (**Ruddick 1981**). The dams were necropsied at day 22 of gestation. MEHP was lethal to some mothers at dosages of 225, 450 and 900 mg/kg bw therefore the others groups was exposed to new doses of 0, 5 100 and 200 mg/kg bw. Dosages of 100 and 200 mg/kg reduced the maternal weight gain of dams in the tr atment when compared to the control group ($p<0.05$). Treatment with 450 mg/kg statistically f cted the ean litter weight of the live pups ($p<0.05$). Examination of the fetal skeleton did not reveal any pronounc d alterations between treated and control groups other than disturbances in the placement of the ste nebrae plates, a 14th and wavy ribs which were observed in both experimental and control fetuses. Visceral anomal es were not seen.

Mice

In a study comparable to a guideline study and performed according to GLP principles, dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% of DEHP (0, 44, 91, 190.6 or 292.5 mg/kg bw/day; > 99% pure) were administered to g oups of 1-CR outbred mice (30-31 per group) throughout gestation (days 0-17) (**Tyl et al., 1988**). Maternal toxicity, indicated by reduced maternal body weight gain, was noted in the two highest dose groups, mainly due to reduced gravid uterine weight. There were no treatment-related effects on the number of corpora lutea, implantation sites per dam, the percent pre-implantation loss, and sex ratio of live pups. The number and percent of resorptions, late foetal deaths, and dead and malformed foetuses were all significantly increased from 0.1%. Foetal weight and the number of live foetuses per litter were significantly reduced from the same dose level. Both the percentage of foetuses with malformations and the percentage of malformed foetuses per litter were significantly increased from 0.05%. The observed external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted, or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and

fused thoracic vertebral centra. The NOAEL for maternal toxicity was concluded to be 0.05% (91 mg/kg bw/day) and for developmental toxicity 0.025% (44 mg/kg bw/day).

Reader (1996) performed a GLP study of the embryo-foetal toxicity in the CD-1 mice by oral gavage administration. Doses of 0, 40, 200 or 1,000 mg DEHP/kg bw/day were administered to groups of 15 pregnant mice from day 6 to 15 of gestation. A control group of 30 pregnant mice received a vehicle (0.5% carboxymethylcellulose containing 0.1% Tween 80). Litter parameters following necropsy of the females on day 17 of gestation revealed low numbers of viable young, high numbers of resorptions, and a greater extent of post-implantation loss for females given 1,000 mg/kg bw/day than in the control group. Cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney anomalies were observed. At 200-mg/kg bw/day, there was a slightly higher incidence of foetuses with intra-muscular or nasal haemorrhage or dilated orbital sinuses. There also was a small number of foetuses with anomalous innominate or azygous blood vessels. From this study a NOAEL of 200-mg/kg bw/day can be derived for maternal toxicity and a NOAEL of 40-mg/kg bw/day for developmental toxicity.

DEHP was given to female ICR mice (8 to 16 weeks old) at dietary levels of 0, 0.05, 0.1, 0.2, 0.4 or 1.0% (equivalent to 0, 70, 190, 400, 830 and 2,200 mg/kg bw, purity not specified) from day 1 to 18 of ge tation (**Shiota and Nishimura, 1982**). On day 18 the animals were killed. The average weight of li e foetuse was decreased and the incidence of malformed foetuses was significantly higher from 400 mg/kg bw The most common malformations were neural tube defects (exencephaly and spina bifida), malformed tail, gastroschisis and club foot. The NOAEL for maternal and developmental toxicity was 70 mg/k bw of DEHP.

NTP (1991) evaluated the developmental toxicity of **MEHP** in CD-1 mice ed a diet ontaining the chemical on gd 0–17. Groups of 25–27 mice received doses of 0, 0.017, 0.035, 0.07, o 0.14% MEHP in feed. Average doses were reported as 0, 35, 73, 134, or 269 mg/kg bw/day MEHP. Doses were s lected t be approximate molar equivalents to the DEHP doses studied by Tyl et al. (1988) in the same m use str in using a similar protocol. Maternal body weights and food and water consumption were rec rded throughout the treatment period. At scheduled sacrifice on gd 17, the numbers of resorptions and d ad or live fetuses were recorded. All fetuses were weighed, and live fetuses were sexed and examined for external, iscer l, and skeletal malformations. MEHP-exposed females exhibited no clinical signs of maternal toxicity, and food and water consumption were similar to those of controls. There was a decrease in the adjusted b dy weight gain of mice in the 269 mg/kg bw/day dose group. The relative liver weights of mice fed a diet containing 134 and 269 mg/kg bw/day increased. The maternal NOAEL was stated to be 134 mg/kg bw/day. The percent litters with resorptions increased at all dose levels. The numerical values increased in a dose elated manner, reaching 77% in the high-dose group. A significant linear decrease in average number of liv fetuses per litter was observed with increasing dose level; values for the 73, 134, and 269 mg/kg bw/day do e groups were significantly different from controls by pair wise comparison. Fetal malformations were observed in a significantly higher percentage of litters at dose levels of 73 mg/kg bw/day and greater and in a significantly higher percentage of fetuses at doses of 134 mg/kg bw/day and higher. MEHP exposure was associated with an increase in skeletal and visceral malformations, with the latter increase primarily due to cardiovascular malformations. A NOAEL for developmental toxicity was not observed in this study. The LOAEL (based on incidence of litters with resorptions) was 35 mg/kg bw/day MEHP. The Panel has high confidence in the quality of this study and its ability to identify the developmental LOAEL for oral ex osure.

Rabbits

No developm ntal t dies have been performed in rabbits given DEHP.

Other developmental toxicity studies

Oral

Rat

Two multi-generation studies (Schilling et al., 2001 and Wolfe and Layton, 2004; see section 5.9.1.1.) in rats give important information on development as well. Based on Wolfe and Layton (2004) a NOAEL of 4.8 mg/kg/day for developmental effects on the testis is deduced.

The reproductive effects of in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP) in adult male offspring rats were investigated (**Andrade et al., 2006b**). The selected endpoints included reproductive organ weights, testicular function, hormonal status, sexual behaviour and fertility. Two wide ranges of doses, low and high, were tested. Female Wistar rats were treated daily with DEHP and peanut oil (vehicle control) by gavage from gestation day 6 to lactation day 21. The low-doses were 0.015, 0.045, 0.135, 0.405 and 1.215 mg DEHP/kg body weight (bw) /day, and the high-doses were 5, 15, 45, 135 and 405 mg DEHP/kg bw/day. A reduction in

daily sperm production of 19-25% in relation to control was observed in animals exposed to 15, 45, 135 and 405 mg/kg/day. Quantitation of specific cell types shows that the observed effects in daily sperm production are not related to changes in the number of Sertoli cells or their capability to support early stages spermatocytes. A low incidence of cryptorchidism was observed in DEHP exposed groups with a lowest observed adverse effect level of 5mg/kg/day. Serum testosterone concentration was similar to control at most doses but was significantly increased at 0.045, 0.405 and 405 mg DEHP/kg/day. In spite of this effect, the weight of seminal vesicle with coagulating glands was significantly reduced at 405 mg/kg/day. Testis, epididymis and prostate weights were similar among groups. Fertility and sexual behaviour were not affected by DEHP treatment at any dose. Overall, our results show that in utero and lactational DEHP exposure reduces daily sperm production and has the potential to induce reproductive tract abnormalities (of which cryptorchidism seems to be the most sensitive in our rat strain) in male offspring rats. The lowest observed adverse effect levels (LOAELs) for these effects were 15 and 5 mg/kg/day, respectively. Therefore, the no observed adverse effect level (NOAEL) for this study can be set at 1.215 mg/kg/day.

Time-mated Wistar rats were gavaged from GD7 to PND 16 with doses of DEHP from 3 to 900 mg/kg bw/d (**Christiansen et al., 2010**). Male pups were investigated besides others for anti-androgenic effects like anogenital distance (AGD), nipple retention (NR), external genital development, organ weights and histopathology, gene expression of androgen-regulated genes. At doses >/= 10 mg/kg bw/d ad erse effects like reduced AGD, increased NR, reduced weight of levator ani/bulbocarvernosus muscles nd prosta e as well as mild external genitalia dysgenesis were observed. At higher doses histopathological effect on the testes, reduced testis weight and expression of androgen-regulated genes in the prostate occurred. The NOAEL and LOAEL for male reproductive toxic effects were 3 and 10 mg/kg bw/d, respectively.

Carbone et al. (2010) exposed Wistar rats (3 dams per group) to 0, 3, 30 mg DEHP/kg bw/d via drinking water from GD1 till PND21. Male offspring (10 per group) were examined on PND30. Whereas no adverse effects were observed at 3 mg/kg bw/d undescendent testis, decreased te tis weigh increased hypothalamic GABA and reduced serum FSH were observed in the 30 mg/kg bw/d dose gro p. Body weight, serum LH and hypothalamic aspartate were not affected.

Akingbemi at al. (2001) studied effects of oral exposure to DEHP on male steroidogenesis in Long-Evans rats using several different exposure regimes. Hormone level (testosterone and LH) were determined in vivo in serum, and Leydig cells were isolated and cultured for analyses of in vitro androgen biosynthesis. Pregnant dams (n=7) were administered 100 mg/kg and day of DEHP by gavage during gestation day 12 to 21. Serum levels of testosterone and LH were significantly reduced in the offspring at 21 and 35 days of age (approximately to 70 and 40% of controls levels, respectively), but n t at 90 days, as measured in 9-18 randomly selected male pups per group. In Leydig cells isolated from 18 pups, he testosterone production was reduced at day 21 (by approximately 50%), but not later. After exposur of lactating dams to 100 mg/kg and day (n = 7) during postnatal day (PND) 1 to 21 by gavage, serum concentrations of testosterone in the offspring were reduced at day 21, but not at day 35 and 90 post-exposure. No effects were seen on LH. Prepubertal rats (n=10) were gavaged with 0, 1, 10, 100 or 200 mg/kg and day for 14 days during either PND 21-34 or 35-49. No effects were observed on serum hormone levels, but the Leydig cells were affected by DEHP as indicated by decreased in vitro testosterone production and inhibited steroidogenic enzymes (measured in isolated Leydig cells) after exposure to 100 or 00 mg/kg and day (exposure PDN 21-34), or 10, 100 or 200 mg/kg and day (exposure PND 35-48). When prepu ertal rat were exposed as above, but for 28 days (PND 21-48), increased concentrations of serum testos erone, inter titial fluid testosterone, and serum LH were observed (30-40 % at 10, 100 and 200 mg/kg and day). S milarly, the testosterone production was dosedependently increased in isolated Leydig cells obtain d from hese rats. When young adult rats were exposed as above for 28 days (PND 62-89), no effects were observed on any parameter. This study shows that the younger the rats are, the more sensitive they are to the effects of DEHP. Exposure of the dams during pregnancy or at the first postnatal weeks to 100 mg/kg and day red ced the serum levels of testosterone in male offspring. Effects on the Leydig cells were indicated at even lower exposure (10 mg/kg and day), but the relevance of the in vitro assay is not clear. The LOAEL for effects of DEHP on the serum concentration of testosterone in very young rats is 100 mg/kg and day.

Moore et al. (2001) studied the effects of DEHP (0, 375, 750 or 1,500 mg/kg and day, by gavage) on male reproductive system development and sexual behaviour in Sprague-Dawley rats (n = 5-8/group). The exposure started at gestation day 3, ended at postnatal day 21, and male pups were examined at PND 21, 63 and 105. Numerous effects, including those normally observed after high doses of DEHP, such as malformations and reduced weights of organs related to the male sexual system, were observed in the pups at the highest doses. The lowest dose was a LOAEL (375 mg/kg and day), with findings of adverse effects on aerola and nipple retention, as well as testis and anterior prostate weight (reductions). Although not statistically significant, there were indications of effects on sexual behaviour at PND 105 in all dose groups (inactivity in 3 of 7 low dose males when kept together with females).

Parks et al. (2000) studied the effects of DEHP on male reproductive parameters in Sprague-Dawley rats. Sprague-Dawley rats were randomly assigned to groups that were gavage dosed with 0 (corn oil vehicle) or 750 mg/kg bw/day DEHP from GD 14 (GD 1 = day after mating) until necropsy. Rats were killed and necropsied on GD 17, 18, or 20 or PND 2 (PND 1 = day after birth). The study was conducted in 2 blocks, and a total of 4–5 litters per group were examined at each necrospy period. At GD 17, 18, and 20 and PND 2, 1 testis from 2 or 3 males/litter was incubated in media for 3 hours to determine ex vivo testosterone production, and the other testis was used to measure testosterone content. In GD 17, 18, and 20 males, testosterone levels were also measured in the carcasses from which testes were removed (n = 18–20 per group). Testosterone levels were measured by RIA. One testis from each of 4 DEHP-treated and 6 control PND 2 males was fixed in 5% glutaraldehyde for histopathological examination. One testis from each of 4 control and 5 DEHP-exposed PND 20 males and an unspecified number of DEHP-exposed PND 3 males from a parallel study was stained for 3β-hydroxysteroid dehydrogenase, which is specific for Leydig cells. Anogenital distance was measured in all male and female offspring on PND 2. Litter means were used in statistical analyses. Data were analyzed by ANOVA followed by 2-tailed *t*-tests if ANOVA resulted in significant findings. Testicular histopathological findings were analyzed by Fisher exact test. Maternal weight gain during gestation was significantly reduced in the DEHP-treated group. Number of live pups at birth was not significantly affected by DEHP treatment. Ex vivo testicular testosterone production in GD 17, 18, and 20 and PND 2 offspring from DEHP-exposed groups was significantly lower compared to control groups. Testicular testosterone content in DEHP-exposed offspring and pups was reduced by 60–85% compared to controls examined at each necropsy period; the effect was statistically significant at all time points except GD 20. [It appears a footnote regarding GD 20 is missing in Table 1 of the study.]Whole body testosterone levels were significantly lower in DEHP-exposed fetuses on GD 17 (71% lower than controls) and 18 (47% lower than controls), but the reduction on GD 20 was not significant. Significant reductions in testis weight were noted in the DEHP group on GD 20 (18% lower than controls) and PND 2 (49% lower than controls). Body weights of DEHP-exposed pups were described as 23% lower than controls on PND 2, but statistical significance was not achieved. Testis weights adjusted for body weights were significantly decreased in PND 2 pups exposed to DEHP. Anogenital distance was significantly reduced by 36% in PND 2 males compared to controls but was not affected in female pups exposed to DEHP. Histopathological examination of PND 2 testes of DEHP-treated rats revealed an increased number of enlarged and multinucleated gonocytes and aggregates of hyperplastic Leydig cells. 3β-Hydroxysteroid dehydrogenase staining confirmed the presence of Leydig cell aggregates in DEHP-exposed males on GD 20 and PND 2. In contrast, 3β-hydroxysteroid dehydrogenase staining revealed an even dispersion of Leydig cells and less intense staining in testes of control fetuses and pups. The study authors concluded that treatment with 750 mg/kg bw/day DEHP inhibited testosterone production in male pups during the period of sexual differentiation, and this inhibition was a likely cause of malformations observed in other studie

Gray et al. (1999) investigated the reproductive effects of ten known or suspected antiandrogens, including flutamide, Vinclozolin, dibuthyl phthalate (DBP) and DEHP. Eight pregnant Sprague Dawley dams were administered DEHP (750 mg/kg bw/day > 99% pure) in corn oil by gavage from gestation day 14 to day 3 of lactation. The male offspring was examined for abnormalities (retained nipples, cleft phallus, vaginal pouch, and hypospadias). The animals were also examined internally (ectopic or atrophic testes, agenesis of the gubernaculum, epididymides, sex accessory glands, and ventral prostate, epididymal granulomas, hydronephrosis, an enlarged bladder with stones). Weights measured included body, pituitary, adrenal, kidney, liver, ventral prostat seminal vesicle (with coagulating gland and fluid), testis, and epididymis. Gonads and sex accessory tissues were examined microscopically. DEHP was considerably more toxic than was DBP to the reproductive system of the male offspring. The gestational and lactational exposure induced a statistically significantly increased incidence of both reproductive and non-reproductive malformations including decreased anogenital distance, areolas (88%), hypospadias (67%), vaginal pouch (45%), ventral prostate agenesis (14%), testicular and epididymal atrophy or agenesis (90%), and retained nipples in examined pups. In addition, several 8 day old pups displayed haemorrhagic testes by gross examination. In adult offspring (5 months old) the weight of the gonads, accessory sex organs, and the Levator ani-bulbocavernosus were statistically significantly decreased. Gray and coworkers found that the chemicals investigated could be clustered into three or four separate groups, based on the resulting profiles of reproductive effects. DBP and DEHP induced a higher incidence of testicular and epididymal abnormalities, including atrophy and agenesis, which is not generally found with flutamide or Vinclozolin even at high dose levels. A LOAEL of 750-mg/kg bw/day is derived from this study.

In order to define the dose-response relationship between di(2-ethylhexyl) phthalate (DEHP) and the Phthalate Syndrome of reproductive alterations in F1 male rats, **Gray et al. (2009)** dosed Sprague-Dawley (SD) rat dams by gavage from gestational day 8 to day 17 of lactation with 0, 11, 33, 100, or 300 mg/kg/day DEHP (71–93 males per dose from 12 to 14 litters per dose). Some of the male offspring continued to be exposed to DEHP via gavage from 18 days of age to necropsy at 63–65 days of age (PUB cohort; 16–20/dose). Remaining males were

not exposed after postnatal day 17 (in utero-lactational [IUL] cohort) and were necropsied after reaching full maturity. Anogenital distance, sperm counts and reproductive organ weights were reduced in F1 males in the 300 mg/kg/day group and they displayed retained nipples. In the IUL cohort, seminal vesicle weight also was reduced at 100 mg/ kg/day. In contrast, serum testosterone and estradiol levels were unaffected in either the PUB or IUL cohorts at necropsy. A significant percentage of F1 males displayed one or more Phthalate Syndrome lesions at 11 mg/kg/day DEHP and above. The study was able to detect effects in the lower dose groups only because it examined all the males in each litter rather than only one male per litter. Power calculations demonstrate how using multiple males versus one male/litter enhances the detection of the effects of DEHP. The results at 11 mg/kg/day confirm those reported from a National Toxicology Program multigenerational study which reported no observed adverse effect levels-lowest observed adverse effect levels of 5 and 10 mg/kg/day DEHP, respectively, via the diet.

Srivastava et al. (1989) dosed groups of 21 pregnant albino rats (strain not specified) on day 6-15 of gestation with 0 or 1,000 mg DEHP/kg bw by gavage. On day 20 of gestation all pregnant rats were killed and seven litters from each group were used for standard teratology studies, the remaining 14 litters were used fo a study of liver enzyme activities and determination of DEHP in liver tissue. There was no significant difference in the number of total live foetuses between control and treated animals. No gross or skeletal abnormalitie were observed in the foetuses of the control or DEHP-exposed animals (no data were shown). Significant amounts of DEHP were, however, found in foetal livers and foetal relative liver weights were increased, whereas the activ ty of mitochondrial succinate dehydrogenase, ATPase, malate dehydrogenase and cytochrome c oxidase was decreased. The authors concluded that maternal exposure to DEHP during pregnancy could dversely affect the foetal livers. These results also indicate that DEHP can cross the placental barrier.

The effects on the testicular development in the offspring exposed to DEHP in utero were studied by **Tandon et al. (1991)**. Groups of six pregnant rats were given vehicle (ground nut oil) or DEHP (1,000 mg/kg bw/day; purity not specified) by gavage, during the entire gestation period Birth weight of all pups and body weight gain of two randomly selected male pups from each litter were recorded at day 7 15, 31, 61 and 91 days of age. Absolute and relative testes weights were significantly reduced t day 31 but normalised at day 61 and 91. The offspring of rats exposed to DEHP during the gestational period exhibited a significant increase in the activities of testicular lactate dehydrogenase (LDH) and gamma-glutamyl transpeptidase and a decrease in sorbitol dehydrogenase at the age of 31 days, which was persistent up to the age of 61 days. The concentration of epididymal spermatozoa was significantly reduced day 91, th only day it was measured (5.04 ± 0.24 million in DEHP-treated versus 6.48 ± 0.35 in controls).

The **Wilson (2007)** study was designed to test the hypothesis that gubernacular lesions would be more prevalent in the DEHP-treated (750 mg/kg/day, g stational days 14–18) Wistar male than in the SD rat offspring, whereas the SD rat would display a higher incidence of epididymal agenesis. As hypothesized, striking differences were seen in the incidences of epididymal (67% in SD versus 8% in Wistar) and gubernacular lesions (0% in SD versus 64% in Wistar) among the two strains. In addition, fetal androgen and insl3 mRNA levels differed among the strains. SD fetal males had higher insl3 mRNA and lower T levels than Wistar males. The ratio of ins l3 mRNA to T differed among DEHP ated SD and Wistar fetal males, indicating that the steroidogenic pathway was more affected in the SD strain than in the Wistar strain. Taken together, these results suggest that the different malformat on profil s produced by in utero phthalate treatment arise, at least in part, from strain differences in fetal Leydig ce l function and the manner in which these cells respond to DEHP treatment.

Pregnant Spr gue Dawley rats were gavaged on gestation days (GD) 14–18 with vehicle control, 500 mg/kg DBP, 500 mg/kg DEHP, or a combination of DBP and DEHP (500 mg/kg each chemical; DBP + DEHP) (**Howdeshell 2007**); the dose of each individual phthalate was one-half of the effective dose predicted to cause a 50% incidence of epididymal agenesis. In experiment one, adult male offspring were necropsied, and reprod ctive malformations and androgen-dependent organ weights were recorded. In experiment two, GD18 testes were incubated for T production and processed for gene expression by quantitative real-time PCR. The DBP + DEHP dose increased the incidence of many reproductive malformations by > 50%, including epididymal agenesis, and reduced androgen-dependent organ weights in cumulative, dose-additive manner. Fetal T and expression of insl3 and cyp11a were cumulatively decreased by the DBP + DEHP dose. These data indicate that individual phthalates with a similar mechanism of action, but with different active metabolites (monobutyl phthalate versus monoethylhexyl phthalate), can elicit dose-additive effects when administered as a mixture.

Howdeshell (2008) characterized the dose-response effects of six individual phthalates (BBP, DBP, DEHP, diethyl phthalate [DEP], diisobutyl phthalate [DiBP], and dipentyl phthalate [DPP]) on gestation day (GD) 18 testicular testosterone production following exposure of Sprague-Dawley rats on GD 8–18. BBP, DBP, DEHP, and DiBP were equipotent (ED50 of 440 ± 16 mg/kg/day), DPP was about threefold more potent (ED50 5 130 mg/kg/day) and DEP had no effect on fetal testosterone production. they hypothesized that coadministration of

these five antiandrogenic phthalates would reduce testosterone production in a dose-additive fashion because they act via a common mode of toxicity. In a second study, dams were dosed at 100, 80, 60, 40, 20, 10, 5, or 0% of the mixture. The top dose contained 1300 mg of total phthalates/kg/day including BBP, DBP, DEHP, DiBP (300 mg/kg/day per chemical), and DPP (100 mg DPP/kg/day). This mixture ratio was selected such that each phthalate would contribute equally to the reduction in testosterone. As hypothesized, testosterone production was reduced in a dose-additive manner. Several of the individual phthalates and the mixture also induced fetal mortality, due to pregnancy loss. These data demonstrate that individual phthalates with a similar mechanism of action can elicit cumulative, dose additive effects on fetal testosterone production and pregnancy when administered as a mixture.

Influence of di-(2-ethylhexyl) phthalate (DEHP) on testicular development was studied by oral administration of DEHP at doses of 500 and 1000 mg/kg/day to pregnant rats on gestational days (G) 7 to 18 (**Shirota et al., 2005**). Ethinyl estradiol (EE) at dose levels of 0.25 and 0.5 mg/kg/day was used as a reference substance. Each 5-6 pregnant rats were sacrificed and their fetuses were examined on G12, 14, 16, 18 and 20. Fetal de ths averaging 20-36% were observed at every examination in the group receiving 1000 mg/ kg of DEHP. Inc eases of fetal deaths over 50% were also observed in the reference group that received 0.5 mg/kg of EE. Microscopic examination of the fetal testis in groups treated with DEHP revealed degeneration of germ ells in G16 fetuses and localized proliferation or hyperplasia of interstitial cells in G18 and 20 fetuses. Germ cells having more than two nuclei were observed in a few cases including the control testes of G14 fetuses. Th se multinucleated cells were observed frequently in G20 fetuses treated with DEHP. Examination of testes of naturally delivered offspring of dams treated with 1000 mg/kg of DEHP at 7 weeks of age revealed sc ttered atr phy or dilatation of seminiferous tubules. Another experiment was carried out to confirm the do e of DEHP affecting testicular development and spermatogenesis. DEHP was given to pregnant rats at d ses of 125, 250 and 500 mg/kg/day during G7-18. Similar histopathological changes were observed in fetal test of the group exposed to 500 and 250 mg/kg of DEHP, but not in those exposed to 125 mg/kg. In po tnatal xamin tions, however, no abnormality was found in the testes at 5 and 10 weeks alter birth in any of the reated groups. Furthermore, no abnormal findings were observed in the function of sperm, sperm counts and sperm morphology in the offspring of the group treated with DEHP during the fetal period at 10 weeks of age. Thu , 125 mg/kg/ day is considered the no-observed-effect-level of DEHP on testicular development of rats by xposure in utero during the period of organogenesis.

Gray et al. (2000) examined the effect of perinatal phthalate xposure in rats. Sprague-Dawley rats were gavage dosed with 0 (corn oil vehicle) or 750 mg/kg bw/day DEHP (99% purity) from GD 14 (GD 1 = day sperm detected) to PND 3 (PND 1 = postcoital day 23). The experiment was repeated with a second block of animals. In each block of the experiment, there were 7–9 reat d dams and 9–10 control dams. Parameters examined in pups (period examined) included body weight (PND 2), anogenital distance (PND 2), testicular histology (PND 2, 9–10, and 13, 3–5 months, and 4 7 months), areolas/nipples (PND 13), preputial separation (beginning on PND 28), mating behavior (adulthood), abnormalities of reproductive organs (3–5 months and 4–7 months), and sperm counts. Statistical analyses were b sed on litters, and blocks were pooled in cases of identical results. Analyses included 1-way ANOVA followed by post hoc *t*-tests when statistical significance was obtained. Anogenital distance and rgan weight data were covaried with body weight. Categorical data were analyzed by Fisher exact test or hi-squared test. DEHP treatment resulted in a small reduction in maternal body weight gain. Litter weight at birt was significantly reduced by 15% in the DEHP group, but there was no effect on number of live pups at birth. In DEHP-t eated males on PND 2, anogenital distance was significantly decreased by ~30%, with or without adjustment for body weight, and paired testis weights were significantly decreased by 35%. There was no ffect on anogenital distance in female pups. Histological examination of testes from DEHP-treat d rat on PND 2–3 revealed focal interstitial hemorrhage and multinucleated gonocytes containing 3–5 nuc ei or undergoing degenerative changes. Hemorrhagic testes were observed in 7 DEHP-treated males from 3 litters t PND 8–9. Histological examination of testes on PND 9–10 revealed evidence of focal hemorrhage in some test s and extensive coagulative necrosis in other testes of DEHP-treated rats; loss of seminiferous pithe ium was observed in areas with hemorrhage or necrosis. Areolas were observed in 87% of DEHP-treated male pups versus none in control pups. DEHP treatment did not delay the age of preputial separation, but preputial separation was incomplete due to malformations in 19 of 56 treated pups. DEHP did not appear to affect sexual behavior in adult rats, except that males with malformed penises were unable to achieve intromission. At necropsy, 45 DEHP-treated adult rats from 15 litters were assessed for malformations of reproductive organs, which were observed in 82% of DEHP-treated males. The types of malformations included permanent nipples, clefting of phallus and hypospadias, vaginal pouches, agenesis of prostate, seminal vesicles, or coagulating glands. Sperm production and numbers were said to be unaffected by DEHP treatment [data not shown]. Testicular defects included hemorrhage, granuloma, fibrosis, reduced or atrophy, and non-descent associated with abnormal gubernacula or ligaments. Significant reductions in weight were observed for all male reproductive organs including testis, levator ani plus bulbocavernosus muscle, seminal vesicle, prostate, penis,

and epididymis. Liver, pituitary, kidney, and adrenal weights were not affected by DEHP treatment. Serum testosterone levels were unaffected in DEHP-treated rats. The study authors concluded that 750 mg/kg bw/day DEHP severely alters sexual differentiation in an anti-androgenic manner.

Culty (2008) examined the effects of fetal exposure to a wide range of di-(2-ethylhexyl) phthalate (DEHP) doses on fetal, neonatal, and adult testosterone production. Pregnant rats were administered DEHP from Gestational Day (GD) 14 to the day of parturition (Postnatal Day 0). Exposure to between 234 and 1250 mg/kg/day of DEHP resulted in increases in the absolute volumes of Leydig cells per adult testis. Despite this, adult serum testosterone levels were reduced significantly compared to those of controls at all DEHP doses. Organ cultures of testes from GD20 rats exposed in utero to DEHP showed dose-dependent reductions in basal testosterone production. Surprisingly, however, no significant effect of DEHP was found on hCG-induced testosterone production by GD20 testes, suggesting that the inhibition of basal steroidogenesis resulted from the alteration of molecular events upstream of the steroidogenic enzymes. Reduced fetal and adult testosterone production in response to in utero DEHP exposure appeared to be unrelated to changes in testosterone metabolism. In view of the DEHP-induced reductions in adult testosterone levels, a decrease in the expression of steroidogenesis related genes was anticipated. Surprisingly, however, significant increases were seen in the expressi n of Cyp1 a1, Cy17a1, Star, and Tspo transcripts, suggesting that decreased testosterone production after irth could not be explained by decreases in steroidogenic enzymes as seen at GD20. These changes may reflect n increased number of Leydig cells in adult testes exposed in utero to DEHP rather than increased g ne expression in individual Leydig cells, but this remains uncertain. Taken together, these results demonstr te that in utero DEHP exposure exerts both short-term and longlasting effects on testicular steroidogenes s that might involve distinct molecular targets in fetal and adult Leydig cells.

Borch et al. (2005) evaluated early testicular effects of perinatal exposure o DEHP with or without diethylhexyl adipate in Wistar rats. In the first experiment, pregnant females were tre ted by gavage with vehicle, DEHP 750 mg/kg bw/day or DEHP 750 mg/kg bw/day + diethylhexyl adipate 400 mg/kg bw/day beginning on GD 7 (plug = GD 0; n = 18/dose group). Chemicals were of 99% purity. On GD 21, 8 d ms/group were killed and fetal testes were harvested. The remaining 8 dams/group continued t receive treatment until PND 17. These animals were permitted to litter. Male offspring were killed on PND 26 (bir h = PND 0), and testes were harvested. A second experiment used 20 pregnant animals in each of 4 do e groups: vehicle control, DEHP 300 mg/kg bw/day, DEHP 750 mg/kg bw/day, and DEHP 750 mg/kg w/day + diethylhexyl adipate 400 mg/kg bw/day. Treatment was from GD 7 through PND 17. On PND 22 3 males/litter were killed and testes were harvested. On PND 190, 1 or 2 males/litter were killed and tes es h ves d. Of the testes collected on GD 21, 14–19/dose group (2–4/litter) were fixed in formalin, embedded in paraffin, and sections were stained with hematoxylin and eosin for light microscopy. Ten testes/dose group (1 r 2/litter) from PND 22 and PND 26 animals were processed in the same manner. Another 10 testes rom these age groups as well as 16 testes/dose group (1 or 2/litter) were fixed in Bouin fluid d st ined w th hematoxylin and eosin for light microscopy [embedding material not specified]. Tubule diameters were measured, and a 10% increase over the control maximum was defined as enlarged. Terminal deoxynucl otidal transferase- mediated dUTP nick-end labeling (TUNEL) staining was performed using a c mmer ial kit, and immunostaining was performed for caspase-3, proliferating cell nuclear antigen (PCNA), histone H3, anti-Müllerian hormone, 3β-hydroxysteroid dehydrogenase, vimentin, and smooth muscle actin. Caspase-3 activity was measured in 10 testes/dose group (from 5–10 litters/group) at GD 21, PND 22, and PND 26 [The method was described only by reference to another paper.] DNA laddering was assessed based on relative fluorescence of DNA ladders on gels. Statistical analysis was by ANOVA with post hoc Dunnett test or by Kruskall-Wallis test. Litter was included as a factor in the ANOVA. In testes evaluated on GD 21, vacuolization of Sertoli cells, shedding of gonocytes, reduced interstitial cell cytoplasm, and enlarg d tu ules were identified in offspring of all dams exposed to DEHP 750 mg/kg bw/day, regardless of diethylhexyl adipate co-exposure, compared to 0–14% of dams exposed to vehicle. Leydig cell hyperplasia was identifi d in offspring of more dams with DEHP treatment than control dams [statistical analysis not shown]. The num er of histone H3-positive cells per testis section was not altered by treatment. [Other immunohistochemistry results were not quantified but were not reported as affected by treatment.] Staining for anti-Müllerian hormone to identify Sertoli cells showed positive cells within Leydig cell clusters, outside the tubules. DNA laddering was increased by DEHP treatment, although TUNEL-positive cells and caspase-3-positive cells were not increased by maternal DEHP 750 mg/kg bw/day. On PND 26, tubules without spermatocytes were found in all litters exposed to DEHP compared to 29% of control litters [statistical analysis not shown]. Malformed tubules were identified in 17–29% of DEHP exposed litters compared to none of the control litters. There were no effects of DEHP treatment on any of the measures of apoptosis on PND 22, 26, or 190, although the authors indicated that "a few animals in the treated groups had very high numbers of TUNEL positive cells, presumably spermatocytes." The authors concluded that the development of dysgenic tubules in response to DEHP exposure was related to interstitial changes occurring during gestation, including the presence of Sertoli cells in the interstitium. They believed that Sertoli cell dysfunction in the fetal period might underlie

the focal testicular dysgenesis seen in older animals. The authors proposed that the lack of alteration in Sertoli cell structure in prepubertal rats in this study might reflect recovery from DEHP, which was last administered on PND 17.

Female Wistar rats were treated daily with DEHP and peanut oil (vehicle control) by gavage from gestation day 6 to lactation day 21 (**Andrade 2006a and b**, **Grande 2006**, **Grande 2007**). The low doses were 0.015, 0.045, 0.135, 0.405 and 1.215 mg/kg/day, and the high doses were 5, 15, 45, 135 and 405 mg/kg/day. At the dose levels tested, DEHP had no statistically significant effect on prenatal and postnatal body weight gain of dams. Litter size, sex ratio, postimplantation losses, and number of viable pups were also unaffected by treatment. Pup birth and weaning weight were similar in all groups, and no signs of toxicity were observed in dams and offspring. DEHP had no effect on brain, spleen, thymus, ovary, and thyroid weights of dams. A significant increase in liver and kidney weights was detected at the highest dose level (405 mg/kg/day). In the female offsprings, a significant delay in the age at vaginal opening at 15 mg DEHP/kg/day and above, as well as a trend for a delay n the age at first estrus at 135 and 405 mg/ DEHP/kg/day, was observed. Anogenital distance and nippl development were unaffected. A normal pattern of estrous cyclicity with no hormonal alteration (serum estradiol and progesterone) was observed. A statistically significant increase in tertiary atreti folli les was observed at the highest dose (405 mg DEHP/kg/day). Morphometric analysis indicated that uterus and vagina luminal epithelial cell height were unaffected by treatment. An increase in the number of ovari n atretic tertiary follicles was the only effect observed in adult female offspring exposed *in utero* and during lactation to DEHP. The NOAEL for female reproductive development may be set at 5 mg/kg/day. In male off prings, nipple retention and reduced anogenotal distance, both sensitive markers of anti-androgenic effects during development, were only seen in males exposed to the highest dose (405 mg kg/day). Delayed preputial separation was observed in animals exposed to 15 mg/kg/day and higher oses Histopa hological examination of the testis on postnatal days (PND) 1 and 22 revealed changes at 135 and 405 mg DEHP/kg/day. The most prominent finding on PND 1 was the presence of bi and multinucleated gonocy e . On PND 22 signs of reduced germ cell differentiation in seminiferous tubules of exposed anim ls were o served. The current results show that DEHP acts as an anti-androgen at a high dose exposure (405 mg/kg/day). At the adult age, a reduction in daily sperm production of 19-25% in relation to control was obse ved i nimals exposed to 15, 45, 135 and 405 mg/kg/day. Quantitation of specific cell types shows that the observ d effects in daily sperm production are not related to changes in the number of Sertoli cells or their apability to support early stages spermatocytes. A low incidence of cryptorchidism was observed in DEHP exposed groups with a lowest observed adverse effect level of 5 mg/kg/day. Serum testosterone concentration was similar to control at most doses but was significantly increased at 0.045, 0.405 and 405 mg DEHP/kg/day. In spite of this effect, the weight of seminal vesicle with coagulating glands was significantly reduced a 405 mg/kg/day. Testis, epididymis and prostate weights were similar among groups. Fertility and sexual behav our were not affected by DEHP treatment at any dose. Overall, the results show that in utero and lactati nal DEHP exposure reduces daily sperm production and has the potential to induce reproductive tract abnormalities (of which cryptorchidism seems to be the most sensitive in our rat strain) in male offspring rats. The lowest observed adverse effect levels (LOAELs) for these effects were 15 and 5 mg/kg/day, respective y. Therefore, the NOAEL For male reproductive development can be set at 1.215 mg/kg/day.

However, these results also indicate that other subtle developmental effects occur at lower DEHP doses. In males on PND 1, aromata e activity was inhibited at low doses and increased at high doses resulting in a non-monotonic dose-response. Inhibition was statistically significant at 0.135 and 0.405 mg DEHP/kg/day, while increased activity was observed at 15, 45, and 405 mg/kg/day. In contrast to findings on PND 1, aromatase activity at weaning (PND 22) was more affected in females than in males. An increase in aromatase activity was observed a only one dose in males (0.405 mg/kg/day) while an increase in activity was observed at all doses in the femal s except regard to the age at which effects are manifested.

Pregnant Wistar rats were orally (gavage) exposed to 0, 0.25 or 6.25 mg DEHP/kg bw/d throughout gestation and l ctation (**Wei et al., 2012**). Offspring were investigated for effects on renal histology, renal function and blood pressure (week 3, 15, 21, 33). DEHP exposure affected renal histology (e.g. number of nephrons, glomerular volume) and function (creatinine clearance) in all dose groups. Effects on kidney weight and blood pressure were inconsistent. Renal protein expression of renin and angiotensin II was reduced at birth and increased a weaning, without clear dose-response. DEHP-exposure also affected the expression of some genes involved in renal development. This is the first study reporting kidney toxicity of DEHP at very low doses which needs confirmation from further studies as other studies on developmental toxicity did not observe kidney toxicity in this low dose range. Additionally the partially observed differences between sexes and different findings at the investigated time points need further clarification.

Pregnant Wistar rats were orally (gavage) exposed to 0, 1.25 or 6.25 mg DEHP/kg bw/d throughout gestation and lactation (**Lin et al., 2011**). Offspring (n</=6 male and 6 female per dose group) were investigated for

effects on body weight, glucose and insulin tolerance, beta cell morphometry and function. In all treatment groups effects on beta cells ultrastructure, beta cells mass and pancreatic insulin content were observed as well as alterations in the expression of genes involved in pancreas development and beta cells function at weaning. Adult female offspring revealed elevated blood glucose, reduced serum insulin, impaired glucose tolerance and insulin secretion. Male offspring had increased serum insulin, but there were no significant differences in blood glucose at fasting and during glucose tolerance test. Additionally body weight was reduced during weaning and till adulthood. These preliminary findings in a reduced number of offspring need confirmation from further studies.

Mice

In a continuous breeding study, comparable to a guideline study and performed according to GLP principles, DEHP (> 99% pure) was given to CD-1 mice (20 animals of each sex per dose group and 40 control anima of each sex) at dietary levels of 0, 0.01, 0.1, or 0.3% (equivalent to 0, 14, 141, and 425 mg/kg bw/day, respectively) (Lamb et al., 1987). Both male and female mice were exposed during a 7-day premating period and wer then randomly grouped as mating pairs. The dosing continued during the 98-day cohabitation period and thereafter for 21 days during which final litters were delivered and kept for at least 21 days. Reproductiv function was evaluated by measuring the number of litters per breeding pair, number of pups per litter proport on of pups born alive, and mean pup weight. Exposure to 0.1% DEHP produced a dose-dependent and significant decrease in the number of litters as well as the number and proportion of pups born alive. N pairs w e fertile at 0.3%. At a diet of 0.3% DEHP caused an increased liver weight (both absolute and relative) and significantly reduced weights of the reproductive organs in parental animals of both sexes (test s, epididymis, prostate, and seminal vesicles in males and ovaries, oviducts, and uterus in females). All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. In addition this dose level also caused decreased sperm motility and sperm concentration and an increased incidence of a normal sperm forms. DEHP did not significantly decrease body weight gain in the high-dose group A cr ssove mating trial conducted with F0 mice showed a decrease in fertility both for treated males and for treated fem les, with a complete loss of fertility in the females. Four litters out of twenty were born to treated males mated to control females; in addition, the proportion of pups born alive was decreased. No pups w re born when dosed females were mated to control males. The NOAEL for maternal and developmental t xicity was equivalent to 600 and 20-mg/kg bw/per day, respectively.

In a dietary 2-generation study (comparable to a g ideline study and performed according to GLP principles) in CD-1 mice, DEHP was given in the diet at 0.0, 0 01, 0.025, and 0.05% (equivalent to 0, 19, 48 and 95 mg/kg bw, respectively) to CD-1 mice (**NTIS, 1988**). DEHP reatment did not affect the number of implantation sites per dam, the percent fertile matings, th preg ancies with live litters on pregnancy day 1, or the percent viable litters through gestation to postnatal day 4. The F1 generation was mated within dose groups at sexual maturity and F2-offsprings were evaluated for v ability and growth at postnatal day 4. For F1-litters, the percentage of prenatal mortality was increased at the high dose (9% versus 26.4%). During the neo-natal period, the percent of viable pups was significantly d creased at 0.05% DEHP. No other effects of DEHP were observed upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testes decent or vaginal opening or pontaneous locomotor activity) on postnatal days 14, 21 or 50/day. Treatment-related lesions were n t observed in the dams and no maternal LOAEL was established. The NOAEL for parental toxi ity nd for F2-offspring was 0.05% DEHP (95 mg/kg bw/day), the highest dose tested. The LOAEL for F of spring was 0.05% (95 mg/kg bw/day) (NTIS, 1988).

Tanaka (2002) xamined neurobehavioral toxicity in mice exposed to DEHP (> 97% purity) during prenatal deve opment. At 5 weeks of age, 10 CD-1 mice/sex/group were fed diets containing 0, 0.01, 0.03, or 0.09% DEHP for 4 weeks prior to mating and during a 5-day mating period that began at 9 weeks of age. Females continued to receive the control or DEHP-containing diets throughout the gestation and lactation periods. The authors converted DEHP doses to a mg/kg bw/day basis. Each female was mated to 1 male, and the females were ll wed to litter and rear their offspring. At birth (PND 0), litter size, litter weight, and sex ratio were determined. Offspring were individually weighed, and postnatal survival was monitored during the lactation period. Neurobehavioral parameters examined in all offspring during the lactation period included surface righting (PND 4 and 7), negative geotaxis (PND 4 and 7), cliff avoidance (PND 7), swimming behavior (PND 4 and 14), and olfactory orientation (PND 14). Weaning occurred at 4 weeks of age, and 1 male and 1 female per litter were selected to continue receiving treatment until 9 weeks of age. [Though not specified, it is assumed that the offspring from each treatment group received the same doses as their parents.]. At 7 weeks of age, the F1mice were tested using a Biel type water T-maze. Exploratory behavior was assessed using an animal movement analyzing system in 3-week-old mice from the F1generation and 8-week-old mice from the F0and F1generations. Statistical analyses included Bonferroni multiple comparison, ANOVA, Kruskall-Wallis test, chi-

squared test, Fisher exact test, Wilcoxon sign test, and/or Jonckheere test. [It does not appear that statistical analyses were conducted on a per litter basis.]. In F0mice, DEHP treatment had no effect on body weight gain, movement, or exploratory activity. As a result of non dose-related failures to become pregnant or abortions in 1–2 dams of the low- and mid-dose groups, 8–10 litters were available for evaluation in each treatment group. There were no significant effects on sex ratio or litter size or weight at birth. A 7% decrease in body weight in male offspring of the low-dose group compared to control males on PND 0 was the only significant body weight effect observed in offspring. Significant reductions in survival were noted in the high-dose group for female offspring from PND 4 to 14 and for total offspring from PND 4 to 21. Percentages of total surviving offspring at PND 21 were 98.4% in the control group and 92.8% in the high-dose group. Time for surface righting was significantly delayed in females of the low- and mid-dose groups on PND 4, in males of the high-dose group on PND 7, and in females of the low-dose group on PND 7. There were no other significant findings in neurobehavioral parameters examined during the lactation period [data not shown]. Compared to controls, the e were no adverse effects in water T-maze performance in treated animals at 7 weeks of age, and movement and exploratory behavior were not affected by treatment at 3 or 8 weeks of age. The study authors conclud d that "few adverse effects on several behavioral parameters were produced at the high-dose level of DEHP in he present study. "

Female C3H/N mice were exposed via diet to DEHP (0, 0.05, 5, 500 mg/kg bw/d) for 8 weeks (**Schmidt et al., 2012**). In study part one the mice were mated one week after start of exposure and expo ure continued till the end of lactation, offspring were grown up till PND 84 and mated with untreated males. In tudy part two the mice were mated with untreated males at the end of the 8-weeks exposure period. DEHP int ke lead to an increase in food intake, body weight and visceral fat tissue in female C3H/N mice. Similar effects were observed in offspring. However, these effects did not always clearly correspond wi h dos . Early embryo development was impaired, even if the mothers were only exposed before mating: DEHP t eated nimals revealed an increased number of degenerated blastocytes, however without clear dose resp n e. The abortion rate was 100% in the 500 mg/kg bw/d group, but not affected at lower doses. Opposite find ngs on body weight and fat tissue in mice were reported by Pocar et al. (2012). Pregnant CD-1 mic were orally (gavage) exposed to 0, 0.05, 5.0 or 500 mg DEHP/kg bw/d throughout gestation and lactation (**Pocar et al 2012**). Offspring were investigated for effects on pituitary-gonadal axis. At the highest dose gr up fertility was dramatically reduced, only 1 out of 10 dams was able to deliver, post-implantational losses wer significantly increasesd. DEHP exposure at 0.05 and 5.0 mg/kg/d resulted in decreased body weight at PND21 and 42 in males and females and reduced abdominal fat in females but not in males. These effects did not reveal a clear dose-response relation. Sperm count and sperm viability was decreased in treated animals without clear dose-response relationship.DEHP affected in vitro oocyte maturation and evelopmental competen e in adult female offspring. Expression of steroidogenesis-related genes in the gonads and of gonadotropin mRNA n the pituitary were also affected in treated animals. Due to these contradictory findings in mice fur her inves igations are necessary to evaluate the possible correlation of gestational/lactational DEHP exposure and body weight and fat tissue development.

In vitro study

In the **Bowden study (2000)**, DEHP as administered by the oral route (gavage) to pregnant CD rats on Days 6-11 of gestation at a generally recognised limit dosage of 1000 mg/kg/day. On Day 11 of gestation serum was obtained via the do sal aorta pproximately 1.5 hours after the final administration of DEHP. The influence of this serum upon growth and development of embryos in vitro was assessed in Day 9 embryos from rats of the CD strain. Embryos wer ultured for a period of approximately 48 hours at a temperature of 38.0 ± 1.0 °C. It was observed in th s xploratory investigation that treatment with di-(2-ethylhexyl) phthalate at a dosage of 1000mg/kg/day produced no adverse effects in pregnant female rats or any apparent reduction in the level of zinc in their serum. Culture of embryos in serum from these treated rats, however, produced adverse effects on embryonic growth, development and morphology in vitro. It was concluded, therefore, that the rat whole embryo culture est system is sensitive to detecting teratogenic agent(s) present in serum from DEHP-treated pregnant rat .

The influence of the main metabolites of di-(2-ethylhexyl) phthalate (DEHP) upon growth and development in vitro, was assessed in Day 9 embryos from rats of the CD strain (Bowden 2001). The materials tested were mono-2-ethyl-1-hexylphthalate (MEHP), metabolites I, V, VI and IX. In part 1 of the main study MEHP was added to the culture medium at nominal concentrations of 0.01, 0.04, 0.2, 1, 5 and 10 µmole/ml, in both the presence and absence of metabolic activation. S-9 metabolic activation mixes were prepared from animals treated with Arochlor 1254 or DEHP. In part 2 of the main study metabolites I, V, VI and IX were each added to the culture medium at nominal concentrations of 0.002, 0.01, 0.04, 0.2, 1 and 5 µmole/ml in the absence of metabolic activation. In Part 3 of the study DEHP and the metabolites 2-ethyl hexanol and 2-ethyl hexanoic acid were investigated. All embryos were evaluated alter approximately 48 hours in culture. Data was compiled according to the achieved concentrations for discussion and interpretation of the results. It was observed in this

exploratory investigation that embryos cultured in the presence of MEHP at concentrations in the region of 0.6 - 1.0µmole/ml exhibited adverse effects on embryonic growth, development and morphology in vitro both in the presence and absence of metabolic activation; slight effects were apparent at lower concentrations in the presence of DEHP-derived S-9. Generally more effects were apparent in the presence of metabolic activation, the DEHP-derived S-9 producing slightly greater effects than the Arochlor-derived S-9. At concentrations of 4imole/ml and above embryos had failed to survive to the end of the treatment period, both in the presence and absence of metabolic activation. Embryos cultured in the presence of Metabolite I exhibited only marginal adverse effects on embryonic development at concentrations of 0.023-0.029µmole/ml, adverse effects on morphology were apparent at 0.023µmole/ml and above. Embryos cultured in the presence of Metabolite V exhibited no adverse effects on embryonic growth or development in vitro at concentrations up to 0.408 µmole/ml. Adverse effects on embryonic morphology were apparent at 0.399µmole/ml and above. Embryos cultured in the presence of Metabolite VI exhibited no adverse effects on embryonic growth, development or morphology in vitro at concentrations up to 0.091 µmole/ml. At 0.3 µmole/ml only a marginal effect on embryonic morphology was observed. Embryos cultured in the presence of Metabolite IX exhibited no advers effects on embryonic growth or development in vitro at concentrations up to 0.05. tmole/m1, slight adve se effects were observed at 0.17µmole/m1. Adverse effects on morphology were apparent at 0 0004µmole/ml and above. The types of malformations observed in this study were also seen in a previous study using DEHP-treated rats in particular abnormalities of the otic system and fore limb buds. A comparison of the data for each metabolite suggests the following order of potential toxicity: IX >I>VI>V>MEHP. Embryos cultured in the presence of DEHP exhibited no adverse effects on growth, development or morp l gy at concentrations up to 0.19µmole/ml. Adverse effects on embryonic growth and morphology were apparent at 0.85µmole/ml and above, whilst adverse effects on development were apparent at 2.01. µmol /ml and ab ve. Embryos cultured in the presence of 2-ethyl hexanol at concentrations up to 0.09 µmole/ml exh bited no adverse effects on growth, development or morphology. A slight adverse effect on development s app rent at 0.16 - 0.23 µmole/ml. Adverse effects on embryonic growth, development and morphol gy were pparent at 0.991µmole/ml, in addition half of the embryos had failed to survive to the end of the reatmen period. Embryos cultured in the presence of 2-ethyl hexanoic acid at concentrations up to 1 1 µmole/ml exhibited no adverse effects. At 4.364 - 5.131.1mole/ml adverse effects on growth, development nd morp ology were apparent. A comparison of these data suggest the following order of potential toxicity: DEHP 2-ethyl hexanol > 2-ethyl hexanoic acid.

The following information is taken into acco nt f an hazard / risk assessment:

There are various epidemiological studie examining the reproductive effects of DEHP. These studies largely examine the relationship between urine levels of he DEHP metabolites and various effect parameters. Although some of these studies indicated adverse ffects of DEHP on reproductive functions and the developing organism, associations in these studies were weak, co tradictory, or confounded by exposure to other substances. In agreement with the EU Risk A sessment therefore the risk assessment is based on experimental data.

A number of human studies have attempted to link maternal MEHP levels with gestation length, onset of puberty and AGD. However, th e studies, which were largely negative, are considered inadequate as they generally lacked an adequate ontrol group and were of small sample size.

Developmental stud es in experimental animals comprise single and multiple generation exposure largely by the oral route and predomin tly in rodents. There are no dermal studies, only a single inhalation study and few studies using parente al routes of exposure. There are no developmental studies in primates.

Num rous stud es have shown that DEHP is embryotoxic in rats at doses close to maternally toxic levels. In mic sev ral studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those produ ng observable evidence of toxicity to the dams. Developmentally induced effects are also seen at low doses in multigenerational studies. A well conducted oral 3-generational study in rats derived a NOAEL for developmental toxicity of 4.8 mg/kg bw/d based on the finding of small male reproductive organs at 14 mg/kg bw/d (Wolfe and Layton, 2004). At higher levels of exposure, effects on in utero survival, reduced AGD, undescended testes, retained nipples/areolae, incomplete preputial separation and disruption of spermatogenesis in offspring were evident. In this study, testicular abnormalities in the F1 and F2 generations were much more severe than in F0, indicating the developmental phases were more sensitive to the testicular toxicity of DEHP. The critical study for developmental toxicity is considered to be Wolfe and Layton (2004). For developmental effects, the NOAEL is 100 ppm (4.8 mg/kg bw/d) and the LOAEL is 1000 ppm (14 mg/kg bw/d), based on effects on the male reproductive organs.

Value used for CSA (route: oral): NOAEL: 4.8 mg/kg bw/day

Toxicity to reproduction: other studies

MALE REPRODUCTIVE TRACT TOXICITY

Testicular toxicity in young and adult animals

Testicular effects have been observed in several repeated dose toxicity studies in rats (Poon et al., 1997; NTP, 1982c; Gray et al., 1977; Ganning et al., 1987, 1990; David et al., 2000c). These studies are described in more detail, including systemic effects, in the section on Repeated dose toxicity.

Oral

Rat

Young and adult rats (1, 2, 3, 6, and 12 weeks of age) were given five daily oral doses of di(2-ethylhexyl) phthalate (DEHP) (0, 10, 100, 1000, 2000 mg/kg) and histological changes in the testes were examined 24 hr after the last dose (**Dostal et al., 1988**). Relative testis weights were reduced at doses of 1000 mg/kg in 1, 2, 3, and 6-week-old but not in 12-week-old rats, while doses of 2000 mg/kg were fatal to suckling rats and c used decreased relative testis weight but not death in 6- and 12-week-old rats. In neonatal rats (1 week old) DEHP (1000 mg/kg) caused a 35% decrease in Sertoli cell numbers while 2- and 3-week-old rats sh wed losses of spermatocytes but not of Sertoli cells. The 6- and 12-week-old rats showed loss of both spermatids and spermatocytes at 1000 and/or 2000 mg/kg. Total testicular zinc concentrations were decr ased in 12-week-old but not in suckling (3-week) or weaned (6-week) rats. The results support the hyp th is tha the Sertoli cell is the primary testicular target of phthalate ester toxicity since effects were obs rved a an age when only Sertoli cells were present. Fertility was assessed in mating trials in adult male rat after neonat l exposure to DEHP on Days 6-10. Although Sertoli cell number was reduced 24 hr after the last d se, the numbers were normal at 6 and 13 weeks of age. However, at 6 weeks there was a dose-related decr ase in m tura ion of the spermatids in the tubules. There were no consistent changes in fertility, implantation rate, or numbers of live fetuses in untreated females mated with the DEHP-treated males. However, there were decrease in testis weight and testicular spermatid numbers at 13 and 19 weeks but not at 11, 12, 16, or 23 we ks f age. Therefore, a loss of Sertoli cells due to DEHP exposure neonatally did not affect the fertility of the rats as adults, but may have caused subtle effects on sperm production.

Male Sprague-Dawley rats (4, 10, or 15 weeks of age, 8 animals per group) were used to study the age-dependent effects on male reproductive organs (**Gray and Gangolli, 1986**). The rats were given 2,800 mg/kg bw of DEHP (purity not specified) orally for 10 days Administration to 4-week-old rats produced a marked reduction in absolute weights of the test s, sem nal esicles, and prostate. There was only a slight reduction in testis weight in 10-week-old rats but th seminal vesicle and prostate weights were significantly reduced. DEHP had no effect in 15-week-old rats. Histo ogically the testes of the 4-week-old rats showed severe atrophy affecting virtually all the tubule . These we populated only by Sertoli cells, spermatogonia, and occasional primary spermatocytes. In the 0-week-old rats, these histological changes were evident in 5 to 50% of the tubules, the remainders appearing essent ally normal. No histological abnormalities were seen in testes from the 15-week-old rats. In the same study, the effects of MEHP on Sertoli cell function were studied in immature rats by measuring the se retion of seminiferous tubule fluid and androgen binding protein. A single dose of 1,000 mg/kg bw of MEH reduced fluid and protein production to around 50% of the concurrent control group and to 25% after three repe ted doses.

To study the diffe ing response between immature and mature male rats, **Sjöberg et al. (1985)** carried out a series of experim nts. Groups of 8 male Sprague-Dawley rats (25, 40 or 60 days old) were dosed with 0 or 1,000 mg/kg bw f DEHP in corn oil by gavage for 14 days. After sacrifice liver, testes, ventral prostate, and seminal vesicles were removed, cleaned from fat and weighed. The left testis and epididymis were fixed in Bouin's fluid for his opathological examination. The liver weight was significantly increased in all three age groups. The absolute t sticular weight was significantly decreased; histopathological examination showed severe testicular damage in the 25-day-old rats, whereas the older animals were unaffected. In the youngest age group, there was a marked reduction in the number of germ cells, a high occurrence of degenerating cells, and a reduction of the tubular diameter. There also was a marked reduction in the number of spermatogonia.

Sjöberg et al. (1986) studied the age-dependent testis toxicity of DEHP (1,000 and 1,700 mg/kg bw in the diet for 14 days) in rats at 25, 40 and 60 days of age. Body weight gain was retarded in all dosed groups and testicular weight was markedly reduced in 25- and 40-day-old rats given 1,700 mg/kg bw. Severe testicular damage was shown for the 25-day and 40-day-old rats at both dose levels. No changes were found in the 60-day-old rats. The authors propose that the difference in response to DEHP to male rats of different age may be due to a higher oral absorption of the DEHP-derived metabolite MEHP in younger animals (Sjöberg et al., 1985).

Parmar et al. (1986) found that DEHP affects spermatogenesis in adult male albino rats. Groups of 6 adult

male rats were administered 0, 250, 500, 1,000 or 2,000 mg DEHP/kg bw (purity not specified) in groundnut oil by gavage for 15 days. Body weight, testicular weight, sperm concentration, and activity of several testicular enzymes were determined. In the 2,000-mg/kg bw group, both absolute and relative weights of the testes were significantly reduced. In all dosed groups, sperm counts were significantly reduced in a dose dependent manner from 250 mg/kg bw. The activities of γ-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) were significantly increased at doses from 500 mg/kg bw, sorbitol dehydrogenase (SDH) was decreased from 1,000 mg/kg bw, and acid phosphatase was reduced at 2,000 mg/kg bw. The activity of β-glucuronidase was significantly increased at 2,000 mg/kg bw. The authors suggested that DEHP can affect spermatogenesis in adult rats by altering the activities of these enzymes responsible for the maturation of sperms and that the reduced number of sperms may be responsible for the antifertilic effects of DEHP. The authors also concluded that even at 250 mg/kg bw DEHP causes a decrease in testicular function after short-term dosing. A LOAEL of 250 mg/kg/day DEHP is derived from this study.

To further understand the mechanisms responsible for the enhanced sensitivity of the testes of developing animals to DEHP, the activities of the testicular enzymes associated with spermatogenesis including LDH, GGT, SDH, β-glucuronidase, and acid phosphatase were studied in a similar study investigating the oral effect of DEHP on 25 day old male Wistar rats (**Parmar et al., 1995**). Doses of 0, 50, 100, 250 or 500 mg/kg bw of DEHP (purity not specified) in groundnut oil were given for 30 consecutive days to 6 male rats per dose group. There was an exposure-related and significant decrease of absolute and relative testicular weight at all dose levels. From 50 mg/kg also a dose-dependent and significant increase in the activities of LDH and GGT was noted while that of SDH decreased. β-glucuronidase increased at 250 or 500 mg DEHP/kg, while acid phosphatase decreased at the same dose levels. The administration also resulted in marked destructive changes in the advanced germ cell layers and marked degrees of vacuolar degeneration in the testes at 250 and 500 mg/kg bw. The significant alterations in the activities of SDH, LDH, and GGT occurred thus at much lower DEHP levels and prior to the histopathological changes. The Leydig cells and the fibroblasts appeared normal. A LOAEL for young rats of 50 mg/kg bw/day is derived from this study for effects on absolute and relative testis weight, and reduced testicular enzyme activities.

In a 90-day study performed according to OECD Guidelines and the principles of GLP mild to moderate seminiferous tubular atrophy and Sertoli cell vacuolation were observed in the testes of young male Sprague-Dawley rats (**Poon et al., 1997**). Groups of 10 young male per dose level were given 0, 5, 50, 500 or 5,000-ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw) per day in the diet for 13 weeks. The rats were 105-130 g (approximately 32-37 days) at initiation of dosing and reached sexual maturity at 70 days (Charles River, 2000). The method for preparing testicular tissue included Zenker's fluid fixation, paraffin embedding and haematoxylin and eosin staining., The histopathological slides were controlled blindly. No clinical signs of toxicity were observed. Feed consumtion and body weight gain were not affected. At 5,000-ppm, rats of both sexes had significantly increased absolute and relative liver weights and relative kidney weight. In the 500-ppm dose group, a high incidence of minimal to mild Sertoli cell vacuolation was observed in 7 out of 10 rats. No other effects were noted at this dose level. At 5,000-ppm, the absolute and relative testis weights were significantly reduced. Microscopic examination revealed a mild to moderate, bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules in 9 out of 10 rats. The incidence and severity of seminiferous tubular atrophy were similar to those found in a following study on di-n-octyl phthalate with a positive control group fed a diet containing 5,000-ppm DEHP. The progressive increase in vacuolation of Sertoli cells plus injury and loss of germinal epithelium and spermiogenesis in a treatment-related fashion is regarded as a powerful evidence that the changes observed were not artifactual and that the conclusions were not compromised by the technology employed. A NOAEL of 50-ppm DEHP in the diet (3.7 mg/kg bw/day) is derived from the study.

Fischer 344 rats (10 animals/sex/group) were given 0, 1,600, 3,100, 6,300, 12,500 or 25,000-ppm (0, 80, 160, 320, 630 or 1,250 mg/kg/day) of DEHP (> 99.5% pure) in the diet for 13 weeks prior to an oncogenicity study (**NTP 1982**). The mean body weight gain of male rats was depressed (29%) in males at 25,000-ppm relative to controls. Testicular atrophy was observed in all males fed 25,000-ppm and was present, but less pronounced in males fed 12,500-ppm (630 mg/kg/day). No other compound-related histopathological findings were observed. A NOAEL of 320 mg/kg/day DEHP is derived from this study.

In a study reported by **Gray et al. (1977),** groups of 15 male and female Sprague-Dawley rats were exposed to DEHP via incorporation in the diet at concentrations of 0, 0.2, 1.0 or 2.0% (0, 143, 737 or 1,440 mg/kg bw/day in males) from 1 up to 365 days. The absolute testicular weight in mid- and high-dose rats was lower than compared to control rats while the relative weights were increased. Histological examination revealed a severe seminiferous tubular atrophy and cessation of spermatogenesis related to the dietary level of DEHP. These changes were demonstrated from week 2. A LOAEL of 143 mg/kg/day DEHP is derived from this study.

In an oncogenicity study, performed according to EPA guidelines and in conformity with the principles of GLP,

F-344 rats (70 males and females/group; approx. 6 weeks old at initiation of dosing) were administered DEHP (99% pure) at dietary concentrations of 0, 100, 500, 2,500 or 12,500-ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day) for at least 104 weeks (**David et al., 2000c, 2001**). An additional group (55/sex) was administered 12,500-ppm DEHP for 78 weeks, followed by a recovery period of 26 weeks. An increased incidence of clinical abnormalities was observed in males from the two highest dose groups and the recovery group, significant at the highest dose level. There also was a decreased survival and decreased body weight gain in both sexes at these dose levels, significant only at the highest dose level. In males that died or were sacrificed in extremis during the study, there was an increased (not statistically significant) incidence of small and/or soft testis, small epididymis and/or seminal vesicle. At study termination a dose-related increase of small and/or soft testis was observed in the 2,500 and 12,500-ppm group. Also the recovery group had an increased incidence of small or soft testis. An increased incidence (not significant) of aspermatogenesis was present at 2,500-ppm in unscheduled deaths, t interim sacrifice, and at study termination. At 12,500-ppm, the absolute and relative testis weights were significantly decreased with associated increased incidence of bilateral aspermatogenesis in all males accompanied by hypospermia in the epididymis and decreased incidence of interstitial cell neoplasms 3/10 compared to 9/10 in control group). In the pituitary, an increased number of castration cells were observ d in 30/60 males compared to 1/60 of the control males. There was no indication in rats killed at study te min tion that DEHP-related changes in the testes and pituitary were reversible upon cessation of DEHP-exposur . Due to the dose-related serious effects on the testicles, a NOAEL of 500-ppm corresponding to 28.9 mg/kg/day can be derived for testicular effects.

In a 102 week-study, adult male Sprague-Dawley rats were exposed to DEHP via incorpora on in the diet at dose levels of 0, 0.02, 0.2 or 2% (0, 7, 70 or 700 mg/kg bw/day) (**Ganning et al., 1987, 1990**). In all dose-groups, DEHP exerted a pronounced effect on the function of the testes a er pr longed treatment, consisting of inhibition of spermatogenesis and general tubular atrophy. The LOAEL was 0.02% in the diet (7 mg/kg bw/day), the lowest dose administered. The study was, however, designed to study he effe ts of phthalates on the liver and the information on testicular effects is very limited. Therefor the study results cannot be included in the risk assessment.

In the following oncogenicity study, Fischer 344 rats (50 animals/s x/group; initial body weight just above 200 mg for males and around 150 mg for females) were giv n 0 ,000 or 12,000-ppm (0, 322 or 674 mg/kg/day for males) DEHP (> 99.5% pure) in the diet for 103 weeks (**N P, 1982**). Mean daily doses of DEHP were 322 and 674 mg/kg body weight per day for low- and high-dose male rats, respectively. The survival rate was unaffected. At the end of the study, mean body weights of osed ale rats and high-dose female rats were marginally to moderately lower than those of the corresponding ontrols. Food consumption was slightly reduced in rats of either sex. Interstitial-cell tumours of th testis were bserved in a statistically significant negative relation to dose. There was a statistically significa t increase in bilateral tubular degeneration of the seminiferous tubules and atrophy in the testes. The incid nces were 1/49 (2.0%) in the control, 2/44 (5%) in the low-dose, and 43/48 (90%) in the high-dose group. Histologically, the seminiferous tubules were devoid of germinal epithelium and spermatocytes (tissues had been preserved in 10% buffered formalin and embedded in paraffin). Only Sertoli cells were seen on tubular basem nt membranes. Interstitial cells were somewhat prominent. In high-dose males, the incidence of hypert phy of the anterior pituitary was significantly increased (45% compared with 2% of controls). A LOAEL of 322 mg/kg/day DEHP is derived from this study. No other toxic lesions were associated with compound administratio .

Mouse

Swiss (CD-1) mice (20 animals of each sex) were dosed with 0.30% DEHP (150 mg/kg bw/day; purity not speci ied) n the diet (**Morrissey et al., 1988**). Continuous breeding studies were used to evaluate reproductive performance ove a 98-day cohabitant period. Mice were separated by sex during the first 7 days of DEHP treatm nt. After detection of an adverse effect of DEHP treatment, a 1-week crossover mating trial was carried out between previously treated males and control females. Reproductive ability was assessed at 10 weeks of age in a single breeding trial over a 7-day period. Necropsy for the treated males included organ weights, percentage motil sperm, sperm concentration, and percentage abnormal sperm. In DEHP treated mice, there was a reduction in epididymal and testicular weights, sperm motility, and sperm concentration and an increased number of abnormal sperm cells. No further details are given.

In a study performed according to EPA guidelines and the principles of GLP, B6C3F1 mice (70-85 of each sex/dose group, about 6 weeks of age at the initiation of the study) were administered DEHP daily in the diet at concentrations of 0, 100, 500, 1,500 and 6,000-ppm for 104 weeks (0, 19.2, 98.5, 292.2 or 1,266 mg/kg/day) (**David et al., 2000c; Moore, 1997**). One additional group (55 males) were administered 6,000-ppm DEHP for 78 weeks, followed by a 26-week recovery period. At 1,500-ppm, there was a significant decrease in testicular weight, with an increased incidence and severity of bilateral hypospermia and an associated increased incidence of immature/abnormal sperm forms and hypospermia in the epididymis. At the highest dose level, there was a

statistically significant decrease in survival, treatment-related clinical signs and a significantly reduced body weight gain. In the recovery group, the effects of DEHP in the kidney and testis were at least partially reversible following cessation of exposure. The NOAEL for testicular effects in this study is 500-ppm corresponding to 98.5 mg/kg.

In an oncogenicity study performed according to GLP principles, B6C3F1 mice (50 animals/sex/group) were given 0, 3,000 or 6,000-ppm of DEHP (> 99.5% pure) in the diet for 103 weeks (**NTP, 1982**). Mean daily ingestion of DEHP was calculated to 672 and 1,325 mg/kg bw for low- and high-dose males, respectively. The survival rate was unaffected. In 14% of the high-dose males bilateral seminiferous tubular degeneration and testicular atrophy were observed. This lesion was also found in one control male mouse and in two low-dose males. A NOAEL of 672 mg/kg/day DEHP is derived from this study.

Primate

Male marmosets were treated daily with 0, 100, 500, or 2500 mg/kg DEHP by oral gavage for 65 wk from weaning (3 mo of age) to sexual maturity (18 mo) (**Tomonari 2006, Kurata 2003c**). No treatment-related changes were observed in male organ weights, and no microscopic changes were found in male gonads or secondary sex organs. Sperm head counts, zinc levels, glutathione levels, and testicular enzyme activities were comparable between groups. Electron microscopic examination revealed no treatment-related abnormalities in Leydig, Sertoli, or spermatogenic cells. Histochemical examination of the testis after 3β hydroxysteroid dehydrogenase (3β-HSD) staining did not reveal any alterations in steroid synthesis in the Leydig cells. Thus, although marmoset monkeys were treated with 2500 mg/kg DEHP, throughout the pre- and periadolescent period, no histological changes were noted in the testes. No increases in hepatic peroxisomal enzyme activities were noted in treated groups; isolated hepatic enzyme activities (P-450 contents, testosterone 6β-hydroxylase, and lauric acid ω-1ω-hydroxylase activities) were increased in males of either the mid- or high-dose groups, but no consistent dose-related trend was observed.

In a 13-week oral study performed according to GLP principles, mature male marmosets (4/sex/group, from 13 or 14 months of age) were given daily doses of 0 (corn oil), 100, 500 or 2 500 mg/kg DEHP (purity not specified) in corn oil (**Kurata et al., 1998**). The body weight gain was significantly suppressed in males administered 2,500 mg/kg. Dose-related decreases in spleen weight were observed in all dosed males. Other organ weights, including liver, testes, and pancreas, were not different from the control weights. In the DEHP dosed groups there was a significant rise in the total and free cholesterol and phospholipid levels in administration week 4. In week 13, only the total cholesterol value in the 500 mg/kg males was different from the control value. A clear rise in blood testosterone and oestradiol concentrations in all groups, including controls, were concluded to be hormonal changes accompanying sexual maturity occurring at the age of about 12 months. CLEA Japan, Inc, the animal supplier, have communicated that male marmosets are sexually mature at around 7 months and mate at around 12 months (CLEA, 2000).

Inhalation

Rat

Kurahashi (2005) researched the effects of inhalation of DEHP on testes of pre-pubertal rats. Our results showed that inhalation of DEHP by 4-wk-old male Wistar rats at doses of 5 or 25 mg/m3, 6 h per day, for 4 and 8 wk significantly increased the concentration of plasma testosterone and weight of seminal vesicles. However, the concentration of luteinizing hormones (LH), follicular stimulating hormone (FSH) and the expression of mRNAs of androgen biosynthesis enzyme, cytochrome P450 cholesterol side-chain-cleavage enzyme (P450 cc), 3beta-hydroxysteroid deshydrogenase (3beta-HSD), cytochrome P450 17aplha-hydroxylase/17, 20 lyase (CYP17) and aromatase (CYP19) did not change. Rats with precocious testes did not increase in any of the DEHP groups. The estimated effective dose in this study was less than those reported in previous studies using oral dosing. This study showed that inhaled DEHP increased plasma testosterone concentrations in pre-pubertal rats and suggested that their effects were more sensitive to inhalation than oral dosing.

Testicular toxicity in neonatal animals

Oral

Rat

The effects of DEHP, MEHP and 2-ethylhexanol (2-EH) were determined on gonocytes and Sertoli cell morphology, Sertoli cell proliferation, and expression of cell cycle markers in neonatal rats (three-day old, CD Sprague-Dawley) (**Li et al, 2000**). A single bolus dose of DEHP (20, 100, 200 and 500 mg/kg) was given in corn oil to five pups per group. Diethyl phthalate (DEP: 500 mg/kg) served as the non-toxic control. MEHP (393 mg/kg), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. The doses of MEHP and 2-EH were molar equivalent with 500 mg/kg DEHP. In this dose-response study, all pups were killed 24 hours

after dosing. A time-course study was conducted following a single dose DEHP (200 mg/kg), where the pups were killed after 6, 9, 12, 24 or 48 hours. Biochemical analyses was performed for serum FSH levels, Sertoli cell proliferation (as BrdU labelling; BrdU administered 3 hours before euthanasia), cell cycle regulators cyclin D1, D2, D3, p27kip1 proteins and cyclin D2 mRNA in the testes. Morphological examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 hours post-treatment with DEHP (100-500 mg/kg). With 200 mg/kg DEHP these effects were first determined 12 h after treatment, and persisted for 48 hours. Effects on Sertoli cell morphology were not detailed in the report. MEHP (single dose group) induced effects on gonocytes similar to DEHP. BrdU-labelled Sertoli cells were dose-dependently decreased from 100-500 mg/kg DEHP. No marked difference in BrdU-labelled Sertoli cells was marked with 20 mg/kg DEHP, DEP and vehicle controls. Serum levels of FSH were not affected by DEHP treatment (200 and 500 mg/kg). MEHP also caused a significant decrease in BrdU-labelled Sertoli cells. D2 mRNA was specifically down-regulated by DEHP in a dose-dependent manner (200 and 500 mg/kg only doses reported), and this decrease was manifest as a small, transient but reproducible reduction in the amount of cyclin D2 pro ein with 200 mg/kg DEHP (only dose reported). The effects of MEHP and 2-EH were not determined. 2-EH w s w tho t effect on testicular cell morphology, or Sertoli cell proliferation. A NOAEL for young pups of 20 mg/kg is derived for effects on altered gonocyte morphology and decreased Sertoli cell proliferation by a single or l dose of DEHP.

Di-(2-ethylhexyl) phthalate (DEHP) was administered to 3- to 5-day-old male Sprague Dawley r ts by daily oral gavage of 300 or 600 mg/kg/day for 21 days (**Cammack et al., 2003**). Histopathological evaluation and organ weight measurements were performed on some animals after 21 days of dosing (primary group) and later on the recovery group animals that were held without further treatment until sexual maturity at approximately 90 days of age. Testicular changes, consisting of a partial depletion of the germin l epithelium and/or decrease in diameter of seminiferous tubules, were present in all animals of the 300- and 600-mg/kg/day groups after the 21-day dosing period. Testes weight decreased and liver weight increa ed in hese n mals. In the recovery animals, a residual DEHP-induced decrease in seminiferous tubule diamet r was pre ent in the testis of several animals dosed orally at 300 and 600 mg/kg/day. There was no germinal cell depleti n or Sertoli cell alteration observed in any dose group at any time. Notably, no effects on sperm coun sperm morphology, or sperm motility were observed at 90 days of age in any of the groups.

Other routes

Di-(2-ethylhexyl) phthalate (DEHP) was administered to 3- to 5-day-old male Sprague-Dawley rats by daily intravenous injections of 60, 300, or 600 mg/kg/day or by daily oral gavage of 300 or 600 mg/kg/day for 21 days (**Cammack 2003**). Histopathological evaluatio and organ weight measurements were performed on some animals after 21 days of dosing (primary group) nd later on the recovery group animals that were held without further treatment until sexual maturity a approximately 90 days of age. No effects of any type were observed in animals treated intravenously with 60 mg/kg/day. Testicular changes, consisting of a partial depletion of the germinal epithelium and/or dec ease in d ameter of seminiferous tubules, were present in all animals of the 300- and 600-mg/kg/day groups after the 21-day dosing period. Testes weight decreased and liver weight increased in these animals. Testes changes were d se-related and generally more severe among animals dosed orally versus intravenously. In the recovery animals, a residual DEHP-induced decrease in seminiferous tubule diameter was present in the testis of several animals dosed orally at 300 and 600 mg/kg/day, but not in animals dosed intravenously. There was no germinal cell depletion or Sertoli cell alteration observed in any dose group at any time. Notabl , no effects n sperm count, sperm morphology, or sperm motility were observed at 90 days of age in any of the group .

Effects on the evelopment of the male reproductive tract

Rat

In the **Ge study (2007),** the potential of phthalate exposure to advance or delay the timing of puberty was assess d. Male Long-Evans rat pups were chronically subjected to low or high doses of DEHP, with the androgen-driven process of preputial separation serving as an index of pubertal timing. Rats were treated with 0, 10, 500, or 750 mg/kg body weight DEHP for 28 days starting at day 21 postpartum. The average age at which the animals completed preputial separation was measured in each group. The age of preputial separation was 41.5 +/- 0.1 days postpartum in controls (vehicle). The 10 mg/kg DEHP dose advanced pubertal onset significantly to 39.7 +/- 0.1 days postpartum, whereas the 750 mg/kg DEHP dose delayed pubertal onset to 46.3 +/- 0.1 days postpartum. The 10 mg/kg DEHP dose also significantly increased serum testosterone (T) levels (3.13 +/- 0.37 ng/mL) and seminal vesicle weights (0.33 +/- 0.02 g) compared with control serum T (1.98 +/- 0.20 ng/mL) and seminal vesicle weight (0.26 +/- 0.02 g), while the 750 mg/kg dose decreased serum T (1.18 +/- 0.18 ng/mL) as well as testes and body weights. Direct action of the DEHP metabolite, monoethylhexylphthalate (MEHP), on Leydig cell steroidogenic capacity was investigated in vitro. MEHP treatment at a low

concentration (100 mM) increased luteinizing hormone-stimulated T production, whereas 10 mM concentrations were inhibitory. In conclusion, data from the present study indicate that DEHP has a biphasic effect on Leydig cell function, with low-dose exposure advancing the onset of puberty. High doses of DEHP, which are anti-androgenic, may also be outside the range of real environmental exposure levels.

The **Noriega study (2009)** was designed to determine if the dose response to DEHP was non-monotonic, as hypothesized. Pubertal administration of DEHP delayed the onset of puberty and reduced androgen-dependent tissue weights in both Long-Evans (LE) and Sprague-Dawley (SD) male rats 300 and 900 mg DEHP/kg/day. These effects were generally of greater magnitude in LE than SD rats. By contrast, alterations in testis histopathology (300 and 900 mg/kg/day) were more severe in SD than in LE rats. Taken together, these results suggest that DEHP may be acting on the pubertal male rat testis via two modes of action; one via the Leydig cells and the other via the Sertoli cells. Treatment with DEHP generally reduced serum testosterone and increased serum luteinizing hormone (LH) levels, demonstrating that the reduction in testosterone was due to th effect of DEHP on the testis and not via an inhibition of LH from hypothalamic-pituitary axis. Testost rone production ex vivo (with and without human chorionic gonadotropin stimulation) was consisten ly reduc d in males at the time of puberty and shortly thereafter. DEHP treatment did not accelerate the ag at p berty or enhance testosterone levels at 10 or 100 mg/kg/day in either LE or SD rats, as some have hypothesized Taken together, these results do not provide any evidence of a non-monotonic dose response to DEHP during puberty.

Non-human primates

McKinnell et al. (2009) exposed marmosets in utero (weeks 7 -15 of gestation) or for 14 day after birth to 500 mg/kg bw MBP. Male offspring exposed in utero were investigated at birth (days 1 -5) or in adulthood (weeks 18 -21) for testicular development and function. Additionally 5 pairs of c twin (n=5 exposed and n=5 controls) exposed for 14 days after birth were investigated immediately after end of exposure or testicular development and function. No effects on testis development/function or testicular dysg nesis were measurable. Some effects on germ cell development were found, but these effects were inconsistent and of uncertain significance.

Effects on endocrine system modulation (Fetal testis ster idogenesis)

Moreover, if the rat is any guide, then adult human Leydig ells may be relatively insensitive to the effect of phthalates. Thus, treatment of prepubertal rats with 200 mg/kg/d diethyl hexyl phthalate (DEHP) from postnatal d 21–35 caused a 50% reduction in serum test terone le els (Akingbemi 2001), whereas the same or higher doses administered to adult rats had little or no effect (Akingbemi 2001, Agarwal 1986). Although one study has shown a massive inhibitory effect (>90%) of MEHP on LH-stimulated testosterone production by adult rat primary Leydig cells over 2 h of cultur , this was found only after exposure to 1 mM MEHP, and no effect was found with a 10-fold lower dose (Jones 993).

In contrast to the data in humans, there is unequivocal evidence that certain phthalates can profoundly inhibit testosterone production by the fetal rat te tis. Thus, administration of phthalates to pregnant rats during the last week or so of gestation results in reduced AGD and reproductive malformations, including hypospadias, in the Long Evans (Gray 1999) Sprague-Dawley (Carruthers 2005) and Wistar (Ema 1998) strains of rat. These changes are consist nt with reduced fetal androgen exposure, and this has been demonstrated directly after in utero exposure to a number o phthalates, including DEHP (Wilson 2004, Gray 2000, Parks 2000), DBP (Wilson 2004, Fisher 2003, Mylchreest 2002, Schultz 2001), butyl benzyl phthalate (Wilson 2004), diisobutyl phthalate (Boberg 2008), diisononyl phthalate (Gray 2000), and diisoheptyl phthalate (McKee 2006). Several of these compounds have been shown to cause dose-dependent suppression of fetal testicular testosterone production, and for the mo t potent (DEHP and DBP), this occurs at doses above 100–250 mg/kg/d. Other phthalates, such as diethyl ph halate, dimethyl phthalate, dioctyl phthalate, and diisodecyl phthalate, do not affect fetal rat testicular testost one production or AGD (Gray 2000, Howdeshell 2008).

Mole ular analyses in fetal rat testes after in utero exposure to phthalates has shed light on the potential mechanisms via which phthalates suppress testicular testosterone production. Several of the key genes involved i teroidogenesis are down-regulated after in utero exposure to DBP or MEHP. These genes are StAR, HMG-CoA synthase, and SRB1 (all involved in cholesterol uptake/transport), and the steroidogenic enzymes Cyp11a, 3beta-Hsd, and Cyp17 (Schultz 2001, Lahousse 2006, Lehmann 2004, Plummer 2007). Suppression of these various enzymes provides a convincing explanation for the phthalate-induced reduction in fetal testicular testosterone production.

Based on present evidence, it appears that all of the phthalates that affect testosterone production by the fetal testis do so by similar mechanisms, although the dose-response characteristics may differ; in this regard, DBP and DEHP are the most potent and are approximately equipotent, based on the above-cited studies.

In contrast to the consistent effects of DBP and DEHP on fetal testicular testosterone production in the rat, data for exposure of fetal mice to DBP or DEHP have produced equivocal results. A detailed study showed that administration of single or multiple doses of DBP (up to 1500 mg/kg/d) or MEHP(up to 1000 mg/kg/d) to pregnant mice did not reduce testicular testosterone levels or affect the expression of the steroidogenic enzyme genes, as seen in the rat (Gaido 2007); this was shown in two strains of mice (C57Bl6, G3H/HeJ).

In contrast, a recent study in C57Bl6 mice treated with 100, 200, or 500 mg/kg/d DEHP from e12–e17 reported that this dose-dependently induced hypospadias on e19, with males from the top dose group exhibiting a 75.7% incidence of hypospadias and a 13% reduction in AGD (Liu 2008). In addition to conflicting with the study by Gaido et al. (Gaido 2007), this study also conflicts with data for the rat exposed to similar levels of DBP or DEHPbecause effects on AGD (measured postnatally) are of similar magnitude to those reported for the mice, but the rates of hypospadias reported are considerably less in the rats, ranging from 12–37% (Gray 2000, Fis er 2003, Mylchreest 2000), an observation that is explained by the relatively poor suppression of testost rone levels by DBP in rats during the masculinization programming window (Scott 2008).

No other toxicological studies involving fetal exposure of mice to phthalates that cause fetal testis effec in rats have reported hypospadias (Heindel 1989, Shiota 1982), although it is uncertain whether or not this was specifically sought. Another study has reported that DEHP can reduce insulin-like factor 3 mRNA expression by fetal mouse Leydig cells in vivo and in vitro, but effects on steroidogenesis were not s udied (Song 2008). The conflict over fetal testis effects of phthalates in mice is not clarified by studies on isolated Leydig cells from postnatal mice because positive effects on steroidogenesis have been reported (G nn rsson 2008), whereas negative effects of MEHP have been reported on MA-10 tumor Leydig cells (Dees 2001). However, a very recent study (Lehraiki 2009) perhaps reconciles these disparate findings for phthalate effects in mice. It shows that whether MEHP has inhibitory or stimulatory effects on steroidogenesi in fetal mouse testes cultured over 1–3 d depends on fetal age, culture duration, and the presence/ absence f LH Notably, no inhibition of basal testosterone production was observed at any age, but in particular at e13.5 (corresponding to part of the masculinization programming window), and this is in marked contrast to the studies in rats.

Johnson et al. (2012) concluded that recent studies on fetal testis xenog afts suggest that the human fetal testis responds like the mouse and is refractory to phthalate induced inhibtion of testosterone production. This conclusion is based on experimental data with xenocrafted fetal testis of humans, rats and mice reported by Mitchell et al. (2012) and Heger et al. (2012).

FEMALE REPRODUCTIVE TRACT TOXICITY

Ovarian toxicity

Rat

In a study comparable to a guid line study, regularly cycling Sprague-Dawley rats (6-9 in each study group) were dosed daily with DEHP (> 99% pure) at 2,000 mg/kg bw in corn oil by gavage for 1-12 days (**Davis et al., 1994**). Ovarian morphol gy and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and progesterone levels were analysed. DEHP treatment resulted in prolonged oestrus cycles compared to a control group. DEHP also suppressed or delayed ovulation by the first prooestrus/oestrous after the metoestrus-initiated dosing. Histopathological evaluation of the ovaries showed that 7 out of 10 DEHP-exposed rats had not ovulated by vaginal oestrous in contrast to 13 out of 13 control rats which ovulated by vaginal oestro s Pre-ovulatory follicles were quantitatively smaller in DEHP-exposed rats than in controls due to smaller ranulosa cells. Suppressed serum oestradiol levels caused a secondary increase in FSH levels and did not timulate the LH surge necessary for ovulation. According to the authors, these results suggest that DEHP-t eatm nt causes hypo-oestrogenic anovulatory cycles and polycystic ovaries in adult female rats.

The effects of in vivo administered DEHP (1,500 mg/kg bw orally for 10 consecutive days) on in vitro ovarian steroid profiles in immature and cycling female rats have been studied by **Laskey and Berman (1993).** Groups of 20 and 21 mature female Sprague-Dawley rats were administered 0 or 1,500 mg DEHP/kg bw in corn oil for 10 days. On day 5 before dosing and daily during dosing, the stage of the oestrus cycle was determined for all animals. The day after the final dosing the animals were killed and ovaries, adrenals and serum were used to determine rates of steroid production. No ovary weight differences were noted in the control cycling animals or between the control and DEHP-treated rats. The alterations caused by DEHP in the in vitro ovarian steroidogenic profile were most apparent in rats during dioestrus and oestrous. In the DEHP dosed animals the incidence of animals in prooestrus was clearly reduced from day seven to day ten of dosing. In cultures of adrenals and serum no significant differences in rates of steroid production were observed. In the ovary cultures, di-oestrus rats dosed with DEHP had significantly higher testosterone and oestradiol production, and in rats in oestrus the oestradiol production was significantly lower in DEHP-dosed females. There were no significant differences in

the steroid production of rats in prooestrus (only two dosed animals). The authors conclude that DEHP treatment alters the oestrus cycle and causes concentration changes of testosterone and oestradiol in rats in dioestrus.

Whole ovary cultures from cycling Sprague-Dawley rats fed 1,500 mg/kg bw/day of DEHP (purity not specified; in corn oil) by gavage for 10 days were used to evaluate if DEHP altered steroidogenic profiles (**Berman and Laskey, 1993**). Ovaries were removed and cultured for one hour. Steroidogenic profiles of progesterone, testosterone, and oestradiol release into the medium were measured using radioimmunoassay techniques. Dioestrous ovaries produced more oestradiol after DEHP administration and oestrus ovaries significantly less oestradiol; proestrous ovary production was not significantly changed. Testosterone production was significantly increased only in dioestrous. DEHP had no significant impact on progesterone production or serum levels of progesterone and oestradiol in treated rats.

Monkey

Female marmosets were treated daily with 0, 100, 500, or 2500 mg/kg DEHP by oral gavage for 65 wk from weaning (3 mo of age) to sexual maturity (18 mo) (**Tomonari 2006, Kurata 2003**). Increased ovarian nd uterine weights and elevated blood estradiol level were observed in higher dosage groups, 500 and 2500 mg/kg. These increased weights were associated with the presence of large corpus luteum, a comm n finding in older female marmosets. Although an effect on the ovary cannot be completely ruled out, no abnorm l histological changes were observed in the ovaries or uteri in comparison to controls. No increases in hepatic p roxisomal enzyme activities were noted in treated groups; isolated hepatic enzyme activities (P-450 contents, testosterone 6β-hydroxylase, and lauric acid ω-1ω-hydroxylase activities) were increased in females of e her the mid- or high-dose groups, but no consistent dose-related trend was observed.

In vitro

The female reproductive toxicity of di-(2-ethylhexyl) phthalate and its active m tabolite mono-(2-ethylhexyl) phthalate (MEHP) is attributed to suppression of ovarian granulos cell estr diol production. In these studies, several structurally related phthalates (0-200 microM) and Wy 14,6 3 (0-100 microM) were compared to MEHP for their effects on granulosa cell estradiol production and transcript leve s of cytochrome P450 enzyme CYP 19, also known as aromatase (P450arom), the rate-limiting enzym in th conversion of androgens to estrogens (**Lovekamp and Davis, 2001**). Granulosa cells were obt ined from 28-day-old Fisher 344 rats and were cultured for 48 h. Test chemical or DMSO was added at the time of culture, along with testosterone as a substrate for aromatase. 17beta-Estradiol production was m sured by standard radioimmunoassay, mRNA was measured by fluorescent RT-PCR, and protein was measured y Western blot analysis. MEHP was unique among the phthalates in its ability to decrease estradiol production, while Wy-14,643 had effects similar to MEHP at 100 microM. MEHP and Wy-14,643 also significantly decreased aromatase mRNA levels. The decrease in mRNA was concentration dependent and was p ralleled by a decrease in aromatase protein. MEHP did not alter levels of CYP 11A1, the cholesterol side-chain cl vage enzyme (P450scc). Treatment with a cAMP analogue increased expression of P450scc in the p esence of MEHP (100 to 200 microM) while the decrease in aromatase remained. Thus, these studies suggest that MEHP is distinct from several structurally related phthalates but similar to the peroxisome proliferat Wy-14,643 in its action on granulosa cell estradiol production. Moreover, the suppression of e tradiol by MEHP is likely mediated through its action on aromatase transcript levels independent of cAMP-stimul ted regulation.

Effects on the development of the female reproductive tract

Rat

Ma (2006 evaluated the effects of inhaled di(2-ethylhexyl) phthalate (DEHP) on the onset of puberty and on pos pubertal reproductive functions in pre-pubertal female rats. DEHP was administered by inhalation at doses f 0, 5 and 25 mg/m3 to groups of female rats for 6 h/day, 5 contiguous days/week from postnatal days (PNDs) 22 to 41 and to PND 84. The onset of puberty was determined by daily examination for vaginal opening (VO) and first estrous cycle. Reproductive function was evaluated by observing estrous cyclicity from PNDs 49 to 84. Upon completion of exposure, the rats were sacrificed at PND 42 and PNDs 85–88 during the diestrous stage. DEHP exposure advanced the age of VO and first estrous cycle, and serum cholesterol, luteinizing hormone, and estradiol levels were significantly elevated in the 25-mg/m3 DEHP group. Irregular estrous cycles were observed more frequently in DEHP exposed rats, and serum cholesterol decreased in DEHP-exposed rats in adulthood; RT-PCR showed that the expression of aromatase mRNA, encoding a rate-limiting enzyme that catalyzes the conversion of testosterone to estradiol, was elevated in the 25-mg/m3 DEHP group. These data suggest that inhaled DEHP may advance the onset of puberty and alter post-pubertal reproductive functions.

MECHANISTIC STUDIES

Role of zinc, testosterone and vitamin B12

Seven immature male Crj: Wistar rats (30 days, 75-90 g) per group were orally dosed with DEHP (2,000 mg/kg bw/day) for 0, 1, 3, 6 and 10 days (**Oishi, 1986**). Organ weights were significant decreased: testes by day 3; Seminal vesicle by day10; ventral prostrate by day 3. Testicular morphology was normal on day 1 but changes occurred for longer exposures. By day 3: number of spermatocytes and spermatids were decreased in some seminiferous tubules; day 6: active spermatogenesis was rarely found, seminiferous tubules contained necrotic debris and variable numbers of multinucleated gaint cells. By day 10, all seminiferous tubules had shrunken. Zinc concentration in the testes significantly decreased by by day 6 and 10, and by day 10 in the ventral prostrate. The zinc content was not affected in the seminal vesicle and serum. Specific activities of some zinc containing enzymes such as carbonic anhydrase, alcohol dehydrogenase and aldolase significantly decreased by day 10. The author concludes that several testicular cell-specific enzymes appear to be useful biochemical markers of testicular injury. However, these changes occurred after or simultaneously with massive histological or morphological changes rather than prior to such changes.

In a study comparable to a guideline study, groups of 48 male F344 rats were maintained on synthetic diets containing 2-ppm (low), 20-ppm (normal) or 200-ppm (high) zinc (**Agarwal et al., 1986b**). Aft r one we k of acclimation to the various diets, groups of 12 rats from each dietary regimen were gavaged for 13 onsecutive days with 0, 330, 1,000 or 3,000 mg/kg bw of DEHP (> 99% pure). Organ weights of testi epididymis, prostate, and seminal vesicles were not affected by DEHP in rats at normal- and high zinc diet but were significantly and dose-dependently reduced in rats on low-zinc diet. The combination of low-zinc diet plus 1,000 or 3,000 mg/kg bw of DEHP caused dose-dependent tubular degeneration and atrophy. Se en young male Crj: Wistar rats (115 g) per group were orally dosed with DEHP (2,000 mg/kg bw/day) for 10 days (Oishi, 1986). Zinc sulphate (9 mg/kg) was co-administered intraperitoneally. DEHP caus d a significant reduction in testes weight and testicular zinc concentration. Co-administration of zinc did no signi icantly prevent the DEHP-induced effects.

The involvement of testosterone in the testicular atrophy caused by DEHP was examined by co-administration of testosterone (1 mg/kg bw) subcutaneously along with 2,000 mg/kg bw of DEHP (purity not specified) in groundnut oil to adult male Wistar rats (150-200 g) for 15 days (**Parmar et al., 1987**). Administration of DEHP was found to significantly reduce relative and absolute te tes weights and the sperm count (approximately 75%), and also significantly increase the activity of GGT, LDH and β-glucuronidase and to decrease the activity of SDH and acid phosphatase. Co-administration of tes oster ne seemed to normalise the testes weights, sperm count and the activity of testicular enzymes. The role of testo terone in testis toxicity of DEHP is not fully elucidated. Several reports refer to increased or decr sed estosterone levels in plasma and testicular tissue.

Curto and Thomas (1982) examined changes in te tes and sex accessory weight as well as gonadal zinc in sexually mature rats and mice injected with various doses of DEHP or MEHP (purities not specified). Intraperitoneal and subcutaneous routes of admin stration were used to avoid hydrolyzation in the gastrointestinal tract and to exclude phthal te induced reduction in the gastrointestinal absorption of zinc. Male Swiss-Webster mice (number not stated) received one of the following dose regimens: a) daily sc injections of 1, 5 or 10 mg/kg MEHP for 5 day b) daily sc injections of 5, 10 or 20 mg/kg MEHP for 10 days; c) daily ip injections of 50 or 100 mg/kg MEHP or DEHP for 5 days; or d) alternate daily ip injections of 50 or 100 mg/kg MEHP or DEHP for 20 days (10 injections). Male Sprague-Dawley rats (number not specified) received daily ip injections of 50 or 00 mg/kg MEHP or DEHP for 20 days (10 injections). In mice, no significant alterations in testicular weight, seminal vesicle or anterior prostate weight or zinc levels occurred. Rats revealed significant reductions in both gonad l and prostate gland zinc. Rats injected with MEHP (50 mg/kg) showed a 37% decrease in prostat zinc; DEHP (100 mg/kg) caused a 33% decrease in prostatic zinc and a significant loss of testicular zinc 31%). The results indicated that the male rat is more sensitive to DEHP- or MEHP-induced effects on male gonads than the male mouse. It was also shown that sc or ip injected DEHP or MEHP caused gonadal zinc depletion, thus eliminating altered intestinal absorption as the cause for species differences.

Two strains of mice (Jcl: ICR and Crj: CD-1, four weeks old), were fed diets containing 0, 0.1, 0.2, 0.4 or 0.8% (approximately 300, 600, 1,200 mg/kg bw/day) of DEHP (purity not specified) for two weeks (**Oishi, 1993**). In ICR-mice, testicular weights were unchanged by DEHP treatment at all concentrations when compared to controls. In CD-1 mice, testicular weights were significantly reduced from a dose level of 0.2%. The testicular zinc content was statistically significantly reduced in both strains at dose levels of 0.4 and 0.8%. Testicular activities of lactate dehydrogenase isoenzymes (LDH-X) were significantly reduced in CD-1 mice from a dose level of 0.2%, while a significant reduction of testicular LDH-X activity in ICR mice was observed only at a dose level of 0.8%. The primary metabolite, MEHP, was significantly increased in blood samples of CD-1 mice at 0.8% when compared to ICR mice suggesting that toxicokinetics differences may explain some of the shown strain differences in susceptibility to DEHP testicular toxicity.

A study of the influence of the vitamin B12 derivative adenosylcobalamin on testicular toxicity of DEHP was performed in young male Crj: Wistar rats (30 days; 86-100 g) (**Oishi, 1994**). Groups of 8 animals each were

treated for 7 days. DEHP (0 and 2,000 mg/kg bw/day) was given orally and co-administration of adenosylcobalamin or methylcobalamin, both 0.5 mg/kg, was administered intraperitoneally. DEHP significantly reduced body, testis and prostate weights, inhibited active spermatogenesis, reduced the activity of testicular specific lactate dehydrogenase, and decreased the levels of testicular zinc, magnesium and potassium. Co-administration of adenosylcobalamin, but not methylcobalamin, prevented the DEHP-induced effects.

Apoptosis induction

Expression of apoptosis-related proteins FasL, Fas and Caspase-3, as well as DNA fragmentation were examined in mouse testis 12 h after exposure to 4–0.004 mg/g bw di(2-ethylhexyl) phthalate (DEHP) (**Ichimura 2003**). Immunocytochemical examination of the highest dose (4 mg/g DEHP) mouse revealed a distribution of FasL in Sertoli cell and Fas in nearby spermatocyte, and Fas and Caspase-3 in the same spermatocyte. Fas-positive spermatocytes had a DNA-fragmented nucleus detectable by terminal deoxynucleotidyl transferase-mediated fruorescein-dUTP nick end labeling (TUNEL) method. After exposure to 4, 0.4, 0.04 or 0.004 mg/g DEHP the maximum number of nuclei with fragmented DNA per 0.5µm testis section was 22, 7, 5 and 3, respect vely. In unexposed control the maximum number was 3. To further estimate total amount of the fragment d DNA in testis of the exposed mouse, the extracted DNA fragments were analyzed by agarose gel electrophor sis. The amount of fragments in the first three steps of the DNA ladder was estimated by a photo densitometry. In the highest dose mouse (4 mg/g DEHP), the fragmented DNA was 2.2 times as much in the control In lower dose mouse (0.4, 0.04 or 0.004 mg/g DEHP), it was 1.1 times as much in the control. Taken together, these observations suggest that a single oral exposure to DEHP as low as 0.04 mg/g m h be effe tive to testicular DNA fragmentation and apoptosis.

Age-dependency

Dostal et al. (1988) studied the age-dependency of testicular effects (study comparable to a guideline study) in rats of different age. Oral doses of 0, 10, 100, 1,000 or 2,000 mg/kg bw of DEHP (> 99% pure) were given daily by gavage in corn oil for 5 days to Sprague-Dawley rats (7-10 animals per g oup) at 1, 2, 3, 6 and 12 weeks of age. Absolute and relative testis weights were significantly redu ed at dos s of 1,000 mg/kg bw/day in 1, 2, 3 and 6- week-old but not in 12-week-old rats compared to controls f the same age. Doses of 2,000 mg/kg bw/day were fatal to suckling rats and caused decreased relativ testi weight but no lethality in 6- and 12-week-old rats. The number of Sertoli cell nuclei per tubule was red ced by 35% at 1,000 mg/kg in neonatal rats; two- and three-week old rats showed loss of spermatocytes but not of Sertoli cells. At 1,000 and 2,000 mg/kg also loss of spermatids and spermatocytes was shown in 6- nd 12 w k old rats.

Metabolites of DEHP

The formation of the monoester is an important step in the intestinal absorption of the orally ingested phthalates. In pubertal and adult animals, the t ticu r toxicity of DEHP appears to be mediated by the monoester MEHP. Studies mainly focusing on thi or other metabolites are therefore compiled separately.

To establish the compound or c mpounds responsible for the testicular damage after oral administration of DEHP, **Sjöberg et al. (1986)** administered DEHP and five of its major metabolites (MEHP, 2-ethylhexanol and three identified metabolites (V, VI, or IX) of MEHP) for five days. Groups of 6 male Sprague-Dawley rats (35 days old at the start of the experiment) were given 2.7 mmol kg bw of DEHP (1,055 mg /kg bw) or one of the metabolites. A counting of degenerated cells per tubular cross section was carried out. No testicular damage was observed fol owing oral doses of DEHP or 2-EH. The number of degenerated spermatocytes and spermatids was increased in animals which received MEHP; no such effects were seen in animals given the MEHP-derived metab lit s.

Dalgaard et al. (2001) studied the effects of MEHP on 28-day old male rats, by looking at testicular orph logy and apoptosis, and expression of some cellular markers (vimentin filaments, the androgen receptor, and a gen coding a Sertoli cell secretory product) 3, 6 or 12 hours (n=12) after a single oral dose of 400 mg/kg MEHP. At 3-12 hours, vimentin filaments in Sertoli cells had collapsed, and the expression of the apoptosis gene Caspase-3 was increased. However, there were no other indications of apoptosis as measured by DNA ladder analyses or tunel staining. The expression of TRPM-2 (coding a Sertoli cell secretory product) was transiently increased at 3 hours. At 3 hours there were no histological signs of toxicity, but at 6 and 12 hours the tubuli were disorganised and germ cells detached and sloughed into the lumen of the seminiferous tubules. The results support the Sertoli cells being early targets for MEHP toxicity.

The testicular toxicity of DEHP (> 98% pure) was studied in male Wistar rats (26 days old, 6 animals per group) after a single oral dose of 2,800 mg/kg bw (**Teirlynck et al., 1988**). In the same experiment, MEHP was given in doses of 400 or 800 mg/kg bw. The doses were selected in accordance to previous data showing that oral administration of 2,800 mg/kg bw of DEHP and 400 mg/kg bw of MEHP leads to similar MEHP plasma levels. Seven days after dosing the rats were killed and the testicular zinc concentration was measured. The severity of

the histopathological lesions was graded on the basis of the percentage of seminiferous tubules affected. The diameter of the seminiferous tubules was also measured. The rats showed testicular atrophy 7 days after dosing, as indicated by a significant reduction in relative testicular weight. Histological examination revealed a "dose-dependent" increase in the number of atrophic seminiferous tubules with decreased diameters of the seminiferous tubules and loss of spermatids and spermatocytes. The study suggests that MEHP is more toxic to the testes than DEHP. A significant reduction of the testicular zinc concentration was observed in DEHP treated rats and in rats given MEHP doses of 800 mg/kg bw, but not at doses of 400 mg/kg bw. The concentration of the follicle-stimulating hormone (FSH) in serum was determined but no treatment-related alteration was observed. The authors suggest that the toxic effects of MEHP are not secondary to inhibition of pituitary gonadotropin secretion and that the absence of an elevation of FSH suggests that the function of the Sertoli cells is preserved.

Prepubertal male Fischer rats (28-day-old; number not stated) were given a single 2,000-mg/kg dose of MEHP (95% pure) in corn oil by gavage at a volume equal to 4 ml/kg (control rats received a similar volume of corn oil) to study the effect on germ cell apoptosis in testes (**Richburg and Boekelheide, 1996**). Prelimina y experiments had also suggested that phthalates may cause alterations in the rat Sertoli cell cytoskeleton particularly the intermediate filament vimentin. The rats were killed at 3, 6 and 12 hours after treatment. From each rat, one testis was rapidly frozen in liquid nitrogen and the other was cut in halves for mmersion fixation in Bouin´s fixative and in neutral buffered formalin. Cryosections were stained with a monoclon l antibody to bovine vimentin. In situ Tunel staining was used to stain for DNA. The number of apop otic germ cells was counted in 100 randomly selected seminiferous tubules of testis cross-sections from each of four different rats. MEHP induced collapse of Sertoli cell vimentin filament 3 hours after MEHP administration Six and 12 hours after MEHP exposure, intense vimentin staining surrounding the nucleus was seen, s ggesting that vimentin filament had collapsed toward the Sertoli cell nucleus. The incidence of apoptotic events observed in 100 seminiferous tubule cross sections of testes from each of four rats was count d and t bulated into categories. In control testes, 44.5% of the seminiferous tubule cross-sections did not con ain ny apoptotic cells. However, 3 hours after MEHP treatment, the number of tubule cross sections with no in idence of apoptosis significantly increased to 63.3%. This shift was reflected by a significant decreas in the incidence of tubules containing 1-3 apoptotic cells per cross section at 3 hours. Cross sections of the emin f rous tubules from the 6- and 12-hours groups showed a dramatic increase in the number of ap ptotic event as evident by the increased incidence of seminiferous tubules which contained high categories of p ptotic germ cells and a decrease in the incidence of seminiferous tubule cross sections that contained no apoptosis. The authors suggest that the MEHP-induced collapse in vimentin filaments may lead to alterations in germ cell apoptosis by a disruption in contact-mediated communication between the Sertoli cells and germ cells and that the normal physiological incidence of germ cell apoptosis decreased as early as 3 hours after exposure to MEHP.

Four phthalate diesters, DEHP, DPP (d n-pentyl hthalate), DOP (di-n-octyl phthalate), and DEP (diethyl phthalate) were investigated in viv for effects on Leydig cell structure and function (**Jones et al., 1993**). The study was performed due to earlier study results indicating that communication and control exists between Sertoli and Leydig cells which ppear to e of a paracrine nature. The corresponding monoesters were investigated in vitro (MEHP, MPP MOP, and MEP). The in vivo study was performed by giving 2,000 mg/kg bw by oral gavage on t consecutive days to 3 male Wistar rats (6-8 weeks old, 200-300 g bw) per phthalate. The rats were sacrif ced 24 hours after the final dose. Testicular tissues were studied by light and electron microscopy after gl taraldehyde perfusion fixation, Taab embedding and toluidine blue staining (a highly reliable technique in preparing testis tissue for identifying testicular toxicity). The in vitro study was performed with primary cultures of Leydig cells incubated with 1,000-µM monoester for 2 hours. Phthalate esters exerted a direct effect on L ydig cell structure and function as determined by testosterone output with correlation of the in vitro and in viv effects of MEHP and DEHP, respectively. The changes observed in vivo were present in all animals in each group. Leydig cells stained more densely than other cell types, generally displaying an elongate profil often with thin lamellar processes. In Leydig cell cytoplasmatic ultrastructure, several subtle but highly significant alterations were produced. DEHP administration also resulted in slight rarefaction or vacuolation of a few Sertoli cells in seminiferous tubules, while treatment with DOP or DEP produced no change in seminiferous tubul r structure or Leydig cell morphology. Exposure to DPP produced the most severe changes in Sertoli cells but no changes in Leydig cells. In the in vitro study, MEHP and MPP produced marked effects on structure and function including decreased LH-stimulated secretion of testosterone from Leydig cells incubated with MEHP while MOP caused decreased secretion and MEP was without effect. The results show that DEHP may exert a direct effect on Leydig cell structure and function and that DEHP and MEHP produce similar changes both in vivo and in vitro both in Leydig cells and in Sertoli cells. The authors concluded that a malfunction of Leydig cells likely affects the physiology of adjacent Sertoli cells. The authors also concluded that different phthalates may exert changes that are unique to one or common to both cell types.

The effects of DEHP, MEHP and 2-ethylhexanol (2-EH) were determined on gonocytes and Sertoli cell morphology, Sertoli cell proliferation, and expression of cell cycle markers in neonatal rats (three-day old, CD

Sprague-Dawley) (**Li, 2000**). A single bolus dose of DEHP (20, 100, 200 and 500 mg/kg) was given in corn oil to five pups per group. Diethyl phthalate (DEP: 500 mg/kg) served as the non-toxic control. MEHP (393 mg/kg), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. The doses of MEHP and 2-EH were molar equivalent with 500 mg/kg DEHP. In this dose-response study, all pups were killed 24 hours after dosing. A time-course study was conducted following a single dose DEHP (200 mg/kg), where the pups were killed after 6, 9, 12, 24 or 48 hours. Biochemical analyses was performed for serum FSH levels, Sertoli cell proliferation (as BrdU labelling; BrdU administered 3 hours before euthanasia), cell cycle regulators cyclin D1, D2, D3, p27kip1 proteins and cyclin D2 mRNA in the testes. Morphological examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 hours post-treatment with DEHP (100-500 mg/kg). With 200 mg/kg DEHP these effects were first determined 12 h after treatment, and persisted for 48 hours. Effects on Sertoli cell morphology were not detailed in the report. MEHP (single do e group) induced effects on gonocytes similar to DEHP. BrdU-labelled Sertoli cells were dose-dependently decreased from 100-500 mg/kg DEHP. No marked difference in BrdU-labelled Sertoli cells was marked with 20 mg/kg DEHP, DEP and vehicle controls. Serum levels of FSH were not affected by DEHP treatment (00 nd 500 mg/kg). MEHP also caused a significant decrease in BrdU-labelled Sertoli cells. D2 mRNA was sp cifically down-regulated by DEHP in a dose-dependent manner (200 and 500 mg/kg only doses reported), and thi decrease was manifest as a small, transient but reproducible reduction in the amount of cyclin D2 protein with 200 mg/kg DEHP (only dose reported). The effects of MEHP and 2-EH were not dete mined. 2 EH was without effect on testicular cell morphology, or Sertoli cell proliferation. A NOAEL for young pups of 20 mg/kg is derived for effects on altered gonocyte morphology and decreased Sertoli cell pr f ation y a single oral dose of DEHP.

In vitro studies

Grasso et al. (1993) studied the effects of DEHP and MEHP on cultured rat Sertoli cells. The Sertoli cells were obtained from Fischer 344 rats (13-82 days of age) and cultured in the presence of MEHP at concentrations ranging from 0.001 to 100 µM. MEHP was found to specifically reduce the ability of FSH to stimulate cAMP accumulation in rat Sertoli cells. This inhibition by MEHP of FSH-stimulated cAMP accumulation had a lag period of 6 hours and reached a maximal inhibition of 40 60% after 24-hours. Preincubation of Sertoli cells for 24 hours with 100 µM DEHP had no effect on FSH binding The authors concluded that the ability of certain phthalate esters to disrupt the FSH-linked signal tran ducti n pathway in primary Sertoli cell cultures by a mechanism, located at the cell membrane, is likely to b a part of the mechanism responsible for their testicular toxicity.

Lehraki (2009) used organ culture of fetal teste at different stages of development to analyze the direct effects of phthalates on both steroidogenesis and gonocy e development and to determine if the effects of MEHP on these functions reported in the rat can b extended to other mammalian species. We defined specific periods of sensitivity of the fetal mouse testis to MEHP f r these two functions and showed that the effects of phthalates on steroidogenesis vary with the developmental stage. Conversely, the strong deleterious effects of phthalates on germ cells were constantly pres nt during the active phases of gonocyte development and thus share no relationship with the steroidogenic st tus. Moreover, all the effects of phthalates were unchanged in testes from mice deficient for estrogen (ERaKO or ERbKO) or androgen (Tfm) receptors. In conclusion, these results demonstrate that ph halates impair mouse fetal germ cell number similarly to other mammalian species, but are neither estrogenic nor antiandrogenic molecules because their effects do not involve, directly or indirectly, ER or AR.

Sjöberg et al. (1986) investigated the ability of DEHP, 2-EH, MEHP and metabolites V, VI, and IX to induce germ cell detachment from mixed primary cultures of Sertoli and germ cells. Only exposure to MEHP (10 µM for 24 hours or 1 200 µM for 48 hours) caused a significantly higher degree of germ cell detachment.

Grasso et al. (1993) studied the effects of DEHP and MEHP on cultured rat Sertoli cells. The Sertoli cells were ob ained from Fischer 344 rats (13-82 days of age) and cultured in the presence of MEHP at concentrations ranging from 0.001 to 100 µM. MEHP was found to specifically reduce the ability of FSH to stimulate cAMP cumulation in rat Sertoli cells. This inhibition by MEHP of FSH-stimulated cAMP accumulation had a lag period of 6 hours and reached a maximal inhibition of 40-60% after 24-hours. Preincubation of Sertoli cells for 24 hours with 100 µM DEHP had no effect on FSH binding. The authors concluded that the ability of certain phthalate esters to disrupt the FSH-linked signal transduction pathway in primary Sertoli cell cultures by a mechanism, located at the cell membrane, is likely to be a part of the mechanism responsible for their testicular toxicity.

Cell cultures of Sertoli cells were also used to study lactate and pyruvate production after adding MEHP or DEHP (**Moss et al., 1988**). MEHP (0.1-200 µM) produced a concentration-dependent stimulation of lactate, but not pyruvate production over a 24-hour treatment period and an increase in the ratio of lactate/pyruvate

concentration in the culture medium. DEHP had no such effects. The developing germ cells cannot utilise glucose to maintain ATP levels and are apparently dependent on a supply of lactate and pyruvate produced by Sertoli cells under control by FSH. The authors conclude that loss of germ cell in phthalate-induced testicular atrophy is not due to inhibition of energy substrate production by the Sertoli cells and that stimulation of lactate production may be a useful in vitro marker for phthalate esters that cause testicular injury.

Li et al. (1998) studied the effects of MEHP and DEHP on neonatal Sertoli cells and gonocytes (primitive spermatogonia) maintained in hormone- and serum-free coculture. They found that MEHP induced gonocyte detachment from the Sertoli cell monolayers in a time and dose-dependent manner. The cocultures of Sertoli cells and gonocytes were prepared from testes of 2-day-old male rat pups. Final concentrations of 0.01, 0.1, or 1.0 μM MEHP (≥ 99% pure) was added to the cocultures. DEHP (≥ 99% pure) was used as a negative control. At a dose of 0.1 μM MEHP, gonocytes rounded up and started to detach from cocultured Sertoli cells after 24 hour of exposure. At 1.0 μM, MEHP caused a rapid detachment of gonocytes detectable after 12 hours of exposure. No morphological changes were found in cultures treated with vehicle alone or with DEHP added at a 10-fold higher concentration than the maximal dose of MEHP. When cultures were labelled with BrdU (5 bromo-2'-deoxyuridine) few labelled cells could be found in the cultures treated with 1.0 μM MEHP compared to controls. No visually detectable increase in labelling could be observed in cultures simult neously reated with FSH and 1.0 μM MEHP. Labelling indices in cultures treated with 0.1 or 1.0 μM MEHP were ignificantly lower than that in the vehicle-treated controls, reflecting decreases in Sertoli cell proliferation of 33.6 and 83.6%, respectively, over controls. The labelling indices of cultures treated with 10 μM MEHP was however, significantly higher than that of the vehicle-treated controls. The study results show that MEHP directly targets the Sertoli cells and impairs their proliferation and that MEHP also may affect the int raction of gonocytes with Sertoli cells and/or target gonocytes directly. The findings also show that phthalate-induced changes in germ cell-Sertoli cell adhesion may occur during early postnatal development in rats.

In this study (**Li 2003**), organ cultures of fetal and neonatal rat testes were used to assess the in vitro effect of MEHP on seminiferous cord formation in Embryonic Day 13 (E13) testes and on the development of E18 and Postnatal Day 3 (P3) testes. Interestingly, MEHP had no effect on cord formation in the organ cultures of El 3 testes, indicating that it has no effect on sexual differentiation of the indifferent gonad to testis. Consistently, the expression of a Sertoli cell-specific protein, mullerian inhibit ng substance (MIS), or the number of gonocytes did not change in E13 testes after MEHP treatment. In con rast, MEHP decreased the levels of MIS and GATA-4 proteins in Sertoli cells and impaired Sertoli cell proliferation in the organ cultures of E18 and P3 testes. These results suggest chat MEHP negatively influenc prolifer ion and differentiation of Sertoli cells in both fetal and neonatal testes. In addition, MEHP treatment did not alter the number of gonocytes in E18 testes, whereas the number of gonocytes in P3 testes decreased in a dose dependent manner, apparently due to enhanced apoptosis. These results suggest that MEHP adver ely affect the gonocytes, which are mitotically active and undergoing migration and differentiation in ne atal estes, but it has no effect on fetal gonocytes that are mitotically quiescent.

The in vitro effects of a 24 hour exposure of a Leydig cell line to MEHP were studied by means of electron microscopy (EM) and by measuring progesterone production and cell viability (**Dees et al., 2001**). At a concentration of 1 μM MEHP, the first signs of toxicity appeared, as indicated by morphological changes involving nuclei (h terochromatin, euchromatin and large nucleoli), mitochondria (generally condensed, but some were swollen or had an abnormal form and contained degenerated christae) and presence of moderate numbers of l pid droplet At 10 μM MEHP, the cell shape was affected (large and round to oval), SER was lost, large vacuole and n merous lipid droplets were present in the cytoplasm, and some mitochondria were seen in close app sitio to the lipid droplets. At 100 μM MEHP, the effects were more severe and some apoptotic cells were seen A more general cell death was observed at 1-3 mM, as determined both by structure and a cell viability assay (MTT). Progesterone production was reduced in the 1-10 μM range (by approximately 50%), returned to normal values at 100 μM, and ceased when cell started to die. The authors discuss a mechanism wher the mitochondria are the first targets of the toxicity, they then fuse with lipid droplets and degrade. The study onduct seems proper, and the study pinpoints Leydig cells as potential sensitive targets for the DEHP-metabolite MEHP. However, the relevance for DEHP toxicity is not clear.

Lovekamp and Davis (2001) studied the in vitro effects of a 48 hours exposure period to 0-200 μM MEHP on primary rat granulosa cells. The authors find dose-dependent effects of MEHP on the levels of aromatase RNA, which is decreased as from exposure to 50 μM MEHP, and on the amount of aromatase protein and estradiol production (as from 100 μM MEHP). The relevance to DEHP toxicity is not clear.

Lague (2008) reports that testosterone does increase Insl3 mRNA levels in a Leydig cell line and primary Leydig cells. Testosterone activated the activity of the Insl3 promoter from different species. In addition, the testosterone-stimulating effects on Insl3 mRNA levels and promoter activity require the androgen receptor. The testosterone-responsive element to the proximal Insl3 promoter region has also been mapped. This region,

however, lacks a consensus androgen response element, suggesting an indirect mechanism of action. Mono-(2-ethylhexyl) phthalate (MEHP) represses Insl3 transcription, at least in part, by antagonizing testosterone/androgen receptor action. All together our data provide important new insights into the regulation of Ins13 transcription in Leydig cells and the mode of action of phthalates.

The effects of MEHP on granulosa cell function were studied in vitro (**Treinen and Heindel, 1992**). It was shown that MEHP inhibited FSH- but not forskolin-, isoproterenol-, or cholera toxin-stimulated granulosa cell cAMP accumulation in vitro. MEHP also inhibited FSH-stimulated progesterone production, a cAMP-dependent process. Similar to MEHP, the protein kinase C activator (TPA) has been shown to inhibit rat granulosa cell cAMP accumulation in a FSH specific manner, and decrease FSH-stimulated progesterone production.According to the authors, these data indicate that the inhibitory effects of MEHP on granulosa cell function are independent of phorbol ester-sensitive PKC activation.

Justification for classification or non classification

Classification according to:

Regulation (EC) No 1272/2008 Annex VI Table 3.1: Reprotoxic 1B

Regulation (EC) No 1272/2008 Annex VI Table 3.2: Repr. Cat. 2; R60-61

5.10. Other effects

5.10.1. Non-human information

5.10.1.1. Neurotoxicity

The results of experimental studies are summarised in the following table:

Table 47 Overview of experimental studies on neurot xicity

Method	Re ults	Remarks	Reference
rat (Fischer 344) female subacute (oral: gavage) 50, 150, 500 or 1,500 mg/kg bw/day (actual ingested) Exposure: 14D (daily) equivalent or similar to OECD Guideline 424 (Neuro oxicity Study in Rodents)	NOAEL : 500 mg/kg bw/day (a tual dose received) (female) (no effect at the highest dose treated)	2 (reliable with restrictions) key study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Moser VC, Cheek BM, MacPhail RC (1995) Moser VC, MacPhail RC, Gennings C (2003)
rat (Fisch r 34) female acut (o al: gavage) 150 500 1,500 or 5,000 mg/kg/day (nom nal conc.) Exposure: one single treatment Fonctional Observation Battery and motor activity measurements after a single exposure	NOAEL : 1500 mg/kg bw/day (actual dose received) (female) (clinical signs (ptosis, piloerection, slight lacrimation, hypothermia) observed at 5000lg/kg in 2/8 females)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): bis(2-ethylhexyl) phthalate**	Moser VC, Cheek BM, MacPhail RC (1995) Moser VC, MacPhail RC, Gennings C (2003)

The neurobehavioural effects were tested in rats by a functional observational battery (FOB) and motor activity measurements before exposure, at specified times after a single dose exposure, and during and after a 14-day repeated dose exposure (Moser et al., 1995). Female Fischer 344 rats (number not given) were administered 150, 500, 1,500 or 5,000 mg/kg/d of DEHP (> 99% pure) (single dose study), or 50, 150, 500 or 1,500 mg/kg/d of

DEHP (repeated exposure, 14 days) in corn oil by gavage. The FOB included following measures: autonomic, activity, excitability, neuromuscular, sensorimotor, and physiological measures. Motor activity was measured in a maze. The FOB was performed on each rat just prior to the first dose.

Thereafter, the FOB followed by motor activity assessments was conducted at 4 and 24 hours after exposure (single dose study), and on day 4 and 9 (before the daily dose) and 24 hours after the last dose. No lethality occurred. A single administration of the highest dose produced pronounced signs of general debilitation in two rats 24 hours after dosing. No changes in body weight were observed in either study.

No functional domain was overall affected in either study.

In a BASF study (2001) realized according to the EPA OPPTS 870.6200 guideline and in compliance with Good Laboratory Practices, F2 pups from a 2-generation study were tested for neurotoxicity in a Neurotoxicity Screening Battery, a Functional observation battery, a FOB Motor activity assessment and a Water maze test.

No sign of neurofunction impairment was noted in any of the various parameters of the Water Maze T st, the Functional Observation Battery, and the Motor Activity Assessment.

The following information is taken into account for any hazard / risk assessment:

Female Fischer 344 rats treated with a single oral dose (up to 5000 mg/kg bw) or with epeated doses (up to 1500 mg/kg bw per day for 14 days) of di(2-ethylhexyl) phthalate showed no neurobehavioural effects, as evaluated by functional observational battery and motor activity testing.

Value used for CSA (route: oral): NOAEL: > 1500 mg/kg bw/day

5.10.1.2. Immunotoxicity

The following is quoted from the review article of Kimber and Dearman in 2010 (An assessment of the ability of phthalates to influence immune and allergic responses).

Effects of phthalates on antibody expression in vivo

The putative ability of certain phthalates to act as adjuvants for the development of immune responses to known protein allergens has been investigated in anim l (almost xclusively mouse) models. In considering the conduct and interpretation of such models it is important o appreciate that the immunological response of greatest moment in the development of allergic s nsitis tion and of atopic allergic disease (and therefore the event of greatest relevance when considering th possible contribution of the putative adjuvant activity of phthalates in driving susceptibility to allergy) is the p oduction of specific IgE antibody. This is the primary effect molecule of atopic allergy. During the acqui ition of sen itisation IgE antibody distributes systemically and associates, via specialised plasma membrane receptors, with mast cells. As described above, subsequent encounter with the inducing allergen causes mast c ll activa ion resulting in inflammation and the signs and symptoms of an allergic reaction. Although specific IgE antib dy is therefore the most relevant marker of allergic sensitisation, it is produced in very small amounts and for this (and other reasons) is difficult to measure accurately. As a consequence, other antibody read-outs are commonly used in addition to, or as surrogates for, specific IgE. Those most commonly used in mouse models are total plasma levels of IgE immunoglobulin (as opposed to specific IgE ntibody), a d the induction of IgG1 antibody responses. Total plasma levels of IgE are frequently elevated during imm ne responses to allergens, and IgG1 antibody production is regulated by mechanisms similar to those hat control IgE responses (Snapper et al., 1988). It is of some importance for the interpretation of data der ved from murine models that IgG1 is not only considerably less effective at stimulating mast cell degr nulation than is IgE (Ovary, 1982), but also IgG1 antibody levels do not always mirror exactly the same pattern s IgE antibody responses. Thus, we have observed very different dose response profiles for IgG1 anti-OVA antibody responses compared with anti-OVA IgE antibody responses following subcutaneous admin stration of DEHP to mice (Dearman et al., 2008a). A lack of correlation between IgG1 and IgE specific tibody production was recorded also for detergent enzymes and food proteins (Sarlo et al., 2005).

Another issue is the dose metric to be used for comparison between different types of exposures. Although dose/unit body weight is accepted generally for comparisons between species, and for routes of exposure such as oral or intraperitoneal (ip), it is unclear how appropriate this metric is for consideration, for example, of topical exposure. For the current comparisons we have therefore elected to use total dose per application as the dose metric for comparison, given that all experiments have utilised the same experimental animal (the mouse).

Experimental approaches have included examination of the ability of various phthalate monoesters and diesters (administered by a variety of routes of exposure) to influence specific antibody responses to a reference protein allergen, ovalbumin (OVA), or to a reference immunogen (keyhole limpit haemocyanin; KLH) (Larsen

et al., 2001a; 2001b; 2002; 2003; 2007; Lee et al., 2004; Larsen and Nielsen, 2007; 2008; Dearman et al., 2008a; 2008b; 2009; Hansen et al., 2007). The impact of phthalates on the total serum concentration of IgE has also been investigated (Butala et al., 2004; Lee et al., 2004; Dearman et al., 2009). In many, but not all, instances the strain of mouse that is most susceptible to the development of IgE-mediated reactions, the BALB/c strain mouse, has been utilised.

The ability of phthalates to display adjuvant properties under these experimental conditions appears to be influenced very markedly by the route through which exposure to the phthalate is effected. Subcutaneous exposure to some phthalates appears to be particularly effective at causing augmentation of antibody responses to OVA, with relatively low doses of phthalate (0.1 mg-40 mg phthalate per dose) impacting on the vigour of IgG1 and/or IgE antibody responses. When the adjuvant effects of various monophthalates were studied , for some chemicals (mono-2-ethylhexyl phthalate [MEHP], mono-n-octyl phthalate [MNOP] and mono-iso-nonyl phthalate [MINP]), both inhibitory "immunosuppressive" effects (statistically significant decreases in antibody production) and adjuvant effects (statistically significant increases in antibody production) were observed a different doses within the same experiment (Larsen et al., 2001a). Thus, for example, subcutane us tre tment of mice with MINP at 20 mg inhibited anti-OVA IgE antibody production; whereas 2 mg of the same chemical enhanced IgE antibody responses. Moreover, in the same experiment 0.2 mg was found to i hibit anti OVA IgG1 antibody production. Only inhibitory effects on IgE and IgG1 antibody levels were recorded following exposure to 2 mg mono-iso-decyl phthalate (MIDP), with administration of either 20 mg or 0.2 mg of phthalate being without impact on antibody production.

Using an identical experimental system, and similar concentration ranges, subcutan ous injection of mice with monobenzyl phthalate (MBzP), or mono-n-butyl phthalate (MBP), was without effect on either IgG1 or IgE antibody responses (Larsen et al., 2001a). The same investigators have also examined the immunomodulatory effects of various diester phthalates (Larsen et al., 2001b; 2002; 2003). Inhibitory activity was not seen with any of these compounds. However, treatment with 40 mg DEHP was reported to cause increased expression of IgG1 anti-OVA antibody, although it was without effect on IgE anti-OVA antibody levels (Larsen et al., 2001b). Other diester phthalates such as di-n-octyl phthalate (DNOP), DINP and DIDP augmented both IgG1 and IgE anti-OVA antibody responses at certain doses (Larsen et al., 2002). It is not clear, however, why in a subsequent publication (Larsen and Nielsen, 2008) the same investigator reported effects with the same compounds on IgG1 antibody production, in the absence of changes in IgE antibody. In the same experimental system, subcutaneous injection of phthalates that are structurally similar to DEHP, including BBP, bis-(2-ethylhexyl) terephthalate (DOTP) and trioctyl trimellitate (OTM) w s found not to influence either IgG1 or IgE antibody responses (Larsen et al., 2003; Larsen and Nielsen 2008). The authors speculated that the length of the carbon side chains is important in dictating the biologic l activity of phthalates in this respect; the suggestion being that a total of 16 carbon atoms distributed b tween th 2 ester groups confers maximum adjuvant activity (Larsen and Nielsen, 2008).

Intraperitoneal (ip) route of administration of phthalates has also been reported to have adjuvant effects at relatively low doses. Thus, in l 9/Sv strain mice ip injection of DEHP (100 mg) with the reference allergen OVA resulted in a significant incre in IgG1 anti-OVA antibody, but was without effect on IgE antibody responses (Larsen and Nielsen, 2008). These data are comparable with previous observations that subcutaneous injection of DEHP with OVA in BALB/c strain mice enhanced specific IgG1 antibody without impacting on specific IgE respons s (Larsen et al., 2001b).

Using a somewha different protocol, other investigators have shown that ip administration of either DEHP or DINP (40 to 125 mg) to BALB/c strain mice enhanced the total serum concentration of IgE, and also increased level of specif IgE antibody provoked by subcutaneous injection of the immunogen KLH (Lee et al., 2004). Di- so-nonyl phthalate proved more effective than did DEHP for the augmentation of IgE anti-KLH antibody respon es. In our own laboratory, and using a similar protocol, we also found that ip injection of DINP, but not of DEHP was associated with an increase in IgG1 anti-KLH antibody production (Dearman et al., 2009).

In con rast, the general experience has been that exposure of mice to phthalates via routes that more closely r flect conditions of human contact (that is either oral or topical exposure), is without impact on antibody responses, or is considerably less effective, at mediating adjuvant-like effects. Oral exposure of BALB/c strain mice to 50 mg per dose DEHP or DINP was without influence on the total serum concentration of IgE, or on levels of IgG1 anti-KLH antibody induced by concurrent subcutaneous administration of the antigen (Dearman et al., 2009). The doses of phthalates used in those experiments, and that were without effect on antibody production or IgE levels, did, however, display significant systemic effects, measured as a function of increases in liver weight (Dearman et al., 2009). Topical exposure of B6C3F1 mice to high doses (up to 100 mg) of DEHP, BBP, DINP or di-iso-hexyl phthalate (DIHP) also failed to alter the total serum concentration of IgE antibody, despite being associated with significant elevations in liver weight (Butala et al., 2004). Using a similarly high dose (50 mg) of DEHP delivered topically (with concurrent subcutaneous injection of the

reference allergen OVA) we did not see any impact on the development of anti-OVA IgG1 or IgE antibody responses. However, in the same series of experiments a high dose of BBP (100 mg) significantly enhanced IgG1 anti-OVA antibody responses in the absence of any effect on IgE antibody production (Dearman et al., 2008a; 2008b). In interpreting these data it is important to appreciate that topical administration of chemicals to mice in the absence of occlusion will inevitably result in significant oral exposure as the result of grooming.

There is also some information available about the impact of inhaled phthalates on immune function in mice. Inhalation exposure to DEHP (up to 13 mg/m3) together with OVA had similar effects to that of simultaneous subcutaneous or ip administration of the two materials (Larsen et al., 2007). That is, increased IgG1 anti-OVA antibody levels were found in the absence of effects on IgE antibody production. Airway exposure to MEHP (the major metabolite of DEHP), had apparently identical effects to those seen with DEHP, but at considerably lower doses than those required for the parent compound (0.03 mg/m3) (Hansen et al., 2007). The authors calculat d that, with respect to extrapolation to human airways, there was a margin-of-safety of approximately 50 (Larsen et al., 2007). They therefore drew the conclusion that "realistic" DEHP exposure levels likely to be encoun red in the environment would not be expected to cause adjuvant effects in humans, or to result in all rgic inflammation of the lung (Larsen et al., 2007).

Taken together, these experimental studies reveal that the route of administration of phthalate is of considerable importance, and appears to have a decisive influence on whether immunomodulatory ffects are induced. Comparatively low doses of phthalates affect antibody parameters when admini tered only by routes of exposure (subcutaneous or ip) that do not reflect, and are not generally relevant f , pportunities for human contact with phthalates . Although these are among the routes are favoured for expe imental immunisation (van Zijverden and Granum, 2000) and, in the case of subcutaneous administration, for the purposes of vaccination, neither are routes by which individuals would normally be expected to encounter phthalates in the domestic or occupational environment.

This interpretation is consistent with recent investigations in which potential routes of exposure to the most commonly used phthalates have been estimated employing scenario-based approaches (Wormuth et al., 2006). It was found that with respect to DEHP and DIBP, infants and toddlers receive the majority of their exposure orally through the ingestion of both dust and food, while in older children and in adults exposure is primarily through the diet. Similarly for DINP and DIDP, it is estimated th t exposure of children results primarily from oral contact and ingestion (mouthing as well as ingestion of dust) For adults, exposure to DIDP is also largely by the oral route (from food and to a lesser extent dust) with de mal and inhalation pathways making smaller contributions. In contrast, in the case of DINP, it is the skin and inhalation pathways that make the largest contributions to adult exposure patterns with l wer levels encountered via oral ingestion. For BBP, oral ingestion is the most important route of exposure for children, with inhalation exposure assuming greater important among adults (Wormuth et al 2006). The data summarised above from studies in mice indicate clearly that the oral exposure to phthalates is substantially less effective at causing adjuvant-like effects than are either subcutaneous or ip expo ures.

In reconciling effects seen in exp rimen al models with potential hazards to human health it is relevant to consider the nature of djuvant effects observed with phthalates in mice. Particularly in more recent studies, evaluations of effec s on antibody production have been confined only to consideration of IgG1 responses. As discussed previously, although IgG1 antibody production in the mouse is often used as a surrogate for IgE antibody responses, IgG1 is onsiderably less effective at inducing murine mast cell degranulation than is IgE, and in humans the class of antibody that mediates allergic and anaphylactic reactions is (almost exclusively) IgE (Lehrer and V ughan 1976; Ovary, 1982; Oshiba et al., 1996). With respect to human health a strong case can be made, ther fore that an induced elevation in mice of IgG1 antibody production, in the absence of effects on IgE antibody responses, is of lesser concern than would be a significant elevation in IgE levels .

One oth r aspect of the impact of phthalates on immune responses requires consideration. As summarised above, it i clear that under some circumstances, and at some doses, certain phthalates were reported to display what were described as "immunosuppressive" effects (inhibition of IgG1 and/or IgE antibody production), (Larsen et l 2001a). This inhibitory activity was not exclusively a consequence of generalised systemic adverse effects as depression of antibody responses was not observed exclusively at maximal doses. One can, of course, speculate in general terms about variable dose-related effects of chemicals on the orchestration of immune responses, and in particular on immunoregulatory elements. It is nevertheless difficult to develop a plausible and coherent hypothesis to explain why in some circumstances certain phthalates appear to inhibit antibody production at lower doses, while augmenting the same responses at higher doses. It might be argued that at different dose levels such phthalates are acting selectively on distinct cellular vectors of immune function resulting in unexpected dose-related influences on immune responses. While it is not possible presently to discount such dose-selective effects, an alternative, and to our minds more plausible, explanation is that the readout of antibody production that has been used for evaluation of the potential impact on phthalates on the immune system is

subject to natural fluctuations and perturbations that might not necessarily be treatment-related. If this is indeed the case then there is clearly need for some caution in defining the putative immunomodulatory properties of phthalates.

Effects of phthalates on other immune and inflammatory parameters in vivo

Although the majority of studies investigating the potential of phthalates to impact on immune function have focused on consideration of induced changes in antibody responses, less commonly, other readouts have been used. One such is the activity of discrete functional sub-populations of T lymphocytes that collectively dictate the quality and vigour of adaptive immune responses. A seminal observation, made over two decades ago, is that the induction and maintenance of IgE antibody responses is controlled by functional subpopulations of T helper (Th) cells. It quickly became clear that the successful maturation of activated B lymphocytes into IgE prod cing cells, and a sustained IgE antibody response, requires the preferential development of the Th2 sub-population f Th cells. These cells produce an array of cytokines including interleukins 4, 5 and 13 (IL-4, IL-5 and L 13) that promote IgE antibody production and facilitate IgE-mediated allergic inflammation (Finkelman et al., 1988a; 1988b; Mosmann et al., 1991). The other major functional sub-population of Th cells is designated Th1 ells. These cells display a different pattern of cytokine production, including, importantly, interf ron-γ (IFN-γ that antagonises the genesis of IgE antibody responses. Moreover, Th1 cells and their cytokine pr ducts ser e to constrain and inhibit immediate-type allergic responses effected by IgE antibody (Finkelman et l., 1988a; 1988b; Mosmann et al., 1991).

Against this background it is legitimate therefore to consider whether phthalates h ve any p ential to impact on Th cell function, and in particular to influence or perturb the balance between Th1 and Th2 cells and their soluble cytokine products. Although relatively few studies of phthalates have sought to examine Th1- and Th2-type responses as a function of differential cytokine expression, there is available some information.

Exposure of BALB/c strain mice to DEHP or to DINP (40 to 125 mg) by ip injection was reported to increase production of the Th2-type cytokine IL-4 by KLH-primed lymph node cells (LNC) (Lee et al., 2004). Some evidence for increased levels of IL-4 production by draining LNC associated with exposure to phthalates was also described by Maruyama et al (2007). They sensitised CD-1 strain mice topically to the experimental contact allergen fluorescein isothiocyanate (FITC). The FITC was fo mulated either in a 1:1 mixture of acetone and various low molecular weight phthalate esters (DEP, DPP nd DBP), or with acetone alone. Compared with mice that had been exposed to FITC in the absence of phthal tes, those that had received phthalates topically were reported to produce increased amounts of IL-4 y drai ing LNC (Maruyama et al., 2007). The impact of phthalates on cytokine expression has also been investigated following inhalation exposure. In one series of experiments BALB/c strain mice were xposed y inhalation to OVA with or without DEHP. In this instance mice that received DEHP displayed no only elev ted levels of IL-5 (a type 2 cytokine), but also increased amount of the Th1 product IFN-γ (L rsen et al. 2007).

Finally, the impact of phthalates has bee considered also following oral administration. Dietary exposure of rats to 12,000 ppm of DEHP was fo nd to alter the balance of Th1/Th2 cytokines in the liver towards a preferential Th2 type phenotype. In the context f these experiments, however, the phthalate-induced Th2 response had an ameliorating effect on the tissue damaging Th1 response that was being provoked by the intraperitoneal administration of Mycobacter um bovis purified protein derivative (PPD) (Badr et al., 2007).

It is important to acknowledge, however, that in other studies exposure of rodents to relatively high doses of DEHP and variou other phthalates either by topical treatment, or via gavage, have failed to impact on cytokine expression (B tala et al., 2004; Piepenbrink et al., 2005). In the study by Piepenbrink et al (2005), other immune parameters (lymphoid organ weights, thymus histology, antibody levels) were also found to be unaffected by ora administrati n of DEHP. In contrast, other authors have demonstrated that dietary exposure of C57BL/6 strain mice to relatively low levels of DEHP was immunosuppressive, resulting in a marked atrophy of both thymus and spleen (Yang et al., 2000).

In oth r investigations attention has focused instead on examination of whether exposure to phthalates is able to i fluence inflammatory processes. There are several studies which suggest that exposure to DEHP can enhance ongoing inflammatory responses. In a rat model of OVA-induced allergic asthma, gavage exposure to DEHP was associated with enhanced inflammatory cell infiltration in OVA-challenged airways (Yang et al., 2008). Similar general conclusions were drawn from the use of another rodent model, in this case of atopic dermatitis. Mice of NC/Nga strain develop signs and symptoms of atopic dermatitis. Using these animals it was found that ip exposure to DEHP exacerbated skin lesions induced by intradermal injection of house dust mite allergen. These changes were associated with increased expression of cutaneous chemokines such as macrophage inflammatory protein-1a (MIP-1a) (Takano et al., 2006). Furthermore, the same group reported that maternal ip exposure to DEHP during the neonatal (but not the fetal) period aggravated skin lesions in mite

allergen-sensitised male offspring, but not in female offspring (Yanagisawa et al., 2008). The biological significance of these observations are as yet uncertain.

It can be concluded, therefore, that investigations of the effects of phthalates upon various immune and inflammatory responses in experimental animals have yielded conflicting, and somewhat variable, results ranging from potentiation of immune or inflammatory responses, to the absence of any effect, to inhibitory or immunosuppressive activity. Although the available evidence suggests that certain phthalates, when delivered at appropriate doses, and via an appropriate route, can impact on immune and inflammatory function in rodents, it is clear that as yet no consistent pattern has emerged. There is clearly a case to be made for the design of more definitive animal studies that will allow development of a more detailed understanding of whether and to what extent, and under what conditions, certain phthalates are able to effect meaningful changes in immune function. In advance of that and access to more detailed information it is appropriate to question whether there are any other relevant data available.

Effects on the elicitation of clinical symptoms in asthmatics

There has been a single study in which the influence of inhalation exposure to phthalates on immune responses in humans has been examined (Deutschle et al., 2008). Patients allergic to house dust mite and healthy (non-allergic) controls were challenged (nose-only) with atmospheres containing low (0.41 mg/g) or high (2.09 mg/g) levels of DEHP. Clinical symptoms were scored, nasal secretions monitored for cytokine and chemokine expression, and a transcriptional analysis was performed on nasal biopsy samples. Nasal exposure to the dust was without effect on clinical symptoms for either group. House dust mite allergic patients, but not nonallergic individuals, responded to challenge with the DEHPlow dust with up-regulation of a number of markers of inflammation including IL-6 and granulocyte-colony stimulating factor (G-CSF). Exposure to the DEHPhigh dust attenuated the response, down-regulating levels of inflammatory cytokines. These are interesting observations, but as the authors acknowledge, a short term exposure protocol (3 hrs) was used and it is therefore difficult to extrapolate from these data to normal environmental exposure patterns. Moreover, as the subjects were not challenged with house dust mite allergen alone, it was not possible in this study to distinguish between the effects due to low dose DEHP, and those due to house dust mite allergen alone.

In vitro effects

Finally, attempts have also been made to characterise in vitro the ability of various phthalates to impact on immune function. There are various investigations in which the impact of phthalates on primary immune cells, or immune cell lines, has been characterised in culture. In common with other aspects of toxicology, it is particularly important when using in vitro cell culture systems to demonstrate that any observed effects are not due to non-specific toxicity or other artefacts, and that biological significance is ascertained before extrapolation to definition of human health risks. In some in vivo experiments, inhibitory effects have been recorded, although in others immunostimulatory effects have been seen. There is some evidence that in vitro exposure of allergen-primed LNC to DEHP, or to DINP, was able to enhance the expression of IL-4, but was without effect on the type 1 cytokine IFN-g (Lee et al., 2004). Similar phthalate-induced effects on IL-4 expression by a murine thymoma line (EL4) were reported by the same authors (Lee et al., 2004). In contrast, we failed to see any impact of phthalates on cytokine expression using a very similar experimental system (Dearman et al., 2009). In a rather different approach the impact of culture of the human monocytic cell line THP-1, or peripheral blood mononuclear cells (MNC) from allergic and non-allergic donors, with 6 different monophthalates on cytokine mRNA expression levels was examined (Glue et al., 2002). There was no effect of the monophthalates on cytokine expression by the THP-1 cells, or on MNC derived from non-allergic individuals, although increased expression of IL-4 in MNC from allergic individuals was reported for MNBP only (Glue et al., 2002). There are other reports that certain monophthalates promote the production by epithelial cell lines of inflammatory cytokines such as IL-6 and IL-8 (Jepsen et al., 2004). In these experiments, high doses of phthalate resulted in inhibition of cytokine production. Some phthalates, particularly DEHP and MEHP, have been shown to augment histamine release by human basophils isolated from peripheral blood MNC; results interpreted as suggesting that phthalates may have an adjuvant effect on the elicitation of allergic responses (Glue et al., 2005). The physiological significance of this finding is, however, called into question by the observation that concentrations of DEHP reflective of likely human exposure levels failed to provoke clinical symptoms in allergic individuals (Deustchle et al., 2008).

A variety of other authors have demonstrated that culture of inflammatory cells such as macrophages with phthalates has various inhibitory effects, such as decreasing nitric oxide or tumour necrosis factor-a production, consistent with potentially immunosuppressive effects (Hong et al., 2004; Ohnishi et al., 2008). There is also literature that suggests that phthalates such as MEHP induce apoptosis in B cells (the cells that are responsible for the production of immunoglobulin), indicative of down-regulation of antibody responses (Schlezinger et al.,

2004; 2007; Bissonnette et al., 2008). The general biological relevance of these observations is uncertain, as is their relationship, if any, to the immunomodulatory properties of phthalates.

The following information is taken into account for any hazard / risk assessment:

The weight of evidence suggests that, although some phthalates may have an intrinsic ability to modify adaptive immune responses (under strictly defined conditions, and via mechanisms that have yet to be elucidated), there is no evidence to suggest that these chemicals have a consistent and proven ability to enhance allergic sensitisation under conditions of exposure that are relevant for human health. It is premature therefore to implicate phthalates as having contributed to the increasing prevalence of atopic allergy and asthma.

5.10.1.3. Specific investigations: other studies

Summary of studies investigating the effects of DEHP on thyroid

Phthalates are commonly used plasticizers known for their effect on reproduction. In order to evaluate he endocrine effects of di(2-ethylhexyl)phthalate (DEHP), here are presented summaries of studies, which ave reported the effects of DEHP on the thyroid gland.

Chronic/long-term studies

One single long-term study indicates DEHP effects on thyroid (NTP carcinogenicity study, 1982). After DEHP treatment at 6000 ppm in diet in male mice, a slight increase in carcinomas and a en mas of C-cells is observed. No effect is observed in female mice or in male or female rats.

All other reliable and complete chronic and long-term studies indicate no DEHP-related effect on thyroid histology or on thyroid weight in rats and in mice up to dose-levels of 12000 ppm (NTP 1982, Moore 1999, Voss 2005).

In the Kurata study (2003), DEHP had no effect on thyroid histology or on blood concentration of T3 and T4 after treatment of marmoset for 65 weeks at a dose-level of 2500 mg/kg/d.

Subacute/subchronic studies

In the Poon sub-chronic study (1997), rats treated wi h DEHP at doses of 5000 ppm in diet for 13 weeks showed moderate histological alterations of thyroid (decrease in follicular size, mild decrease of colloid density). Effects on testes and on liver have been highlighted at l wer n ntrations.

Female rats intraperitoneally treated with a DEHP emulsion at dose of 75 µg/kg once every two days for 14 days (total of 7 injections) showed an increa e of bloo concentrations of T3 and T4 and a thyroid hyperplasia. No effect on blood concentration of TSH h s been observed compared with control rat. These modifications were reversible after a 7-day no-treatment period (Gayathri 2004).

Oral DEHP treatment in pregn nt rats from gestation day 6 to post-partum day 21 a concentrations between 0.015 and 405 mg/kg did not ind ce any effect on the thyroid weight of dams or offspring, indicating no toxicity on the organ development (Grande 2007).

Specific investigati ns

Three articles present he in vivo specific action of DEHP on thyroid function.

After a 21-day treatm nt in rat at doses of 1 or 2% in diet, a decrease in plasma concentration of T4 has been observed. How ver, no effect on plasma concentration of T3 has been shown (Hinton 1986). The microscopy pre ented marked utrastructural alterations indicating an hyperactivity (increase in the lysosomes number and size, nlargement of the Golgi apparatus, mitochondrial damages).

Price (19 7) administered DEHP at concentrations of 0, 50, 200, 1000 mg/kg/d in diet to male Wistar rats during 3 mon hs and observed marked alterations in the thyroid (changes follicule size and shape).

Hinton's and Price's conclusions have to be put in perspective with the lack of available data in the publication (details on sample or on animals, no indication on analytical method, no individual data) and with methodological deficiencies (number of animals insufficient: n=4 for 2% group, n=6 for 1% group and control group).

In a study investigating the effect of DEHP on serum levels of thyroid hormones, intact male Sprague-Dawley rats and thyroidectomised male rats with parathyroid replants (5-6 animals per group) were given 1,200 mg/kg bw/day of DEHP by gavage in corn oil for 7 days (Badr, 1992). One group of intact and one group of thyroidectomised rats were subcutaneously administered T4 in saline (400 and 200 µg/kg bw/day, respectively). A vehicle group was included for each treatment group. Total concentration of T4 and T3 as well as free T3

(fT3) in serum was measured by solid phase radioimmuassay. In intact rats, DEHP did not alter serum levels of the thyroid hormones when compared to controls. When thyroidectomised rats were supplemented with T4, the serum hormone levels were elevated when compared to intact controls. DEHP lowered these levels significantly when given to either intact or thyroidectomized rats supplemented with T4. Hormone levels were below the detection limit in thyroidectomized rats given corn oil or DEHP. The authors suggested that DEHP enhances the metabolism and/or excretion of thyroid hormones.

In the Howarth article (2001), rats were treated for 14 days with DEHP at 1% in diet. The thyroids of animals treated with DEHP showed evidence for hyperactivity, as indicated by a reduction in follicular size and increase in the proportion of follicular cells with a columnar appearance. However, Howarth's investigations were focused on DEHP effects on hepatic enzyme activity and no investigation of the thyroid function has been realized.

Three authors have studies the mechanistic in vitro effects of DEHP on thyroid

Wenzel (2005) presents the DEHP treatment effect on modulation of iodide uptake mediated by the sodium/iodide symporter (NIS) in FRTL-5 rat thyroid follicular cells. A significant increase in iod uptake was observed at non-cytotoxic concentrations (10e-2 M).

Breous (2005) has observed no DEHP related effect on the transcriptional activity of s dium/iodide symporter (NIS) in PC Cl3 cells.

The TH-like activity (TH=thyroid hormon) of DHEP was evaluated in an in vitro assay (Thyroid hormone-dependent GH3 cell growth or T-screen) using rat pituitary GH3 cells (Ghisari 2009) A decrease in the cell growth was observed, indicating a possible antagonist effect on T3. This result confirms the observations of the Sugiyama study (2005) in the thyroid hormone (TH) screening assay in a transduced Xenopus laevis cell line.

Human data

Three authors presented human data.

Meeker (2007) has studied in 408 men the association between the urinary concentrations of mono(2-ethylhexyl) phthalate (MEHP) and other phthalate monoester metab lite , along with serum levels of free thyroxine (T4), total triiodothyronine (T3), and thyroid-stimulating h rmone (TSH). According to the author, an inverse association between MEHP urinary concentrations and free T4 and T3 serum levels was found.

Rais-Bahrami (2004) found no effect of DEHP exposure as neonates in adolescents (14-16 year old, n=13 males and 6 females) on thyroid hormone blood level

Huang (2007) has studied in 76 pregnant women he association between urinary concentration of five phthalate monoesters and serum levels hormones (including Thyroid hormones). No association has been observed.

Conclusion

Some short-term and mechanistic studi s on DEHP indicated an effect on the thyroid function (hyperactivity, moderate to marked histological alterations), but a high number of reliable long-term studies were realized on different species and did not show any effect on the thyroid histology or function. From a mechanistic point of view, metabolism o thyroid hormones occurs through a variety of pathways in different organs before excretion of metabolites through the biliary system and faeces or via the kidneys. In brief, thyroid hormones are deiodinated and conjugated with glucuronates and sulphates. The deiodination occurs in different organs (kidneys liver h art, placenta), but the conjugation with glucuronate and sulphate and mainly occurs in liver (Cur an 1991). ike the other peroxysomes proliferators, phthalates have been found to increase the expression of n mer us hepatic enzymes, including the UDP-glucuronosyltransferase (Moody, 1994), leading to an increase of the peripherical thyroid hormone metabolism. The resultant compensatory increase in thyroid function may theorically lead to thyroid hyperactivity, hyperplasia and after chronic treatment even to thyroid neoplasia (Wu 1994) In an other hand, PPARα expression in humans is about 1/10 times less than that in rodents (Palmer 1998, Ito 2008). The effects observed in human are expected to be 10 times lower than those observed in rodents. In conclusion, a direct effect of DEHP on the thyroid gland is very unlikely. The potential of DEHP to induce effect on the thyroid gland may certainly be indirect and caused by the increased thyroid hormones metabolism.

Mechanism of carcinogenicity

The mechanisms through which peroxisome proliferators (PPs) such as DEHP induce liver tumours in rodents have been extensively studied and discussed in the last years. A brief overview of the current opinion on mechanisms and the significance for humans will be outlined here, for further details are referred to relevant reviews and criteria documents (Doull et al, 1999; Cattley et al., 1998; Youssef and Badr, 1998; Wolfgang et al., 1996; IARC, 1995; ATSDR, 1993; Bentley et al., 1993; ECETOC, 1992; WHO, 1992).

Negative results have been obtained in the majority of the genotoxicity studies on DEHP, MEHP and 2-EH. More conclusive positive results were obtained on cell transformation, induction of aneuploidy, and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic substances such as tumour promoters and peroxisome proliferators. Taken together all the results, both negative and positive, DEHP and its major metabolites are considered to be non-genotoxic substances. The results of tumour initiating and/or promoting studies indicate that DEHP have no tumour initiating activity, positive promoting activity in mice liver and a weak or no promoting activity in rat liver.

In the past, generally, three mechanisms have been proposed to account for liver carcinogenesis induced by DEHP and other PPs in rodents:

1) Induction of peroxisome proliferation leading to oxidative stress and generation of electrophilic free radicals, and

2) Increased hepatocyte proliferation/ suppression of hepatocellular apoptosis which could lead to fix tion f a previously existing DNA-damage, enhancing the conversion rate of initiated cells to tumor cells as we l as increasing the susceptiblity of hepatocytes to replication and subsequent neoplastic transformation

3) Recently a third mechanism through activation of peroxisome proliferator-activated recept rs (PPARa) has been accepted by most of the experts in this field.

Peroxisomes proliferation

Peroxisomes are cytoplasmic organelles present in all cell types and contain a numb r of hydrogen peroxide generating oxidases, catalase (which catalyses the degradation of hydrogen peroxide), nd a fatty acidb-oxidation enzyme system. Peroxisome proliferation is characterised by increased peroxisome volume density (resulting from an increase primarily in the number of peroxisomes, although size may also be increased), changes in morphology, and induction of peroxisomal enzyme activities.

Other reported effects of PPs in hepatocytes of rats and mice include mitochondrial proliferation (with changes in enzyme activities), increase in the number of lysosomal bodies (with changes in enzyme activities and lipofuscin deposition), and induction of some microsomal enzyme activities. While marked effects have been observed in hepatocytes, only minor effects have been obse ved in certain other tissues. The term PPs covers substances, which in rodents induce peroxisome prolif ratio and liver enlargement (hepatomegaly), the latter being due to both hepatocyte hyperplasia (incr sed repli ative DNA synthesis and cell division) and hypertrophy. Hepatomegaly and peroxisome proliferation is an early event during exposure to DEHP and has been observed in rats from about 14 day of exposur and throughout the exposure period at dose levels from 0.05% DEHP in the diet.

It has been suggested that liver tumour fo mation following prolonged administration of PPs arises from a sustained oxidative stress to ro ent hepatocytes due to an imbalance in the production and degradation of hydrogen peroxide. The imbalance in the hydrogen peroxide production and degradation might be a result of that catalase is induced to a much less r extent than peroxisomalb-oxidation enzymes. The increased level of hydrogen peroxide in hepatocytes might, either directly or via other reactive oxygen species (e.g., hydroxyl radicals) cause DNA damage and subsequent neoplastic transformation, or apoptosis, e.g. by oxidative damage to intracellular membranes w ich then can lead to increased cell turnover thus increasing the probability of spontaneous tumour f rmation or to cell death.

However, there is vidence suggesting that the level of oxidative damage in vivo may be too low to account entirely for the arcinogenicity of PPs. Following prolonged administration (up to 79 weeks) of DEHP to rats (Tamura e al., 1990a,b), a 20-fold increase in peroxisomalb-oxidation activity was found after 2-4 weeks of treatment with a gradual decrease from week 20 to week 79 but remaining at an 8-10-fold higher level than control vels. Catalase activity increased (2-3-fold) after short-term treatment and remained at this level th oughout the treatment period. The hepatic hydrogen peroxide level also increased but only 1.2-1.7-fold. As the he atic hydrogen peroxide levels increased only slightly and did not correspond to the increase in p oxisomalb-oxidation activity, these results indicate that a large part of the hydrogen peroxide produced by peroxisomalb-oxidation could be rapidly scavenged by catalase. This could be explained by the fact that the maximal hepatic catalase activity in vitro is thousands of times greater than the correspondingb-oxidation activity in untreated animals. After treatment with peroxisome proliferator, the maximal activity of catalase is still thousands of times greater, even though catalase is only up-regulated 2- 3-fold and acyl-CoA oxidase increases as much as 20-fold. This indicates that liver tumours produced after long-term administration of DEHP might not be due only to the oxidative stress introduced by the enhanced peroxisomalb-oxidation. This conclusion is strengthened by the fact, that even when oxidative stress was induced by the administration of buthionine sulfoximine at a dose that drastically lowered the endogenous glutathione pool in liver, the potent peroxisome proliferator, nafenopin, failed to induce unscheduled DNA synthesis, or increase the level of DNA

single strand breaks in hepatocytes from animals treated in vivo (Nilsson et al., 1991). These authors also suggested, that the modest increase in the levels of the oxidation product 8-hydroxydeoxy-guanosine (8-OHdG), and that was found in liver DNA from animals treated with the potent peroxisome proliferators over long periods of time (Kasai et al., 1989), most probably represents an artifact from isolation of the DNA where no precautions had been taken to prevent e.g. OH-radicals to be formed through Fenton-like reactions (Nilsson et al., 1991).

In conclusion, induction of peroxisome proliferation alone is not enough to explain the hepatocarcinogenic activity of DEHP in rodents.

Activation of PPARα

Recent investigations have demonstrated the central role of a class of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), in mediating the effects of PPs (reviewed by Cattley et al., 1998). I the presence of PPs or fatty acids, the PPAR receptors induce the transcriptional regulation of PP-responsive genes.

Out of four different subtypes, the subtype PPARα that is strongly expressed in tissues catabolizing fatty acids (liver, digestive mucosa, kidney proximal tubules, muscle and retina), seems to be the most importan wi h respect to processes associated with peroxisome proliferation. The expression of PPARα may be, to some extent, affected by glucocorticoids (Lemberger et al., 1994, 1996), providing an explanation of the previously observed effects on peroxisome proliferation induced by fasting and stress caused e.g. by hypothermia (de Duve, 1983; Reddy and Lalwani, 1983). Gene transcription is affected through a heterodimeri re ptor omplex involving PPARα and the retinoid X receptor (RXR) that is activated by PPs and 9-cis-retinoi acid (present endogenously). The activated receptor complex regulates transcription via binding to the promoter regions of peroxisome responsive genes, e.g. those involved in the ß-oxidation of fatty acids. However, the fact that potent PPs will act as hypolipidemic drugs in man without causing detectable peroxisome proliferation seems to indicate, that other genes are also affected by PPARα , e.g. the promoter regions of the human apolipoprotein genes (apo A-I, apo A-II, apo C-III). The fact that long-term administration of DEHP in the feed down to a concentration of 200 ppm increases the dolichol content of lysosomal membranes (Edlund et al., 1986) also bears evidence of the multifaceted effects induced by PP .

When DEHP (1.2% in the diet) was administered to Sv/129 mice entirely lacking the PPARα , none of the responses typical for peroxisome induction (such as in reas in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly) found in t e wild type mouse could be detected (Ward et al., 1998). Further, whereas the wild type mouse fed DEHP exhibited typical lesions in liver, kidney and testis, no signs of liver toxicity was detected in the knockout mouse". On the other hand, evidence of lesions in kidneys and testes were also found in the latter, ndicating PPARα independent pathways for induction of toxicity in these organs. In another study with PPARα null mice fed the potent peroxisome proliferator, Wy-14,643 at 0.1% in the diet for 11 months, no indication of r plicative DNA synthesis in the liver, and no increase in the incidence of liver tumors were observed In contrast, after 11 months administration, 100% of the wildtype mice exhibited multiple hepatocellular neoplasms (Peters et al., 1997). Although it is established that PPARα could mediate liver cell proliferation and h patocarcinogenesis of the studied PPs, the pathways involved have not been elucidated.

A report from 2003 Klaunig .E. et al, 2003) provides an in-depth analysis of the state of the science on several topics critical to evalu ting the relationship between the mode of action (MOA) for PPARα agonist, such as DEHP, and the human relevance of related animal tumours. Topics include a review of existing tumor bioassay data, data from animal and human sources relating to the MOA for PPARα agonists in several different tissues, and ase s udies on the potential human relevance of the animal MOA data. The summary of existing bioassay dat discl ses su stantial species differences in response to peroxisome proliferators in vivo, with rodents more respon ve than primates. It is clear from the published studies that humans possess a functional PPARα, and that peroxisome proliferators activate the human receptor. It is clear that some of the genes modulated by these chemi als in humans differ from those regulated by rodent PPARα. The available epidemiological and clinical studi s are inconclusive, but, nonetheless, do not provide evidence that peroxisome proliferators, such as DEHP, cause liver cancer in humans.

In conclusion, the available, studies on transgenic mice, indicate that PPARs may play a central role in mediating the hepatotoxic effects of DEHP, such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly. Also, it has been demonstrated that PPARs is required in mediating the hepatocarcinogenic effects of the PPs Wy-14,643 in mice.

Exposure of Leydig cells to PPs prevented cholesterol transport into the mitochondria after hormonal stimulation and inhibited steroid synthesis, without altering total cell protein synthesis or mitochondrial and DNA integrity (Gazouli 2002).

PPs also reduced the levels of the cholesterol-binding protein peripheral-type benzodiazepine receptor (PBR) because of a direct transcriptional inhibition of PBR gene expression in MA-10 Leydig cells.

MA-10 cells contain mRNAs for PPARalpha and PPARbeta/delta, but not for PPARgamma.

In vivo treatment of mice with PPs resulted in the reduction of both testis PBR mRNA and circulating testosterone levels, in agreement with the proposed role of PBR in steroidogenesis.

By contrast, liver PBR mRNA levels were increased, in agreement with the proposed role of PBR in cell growth/tumor formation in non steroidogenic tissues. However, PPs did not inhibit testosterone production and testis PBR expression in PPARalpha-null mice. These results suggest that the antiandrogenic effect of PPs is mediated by a PPARalpha- dependent inhibition of Leydig cell PBR gene expression.

Species differences

Marked species differences with respect to hepatic response to PPs are apparent, were rats and mice seem t exhibit the highest sensitivity. Guinea pigs and monkeys are relatively insensitive, while Syrian amste s have demonstrated a sensitivity intermediate between these two groups of mammals. In a comparative study (4 days) of rats and hamsters, the liver weight of hamsters was significantly increased only at 1000 mg/kg b.w per day with no significant increases in peroxisomal enzyme activities, whereas a significant and dose dependent (from 100 mg/kg b.w. per day) increase in rat liver weight as well as in peroxisomal enzyme a tivities was observed (Lake et al., 1984). In marmosets, the liver weight was not affected and microscopic examination revealed only a slight increase in peroxisomes following administration of 2000 mg/kg b.w. per day for 14 days. In rats, hepatomegaly, marked peroxisome proliferation, and increased peroxisomal enzyme ctivities was observed following a similar dosage regimen (Rhodes et al., 1986). In another comparative study (21 days) with rats and cynomolgus monkeys, no treatment related changes in liver weight and peroxisomal enzyme activities were observed in monkeys at dose levels up to 500 mg/kg b.w. per day, whereas in rats marked effects on the same parameters were seen at a similar dosage regimen (Short et al., 1987).

In vitro studies using primary hepatocyte cultures from rod nts and primates have supported the in vivo findings. Thus, whereas a number of different PPs have caused peroxisome proliferation in rat and mouse hepatocytes, several investigations have demonstrated a lack of activ ty in primate and human cells (reviewed in: Ashby et al., 1994; IARC, 1995; Elcombe et al., 1997). MEHP, a metab lite of DEHP, did not stimulate peroxisome proliferation in human cells, although a marked respons was obtained in rat hepatocytes (Butterworth et al., 1989).

The potential human response to PPs ha been xamined in liver biopsies obtained from patients treated with hypolipidemic drugs. Morphometric m asuremen s in liver biopsies did not reveal evidence for peroxisome proliferation. The potential carcinogeni risk of hypolipidemic therapy with fibrates (clofibrate and gemfibrozil, both being potent PPs) has been evaluated i two limited clinical trials with no evidence for carcinogenesis obtained (IARC, 1996). No rel vant data are available on humans exposed to DEHP. But in a study where liver biopsies had been obtained from dialysis patients, who are exposed to significant quantities of DEHP leached from PVC dialysis tubings, Ganning t al. (1987) reported an increased number of peroxisomes in exposed individuals. However, this claim was based on two electron micrographs from two different patients, where an apparent increase in the number of peroxisomes was found in one specimen.

In rodent liver hepato egaly and peroxisome proliferation require expression of functional PPARα (Lee et al, 1995). The slight r no responsiveness of human liver to some effects of PPs, such as hepatomegaly and peroxisome proliferation, could be explained by a low level of PPARα found in human livers (1-10% of the level found in ra and mouse liver), as well as observations of genetic variations that render the human PPARareceptor less activ as compared to PPARaexpressed in rodent liver (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt t al., 999).

In conclusion, the available data indicate a quantitative species differences in the response to the hepatic effects of DEHP and in the activation of PPARα.

The effects of the metabolites of DEHP have been studied in primary hepatocyte cultures of rat, rabbit, guinea pig, and monkey (Dirven et al., 1993a). The cell cultures were derived from male Wistar rats, male Dunkin Hartley guinea pigs, male New Zealand rabbits, and male cynomolgus monkeys and cultured in the presence of various concentrations of MEHP (> 99% pure), mono(5-carboxy-2-ethylpentyl) phthalate (metabolite V) (purity: 96%), or mono(2-ethyl-5-oxohexyl) phthalate (metabolite VI) (purity: 99%).

In rat hepatocyte cultures, MEHP and metabolite VI were equally potent in inducing peroxisome proliferation (50% increase in peroxisomal palmitoyl-CoA oxidase activity and microsomal lauric acid ω-hydroxylation activity with 5-15 µM MEHP), while metabolite V was much less potent. In hepatocyte cultures derived from

guinea pigs, rabbits, and monkeys, a 50% increase in peroxisomal palmitoyl-CoA oxidase activity was found after treatment with 408-485 µM MEHP.

No induction of microsomal lauric acid ω-hydroxylation activity was found in these three species.

A primary rat hepatocyte culture system was utilised to determine the proximate peroxisome proliferator(s) derived from DEHP (Mitchell et al., 1985b). DEHP (purity not specified) was administered to rats by gavage and the urinary metabolites were identified and isolated. The major metabolites of the ω-oxidation pathway [mono(5-carboxy-2-ethyl-pentyl) phthalate (metabolite V) and mono(3-carboxy-2-ethylpropyl) phthalate (metabolite I)] and of the (ω-1)-oxidation pathway [mono(2-ethyl-5-hydroxyhexyl) phthalate (metabolite IX) and mono(2-ethyl-5-oxo-hexyl) phthalate (metabolite VI)] together with MEHP and 2-EH were added to primary rat hepatocyte cultures and the effect on peroxisomal enzyme activity was determined. The (ω-1)-oxidation products (metabolite V and IX) produced a large (7- to 11-fold) induction on PCoA oxidation whereas the ω-oxidation products (metabolite I and V) produced little or no effect. Similar effects were observed for the induc ion of cytochrome P-450-mediated LAH.

Hepatocyte proliferation and apoptosis

Hepatocyte proliferation (characterised by increased replicative DNA synthesis and cell division, and hypertrophy) is an important response in rodent liver to PPs and has been implicated in the mechanisms of rodent hepatocarcinogenesis. Hepatocyte proliferation occurs in the tumours that develop in rats and mice after administration of PPs and is seen in lesions that are the direct progenitors of tum rs

Thus, at least for the more potent PPs, a close correlation has been found between induction of sustained replicative DNA synthesis and the potency of various PP with respect to induction of liver tumors (Marsman et al., 1988, 1992). However, the association of cell proliferation with tumour formation in rodent liver is complex and the magnitude of response for hepatomegaly and hepatocyte proliferation is not entirely predictive of eventual tumour yield. For PPs, it is important to differentiate short-term from prolonged stimulation of cell replication. Hepatomegaly is evident during the first few days of administration of PPs and is largely due to transient hepatocyte proliferation that subsides after several days as liver weight reaches a new plateau. Hepatomegaly has also been seen in rodent liver after p olong d administration of some, but not all, PPs.

There is some indications that suppression of hepato ellular apoptosis also occurs during the induction of hepatomegaly. The increase in liver weight is dependen on c ntinued exposure and is reversible upon cessation of exposure and it has been suggested that this r vers l co ld be related to large increases in hepatocellular apoptosis.

An increased rate of cell proliferation c n be a cr ical effect both in tumour initiation, by increasing the frequency of spontaneous mutations and the rate f conversion of DNA adducts into mutations before they are repaired, and in tumour promotion by facili ting the promotion of initiated cells. This effect could be strengthened by suppression of hepatoce lular apoptosis, which lead to increasing the number of mutant hepatocytes susceptible to repli ation and subsequent neoplastic transformation. It has been reported (James 1998, Hagiwara 1990, Uno 1994, Ward 1988), that DEHP and its metabolite MEHP can induce DNA synthesis and inhibit hepatocyte apoptosis in rats and mice hepatocytes in both in vivo and in vitro studies. Also a tumour-promoting activity f DEHP was observed in the liver of mice whereas a promoting activity in the liver of rats is equivocal. As first s ggested by Préat et al. (1986a, 1986b), in addition to cell proliferation PPs appear to have an important role in promoting the selective growth of basophilic preneoplastic foci. Thus, whereas phenobarbital causes n increase in the number of preneoplastic foci in a liver initiated by e.g. diethylnit osamine, a potent PP like nafenopin or WY-14,643 does not increase the number of such foci app eciabl , but causes a great increase in the size of these foci. Further, the basophilic foci induced by PPs seem to ha e a much higher likelihood to progress to hepatocellular carcinomas by boosting the selection of transformed cells (Préat et al., 1986a, 1986b; Cattley and Popp, 1989). Development of foci and adenomas d pends on the continuous exposure to PPs, where cessation of exposure results in disappearance of benign lesion , and continued exposure is essential for progression to malignant tumors (Marsman and Popp, 1994; Miller and Cattley, 1996).

In conclusion, induction of hepatocyte proliferation combined with suppression of hepatocellular apoptosis could play a major role in the hepatocarcinogenicity of DEHP.

5.10.2. Human information

Human biomonitoring determines internal exposure (i.e. body burden) by measuring the chemicals, their metabolites or specific reaction products in human specimens (e.g. urine or blood). Progress in human

biomonitoring has opened up new possibilities in assessing phthalate exposures, because most of the biomarkers used in modern phthalate biomonitoring are specific metabolites generated in the human body (secondary, oxidized metabolites) which are not prone to external phthalate contamination. Furthermore, as biomonitoring represents an integral measure of exposure from multiple sources and routes, biomonitoring data permit a new approach to exposure assessment even when the quantity and quality of external exposures are unknown or ambiguous. Biomonitoring data can also be used to compare exposures of the general population with special subpopulations. This way, although biomonitoring is an integral measure from all sources, special routes or sources of exposure, contributions of exposure routes (e.g. foodstuff) or exposures caused by individual life style can be identified in combination with survey/questionnaire data and/or a selective study design.

Available information on biomonitoring studies is summarized in chapter 9 and in Annexes 2 and 3 of this report.

5.10.3. Summary and discussion of specific investigations

Specific investigations: other studies

Proposed mechanisms of hepatocarcinogenicity:

This part has been cited in the EU Risk Assessment 2008 (p 403-408)

The mechanisms through which peroxisome proliferators (PPs) such as DEHP induce liver tumours in rodents have been extensively studied and discussed in the last years.

A brief overview of the current opinion on mechanisms and the significance for humans will be outlined here, for further details are referred to relevant reviews and criteria documents (Doull et al, 1999; Cattley et al., 1998; Youssef and Badr, 1998; Wolfgang et al., 1996; IARC, 1995; ATSDR, 1993; Bentley et al., 1993; ECETOC, 1992; WHO, 1992).

Negative results have been obtained in the majority of the genotoxicity studies on DEHP, MEHP and 2-EH. More conclusive positive results were obtained on cell transformation, induction of aneuploidy, and cell proliferation. These test systems are, however, also sensitive to several non-genotoxic subs ances such as tumour promoters and peroxisome proliferators.

Taken together all the results, both negative and positive, DEHP and its major metaboli es are considered to be non-genotoxic substances. The results of tumour initiating and/or promoting studies indicate that DEHP have no tumour initiating activity, positive promoting activity in mice liver and a weak or no promoting activity in rat liver.

In the past, generally, three mechanisms have been proposed to account for liver carcinogenesis induced by DEHP and other PPs in rodents:

1) Induction of peroxisome proliferation leading to oxid tive stress and generation of electrophilic free radicals, and

2) Increased hepatocyte proliferation/ suppress of hep tocellular apoptosis which could lead to fixation of a previously existing DNA-damage, enhancing the onversion rate of initiated cells to tumor cells, as well as increasing the susceptiblity of hepatocytes to replica ion and subsequent neoplastic transformation.

3) Recently a third mechanism through activation of peroxisome proliferator-activated receptors (PPARα) has been accepted by most of the experts in this field. The possible mechanisms of hepatocarcinogenicity and the species differences with respec to these hepatic effects of PP will be discussed in this section.

Peroxisome proliferation:

Peroxisomes are cytoplasmic organelles present in all cell types and contain a number of hydrogen peroxide generating oxidases catalase (which catalyses the degradation of hydrogen peroxide), and a fatty acid ß-oxidation enzyme system. Peroxisome proliferation is characterised by increased peroxisome volume density (resulting from an incr ase primarily in the number of peroxisomes, although size may also be increased), changes in m rphol gy, and induction of peroxisomal enzyme activities.

Other repo ted ffects of PPs in hepatocytes of rats and mice include mitochondrial proliferation (with changes in enzyme activitie), increase in the number of lysosomal bodies (with changes in enzyme activities and lipofuscin deposition), and induction of some microsomal enzyme activities. While marked effects have been observed in hepatocy es, only minor effects have been observed in certain other tissues. The term PPs covers substances, which in rodents induce peroxisome proliferation and liver enlargement (hepatomegaly), the latter being due to both hepatocyte hyperplasia (increased replicative DNA synthesis and cell division) and hypertrophy. Hepatomegaly and peroxisome proliferation is an early event during exposure to DEHP and has been observed in rats from about 14 days of exposure and throughout the exposure period at dose levels from 0.05% DEHP in the diet.

It has been suggested that liver tumour formation following prolonged administration of PPs arises from a sustained oxidative stress to rodent hepatocytes due to an imbalance in the production and degradation of hydrogen peroxide. The imbalance in the hydrogen peroxide production and degradation might be a result of that catalase is induced to a much lesser extent than peroxisomal ß-oxidation enzymes. The increased level of hydrogen peroxide in hepatocytes might, either directly or via other reactive oxygen species (e. g., hydroxyl radicals) cause DNA damage and subsequent neoplastic transformation, or apoptosis, e. g. by oxidative damage

to intracellular membranes which then can lead to increased cell turnover thus increasing the probability of spontaneous tumour formation or to cell death.

However, there is evidence suggesting that the level of oxidative damage in vivo may be too low to account entirely for the carcinogenicity of PPs. Following prolonged administration (up to 79 weeks) of DEHP to rats (Tamura et al., 1990a, b), a 20-fold increase in peroxisomal b-oxidation activity was found after 2-4 weeks of treatment with a gradual decrease from week 20 to week 79 but remaining at an 8-10-fold higher level than control levels. Catalase activity increased (2-3-fold) after short-term treatment and remained at this level throughout the treatment period. The hepatic hydrogen peroxide level also increased but only 1.2-1.7-fold. As the hepatic hydrogen peroxide levels increased only slightly and did not correspond to the increase in peroxisomal b-oxidation activity, these results indicate that a large part of the hydrogen peroxide produced by peroxisomal ß-oxidation could be rapidly scavenged by catalase. This could be explained by the fact that the maximal hepatic catalase activity in vitro is thousands of times greater than the corresponding ß-oxidation activity in untreated animals. After treatment with peroxisome proliferator, the maximal activity of catalase is still thousands of times greater, even though catalase is only up-regulated 2- 3-fold and acyl-CoA oxidase increases as much as 20-fold. This indicates that liver tumours produced after long-term administration of DEHP might not be due only to the oxidative stress introduced by the enhanced peroxisomal ß-oxidation. This conclusion is strengthened by the fact, that even when oxidative stress was induced by the administration of buthionine sulfoximine at a dose that drastically lowered the endogenous glutathione pool in liver, the potent peroxisome proliferator, nafenopin, failed to induce unscheduled DNA synthesis, or increase the level of DNA single strand breaks in hepatocytes from animals treated in vivo (Nilsson et al., 1991). These authors also suggested, that the modest increase in the levels of the oxidation product 8-hydroxydeoxy-guanosine (8-OHdG), and that was found in liver DNA from animals treated with the potent peroxisome proliferators over long periods of time (Kasai et al., 1989), most probably represents an artifact from isolation of the DNA where no precautions had been taken to prevent e. g. OH-radicals to be formed through Fenton-like reactions (Nilsson et al., 1991).

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An increased rate of cell proliferation can be a critical effect both in tumour initiation, by increasing the

frequency of spontaneous mutations and the rate of conversion of DNA adducts into mutations before they are repaired, and in tumour promotion by facilitating the promotion of initiated cells. This effect could be strengthened by suppression of hepatocellular apoptosis, which lead to increasing the number of mutant hepatocytes susceptible to replication and subsequent neoplastic transformation. It has been reported recently (James et al., 1998), that DEHP and its metabolite MEHP can induce DNA synthesis and inhibit hepatocyte apoptosis in rats and mice hepatocytes in both in vivo and in vitro studies. Also a tumour-promoting activity of DEHP was observed in the liver of mice whereas a promoting activity in the liver of rats is equivocal. As first suggested by Préat et al. (1986a, 1986b), in addition to cell proliferation PPs appear to have an important role in promoting the selective growth of basophilic preneoplastic foci. Thus, whereas phenobarbital causes an increase in the number of preneoplastic foci in a liver initiated by e. g. diethylnitrosamine, a potent PP like nafenopin or WY-14,643 does not increase the number of such foci appreciably, but causes a great increase in the size of these foci. Further, the basophilic foci induced by PPs seem to have a much higher likelihood to progress to hepatocellular carcinomas by boosting the selection of transformed cells (Préat et al., 1986a, 1986b; at ley and Popp, 1989). Development of foci and adenomas depends on the continuous exposure to PPs, where cessat on f exposure results in disappearance of benign lesions, and continued exposure is essential for progr ssion o malignant tumors (Marsman and Popp, 1994; Miller and Cattley, 1996).

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studies are inconclusive, but, nonetheless, do not provide evidence that peroxisome proliferators, such as DEHP, cause liver cancer in humans.

In conclusion, the available, studies on transgenic mice, indicate that PPARs may play a central role in mediating the hepatotoxic effects of DEHP, such as increase in the number of peroxisomes, induction of replicative DNA-synthesis, and hepatomegaly. Also, it has been demonstrated that PPARs is required in mediating the hepatocarcinogenic effects of the PPs Wy-14,643 in mice.

Species differences

Marked species differences with respect to hepatic response to PPs are apparent, were rats and mice seem to exhibit the highest sensitivity. Guinea pigs and monkeys are relatively insensitive, while Syrian hamsters have demonstrated a sensitivity intermediate between these two groups of mammals. In a comparative study (14 d ys) of rats and hamsters, the liver weight of hamsters was significantly increased only at 1000 mg/kg b. w per day with no significant increases in peroxisomal enzyme activities, whereas a significant and dose-depend nt (f m 100 mg/kg b. w. per day) increase in rat liver weight as well as in peroxisomal enzyme activities was o s rved (Lake et al., 1984). In marmosets, the liver weight was not affected and microscopic examination r vealed only a slight increase in peroxisomes following administration of 2000 mg/kg b. w. per day for 1 days. In r ts, hepatomegaly, marked peroxisome proliferation, and increased peroxisomal enzyme activities was observed following a similar dosage regimen (Rhodes et al., 1986). In another comparative study (21 days) with rats and cynomolgus monkeys, no treatment related changes in liver weight and peroxisomal enzyme activities were observed in monkeys at dose levels up to 500 mg/kg b. w. per day, whereas in rats marked e ects on the same parameters were seen at a similar dosage regimen (Short et al., 1987).

In vitro studies using primary hepatocyte cultures from rodents and primates have supported the in vivo findings. Thus, whereas a number of different PPs have caused peroxisome proliferation in rat and mouse hepatocytes, several investigations have demonstrated a lack of activity in primate and human cells (reviewed in: Ashby et al., 1994; IARC, 1995; Elcombe et al., 1997). MEHP, a metabolite of DEHP, did not stimulate peroxisome proliferation in human cells, although a marked response was obtained in rat hepatocytes (Butterworth et al., 1989).

The potential human response to PPs has been examined in liver biopsies obtained from patients treated with hypolipidemic drugs. Morphometric measurements in liver iopsies did not reveal evidence for peroxisome proliferation. The potential carcinogenic risk of hypolipidemic therapy with fibrates (clofibrate and gemfibrozil, both being potent PPs) has been evaluated in two limited clinical trials with no evidence for carcinogenesis obtained (IARC, 1996). No relevant data are av ilable on humans exposed to DEHP. But in a study where liver biopsies had been obtained from dialys s patients who are exposed to significant quantities of DEHP leached from PVC dialysis tubings, Ganning et l. (1987) reported an increased number of peroxisomes in exposed individuals. However, this claim was based on two electron micrographs from two different patients, where an apparent increase in the numbe of perox somes was found in one specimen.

In rodent liver, hepatomegaly and peroxisome proliferation require expression of functional PPARα (Lee et al, 1995). The slight or no sponsiveness of human liver to some effects of PPs, such as hepatomegaly and peroxisome proliferation, could be explained by a low level of PPARα found in human livers (1-10% of the level found in rat and mo se liver) as well as observations of genetic variations that render the human PPARα receptor less active as compared to PPARα expressed in rodent liver (Tugwood et al., 1996; Palmer et al., 1998; Woodyatt et l., 1999).

In con lu ion, he available data indicate a quantitative species differences in the response to the hepatic effects of DEHP and in he activation of PPARα.

5.11. Derivation of DNEL(s) / DMEL(s)

5.11.1. Overview of typical dose descriptors for all endpoints

Table 48 Available dose-descriptor(s) per endpoint for the submission substance as a result of its hazard assessment

Endpoint	Route	Dose descriptor or qualitative effect characterisation; test type	Reference to selected study (see footnotes for justification)
Acute toxicity	oral	No adverse effect observed discriminating dose: 20000 mg/kg bw	NTP (1982a) (see section 5.2.1.1)
Acute toxicity	dermal	Adverse effect observed LD50: 19800 mg/kg bw	Shaffer CB, Carpenter CP (1945) (see section 5.2.1.3)
Acute toxicity	inhalation	Adverse effect observed discriminating conc.: 10620 mg/m³	Greenough RJ (1981a) (see section 5.2.1.2)
Irritation / Corrosivity	skin	Adverse effect observed slightly irritating	Mürmann P (1987a) (see section 5.3.1.1)
Irritation / Corrosivity	eye	Adverse effect observed slightly irritating	Mürmann P (1987b) (see section 5.3.2.1)
Irritation / Corrosivity	respiratory tract	No study available	
Sensitisation	skin	No adverse effect observed (not sensitising)	Greenough RJ (1981d) (see section 5.5.1.1)
Sensitisation	respiratory tract	No adverse effect observed (not sensitising)	
Repeated dose toxicity	oral	Adverse effect observed NOAEL: 500 ppm (nominal) (equivalent to = 28.9 mg/kg bw/d for males and 36.1 mg/kg bw/d for females) (rat, chronic) Target organs: urogenital: kidneys	David RM, Moore MR, Finney DC (2000a) David RM, Moore MR, Finney DC (2001) (see section 5.6.1.1)
Repeated dose toxicity	dermal (systemic effects)	No study available	
Repeated dose toxicity	dermal (local effects)	No study available	
Repeated dose toxicity	inhalation (systemic effects)	Adverse effect observed NOAEC: 50 mg/m³ (subacute; rat)	Klimisch HJ, Gamer AO, Hellwig J (1992) Klimisch HJ (1990) Klimisch HJ (1988) (see section 5.6.1.2)
Repeated dose toxicity	inhalation (local	Adverse effect observed	Klimisch HJ, Gamer AO, Hellwig J (1992)

Endpoint	Route	Dose descriptor or qualitative effect characterisation; test type	Reference to selected study (see footnotes for justification)
	effects)	NOAEC: 50 mg/m³ (subacute; rat)	Klimisch HJ (1990) Klimisch HJ (1988) (see section 5.6.1.2)
Mutagenicity	in vitro / in vivo	No adverse effect observed (negative)	
Carcinogenicity	oral	Adverse effect observed NOAEL: 500 ppm (nominal) (equivalent to = 28.9 mg/kg bw/d for males and 36.1 mg/kg bw/d for females) (rat, chronic)	David RM, Moore MR, Finney DC and Guest D (200) David RM, Moor MR, Cifone MA, Finney DC and Guest D (1999) (see sec ion 5.8.1 1)
Carcinogenicity	dermal	No study available	
Carcinogenicity	inhalation	No study available	
Reproductive toxicity: effects on fertility	oral	Adverse effect observed NOAEL: 1000 ppm (nominal) (equivalent to approximately 77 mg DEHP/kg bw/day in the F0 animals, and 48 and 46 mg DEHP/kg bw/day in the F1 and F2 animals respectively) (rat, 3-generation)	Wolfe GW and Layton KA (2004) Peckham JC (2003) Blystone CR, Kissling GE, Bishop JB, Chapin RE, Wolfe GW and Foster PMD (2010) (see section 5.9.1.1)
Reproductive toxicity: effects on fertility	dermal	No study avail ble	
Reproductive toxicity: effects on fertility	inhalation	No adverse ffect observed	Klimisch HJ, Gamer AO, Hellwig J (1992) BASF (1990) (see section 5.9.2.1)
Reproductive toxicity: devel pm ntal tox city	oral	Adverse effect observed NOAEL: 100 ppm (nominal) (equivalent to approximately 8 mg DEHP/kg bw/day in the F0 animals and 4.9 and 4.8 mg DEHP/kg bw/day in the F1 and F2 animals, respectively) (rat, 3-generation)	Wolfe GW and Layton KA (2004) Peckham JC (2003) Blystone CR, Kissling GE, Bishop JB, Chapin RE, Wolfe GW and Foster PMD (2010) (see section 5.9.2.1)
Reproductive toxicity: developmental toxicity	dermal	No study available	
Reproductive toxicity:	inhalation	No adverse effect observed	Merkle J, Klimisch HJ, Jäckh R (1988)

Endpoint	Route	Dose descriptor or qualitative effect characterisation; test type	Reference to selected study (see footnotes for justification)
developmental toxicity			BASF (1986) (see section 5.9.2.1)

Justification for endpoint selection:

- Acute toxicity (oral): Key study
- Acute toxicity (dermal): Key study
- Irritation / Corrosivity (skin): Key study
- Irritation / Corrosivity (eye): Key study
- Sensitisation (skin): Key study
- Repeated dose toxicity (oral): Key study
- Repeated dose toxicity (inhalation (systemic effects): Key study
- Repeated dose toxicity (inhalation (local effects): Key study
- Carcinogenicity (oral): Key study
- Reproductive toxicity: effects on fertility (or l): Key s udy
- Reproductive toxicity: developmental toxicity (or l): Key study
- Reproductive toxicity: developm ntal oxicity (inhalation): Key study

5.11.2. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptor for critical health effects

Table 49 DN(M)ELs for workers

Route	Type of effect	Hazard conclusion	Most sensitive endpoint
Inhalation	Systemic effects - Long-term	DNEL (Derived No Effect Level): 1.6 mg/m³	developmental toxicity / teratogenicity (Oral)
Inhalation	Systemic effects - Acute	No hazard identified	
Inhalation	Local effects - Long-term	No hazard identified	
Inhalation	Local effects - Acute	No hazard identified	
Dermal	Systemic effects - Long-term	DNEL (Derived No Effect Level): 3.4 mg/kg bw/day	developmental t xicity / teratogenicity (Oral)
Dermal	Systemic effects - Acute	No hazard identified	
Dermal	Local effects - Long-term	No hazard identified	
Dermal	Local effects - Acute	No hazard identified	
Eyes	Local effects	No hazard identified	

Further explanation on hazard conclusions

- **Inhalation Systemic effects - Long-term:** R lev nt dose descriptor: 4.8 mg/kg bw/d (NOAEL from 3-Generation study in rats); Modificat on of start ng point: x 1/0.38 cubic metre/kg bw (conversion into inhalation NAEC by using an 8-hour espiratory volume for the rat), x 75/75 (correction for 75% bioavailability for inhalation n humans and 75% for oral absorption in adult rats), x 6.7 cubic metre/10 cubic metre (correction for activity driven d fferences of respiratory volume in workers compared to workers in rest) x 7/5 (correction of start ng point for using a NOAEL from a feeding study with exposure at 7/d compared to the worker exposure situation 5 d/w): leads to starting point 11.85 mg/m3; Assessment factors: Interspecies: 2.5 (no allometric scaling factors is used as the oral dose was converted into a NAEC); Intraspecies: 3 (as used in he EU RA of DEHP for potential intraspecies differences for the worker); Exposure duration 1 (the NOAEL is based on a 3-generation study)

- **Inhalation Systemi effects - Acute:** DEHP shows very low acute toxicity (not leading to C&L), no dose-respons dat available to derive DNEL, no hazard identified.

Inh ation Local effects - Long-term: According to the available data, no local effects are expected after long-te m exposure by inhalation.

Inhalation Local effects - Acute: According to the available data, no local effects are expected after short-term exposure by inhalation.

- **Dermal Systemic effects - Long-term:** Relevant dose descriptor: 4.8 mg/kg bw/d (NOAEL from 3-Generation study in rats); Modification of starting point: x 75/5 (correction for 5% bioavailability for dermal exposure in humans and 75% for oral absorption in adult rats) x 7/5 (correction of starting point for using a NOAEL from a feeding study with exposure at 7/d compared to the worker exposure situation 5 d/w): leads to starting point 100.8 mg/kg bw/day; Assessment factors: Interspecies: 4 x 2.5 (factor 4 for allometric scaling from rat to humans; factor 2.5 for remaining uncertainties); Intraspecies: 3 (as used in the EU RA of DEHP for potential intraspecies differences for the worker); Exposure duration: 1 (the NOAEL is based on a

3-generation study)

- **Dermal Systemic effects - Acute:** DEHP shows very low acute toxicity (not leading to C&L), no dose-response data available to derive DNEL, no hazard identified.

- **Dermal Local effects - Long-term:** According to the available data, no local effects are expected after long-term dermal exposure.

- **Dermal Local effects - Acute:** According to the available data, no local effects are expected after short-term dermal exposure.

- **Eyes Local effects:** According to the available data, if at all only minor reversible local effects are expected after exposure of the eyes.

Further explanation on DNEL derivation for workers

Route / Type of effect	DNEL derivation	Assessment factors (AF) for DNEL derivation
Inhalation Systemic effects - Long-term	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEC 11.85 mg/m³	**AF for other int rspecies differences:** 2.5 (ECHA Guidance) **AF for intraspecies differences:** 3 (EU Risk Asseessment Report, see REACH Annex I, 0.5) **Overall Assessment Factor:** 7.5
Dermal Systemic effects - Long-term	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEL 100.8 mg/kg bw/day	**AF for interspecies differences (allometric scaling):** 4 (ECHA Guidance) **AF for other interspecies differences:** 2.5 (ECHA Guidance) **AF for intraspecies differences:** 3 (EU Risk Asseessment Report, see REACH Annex I, 0.5) **Overall Assessment Factor:** 30

Justification for ro te-to-route extrapolation:

- **Inhalation Systemi effects - Long-term:** After oral or inhalative exposure only sytemic but no local effects were observed.

Dermal Systemic effects - Long-term: After oral and dermal exposure especially sytemic toxicity was observ d.

Discussion

NOAEL of 4.8 mg/kg bw/d for reproductive and developmental effects observed in 3-generation oral study in rats (Wolfe and Layton, 2004).

Description	Value	Remark	
Step 1) Relevant dose-descriptor	NOAEL: 4.8 mg/kg bw/day	NOAEL from 3-Generation study in rats	

Description	**Value**	**Remark**	
Step 2) Modification of starting point	0.38 m^3/kg bw 75/75 6.7 m^3/10 m^3 7/5	- Conversion into inhalation NAEC (in mg/m^3) by using an 8-hour respiratory volume for the rat. - Correction for 75%bioavailability for inhalation in humans and 75% fororal absorption in adult rats - Correction for activity driven differences of respiratory volumes in workers compared to workers in rest (6.7 m^3/ m^3). - Correction for use of NOAEL from feeding study with 7d exposure/week compared to worker's exposure 5 d/week	
Step 3) Assessment factors			
Interspecies	2.5	O ly a factor 2.5 is used, and no allometric scaling factors is used	
Intraspecies	3	In the EU RA of DEHP anassessment factors of 3 is used for potential intraspecies differences for the worker (see also see REACH Annex I, 0.5 for justification).	
Exposure duration	1	The NOAEL is based on a 3-Generation study	
Dose r sponse	1		
Quali y of database	1		
DNEL	**Value**		
based on $NOAEL_{rat}$	1.6 mg/m^3 = 4.8 x 1/0.38 x 75/75 x 6.7/10 x 7/5 x1/(2.5 x 3 x 1 x 1 x 1)		

This DNEL of 1.6 mg/m^3 corresponds to an oral intake of 230 µg/kg bw/day (assuming equal absorption rates of 75% for oral and inhalation exposure, 70 kg body weight, respiratory volume 10 m^3 /work day: 1.6 mg/m^3 x 10

m^3/day x 1/70 kg bw).

Long –term – dermal, systemic toxicity

NOAEL of 4.8 mg/kg bw/d for reproductive and developmental effects observed in 3-generation oral study in rats (Wolfe and Layton, 2004).

Description	**Value**	**Remark**	
Step 1) Relevant dose-descriptor	NOAEL: 4.8 mg/kg bw/day	NOAEL from 3-Generation study in rats	
Step 2) Modification of starting point	75/5 7/5	- Correction for 5%bioavailability for dermal exposure in human and 75% fororal absorption in adults rats - Correction for use of NOAEL from feeding study with 7d exposure/week compared to worker's exposure 5 d/week	
Step 3) Assessment factors			
Interspecies	2.5 x 4		
Intraspecies	3	In the EU RA of DEHP anassessment factors of 3 is used for potential intraspecies differences for the worker (see also see REACH Annex I, 0.5 for justification).	
Exposure duration	1	The NOAEL is based on a 2-year study	
Dose response	1		
Quality f dat base	1		
DNEL	**Value**		
based on $NOAEL_{rats}$	3.4 mg/kg bw/day = 4.8 x 75/5 x 7/5 x 1/(10 x 3 x 1 x 1 x 1)		

Table 50 DN(M)ELs for the general population

Route	Type of effect	Hazard conclusion	Most sensitive endpoint
Inhalation	Systemic effects - Long-term	DNEL (Derived No Effect Level): 0.13 mg/m³ (children), 0.17 mg/m³ (adults)	developmental toxicity / teratogenicity (Oral)
Inhalation	Systemic effects - Acute	No hazard identified	
Inhalation	Local effects - Long-term	No hazard identified	
Inhalation	Local effects - Acute	No hazard identified	
Dermal	Systemic effects - Long-term	DNEL (Derived No Effect Level): 0.72 mg/kg bw/day (adults and children)	developmental toxi ity / teratogenici y (Oral)
Dermal	Systemic effects - Acute	No hazard identified	
Dermal	Local effects - Long-term	No hazard identified	
Dermal	Local effects - Acute	No hazard identified	
Oral	Systemic effects - Long-term	DNEL (Derived No Effect Level): 0.036 mg/kg bw/day (children), 0.048 mg/kg bw/day (adults)	developmental toxicity / teratogenicity (Oral)
Oral	Systemic effects - Acute	No hazard identified	
Eyes	Local effects	No hazard identifi d	

Further explanation on hazard conc usions:

- **Inhalation Systemic effect - Long-term:** Relevant dose descriptor: 4.8 mg/kg bw/d (NOAEL from 3-Generation study in rats); Modification of starting point: x 1/1.15 cubic metre/kg bw (conversion into inhalation NAEC by using a 24 hour respiratory volume for the rat), x 75/75 (correction for 75% bioavailability for inhalation in humans and 75% for oral absorption in adult rats) for adults and 75/100 for children; Assessment factors: Interspecies: 2.5 (no allometric scaling factors is used as the oral dose was converted into a NAEC); Intraspecies: 10 (default factor of ECHA REACH Guidance); Exposure duration: 1 (the NOAEL is bas d on a 3-generation study)

- **Inhalation Systemic effects - Acute:** DEHP shows very low acute toxicity (not leading to C&L), no dose-r spons dat available to derive DNEL, no hazard identified.

  Inhalation Local effects - Long-term: According to the available data, no local effects are expected after long-term exposure by inhalation.

  Inhalation Local effects - Acute: According to the available data, no local effects are expected after short-term exposure by inhalation.

- **Dermal Systemic effects - Long-term:** Relevant dose descriptor: 4.8 mg/kg bw/d (NOAEL from 3-Generation study in rats); Modification of starting point: x 75/5 (correction for 5% bioavailability for dermal exposure in humans and 75% for oral absorption in adult rats); Assessment factors: Interspecies: 4 x 2.5 (factor 4 for allometric scaling from rat to humans; factor 2.5 for remaining uncertainties); Intraspecies: 10 (default factor of ECHA REACH guidance); Exposure duration: 1 (the NOAEL is based on a 3-generation study)

- **Dermal Systemic effects - Acute:** DEHP shows very low acute toxicity (not leading to C&L), no dose-response data available to derive DNEL, no hazard identified.

- **Dermal Local effects - Long-term:** According to the available data, no local effects are expected after long-term dermal exposure.

- **Dermal Local effects - Acute:** According to the available data, no local effects are expected after short-term dermal exposure.

- **Oral Systemic effects - Long-term:** Relevant dose descriptor: 4.8 mg/kg bw/d (NOAEL from 3-Generati n study in rats); Modification of starting point: for adults: not necessary same absorption factor for r t and humans: 75%; for children: absorption 100% used: 4.8 x 0.75/1 = 3.6 mg/kg bw/day. Assessment f ctor : Interspecies: 4 x 2.5 (factor 4 for allometric scaling from rat to humans; factor 2.5 for remaining uncertainties); Intraspecies: 10 (default factor of ECHA REACH guidance); Exposure duration: 1 (the NOAEL is based on a 3-generation study)

- **Oral Systemic effects - Acute:** DEHP shows very low acute toxicity (not leading to C&L), no dose-response data available to derive DNEL, no hazard identified.

- **Eyes Local effects:** According to the available data, if at all only minor reversible l cal effects are expected after exposure of the eyes.

Further explanation on DNEL derivation for the general population

Route / Type of effect	DNEL derivation	Assessment factors (AF) for DNEL derivation
Inhalation Systemic effects - Long-term	**DNEL derivation meth d:** ECHA REACH Guidance **Dose descripto starting point:** 4.2 mg/m³	**AF for other interspecies differences:** 2.5 (ECHA Guidance) **AF for intraspecies differences:** 10 (ECHA Guidance) **Overall Assessment Factor:** 25
Dermal Systemic effects - Long-term	**DNEL d rivation method:** ECHA REACH Guidance **Dose escriptor starting point:** NOAEL 72 mg kg bw/day	**AF for interspecies differences (allometric scaling):** 4 (ECHA Guidance) **AF for other interspecies differences:** 2.5 (ECHA Guidance) **AF for intraspecies differences:** 10 (ECHA Guidance) **Overall Assessment Factor:** 100
Oral Systemic effects - L ng-term	**DNEL derivation method:** ECHA REACH Guidance **Dose descriptor starting point:** NOAEL	**AF for interspecies differences (allometric scaling):** 4 (ECHA Guidance) **AF for other interspecies differences:** 2.5 (ECHA Guidance) **AF for intraspecies differences:** 10 (ECHA Guidance) **Overall Assessment Factor:** 100

Justification for route-to-route extrapolation:

- **Inhalation Systemic effects - Long-term:** After oral or inhalative exposure only sytemic but no local effects were observed.

- **Dermal Systemic effects - Long-term:** After oral exposure especially sytemic toxicity was observed.

- **Oral Systemic effects - Long-term:** no route to route extrapolation

Discussion

NOAEL of 4.8 mg/kg bw/d for reproductive and developmental effects observed in 3-generation oral study n rats (Wolfe and Layton, 2004).

Description	**Value**	**Remark**	
Step 1) Relevant dose-descriptor	NOAEL: 4.8 mg/kg bw/day	NOAEL from 3-Generation study in rats	
Step 2) Modification of starting point	1.15 m^3/kg bw75/75 (adults) 75/100 (children)	- Conversion into inhalation NAEC (in mg/m^3) by using a 24-hour respiratory volume for the rat. - no difference in inhalative bioavailability assumed: 75% b oavailability for inh lative exposure in dult humans and 75% for oral absorption in adult rats - correction factor 75/100 for children	
Step 3) Assessment factors			
Interspecies	2.5	Only a factor 2.5 is used, and no allometric scaling factors is used	
Intraspecies	10		
Exp su e duration	1	The NOAEL is based on a 3-Generation study	
Dose response	1		
Quality of database	1		
DNEL	**Value**		
based on $NOAEL_{rats}$	0.17 mg/m^3(0.04 ppm) for adults = 4.8 x 1/1.15 x 75/75 x 1/(2.5 x 10 x 1 x 1 x 1)		

Description	Value	Remark	
	0.13 mg/m^3(0.04 ppm) for children = 4.8 x 1/1.15 x 75/100 x 1/(2.5 x 10 x 1 x 1 x 1)		

Long –term – oral, systemic toxicity

NOAEL of 4.8 mg/kg bw/d for reproductive and developmental effects observed in 3-generation oral study in rats (Wolfe and Layton, 2004).

Description	Value	Remark	
Step 1) Relevant dose-descriptor	NOAEL: 4.8 mg/kg bw/day	NOAEL from 3 - Generation study in rats	
Step 2) Modification of starting point	75/75 (adults) 75/100 (children)	- no difference in oral bioavailability assumed: 75% bioavailability for oral exposure in adult humans and 75% for oral absorption in adult rats - correction factor 75/100 f r children	
Step 3) Assessment factors			
Interspecies	2.5 x 4		
Intraspecies	10		
Exposure duration	1	The NOAEL is based on a 3 -Generation study	
Dose response	1		
Quality of databas			
DNEL	**Value**		
bas d on $NOAEL_{rats}$	0.048 mg/kg bw/dfor adults = 4.8 x 75/75 x 1/(10 x 10 x 1 x 1 x 1) 0.036 mg/kg bw/dfor children = 4.8 x 75/100 x 1/(10 x 10 x 1 x 1 x 1)		

Long –term – dermal, systemic toxicity

NOAEL of 4.8 mg/kg bw/d for reproductive and developmental effects observed in 3-generation oral study in rats (Wolfe and Layton, 2004).

Description	**Value**	**Remark**	
Step 1) Relevant dose-descriptor	NOAEL: 4.8 mg/kg bw/day	NOAEL from 3-Generation study in rats	
Step 2) Modification of starting point	75/5	Correction for 5%bioavailability for dermal exposure in humans and 75% for oral absorption in adult rats	
Step 3) Assessment factors			
Interspecies	2.5 x 4		
Intraspecies	10		
Exposure duration	1	The NOAEL is based on 3-Generation study	
Dose response	1		
Quality of database	1		
DNEL	**Value**		
based on $NOAEL_{rats}$	0 2 mg/kg bw/d = 4 8 x 75/ x 1/(10 x 10 x 1 x 1 x 1)		

6. HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1. Explosivity

Data waiving: see CSR section 1.3 Physico-chemical properties.

Classification according to GHS

Name: bis(2-ethylhexyl) phthalate
Reason for no classification: conclusive but not sufficient for classification

Classification according to DSD / DPD

6.2. Flammability

Data waiving: see CSR section 1.3 Physico-chemical properties.

Classification according to GHS

Name: bis(2-ethylhexyl) phthalate
Reason for no classification (Flammable gases): conclusive but not sufficient for classification
Reason for no classification (Flammable aerosols): c nclus ve but not sufficient for classification
Reason for no classification (Flammable liquids): conclu ive but not sufficient for classification
Reason for no classification (Flammable solids): onclusive but not sufficient for classification

6.3. Oxidising potential

Data waiving: see CSR section 1 3 Physico-chemical properties.

Classification acc rding to GHS

Name: bis(2 ethylhexyl) phthalate
Reason for no classification (Oxidising gases): conclusive but not sufficient for classification
Rea on fo no c assification (Oxidising liquids): conclusive but not sufficient for classification
Reas n for no classification (Oxidising solids): conclusive but not sufficient for classification

7. ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this application for authorisation.

8. PBT AND VPVB ASSESSMENT

8.1. PBT assessment: overall result

The substance is not PBT / vPvB

Justification

While DEHP fulfills criteria for ready biodegradability (including the 10 -day window requirement), results of simulation tests in surface water, sediment and soil are highly variable. Concluding from that, conservative half-lives are derived justifying an overall classification as P. From a BCF determined in fish of 614 and data relia ly demonstrating trophic dilution in food webs, bioaccumulation potential is regarded to be low. While no cotoxic effects were determined in relevant aquatic studies on acute or chronic toxicity, DEHP must be regard d a T from its human health hazard classification for reproductive toxicity: Repr. 1B. Thus, DEHP is regarded as

- P (**not** vP)
- Not B
- T.

In conclusion, DEHP does neither fulfil PBT nor vPvB characteristics.

Likely routes of exposure

DEHP is concluded to be not PBT or vPvB. Therefore no emission characterisation is required.

8.2. Results of detailed PBT / vPvB assessment

8.2.1. Persistence

8.2.1.1. Evidence of non-P / non-vP properti :

8.2.1.1.1. Screening criteria

Not P and not vP based on: readily biod gradabl
In a reliable test on ready biodegradability (Diefenbach, 1994; according to OECD Guideline No. 301 B), DEHP proved to be “readily biodegradable” (82% after 28 days) and fulfilled the “10-days window” criterion.

8.2.1.1.2. Criteria based on Annex XIII of REACH

P but not vP based on T½ ≤ 0 days in marine, fresh- or estuarine water
Considering the high data variability in simulation tests on degradation of DEHP in surface waters it seems to be preferable as a realistic worst case approach to base the half-live of DEHP in surface waters on results of screening tes on biodegradability according to Table R16 -7. If so, degradation rate based on Diefenbach (1994) would set a ha f life of 15 days in surface water. However, considering the variability of the degradations observed in test simulating natural conditions, we assume that a more conservative value is preferable. In conc usion, a half-life for DEHP in surface waters of 50 days (at 12 °C) for readily biodegradable substances failing t e 10 day window is applied for the CSA. This is a conservative approach taking due account of data po nting to lower degradation rates at lower temperatures and taking account of mineralization half-lives rather than p imary biodegradation. The half-life is in line with the one derived by EU-RAR (ECB, 2008).

8.2.1.1.2. Evidence of P or vP properties

From the *simulation studies on sediment* available (including marine sediment), a relevant half-life for DEHP in bulk-sediment (total sediment) of
DT50 = 300 days at 12 °C
was derived.
For soil, while recent studies prove that DEHP is not persistent in soil even under partially anaerobic conditions, still the very conservative approach taken in the earlier EU-RAR (ECB, 2008) is followed: In this work based on the publications avialable at that time a

DT50 (agricultural soil) of 300 d at 12°C
was derived. This conservative approach takes due account of the high variability observed with simulation test data for biodegradation of DEHP in soil.

8.2.1.3. Conclusion on P / vP properties

P not vP :

While half-lives derived for sediment and soil of 300 days would result in a classification of DEHP as very persistent (vP) according to REACH Annex XIII, the value for soil may be regarded as very conservative, and, based on screening data (DEHP was demonstrated to be readily biodegradable fulfilling the 10-day window requirement), DEHP would clearly be regarded as not persistent (not P). Taking into account all degradation data available, DEHP is regarded as persistent, but not very persistent.

8.1.2. Bioaccumulation

8.1.2.1. Evidence of non-B / non-vB properties

Criteria based on Annex XIII of REACH

Not B and not vB based on: BCF ≤ 2,000 L/kg

While high BCF-values were determined for mussel (Mytilus edulis, 2500) and amphibod (Gammarus, 2700), these are deemed not to be relevant: Firstly, actual BCF values are expected to be considerably lower, as these values were based on total radioactivity likely leading to an overestimation of bioconcentration as discussed in EU-RAR (ECB, 2008). In addition, the RAR also states that the elimination half-life for Gammarus was very short (less than 4 days). Secondly, trophic dilution is clea ly evident from the studies summarized in IUCLID section 5.3.1 and this is in line with conclusions drawn within EU-RAR on DEHP. Thus, for CSA and PBT assessment it is scientifically well justified to use th BCF derived from the reliable fish study performed by Mehrle and Mayer (1976).
Value used for CSA and PBT-assessment: BC DEHP) 614 dimensionless (L/kg ww or dimensionless).
The BCF of 614 is clearly below the cut-off criterion as specified in REACH Annex XIII for bioaccumulative substances.

8.1.2.2. Conclusion on B / vB properties
DEHP is not B/vB: The BCF of 614 determi ed in a reliable study on fish bioconcentration is clearly below the cut-off criterion of 2000 as specified in REACH Annex XIII for bioaccumulative substances

8.2.3. Toxicity

8.2.3.1. Evidence of non-T properties

Criteria bas d on Annex XIII of REACH

EC10 or NOE ≥ 0.01 mg/L for marine / freshwater organisms (long-term toxicity)
Long-term effec studies, where test organisms are exposed to DEHP via water, are available for fish, aquatic inver ebrates, algae and microorganism. However, there are no reliable long-term studies indicating effects on organisms exposed to DEHP in water below the apparent water solubility of DEHP. Therefore, it is not c nsidered suitable to specify a chronic NOEC or EC10 for organisms exposed via water phase. Based on ecotoxicity data for DEHP, the T-criterion is not fulfilled.

8.2.3.2. Evidence of T properties
The substance is classified as toxic for reproduction, Repr. 1B (Hazard statement: H360: May damage fertility or the unborn child). Due to this classification DEHP fulfils one of the criteria for T according to REACH Annex XIII.

8.2.3.3. Conclusion on T properties
DEHP is concluded to fulfil criteria for T:
While no long-term ecotoxic effects of DEHP were observed for the aquatic environment if tested below the apparent water solubility for DEHP, it must be regarded as T from its human health hazard classification for

reproductive toxicity: Repr. 1B (Hazard statement: H360: May damage fertility or the unborn child). Due to this classification DEHP fulfils one of the criteria for T according to REACH Annex XIII.

9. EXPOSURE ASSESSMENT

9.0. Introduction

9.0.1. Overview of uses and exposure scenarios and scope of assessment

As already explained above, this CSR is largely built by using relevant parts of the authorisation chemical safety report prepared by the authorisation task force for DEHP (ATF DEHP). The applicant aquired access for the use of relevant parts of the ATF's CSR.

Recycled soft PVC containing DEHP is mainly gained from post-consumer waste by separating PVC fr m other parts, followed by shredding or micronizing the soft PVC. Another manufacturing process includes iss luti n of PVC waste and purification by precipitation.
These solid recyclates, consisting of PVC granulate and containing typically approx [redacted] DEHP (with maximum concentrations up to 20%), are then used for PVC processing and production f articles by PVC converter companies.

The applicants put on the market together approx. [redacted] of soft P C r cycla e containing DEHP. With an average DEHP content of [redacted], this represents approx [redacted] of DEHP. The modelling under the socio-economic assessment predicts higher quantities of DEHP in soft PVC recyclate at below [redacted] for the same amount of recyclate; the difference in these figures stems from the SEA taking an average figure across the EU and taking variations in the lifetime of different articles into account when predicting future concentrations in post-consumer wastes. Quantities of recyclate placed on the market might grow as discussed in the socio-economic assessment [redacted], giving an absolute maximum amount of DEHP contained in soft PVC recyclates at betw en [redacted]
Virgin DEHP contained in articles was estimated to amount to [redacted] in 2012 (EU production [redacted] ICIS data) and import 35 kT in 2010 RAC/SEAC ba kground report (RAC/SEAC, 2012). Furthermore, today DEHP within other soft PVC recyclate is es imat d to a further [redacted] by RPA in the socio-economic assessment.

Use of pure DEHP is not relevant here and not covered by this application. Where recycled soft PVC is used together with the neat substance for processing f soft PVC, users will have to buy from and follow instructions provided by a manufacturer of DEHP that h s obtained the authorisation and within the limits of that authorization (e.g. operating condi ions, risk management measures, end applications covered). If a specific use or application is not covered by the virgin DEHP manufacturer's authorisation, then the converter should not add new virgin DEHP to its fo mulation The user of neat DEHP together with recyclate containing DEHP will have to apply the most stringent risk management measures prescribed for its raw materials. This therefore means, in addition, that he should apply the specific risk management measures aimed at limiting exposure due to the emptying of recyclate bags (PROC 8a and 8b).

The table below lists he use , for which authorisation is requested.

Table 51 Bri f description of the use processes for all identified uses

Id ntified use	Brief description of use process
#1: F mulation of recycled soft PVC containing DEHP in compounds and dry-blends	Formulation of recycled soft PVC containing DEHP in compounds and dry-blends
#2: Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles	Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles

This application seeks authorisation for two uses within a single supply chain: formulation of recycled soft PVC containing DEHP into solid soft PVC mixtures and use of recycled soft PVC containing DEHP (as it is or in these formulations) for production of PVC articles.

The exposure scenarios (with contributing scenarios) established for these uses and for the manufacturing process are listed in the following table.

Use applied for	Use title	ES no.	ES name	Manufacture	Identified uses				Resulting life cycle stage		Sector of Use (SU)	Product Category (PC)	Process Category (PROC)	Article category (AC)	Environmental release category (ERC)
					Formulation	Industrial End use	Professional End use	Consumer end use	Service lif (for articles)	Waste stage					
#1	Formulation of recycled soft PVC containing DEHP in compounds and dry-blends	ES1	Formulation of recycled soft PVC containing DEHP in compounds and dry-blends		x						10, 12	32	1, 2, 3, 4, 5, 8a, 8b, 14, 15		n.a.
#2	Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles	ES2	Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles			x					12	32	2, 3, 4, 6, 8b, 14, 21	13	n.a.
		ES2-SL-P	*Contributing Scenario: Service life professionals: Profes ional handli g of PVC products made from recycled soft PVC containing DEHP Installation of building materials and similar activities) / inhalati n exposure from volatile DEHP / professional PVC footwear)*						*x I/P* *				*21*	*13*	n.a.
		ES2-SL-C	*Cont ibuting Scenario: Service life consum rs: Exposure from consumer articl s made from recycled soft PVC containing DEHP*						*x C*					*13*	n.a.

* This service life scenario was devel ped for professional workers, but equally applies to industrial workers because exposure is not expected to differ between professional and industrial workers for his scenario. n.a.: not applicable

Uses exempted from authorisation:

According to REACH Art. 60, 2 human health risks arising from use of medical devices, which are regulated by Directives 90/385/EEC, 93/42/EEC or 98/79/EC should not be considered when applying for authorisation under REACH.

According to COMMISSION REGULATION (EU) No 143/2011 of 17 February 2011 uses of DEHP in the immediate packaging of medicinal products covered under Regulation (EC) No 726/2004, Directive 2001/82/EC, and/or Directive 2001/83/EC are exempted from authorisation.

The use of DEHP in food contact materials is regulated in COMMISSION DIRECTIVE 2007/19/EC of 30 March 2007.

Uses advised against:

Use of DEHP is prohibited in toys and childcare articles by DIRECTIVE 2005/84/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 14 December 2005, replaced by REACH Annex XVII,, Commission Regulation (EC) 552/2009.

Use of DEHP in cosmetic products is not permitted in the European Union, as regulated in COMMISSION DIRECTIVE 2004/93/EC of 21 September 2004, replaced by REGULATION (EC) 1223/2009 of the European Parliament and of the Council of 30 November 2009.

Use in food contact materials is limited by Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Allowed uses and limit may be found in Annex I, table 1 of this Regulation.

Products included in this Application for Authorisa on:

The only industrial end use included in this application for a thorisation is use of recycled soft PVC containing DEHP for production of PVC articles. The ran of artic es produced from recycled soft PVC is limited and consists mainly of products made for outdoor use such as water-proofing and geo-membranes, profiles and gaskets, vehicle splash guards, traffic cones and traffic sign components, ropes and garden hoses and roofing. Products used in the professional envir nment include industrial flooring, stall mats, noise insulation and other membranes, tarpaulin parts as well as te hnical profiles and gaskets.
Consumer articles made from r cycled soft PVC are mainly (gym, door and car) mats, back layers of flooring, footwear (sandals, clogs and w llington oots, outer soles of shoes), luggage, outdoor seats, and handles.

A description of the articles made from recycled soft PVC and the expected exposure from use of these articles is given in 9.3.2.4. On all accounts, this application does not include the uses of DEHP to produce the following PVC articles:

- erasers
- adu t ys (sex toys and other articles for adults with intensive contact with mucous membranes)
- smal (<1 c) PVC items available in the home environment (without attachment to larger objects), which an be swallowed by small children
- textiles clothing with direct contact to large skin surfaces (worn on bare skin).

S ope of the Assessment

DEHP is classified for reproductive toxicity (Repr. 1B, H360), but is not classified for any other physical, human health or environmental hazard. It is of low acute toxicity and does not exert local effects (irritation, sensitisation).
Therefore, as required by REACH the focus of the assessment is on human health hazards after long-term exposure. An environmental exposure assessment is not performed.

Table 52 Assessment of human health of workers, consumers and the general population via the environment

DNEL for systemic effects via	workers	consumers	men via the environment
inhalation	x	x	
dermal exposure	x	x	
oral exposure		x	x

9.0.2. Assessment of the exposure to the environment

Not relevant for this application for authorisation.

9.0.3. Assessment of exposure of humans via the environment

Exposure via the environment is best reflected in the body burdens of the general population. Main exposure pathway for DEHP is considered to be food, but it is unknown, what contribution comes from DEHP entering the food chain via environmental media and how much is contributed by other pathways, such as food contact materials (see below, 9.0.5.3).
Extensive biomonitoring data are available for DEHP metabolites in urine of the general population. These data are shown and explained in detail below (consumer exposure assessment, 9.0.5.2).
Intake levels calculated from these biomonitoring results are integrating over all possible sources of DEHP for the general public, including exposure via the environment.

It is reasonable to assume that only a small part of the ov r ll exposure to DEHP comes from recyclate. The total exposure assessed by using biomonitoring data is considered a vast overestimation of exposure from recycled soft PVC.

9.0.4. Assessment of exposure to workers

9.0.4.1. Substance specific input data – toxicological basis

DEHP does not have any significan l cal effects on the skin, eyes or respiratory tract and is not a sensitizer. It is therefore not possible to quantify local DNEL values and neither quantitative nor qualitative risk characterisation is required for DEHP for loca effects. Also, DEHP is of low acute toxicity and is not classified for these endpoints.
In agreemen with the opi ion of the Risk Assessment Committee (RAC/SEAC, 2012) reproductive toxicity is the most critical endpoint for evaluating human health hazards. Other effects observed in long-term experimental studie (n phro athy, liver effects) consistently occur at higher doses and thus sufficient protection is provided by ompli nce with the derived DNELs also for these endpoints. Therefore, this assessment focuses on reprodu tive toxic effects of DEHP.

The f llowing systemic, long-term DNELs were derived for DEHP.

Table 53 Long-term DNELs derived to assess human health risks for workers and the general population

Route of exposure	Workers	General population (adults/children)
Inhalation (mg/m^3)	1.6 (corresponding to an external oral exposure of 230 µg/kg bw/day)*	0.17/0.13
Dermal (mg/kg bw/d)	3.4	0.72
Oral (mg/kg bw/d)	Not relevant	0.048/0.036

* This value was derived to compare with biomonitoring data: it represents the oral dose which would lead to the same internal exposure as the inhalative DNEL for workers, using the same basis and assessment factors (assuming equal absorption rates for oral and inhalation exposure, 10 m^3 respiratory volume during a work shift for adult workers and 70 kg body weight - see CSR section 5.11).

All DNELs are based on the multi-generation rat reproductive toxicity study of Wolfe and Layton (2004), where reproductive toxicity was observed in progeny after perinatal exposure. Thus, perinatal exposure is considered to be the most critical time window with respect to the reproductive toxic effects of DEHP and pregnant women are the target population group. Reproductive toxicity after exposure of adult animals occurred at higher doses. Therefore, the DNELs presented above are considered to be conservative and are probably overestimating effects in all other population groups (adult males, children).

A factor of 3 is used for inter-individual differences for workers, (instead of 5, as proposed in the guidance) The REACH legal text requires dossier submitters to consider existing evaluations and to justify any chang from such an evaluation: "Where available and appropriate, an assessment carried out under Communi y legi lation (e.g. risk assessments completed under Regulation (EEC) No 793/93) shall be taken into ac ount in he development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified." (REGULATION (EC) No 1907/2006, Annex I, 0.5). As there is no new dat which j stifies a change in this assessment factor, the REACH Regulation specifies that this is the assessment fac or to be used (in line with the EU Risk Assessment Report (ECB, 2008).

It has been noted that the Committee for Risk Assessment (RAC) very recently propos d slightly different DNELs (ECHA, 2013) for workers. A discussion of the differences and their implications are included in Annex #5# of this report.

According to EU occupational safety legislation, pregnant women are excluded from workplaces with exposure to hazardous substances classified CMR 1. Therefore, use of the worker DNEL based on pregnant women, presented above for assessing workplace exposures is for precautionary reasons only, for situations which should not occur according to EU occupational safety legislati n (EU Directive 92/85/EEC, Art.6). The conservatism of the DNEL becomes also evident when compared with nati nal occupational exposure limits (OEL). OELs in various countries around the world are mostly between 3 and 10 mg/m^3, with short-term (15 min STEL) values between 5 and 80 mg/m^3 (see GESTIS databas). A OEL is set in Germany at 10 mg/m^3 (15 min STEL: 80 mg/m^3) and in France at 5 mg/m^3 (Protois et al., 2007).
The DNELs based on the multi-generation rat reproductive toxicity study of Wolfe and Layton (2004) are also considered conservative as there is con iderable uncertainty about the usefulness of the rat model for investigating reproductive toxicity of phthalates n humans (see also the RAC discussion on lowering the interspecies assessment factor (RAC/SEAC, 2012)) . So far, no convincing evidence has emerged for effects in primate studies similar to the s -called T sticular Dysgenesis Syndrome in rats. A subchronic study with marmosets did not show adverse effects on male reproductive organs (Kurata et al., 1998) and in a recent chronic study with marmosets where exposure was started at an juvenile age, exposure up to 2500 mg/kg bw/day remained without ef ects on male gonads (Tomonari et al., 2006). Furthermore, in a study by McKinnell (2009) perinatal exposure was used t investigate the effect of phthalates on testicular development and function in the marmoset. Monobutyl phthalate (MBP), which is an effective reproductive toxicant in rats, did not affect testis development/fun tion or cause testicular dysgenesis, and no effects emerged by adulthood when applied in a dose of 500 mg/kg bw/day. Some effects on germ cell development were found, but these were inconsistent and of un erta n sig ificance.

Recen tudies where human and rodent xenografts of fetal testes were transplanted into rodent hosts which then were exposed to phthalates (dibutyl phthalate), further support the view that important species differences exist in ster idogenesis and that the rat might be not the most appropriate model for reprotoxic effects of phthalates in humans (Heger et al., 2012; Johnson et al., 2012; Mitchell et al., 2012).

DEHP is well absorbed after oral and inhalation exposure. For rats Laignelet and Lhuguenot (2000 a,b) observed a higher oral absorption under a repeated dose regimen (up to 74% were excreted as metabolites in urine, see section 5.1) compared to the absorption rates observed in studies with single oral application of approx. 50%. Approx. 50% absorption after a single oral dose was also observed in the human toxicokinetic study of Anderson et al. (2011) with 20 volunteers, indicating similar oral absorption in rats and humans.
Substantially less is absorbed percutaneously (see section 5.1 for details).

[1] http://www.dguv.de/ifa/de/gestis/limit_values/index.jsp, data retrieval December 2012

The following absorption rates are used for risk assessment:

Table 54 Absorption rates for DEHP used for risk assessment

	oral	dermal	inhalation
humans (adults/children)	75%/100%	5%	75%/100%
rats	75%	5%	75%

These absorption rates are in agreement with the assumptions used by RAC (RAC/SEAC, 2012). Although no convincing information is available that children have a higher absorption rate compared to adults, in this assessment, in agreement RAC/SEAC (2012) 100% was assumed as a further conservative st p in the assessment.
The only study available (Sjöberg et al., 1985), which investigated possible age-related differences of DEHP absorption by directly comparing absorption in various age groups, reports an increased absorpti n in younger rats (44 and 26% in 25- and 60-day old rats, respectively). But the absorption rate in juvenile animals was similar to that found in other studies and the absorption rate found in adult animals (26%) was un s lly low and in deviation to the results of several other toxicokinetic studies. The observed differenc between age groups thus might be caused by deficiencies of this study. No convincing data are available to conclude on an even higher oral absorption rate in young animals and/or children.

In the EU Risk Assessment Report, for exposure assessment absorption of 100% for children was assumed. But no adjustment for absorption was considered necessary for the NOAEL derived from the study of Wolfe and Layton (2004) for any of the target populations. So, the approach used here is even more conservative than that chosen in the EU Risk Assessment Report.

In agreement with the EU Risk Assessment Report, n adju tment for differences in absorption between inhalation exposure and the oral absorption of experimental animals in the Wolfe and Layton study (2004) is considered necessary here. Again, according to REACH (EGULATION (EC) No 1907/2006, Annex I, 0.5) any deviation from the EU Risk Assessment Repo t has to be justified.

It has been noted that the Committee for Risk As essment (RAC) very recently proposed slightly different absorption rates for humans (ECHA, 2013) A discussion of the differences and their consequences are included in Annex #5# of this report.

It can be concluded that the approach t human health risk characterisation used in this CSR is conservative because

- a DNEL is used which is derived from a study with perinatal exposure and is used for all populations (workers, g neral pu lic) and all age groups, including children,
- the DNEL comes from an animal model (rat), whose relevance for humans is unclear and possibly over y con rvative, as data from primate studies suggest lower risks.
- very conservative approach to oral absorption of DEHP by children was used.

9 0.4.2 General approach for using biomonitoring data

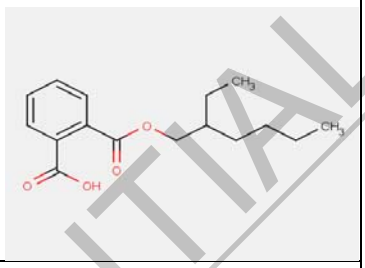
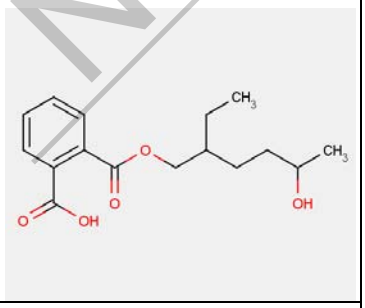
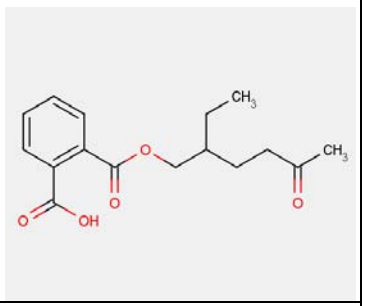
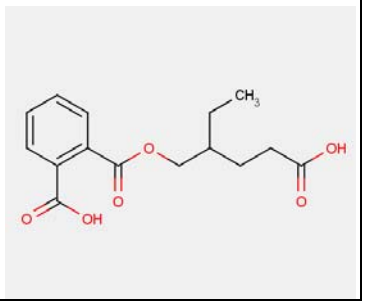
Biomarkers of DEHP exposure

In this assessment biomonitoring data are the key data for assessing exposure. Recalculation from biomarker concentrations (i.e. DEHP metabolites in urine) allow for recalculating the corresponding intake of DEHP, which then can be compared to the DNELs derived for DEHP. Biomonitoring measurements are favoured compared to modelling approaches as they provide real exposure data for the target populations, by integration over all exposure pathways and sources.

For DEHP a wealth of information on exposure is available for both the general population and for occupationally exposed persons. Results from these studies allow for a realistic and complete assessment of exposure, as it provides individual exposure measurements, which integrate contributions from all pathways and

sources (Calafat and McKee, 2006; Koch and Calafat, 2009; Koch et al., 2003; Wittassek et al., 2011). Established biomonitoring methods determine the concentration of DEHP metabolites in urine (Koch et al., 2003). Some or all of the metabolites in the following table are investigated in recent biomonitoring studies.

Table 55 Main urinary metabolites of DEHP determined in biomonitoring studies

Abbreviation	Substance name	Other abbreviations	Structure
MEHP	Mono(2-ethylhexyl)phthalate		
5OH-MEHP	Mono(5-hydroxy-2-ethylhexyl)phthalate	MEHHP	
5oxo-MEHP	Mono(5-oxo-2-ethylhexyl)phthalate	MEOHP	
5cx-MEPP	M no(5-car oxy-2-ethylpentyl)phthalate	MECPP	

Most recent studies measure MEHP and the oxidised metabolites 5OH-MEHP and 5oxo-MEHP (e.g. the DEMOCOPHES project, see below). Other studies in addition include 2cx-MEPP (mono(2-carboxy-2-ethylpentyl)phthalate) and/or 5cx-MEPP.
Most studies measure metabolite concentrations in spot samples (1st morning urine sample), whereas it is generally agreed that 24 h-samples are more reliable for substances with short half-lives, as urinary concentrations depend on the time since the last meal and show substantial variability (Kessler et al., 2012). Concentrations were either expressed on a volume basis (amount per L urine) or as creatinine corrected values

(amount per g creatinine). In the tables in Annexes #2# and #3# showing recent results of biomonitoring studies both types of results are given.

Calculation of DEHP intake

Only for some biomonitoring studies, authors calculated the corresponding DEHP intakes. In order to be able to make use of all available biomonitoring results, in this assessment the intake of DEHP is calculated from the metabolite concentrations found in urine.

Volume-based as well as creatinine-based concentrations are measured and can be used for calculating DEHP intake (see below). When only data on biomarkers in spot samples are available, a correction for 24h urine has to be made, which can be made either based on total daily creatinine excretion or total daily urine volume. Both creatinine excretion and total urine volumes are highly dependent on age, body mass index and gender. Empirical factors are applied to account for these influences (Wittassek et al., 2007a). Intake estimates for children obtained with both methods deviated by approximately a factor of two, the volume method resulting in the higher values (Wittassek et al., 2007a).

Differences between results obtained reflect the methodological uncertainty in converting biomarker concentrations into daily intakes. Wittassek et al. (2007a) considered both methods as equally applicable, none being superior to the other. However, in a later publication Wittassek et al. (2011) state that *"Owing to the rapidly increasing creatinine excretion in developing children, body height and gender-based reference values for daily urinary creatinine excretion were used in the creatinine calculation mode. Corresponding detailed data for the daily urine volume excretion in children were not available. This might be one reason why the values were on average about two times higher with the volume-based model compared with the creatinine-based model",* thus giving preference to the creatinine method.

In order to calculate DEHP intake from urinary concentrations of biomarkers, the general procedure for calculating external exposure from biomonitoring according to Aylward et al. and Wittassek et al. (2007a) is used (see below). Despite the critique expressed above, for children the more conservative volume based calculation method is used, adding to the conservatism of the approach. Therefore, intake values calculated for the general public (children and adults) are derived from volume-based measurements.

For workers, as the creatinine correction approach is the generally accepted method for adults, and some occupational studies report creatinine-based values only creatinine-based values are used for calculating intake levels. Lorber et al. (2011) caution against using creatinine adjusted values for substances like DEHP, as an underestimation might occur in situations where the time of intake is more than 24 h from the time of sampling. But this error is considered to be negligible, when end shift values are measured.

The following formula and input data are used for the calculation:

Table 56 Calculation of DEHP intake from volume based urinary biomarkers according to Aylward et al. (2009)

Volume-based calculation:	$D = (Cu \times UV \times MW_{DEHP})/(MW_{metab} \times BW \times UEF)$		
with			
			value (source)
Daily dose	D	µg/kg bw/ day	(calculated from formula)
Urinary concentration of metabolites	Cu	µg/L	(measured)
Urinary volume (24 h) adults (average men/women)	UV	L/d	1.65 (Aylward et al., 2009)
Urinary volume (24 h) children (6 – 11 a)	UV	L/d	0.66 (Aylward et al., 2009)
Molecular weight of metabolite(s) (average)	MWmetab	g/mol	300 (Aylward et al., 2009)
Molecular weight DEHP	MWDEHP	g/mol	391 (Aylward et al., 2009)
Body weight adults (average men/women)	BW	kg	62.5 (Aylward et al., 2009)
Body weight children (6 – 11 a)	BW	kg	32 (Aylward et al., 2009)
Urinary excretion fraction (mol based)	UEF	(dimensionless)	see below (Anderson et al., 2011)

Table 57 Calculation of DEHP intake from creatinine-based urinary biomarkers according to Aylward et al. (2009)

Volume-based calculation:	$D = (Cu \times CE \times MW_{DEHP})/(MW_{metab} \times BW \times UEF)$		
with			
			value (source)
Daily dose	D	µg/kg bw/ day	(calculated from formula)
Urinary concentration of metabolites	Cu	µg/L	(measured)
Creatinine excretion (24 h) adults (average men/women)	CE	g/d	1.35 (Aylward et al., 2009)
Creatinine excretion (24 h) children (6 – 11 a)	CE	L/d	0.5 (Aylward et al., 2009)
Molecular weight of metabolite(s) (average)	MWmetab	g/mol	300 (Aylward et al., 2009)
Molecular weight DEHP	MWDEHP	g/mol	391 (Aylward et al., 2009)
Body weight adults (average men/women)	BW	kg	62.5 (Aylward et al., 2009)
Body weight children (6 – 11 a)	BW	kg	32 (Aylward et al., 2009)
Urinary excretion fraction (mol based)	UEF	(dimensionless)	see below (Anderson et al., 2011)

UEF is the mol fraction of a metabolite (or the sum of the measured metabolites) of the DEHP dose, which is excreted in the urine. For UEF in most publications the value obtained from the investigation of Koch et al. (2005) was used. A value (overall molar fraction of four metabolites (MEHP, 5OH-MEHP, 5oxo-MEHP, and 5cx-MEPP) excreted within 24 h, median level of three dose levels) of 0.628 was obtained. This value is based on a metabolism study with one individual.

More recently, Anderson et al. (2011) published a toxicokinetic study, which provides a more reliable value for UEF.

Samples were obtained from an open-label, fixed sequence, single oral dose study in 10 male and 10 female subjects. The dosed substance was deuterated di-2-ethylhexylphthalate (D4-DEHP) at two dose levels of 0.31 and 0.78 mg of D4-DEHP. Urine samples were collected at intervals up to 48 h post-dose.

On average (arithmetic mean for two doses) total excretion of all 4 metabolites (MEHP, 5oxo-MEHP, 5OH-MEHP and 5cx-MEPP) was 45.3% of DEHP dose within 24 h and 47.1% within 48 h. The following table gives the excretion values for individual metabolites over 48 h as measured for the lower dose (which is closer to environmental exposure levels) and the UEF used for dose calculations.

Table 58 Urinary excretion of DEHP metabolites in human volunteers (mol based) (Anderson et al., 2011) and derived urinary excretion fractions (UEFs)

Metabolite excretion	
MEHP	6.94%
5OH-MEHP	16.33%
5oxo-MEHP	12.53%
5cx-MEPP	15.9%
UEFs (mol based)	
2 Metabolites: MEHP + 5cx-MEPP	0.228
2 Metabolites: 5OH-MEHP + 5oxo-MEHP	0 289
3 Metabolites: MEHP + 5OH-MEHP + 5oxo-MEHP	0.358
4 Metabolites	0.517

The UEF values derived from the Anderson et al. (2011) study are more conservative (i e. leading to a higher calculated intake level) than that based on the Koch et al. (2005) study (UEF 0.628 for 4 metabolites) Furthermore, these UEF are in agreement with another, recently published toxicokineti stu y (Kessler et al., 2012). In this study with 4 human volunteers, 31% of a single orally dose of DEHP was excreted within 46 h as the metabolites MEHP, 5OH-MEHP and 5oxo-MEHP. This value corresponds well with the 35.8% (i.e. UEF 0.358) found by Anderson et al. (2011).

The following table exemplifies the conservatism of the approach by comparing our intake calculations with that of Frederiksen et al. (2011) for their own dataset.

Table 59 Comparison of the intake calculations by tudy authors Frederiksen et al. (2011) (biomonitoring study on 129 Danish children, see also Annex #3#)

	Sum of 4 DEHP metabolites as reported by study autho s [µg/L urine]	Daily EHP intake as stimated by study authors [µg/kg bw/day]	DEHP intake as estimated by method as described above [µg/kg bw/day]
50th percentile	107.0	4.0	5.6
95th percentile	352 0	10.7	18.3

This approach, as proposed by Aylward et al. (2009), with the UEF derived from Anderson et al. (2011) (called the Aylward/Anderson method in the following) is used for calculating intake for biomarker concentrations in the general public and for worker populations. The calculated intake values are then compared with the respective DNELs of

- 1 6 mg/m , respectively the corresponding external intake via the inhalation route of 0.23 mg/kg bw/day for workers (as the same absorption rate of 75% is assumed for inhalation and oral exposure of dults, the calculated intake can directly be compared with the DNEL analogue intake level of 0.23 mg/kg bw/day) and
- 0.036 and 0.048 mg/kg bw/day for oral exposure of the general population (children and adults, respectively).

DEHP metabolites in blood have short elimination half-lives in the range of few hours. Variability with time in the general public within one day and between days is substantial, as has been shown by Preau et al. (2010). In this study spot and 24h urine samples were gathered from 8 volunteers over the course of one week and 5OH-MEHP was analysed for its within-day, between-day and between-person variability, in dependence of sample type (spot, morning urine, 24h urine). For MEHP both within-day and between days variability was higher than variability between persons. Variability could be reduced to some extent by creatinine adjustment. The authors proposed multiple spot sample measurements over a period of days to assess individual exposure.
Christensen et al. (2012) investigated the variability of DEHP metabolite concentrations in spot sample measurements versus 24h sample measurements. In one (although small, n=8) study population, where both types of samples have been taken, variability (and high end percentile values) were substantially lower in the 24h sample measurements. The authors caution against using high end percentiles of spot sample measurements to

characterise high end long-term intakes in the population.
It can be concluded that high percentile values from a biomonitoring study using spot samples do not reflect the average exposure of individuals with high exposure, but rather constitute singular high exposure levels. In other words: it is unlikely that the person who showed a high value corresponding to the 95 percentile value in one measurement campaign will show the same high level every day during a prolonged time period. Even with 24h urine samples the high day-to-day variability observed by Preau et al. (2010) indicates that high percentile values within one measurement campaign overestimate the exposure of the highly exposed individuals.
Therefore, for the general population, the medians of biomarkers as reported in biomonitoring studies are considered a best estimate of long-term exposure. In addition, 95 percentile values are reported to characterise the high end exposure situation, bearing in mind that these values most probably overestimate the exposure of the 95 percentile of the population.

In occupational studies, in general average values (median, arithmetic or geometric means) are reported only, as they are considered to adequately characterise long-term average exposure. Values used in this assessment generally are "end-of-shift" concentrations, integrating occupational exposures with background exposure from non-occupational sources. Although the same constraints as discussed above apply to the use of upper percentile values from biomonitoring studies with workers, in addition to the estimate of average body burdens an estimate was developed for high end exposures at the workplace (approx. 90th percentile) (see below).

9.0.4.3 Industrial scenarios

General approach

Manufacture is not relevant for this application for authorisation.

The industrial scenarios (see section 9.0.1) covered in this application for authorisation are 1) use of recycled soft PVC containing DEHP for formulation of solid PVC mixtures (use #1 (ES1)) and 2) polymer processing into PVC articles (use #2 (ES2)). Occupational exposure during industrial activities at converters, where workers were engaged in formulating and processing PVC, was assessed in several published and unpublished studies (see below), from which both biomonitoring (DEHP metabolites in urine) and inhalation data (DEHP concentration in workplace air) are available. The industrial activities include handling of free DEHP for formulating and use of liquid formulations (Plastisol). Free DEHP and Plastisol use are not relevant for use of recycled soft PVC containing DEHP. As handling of liquid substance and formulations are expected to give rise to higher exposures than DEHP included in the polymer matrix, use of monitoring results for exposure assessment from these industrial sites is considered to be highly conservative. In addition, the concentration of DEHP in recyclate is up to 20% only, compared to DEHP concentrations of typically 30% in newly formulated soft PVC.

As the worker exposure assessment is mainly based on biomonitoring data from cohorts engaged in various occupational activities, dealing with individual PROCs as contributing scenarios is not feasible in this case.

While there are some facilities exclusively engaged in formulation (i.e. compounding in the context of the plasticiser DEHP) formulation activities are often performed prior to PVC processing in the same plant. Many of the studies discussed in detail in the exposure scenarios below involved exposure during formulation (often described as "mixing" in the sources) and polymer processing of workers from the same plant. As a consequence, a clear distinction between formulation and polymer processing is not always possible and some datasets were included in the assessment of both exposure scenarios.

As explained above the exposure assessment and risk characterisation are largely based on biomonitoring data, since these reflect the combined exposure from inhalation and dermal pathways. Inhalation data will be discussed as a supporting piece of information. A survey was conducted in 2009 at downstream user level to collect all available measurements on air concentrations and corresponding operational conditions and risk management measures at these workplaces (Cadogan et al, 2010). These data have been backed up by available public references (e.g. Protois 2007) and by information retrieved from the German MEGA database (IFA, 2012).

The operational conditions and risk management measures described in the following exposure scenarios represent best practice with respect to current knowledge. It is recognised that they are generally stricter than the conditions present during the biomonitoring studies. For example, wearing gloves is communicated in the exposure scenario for all processes, while the biomonitoring data come from subjects not all of whom wore

gloves, especially in the case of polymer processing. Therefore, the measures communicated in the exposure scenarios can generally be expected to lead to lower exposures than those estimated on the basis of the biomonitoring data. This adds a conservative element to the exposure estimates. A detailed discussion of this issue is included in the respective exposure estimation sections.

End-shift biomarker concentrations are used to evaluate the occupational exposure situation. By the nature of these values they include the background from other sources, e.g. food. Gaudin et al. (2011) did not find differences in DEHP urinary biomarker concentrations of manufacture workers between pre- and post-shift levels, indicating that occupational exposure was negligible compared to the background exposure. End-shift samples are taken in this assessment as a conservative means to assess occupational exposure.

Only some of the authors transformed measured values into intake levels. To exemplify the comparability of these calculations with our approach (as done above with a study in the general population: Frederiksen t al. 2011), in the table below the calculations presented by Hines et al. (2011) are compared with our calculations. In principle the same procedure is applied but due to the urinary excretion rates measured by Anderson et l. (2011) the calculated values obtained here are more conservative than those reported by study authors. Ano her reason for differences may consist in the fact that study authors' calculations used individual creatinine-adjust d values, whereas the calculations here had to be based on group means, as only these values w re given n the publications.

Table 60 Comparison of intake calculations from Hines et al. (2011) with calculations used in this assessment for the same occupational cohorts

	Authors' intake calculations (GM) (µg DEHP/kg bw/day)	Intake calculations according to procedure in this assessment (GM) (µg DEHP/kg bw/day)
Manufacturing	3.2	3.6
PVC compounding	12	16
PVC film	17	22
Vehicle filter	4.3	5.7

Estimation of average and high end exposures

Average values (arithmetic mean, geometric mean, median) are generally reported in biomonitoring studies with occupational cohort (Dirven t al., 1993b; Gaudin et al., 2008; Hines et al., 2011; Hines et al., 2009). Only Gaudin et al (2011) r ported 95 percentiles as well. The average values are considered to express the longer-term exposur m re adequately than higher percentile values (see detailed discussion under 9.0.4.2). Therefore, creatinine-adjusted geometric means (or, where not available, median values) are considered to be the most reliable da a for calculation of intake values. Creatinine-adjustment is generally accepted and well established for cor ection of dilution of urine for adults.

In addition to intake levels calculated from average body burdens estimates are developed to characterise high- nd exposures of workers. In most studies higher percentile values for urinary excretion of metabolites are not given Only Gaudin et al. (2011) reported such values (not differentiated according to industrial sector, therefore these values are not reported in Annex #2#). These values are given in the table below.

Table 61 Relationship between geometric means and 95th percentile values for all exposed workers investigated by Gaudin et al. (2011)

	MEHP pre-shift	MEHP post-shift	5cx-MEPP pre-shift	5cx-MEPP post-shift
GM (µg/g creatinine)	8.7	16.8	27.7	46.6
95th percentile (µg/g creatinine)	47.2	102.7	130.5	277.8
ratio	5.4	6.1	4.7	6.0

Substantial lower ratios between average and high-end exposure were reported by Hines et al. (2011) and Dirven et al. (1993b) (for individual values see Annex #2#): no 90th or 95th percentiles are reported in these studies, but

- ratios between **maximum** values and geometric means for individual metabolit s are in the range of 3.5 to 7.7 for all activities in the study of Hines et a. (2011)
- ratios between **maximum** values and medians for individual metabolites are in the range of.1.4 to 6.1 for all activities in the study of Dirven et al. (1993b).

Taking into account that

- ECHA Guidance on Information Requirements and CSA, R.14 (ECHA, 2010a) stipulates that the 90th percentile of an exposure distribution should be used for risk characterisation (instead of the 95% percentile)
- (although concentrations of different metabolites are related) ratios between geometric mean and the 95th percentile value of total body burdens in th study population of Gaudin et al. (2011) will be lower than the ratios reported above for i dividual metabolites (i.e. 4.7 to 6.1)
- - and the data from Hines et al. (2011) and Dirv n et al. (1993b) point to much lower factors than Gaudin et al.

we therefore use a factor of 4 in this docum t to estimate the 90th percentile intake value from intake values calculated with the geometric means of urinary metabolite concentrations.

Short-term exposure

DEHP is of low acute toxicity nd does not show irritating or other local effects. Therefore, peak exposures are not of particular concern. According to ECHA Guidance on Information Requirement and CSA (ECHA, 2010a), peak exposures may b xtrapolated from shift averages by using a factor of 4, if variability is "not very high". This can reasonably be assumed here, as geometric standard deviations (GSD) were generally in the range of 2 to 4 for most measurements of any of the metabolites in Hines et al. (2011), and the Guidance assumes "not very high" variability when GSD is below 6.

As the highest RCR calculated for occupational exposure is 0.1, based on GM (and 0.4 based on the estimated 90th percen ile value) (see section 10. 1 to 10.3), peak exposures, which exceed shift averages by a factor of 4 wo ld only exce d the systemic long-term DNEL by less than a factor of 2 (0.4 times 4).

This is confirmed by the following consideration:
In the able below the highest maximum levels reported for any workplace in the studies of Gaudin et al. (2011) and Hines et al. (2009) are presented, and intake levels were calculated and compared to the long-term systemic DNEL (surrogate DNEL for external exposure of 230 µg/kg bw/day, see Table 53**Error! Reference source not found.**).

Table 62 Maximum single biomarker concentrations, and respective intake levels

Study	Biomarker concentrations	Intake level, calculated by the Aylward/Anderson method	Comparison to long-term DNEL (230 µg/kg bw/day)
Hines et al., 2009	1759.3 µg/g creatinine * (sum of maximum values for each of 4 metabolites, creatinine adjusted, highest values taken from PVC film production)	96 µg/kg bw/day	< long-term DNEL
Gaudin et al., 2011	1866.6 µg/g creatinine* (sum of maximum values for each of 2 metabolites, creatinine adjusted, highest values taken from wall covering products)	230 µg/kg bw/day	approx. long-term DNEL

* Highest values reported for each metabolite were summed up

In conclusion, taking into consideration the conservatism of the systemic DNELs derived for workers (see discussion above) and the low acute toxicity of DEHP it can be concluded that short-term DNELs for the various exposure pathways would be well above the long-term systemic DNELs and therefore are not expected to be exceeded in the work environment. In addition, for specific activities of short duration (sampling and cleaning activities) in the respective exposure scenarios addition l per onal risk management measures are implemented to further reduce individual inhalation exposure. Short-term exposure situations are therefore considered to be adequately controlled if long-term exposure is safe and re n t considered separately in this assessment.

All biomonitoring data for the work environment sed in this assessment are presented in tabular form in Annex #2#.

The overall data quality of the available ccupational exposure data is considered to be high: the methodology to measure biomonitoring data for DEHP is well developed. The measured data concern the substance in question and cover all relevant industria activities The data are coming from several different sources, regions and companies. Biomonitoring data are supported by reliable air monitoring measurements, among them a recent evaluation of German workplace data covering more than 250 individual measurements for the relevant industrial sectors.

Modelling approach for as essing exposure from using recycled soft PVC material by formulators and converters

Recy led soft PVC containing DEHP is produced by recycling companies and delivered to PVC converters for fur her processing. Recyclate is typically delivered in bags of various sizes as coarse material of low dustiness. The a tivities carried out with recyclate at converter companies are part of the general activities of converters with the xception of the emptying of bags with recyclate and feeding the material into the process, which is unique for use of recycled soft PVC. While one of the biomonitoring studies (Dirven et al, 1993) in fact reflects the use of recycled soft PVC in one of the companies assessed, it cannot be ascertained whether the emptying of bags of recycled soft PVC containing DEHP – from either small bags of 25 kg (PROC 8a) or from big bags with 1,000 kg (PROC 8b) – with dust generation during the processes is completely covered by the biomonitoring data. Exposure from these tasks was therefore modelled.

Inhalation exposure was modelled using ART (Advanced REACH Tool, v.1.5, https://www.advancedreachtool.com) and the 90th percentile was used as the exposure estimate. Exposure was modelled as a task-based concentration (i.e. assumed to take place for 8 h/d) and the modelled estimate can directly be compared with the long-term DNEL.

When applying ART, only those parameters that reduce the exposure estimate are documented in the exposure

scenarios as conditions of use or risk management. The input parameters and the selected values are described in Annex #4#. Briefly, soft PVC recyclate was considered a dry powder (moisture content < 5%) with the dustiness judged to be coarse for the purpose of ART modelling. The DEHP concentration in the recyclate was set to the upper range limit of 20%. The activity relevant here was modelled using the "falling powders" class in ART, with rates depending on the system (manual vs. dedicated facilities). The drop height of the powders was set to > 0.5 m to obtain a conservative estimate.

The estimated inhalation exposure is also conservative since it assumes that DEHP is freely available from the solid PVC matrix.

Dermal exposure during handling of recycled soft PVC powder containing DEHP was modelled with an algorithm described in detail below in the context of professional and consumer service life scenarios. Briefly, dermal exposure is assessed on the basis of the rate of DEHP migration from PVC articles obtained in tests us ng artificial sweat, together with assumptions on the skin surface area in contact with the PVC article as well as assumptions on exposure duration and frequency. The anthropometric input data use the parameter "to al skin surface area/body weight" to prevent undue combination of individual values for skin surface are and body weight. For the full explanation, please refer to section 9.0.5.4 below and Annex #1#.

In contrast to the professional and consumer service life scenarios, however, dermal exposure of industrial workers is towards recycled soft PVC powder (rather than PVC articles) containing DEHP. Sinc the migration rates into artificial sweat evaluated in Annex #1# were all obtained with PVC articles, the e is some uncertainty whether this also applies to DEHP in recycled soft PVC powder:

- Recycled soft PVC can be envisaged to contain a lower percentage of DEHP compared to new PVC articles. While there is no clear relationship between the DEHP concentration in PVC and the migration rate observed with available measured data, a lower migration rate from recycled soft PVC powder may exist in reality.
- The physical state (powder vs. manufactured article) may influence migration and a higher migration rate from recycled soft PVC powder – due to its higher surface area – may be assumed.

In the light of these issues and the lack of any relevant e per mental data on migration from PVC powder, the maximum migration rate (0.83 µg/cm^2 x h) observed in migration experiments with articles rather than the 95th percentile (0.27 µg/cm^2 x h) was chosen for the exposure estimation.

The fraction of the total skin surface area in c ntac with recycled soft PVC powder was derived under the assumption of exposure on the outer pa ts of both hands and arms. Therefore, the mean "recommended value" for hands (5.2%) and arms (15.2%) for male adul s in the Exposure Factors Handbook of the EPA (2011) were added and one half of the sum (10.2% to represent exposure on the outer parts only) was used (the values for female adults are considerably lower: 4 8% and 12.8%, respectively, sum: 17.6%, one half: 8.8%).

Taken together with the total skin surface area assumed for men of 19,400 cm^2, the fraction assumed corresponds to 1,980 cm^2, which i more than two-times higher than the one assumed in ECETOC TRA (v.3) of 960 cm^2 for PROC 8a and PRO 8b (also reflecting the surface area of both hands). The value chosen therefore represents a conservative estima e. A standard working week of 8 h/d on 5 d/w is assumed in the dermal exposure estimate.

The overall c lcul tion flow for estimating dermal exposure is presented in the following table.

Tab e 63 Calculation flow for dermal exposure estimation

Calculation	Parameter	Value	Unit	Source
	Migration rate (maximum value)	0.83	µg/cm^2 x h	Derived below
multiplied by	Total skin surface area/body weight (mean)	280 (adults)	cm^2/kg b.w.	Derived below
multiplied by	Fraction of total skin surface area in contact	0.102	dimensionless	ES-specific
multiplied by	Contact time	8	h/d	ES-specific
multiplied by	Contact frequency	0.714	/d	ES-specific

9.0.4.4 Professional scenarios

This application for authorisation does not cover professional end uses of DEHP. However, professional workers may be exposed to DEHP from PVC articles containing soft PVC recyclate during their service life. In fact, this service life scenario also applies to industrial workers, since exposure from the service life stage (due to handling of PVC articles, working in workshops equipped with PVC flooring, etc.) is considered to be identical for professional and industrial workers. The following table summarises the contributing scenario "Service life – professionals" for exposure scenario ES2, together with the exposure pathways considered.

Table 64 Contributing service life scenarios for professional (and industrial) workers

ES no.	ES name	Exposure pathway
ES2-SL-P	Service life professionals: Professional handling of PVC products made from recycled soft PVC containing DEHP: Installation of building materials and similar activities (PROC21) / inhalation exposure from volatile DEHP / professional PVC footwear) (no PROCs)	Dermal, inh lation

This scenario involves dermal and inhalation exposure. PROC21 has been assigned o ES2-SL-P, which possibly involves exposure to dust. However, soft PVC articles plasticised with DEHP will – by their very nature – not be subjected to dust-generating processes and inhalation exposure from dust is therefore not relevant for this scenario.

Inhalation exposure to volatile DEHP, as included in this scenario, e.g. when working indoors with large PVC surfaces (e.g. industrial and warehouse flooring or polytunnels) being present, will be assessed on the basis of the saturated vapour concentration of DEHP.

Dermal exposure will be assessed on the basis of alg rithms and input data primarily developed for modelling consumer exposure. These are extensively discussed in ectio 9.0.5.4 below and in Annex #1#. Briefly, dermal exposure is assessed on the basis of the 95th per entile for he rate of DEHP migration from PVC articles in tests using artificial sweat, together with assumptions o the skin surface area in contact with the PVC article as well as assumptions on exposure duration and frequency. The anthropometric input data use the parameter "total skin surface area/body weight" to prevent undue comb nation of individual values for skin surface area and body weight. For the full explanation, pl se r fer to section 9.0.5.4 below and Annex #1#.

The overall calculation flow for estimating dermal exposure is presented in the following table.

Table 65 Calculation flow for dermal exposure estimation

Calculation	Para eter	Value	Unit	Source
	Migration rate (95th percentile)	0.27	µg/cm^2 x h	Derived below
multiplied by	Total skin surface area/body weight (mean)	280 (adults)	cm^2/kg b.w.	Derived below
multiplied by	Fraction of total skin surface area in contact		dimensionless	ES-specific
multiplied by	Contact time		h/d	ES-specific
multiplied by	Contact frequency		/d	ES-specific
multiplied by	Clothing penetration factor*	0.35	dimensionless	Derived below
results in	External exposure		µg/kg b.w. x d	

* value based on 90th percentile of median

9.0.5. Assessment of consumer exposure

9.0.5.1. Substance-specific input data

DNELs and absorption factors used for assessing human health risks in the general population are described above (section 9.04, Assessment of exposure to workers).

These DNELs were derived based on observations of effects during the critical perinatal period and therefore are considered most relevant for pregnant women. Due to different assumptions on absorption a slightly lower DNEL is applied to assess exposure of children, although, as the critical window of exposure is perinatal, a higher DNEL might be justifiable for children.

Table 66 DNELs derived to assess human health risks for the general population

Route of exposure	General population (adults/children)	Application
Inhalation (mg/m^3)	0.17/0.13	Used to assess exposure via inhalation (indoor air concentr tions)
Dermal (mg/kg bw/d)	0.72/0.72	Used to assess mod lled exposure from service life of articles
Oral (mg/kg bw/d)	0.048/0.036	1. Used to assess modelled exposure from service life of articles 2. Used to assess intake calculated from biomonitoring studies

The oral DNEL is not only used to evaluate estimated exposure from service life scenarios, but also to evaluate results of biomonitoring studies. As explained above, urinary excretion fractions (UEF) were derived from the human toxicokinetic study of Anderson et al. (2011). T ese fractions were calculated from the observed excretion of DEHP metabolites in urine of volunteers afte oral exposure. Therefore, intake levels calculated back from biomarker concentrations can be directly compared to tolerable intake levels for oral exposure.

9.0.5.2. Principal approach to assess c nsum r exposure

This application for authorisation cover only industrial uses. But consumer exposure to DEHP may occur from using PVC articles containing s ft PVC recy late. These service life scenarios are described below. Parallel methodological approaches are used:
The assessment of consumer exposure to DEHP uses as its main data basis the available biomonitoring data. As outlined above the biomonitoring data reflect DEHP exposures from all possible sources. The main source of exposure to DEHP for the general public is food. The role of food for DEHP exposure and possible sources for DEHP in food are explained elow. As food is the main source of the current DEHP body burden, the assessment using biomonitoring data can be considered an overestimation of the exposure from using PVC articles cont ining soft PVC recyclate and is used as a conservative method to estimate consumer exposure.

The current app oach is based on three pillars:

- expo ure estimation for the general public based on biomonitoring data
  analysis of the contribution of food to overall DEHP exposure
- modelling of exposure from sentinel products chosen to reflect exposure from all possible consumer articles.

The latter step is performed to show that even under very conservative modelling assumptions frequent users of various PVC articles containing soft PVC recyclate will not exceed the DNEL.

9.0.5.2. Biomonitoring data for the general population

Biomonitoring data for European populations of all age groups are used and included in the analysis. The following criteria were applied:

- European populations

- analysis of at least two metabolites
- sampling period > year 2000.

Data from European biomonitoring studies for the general population are tabulated in Annex #3# and are discussed in detail in section 9.3.2.
The overall data quality of the available biomonitoring data is considered to be high: the methodology is well developed and there is a broad range of recent studies available, with actual measurement data for the substance and exposure situation in question, which covers many different European countries with study populations of reliable sizes. Especially the new data emerging from the European research project DEMOCOPHES, although not completely published yet, provide a huge database for body burdens of children and adults from 17 European countries.

9.0.5.3. Exposure from food

This application for authorisation excludes the use of DEHP in food contact materials, which has been subject to EU-wide regulation since 2007 (Commission Directive 2007/19/EC).

Food is considered the main source of DEHP intake (Dickson-Spillmann et al., 2009; Kappenstein et al., 2012; Kommission "Human-Biomonitoring" des Umweltbundesamtes, 2011; Wittassek et al., 2011).

In the duplicate study performed by Fromme et al. (2007), urine biomarker concentrations were analysed in parallel with all food items (duplicate diet study design) taken up by 50 volunteers over the course of 7 days. The median and 95th percentile for measured DEHP intake from food was 2.4 and 4 µg DEHP/kg bw/day. These values were in good agreement with the intake calculated from biomarker urinary concentrations, demonstrating that DEHP intake in this population was mostly from food.

In a follow-up study of this group urine biomarker concentrations were determined in the urine of 22 infants (aged 15 to 22 months) and again content of phthalate in food was analysed in parallel for seven days (duplicate diet study design) (Fromme et al., 2013). The authors defined an "average" and "high" intake scenario, for which the median or 95th percentile value from measurements over seven days for each subject was used. Intake from biomonitoring data was calculated similar to the method used in this report. Daily DEHP intake from food was calculated to be 2.64 and 4.66 µg/kg bw for the average and high scenario, respectively (median of distribution of all individuals) and daily intake calculated from urinary biomarkers was 2.60 and 4.90 µg/kg bw for the average and high scenario, respectively (median of distribution of all individuals). The data suggest that for this age group, food accounts for close to the total DEHP uptake.

RAC/SEAC (2012) considered the recently published data from the UK FSA Total Diet study as a reliable source to estimate the DEHP intake from food. The data were reported by the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in their comments on the Danish Restriction Proposal (CoT, 2011)[2]. Values presented by COT are based on the UK FSA Total Diet Study from 2007 and represent 97.5 percentiles of intake (see table below).

Table 67 Estimates of DEHP exposure from food, based on the UK FSA Total Diet Study

Age Group	97.5th percentile of intake (µg/kg bw/day)
2-years old	6.9 – 9.9
6-7 years old	5.5-6-7 (aged 4-6) 4.6 – 5.2 (aged 7-10)
adults	3.4 – 4

Sioen et al. (2012) performed a dietary exposure assessment for phthalate exposure of preschool children (2.5 to 6 years) and adults in Belgium. In their probabilistic assessment they combined food consumption data from two Belgian databases with measured phthalate concentrations in 550 food products from the Belgian market (sampled between 2009 and 2011). The following table presents the probabilistic intake measurements for children and adults (with consideration of food preparation, median bound method, where values below the limit of quantification (LOQ) was set to ½ LOQ.

[2] see also http://cot.food.gov.uk/pdfs/tox201129.pdf

Table 68 Estimates of DEHP exposure from food in Belgium - Sioen et al. (2012)

Age group	Median intake (µg/kg bw/day)	95th percentile (µg/kg bw/day)
preschool children	3.50	5.38
adults	1.49	2.86

In an additional scenario ("worst case scenario"), the maximum DEHP concentration per food group was used for all calculations. In this scenario, intake levels were higher compared to the probabilistic scenario by a fa tor of 5 to 6.

A recent exposure assessment of DEHP from food in Germany was based on occurrence data and cons mption data from a national food consumption survey. If means of DEHP in individual food items used together with means of consumption data, a total average DEHP exposure from food of 9.3 µg/kg bw/day for adults was calculated, whereas with median values for concentrations in food average exposure was 3.6 µg/kg bw/day (Heinemeyer et al., 2013). The authors also applied a probabilistic model and obtained mean (and 95th percentile values) of 10.2 (10.4) µg/kg bw/day, if median food concentrations were used, and 14.0 (21.3) µg/kg bw/day, when mean food concentrations were used.

In summary, the Belgian data are in agreement with the UK Total Diet Study results. Whether the somewhat lower figures (comparison of 95th percentiles in Sioen et al. (2012) with 97.5th percentiles of the UK data) from Belgium reflect a downward trend with time, are the result of the different percentiles compared, are due to the methodological differences or result from a combination of these factors cannot be assessed. A median exposure of 2-3 µg/kg bw/day for adults is in agreement with the intake calculated from recent biomonitoring data (e.g. based on geometric means from DEMOCOPHES study (see below): 2.8 µg/kg bw/day), leading to the conclusion that the major part of total DEHP intake in this population group is via food.

The major role of food for overall DEHP intake is cor obor ted by a fasting study reported by Wittassek et al. (2011). Three adult volunteers fasted and drank only mineral water for 48 hours. Within the first 24 h DEHP biomarker concentrations in urine sharply decreased by approx. one order of magnitude and remained low (factor 7.5 for medians compared to pre-fasting lev ls) throughout the fasting period. The authors concluded that during fasting no significant DEHP exposure occurred. This is in agreement with analyses of the influence of fasting times in the NHANES dataset of urinary excretion of DEHP metabolites (Aylward et al., 2011).

Contributions by food contact materia s

In a US study, Rudel et al. (2011) inv stigated the influence of food packaging material on phthalate intake. In this intervention study, 20 individuals from 5 families consumed fresh food, which was not canned or packed in plastics for three days. Urine oncentrations of the three primary and secondary DEHP metabolites were measured pre- and p st-intervention and during the intervention. DEHP body burden was (based on geometric means) redu d by 53 t 56%.

In an inv stiga i n of potential contamination pathways of DEHP in cow's milk, Fierens et al. (2012b) found that DEHP con entr tions both in milk from cooling tanks and in machine-milked milk were substantially higher compared to hand-milked samples. This observation points to a relevant contribution to DEHP levels by leaching from se lants, milk tubes or other parts of the system.

Fierens et al. (2012a) investigated food samples and packaging materials from the Belgian market for the p e ence of phthalates. They found DEHP to be present in all 12 samples of packaging materials investigated. The fact that DEHP is still present in food packaging material and contributes to the overall exposure is also supported by a Portuguese study (Pocas et al., 2010).

These data from Belgium and Portugal show that food contact materials continue to play a significant role for exposure to DEHP, clearly implying that the Commission Directive 2007/19/EC is not efficiently implemented.

9.0.5.4. Exposure from consumer articles and indoor sources

Consumer exposure to DEHP from PVC articles containing soft PVC recyclate was assessed for several different pathways and article categories.

Table 69 Contributing service life scenarios for consumers

ES no.	ES name	Exposure pathways
ES2-SL-C	*Contributing Scenario:* *Service life consumers: exposure from consumer articles made from PVC recyclate*	Dermal, oral

The approach for assessing consumer exposure to DEHP from PVC articles containing soft PVC recyclate, including the derivation and discussion of critical input parameters, is fully documented in Annex #1# Briefly, the approach taken for assessing consumer exposure can be described as follows.

- For each exposure scenario, a **sentinel article** is chosen for an in-depth exposure estima ion. Sel ction of the sentinel article (e.g. sandals as sentinel product for PVC footwear with direct skin conta t) is ased on an anticipated high exposure, e.g. due to high skin surface area in contact with the PVC a ticle, high exposure duration, high exposure frequency or a combination of these factors. Other PVC articles also covered under the respective use are specifically mentioned in the exposure scenario and discussed quantitatively in the course of exposure estimation.
- In some exposure scenarios, additional considerations are required for **specific exposure situations**, such as the application of sun lotion when simultaneously wearing beach sandals The discussion of these issues is included in the specific exposure scenarios.
- Exposure to DEHP from PVC articles containing soft PVC recyclate is estimated by **simple calculations** with a limited number of input parameters. No specific software tools (such as ECETOC TRA or ConsExpo) were used.
- Exposure estimation using this approach basically in olves two sets of input parameters: a) **general input parameters** (e.g. anthropometric data, mouthing duration for children and migration rates) that are discussed in this section (see below) and b) **specific input parameters** (e.g. the fraction of the skin surface area that is exposed, duration and frequency of d rmal ontact) that are discussed in the respective exposure scenarios. The overall calculation flow fo ach pathway, with general input values and calculation steps is presented at the end of each section below.
- Consumer exposure is generally estimated n th basis of **95th percentiles for one key input value** (e.g. the migration rate) and mean values f r other input values. This approach prevents extremely overconservative values being derived from the comb nation of several upper percentiles.
- This issue was also considered in the derivation of anthropometric input data, for which undue combination of upper/lower percentiles can actually result in unphysiological values (see e.g. Phillips et al., 1993). As a consequence, dermal exposure estimates rely on the total skin surface area/kg body weight (in cm^2/kg) as a key input param ters (to be multiplied with the fraction of skin surface in contact with PVC article in the specific exposure scenario), rather than on specific percentile values for the skin surface area and the body weight.
- Where a plicable, th following **age groups** are **considered** in the exposure assessment:
  - Adu s (wh e exposure is assumed to equal that of the sensitive subgroup pregnant women)
  - Childr n (based on assumed highest exposure, see below)
    - 6- <12 months old (6-12 months old hereafter)
    - 2- <3 years old (2-3 years old hereafter)

  These age groups for children were selected based on an assessment of potentially high dermal exposure (ased on high total body surface/kg body weight) in combination with a potentially high oral exposure (based on mouthing duration).
- Besides exposure to DEHP through direct contact with PVC articles containing soft PVC recyclate, exposure in the **indoor environment** is addressed by estimating **oral exposure from ingestion of house dust** as well as **inhalation exposure**. This exposure pathway differs from all others in that both the DEHP concentration in house dust and the DEHP concentration in indoor air are potentially affected by all PVC articles containing DEHP that are used indoors. Therefore, these pathways cannot be assigned to a specific exposure scenario and are included in an assessment of the aggregate exposure.

The following sections briefly describe the derivation of the key values used in the exposure assessment.

Anthropometric data

Key anthropometric input data were derived on the basis of data recommended in the ECHA Guidance and the Exposure Factors Handbooks (EFHs) of the U.S. Environmental Protection Agency, available both specifically for children (EPA, 2008) and in a more general form (EPA, 2011). EFHs derive "recommended values" on the basis of a detailed discussion of available sources and "recommended values" were chosen, where available. While it is acknowledged that the EFHs primarily relate to the U.S. population, the difference to Europe is considered small for anthropometric data, especially in light of the fact that populations from different European countries may also differ substantially. In any case, use of the EFH data is also envisaged in the ECHA Guidance (ECHA, 2010b).

The following table shows the key values used for the total skin surface area/body weight, together with th rationale and supporting values.

Table 70 Key values used for the total skin surface area/body weight

Total skin surface area/ body weight	Key value	Rationale	Supporting values
Adults and pregnant women	280 cm^2/kg	Derived from values suggested in ECHA Guidance (ECHA, 2010b): Men: 19,400 cm^2/70 kg = 277 cm^2/kg Women: 16,900 cm^2/60 kg = 282 cm^2/kg	EPA, 2011; Phillips et al., 1993*: US males and females combined, ≥ 18 years: Mean: 284 cm^2/kg Median: 286 cm^2/kg 95th percentile: 329 cm^2/kg Range: 200-351 cm^2/kg Bremmer et al. (2006): Men: 19,100 cm^2/74 kg 258 cm /kg Women: 16,800 cm^2/61 kg = 275 cm^2/kg Andersen et al. (2012), pregnant women: 18,500 cm^2/73.5 kg = 252 cm^2/kg
Children (6-12 months)	490 cm^2/kg	Means for US children aged 6-12 months (recommended values): 4,500 cm^2/9.2 kg = 89 cm^2/kg (EPA, 20 8; 2011)	German KiGGS survey (Mekel et al., 2007; RKI, 2011); calculated from median values for height and body weight (see Annex #1#): Boys and girls combined, 6-12months: 501 cm^2/kg Boys and girls combined, 2-3 years: 450 cm^2/kg EPA, 2008; 2011; Phillips et al., 1993*: US boys and girls combined, 0-2 years: Mean: 641 cm^2/kg Median: 617 cm^2/kg 95th percentile: 846 cm^2/kg Range: 421-1142 cm^2/kg Bremmer et al. (2006) for: 1.5-years: 4,800 cm^2/9.85 kg = 487 cm^2/kg 2.5-years: 5,750 cm^2/12.5 kg = 460 cm^2/kg Tønning et al. (2009) for 2 years: 6,000 cm^2/15.2 kg = 395 cm^2/kg
Children (2-3 years)	440 cm^2/kg	Means for US children aged 2 3 ye rs (recommended values): 6 100 cm^2/13 8 kg = 442 cm^2/kg (EPA, 2008; 2011)	

* The data in EPA (2011) were rounded, but the original data from Phillips et al. (1993) are shown here.

The body weights and total skin surface areas used in the calculation of SA/BW ratios above are also used – where required – in other exposure estimates and are summarised in the following table.

Table 71 Key values for body weight and skin surface area

	Body weight	**Skin surface area**
Adults	70 kg (men), 60 kg (women)	19,400 cm^2 (men), 16,900 cm^2 (women)
Children (6-12 months)	9.2 kg	4,500 cm^2
Children (2-3 years)	13. 8 kg	6,100 cm^2

Note that the values for adults are the ones proposed in the ECHA Guidance (ECHA, 2010b) and the ones f r children are "recommended values" of the U.S. Environmental Protection Agency for the specific age groups (EPA, 2008; 2011).

Dermal exposure assessment
One of the most important values for dermal exposure to DEHP from PVC articles is the DEHP mig ation rate from such articles into artificial sweat. The approach chosen here is based on the statisti al ev luation of all available migration data from the "Surveys of Chemical Substances in Consumer Products" by t e Danish EPA (Andersen et al., 2012; Svendsen et al., 2007; Tønning et al., 2010a; Tønning et al., 2009; Tønning et al., 2010b), rather than using article-specific migration values. The rationale for this approach is di uss d in detail in Annex #1#. Most generally, this approach seeks to derive measured data that are "*reliable and representative for the situation that needs to be assessed*", as required in the ECHA Guidance (ECHA, 2010b).

On the basis of 51 measurements of in vitro DEHP migration from PVC articles into artificial sweat, a
95th percentile of **0.27 µg/cm^2 x h**
was derived and used in the exposure estimation. Mean values of this evaluation (AM, GM and median) were in the range of 0.03-0.08 µg/cm^2 x h.

For some specific exposure scenarios, the PVC article is not n direct contact with the skin. A clothing penetration factor was applied in these situations to a coun for a reduced exposure. While no data could be located for the specific cases evaluated in this dossier, inform tion from the exposure assessment of biocidal products and pesticides was taken into consider tion. G n rally, the "Technical Notes for Guidance" (TNsG) of human exposure to biocidal products (EC 2007; 2 10b) assume a clothing penetration of 50% for non-professionals. This value is very conser ative, as shown by detailed evaluations fully discussed in Annex #1#. On the basis of more than 2,000 individual inner/ uter paired measurement samples from the Pesticide Handlers Exposure Database (PHED), as evaluated by Driver et al. (2007), and taking into the consideration the loading resulting from the migration ra e derived above, we derived
a clothing penetration of 35%
for the exposure assessment in th s dossier. It is considered conservative for several reasons discussed in Annex #1#. The analysis by Dri er et al. (2007) with a mean clothing penetration of 8-12% is supported by a review of Ross et al. (2008), who found pesticide clothing penetration to range between 0.082 and 10% and noted the conservatism of an verage v lue of 10% as employed in several exposure assessments.

The overall ca culation flow for estimating dermal exposure is presented in the following table.

Table 72 Calculation flow for dermal exposure estimation

Calculation	Parameter	Value	Unit	Source
	Migration rate (95th percentile)	0.27	µg/cm^2 x h	Derived above
multiplied by	Total skin surface area/body weight (mean)	280 (adults) 440 (children, 2-3years) 490 (children, 6-12 months)	cm^2/kg b.w.	Derived above
multiplied by	Fraction of total skin surface area in contact		dimensionless	ES-specific
multiplied by	Contact time		h/d	ES-specific
multiplied by	Contact frequency		/d	ES-specific
multiplied by	Clothing penetration factor*	0.35	dimensionless	Derived above
results in	External exposure		µg/kg b.w. x d	

* Only applied in some exposure scenarios, value based on 90th percentile of median

Oral exposure assessment

Oral exposure to DEHP from PVC articles primarily involves mouthing of PVC articles by children, although one adult exposure scenario is also considered. As a consequence, the duration of mouthing by children and the rate of DEHP migration from PVC articles into saliva are the central input values. Again, the following values are discussed in detail in Annex #1#.

The key values for the mouthing duration were derived on the basis of two studies from Europe, one from the Netherlands (Groot et al., 1998; Könemann, 1998 Steenbekkers, 2001) and another one from the United Kingdom (DTI, 2002; Smith and Norris 2003). The mouthing duration derived refers to objects other than dummies, toys and fingers, since DEHP exposure from these categories is not relevant. The following key values were derived.

Table 73 Key values for mouthing duration (children 6-12 months and 2-3 years old)

Daily mouthing time for objects other than dummies, toys and fingers	**Key value**	**Rationale**	**Supporting values**
Children (6-12 months)	25 minutes/ day	UK study: 25 minutes/day (rounded mean, 6-9 months, n=15) 16 minutes/day (rounded mean, 9-12 months, n=17) Dutch study: 9.4 minutes/day (mean, 6-12 months, n=14) 25.7 minutes/day (maximum, 6-12 months, n=14)	9 minutes/day (mean, US children, 0-18 months, n=107, objects other than pacifier, teether or toy, including zero values) 22 minutes/day (mean, US children, 0-18 months, n=46, objects other than pacifier, teether or toy, excluding zero values) Juberg et al. (2001) 4.4 minutes/day (mean, US children, 3-12 months, n=54 soft plastic items*) 17.5 minutes/day (95th percentile, US children, 3-12 months, n=54, soft plastic items*) Babich et al. (2004); Greene (2002); Kiss (2002)
Children (2-3 years)	15 minuets/ day	UK study: 22 minutes/day (rounded mean, 2 years n=39) 15 minutes/day (rounded mean 3 years, n=31) Dutch study: 2.0 minutes/day (maximum, 18-36 months, n=11) 11.6 minutes/day (maximum, 18-36 months, n=11)	2 minutes/day (mean, US children, 19-36 months, n=110, objects other than pacifier, teether or toy, including zero values) 15 minutes/day (mean, US children, 19-36 months, n=18, objects other than pacifier, teether or toy, excluding zero values) Juberg et al. (2001) 3.8 minutes/day (mean, US children, 12-24 months, n=66, soft plastic items, includes soft plastic toys) 13.0 minutes/day (95th percentile, US children, 12-24 months, n=66, soft plastic items*) 4.2 minutes/day (mean, US children, 24-36 months, n=49, soft plastic items*) 18.5 minutes/day (95th percentile, US children, 24-36 months, n=49, soft plastic items*) Babich et al. (2004); Greene (2002); Kiss (2002)

The rationale for selecting these key values is fully discussed in Annex #1#. Briefly, the flowing points support these key values:

- The mean value from the UK study for children 6-9 months old (25 min/day) is taken as the key value for 6-12 months old children, which is a conservative assumption since the value for 9-12 months old children is considerably lower (16 min/day).

- The value is substantially higher than the mean from the Dutch study (9.4 min/day) and corresponds to the maximum of the Dutch study.
- This mean from the UK study is also higher than supporting values from US studies (Babich et al., 2004; Greene, 2002; Juberg et al., 2001; Kiss, 2002), although the latter differ by age boundaries and/or the types of objects covered.
- For children 2-3 years old, the mean for children 3 years of age of the UK study (15 min/day) is chosen as the key value. The higher mean for children 2 years old (22 min/day) is considered to be too conservative for the following reasons:
- It is about 2-times higher than the maximum found in the Dutch study and about 12-times the mean from this study.
- There is some evidence to suggest that the mean for the 2-years old children is unduly affected by a high maximum value (or several of these), since this is the only age group in which the maximum is m re than 8 times the mean (typically, this factor is about 4-5).
- The US values more clearly support the lower of the two values in question.

Recently, data on the mouthing duration were assessed in relation to the entry 52 of Ann x XVII to REACH review for DIDP and DINP (ECHA, 2012c; RAC, 2013). ECHA (2012c) derived mouthing dur tions of 79 (typical) and 126 minutes/day (reasonable worst case) for children 6-12 months old (values for children 12-18 months old were assumed to be much lower: 4.9 and 13.2 minutes/day, respectiv ly). RAC (2013), on the basis of the data in the ECHA report, used a reasonable worst case value of 2 h/d (only slightly different from the 126 min/d derived by ECHA), but did no derive a typical (i.e. mean) estimate. The difference between the means (25 minutes/day assumed here vs. 79 minutes/day in ECHA (2012c)) is entirely due to the fact that ECHA (2012c) – and also RAC (2013) – derived a duration for the mouthing of toys, childcare articles and other articles (only excluding pacifiers), while the values derived here refer to the mouthing of "other objects" (excluding toys, childcare articles and pacifiers, for which the use of DEHP is prohibited and which are not subject to this application).

It must be stressed that applying these values in the exposure estimation implicitly assumes that "objects other than dummies, toys and fingers" are all made of PVC con ining DEHP. This is certainly a very conservative assumption, since many items mouthed are not made of plastic. In fact, the UK study indicates that some articles potentially consisting of PVC (and therefore co e d by this CSR) were only rarely mouthed by UK children. For example "Packaging (prob. soft plastic)" was mouthed 3 times, "handbag" 2 times and "red vinyl purse" 2 times among the 1,665 observations (0.1 0.2%) of th study (DTI, 2002). Similarly, the supporting values for "soft plastic items" taken from a US study (mean : ca. 4 minutes/day, 95th percentiles: 13-18.5 minutes/day, depending on the age group) suggest tha the mean duration for mouthing of PVC articles may be substantially lower than the values derived above.

The other critical input value, th rate of DEHP migration into saliva, was derived from several studies involving human volunteers. This is in line with the approach of CSTEE (1998), who primarily relied on the human volunteer study of the "Dutch Consensus Group" (Könemann, 1998), with additional evidence from Austrian in vivo migration stud es (Fiala t al., 2000; Steiner et al., 1998). These studies were evaluated, together additional data from more recen Japan se (Niino et al., 2003; Niino et al., 2002) and Mexican (Corea-Tellez et al., 2008) studies. The tudies included in the evaluation related to migration of DEHP, DINP and/or DBP. Again, Annex #1# contains a detailed discussion of the underlying data and a justification for the approach taken.

On he basis of the evaluation in Annex #1#, the (rounded) 95th percentile of all phthalates of
3.65 µg 10 cm^2 x min
is taken s the key value for DEHP migration into saliva.

This key value is considered to represent a conservative approach for DEHP migration from PVC articles into saliva for the following reasons (see Annex #1# for details):

- It represents the 95th percentile from a comparatively large number of in vivo migration data (n=21). While it is based on an evaluation of the mean migration values, a detailed evaluation of the only study reporting individual values (Fiala et al., 2000) supports the conservative nature.
- The underlying basis comprises primarily data for DINP. There is some evidence to suggest that the migration rate from PVC articles is lower for DEHP than for DINP. In fact, the key value derived here is more than 5-times higher than the maximum migration rate measured for DEHP in any in vivo test.
- The key value to a large extent reflects migration under conditions facilitating phthalate release. Overall, 15/21 values (71%) used in its derivation were obtained with biting/chewing on the object. Together with

the relative small fraction of time a child spends biting (23-31%, see Annex #1#), this adds another conservative element in the overall exposure estimate.

It should be noted that the value chosen as a "worst case" by CSTEE (1998) is a maximum value from the Dutch study (8.9 µg/10 cm^2 x min), supported by a maximum value from the Austrian study. In relation to the Dutch study, this maximum value was not reached when a different part of the same teething ring was tested as item no. 3 (maximum 5.7 µg/10 cm^2 x min). In the Austrian study, the high maximum was in fact excluded by the authors from calculation of the mean, since the subject bit off pieces of the article, which were extracted together with the saliva. Together with the issues discussed in detail in Annex #1#, these maximum values are therefore considered highly uncertain and the 95th percentile of 3.65 µg/10 cm^2 x min represents a conservative estimate. The unit of µg/10 cm^2 x min is used in agreement with CSTEE (1998), because the contact area for small children is assumed to be 10 cm^2. This value is assumed here for both age groups.

In a recent report in relation to the entry 52 of Annex XVII to REACH review for DIDP and DINP, ECHA (2012c) derived migrations rates of 14 (typical) and 45 µg/cm^2 x h (reasonable worst case) for both DINP and DIDP in toys, childcare articles and other articles (excluding sex toys). The reasonable worst case estimate was also used by RAC (2013) for DINP and DIDP. It is higher than the one used here (3.65 µg/1 cm^2 x min = 21.9 µg/cm^2 x h), primarily for the following reasons:

- The key value used here is entirely based on vivo data, while the reasonable worst case used by ECHA (2012c) and RAC (2013) is based on a single value found in an in vitro study. In fact, the in vivo data reported in ECHA (2012c) suggest a lower in vivo migration rate than in vitro.
- The key value used here applies to DEHP, for which a lower migration rate is suggested by the data discussed above.

Exposure due to mouthing is considered for one article group/sentinel product, because only very few articles made of recycled soft PVC can be mouthed by children. First, many items that are actually mouthed by children are not made of plastic and an even smaller fraction will be made of PVC (see also Annex #1# for details). Second, among those made of plasticised (i.e. soft) PVC only a fraction will be made of recycled soft PVC. Finally, many articles made of recycled soft PVC are not used in the indoor environment and are in fact not accessible to mouthing by children. This e.g. applies to technical articles such as water-proofing and geo-membranes as well as profiles and gaskets, vehicle splash guards, traffic cones and traffic sign components and other products used on road surfaces. Therefore, only one third of the entire mouthing duration for the category of "other objects" (for details, see Annex #1#) is assumed for mouthing articles made of recycled soft PVC (resulting in a fraction of 0.333 for the article group).

The overall calculation flow for estimating oral exposure from mouthing articles is presented in the following table.

Table 74 Calculation flow for oral exposure estimation (mouthing)

Calculation	**Parameter**	**Value***	**Unit**	**Source**
	Migration rate (95th percentile)	3.65	µg/min (per 10 cm^2 standard contact area)	Derived above
multiplied by	Mouthing duration (mean)	25 / 15	minutes	Derived above
multiplied by	Fraction for article group	0.333	dimensionless	Derived above
divided by	Body weight (mean)	9.2 / 13.8	kg	Derived above
results in	External exposure		µg/kg b.w. x d	

* When two values are given, these refer to children 6-12 months old / children 2-3 years old

Exposure in the indoor environment: oral exposure from house dust

The critical input values for this exposure pathway are the amount of dust ingested per day and the DEHP concentration in house dust. The key values derived are briefly presented in this section. A full discussion of

these values and their justification is available in Annex #1#.

For the daily dust ingestion, the ECHA Guidance (ECHA, 2010b) mentions a conservative estimate of 100 mg/d for house dust intake by children (no differentiation by age) on the basis of a report by Oomen et al. (2008). The latter authors derived dust ingestion values of 100 mg/day for children and 50 mg/day for adults as a "*conservative but realistic estimate*" on the basis of estimates from several studies, which, however, were not discussed to any extent. Oomen et al. (2008) also recognise that many of the underlying studies investigated soil (rather than dust) ingestion but nonetheless propose to use these values. In contrast to this approach, EPA (2008; 2011) provides a detailed discussion of available studies, including an identification of key studies along specific criteria. These authors derived "recommended values" both for soil and dust ingestion, as well as combined soil and dust ingestion, with some differentiation by age. In the exposure assessment performed here, dust ingestion is based on the following mean "recommended values" for dust ingestion from EPA (2008; 2011):

- Adults: 30 mg/d
- Children, 6-12 months: 30 mg/d
- Children, 2-3 years: 60 mg/d

The data in EPA (2008; 2011) show that the value of 100 mg/d– as proposed in ECHA (2010b) for children – represents an upper percentile estimate for dust ingestion (no soil) by children 3-<6 years old, while the value of 50 mg/d for adults represents a mean estimate for soil and dust ingestion. The mean values recommended in EPA are used here, because exposure will be estimated on the basis of an upper percentile of DEHP concentrations in house dust (see next paragraph).

Over the past two decades, several studies investigating DEHP concentrations in house dust have been performed and values from many of these studies were included in the exposure assessment performed in the frame of the proposal for restricting the use of DEHP and other phthalates in articles (contained in the opinion of RAC/SEAC, 2012), although mostly cited from Bornehag et al. (2005b). The European studies served in the Phthalate Restriction Proposal Assessment as a basis for assessing oral exposure via ingestion of dust. The following assessment is also based only on studies conducted in Europe, thus excluding studies from countries such as the USA and China. Studies evaluated in the Phthalate Restriction Proposal Assessment (2012) were consulted in original form to identify additional information (e.g. sampling details and maximum values), whenever possible. In addition, several studies not included in the Phthalate Restriction Proposal Assessment (2012) have been evaluated, including results from the German Environmental Survey for Children (GerES 2003-2006), designed to be representative of the houses/apartments of children 3-14 years old, involving 600 samples (Nagorka et al., 2010; Nagorka et al., 2011). Overall, 19 different studies were included in the assessment, with full details again provided in Annex #1#. A differentiation by sample size was included in the evaluation and the results are summarised in the following table.

Table 75 Descriptive statistics for DEHP in European house dusts with differentiation of studies by sample size

	DEHP concentration in dust [µg/g]		
	All studies	**Studies with n > 50**	**Studies with n <=50**
Evaluation based on median values of studies			
Number of medians	19	11	8
Mean of medians	797	530	1,164
Median of medians	604	447	781
90th percentile of medians	1,256	**770**	2,280
95th percentile of medians	2,013	**910**	2,747
Evaluation based on upper percentiles of studies			
Number of upper percentiles*	16	10	6
Mean of upper percentiles	2,271	**1,834**	3,000
Median of upper percentiles	1,770	**1,770**	2,138
90th percentile of upper percentiles	3,795	2,747	5,292
95th percentile of upper percentiles	24,807	3,408	6,177

* Upper percentiles usually refer to 95th percentiles

The studies with a sample size of more than 50 are considered the most reliable and representative ones (see Annex #1# for a detailed discussion). On this basis a DEHP concentration in house dust of (rounded)
1800 µg/g as a 95th percentile
is derived and used in this assessment. This value

- is based on the median of upper percentiles (usually 95th percentiles) from studies with a large sample size (1,770 µg/g),
- is supported by an almost identical mean of upper percentiles from studies with a large sample size (1,834 µg/g) and by an identical median of upper percentiles from all studies (1,770 µg/g),
- is higher than the 95th percentiles from GerES 1998 and GerES 2003/2006, the only two studies that have been designed to be representative (although only of the German population),
- is only very slightly below the 95th percentile observed in Danish day-care centres (1,900 µg/g), but almost twice the 95 percentile in Danish homes (980 µg/g) from the same study (Langer et al., 2010), which represents the one with the most recent, ascertained sampling data (2008),
- is lower than the 95th percentile of approximately 2260 µg/g implicit in the Phthalate Restriction Proposal Assessment (2012), if the weighted intakes are converted to DEHP concentrations in dust. The reason for this difference lies in the fact, that the value derived here is based on studies with a sufficiently large sample size (the mean of upper percentiles from all studies (2,271 µg/g; Table 75) is in fact almost identical to the value used in the Phthalate Restriction Proposal Assessment (2012)).
- primarily represents dust samples obtained prior to the ban of DEHP in toys and childcare articles, suggesting the DEHP concentrations in house dust in 2013 may be considerably lower.

In addition, the data base includes the only study not performed in Germany or one of the Scandinavian countries (Kolarik et al., 2008), as well as the study with the second highest 95th percentile of all studies (Bornehag et al., 2005b).

Overall, the calculation flow for estimating oral exposure from dust ingestion is presented in the following table.

Table 76 Calculation flow for oral exposure estimation (house dust)

Calculation	Parameter	Value	Unit	Source
	DEHP concentration in house dust (95th percentile)	1800	µg//g	Derived above
multiplied by	Dust ingestion (mean)	0.03 / 0.06 / 0.03	g/d	Derived above
divided by	Body weight (mean)	9.2 / 13.8 / 70	kg	Derived above
results in	Oral intake		µg/kg b.w. x d	

* When three values are given, these refer to children 6-12 months old / children 2-3 years old / adult

Exposure in the indoor environment: inhalation exposure

The inhalation exposure assessment is based on a single figure, namely the DEHP concentrat on in indoor air. The Phthalate Restriction Proposal Assessment (2012) used three different approache for asses ing DEHP concentrations in indoor air:

simulations with two different scenarios of realistic rooms (children's playroom and ba h om) furnished with furniture/materials marketed in Denmark (DEHP in PVC flooring and wall paper based on 2001 ("old") and 2010 ("new") data,

calculations according to the method in the EU Risk Assessment Report (ECB, 2008) and

an evaluation of DEHP concentrations measured in indoor air based on a literature survey published in 2007. The means and maximum values from these sources were average in the evaluation.

The results from these various approaches – as reported in the Phthalate Restriction Proposal Assessment (2012) are presented in the following table.

Table 77 Summary of DEHP concentration in ir de ived in the Phthalate Restriction Proposal Assessment (2012)

Approach	Scenario/Method	DEHP concentration [µg/m^3]
Simulations, gas phase only	Children's playroom, new PVC	0.16
	Children's playroom, old PVC	0.81
	Bathroom, new PVC	0.26
	Bathroom, old PVC	0.80
Calculations, gas phase only	Children's playroom	9.4
	Saturated vapour concentration (SVC)	5.3
Literature values, mostly gas phase nd particle-bound	Average of means	0.23
	Average of maximum values	1

The authors noted that the method according to ECB (2008) is rough, since it assumes a fixed emission rate and also recognised that it is above the SVC. The simulated values for children's playrooms (0.16 and 0.81 µg/m^3) are multiplied by a factor of 5 to account for particle-bound DEHP, resulting in "reasonable realistic" and "reasonable worst case concentrations of 0.8 and 5 µg/m^3, which are taken to risk characterisation in the Phthalate Restriction Proposal Assessment (2012).

It is unclear, given that the literature are not discussed to any extent and no justification is provided, why the simulation data with an added factor for particle-bound DEHP was suggested to be more reliable than actual measured data, which mostly reflects gas phase and particle-bound DEHP according to the Phthalate Restriction

Proposal Assessment (2012).

It should be noted that the "reasonable worst case" value derived on this basis (5 µg/m^3) is 5-times higher than the average of the maximum values reported in the literature data evaluated in the Phthalate Restriction Proposal Assessment (2012). In fact, it is higher than any of the maximum values evaluated (about 3.1 µg/m^3, see Annex #1# for details). This proposed "reasonable worst case" value is also about 10-times the maximum value reported for private homes (0.53), offices (0.32) or day-care centres (0.48 µg/m^3, all values gas phase and particle-bound DEHP, n=30) in Sweden in a more recent study (Bergh et al., 2011), the corresponding arithmetic means being 0.21, 0.27 and 0.12 µg/m^3.

Overall, the contribution of DEHP in indoor air to total DEHP exposure is very small (Phthalate Restriction Proposal Assessment (2012), even with the comparatively high value taken to risk characterisation. Therefore a detailed evaluation of all available information is not considered necessary here. Rather, the literature data reported in the Phthalate Restriction Proposal Assessment (2012) are evaluated using the approach applied above for other pathways. All mean and maximum values from the literature survey published in 2007 (Table 24 in the Phthalate Restriction Proposal Assessment (2012)) were included and analysed statistically. The results of this evaluation are presented in the following table.

Table 78 Descriptive statistics for DEHP concentrations in indoor air (based on data in Table 24 in the Phthalate Restriction Proposal Assessment (2012))

	DEHP concentration in indoor air [µg/m^3]	
	Mean	**Maximum**
n	12	12
Minimum	0.060	0.110
AM	0.229	0.982
GM	0.183	0.676
Median	0.180	0.810
75th percentile	0.323	1.100
90th percentile	0.420	2.135
95th percentile	**0.507**	**2.646**
Maximum	0.600	3.130

As noted in the Phthalate Restriction Proposal Assessment (2012), the underlying data primarily reflect both gas phase and particle-bound DEHP. Therefore, an additional factor – as applied to simulation data – is not required. Since some of the underlying data may, however, only represent gas phase concentrations, the 95th percentile of the maximum values is taken as a conservative estimate for exposure to DEHP in indoor air.

On this basis, a DEHP concentration (gas phase and particle-bound) in indoor air of (rounded)
2.6 µg m^3 as a 95th percentile
is derived and used in this assessment.

9.1. Manufacture

Not relevant for this application for authorisation.

9.2. Use #1: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends

9.2.1. Exposure scenario

ES1: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends
Use descriptors related to the life cycle stage and all the uses under it: SU 10,12 – PROC 1, 2, 3, 4, 5, 8a, 8b, 14, 15 – PC 32
List of names of contributing worker scenarios: ES1-W: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends (PROC 1, 2, 3, 4, 5, 8a, 8b, 14, 15)
9.2.1.1 Contributing exposure scenario controlling environmental exposure
not relevant
9.2.1.2 Contributing exposure scenario ES1-W controlling worker exposure
Formulation of recycled soft PVC containing DEHP in compounds and dry-blends (PROC 1, 2, 3, 4, 5, 8a, 8b, 14, 15) Covers the material receipt, preparation, reaction, pell tisati n, bulk transfer and storage, as well as equipment cleaning, and associated maintenance activities as well a use in laboratories.
Product characteristic
Physically dispersed in a solid matrix (PVC) . Solid, low dustiness [OC1] Typical concentration in recycled soft PVC: [redacted] Limit the substance in product t 20 % [OC21]
Amounts used
The biomonitoring studies used as a basis for the exposure estimate (see section 9.2.2) represent a wide-range of companies of different izes. The amounts used are not critical for the exposure estimate derived from these data. For modelling exp sure from emptying bags with recyclate material the following assumptions were made: PROC 8a: 25 kg per b g PROC 8b: 1000 kg per bag.
Frequen y and duration of use/exposure
The frequency and duration of exposure represents typical working patterns in industry (8 h/d, 5d/w). The biomonitoring studies used as a basis for the exposure estimate (see 9.2.2) typically collected pre- and post-shift s mples during a normal working week of five days. Covers daily exposures up to 8 hours (unless stated differently) [G2]
Human factors not influenced by risk management
The biomonitoring studies used as a basis for the exposure estimate (see 9.2.2) reflect exposure from all pathways. For PROCs 8a and 8b modelling was performed for inhalation and dermal exposure.
Other given operational conditions affecting workers exposure
The biomonitoring studies used as a basis for the exposure estimate (see 9.2.2) reflect operational conditions typical for the industry, including higher process temperatures during certain processes. Assumes a good basic standard of occupational hygiene is implemented [G1].

Provide a good standard of general ventilation (not less than 3 to 5 air changes per hour) [E11].
Assumes industrial (unless stated differently) [G26]
Assumes activities are at ambient temperature (unless stated differently) [G17].
For dry blending and compounding: Some operations are carried out at elevated temperature [OC7] in closed systems: 100-150°C; During activities involving operator's action, the temperature is estimated to be maximum 40°C.

Technical conditions and measures at process level (source) to prevent release

The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3)

Process	*PROC*	*Operational Conditions / Risk Management Measures*
Closed continuous process, Indoors	1	Solid, low dustiness [OC1]. Handle substance within a closed syst m [E47]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
General exposures (closed systems); With sample collection; Indoors.	2	Solid, low dustiness [OC1].Handle substance wi hin a closed system [E47]. Sample via a closed loop or other syst m to avo d exposure [E8]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee train g [PPE16].
General exposures (closed systems); With sample collection; Elevated process temperature Indoors, with LEV present.	2	Solid, low dustiness [OC1]. Handle substan e within a predominantly closed system provided with extract ventilation [E49]. Sample via a closed loop or other system to avoid exposure [E8]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16]
Batch closed process exposures; Indoors, with LEV present.	3	Solid, low dustiness [OC1]. Handle substance within a predominantly closed system provid d with extract ventilation [E49]. Sample via a closed loop or oth r system to avoid exposure [E8]. Avoid carrying out activities i volv ng exposure for more than 4 hour [OC28]. Wear chemi ally resist nt gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Batch closed process exposures; Elevated process temperature; Indoors with LEV present	3	Solid, low dustiness [OC1]. Sample via a closed loop or other system to avoid xposure [E8]. Handle substance within a predominantly closed ystem provided with extract ventilation [E49]. Avoid carrying out activities involving exposure for more than 4 hour [OC28]. Wear hemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16]
Batch closed process exposures; Eleva ed process temperature; Indoors	3	Solid, low dustiness [OC1]. Handle substance within a closed system [E47]. Sample via a closed loop or other system to avoid exposure [E8]. Avoid carrying out activities involving exposure for more than 4 hour [OC28]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16]
Batch p oces exposu es; Elevated pro ss temperature; indoor	4	Solid, low dustiness [OC1]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Em tying bags of r cycled soft PVC containing DEHP; Manual; Non-dedicated facility; Indoors with LEV	8a	Solid, low dustiness [OC1]. Provide extract ventilation to material transfer points and other openings [E82]. Do not transfer more than 100 kg/minute. Use in room with a volume of minimum 300 m^3.
Emptying bags of recycled soft PVC containing DEHP; Dedicated facility;	8b	Solid, low dustiness [OC1]. Ensure material transfers are under containment or extract ventilation [E66]. Do not transfer more than 1000 kg/minute. Use in room with a volume of minimum 300 m^3. Ensure that the distance from worker to task is greater than 1 m.

Indoors with LEV		
Drum/batch transfers of solid formulation; Dedicated facility; Indoors with LEV	8b	Solid, low dustiness [OC1]. Ensure material transfers are under containment or extract ventilation [E66]. Clear transfer lines prior to de-coupling [E39]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Production of mixtures or articles by tabletting, compression, extrusion, pelletisation; Indoors with LEV	14	Solid, low dustiness [OC1]. Avoid carrying out activities involving exposure for more than 2 hours. Handle substance within a predominantly closed system provided with extract ventilation [E49]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Quality control in laboratory; Indoors with fume cupboard	15	Handle in a fume cupboard or under extract ventilation [E83] Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Technical conditions and measures to control dispersion from source towards the worker		
The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use ypical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than he working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3).		
Organisational measures to prevent/limit releases, dispersion and exposure		
The biomonitoring studies used as a basis for the exposure estimate reflect conditions f use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3).		
Conditions and measures related to personal protection, hygiene and health evaluation		
The biomonitoring studies used as a basis for the exposur estimate reflect conditions of use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3).		
Additional good practice advice beyond the REACH CSA		
None		

9.2.2 Exposure estimation

9.2.2.1 Environmental exposure

Not relevant for this application for auth risation.

9.2.2.2 Humans via the environment

Based on the biomonitoring r sults reported in section 9.3.2.4, the overall intake of the general population from all sources is calculat d to b 3.57 µg/kg bw/day (geometric mean) (90th percentile: 10.29 µg/kg bw/day) for children and 2.81 µg/kg bw/day (geometric mean) (90th percentile: 8.75 µg/kg bw/day) for adults, based on biomonitoring data from the pan-European project DEMOCOPHES.
These data will lso be used for assessing exposure via the environment. As the biomonitoring data include con ributions from all sources, among them exposure of men via the environment, exposure via the environment can be onsidered to be adequately controlled, if the combined intake from all sources is below the DNEL systemic long-term.

It is r asonable to assume that only a small part of exposure of humans via the environment is attributable to the production and use of recyclate products containing DEHP.

9.2.2.3 Workers exposure

Exposure of industrial workers during formulation has been assessed using biomonitoring data from a variety of different workplaces. The studies were conducted in France (Gaudin et al., 2011), the USA (Hines et al., 2009) and The Netherlands (Dirven et al., 1993b). The datasets with more details on these studies are documented in Annex #2#.
The approach taken is explained in sections 9.0.4.2-9.0.4.3 above. Briefly, the sum of geometric means for all

metabolites was converted to intake levels, using the post-shift metabolite concentrations measured in urine. When samples from different days of the week were available (Dirven et al., 1993b), the end-of-week post-shift samples were used in the calculation of intake levels, to cover any potential build-up during the week.

The results of this evaluation are shown in Table 79, together with information on the types of processes covered. Data on formulation and use of Plastisol are presented as well, although these activities are not carried out with recycled soft PVC. Formulation steps (i.e. "mixing", "compounding") are often performed in the same companies that process polymers so that a separation is impossible and some biomonitoring studies are used in the exposure estimation for formulation and in the exposure estimation for polymer processing reported below (see 9.3.2).

Table 79 Summary of DEHP intakes calculated from biomonitoring data during formulation by the Aylward/Anderson method

Source		**Gaudin et al., 2011**	**Hines et al., 2009**	**Dirven et al., 1993 b****
Type of setting (no. of samples/no. of workers)	**Processing steps covered*****	**Intake (µg/kg bw/d)***		
Plastisol coating (25/5)	Plastisol coating, including preparation of Plastisols	11		
PVC 1 compounding (41/9)	Dry blending, extruding, milling, cooling and packaging	17		
PVC 2 compounding (31/5)		4.7		
PVC compounding (12/12)	Mixing, extruding and mill ng		16	
PVC film production (25/25)	Compounding, mixi g pa e preparation, extrusion, milling nd calendering		22	
PVC boot production (9/9)	Mixing extrusion cooling and storage			23
Cable production (6/6)	Mixing, gran lating, extrusion			13

* Intake based on creatinine-adjusted geometric means of post-shift biomonitoring data; intakes are rounded to two significant figures.

** Geometric mean were not reported by Dirven et al. (1993b) and median values were used instead.

*** Processes related to formulation are underlined.

The intak s der ved range from 4.7-23 µg DEHP/kg bw/d and involved 71 workers with 149 samples from 7 diff rent p ants. The results will be discussed in relation to the representativeness of the underlying data in the following sections.

Very recently Fong et al. (2013) presented results of a biomonitoring study on workers in three PVC production plants in Taiwan producing synthetic leather sheets and pellets (one PVC film factory, two pellet factories). P cess components in all three companies involved material tanks for DEHP, Banbury mixer, plastic roller machine, plastic granulator and product conveyor. The compounding activities took place mostly in closed systems, whereas plastic rolling and granulation were open processes. Temperatures in the mixers reached 170°C. Hot material was transferred to the next (open) processing steps (plastic rolling or granulation), leading to inhalation exposure via evaporation of DEHP. End products were moved on the conveyor and cooled to room temperature before packaging. None of the plants had installed a local exhaust ventilation system at the sites of processing. Workers wore neither gloves nor respirators.
The study included 89 male workers, 66 of them were categorised to be highly exposed (raw material processing), whereas 23 administrative staff were considered subject to low exposure, based on an analysis of working conditions and surroundings.

Geometric means of post-shift urinary concentrations of MEHP, 5OH-MEHP and 5oxo-MEHP in the high exposure group were 25.1, 97.1, and 77.4 µg/g creatinine, respectively, compared to 16.5, 57.1, and 42.8 µg/g creatinine in the low exposure group. From these urinary concentrations of the three metabolites external doses of 15.7 and 9.2 µg/kg bw/day for the high and low exposure group, respectively, can be calculated (with the procedure as described above). With a similar method the authors of the study calculated external doses of 14.0 and 7.6 µg/kg bw/day for the high and low exposure group, respectively, which demonstrates again that the calculation method applied here is conservative.
The exposure level of the high exposure group is lower than the highest level found in the Hines et al. and Dirven et al. studies, (22 and 23 µg/kg bw/day, respectively) and therefore are leading to even lower RCRs.

In addition to biomonitoring measurements the authors determined air concentrations at the workplaces by personal sampling. Geometric mean air concentrations were 32.7 and 5.27 µg DEHP/m^3 for the high and low exposure group, respectively. A strong correlation was found between air concentrations of DEHP and urinary metabolite concentrations of the high exposure group, indicating that air concentrations are good indic tors of overall exposure. Contribution of inhalation exposure to total exposure (as measured by biomonit ring) n the high exposure group was 20.8% and was significantly higher than for the low exposure group (4.8%) indicating that for this group other sources of DEHP intake (outside the workplace) were significant.
Activities at these three plants involved mixing/compounding as well as processing. Although workers were involved in both compounding and processing activities, it can be assumed that the open processing activities led to higher exposures compared to compounding. External doses calculated from th inary metabolite concentrations are well below the DNEL, even without applying RMMs as recomm nded in this CSR for formulation and processing.

Processes and companies covered

The data presented reflect operational conditions typical for the industry, both in relation to the amounts used and high temperatures during specific processes. For example, Dirven et al. (1993b) cover the mixing of DEHP, PVC granules and other additives into a PVC paste for cable production at a process temperature of 200 °C. Similarly, Hines et al. (1999) reported operating temperatures for mixing between 65 and 150°C for mixing in the companies investigated.

More generally, the biomonitoring data cover the two most important compounding (mixing) methods, namely the preparation of plastisols and dry-blending. A hird method (Banbury blending) is considered “rather obsolete, but occasionally associated with calendering (OECD, 2009).

The data can also be considered representative in terms of the type of companies included and operational conditions. For example, the company inv lved in plastisol coating consumed about 100 tons of DEHP per year (Gaudin et al., 2011) and can therefore b considered a small site according to the criterion of <250 t plasticiser per year in the OECD Emission Scenario Document on Plastic Additives (OECD, 2009). One of the “PVC compounding” companies, in contrast used about 3,000 t DEHP per year (Gaudin et al., 2011) and certainly represents a larger site.

One of the drawbac s of the data basis is the limited geographical coverage, with data from only two European countries and addition l data from the USA. Therefore, additional data on occupational inhalation exposure (i.e. DEHP concentrati s in workplace air) from European countries were surveyed and are summarised in Table 80. The data relate to processes that can primarily be described as “formulation”, although a clear distinction between fo mul tion and processing is often impossible (see 9.3.2 for additional data on processing). It is noteworthy that most of the data are at least two decades old and more recent data are only available from the German MEGA database (IFA, 2012; see below). The EU RAR (ECB, 2008) discusses some more data, which are even older and are not included here.

Table 80 Summary of DEHP concentrations in workplace air: formulation

Process (source)	No. of samples	No. of companies	DEHP concentration in air (µg/m³)			Location, Year
			Ambient sampling	Personal sampling		
			Max.	Max.	Mean	
Compounding (Vainiotalo and Pfäffli, 1990)	5	1			20	Finland, before 1990
Mixing, PVC boot production (Dirven et al., 1993b)	16	1		1214	261	The Netherlands, before 1993
Mixing, cable production (Dirven et al., 1993b)	8	1		809	180	The Netherl nd before 1993
Compounding (Protois et al., 2007)	12/10*	2	163	1889		France, 2005
Mixing (IFA, 2012)	23	12	36.8**			Germany, 2000-2011

*Number of ambient/personal samples;

** 95th percentile (maximum not reported), 90th percentile is 28.8 µg/m³; although th overall data are differentiated by ambient and personal sampling, the data structured by process ("mixing" reported here) are not.

These published data indicate that compounding as well as certain mixing and extrusion processes resulted in the highest exposures obtained with personal sampling. All these processes are covered by the biomonitoring data used in the exposure assessment, in the case of the Dutch study certainly and in the case of the French study possibly covering the same companies. The Dutch study is he only one that specifically measured DEHP concentrations in the air of mixing departments (differentiated from the extruding department).

Protois and co-workers (2007) realised that the higher number of ambient than personal samples contravened the principles of their usual procedures with a preference fo pers nal samples. However, they stated that this reflects the fact that the industrial sectors involv d are ha cterised by a high degree of automation with very few workers actually present on the work fl r

A survey of the European PVC process ng indust y involving 30 companies, largely confirmed these data on inhalation exposure. Generally, only individual samples were available and the highest concentration obtained for some mixing operations wa 1200 µg/m (Cadogan 2010), which is in line with the data reported in Table 80.

On the basis of these data, proce ses leading to the highest inhalation exposure are adequately covered by the biomonitoring data. The Dutch study found no clear correlation between the DEHP concentration in the air and metabolite concentrations in urine, but this may have been the result of the limited sampling duration for air monitoring (2-4 h) nd high i traday variation (Dirven et al., 1993b). From the data available it can be concluded that average air conc ntrations are below 300 µg/m³ during DEHP formulation.

Dermal exposure data re very scarce and only relate to exposure of the hands (e.g. some individual measurem nts performed by Protois et al. (2007)). These data do not allow a comprehensive assessment of dermal exposure and ultimately support the use of biomonitoring data in the exposure assessment of DEHP at the workplace.

Risk management measures covered

The biomonitoring data (and the inhalation exposure data) were obtained in companies typical of the polymer processing industry. The processes run in these companies can be considered to be representative of the industry.

Apart from these forms of containment typical for some of the processes, none of the biomonitoring studies employed in the exposure assessment (Dirven et al., 1993b; Gaudin et al., 2011; Hines et al., 2009) reported specific RMMs in place during the measurements, such as LEV. While it cannot be completely excluded that such measures were in place in some of the processes (or specific steps), these studies focused on individuals that the authors believed to have a high potential exposure. For example, Gaudin et al. (2011) conducted on-site visits before sampling in order to identify sectors within the plants where workers could be exposed and other with no exposure (serving as controls). Similarly, Hines et al. (2009) identified workers involved in processes

using DEHP as potentially exposed. Appropriate selection of those considered to be exposed was confirmed by Gaudin et al. (2011), who found higher metabolite concentrations in exposed workers compared to controls.

Hines et al. (2009) specifically addressed the relationship between glove use and metabolite concentrations in urine. They noted that chemical-resistant gloves were primarily worn during DEHP manufacture (89% of workers) and PVC film production (36%), implying that they are less often worn during compounding (i.e. formulation; specific figures, however, are not reported). This information suggests that the biomonitoring data reflect the practice of RMMs then in place and are not derived from an unrepresentative subset with an unusual high-level use of RMMs.

The operational conditions and risk management measures communicated in the exposure scenario above (in particular the handling of the substance within predominantly closed systems or under containment for almos all processes and the wearing of gloves for all processes) certainly represent more strict conditions compared to the ones present during the biomonitoring studies. It can therefore be assumed that the exposure will be lower th n the one estimated on the basis of the biomonitoring data.

The methodology applied here for the conversion of biomonitoring data to intakes yields o servative exposure estimates, as already discussed in section 9.0.4.3. The comparison with calculations performed by Hines et al. (2011) shows that our approach consistently results in higher estimates than the one by he author with both methods being based on geometric means.

The highest of the intakes calculated from the biomonitoring data (23 µg/kg bw/d, se Table 79 above) is chosen as the value taken to risk characterisation. Again, background exposure is included in the intake calculation, further adding to the conservatism of the approach.

Average exposure of industrial workers to DEHP from formulation is estimated to be 23 µg/kg bw/d, based on biomonitoring data.

(conservatively estimated 90th percentile of intake (see chapter 9.0.4.3): 92 µg/kg bw/d).

Exposure modelling for emptying bags with recycl te

As explained in chapter 9.0.4 two tasks specific to the use of recycled soft PVC, i.e. the emptying of either small or big bags of recycled soft PVC, which repres nts he first step in using recyclate material at the converter companies, might not be adequately covered by he biomonitoring data. Exposure during these tasks was therefore modelled using ART for inhal tion exp sure and the algorithm also used for consumers for dermal exposure (see section 9.0.4.).
In most cases, recyclate is delivered in big bags (1000 kg), which are emptied at the converters using automated bottom discharge systems (the ags are p aced on supports and are emptied by opening a valve at the bottom of the system) (PROC 8b). Alternativ ly big bags are emptied using aspiration system, where the material is sucked in under redu ed pressure. As the first option is related to potentially higher exposure, PROC 8b is modelled with ART as falling solid, by considering the conditions of the technical equipment of emptying big bags. As in some ca es also manually opening of small bags (25 kg) may occur, this activity is also modelled (PROC 8a).

Inhalati exp sure from these two activities (emptying bags of recycled soft PVC represented by PROC 8a and 8b, respect vely was modelled under the assumption of conditions of use and risk management measures as described in the exposure scenario in section 9.2.1 above. The estimates are shown in Table 82 below and full details re provided in Annex #4#.

Dermal exposure was calculated with the algorithm described in section 9.0.4.3, using a maximum migration rate for DEHP migration from PVC powder. The skin surface area in contact with the powder was assumed to be 10.2% of the total skin surface, roughly representing one half of both hands and both arms (see Annex #1#). This is considered to be a conservative assumption. A default working week (8 h/d, 5 d/w) was assumed. The results are shown in the following table. Exposure to the bare skin is assumed, therefore no clothing factor is applied.

Table 81 Dermal exposure to DEHP from emptying bags of recycled soft PVC

Calculation	Parameter	Value	Unit	Source
	Migration rate (maximum)	0.83	µg/cm^2 x h	Derived below
multiplied by	Total skin surface area/body weight (mean)	280 (adults)	cm^2/kg b.w.	Derived below
multiplied by	Fraction of total skin surface area in contact	0.102	dimensionless	ES-specific
multiplied by	Contact time	8	h	ES-specific
multiplied by	Contact frequency	0.714	/d	ES-specific
results in	External exposure	0.135	mg/kg b.w. x d	

Table 82 Modelled DEHP exposure for emptying small (PROC 8a) or big bags (8b) of recycled soft PVC

	PROC 8a	PROC 8b
Inhalation exposure [mg/m^3] (90th percentile)	1.3	0.48
Dermal exposure [mg/kg b.w. x d] (based on maximum migration rate)	0.135	0.135

These estimates are conservative because

- inhalation exposure
  - was estimated using the 90th percentile,
  - assumes that DEHP is freely available from the PVC matrix and
  - a high drop height (> 0.5 m) was assumed also for PROC 8a, for which near field exposure (> 1 m distance to the source) was modell d
- dermal exposure
  - assumed the maximum migration rate observed in any of the studies using PVC articles (with simultaneously application of sun tion (see section 9.3.2.4.2 for the conservative nature of this figure) and
  - assumed a high skin surface area in contact with the soft PVC powder
- both inhalation and dermal exposure assumed continuous exposure for 8 h/d on 5d/w.

9.3. Use #2: Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles

9.3.1. Exposure scenario

9.3.1 ES2: Industrial use of recycled soft PVC containing DEHP in polymer processing by calenderi g, extrusion, compression and injection moulding to produce PVC articles
Use descriptors related to the life cycle stage and all the uses under it: SU 3, 12 – PC 32 – PROC 2, 3, 4, 6, 8a, 8b, 14, 21 – AC 13
List of names of contributing worker scenarios: ES2-W: Industrial use of recycled soft PVC containing DEHP in polymer processing by cale dering, extrusion, compression and injection moulding to produce PVC articles (PROC 2, 3, 4, 6, 8a, 8b 14, 21) ES2-SL-P: Service life professionals: Professional handling of PVC products made from recyclate: Installation of building materials and similar activities (PROC21) / inhalation exposure from volatile DEHP / professional PVC footwear) (no PROCs)
List of names of contributing consumer scenarios: ES2-SL-C: Service life consumers: Exposure from consumer articles made from recycled soft PVC containing DEHP (AC13)
9.3.1.1 Sub-scenarios exposure scenario controlling environmental exposure
not relevant
9.3.1.2 Contributing exposure scenario ES2-W contr ll ng worker exposure
Industrial use of recycled soft PVC containing DEHP n polymer processing by calendering, extrusion, compression and injection moulding to produc PVC rt les (PROC 2, 3, 4, 6, 8a, 8b, 14, 21) Covers the transferring and processing of formula ions containing DEHP as well as equipment cleaning, and associated maintenance activities. Includes low nergy manipulation of the resulting polymers
Product characteristic
Physically dispersed in the so id formulation (PVC) Solid, low dustiness [OC1]. Typical concentration in recycled oft PVC [redacted] Limit the substanc in product to 20 % [OC21]
Amounts used
The amoun s used are n t critical for the exposure estimate. The biomonitoring studies used as a basis for the exposure estimate (e section 9.3.2) represent a wide-range of companies of different sizes. For mod lling exposure from emptying bags with recycled soft PVC material the following assumptions were m de: PROC 8a: 25 kg per bag PROC 8 : 1000 kg per bag.
Frequency and duration of use/exposure
The frequency and duration of exposure represents typical working patterns in industry (8 h/d, 5d/w). The biomonitoring studies used as a basis for the exposure estimate (see 9.3.2) typically collected pre- and post-shift samples during a normal working week of five days. Covers daily exposures up to 8 hours (unless stated differently) [G2]
Human factors not influenced by risk management
The biomonitoring studies used as a basis for the exposure estimate (see 9.3.2) reflect exposure from all pathways. For PROCs 8a and 8b modelling was performed for inhalation and dermal exposure.

Other given operational conditions affecting workers exposure

The biomonitoring studies used as a basis for the exposure estimate (see 9.3.2) reflect operational conditions typical for the industry, including higher process temperatures during certain processes.

Assumes a good basic standard of occupational hygiene is implemented [G1].
Provide a good standard of general ventilation (not less than 3 to 5 air changes per hour) [E11].
Assumes industrial (unless stated differently) [G26]
Assumes activities are at ambient temperature (unless stated differently) [G17].
For calendaring, compounding, extrusion, compression and injection moulding: some operations are carried out at elevated temperature in closed systems: up to 180°C. Some activities involving operator's action take place at temperatures higher than ambient: the maximum temperature in those cases is estimated to be at 40°C.

Technical conditions and measures at process level (source) to prevent release

The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use typical for th industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from which the biomonitoring data were obtain d (see 9.0.4 3).

Process	*PROC*	*Operational Conditions / Risk Management Me sures*
General exposures (closed systems); Indoors with LEV	2	Solid, low dustiness [OC1]. Handle substance within predominantly closed system provided with ventilation [E49]. We r chemically resistant gloves (tested to EN374) in combination w th basi mployee training [PPE16]
Batch closed process exposures; Indoors with LEV	3	Solid, low dustiness [OC1]. Handle substance within predominantly closed system provided with ventilation [E49]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16]
Batch process exposures; Indoors with LEV	4	Solid, low dustiness [OC1].Handle substance within predominantly closed system pr vided with ventilation [E49]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16]
Calendering operations, Indoors with LEV	6	Solid, l w dustiness [OC1].Handle substance within predominantly closed sys em provided with ventilation [E49]. Wear chemically resistant gloves (sted to EN374) in combination with 'basic' employee training PPE16]
Emptying bags of recycled soft PVC; Manual; Non-dedicated facility; Indoors with LEV	8a	S lid, low dustiness [OC1]. Provide extract ventilation to material transfer points and other openings [E82]. Do not transfer more than 1 0 kg/minute. Use in room with a volume of minimum 300 m^3.
Emptying bags of recycled s f PVC; Dedicated facility Ind ors with LEV	8b	Solid, low dustiness [OC1]. Ensure material transfers are under containment or extract ventilation [E66]. Do not transfer more than 1000 kg/minute.Use in room with a volume of minimum 300 m^3. Ensure that the distance from worker to task is greater than 1 m.
Extr sion, tabletting, com ression, pelletisation operations, Indoors with LEV	14	Solid, low dustiness [OC1]. Avoid carrying out activities involving exposure for more than 2 hours. Handle substance within a predominantly closed system provided with extract ventilation [E49]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].
Low energy manipulation of articles such as cutting, welding, gluing, Indoor with LEV	21	Solid, low dustiness [OC1]. Handle substance within a predominantly closed system provided with extract ventilation [E49]. Wear chemically resistant gloves (tested to EN374) in combination with 'basic' employee training [PPE16].

Technical conditions and measures to control dispersion from source towards the worker
The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3).
Organisational measures to prevent/limit releases, dispersion and exposure
The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the working conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0.4.3).
Conditions and measures related to personal protection, hygiene and health evaluation
The biomonitoring studies used as a basis for the exposure estimate reflect conditions of use typical for the industry at the time. Conditions of use communicated in this exposure scenario are stricter than the worki g conditions, which were in place at the sites from where the biomonitoring data were obtained (see 9.0 4 3).
Additional good practice advice beyond the REACH CSA
None
9.3.1.3 Contributing scenario ES2-SL-P controlling worker exposure
ES2-SL-P: Service life professionals: *Professional handling of PVC products m de from recycled soft PVC containing DEHP: Installation of building materials and similar activities (PROC21) / inhalation exposure from volatile DEHP / professional PVC footwear) (no PROCs)* Professional handling of PVC products containing *recycled soft PVC containing DEHP*: Covers installation of building materials and similar activities (PROC 21), while working in workshops with large PVC surfaces (inhalation exposure to volatile DEHP) and simultaneously wearing PVC footwear (e.g. waterproof boots, clogs) (no PROC)
Handling of PVC products containing DEHP by craftsm n, such as the installation of roofing. Typical tasks include manual cutting, assembling and disassembling soft PVC articles, which are considered to lead to dermal exposure only. The same craftsmen are assumed to wea PVC footwear (e.g. waterproof boots) and additional inhalation exposure may exist in specific situations, g. for workers occupied in rooms with large PVC surfaces, such as large-scale PVC flooring or polytunnels. These exposures are primarily relevant for professional workers, but similar situations may exist for industrial workers and are therefore also covered by this scenario. DEHP is only contained in plasticised PVC articles (containing recycled soft PVC), which are not handled by dust-generating processes, also describ d by PROC 21.
Article characteristic
Articles handled are made of PVC (pla tic). This contributing scenario covers the handling of PVC articles containing recycled soft PVC primarily by craftsmen, such as roofing and flooring products, technical profiles and gaskets as well as waterproofing and insulation membranes. It also covers PVC a ticles containing soft PVC recyclate handled by workers in other professions, e.g. roadmen, landsc pe gardeners and shop assistants, such as: outdoor products (e.g. garden hoses and tubes), automotive and traffic-related products (e.g. traffic cones and components of traffic signs) and many other items (e.g stall mats for cows and vehicle splash guards, as exposure (in terms of skin surface exposed and/or exposur duration/frequency) is obviously lower compared to daily dermal contact for several hours to building mater als by craftsmen. This con ributing scenario includes waterproof footwear (boots with inner lining and worn with socks), as worn e.g. by professional craftsmen,. It also covers non-waterproof footwear, such as clogs and safety shoes worn in the professional environment, e.g. by hospital staff, pool attendants etc.(worn against bare skin), The possibility of dermal exposure to DEHP from articles is assessed with a DEHP-specific migration rate of 0.27 µg/cm^2 x h (95th percentile, see Annex #1#). The possibility of DEHP inhalation exposure from articles is assessed with the saturated vapour concentration (SVC) of DEHP of 5.3 µg/m^3 (ECB, 2008).
Amounts (contained in articles) present at workplace
This parameter is not critical for the exposure assessment, since this is based on the migration rate (dermal exposure) and the SVC (inhalation exposure).
Frequency and duration of use/exposure

Covers daily exposures up to 8 hours (unless stated differently) [G2].
Human factors not influenced by risk management
Exposure of the inside of both hands is assumed for dermal exposure when handling PVC articles containing recycled soft PVC. Exposure of the entire feet and one half of the legs is assumed when wearing PVC boots.
Other given operational conditions affecting workers exposure
Assumes use at not more than 20°C above ambient temperature, unless stated differently [G15].
Conditions and measures at level of article production to prevent release during service life
None
Technical conditions and measures to prevent release (at source) from processing of articles
None
Technical conditions and measures to control dispersion from source towards the worker
None
Organisational measures to prevent /limit releases, dispersion and exposure
None
Conditions and measures related to personal protection, hygiene and health evaluation
Workers are considered to wear socks when wearing boots made of PVC. Furthermore, boots are manufactured with an inner textile lining. Both conditions result in the application of a clothing penetration factor (see Annex #1#).
Additional good practice advice beyond the REACH CSA
None
9.3.1.4 Contributing scenario ES2-SL-C controlling consumer exposure
ES2-SL-C: Service life consumers: Exposure from c nsumer articles made from recycled soft PVC containing DEHP
ES2-SL-C covers dermal exposure from consum r contact with PVC articles containing recycled soft PVC. For some PVC articles, oral exposure (primarily by mo thing) is addressed as well. Exposure is estimated with a sentinel product approach (see 9.0.5.4 with the ollowing 4 groups (X-1 - X-4) and their sentinel articles: X-1: Gym mats, X-2: Handles, X 3: Pla tic sandals , X-4: Seating for outdoor use. The specific articles covered by each group are mentioned in the exposure estimation section (see 9.3.2.4). On all accounts, this application does not include the uses of DEHP to produce the following PVC articles: - erasers - adult toys (ex toys and other articles for adults with intensive contact with mucous membranes) - small (<10 cm) PVC items available in the home environment (without attachment to larger objects), which can be swall wed by small children - textiles/clothing with direct contact to large skin surfaces (worn on bare skin) This scenari als co ers oral (via house dust) and inhalation exposure in the indoor environment that cannot be attributed to specific articles.
Article haracteristic
Articles are made of soft PVC containing soft PVC recyclate (plastic). The possibility of dermal exposure to DEHP from consumer articles is assessed with a DEHP-specific migration rate of 0.27 µg/cm^2 x h (95th percentile). The possibility of oral exposure to DEHP from articles is assessed with a DEHP-specific migration rate of 3.65 µg/10 cm^2 x min (95th percentile); see 9.0.5.4 and Annex #1# for details.
Amounts (contained in articles) present at workplace
This parameter is not critical for the exposure assessment. Exposure assessment is based on biomonitoring data from studies in the general population and therefore reflects current practice of use of consumer articles without specific restrictions or conditions. Furthermore, for specific article groups (and their sentinel products), exposure modelling is performed, which is based on the migration rate.

Frequency and duration of use/exposure
Exposure assessment is based on biomonitoring data from studies in the general population and therefore reflects current practice of use of consumer articles. For exposure modelling of specific article groups, frequency and duration of exposure differ depending on the specific article group (X-1 – X-4). Values for these parameters have been derived to represent typical conditions for the European population and are discussed in detail in section 9.3.2.4.
Human factors not influenced by risk management
Exposure assessment is based on biomonitoring data from studies in the general population and therefore reflects current practice of use of consumer articles. For exposure modelling of specific article groups, human factors (primarily the skin surface area assumed to be in contact with the article) differ depending on the specific article group (X1 – X4). Values for dermal and oral exposure determinants have been derived to represent typical conditions for the European population and are discussed in detail in section 9.3.2.4. In the exposure modelling exercise, three age groups (adults, children 6-12 months old, children 2-3 years old) are considered to reflect potentially high exposures. The age groups of children were selected based on high skin surface area per kg body weight and high mouthing durations (see Annex #1# for details).
Other given operational conditions affecting consumer exposure from article service life
None
Conditions and measures at level of article production to prevent release during service life
None
Conditions and measures related to information and behavioural advice to consumers
None
Conditions and measures related to personal protective equipment and hygiene
None
Additional good practice advice beyond the REACH CSA
None

9.3.2. Exposure estimation

9.3.2.1. Environmental exposure

Not relevant for this application for authorisation.

9.3.2.2. Humans via the environment

Based on the biomonitoring results reported in section 9.3.2.4, the overall intake of the general population from all sources is calculated to be 3.57 µg/kg bw/day (geometric mean) (90th percentile: 10.29 µg/kg bw/day) for children and 2.8 µg/kg bw/day (geometric mean) (90th percentile: 8.75 µg/kg bw/day) for adults, based on biomonitoring data from the pan-European project DEMOCOPHES.
These data will also be used for assessing exposure via the environment. As the biomonitoring data include contributions from all sources, among them exposure of humans via the environment, exposure via the environment can be considered to be adequately controlled, if the combined intake from all sources is below the DNEL systemic long-term.

It is reasonable to assume that only a small part of exposure of humans via the environment is attributable to the production and use of recyclate products.

9.3.2.3. Workers exposure

9.3.2.3.1 ES2-W: Industrial use of recyclate in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles

Exposure of industrial workers during polymer processing has been assessed using biomonitoring data from a variety of different workplaces. The studies were conducted in France (Gaudin et al., 2011), the USA (Hines et al., 2009) and The Netherlands (Dirven et al., 1993b). The datasets with more details on these studies are documented in Annex #2#. Some of these data – but not all (e.g. polymer moulding and vehicle filter manufacturing) – have already been discussed for formulation (see 9.2.2), since mixing and processing often take place in the same plant and cannot clearly be distinguished in the context of the biomonitoring data.

The approach taken is explained in sections 9.0.4.2-9.0.4.3 above. Briefly, the sum of geometric means for all metabolites was converted to intakes, using the post-shift metabolite concentrations measured in urine. When samples from different days of the week were available (Dirven et al., 1993b), the end-of-week post-shift samples were used in the calculation of intake levels, to cover any potential build-up during the week.
The results of this evaluation are shown in the Table below, together with information on the types of processes covered.

Table 83 Summary of DEHP intakes calculated from biomonitoring data during polymer processing by the Aylward/Anderson method

Source		**Gaudin et al., 2011**	**Hines et , 2009**	**Dirven et al., 1993b ****
Type of setting (no. of samples/no. of workers)	**Processing steps covered**	**Intake (µg/kg bw/d)***		
Plastisol coating (25/5)	Plastisol coating, including preparation of Plastisols	11		
PVC 1 compounding (41/9)	Dry blending, ext uding, milling, cooling and pa kaging	17		
PVC 2 compounding (31/5)		4.7		
PVC compounding (12/12)	Mixing extruding and milling		16	
Polymers moulding (41/9)	Moulding to produce flexible pipes for ve icles	4.8		
Wall coverings products (78/1)	Pr nting flat vinyl and expanded vinyl wallpaper with inks containing plastisols	13		
PVC film production (25/25)	Compounding, mixing, paste preparation, extrusion, milling and calendering		22	
PVC boot prod ction (9/9)	Mixing, extrusion, cooling and storage			23
Cabl production (6/6)	Mixing, Granulating, extrusion			13
Vehi le filter manufacturing (18/18)	Dispersing plastisol		5.7	

* Intake based on creatinine-adjusted geometric means of post-shift biomonitoring data; intakes are rounded to two significant figures.

** geometric means were not reported by Dirven et al. (1993b) and median values were used instead;

The intakes derived range from 4.4-23 µg DEHP/kg bw/d and involved 116 workers with 286 samples from 10 different plants. The results will be discussed in relation to the representativeness of the underlying data in the following sections. (Although Plastisol use is not relevant for processing recycled soft PVC, the respective results are presented in the table above for comparison.)

As discussed in chapter 9.2.2, a new study by Fong et al. (2013) presents the results of a biomonitoring study on

workers in three PVC production plants in Taiwan, producing synthetic leather sheets and pellets (one PVC film factory, two pellet factories). Both mixing and processing activities were involved at all sites. Details on the study can be found in section 9.2.2. Geometric means of post-shift urinary concentrations of MEHP, 5OH-MEHP and 5oxo-MEHP in the high exposure group were 25.1, 97.1, and 77.4 µg/g creatinine, respectively, compared to 16.5, 57.1, and 42.8 µg/g creatinine in the low exposure group of administrative rather than plant workers. From these urinary concentrations of the three metabolites, external doses of 15.7 and 9.2 µg/kg bw/day for the high and low exposure group, can be calculated (with the procedure described above). Using a similar method, the authors of the study calculated external doses of 14.0 and 7.6 µg/kg bw/day for the high and low exposure group, respectively, which demonstrates again that the calculation method applied here is conservative. In addition to biomonitoring measurements, the authors determined air concentrations at the workplaces by personal sampling. Geometric mean air concentrations were 32.7 and 5.27 µg DEHP/m^3 for the high and low exposure group, respectively. A strong correlation was found between air concentrations of DEHP and urinary metabolite concentrations of the high exposure group, indicating that air concentrations are good indi at rs of overall exposure. The contribution of inhalation exposure to total exposure (as measured by biomonitoring) in the high exposure group was 20.8% and was significantly higher than for the low exposure group (4.8% , indicating that for this group other sources of DEHP intake (outside the workplace) were significant

Activities at these three plants involved mixing/compounding as well as processing. Although workers were involved in both compounding and processing activities, it can be assumed that the open processing activities led to higher exposures compared to compounding. External doses calculated from th urinary metabolite concentrations are in the range of results from other biomonitoring studies, although it is noteworthy, that body burdens are lower than those measured by Hines et al. (2009) for film production. They are well below the DNEL, even without applying RMMs as recommended in this CSR for formulation and processing.

Processes and companies covered

The biomonitoring data cover a wide range of different processing steps, such as plastisol coating, extrusion, calendering and moulding. The data reflect operational conditions typical for the industry, both in relation to the amounts used and high temperatures during specific pr cesses For example, Dirven et al. (1993b) cover temperatures up to 170°C in boot production and Gaudin t al. (2011) cover temperatures up to 120°C for compounding and 140°C for polymer moulding. Simi rly, Hines et al. (1999) cover various processes with elevated temperatures, e.g. >150 °C for calend ring extr sion and milling operations. These authors also noted that the milling and calendering operations in their study involved heated materials with large surfaces, thus potentially leading to high exposures.

More generally, the data cover calender ng and p astisol coating operations, which are considered open processes, while others are only partly open (e g. some extrusion processes) or closed (moulding) processes (OECD, 2009). In addition, all he proce ses treated in this document as the major types of processing of plasticised polymers (calendering, extrus on, plastisol and moulding applications) are covered by the biomonitoring data, while other types are characterised as “small to very small applications” (OECD, 2009). Nonetheless these will be discussed below in the context of data on inhalation exposure.

Injection moulding, compression moulding and extrusion blow moulding are common processes used at various plants when processing PVC recyclate. While “polymer moulding” is covered by the data reported by Gaudin et al. (2011), th specific type of moulding process is not identified. Nonetheless, all moulding processes are considered clo ed processes in OECD (2009). It is therefore reasonable to assume that exposure during these moulding ctivi ies is not higher (and most probably much lower) than the highest exposure evident in the biomonit ring data during open or semi-open processes, such as extrusion.

At many ites, production waste is recycled at the same site. In general, it is grinded before reuse for processing nto ar icles. The grinding of production waste leads to the generation of coarse material. Due to the nature of soft PVC, grinding will not lead to the generation of fine dusts. Exposure resulting from these types of operations is therefore expected to be low and is certainly lower compared to the ones covered by the highest biomonitoring values.

As discussed in section 9.2.2.3, the data can also be considered representative in terms of the type of companies included and operational conditions. For example, the company involved in plastisol coating consumed about 100 tons of DEHP per year (Gaudin et al., 2011) and can therefore be considered a small site according to the criterion of <250 t plasticiser per year in the OECD Emission Scenario Document on Plastic Additives (OECD, 2009). One of the “PVC compounding” companies, in contrast, used about 3,000 t DEHP per year (Gaudin et al., 2011) and certainly represents a larger site.

One of the drawbacks of the data basis, as discussed in section 9.2.2.3, is the limited geographical coverage, with data from only two European countries and additional data from the USA. In addition, despite the wide variety of polymer processes covered by the biomonitoring data, there may be some other processes not covered. For these reasons, additional data on occupational inhalation exposure (i.e. DEHP concentrations in workplace air) from European countries were surveyed and are summarised in the table below. It is noteworthy that most of the data are at least two decades old, but more recent data are available from the German MEGA database (see below). The EU RAR (ECB, 2008) discusses some more data, which are even older and are not included here.

Table 84 Summary of DEHP concentrations in workplace air

Process (source)	No. of samples	No. of companies	DEHP concentration in air (µg/m³)			Location, Year
			Ambient sampling	Personal sampling		
Primarily formulation			**Max.**	**Max.**	**Mean**	
Compounding (Vainiotalo and Pfäffli, 1990)	5	1			20	Finland, before 1990
Mixing, PVC boot production (Dirven et al., 1993b)	16	1		1214	261	The Netherlands, before 1993
Mixing, cable production (Dirven et al., 1993b)	8	1		809	180	The Netherlands, before 1993
Compounding (Protois et al., 2007)	12/10*	2	163	1889		France, 2005
Primarily processing						
Injection moulding (Vainiotalo and Pfäffli, 1990)	2	1			20	Finland, before 1990
Injection moulding (Protois et al., 2007)	18 / 4	2	424	180		France, 2005
Extrusion (Vainiotalo and Pfäffli, 1990)	4	1			50	Finland, before 1990
Extrusion (Vainiotalo and Pfäffli, 1990)	5	1			300	Finland, before 1990
Extrusion, PVC boot production (Dirven et al., 1993b)				278	120	The Netherlands, before 1993
Extrusion, cable production (Dirven et al., 1993b)				1266	239	The Netherlands, before 1993
Extrusion (calendering, blowing, profiling) (Protois et al., 2007)	29 / 10	4	2470	265		France, 2005
Calendering (Vainiotalo and Pfäffli, 1990)	7	1			500	Finland, before 1990
Plastic processing, e.g. calendering, extrusion, bonding, plastic welding (HVBG, 1999)	85	31	5930 80			Germany, 1991-1995 (95th percentile and median)§
Plastic industry, manufacture (IFA, 2012)	113	36	708			Germany, 2000-2011 (95th percentile)** §

Plastic industry, processing (IFA, 2012)	142	42	944			Germany, 2000-2011 (95th percentile)** §
Hot embossing (Vainiotalo and Pfäffli, 1990)	5	1			50	Finland, before 1990
Welding (Vainiotalo and Pfäffli, 1990)	4	1			300	Finland, before 1990
Thermoforming (Vainiotalo and Pfäffli, 1990)	2	1			20	Finland, before 1990
High-frequency welding (Vainiotalo and Pfäffli, 1990)	2	1			<20	Finland, befo e 19 0
Thermal welding (Protois et al., 2007)	11 / 5	2	40	50		Fran e, 2005

*Number of ambient/personal samples;

** the median is not reported since more than 50% of the values are below the limit f detection; § although the overall data are differentiated by ambient and personal sampling, the data structured by industrial sectors are not.

These published data indicate that compounding as well as certain mixing and extrusion processes resulted in the highest exposures obtained with personal sampling. All these processes are covered by the biomonitoring data used in the exposure assessment, in the case of the Dutch study certainly and in the case of the French study possibly covering the same companies. Protois and co-workers (2007) realised that the higher number of ambient than personal samples contravened the principles of thei usual procedures with a preference for personal samples. However, they stated that this reflects the fact the industrial sectors involved are characterised by a high degree of automatisation with very few workers actu lly pr sent in the workshops.

A survey of the European PVC processing indu try inv lv ng 30 companies, largely confirmed these data on inhalation exposure. Generally, only individ al samples were available for specific processes with highest concentrations obtained for some mixing (1200 µg/m^3) and calendering (2000 µg/m^3) operations, which are in line with the maximum values reported

The German data clearly indicate a decline in the 95th percentile of DEHP concentrations in workplace air from 5930 (sampling 1991-1995) to ess than 000 µg/m^3 (2000-2011). The 90th percentiles in the industrial sectors shown above decreased over the ame p riods from 2450 to less than 500 µg/m^3 (HVBG, 1999; IFA, 2012), as shown in Table 84 and Table 85. This finding is important, since the underlying data were obtained with the same methodology, i.e. the one employed by the German Social Accident Insurance Institutions ("Berufsgenossenschaften" in German) and collected in the MEGA database ("Messdaten zur Exposition gegenüber Gefahrstoffen am Arbeitsplatz" in German).

The most recent evalu tion of the data contained in the MEGA database (IFA, 2012) presented several degrees of differen iation for the sampling period 2000-2011. The data representative for whole-shift (>6h) exposures are pre ented n the able below. Limits of quantifications in these measurements were between 13 and 60 µg/m^3.

Table 85 DEHP concentrations at German workplaces 2000-2011 – by type of sampling and industrial sector (evaluation of the MEGA database, IFA, 2012)

	Number of measurements/companies	Measurements < LOQ (%)	Median (µg/m^3)	90th percentile (µg/m^3)
All data combined	533/206	59.8	- (more than 50% of values below LOQ)	229
Differentiated according to type of sampling				
Personal sampling – without extended data collection*	76/51	59.2	- (more than 50% of values below LOQ)	104
Personal sampling – with extended data collection	116/41	53.4	- (more than 50% of values below LOQ)	219
Ambient air – without extended data collection	120/74	61.7	- (more than 50% of values below LOQ)	96
Ambient air – with extended data collection	122/62	59.0	- (more than 50% of values below LOQ)	338
Differentiated according to industrial sector				
Production and processing of rubber	27/10	44.4	15	183
Metal industry	28/14	46.4	14	132
Electrical engineering / electrical industry	41/19	85.4	- (more than 50% of values below LOQ)	24
Construction industry	22/13	95.5	- (more than 50% of values below LOQ)	- (more than 95% of values below LOQ)
Wood processing	17/4	58.8	- (more than 50% of values below LOQ)	116
Textile industry	32/19	56.3	- (more than 50% of values below LOQ)	252
Plastic industry, manufacture	113/36	46.9	17	464
Plastic industry, processing	142/42	59.2	- (more than 50% of values below LOQ)	273
Other sectors	111/56	65.8	- (more than 50% of values below LOQ)	109

* It is not quite clear from the publication to what the German term “Erfassung” (collection, compilation, gathering of information/data) refers to. However, MEGA data are often obtained from measurements that also collect information on many other conditions, e.g. on the specific company, air exchange rates, type of ventilation etc. Therefore, it can be assumed that the differentiation refers to whether these types of data have been collected or not in addition to the actual air monitoring

Table 86 DEHP concentrations at German workplaces 2000-2011 – various industrial activities relevant to the plastics industry (evaluation of the MEGA database, IFA, 2012)

	Number of measurements/companies	Data < LOQ (%)	Median (µg/m^3)	90th percent le (µg/m^3)
Extrusion	63/37	76.2	- (more than 50% of values below LOQ)	76.5
Bonding	14/6	28.6	- (more than 50% of values below LOQ)	358
Calendering	44/10	22.7	86	904
Welding of plastics	80/33	43.8	26	540
Storage	10/8	80.0	17	14
Mixing	23/12	69.6	- (more than 50% of values below LOQ)	28.8
Packaging, foil shrieking	12/4	66.7	- (more than 50% of values below LOQ)	24
Other activities	157/85	72.6	- (more than 50% of values below LOQ)	65.9

On the basis of the more recent German data, calendering leads to the highest inhalation exposure (90th percentile: 904 µg/m^3), a find ng that is supported by the French data (although many different processes are summarised in one f gure). This is not surprising since calendering operations are typically carried out in open processes (OECD, 2009). Calendering operations are adequately covered by the biomonitoring data. The latter result in a cal ulat d DEHP intake for processes involving calendering (22 µg/kg bw/d) that are practically identi al t the highest DEHP intake calculated from biomonitoring data (23 µg/kg bw/d) related to various other pro esses, uch s mixing and extrusion (Exposure of industrial workers during polymer processing has been asses ed using biomonitoring data from a variety of different workplaces. The studies were conducted in France (Gaudin et al., 2011), the USA (Hines et al., 2009) and The Netherlands (Dirven et al., 1993b). The datasets with m re details on these studies are documented in Annex #2#. Some of these data – but not all (e.g. polymer mould ng and vehicle filter manufacturing) – have already been discussed for formulation (see 9.2.2), since mixing and processing often take place in the same plant and cannot clearly be distinguished in the context of the biomonitoring data.

The approach taken is explained in sections 9.0.4.2-9.0.4.3 above. Briefly, the sum of geometric means for all metabolites was converted to intakes, using the post-shift metabolite concentrations measured in urine. When samples from different days of the week were available (Dirven et al., 1993b), the end-of-week post-shift samples were used in the calculation of intake levels, to cover any potential build-up during the week.
The results of this evaluation are shown in the Table below, together with information on the types of processes covered.

Table 83). While these latter processes show comparatively low DEHP concentrations in workplace air in the most recent German data, the high DEHP intakes calculated from biomonitoring data may reflect a higher dermal exposure.

The Dutch biomonitoring study found no clear correlation between the DEHP concentration in the air and metabolite concentrations in urine, but this may have been the result of the limited sampling duration for air monitoring (2-4 h) and high intraday variation (Dirven et al., 1993b). From the data available it can be concluded that even two decades ago average air concentrations were below 500 µg/m^3 during DEHP processing, and are expected to be well below 300 µg/m^3 these days. Maximum values from personal sampling in general do not exceed 2000 µg/m^3.

Dermal exposure data are very scarce and only relate to exposure of the hands (e.g. some individual measurements performed by Protois et al. (2007)). These data do not allow a comprehensive assessm nt of dermal exposure and ultimately support the use of biomonitoring data in the exposure assessment of DEHP a the workplace.

Risk management measures covered

The biomonitoring data (and the inhalation exposure data) were obtained in companies ypical of the polymer processing industry. The processes run in these companies can be considered to be representative of the industry, i.e. some processes are known to be closed processes, while others are semi-open r open p cesses (see above and OECD (2009).

Apart from these forms of containment typical for some of the processes, n ne of the biomonitoring studies employed in the exposure assessment (Dirven et al., 1993b; Gaudin et al., 201 ; H nes et al., 2009) reported specific RMMs in place during the measurements, such as LEV. While it cannot be completely excluded that such measures were in place in some of the processes (or specific processing steps), these studies focused on individuals that the authors believed to have a high potential exp sure For example, Gaudin et al. (2011) conducted on-site visits before sampling in order to identify sectors within the plants where workers could be exposed and other with no exposure (serving as controls) Similarly, Hines et al. (2009) identified workers involved in processes using DEHP as potentially exp sed. Appropriate selection of those considered to be exposed was confirmed by Gaudin et al. (2011) who fo nd higher metabolite concentrations in exposed workers compared to controls.

Hines et al. (2009) specifically addressed the rel tion hip between glove use and metabolite concentrations in urine. They noted that chemical-resistant gloves were primarily worn during DEHP manufacture (89% of workers) and PVC film production (36%) implying that they are less often worn during other types of processing (although specific f gures are not reported). These authors did not observe an effect of glove use (yes vs. no) on post-shift metabolite concentr tions during PVC film production, but this finding is somewhat limited due to the relatively small sample size Nonetheless, these data suggest that the biomonitoring data reflect the practice of RMMs then in place and are not derived from an unrepresentative subset with an unusual pattern of high-level RMMs.

The operational cond ions and risk management measures communicated in the exposure scenario above (in particular the handling of the substance within predominantly closed systems with extract ventilation for almost all processes and he wearing of gloves for all processes) certainly represent more strict conditions compared to the ones presen during the biomonitoring studies. It can therefore be assumed that the actual exposure will be low r than the one estimated on the basis of the biomonitoring data.

The m thodology applied here for the conversion of biomonitoring data to intakes yields conservative exposure estimates, as already discussed in section 9.0.4.3. The comparison with calculations performed by Hines et al. (2011) show that our approach consistently results in higher estimates than the one by the authors, with both methods being based on geometric means. The highest of the intakes calculated from the biomonitoring data (23 µg/kg bw/d, see above) is chosen as the value taken to risk characterisation. Again, background exposure is included in the intake calculation, adding to the conservatism of the approach.

Average exposure of industrial workers to DEHP from polymer processing is estimated to be 23 µg/kg bw/d.
(conservatively estimated 90th percentile of intake (see chapter 9.0.4.3): 92 µg/kg bw/d).

Exposure modelling for emptying bags of recycled soft PVC

As explained in chapters 9.0.4 and 9.2.2.3 two tasks specific to the use of recycled soft PVC, i.e. the emptying of either small or big bags of recycled soft PVC, which represents the first step in using recyclate material at the converter companies, are not or not adequately covered by the biomonitoring data. Exposure from these tasks was therefore modelled using ART for inhalation exposure and the algorithm also used for consumers for dermal exposure (see section 9.0.4.).
In most cases, recyclate is delivered in big bags (1000 kg), which are emptied at the converters using automated bottom discharge systems (the bags are placed on supports and are emptied by opening a valve at the bottom of the system) (PROC 8b). Alternatively, big bags are emptied using aspiration system, where the material is sucked in under reduced pressure. As the first option is related to potentially higher exposure, PROC 8b is modelled with ART as falling solid, by considering the conditions of the technical equipment of emp ying big bags. As in some cases also manually opening of small bags (25 kg) may occur, this activity is also modell d (PROC 8a).

Details of the modelling assumptions were already described in section 9.2.2.3 and the inhal tion expo ure modelling is documented in Annex #4#. The modelling results are identical to those already pre ented in section 9.2.2.3 and are repeated here for ease of comparison.

Table 87 Modelled DEHP exposure for emptying small (PROC 8a) or big bags (8b) of recycled soft PVC

	PROC a	PROC 8b
Inhalation exposure [mg/m^3] (90th percentile)	1.3	0.48
Dermal exposure [mg/kg b.w. x d] (based on maximum migratio rate	0.135	0.135

These estimates are conservative for several reasons (see the discussion in section 9.2.2.3).

9.3.2.3.2 ES2-SL-P: Professional handling of PVC pr ucts made from recycled PVC containing DEHP: Installation of building materials and simil r activities / inhalation exposure from volatile DEHP / professional PVC footwear)

As outlined in section 9.0.4.4 abo e, this scenario covers dermal exposure from handling PVC products and wearing PVC footwear as well as inhalation exposure (see below). PROC21 was assigned to the activities in the scenario, because handling of these products involves activities such as manual cutting (e.g. of PVC flooring material) as well as assembly/dis ssembly of articles (e.g. of extruded technical profiles and gaskets), as described in the ECHA Guidance on IR and CSA, R.12. These authors consider possible exposure to fibres, metal fumes or dust and it should be noted that dermal and inhalation exposure modelled in the ECETOC TRA tool was largely developed fo the metal industry (ECETOC, 2009). It is therefore not relevant to the activities covered here. More ge erally, inhalation exposure to dusts is not applicable, since soft PVC articles plasticised with DEHP will – by their very nature – not be subjected to dust-generating processes.

This scena io al o covers all other activities, during which professional (and industrial) workers handle PVC arti les, e g. during installation of roofing, waterproofing membranes for outdoor use, geo-membranes, noise i sulat on membranes, hoses and tubes for industrial use, (parts of) tarpaulins and vehicle splash guards, technical rticles for agriculture (stall mats for cows) as well as traffic-related products (traffic cones, components of traffic signs and traffic safety products used on road surfaces), but also during the collection and transport of PVC waste.

Dermal exposure is assessed with the algorithm introduced in section 9.0.4.4 above. Two specific dermal exposures are assessed:

- Dermal exposure from handling PVC articles containing soft PVC recyclate with exposure to the inside of both hands being assessed ("handling").
- Dermal exposure from wearing PVC footwear. Due to the larger skin surface area, PVC boots are assessed here under the assumptions of socks being worn (wearing other footwear without socks are addressed qualitatively towards the end of this section).

The ES-specific values that enter the calculation are:

"Handling":

Fraction of total skin surface area in contact with the article: 2.6%, "recommended value" in EPA (2011) is 5.2% for hands (men; women: 4.8%); half of this value (2.6%) is assumed for exposure of the inside of both hands.

Contact time and frequency: 8 h/d on 5 d/w for a typical occupational scenario

Clothing penetration factor: 1 (exposure to bare hands, no gloves assumed)

"Wearing boots":

Fraction of total skin surface area in contact with the article: 23.25%, "recommended values" in EPA (2011) are 33.1% for both legs (men; women: 32.3%; half of this value (16.55%) used) 6.7% for both feet (mean; women: 6.6%), resulting in the total of 23.25% that is assumed for exposure when wearing PVC boots.

Contact time and frequency: 8 h/d on 5 d/w for a typical occupational scenario

Clothing penetration factor: 0.35 (socks are assumed to be worn when wearing boots)

Note that the combined "handling" and "wearing" scenario probably only applies to very specific situations, e.g. when a farmer wearing PVC boots simultaneously handles stall mats made of recycled soft PVC. Most craftsmen handling recycled soft PVC articles, such as traffic cones or components of traffic signs will not wear PVC boots. This scenario also covers other professional (and industrial) activities in which e worker has hand contact with recycled soft PVC articles and also simultaneously wears PVC foo wear. A possible scenario includes pool attendants wearing plastic sandals or clogs (both without s cks, ee qualitative assessment below), while simultaneously handling PVC articles, such as hoses and tubes However, the assumption of continuous exposure during the entire working day is certainly conservative for the handling aspect of this scenario.

The dermal exposure estimate for ES2-SL-P is shown in the following table.

Table 88 Dermal exposure estimation for ES2-SL P

Calculation	Parameter	Value "handling"	V lue "wearing"	Unit	Source
	Migration rate (95th percentile)	0.27	0.27	µg/cm^2 x h	Derived above
multiplied by	Total skin surface area/body weight (mean)	280 (adults)	280	cm^2/kg b.w.	Derived above
multiplied by	Fraction of total skin surface area in contact	0.026	0.2325	dimensionless	EPA, 2011
multiplied by	Contact time	8	8	h	ES-specific
multiplied by	Contact frequency	0.714	0.714	1/d	ES-specific
mult pli d by	Clothing penetration factor	1	0.35	dimensionless	Derived above
results in	**External exposure**	**11.2**	**35.1**	**µg/kg bw/d**	
	combined	**46.3**		**µg/kg bw/d**	

This exposure estimate is considered to be conservative for the following reasons:

- The underlying migration rate represents the 95th percentile from 51 measurements of in vitro DEHP migration from PVC articles into artificial sweat.
- The estimate assumes continuous contact to PVC articles over the entire shift, certainly a conservative assumption for this scenario.
- The conservative estimates for "handling" and "wearing" are combined.

- The estimate assumes that gloves are not worn during these activities.

Dermal exposure to DEHP from professional handling of PVC products containing soft PVC recyclate**, while simultaneously wearing PVC footwear** containing soft PVC recyclate **is estimated to be 46.3 µg/kg bw/d.**

As indicated above, this scenario also covers wearing plastic sandals or clogs while handling PVC articles. For these footwear items, wearing of socks – e.g. by pool attendants, cannot be assumed and a clothing penetration factor cannot be applied. However, the skin surface area in contact with the article is substantially lower than for boots. Taking the mean recommended values in EPA (2011), the skin surface area of both feet (conservatively assumed to be in contact with sandals and clogs) is 6.7% for men, which slightly lower than the skin surfac area assumed for boots, when corrected for the clothing penetration factor (23.25% * 0.35 = 8.1%). Dermal exposure when wearing plastic sandals or clogs is therefore expected to be lower than exposure when wearing oots As a consequence of these considerations, handling recycled soft PVC articles and simultaneously wearing andals of clogs made of recycled soft PVC is also covered by this scenario.

While this scenario primarily relates to professional workers manually handling PVC articles containing soft PVC recyclate, while simultaneously wearing PVC footwear (both activities resulting in derma exposure), situations may exist in which these same workers are also exposed via inhalation. Such si uations can be envisaged e.g. for storekeepers in HVAC retail outlets who manually handle PV articles and wear PVC footwear (dermal exposure to DEHP migrating from the article, addressed above) th t are stored on-site (inhalation exposure to DEHP evaporating from the articles).

The inhalation pathway alone is also relevant for professional and indus rial worke s not manually handling PVC articles containing soft PVC recyclate and/or wearing PVC footw ar. Examples nclude:

- Workers in any indoor occupational environment fitted with PVC a ticles containing soft PVC recyclate (e.g. flooring and HVAC ducts),
- Agricultural workers in polytunnels made of PVC containing soft PVC recyclate.

All these workers are assumed to be exposed to apou -phase DEHP only. Therefore, the saturated vapour concentration of DEHP can be used in the exposure ass ssm nt, which was given in the RAR (ECB, 2008) as 5.3 µg/m^3.

Inhalation exposure to DEHP from working i workrooms fitted with PVC products containing soft PVC recyclate is estimated (based on saturated vap ur concentration, worst case assumption) to be 5.3 µg/m^3.

9.3.2.4 Consumer exposure from article service life

9.3.2.4.1 Exposure assessment based on biomonitoring data

Biomonitoring data
Biomonitoring data from European studies of the last decade are presented in Annex #3#.
Studies compli nt with the following criteria were included:

- European populations
- ana ysis f at least two metabolite (recent studies mostly analyse three or four metabolites, see above)
- s mpling period > 2000.

Time trend analysis
Time eries studies show a clear and constant downward trend of DEHP metabolite concentrations in the general p pulation; therefore more emphasis is given on more recent data.
The sample pool from the German Environmental Specimen Bank provides a unique opportunity to investigate time trends over longer sampling periods. Samples have been taken and stored under controlled and standardised conditions. 24-h urine samples from young German adults were taken in the years 1988 to 2008 and were analysed by Wittassek et al. (2007b) and Göen et al. (2011) with respect to DEHP metabolites. Results from these analyses are shown in Figure 1.

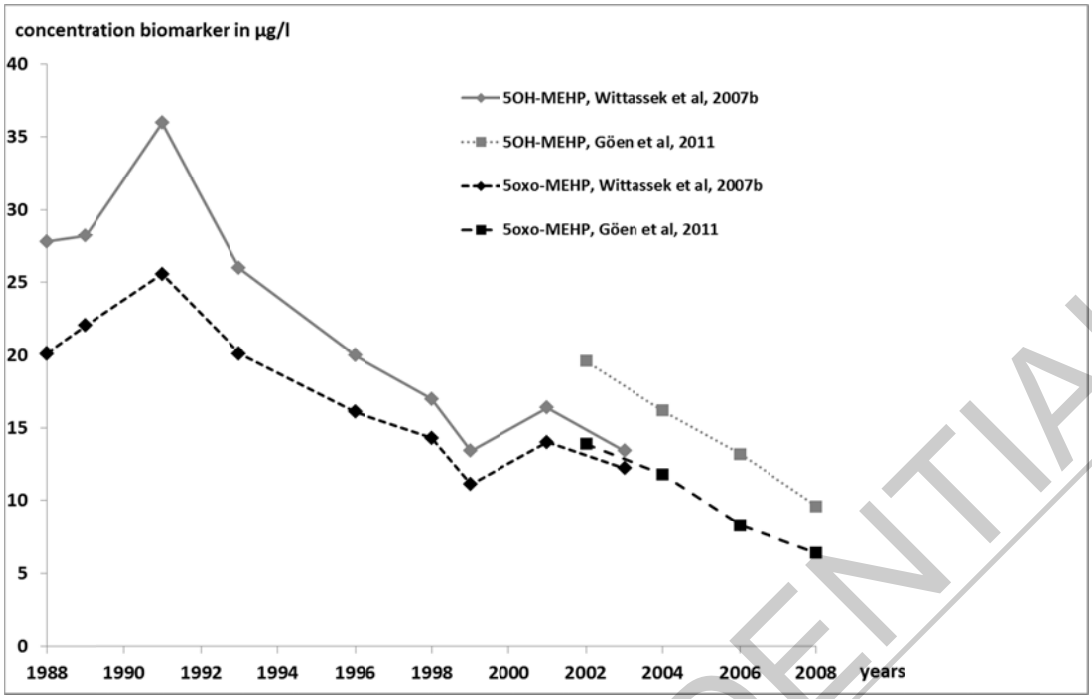


Figure 1 Time trend for median urinary concentrations (µg/L 24 h-urin) of 5-OH-MEHP and 5oxo-MEHP in samples from the German Environmental Specimen Bank (d ta from W ttassek et al. (2007b) and Göen et al. (2011))

These investigations of samples from the German Envi onm ntal Specimen Bank consistently demonstrate the decline of body burdens in the German populat on, which is roughly parallel with the reduction in manufacture and use volumes in Europe. Regulations imposed n use of DEHP

- ban of DEHP in toys and ch ldcare a ticles and in cosmetic products
- restriction of DEHP in food contact materials

are expected to have influenced only the most recent years of this data compilation. It can be expected that the further reduction of DEHP use the listing of DEHP in REACH Annex XIV, and the impact of the existing regulations has led to a further decline of body burdens. This view is supported by the most recent results from biomonitoring studies (see below)

Age-dependence of body burden

Biomonitoring data are mostl available for adults and for children aged 4 and older. According to the age-differentiated analysis of Be ker et al. (2009) concentrations of DEHP metabolites in urine are highest in 6 to 8 year olds, bu lev ls in 4 to 6 years old children were only slightly lower.

As few data ar available for younger children it is noteworthy that Fromme et al. (2011) measured DEHP and metabolite in mothers' milk and calculated from these data the intake of breast-fed babies. Using median and 95t percentile values, the authors estimated an "average" and "high" daily DEHP intake for exclusively breast-fed inf nts of 0.6 and 2.1 µg/kg bw/day, respectively. This estimated intake is substantially lower than intakes calculated for older children based on urinary metabolite concentrations. This is in agreement with low urinary metab lite concentrations found by Carlstedt et al. (2013) in urine of 2 to 6 month old babies.

In a r cent study of this group, urinary biomarker concentrations were determined in the urine of 22 infants (aged 15 to 22 months) and again, the content of phthalates in food was analysed in parallel for seven days (duplicate diet study design) (Fromme et al., 2013) (for details see chapter 9.0.5). Daily DEHP intake from food was calculated to be 2.64 and 4.66 µg/kg bw for the average and high scenario, respectively and average daily intake calculated from urinary biomarkers was 2.60 and 4.90 µg/kg bw for the average and high scenario, respectively. The data show that for this age group, food accounts for close to the total DEHP uptake.

It can be concluded from these studies that exposure young children to DEHP is lower than at older ages (> 3 years)

Biomonitoring data

Detailed data from all evaluated studies are provided in Annex #3# and are presented graphically in Figure 2 and Figure 3 for adults and in Figure 4 and Figure 5 for children. Although heterogeneous with regard to provenance and size of study population, methods used, numbers of metabolites measured and other parameters, more recent studies show lower body burdens than older ones.

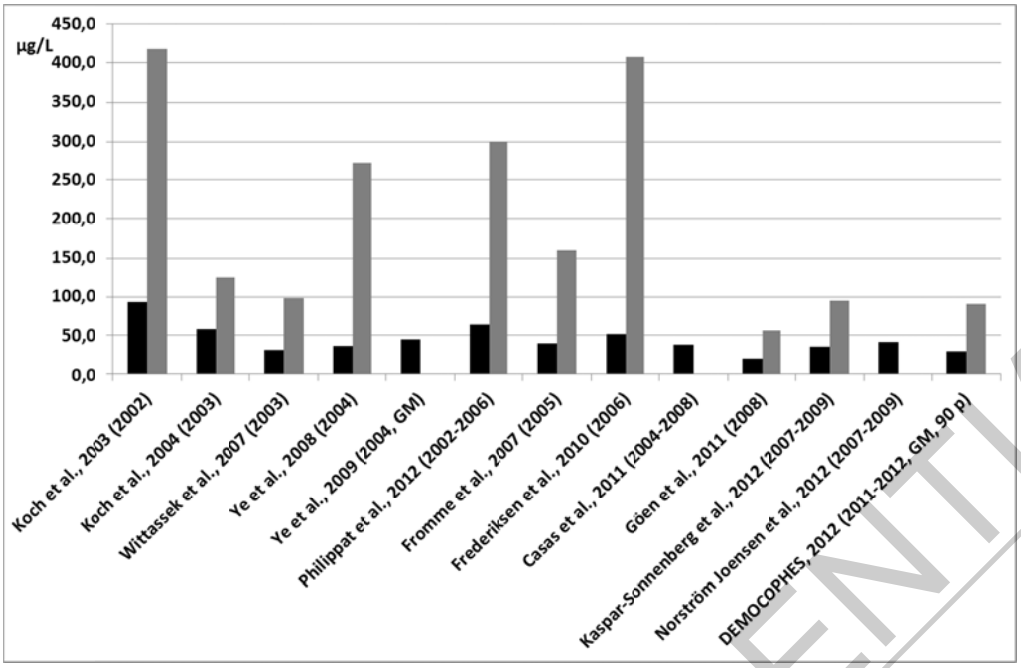


Figure 2 50 and 95 percentiles (black and grey, respectively of the sum of 3 DEHP metabolites (MEHP, 5OH-MEHP and 5oxo-MEHP) in urine of adults (in µg/L), as available from the listed studies

Remark: As indicated, the data of Ye et al. (2009) and DEMOCOPHES (2012) represent geometric means, not 50 percentiles and DEMOCOPHES (2012) calculated the 90. percentile

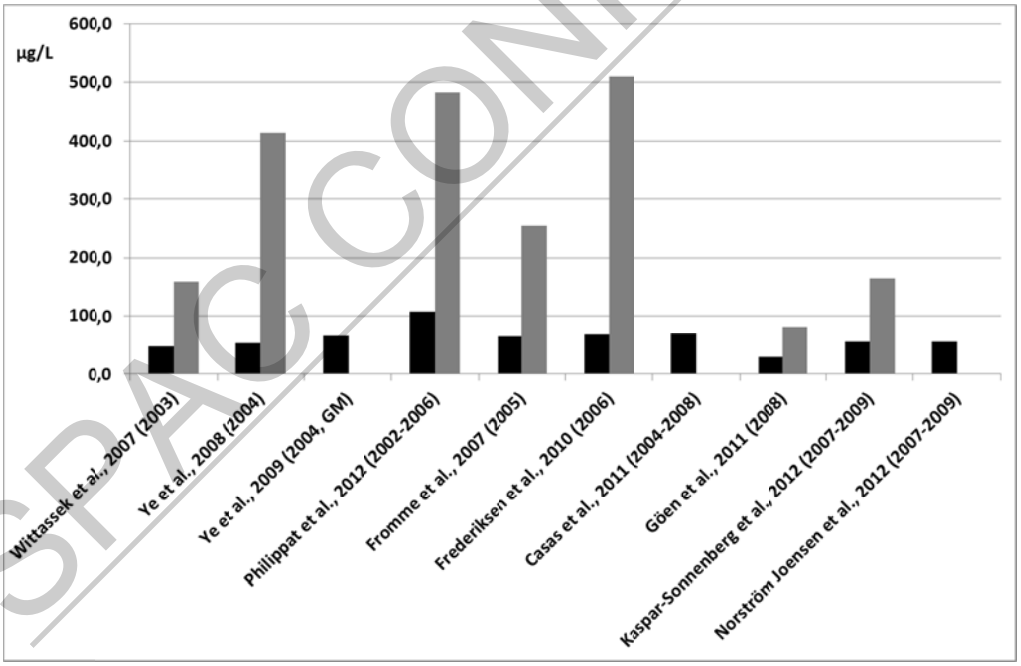


Figure 3 50 and 95 percentiles (black and grey, respectively) of the sum of 4 DEHP metabolites (MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP) in urine of adults (in µg/L), as available from the listed studies

Remark: As indicated, the data of Ye et al. (2009) represents the geometric mean, not the 50 percentile

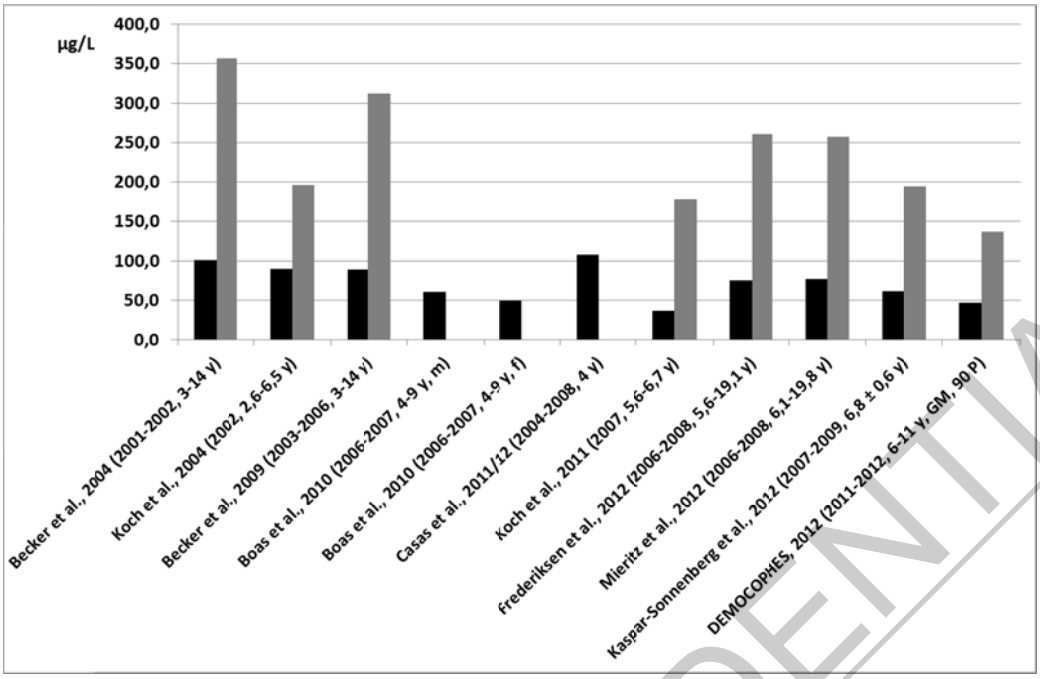


Figure 4 50 and 95 percentiles (black and grey, respectively) of he sum of 3 DEHP metabolites (MEHP, 5OH-MEHP and 5oxo-MEHP) in urine of children (in µg L), as a ailable from the listed studies

Remark: As indicated, the data DEMOCOPHES (2012) r present the geometric mean, not the 50 percentile and the 90, not the 95 percentile

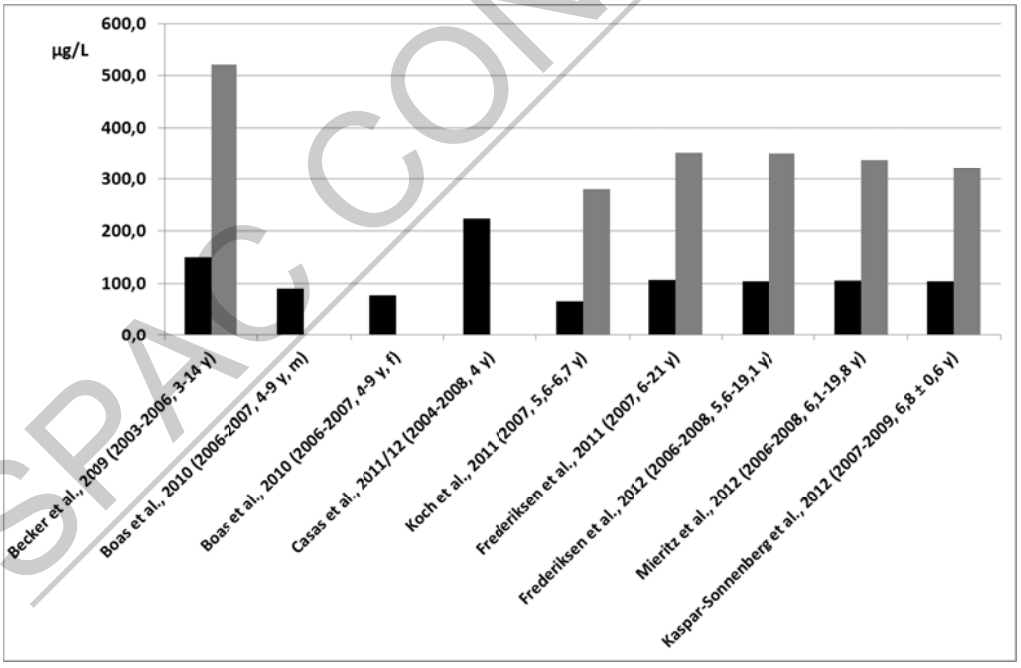


Figure 5 50 and 95 percentiles (black and grey, respectively) of the sum of 4 DEHP metabolites (MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP) in urine of children (in µg/L), as available from the listed studies

In the following figures intakes calculated with the Aylward/Anderson method, based on geometric means or median values, and on 95 (or 90) percentiles from these studies are presented.

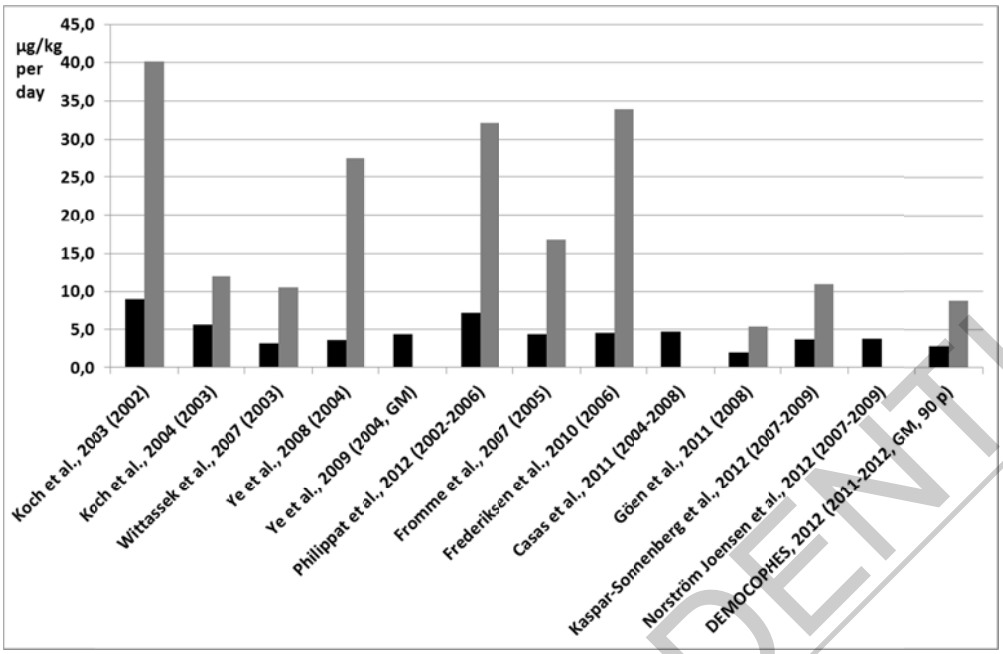


Figure 6 50 and 95 percentiles (black and grey, resp ctively) of calculated daily DEHP intakes (in µg/kg per day, calculated with Aylward/Anderson method) fo adult

Remark: As indicated, the calculation derived from Ye et al. (2009) and DEMOCOPHES (2012) is based on geometric means, not 50 percentiles and that fo DEMOCOPHES (2012) on the 90, not the 95 percentile

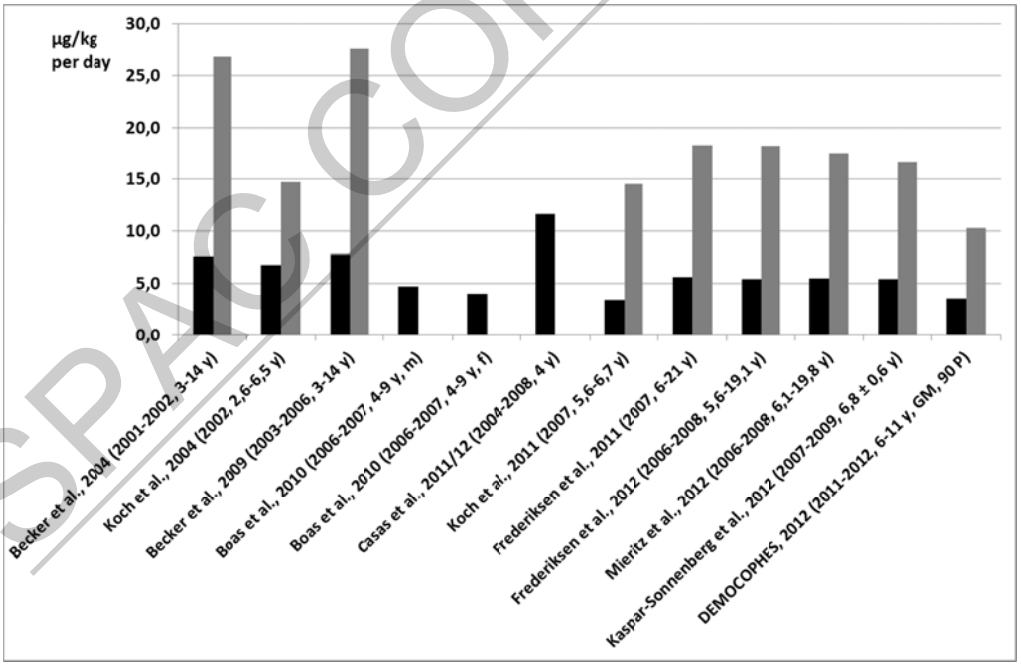


Figure 7 50 and 95 percentiles (black and grey, respectively) of calculated daily DEHP intakes (in µg/kg per day, calculated with Aylward/Anderson method) for children

Remark: As indicated, the calculation derived from DEMOCOPHES (2012) is based on the geometric mean, not

the 50 percentile and on the 90, not the 95 percentile
Table 89 shows the data from the most recent and comprehensive biomonitoring project, the European research project DEMOCOPHES (DEMOCOPHES, 2012; DEMOCOPHES, 2013; Schwedler et al., 2013; UBA, 2012)). Scientists from seventeen European countries participated in this project with the aim to recruit and investigate 120 mother-child pairs per country (except for Cyprus and Luxembourg: 60 mother-child pairs, due to small population). In total, data for 1844 mother-child pairs from 17 European countries were obtained.

Morning urine samples were taken during the time period September 2011 to February 2012. Participating women were in childbearing age (20 to <45 years) and their participating children aged 6-11 years. Urine was analysed according to standardised methods for the concentration of the DEHP metabolites MEHP, 5OH-MEHP and 5oxo-MEHP and multivariate regression analysis (ANOVA) was applied to all data to identify potenti l covariates. The sum of DEHP metabolites measured is reported within the project as volume-based concentrations, as reliable creatinine concentrations could not be obtained from each country.

Table 89 Results of DEMOCOPHES project: Pan-European dataset of 1844 mother-child pairs on rinary concentrations of DEHP metabolites.

Urinary concentrations	**Children**	**Mothers**
Geometric Mean (95% CI) of sum* of urinary DEHP metabolites [µg/L]	47.6 (46.0-49.3)	29.2 (28.1-31.3)
P90 (95% CI) of urinary DEHP metabolites [µg/L]	137 (126-150)	91 (84-100)
P90/P10	8	10

* DEHP metabolites measured: MEHP, 5-OH-MEHP and 5-oxo-MEHP

Some between-study (between-country) variability was observed in he DEMOCOPHES study. For example, slightly lower geometric mean values were observed in the Germ n study population: 21.6 µg/L urine for mothers and 39.2 µg/L for children.

The DEMOCOPHES study is one of the most compre ensi e biomonitoring efforts ever and provides a Europe-wide assessment of body burdens of the general populati n. Its results are in agreement with other biomonitoring studies from Germany and Denmark (see figures above and Annex #3#). Also, a very recent biomonitoring studies of Danish children, not yet included in Ann x #3#, reports comparative exposure levels (Langer et al., 2013). In this study, urinary DEHP metabolite concentrations did not correlate with indoor dust concentrations of DEHP, which is a further indication tha indoor s urces are not a prime source of exposure.

The values from the pan-European datas t of the DEMOCOPHES study are used to calculate the corresponding intakes (with the Aylward/Anderson method). These intake values are used for risk assessment.

Table 90 Intake values calculated from data of the DEMOCOPHES project, based on Aylward/Anderson method

Intake values [µg/kg bw/day]	**Children**	**Mothers**
based on GM of sum f urinary DEHP metabolites	3.57	2.81
based on P90 o urinary DEHP metabolites	10.29	8.75

Results from biomonitoring studies comprise exposure from all exposure pathways and all sources, including fo d nd food contact materials and medical devices. It is reasonable to assume that only a small part of the measu ed body burden is actually originates from manufacture and use of recyclate products.

9.3.2.4.2 Exposure assessment based on exposure modelling

This exposure estimation covers all articles made of recycled soft PVC for consumer use. In addition to using the biomonitoring data for exposure assessment, consumer exposure to DEHP from PVC articles containing soft PVC recyclate was modelled on the basis of the methodology described in section 9.0.5.4 and – in more detail – in Annex #1#. PVC articles are grouped by their exposure characteristics (taking account of exposure pathways, frequency and duration of contact as well as contact surface area in the case of dermal exposure) and a sentinel product is chosen for each group (Table 91). Sentinel products are supposed to have the highest exposure within

a group of articles.

Group X-0 differs from the other groups in that direct dermal or direct oral exposure (via migration of DEHP from these articles and mouthing) is considered insignificant for the articles of this group. From the lack of direct contact, it can clearly be concluded that any exposure would be substantially lower than that of other article groups.

In addition to the exposure resulting from direct contact, it is considered that all articles may contribute to inhalation exposure and oral exposure via house dust. The DEHP concentrations in indoor air and house dust cannot be allocated to specific PVC articles, but are rather the result of DEHP migration (or DEHP otherwise being set free) from all articles combined (i.e. all articles covered by groups X-0 – X-4).

Table 91 Overview of consumer exposure estimation groups

Acronym*	**X-0**	**X-1**	**X-2**	**X-3**	**X 4**
Exposure pathway	No direct contact	Dermal	Dermal, oral	Dermal	Dermal
Dermal contact area	N/A	M	L-M	M	M-H
Duration/frequency of dermal contact	N/A	L	M-H	H	H
Oral contact area	N/A	N/A	L	N A	N/A
Frequency of oral contact	N/A	N/A	L	N/A	N/A
Sentinel article	Flooring with PVC back-coating**	Gym ma s	H ndles	Plastic sandals	Seating for outdoor use

* The acronyms X-0-X-4 are explained below; : Low M Medium; H: High; N/A: Not applicable

** A sentinel product is formally assigned, but this group covers exposure representing all articles (see above)

Note that oral exposure primarily relate to mouthing of articles by children. Exposure via this pathway depends, among others, on the mouthing dura ion, which however, cannot be assigned to specific PVC articles. However, it is obvious that mouthing arti les made of recycled soft PVC is less frequent than mouthing other PVC articles, since recycled soft PVC article are prim rily used outdoors and in technical products rather than consumer products (also see section 9.0.5.4) Therefore, the overall mouthing duration derived in section 9.0.5.4 and discussed in detail in Annex #1# was reduced to one third and only applied to article group X-2. Please note that this application doe not cover any items which are suitable for swallowing (i.e. small (<10 cm) soft PVC items available in the home environment, (without attachment to larger objects).

Group X-0: Expo ure from carpets with PVC-backing and similar products (no direct contact)

Sen inel p oduct: Flooring with PVC back-coating

Rational for selection: Potentially high area

Other products: Any other products without significant dermal or oral contact, such as technical profiles and gaskets and footwear outer soles,

As already explained above, this group covers articles made of recycled soft PVC without appreciable direct dermal contact or the potential of being mouthed by children (back-coating of flooring, outer soles of shoes, inner layers of garden hoses, technical profiles and gaskets, roofing). In fact, some of these PVC articles will rarely be accessible to consumers in the indoor environment (e.g. technical profiles and gaskets). PVC articles mentioned here are considered to have a negligible potential for direct dermal or oral contact. For example, consumers do not come in direct contact with the PVC of flooring with PVC back-coating and will only very

rarely (if at all) touch technical profiles and gaskets installed in the home. Where occasional contact with some of the PVC articles covered by this group can be anticipated, frequency, duration and/or the skin surface area exposed is considered to be much lower than for the PVC articles covered by groups X-1 – X-4 above. As a consequence, dermal exposure from these PVC articles is estimated to be considerably less than the exposure for groups X-1 – X-4 and is not considered here quantitatively.

X-1: Dermal exposure from PVC gym mats and similar articles

Sentinel product: Gym mats

Rationale for selection: Medium contact area for this group of articles

Other products: Door mats, car mats

This group covers dermal exposure from gym mats made of recycled soft PVC and similar articles. The exposure frequency and contact time is considered medium, while the contact area is considered medium to high. Oral exposure from these products is considered practically negligible and is not considered in the exposure estimation.

The respective general input values were derived in section 9.0.5.4 and are discussed in detail in Annex #1#. Specific input values are shown in the following table, together with their rationale and the exposure estimation result.

Table 92 Dermal exposure to DEHP from gym mats and similar articles

Parameter	Value	Unit	Rationale	Source
Migration rate	0.27	µg/cm^2 x h	95th percentile	See Ch. 9.0.5.4
Adults				
Total skin surface area/body weight	280	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.19	-	Mean fractions for body parts from EPA (2011; see Annex #1#); one half of the hands and legs (19% of the total skin surface area) is assumed to be in contact with the sentinel article (gym mat) during exercise; the higher value for men is assumed here. The surface area assumed is (19,400 cm^2 * 19% =) 3,686 cm^2, which is higher than the surface area assumed in the Phthalate Restriction Proposal Assessment (2012) for a similar article (balance balls: 1,227 cm^2). This appear reasonable, since a higher fraction may be in contact in the case of mats compared to balls. A higher value of 25% of the total body surface area was assumed for sleeping mats (sports use) in Danish EPA Survey 117 (Andersen et al., 2012), according to the authors corresponding to the "upper or lower side of the body" (no additional explanation), obviously assuming a person lying flat on the mat. This is considered too high for people doing exercises.	EPA (2011)

Parameter	Value	Unit	Rationale	Source
Contact time	1	h (per day)	Time use surveys in 14 EU member states indicate an average of 28 and 41 minutes/d for sport activities for women and men, respectively aged 15-24 (all countries combined, range: 21-59 min/d); other time use surveys indicate substantially shorter durations for "sports and outdoor activities except walking and hiking" of less than 30 min/d. Time use survey data were not collected for use in exposure estimation, but a rounded value of 60 min/d is assumed here for the sentinel article (gym mats); this value is 3-times higher than the one used in the Phthalate Restriction Proposal Assessment (2012) for balance balls and training balls (10 minutes each). An identical values of 1h/d was assumed for sleeping mats (sport use) in Danish EPA Survey 117 (Andersen et al., 2012).	EUROSTAT (2008; 2012b)
Contact frequency	1	1/d	Daily use is assumed as a conservative approach	
External exposure	**14.4**	**µg/kg x d**	Calculated value	
Children, 6-12 months				
Total skin surface area/body weight	490	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.13	-	The data in Annex #1# show that the fraction for children of this age group is 13%. The surface area assumed is (4,500 cm^2 * 13% =) 585 cm^2.	EPA, 2011; Annex #1#
Contact time	1	h (per day)	No information is available for this parameter for this age group; the rationale is therefore that the child is associated with the parent and is in contact with the article for the same duration.	
Contact frequency	1	1/d	Daily use is assumed as a conservative approach	
External exposure	**17.2**	**µg/kg x d**	Calculated value	
Children 2-3 years				
Total skin surface area/body weight	440	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.15	-	The data in Annex #1# show that the fraction for children of this age group is 15%. The surface area assumed is (6,100 cm^2 * 15% =) 915 cm^2, which is higher than the surface area assumed in the Phthalate Restriction Proposal Assessment (2012) for a similar article (balance balls: 551 cm^2) for 2-year old children.	EPA, 2011; Annex #1#

Parameter	Value	Unit	Rationale	Source
Contact time	1	h/d	No information is available for this parameter for this age group; the rationale is therefore that the child is associated with the parent and is in contact with the article for the same duration.	
Contact frequency	1	per day	Daily use is assumed as a conservative approach	
External exposure	**17.8**	**µg/kg x d**	Calculated value	

These values are considered to represent a conservative estimate of DEHP exposure from PVC flooring, since the estimate

- assumes that DEHP migrates from all PVC articles at a high rate (95th percentile ch sen)
- considers daily contact, while such exercises may often be less frequent
- is based on contact of the bare skin with the PVC article and
- assumes a higher skin surface area in contact with the article and a higher duration than a similar (although not identical) scenario in the Phthalate Restriction Proposal Assessment 2012)

This exposure estimate also covers similar PVC articles in this gr up. For e ample, door mats are covered since both the skin surface area in contact and the duration of contac will e low r. Direct contact of the bare skin with door mats will be a rare event, but the exposure estimate als cover the unlikely event of a child crawling on a door mat for one hour each day. Car mats are expe ted to give rise to negligible dermal or oral exposure. It is included here for similarity of the products (mats) and xposure pathways (dermal contact by feet), but it is clear from the calculations performed that dermal exp sure from car mats is also covered by this scenario.

X-2: Dermal and oral exposure from smaller PVC articles containing soft PVC recyclate

Sentinel product: Handles

Rationale for selection: Frequent hand contact and easily accessible for mouthing by children

Other products: Luggage, tarpaulin (parts), ropes for garden use and tubes

This group covers dermal exposure from PVC articles with direct skin contact to hands. The exposure frequency and contact time is onsidere medium-high, while the contact area is considered low-medium. Oral exposure of children is possible by mouthing these articles. Frequent (daily) dermal contact is considered here for both groups of ch ldren although this is considered unlikely, taking into consideration the type of products.

The r spe tive eneral input values were derived in section 9.0.5.4 and are discussed in detail in Annex #1#. Sp ific input values are shown in the following table, together with their rationale and the exposure estimation result

Table 93 Dermal exposure to DEHP from smaller PVC articles containing soft PVC recyclate

Parameter	Value	Unit	Rationale	Source
Migration rate	0.27	µg/cm^2 x h	95th percentile	See Ch. 9.0.5.4
Adults				
Total skin surface area/body weight	280	cm^2/kg	Mean	See Ch. 9.0.5.4

Parameter	Value	Unit	Rationale	Source
Fraction of total skin surface area in contact	0.026	-	Mean fractions for body parts from EPA (2011; see Annex #1#); one half (50%) of the hands (i.e. the inside of both hands) is assumed to be in contact with the sentinel article (handles); the higher value for men is assumed here. The surface area assumed is (19,400 cm^2 * 2.6% =) 504 cm^2, which is higher than the surface area assumed in Phthalate Restriction Proposal Assessment (2012) for one half of both hands for a different article (366 cm^2, training balls).	EPA, 2011
Contact time	1.5	h (per day)	Time use surveys from many different European countries indicate an average time for cleaning, handicraft and gardening (all considered potentially relevant for handles) of 54, 14 and 12 min/d, respectively (sum: 0 min/day; women aged >65, all co ntries combined, range: 54-110 min/d) and of 43-76 min/d for all adults (men and women, ll age groups, for similar activiti s). Time use survey data were not designed for us in exposure estimation, but a rounded v lue f 90 min/d is assumed here for the sentine article (handles) on the basis of t e upper end values above (upper end chosen to refl ct the variety of differen articl s with handles). The Phthalate Restriction Proposal Assessment (2012) does not con ain a suitable article for comparison.	EUROSTAT (2008; 2012b)
Contact frequency	1	1/d	D ily use is assumed	
External exposure	**2.95**	**µg/kg x d**	C lculated value	
Children, 6-12 months				
Total skin surface area/body weight	490	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.027	-	The data in Annex #1# show that the fraction for children of this age group is 2.7%. This age group is not assessed in the Phthalate Restriction Proposal Assessment (2012)	EPA (2011)
Contact time	1.5	h (per day)	No specific data for children, but the value for adults is assumed here with the rationale that small children will be close to their parent and be in contact with articles handled by their parent.	EUROSTAT (2008; 2012b)
Contact frequency	1	1/d	Daily use is assumed	
External exposure	**5.36**	**µg/kg x d**	Calculated value	
Children, 2-3 years				
Total skin surface area/body weight	440	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total	0.024	-	The data in Annex #1# show that the fraction	EPA (2011)

Parameter	Value	Unit	Rationale	Source
skin surface area in contact			for children of this age group is 2.4%. The Phthalate Restriction Proposal Assessment (2012) does not contain a suitable article for comparison.	
Contact time	1.5	h (per day)	No specific data for children, but the value for adults is assumed here with the rationale that small children will be close to their parent and be in contact with articles handled by their parent.	EUROSTAT (2008; 2012b)
Contact frequency	1	1/d	Daily use is assumed	
External exposure	**4.28**	**µg/kg x d**	Calculated value	

These values are considered to represent a conservative estimate of dermal DEHP exposure from smaller PVC articles containing soft PVC recyclate, since the estimate

- assumes that DEHP migrates from all PVC articles at a high rate (95th percen il chosen)
- considers a prolonged contact time (activities such as gardening, cleaning and andicraft are all assumed to involve working with tools with handles made of recycled soft PVC)
- assumes, although unlikely, frequent use of the products by children.

This exposure estimate also covers similar PVC articles in this gr up. Whil some of these articles are large, such as tarpaulins, only parts of these articles are made of recycled oft PVC (such as tarpaulin strings) and exposure duration and/or exposure frequency will be lower than ssum d for handles. The same applies to other articles covered by this article group, such as luggage, r pes for garden use and garden hoses and tubes, which are all covered due to a shorter contact frequency/duration resulting in lower exposure. In fact, direct contact with PVC in garden hoses for private use is unlikely, ince recycled soft PVC is only used in the inner layer.

In addition to dermal exposure, articles made of recycled ft PVC belonging to this group may be mouthed by small children and exposure via this pathway will therefore be estimated. The respective input values were derived in section 9.0.5.4 and are discu sed in de ail in Annex #1#. The results of this estimation are shown in the following table.

Table 94 Oral exposure to DEHP from mouthing smaller PVC articles containing soft PVC recyclate

Parameter	Value	Unit	Rationale	Source
Migration rate	3.65	µg/min (per 10 cm^2 contact area)	95th percentile	See Ch. 9.0.5.4
Children, 6-12 months				
Mouthing duration	25	minutes/d	Mean	See Ch. 9.0.5.4
Fraction for article group	0.33			See Ch. 9.0.5.4
Body weight	9.2	kg	Mean	See Ch. 9.0.5.4
Oral intake	**3.27**	µg/kg x d	Calculated value	
Children, 2-3 years				
Mouthing duration	15	minutes/d	Mean	See Ch. 9.0.5.4
Fraction for article group	0.33			See Ch. 9.0.5.4
Body weight	13.8	kg	Mean	See Ch. 9.0.5.4
Oral intake	**1.31**	µg/kg x d	Calculated value	

These values are considered to represent a conservative estimate of DEHP exposure from mouthing smaller PVC articles containing soft PVC recyclate, since the estimate

- assumes that DEHP migrates from all PVC articles at a high rate (95th percentile chosen)
- is based on migration data for DINP despite some indications that DEHP in fact shows a lower migration
- is based on migration data facilitating phthalate release (biting/chewing in *in vivo* studies)
- is based on a mouthing duration for "objects other than dummies, toys and fingers", assuming that these are all made of recycled soft PVC containing DEHP.

These issues are discussed in more detail in the context of the input data in Ch. 9.0.5.4 and Annex #1#.

As mentioned in Ch. 9.0.5.4, DEHP exposure from mouthing PVC articles cannot be differentiated by article group and will mostly not come from recycled soft PVC. Therefore, the entire mouthing duration (as a key input value) has been reduced to one third of the total mouthing duration for "objects other than dummies, toys and fingers"

X-3 Dermal exposure from PVC footwear with direct or indirect skin contact

Sentinel product: Plastic sandals

Rationale for selection: High exposure duration and frequency and direct skin contact

Other products: Clogs, slippers, waterproof boots with indirect skin contact

This group covers dermal exposure from PVC footwear with direct skin contact. The exposure frequency and contact time is considered high and the contact area is considered medium. Oral exposure is considered negligible. This group also covers waterproof boots that are in indirect contact with the skin. While waterproof

boots have a higher contact area than e.g. sandals, there is no direct skin contact since waterproof boots have an inner non-PVC lining and socks are worn inside. A qualitative assessment is included at the end of this section.

Exposure is estimated only for adults and children 2-3 years of age. Younger children 6-12 months of age will only rarely (if at all) wear the sentinel product (sandals), since children typically only start to walk after 12 months of age (Cohen Hubal et al., 2000). Even considering children that walk at an earlier age, these can be considered not to wear clogs, slippers, plastic sandals, but rather shoes providing better foothold. Actually, no products made from recyclate are known for these age groups, but they are included in the exposure assessment for precautionary reasons.

The respective general input values were derived in section 9.0.5.4 and are discussed in detail in Annex #1#. Specific input values are shown in the following table, together with their rationale and the exposure estimati n result.

Table 95 Dermal exposure to DEHP from PVC footwear with direct skin contact

Parameter	Value	Unit	Rationale	Source
Migration rate	0.27	µg/cm^2 x h	95th percentile	See Ch. 9.0.5.4
Adults				
Total skin surface area/body weight	280	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.033	-	Mean fractions for body parts from EPA (2011; see Annex #1#); the soles of both feet are assumed to be in contact with the sandals (sentinel product); taken as one half of the fraction for feet (3.3%). The surface area assumed is (19,400 cm^2 * 3.3% =) 640 cm^2, which is higher than the surface area assumed in the Phthalate Restriction Proposal Assessment (2012) for plastic sandals (444 cm^2).	EPA (2011)
Contact time	8	h (per day)	The value of 16 hours assumed in he Phthalate Restriction Proposal Assessment (2012) is considered too h gh, since adu ts spent about 9 hours sleeping (EPA, 2 11; BAGS, 1995; EUROSTAT, 2008; 2012b); for the assumed cont ct frequency (wearing sandals every day), his is c nsidered too long and the contact time is educed to 8 h, a value chosen for footwear (shoes, boots) in ECETOC TRA (v.3).	ECETOC (2012)
Contact frequency	1	1/d	Daily u e is assumed	
External exposure	**20.0**	**µg/kg x d**	Calculated value	
Children, 2-3 years				
Total skin surface area/body weight	440	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.033	-	The data in Annex #1# show that the fraction for children in this age group is similar to adults. The surface area assumed is (6,100 cm^2 * 3.3% =) 201 cm^2, which is higher than surface area assumed in the Phthalate Restriction Proposal Assessment (2012) for plastic sandals (170 cm^2).	EPA, 2011
Contact time	8	h (per day)	The value of 10 hours assumed in the Phthalate Restriction Proposal Assessment (2012) is considered too high, since children of this age spent about 12 hours sleeping (EPA, 2008; 2011; BAGS, 1995); the value used here assumes that children wear plastic sandals for one third of the day and is in agreement with the ECETOC TRA (v.3) default for footwear (shoes, boots), although dermal exposure from these articles is only assessed for adults in ECETOC TRA.	ECETOC (2012)
Contact frequency	1	1/d	Daily use is assumed	
External exposure	**31.4**	**µg/kg x d**	Calculated value	

These values are considered to represent a conservative estimate of DEHP exposure from PVC footwear with direct skin contact, since the estimate

- assumes that DEHP migrates from all PVC articles at a high rate (95th percentile chosen)
- is based on a high exposure duration of 8 h/d and frequency (daily use).

These issues are discussed in more detail in the context of the input data in Ch. 9.0.5.4 and Annex #1#.

The Phthalate Restriction Proposal Assessment (2012) estimated a higher worst case exposure to DEHP from plastic sandals of up to 72 µg/kg x d (external exposure) for 2-years old children (3.6 µg/kg x d internal exposure/5% dermal absorption). This higher exposure estimate is largely due to the fact that the Phthalate Restriction Proposal Assessment (2012) applied a migration rate one order of magnitude higher than the migration rate used here and the migration value of 2.29 mg/kg can be corrected to 3.3 µg/cm^2 x h on the basis of the original data in the assessment). This migration rate of 2.29 mg/kg is assumed for the strap of sandals in contact with parts of the feet with sunscreen applied. This approach is not followed here for the following reasons:

- This migration rate was not actually measured. Rather, a factor of 149.5 for facilitated DEHP release under the influence of sunscreen with dynamic extraction was derived from two replicate *in vitro* measurements on the sole of one pair of sandals only (this limitation is also acknowledged in the Phthalate Restriction Proposal Assessment (2012)). Note that these data were included in the analysis of migration data in Annex #1#, Appendix A-1 upon which the 95th percentile applied here was based.
- This derived factor for the soles of sandals was multiplied with a migration rate obtained for straps of different sandals (with replicate measurements often showing high variation) and used in the exposure estimation for these other sandals. Since the latter were differentiated by age groups (i.e. sandals for 2-years old children and adults), different migration rates for different age groups were applied (differing by a factor of 3.5 for the worst case calculations). Such a differentiation of the migration rate, which is more a function of inherent properties and environmental conditions than of the age of the person wearing the sandal, is not evident in the original migration data (2010b), which do not show a systematic difference between sandals for children and adults.
- The two replicate migration measurements with sunscreen under dynamic conditions, on which the factor of 149.5 is based, show a high degree of variation with a coefficient of variation of 70% (coefficient of variation of DEHP concentration in these two replicates: 3%) and are thus to be considered uncertain. The authors of this study noticed that "*the analysis uncertainty on the results of the analyses in duplicate for the additional tests* [e.g. those with sunscreen under dynamic conditions] *is much higher than on the other migration analyses. During the tests with sun lotion the reason might be that it was not possible to ensure that the sun lotion was homogenously applied on the sample before the migration analyses.*" (Tønning et al., 2010b)
- The experimental design (application of sunscreen to the article leading the problems as described above) is difficult to extrapolate to realistic use conditions (application of sunscreen to the skin).
- It is questionable, whether dynamic extraction conditions actually represent the actual use conditions for the exposure duration (8 h/d and frequency (daily) assumed here, which may be more static. In this context, it should be noted that the migration rate with sunscreen under static conditions was lower than the migration rate applied here.
- Finally, the application of the "sunscreen effect" implies that adults and children either a) spend 10-16 h/d outdoors, a figure unsupported by data (see EPA (2008; 2011) or b) apply sunscreen even when indoors, which is unlikely. In either case, the approach also assumes that the "sunscreen effects" is effective over the entire duration.
- Overall, before data can be used for risk assessment purposes, it is essential that these data are "*reliable and representative for the situation that needs to be assessed*", as is required by ECHA Guidance (ECHA, 2010b); the data available for the sunscreen effect do not meet these pre-requisites of the Guidance.

The underlying data are discussed in detail in Annex #1# and its Appendix A-1.

Other footwear covered in this group (e.g. clogs, slippers) may differ from sandals by their potentially higher skin surface contact area (all feet as opposed to half of the feet applied for sandals). This would lead to a two-fold higher exposure compared to the one derived above. In fact, sandals were not just chosen as the sentinel product because exposure from sandals was specifically addressed in the Phthalate Restriction Proposal Assessment (2012), but because clogs and slippers may more often be worn with socks on and/or may have an additional inner lining preventing direct contact of PVC with the skin. The application of a clothing penetration

factor of 0.35 (see section 9.0.5.4) would ultimately reduce exposure by a factor of more than 2, leading to a lower exposure than derived here.

Overall, the conservative assumptions of direct skin contact for 8 h/d and daily use are believed to cover other footwear (clog, slippers) as well, which may occasionally be worn on the bare skin. Again, it is emphasized that no footwear products from recyclate materials made for children are known. Exposure considerations for children are included here for precautionary reasons only.

In addition, waterproof boots are covered by this article group. Application of a clothing penetration factor can also be assumed for waterproof boots, since socks will be worn in such type of footwear and the boots are always produced with an inner textile lining. However, the skin contact area is higher for boots and leads to a slightly higher exposure in adults compared to sandals and clogs (see section 9.3.2.3.1 for a discussion in the context of a professional service life scenario). Nonetheless, sandals were chosen as sentinel products to address a potentially high exposure for children. In contrast to adults, the relative fraction of the total skin surface area is small f r the legs of children (25.3% for 2-3 years old children vs. 33.1% in male adults), while the relative fr ction f feet is about the same (6.3% and 6.7%, respectively). As a consequence, the skin surface area in contact with b ots for children 2-3 years old is (one half of the legs (12.65%) + entire feet (6.3%) = 18.95%, multiplied by the clothing penetration factor (0.35) =) 6.6%. This values is only 2-times higher than the value applied for sandals. While this would suggest a higher exposure to DEHP from waterproof boots compared to sand ls, both the contact frequency and the contact duration are lower than the values applied for sandals above (daily use for 8 hours a day). For example, an average of every second day with rain (where waterpro f bo ts may be worn) can be assumed for the frequency on the basis the "Urban Audit" (core city section) data (EUROSTAT, 2012a) and a mean “recommended value” 1.3 h/d (EPA, 2008; 2011; “time spent outdoors”) can be assumed as a surrogate for the duration of wearing waterproof boots. The lower frequency and duration of wea ing waterproof boots compared to wearing sandals clearly outweigh the higher skin surface area in co tact associated with wearing boots.

Overall, the calculated exposure to DEHP from waterproo boots would be lower than the one calculated for wearing plastic sandals. Waterproof boots are therefore also c vered by this exposure scenario.

X-4: Dermal exposure from PVC articles containing soft PVC recyclate (prolonged contact)

Sentinel product: Seating fo ou door use

Rationale for selection: Frequen and prol nged dermal contact with large surfaces assumed

Other products: None

This group covers dermal exposure from prolonged contact with large PVC surface. Exposure frequency and contact time as well as the contact area are considered high. Oral exposure from these products is considered practically negligible and is not considered in the exposure estimation.

The respective gener l input alues were derived in section 9.0.5.4 and are discussed in detail in Annex #1#. Specific input v lues are shown in the following table, together with their rationale and the exposure estimation result.

Table 96 D rmal exposure to DEHP from PVC articles containing soft PVC recyclate (prolonged contact)

Parameter	**Value**	**Unit**	**Rationale**	**Source**
Migr tion rate	0.27	µg/cm^2 x h	95th percentile	See Ch. 9.0.5.4
Adults				
Total skin surface area/body weight	280	cm^2/kg	Mean	See Ch. 9.0.5.4

Fraction of total skin surface area in contact	0.22	-	Mean fractions for body parts from EPA (2011; see Annex #1#); slightly more than one fifth (22%) of the total skin surface area is assumed to be in contact with the sentinel article (outdoor seating), based on one fourth of trunk, arms and legs being in contact; the higher value for men is assumed here. A slightly higher value of 25% of the total body surface area was assumed for sleeping mats in Danish EPA Survey 117 (Andersen et al., 2012), which is reasonable since lying on a sleeping mat would involve a higher contact area than sitting (assumed for outdoor seating).	EPA (2011); Annex #1#
Contact time	5	h (per day)	Mean; US adults, total time spent outdoors, "recommended value": 281 min/d (4.7 h/d) for adults 18-64 y s of age and 298 min/d (5.0 h/d) for adults >64 years of age; the mean for German adults (20-75 years of age) is lower (3 h/d); the higher US value is as umed because it represent an EPA "recommended valu ".	EPA (2008; 2011); BAGS (995)
Contact frequency	0.5	1/d	Average ba ed on EUROSTAT's "Urban Audit" (ore city secti n), which reports the number f days with rain per annum; the 2007 2009 data for 238 European i i s identify 154 (AM) and 163 (m dian) days/year with rain. The range of 40 (Las Palmas, Spain) to 271 da s/year (Derry, Northern Ireland) with rain reflects the different climatic conditions across Europe. Every second day appears to be a reasonable assumption for a "European average" of days with rain, and – as a consequence – for days without rain as well, which are assumed here for sitting outside.	EUROSTAT, (2012a)
Clothing penetration factor	1		No clothing is assumed to reflect contact with the bare skin. This certainly represents a conservative assumption, since most people will wear at least some clothes when sitting outside.	See Ch. 9.0.5.4
Exte nal exposure	**41.6**	**µg/kg x d**	Calculated value	
Children, 6-12 months				
Total skin surface area/body weight	490	cm^2/kg	Mean	See Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.18	-	The data in Annex #1# show that the fraction is lower for this age group than for adults.	EPA (2011); Annex #1#

Contact time	2.3	h (per day)	Mean; US children 6-12 months, total time spent outdoors, "recommended value": 139 min/d (2.3 h/d); the mean for German children (< 1year of age) is lower (1 h/d), but might also include children 1-6 months old; the higher US value is assumed, because it is more specific for the age group and represents an EPA "recommended value".	EPA (2008; 2011); BAGS (1995)
Contact frequency	0.5	1/d	Same as for adults	
Clothing penetration factor	1		Same as for adults	See Ch. 9.0.5 4
External exposure	**27.4**	**µg/kg x d**	Calculated value	
Children, 2-3 years				
Total skin surface area/body weight	440	cm^2/kg	Mean	S Ch. 9.0.5.4
Fraction of total skin surface area in contact	0.20	-	The data in Annex #1# show hat the fraction is lower for this ag group han for adults.	EPA (2011); Annex #1#
Contact time	1.3	h/d	Mean; US children 2- <3 y ars, total time spent outdoors, "recommended value": 76 min/d (1.3 h/d); the mean for Germa children (1-3 years of age) is higher (2 /d), the lower US value of is assumed b ause it is more specific for the age group and represents an EPA "r commended value".	EPA (2008; 2011); BAGS (1995)
Contact frequency	0.5	p r day	Same as for adults	
Clothing penetration factor	1		Same as for adults	See Ch. 9.0.5.4
External exposure	**15.4**	**µg/kg x d**	Calculated value	

These values re considered to represent a conservative estimate of DEHP exposure from seating for outdoor use, since he e timate

- assumes tha DEHP migrates from all PVC articles at a high rate (95th percentile chosen)
- considers a prolonged contact time of up to 5 hours on every second day

Exposure via house dust and inhalation exposure

In addition to direct oral and dermal exposure from consumer articles estimated above, articles made of recycled soft PVC may contribute to exposure indirectly by migration of DEHP to house dust and volatilisation into indoor air. As exposure from these sources cannot be attributed to specific articles, its potential contribution to total exposure is addressed here and added to the overall exposure from all sentinel products.

As discussed in section 9.0.5.4, articles made of recycled soft PVC are primarily used outdoors and in technical products rather than consumer products. The data reported below, however, refer to DEHP from all PVC articles used in the indoor environment and therefore overestimate the contribution of DEHP from recycled soft PVC

articles.

The respective input values were derived in section 9.0.5.4 and are discussed in detail in Annex #1#. For DEHP intake via house dust, the exposure estimation for the three age groups considered here is shown in the following table.

Table 97 Oral DEHP exposure via house dust

Parameter	Value	Unit	Rationale	Source
DEHP concentration in house dust	1800	µg/g	Mean and median of upper percentiles of studies with more than 50 samples	See Ch. 9.0 5.4
Adults				
Dust ingestion	0.03	g/d	Central tendency, U.S. EPA recommended value for this age group, US	See Ch. 9.0.5.4
Body weight	70	kg	Mean	See Ch. 9.0.5.4
Oral intake	**0.771**	**µg/kg x d**	Calculated value	
Children, 6-12 months				
Dust ingestion	0.03	g/d	Central tendency, U S. EPA r commended value for this age group, US	See Ch. 9.0.5.4
Body weight	9.2	kg	Mean	See Ch. 9.0.5.4
Oral intake	**5.87**	**µg/kg x d**	Calculated alue	
Children, 2-3 years				
Dust ingestion	0.06	g/d	C ntr l tendency, U.S. EPA recommended value for his age group, US	See Ch. 9.0.5.4
Body weight	13.8	kg	M an	See Ch. 9.0.5.4
Oral intake	**7.83**	**µg/kg x d**	Calculated value	

A DEHP concentration (gas phase and particle-bound) in indoor air of
2.6 µg/m^3 as a 95th percentil
was derived in section 9 0 5 4 and is used in this assessment.

This exposure estimate is critically dependent on the general input values (DEHP concentrations in house dust and dust ingestion). The rationale for these values is explained in detail in section 9.0.5.4 and in Annex #1#, which als include a discussion of these parameters.

Combined consumer exposure

The biomonitoring data already integrate exposure from all different sources and thus also represent combined exposure from all PVC articles (including those made from recycled soft PVC). As discussed earlier, intakes derived from biomonitoring data – in addition to any exposure to DEHP from PVC articles – also reflect exposure to DEHP from food and other sources (e.g. toys until prohibition of DEHP in these items).

As another pillar of estimating consumer exposure to DEHP from PVC articles, modelling was performed using a sentinel product approach with four different groups (X-1 – X-4). In addition to these groups, inhalation exposure and oral exposure via house dust ingestion were estimated separately, since DEHP concentrations in these media cannot be assigned to a specific article (and in fact reflects DEHP exposure from all PVC articles, not just the ones made of recycled soft PVC).

The exposures estimated above are summarised in the following table . Inhalation exposure (2.6 µg/m^3, corresponding to an intake of about (2.6 µg/m^3 x 20 m^3/d / 60 kg =) 0.9 µg/kg bw/d for adults) is low. Dermal exposure is not easily compared to other pathways, as percutaneous absorption is substantially less (5%) than oral or inhalation absorption (75%) (chapter 9.0.4.1). Therefore, other DNELs apply and summing up over the three exposure pathways is preferably done based on pathway-specific RCRs (see chapter 10.3.1).

Table 98 Combined consumer exposure from simultaneous exposure to all sentinel products: results of the modelling approach*

Pathway	**X-1**	**X-2**	**X-3**	**X-4**	**IND****	**Sum**
Adults						
Inhalation [µg/m^3]					2.6	**2.6**
External dermal exposure [µg/kg bw/d]	14.4	2.95	20.0	41.6		**79.0**
Oral intake [µg/kg bw/d]					0.771	**0.771**
Children, 6-12 months						
Inhalation [µg/m^3]					2.6	**2.6**
External dermal exposure [µg/kg bw/d]	17.2	5.36	-	27.4		**50.0**
Oral intake [µg/kg bw/d]		3 27			5.87	**9.14**
Children, 2-3 years						
Inhalation [µg/m^3]					2.6	**2.6**
External dermal exposure [µg/kg bw/d]	17.	4.28	31.4	15.4		**69.0**
Oral intake [µg/kg bw/d]		1.31			7.83	**9.14**

* All interim calculation results were round d to three significant figures.

** Exposure from DEHP in ho se dust a d air in the indoor environment

The modelling approach shows that articles covered by X-4 (i.e. seating for outdoor use as the sentinel product) lead to the largest d rmal exposure for adults and the older children (small children are expected to spend less time in outdoor sea ing (see the discussion of article group X-4 above).

If the restrict d dermal absorption is taken into account (assumed dermal absorption being a factor of 75/5 = 15 lower than oral ab orp ion), then the modelling approach results in a combined exposure of the elder children that i up o a f ctor of 1.5 higher than the total intake derived from biomonitoring data based on the 90th per entile from he DEMOCOPHES study (see section 9.3.2.4.1) (or up to a factor of 5 based on GM values) (110 µg kg bw/d divided by 15 + 9.15 µg/kg bw/day for oral exposure = 16.5 µg/kg bw/day compared to 10.3 µg/kg bw/day – 90th percentile for children from DEMOCOPHES). If it is considered that the main source of the b dy urden found in biomonitoring studies is intake from food, then the modelling of exposure from consumer articles results in estimates more than one order of magnitude higher compared to an estimate of exposure from consumer articles based on biomonitoring results. Note that the values derived above for the indoor environment (most notably the comparatively high oral intakes via house dust) do not specifically refer to DEHP from recycled soft PVC, since the DEHP concentration in house dust cannot be separated for recycled and virgin PVC.

This finding testifies to the very conservative nature of the modelling approach that can be summarised as follows:

- The modelling approach assumes DEHP migration from the PVC articles at the 95th percentile (both for dermal and oral exposure (mouthing)) and this conservative input parameter has been applied to all articles. In other words, all sentinel articles evaluated are assumed to show such a high migration.

- In fact, average (geometric mean as well as median) migration rate into artificial sweat is about one order of magnitude lower than the 95th percentile. In the case of the migration into human saliva, average migration rates are about 3-times lower than the 95th percentile value (see Annex #1# for details).

- The migration rate was derived from studies with new PVC material extracted (in vitro into artificial sweat) or mouthed (in vivo into human saliva) once for different durations. This initial migration rate from new material is applied to all scenarios above, while it can be reasonably assumed that the migration ra e will decline over periods of weeks and months relevant for chronic exposure.
- All sentinel products are assumed to be made of recycled soft PVC and all these PVC articles re a sumed to contain DEHP as a plasticiser for the purpose of this assessment. While this is a necessary ass mption in the context of this application for authorisation, it may be responsible for the higher mod lled xposure, when compared with biomonitoring data. For example, exposure via mouthing i estimated under the assumption that all objects mouthed are made of recycled soft PVC containing DEHP. A discussed in detail in Annex #1#, there is substantial evidence that this is hardly the case.

10. RISK CHARACTERISATION

10.1. Manufacture

Not relevant for this application for authorisation.

10.2. Use #1: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends

10.2.1. Human health

10.2.1.1. Workers

The exposure estimation for workers is primarily based on biomonitoring data from PVC formulation and processing companies, where neat DEHP was used as well. These studies assessed exposure from all sources, i.e. also from diet as a very important source. The RCRs for formulation is shown in the following table.

Table 99 RCRs based on biomonitoring data for ES2-W: Formulation of recycled soft PVC containing DEHP in compounds and dry-blends

Statistical basis*	Geometric mean	90th percentile
Intake based on urinary DEHP metabolites [µg/kg bw/d]	23	92
DNEL [µg/kg bw/d]	230	230
RCR	**0.1**	**0.4**

* See section 9.0.4.3 for details

The RCRs are clearly below 1 and the risk is therefore adequately controlled.

As detailed in section 9.2.2.3 above, the underlying exposure estimate and, as a consequence, the risk characterisation is considered conservative for the following reasons:

- The risk characterisation is based on biomonitoring data that consider exposure from all sources including diet.
- The basic studies measured the body burden of workers in PVC formulation and processing plants, where free DEHP was used as well. Exposure from recyclate, where DEHP is enclosed in the PVC matrix is considered to be lower.
- Furthermore, the typical concentration of DEHP in recyclate is [redacted] (maximum 20%), whereas higher concentrations are present in virgin PVC.
- The maximum intake calculated from biomonitoring studies was used in risk characterisation.
- The methodology applied for the conversion of biomonitoring data (DEHP metabolites in urine) consistently leads to higher calculated intake values than the methodology applied by the authors of biomonitoring studies (where available).

  The operational conditions and risk management measures detailed in the relevant exposure scenario are stricter than the conditions in place at the plants where the biomonitoring data were obtained from.

For two specific tasks involving the emptying of bags of recycled soft PVC, which were potentially not completely covered by the biomonitoring data, inhalation and dermal exposure were modelled. The resulting risk characterisation is shown in the following table.

Table 100 Risk characterisation for PROC 8a and 8b (emptying bags with recycled soft PVC)

	PROC 8a	PROC 8b
Inhalation exposure [mg/m^3]	1.3	0.48
DNEL inhalation [mg/m^3]	1.6	1.6
RCR inhalation	0.81	0.30
Dermal exposure [mg/kg b.w. x d]	0.135	0.135
DNEL dermal [mg/kg b.w. x d]	3.4	3.4
RCR dermal	0.039	0.039
RCR total	**0.85**	**0 34**

The exposure estimates were modelled using conservative assumptions, such as complet availability of DEHP from recycled soft PVC in the inhalation exposure estimate (see section 9.2.2.3 f details). A higher RCR is calculated for PROC 8a, but all RCRs are below 1 and the risk is therefore ad quat ly controlled.

10.2.1.2. Consumers

There is no consumer exposure during formulation.

10.2.1.3. Indirect exposure to humans via the environment

Exposure via the environment can be assessed based on he biomonitoring data. As the body burdens measured include all sources of exposure, exposure via the environment being one of them, the RCRs calculated from biomonitoring data (see Table 104) are equally relevant for assessing exposure via the environment. It can be concluded that exposure from this source is adequately controlled.

10.2.2. Environment

Not relevant for this applicatio for auth risation.

10.3. Use #2: Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles

10 3.1. Human health

10.3.1.1 Workers

The exposure estimation for workers is primarily based on biomonitoring data that assess exposure from all s rces, i.e. also from diet as a very important source. The RCR for polymer processing is shown in the following table.

Table 101 RCRs based on biomonitoring data for ES2-W: Industrial use of recycled soft PVC containing DEHP in polymer processing by calendering, extrusion, compression and injection moulding to produce PVC articles

Statistical basis*	Geometric mean	90th percentile
Intake based on urinary DEHP metabolites [µg/kg bw/d]	23	92
DNEL [µg/kg bw/d]	230	230
RCR	**0.1**	**0.4**

* See section 9.0.4.3 for details

The RCRs are clearly below 1 and the risk is therefore adequately controlled.

As detailed in section 9.3.2.3 above, the underlying exposure estimate and, as a consequence, th risk characterisation is considered conservative for the following reasons:

- The risk characterisation is based on biomonitoring data that consider exposure fr m ll sources including diet.
- The basic studies measured the body burden of workers in PVC formulation and p ocessing plants, where free DEHP was used as well. Exposure from recyclate, where DEHP is nclos d the PVC matrix is considered to be lower.
- Furthermore, the typical concentration of DEHP in recyclate is [redacted] (maximum 20%), whereas higher concentrations are present in virgin PVC.
- The maximum intake calculated from biomonitoring studies was used in ris characterisation.
- The methodology applied for the conversion of biomonit ring data (DEHP metabolites in urine) consistently leads to higher calculated intake values than the m thod logy applied by the authors of biomonitoring studies (where available).
- The operational conditions and risk management m a ures detailed in the respective exposure scenario are considered to be stricter than the conditions in plac at the plants where the biomonitoring data were obtained from.

For two specific tasks involving the emptying of bag of recycled soft PVC, for which the biomonitoring data were possibly not adequate, inhalation nd derma exposure were modelled. The resulting risk characterisation is shown in the following table.

Table 102 Risk characterisation for PROC 8a and 8b (emptying bags with recycled soft PVC)

	PROC 8a	PROC 8b
Inhalation exposure [mg/m^3]	1.3	0.48
DNEL inhala ion [mg/m^3]	1.6	1.6
RCR inhal tion	0.81	0.30
Derm l exposure [mg/kg b.w. x d]	0.135	0.135
DNEL dermal [mg/kg b.w. x d]	3.4	3.4
RCR dermal	0.039	0.039
RCR total	**0.85**	**0.34**

The exposure estimates were modelled using conservative assumptions, such as complete availability of DEHP from recycled soft PVC in the inhalation exposure estimate (see section 9.2.2.3 for details). A higher RCR is calculated for PROC 8a, but all RCRs are below 1 and the risk is therefore adequately controlled.

Besides exposure of industrial workers during polymer processing, an additional contributing scenario relating to service life exposure of professionals was assessed within this exposure scenario. This relates to combined dermal and inhalation exposure (ES2-SL-P) .

The RCR calculation for the contributing scenarios is shown in the following table.

Table 103 RCRs based on modelling for contributing professional service life scenarios

ES2-SL-P: Service life professionals: Professional handling of PVC products made from recycled soft PVC containing DEHP: Installation of building materials and similar activities / inhalation exposure from volatile DEHP / professional PVC footwear)	
Dermal exposure [µg/kg bw/d]	46.3
DNEL [µg/kg bw/d]	3400
RCR	**0.014**
Inhalation exposure [µg/m^3]	5.3
DNEL [µg//m^3]	1600
RCR	**0.0033**
RCR, dermal and inhalation combined	**0.017**

All RCRs are clearly below 1 and the risk is therefore adequately controll d.

As detailed in section 9.3.2.3 above, the underlying exposure estimate and, as a consequence, the risk characterisation is considered conservative, primarily for the following reasons:

- The underlying migration rate for dermal exposure represents he 95th percentile from 51 measurements of in vitro DEHP migration from PVC articles into artificial sw at.
- The estimates assume continuous contact to PV articles ov r the entire shift and a continuously high migration from the entire article in contact with the skin.
- For inhalation exposure covered in ES2-SL-P, t e exposure estimate is based on the saturated vapour concentration of DEHP.

In relation to dermal exposure the low RCR of 0 014 indicates that an RCR well below 1 (0.014/0.35 = 0.04) would also result if no clothing penetra ion factor is applied (i.e. direct skin contact). This points to the fact that direct skin contact to PVC articles i con act with smaller fractions of the skin surface (e.g. feet, hands, trunk), results in RCRs below 0.1, although this is only covered here for the wearing of clogs by pool attendants and similar professional settings.

10.3.1.2. Consumers

ES2-SL-C: Service life Con umers: Service life consumers: Exposure from consumer articles made from recycled soft PVC c ntaining DEHP

Consumer exposure es imation is primarily based on biomonitoring data that assess exposure from all sources, i.e. fr m a l PVC articles, but also from diet. The risk characterisation based on biomonitoring data therefore rep esents a combined risk characterisation.

Risk characterisation based on biomonitoring data - combined consumer risk characterisation

The f llowing table shows the RCRs based on intake estimates using the geometric means (GM) and 90th percentiles of urinary DEHP metabolites as a conservative approach.

Table 104 RCRs for ES2-SL-C based on biomonitoring data for the general population

	Children	**Adults**
Intake based on <u>GM</u> of sum of urinary DEHP metabolites		
Intake [µg/kg bw/d]	3.57	2.81

DNEL [µg/kg bw/d]	36	48
RCR based on GM intake	**0.099**	**0.059**
Intake based on 90th percentile of urinary DEHP metabolites		
Intake [µg/kg bw/d]	10.29	8.75
DNEL [µg/kg bw/d]	36	48
RCR based on 90th percentile intake	**0.29**	**0.18**

The underlying biomonitoring data come from various recent European studies, including studies from Denmark and Germany. Especially the pan-European research project DEMOCOPHES provides biomonitoring dat for children and adults from 17 European countries, thus providing a unique basis for assessing the internal burden of DEHP.
The RCRs both for children and adults calculated based on the results from the DEMOCOPHES study ar clearly below 1 and the risk is therefore adequately controlled.
Results from the biomonitoring studies comprise exposure from all exposure pathways and all s urces, including food and food contact materials and medical devices. It is reasonable to assume that only small part of the measured body burden is actually originates from manufacture and use of recycl e pr duc

Risk characterisation for exposure from consumer articles based on m d lling – sentinel approach

An article-specific risk characterisation based on the exposure est mate from biomonitoring data is not feasible. In order to allow for evaluating potential high exposures from specific articles or groups of articles a modelling approach has been used in addition to the exposure assessment based n b omonitoring data. Results of this exposure modelling for specific article groups are presented in Table 105.

Table 105 RCRs for ES2-SL-C: based on modelling exposure from consumer articles made from recycled soft PVC containing DEHP *

Pathway	**X-1**	**X-2**	**X3**	**X4**
Adults				
Inhalation exposure [µg/m3]				
DNEL [µg/m3]				
RCR				
Dermal exposure [µg/kg bw/d]	14.4	2.95	20.0	41.6
DNEL [µg/kg bw/d]	720	720	720	720
RCR	0.020	0.00041	0.028	0.058
Oral exposure [µg/kg bw/d]				
DNEL [µg/kg bw/d]				
RCR				
Total RCR	**0.020**	**0.00041**	**0.028**	**0.058**
Children, 6-12 months				
Inhalation exposure [µg/m3]				
DNEL [µg/m3]				
RCR				
Dermal exposure [µg/kg bw/d]	17.2	5.36		27.4
DNEL [µg/kg bw/d]	720	720		720
RCR	0.024	0.0074		0.038
Oral exposure [µg/kg bw/d]		3.27		
DNEL [µg/kg bw/d]		36		
RCR		091		
Total RCR	**0.024**	**0.098**		**0.038**
Children, 2-3 years				
Inhalation exposure [µg/m3]				
DNEL [µg/m3]				
RCR				
Dermal exposure [µg/kg bw/d]	17.8	4.28	31.4	15.4
DNEL [µg/kg bw/d]	720	720	720	720
RCR	0.025	0.0059	0.044	0.021
Oral exposur [µg/kg bw/d]		1.31		
DNEL [µg/kg bw/d]		36		
RCR		0.036		
Total RCR	**0.025**	**0.042**	**0.044**	**0.021**

* All interim calculation results were rounded to three significant figures and RCRs are given with two signifi ant figures.

All RCRs for the individual article groups/sentinel products are clearly below 1 and the risk is therefore adequately controlled. In fact, all RCRs are below 0.1.

As detailed in section 9.3.2.4.2 above, the underlying exposure estimate and, as a consequence, the risk characterisation is considered conservative for reasons discussed in the context of the specific article groups. As a general conservative assumption, the underlying migration rates for dermal contact and oral exposure via mouthing represent the 95th percentiles.

The RCRs based on modelling for all sentinel products covered by ES2-SL-C are summarised in the following

table. This table includes DEHP exposure via ingestion of house dust and inhalation of indoor air (together abbreviated "IND" in the table) in addition to dermal and oral exposure for groups X-1 – X-4 assessed by the sentinel product approach.

Table 106 RCRs based on modelling exposure to DEHP from all articles covered by this application for authorisation*

Pathway	**X-1 – X-4**	**IND****	**SUM**
Adults			
Inhalation exposure [µg/m3]		2.6	2.6
DNEL [µg/m3]		170	170
RCR		0.015	015
Dermal exposure [µg/kg bw/d]	79.0		79.0
DNEL [µg/kg bw/d]	720		720
RCR	11		0.11
Oral exposure [µg/kg bw/d]		0.771	0.771
DNEL [µg/kg bw/d]		48	48
RCR		0.016	0.016
Total RCR	0.11	0.031	**0.14**
Children, 6-12 months			
Inhalation exposure [µg/m3]		2.6	2.6
DNEL [µg/m3]		130	130
RCR		0.020	0.020
Dermal exposure [µg/kg bw/d]	50.0		50.0
DNEL [µg/kg bw/d]	720		720
RCR	0.069		0.069
Oral exposure [µg/kg bw/d]	3.27	5.87	9.18
DNEL [µg/kg bw/d]	36	36	36
RCR	0.091	0.16	0.25
Total RCR	0.16	0.18	**0.34**
Children 2-3 years			
Inhalation xposure [µg/m3]		2.6	2.6
DNEL µg/m3]		130	130
RCR		0.020	0.020
Dermal exposure [µg/kg bw/d]	69.0		69.0
DNEL [µg/kg bw/d]	720		720
RCR	0.096		0.096
Oral exposure [µg/kg bw/d]	1.31	7.83	9.15
DNEL [µg/kg bw/d]	36	36	36
RCR	0.037	0.22	0.25
Total RCR	0.13	0.24	**0.37**

* All interim calculation results were rounded to three significant figures and RCRs are given with two significant figures

** Exposure from DEHP in house dust and air in the indoor environment

All RCRs are well below 1 and the risk is therefore adequately controlled.

As already discussed above, the underlying exposure estimate and, as a consequence, the risk characterisation is considered conservative for several reasons. In the combined risk characterisation presented here, an additional conservative element is introduced by assuming that a person is always in contact with PVC articles containing soft PVC recyclate, all of which contain DEHP, and that all these articles show a very high migration rate (95th percentile) for prolonged periods of time. It is noteworthy that the unspecific contribution from house dust and inhalation ("IND" in table above) amounts to more than 50% of the total modelled exposure for all age groups, which can be taken as a further indication that actual exposure from these article groups is low.

The fact that RCRs are still below 1 in this combined risk characterisation using the model ng appro ch demonstrates that the risk from DEHP exposure from articles covered by this application for authorisation is adequately controlled.

10.3.1.3. Indirect exposure to humans via the environment

Exposure via the environment can be assessed based on the biomonitorin data As the body burdens measured include all sources of exposure, exposure via the environment being one of h m, the RCRs calculated from biomonitoring data (see Table 104) are equally relevant for assessing exposur via the environment. It can be concluded that exposure from this source is adequately controlled

10.3.2. Environment

Not relevant for this application for authorisation.

10.4. Overall exposure (combined for all exposure routes)

10.4.1. Human health (combined for all exposure routes)

Based on the risk characterisa ion in pr vious chapters, DEHP in recycled soft PVC materials is considered as safe (no risk) for workers and c nsumer at any time of the formulation and use within industrial, professional or private settings. The underlying biomonitoring data integrate exposure from all sources and pathways and thus represent an overall exposure (even from sources not subject to this application for authorisation). In addition, a very conservative modelling approach for consumer exposure from articles has been used as supporting evidence, in which exposure from all groups of PVC articles containing soft PVC recyclate is assumed.

10.4 2. Envi onment

Not r le ant for this application for authorisation.

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Annex #1# Modelling of Consumer exposure from articles

General considerations

Exposure estimation for consumers generally follows a precautionary approach by using complete release approaches or upper percentiles (90th or 95th percentiles) of input values, although ECHA guidance documents (ECHA, 2008; 2010b) do not specify a percentile to be used. However, the basis of these input values and the percentiles they represent is not always adequately described in the literature and may sometimes lead to a combination of upper percentiles, resulting in unrealistic values. This problem has also been acknowledged in the framework of exposure assessment for biocidal products (EC, 2007), which applies 75th percentiles (for data of sufficient quality) whenever different exposures are combined rather than 90th or 95th percentiles. More specifically, the authors state that "*simple addition of percentiles for the routes, phases and cycles of exposure, exposure times or amounts used, and cumulative exposures, has the clear potential to provide an unacceptable estimate of exposure. The assessor needs to take great care to avoid gross errors in combining exposure*" (EC, 2007).

In relation to consumer exposure, such errors are possible in a number of different ways. For example, combining the upper percentile (or even maximum) input values for migration rates, skin surface areas and use frequency may reflect a situation that only exists in such highly exceptional circumstances. The same considerations basically apply to anthropometric measures (body weights, skin surface areas, inhalation volumes etc.), where a combination of upper percentiles – sometimes from different sources –leads to unrealistic values. For example, it is not uncommon to use a skin surface area from one source and the body weight from another source, sometimes from different continents (Europe, USA) and without identification as to which percentiles the values chosen represent. Yet, both parameters are highly correlated (EPA 2008, 2011) and skin surface areas/kg body weight have been derived (see section "Key values for dermal exposure assessment" below for details).

As a consequence of these considerations, exposure was generally estimated in this dossier on the basis of 95th percentiles for one key input value (usually the migration rate) and mean values for other input values. For example, 95th percentile for the migration rate is multiplied by mean values for the skin contact area and the contact duration in the case of dermal exposure.

For each exposure scenario, a sentinel article was chosen for an in-depth exposure estimation and risk characterisation. The sentinel article approach is based on the selected sentinel article representing the worst-case scenario for that group of articles, i.e. the article with the greatest potential for the highest consumer exposure. Other PVC articles also covered under the respective group are specifically mentioned in the exposure scenario and discussed quantitatively in the course of exposure estimation.

Population groups considered

Children differ from adults in relation to some anthropometric measures and exposure determinants, so that the exposure assessment has to be differentiated as well. Where applicable, the following sub-groups are considered in the exposure assessment and risk characterisation:

- Adults (whose exposure is assumed to equal that of the sensitive subgroup pregnant women)
- Children (based on assumed highest exposure, see below)
- 6- <12 months old (6-12 months old hereafter)
- 2 - <3 years old (2-3 years old hereafter)

For children, the age-group considered is of critical importance, since many of the input values required for exposure estimation rapidly change during childhood development. For example, the relative skin surface area (i.e. skin surface/kg body weight) is highest at a very young age and decreases consistently during childhood (Cohen Hubal et al., 2000), a fact that must be considered during dermal exposure assessment. However, the mouthing duration of objects other than toys, fingers and childcare articles – an important input parameter for oral exposure assessment– shows a somewhat different pattern (
Figure 8). Since oral and dermal exposure will be considered in an aggregated assessment for each exposure scenario (where appropriate), great care must be taken to select clearly defined age groups (the underlying figures will be discussed in more detail below).

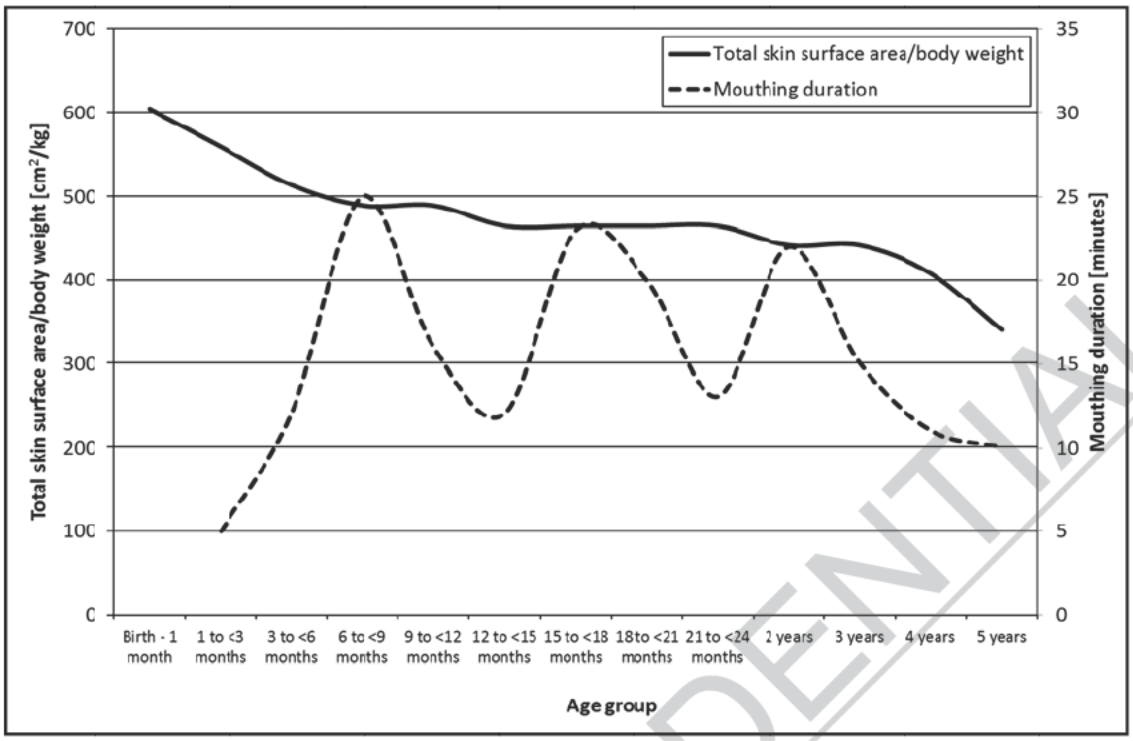

Figure 8 Development of SA/BW ration and mouthing duration („other objects“) with age during childhood development (generated from data in EPA (2011) and DTI (2002))

The “wave pattern” for mouthing “other objects” may refle t the changing pattern of objects mouthed (fingers, toys, dummies/soothers and other objects), which is shown in detail in
Figure 10 below. Note that the mouthing duratio in this f gure relates to “other objects” only (excluding e.g. toys) and is therefore different than value derived y other authors. The issues of mouthing duration and objects covered are discussed in the section “K y values for oral exposure assessment” below.

Based on these data, children of he age gr up 6 12 months old (EPA standard age category) are selected as the age group with the potentially highest exposure. This age group, however, shows a considerably lower house dust intake than older children (EPA, 2008; 2011), as will be discussed in more detail below. Therefore, 2-year old children, who still show consid rable mouthing according to the UK data (see
Figure 8), are also as essed.
Overall, children 9 12 month old and children 2-3 years old are considered. The age groups correspond to U.S. EPA’s age groups, f r which many exposure determinants have been derived.

In the exposu e modelling assessment in the Danish Phthalate Restriction Proposal (as included in the opinion of RAC/SEAC, 2012, further referred to in this document as Restriction Proposal Assessment, 2012) for DEHP and other phth lates in articles), oral exposure from mouthing of an eraser (but not other articles) by considerably older children (6/7 years) was also assessed. The mouthing duration assumed was 60 minutes/day on the basis of a Danish EPA survey (Svendsen et al., 2007), which, however, provides no source for this figure. As shown bel w av ilable studies on mouthing duration usually involved young children up to 3 years old and only one study (DTI, 2002) exists for children up to 5 years old. These show a considerably lower mean mouthing duration (10 minutes/day) for „other objects“ as well as a considerably lower skin surface area/body weight than younger children (
Figure 8), and – as a consequence – children 3-5 years old are considered to be less exposed.
The rationale behind the selection of school children (6/7 years) may well have been that they are more likely to mouth school supplies such as erasers than younger children. However, US data show that the mouthing prevalence of „crayons, pencils, erasers“ is about the same for children 1 year old (19%), 2 years old (17%) and children 6 years old (18%; EPA, 2008).

Sources of anthropometric data

Many different sources report anthropometric data and sometimes also contain recommendations for values to be used in exposure assessment (see e.g. BAGS, 1995; Bremmer et al., 2006; ECHA, 2010b). These sources and the recommendations given therein, however, are often based on the Exposure Factors Handbooks (EFHs) of the U.S. Environmental Protection Agency, available both specifically for children (EPA, 2008) and in a more general form (EPA, 2011). These EFHs are unique in the amount of data reported and the degree of statistical analysis. Therefore, the most recent versions of the EFH are used here for anthropometric input values in addition to data recommended in the ECHA Guidance (ECHA, 2010b). These data have the additional advantage that values are available for defined EPA age groups, i.e. almost all values exist for the same age groups. While it is acknowledged that the EFHs primarily relate to the U.S. population, the difference to Europe is considered small for anthropometric data, especially in light of the fact that populations from different European countries may also differ substantially. In any case, use of the EFH data is also envisaged in the ECHA Gu dance (ECHA, 2010b).

Key values for dermal exposure assessment

Exposed skin surface and body weights

As already mentioned above, there is a danger of excessive combination of high percentile valu s. As a simple example, the German source also quoted as a reference in the ECHA Guidance (ECHA, 2010b) recommends to use the 5th percentile of the body weight as a reasonable worst case assumption. F r the total skin surface area, the 95th percentile is recommended for reasonable worst case scenarios in the same ource (BAGS, 1995). If these values are combined in dermal exposure estimation, they imply a value of 454 cm^2 total skin surface/kg body weight (SA/BW ratio) for 30-40 year old women. Such a high value has never been observed for adults when directly measured (i.e. when both skin surface area and body w ight da a were available for the same subjects). The maximum SA/BW ratio for adults (18 years or older) was 351 cm^2/kg body weight, i.e. only about 75% of the value presented above (EPA, 2011; Phillips et al., 1993) The ba ic problem is that body weight and total skin surface area are sometimes treated as independent variables when they are in fact highly correlated. As a consequence of disregarding this physiological fact, an "*unrealis ic body type*" (Phillips et al., 1993) is assumed when e.g. an upper percentile for the skin surf ce ar a is combined with a lower percentile (or even the median) for body weight. This example is somewhat self- ident, but illustrates the problems associated with undue use of percentiles in exposure assessment.

As a practical example, a training exercise in th co text of RAPEX risk assessments and provided by DG SANCO (2006) estimated DEHP exposure from air mattresses. The author assumed a total skin surface area of 7,900 cm^2 and a body weight of 16 kg f r 5 years old boys. The sources of these values and the percentiles they represent are not given, but the assume body we ght is described as "lower end of the normal range" in the source (DG SANCO, 2006), probably wi h the intention of a "reasonable worst case" estimate. Available data indicate that a body weight of 16 kg represents the 5th percentile for 5-years old boys in the USA and Germany (EPA, 1985; 2008; 2011; RKI, 2011), wi h the assumed skin surface area approximately representing the median. Because body weight and skin urface area are highly correlated, it may well be questioned whether even a single 5-years old boy with these body features exists. The resulting SA/BW ratio of (7,900 cm^2/16 kg =) 494 cm^2/kg has nev r been observed for children of this age with direct SA/BW measurements, as is evident from the graphical presentation of individual values in Phillips et al. (1993).

In order to avoid such problems, the total skin surface area/kg body weight ratios (SA/BW), as calculated from proposed default values for total skin surface area and body weight, were used as a key input value for dermal expo ure assessment. The values derived were checked for consistency with direct SA/BW data (EPA, 2008; 2011; Phillips et al., 1993) and additional sources. Direct SA/BW ratios were derived from a population of 401 individuals for which total skin surface area, body weight and height data were available and SA/BW data were calculated for every individual. These data and their use in exposure assessment have been analysed in detail lsewhere (EPA, 1985; Phillips et al., 1993).

For adults (including pregnant women), the key value is derived from the skin surface area and body weight suggested in the ECHA Guidance for Consumer Exposure Estimation (ECHA, 2010b). The resulting values for men and women are almost identical and are supported by a variety of supporting values. In particular, the key value is almost identical to the direct SA/BW data (EPA, 2011; Phillips et al., 1993).

For children, however, available data agree less well. The direct SA/BW ratios for small children (EPA, 2008; 2011; Phillips et al., 1993) are considerably larger than all other data, including the value derived from recommended surface areas and body weights for young children as reported in the EFH. The differences are most probably due to the fact that SA/BW ratios decrease rapidly during childhood development. Thus, there is a

strong negative correlation between age and SA/BW ratios for children, while there is no correlation for adults ≥ 18 years of age (Phillips et al., 1993). The direct SA/BW data for children up to 2 years of age shown in 107below also include a substantial fraction of children less than 6 months old. The raw data (presented in EPA, 1985) and the analysis by Phillips et al. (1993) indicate that children less than 6 months of age have particularly high SA/BW ratios of up to 1142 cm^2/kg.

Anthropometric data from the German KiGGS ("Kinder- und Jugendgesundheitssurvey", 2003-2006; RKI, 2011) also show the clear decline in the SA/BW ratio during the first months and years of life. From the median body weight and height data of this representative survey, values for SA as well as SA/BW were calculated in Appendix A-2 using equations and constants suggested in EPA (2008). The calculated SA/BW ratios are in good agreement with the EFH data used as key values for children.

The values derived by Bremmer et al. (2006) for children 1.5 and 2.5 years old are slightly lower than the key value for 6-12 months old children, which however, is in line with a decreasing SA/BW ratio with increasing age (key value for 6-12 months old children). The key value for children 2-3years old agrees well with the data derived by Bremmer et al. (2006), their slightly higher values could be due to the fact that they used 25th percentiles for body weights and surface areas.

The value derived by Tønning et al. (2009), however, is substantially lower, not only than the key value for 2-3 years old children, but also than the values derived by Bremmer et al. (2006) for about the same age group. This is most probably due to the fact that Tønning et al. (2009) used different sources for the skin surface area and the body weight, which related to slightly different age groups and were probably relating to different populations.

Table 107 Key values for the total skin surface area/body weight ratio

Total skin surface area/ body weight	**Key value**	**Rationale**	**Supporting values**
Adults and pregnant women	280 cm^2/kg	Derived from values suggested in ECHA Guidance (ECHA, 2010b): Men: 19,400 cm^2/70 kg = 277 cm^2/kg Women: 16,900 cm^2/60 kg = 282 cm^2/kg	EPA, 2011; Phillips et al., 1993*: US males and females combined, ≥ 18 years: Mean: 284 cm^2/kg Median: 286 cm^2/kg 95th percentile: 329 cm^2/kg Range: 200-351 cm^2/kg Bremmer et al. (2006): Men: 19,100 cm^2/74 kg 258 cm /kg Women: 16,800 cm^2/61 kg = 275 cm^2/kg Andersen et l. (2012), pregnant women: 18,500 cm^2/73 5 kg = 252 cm^2/kg
Children (6-12 months)	490 cm^2/kg	Means for US children aged 6-12 months (recommended values): 4,500 cm^2/9.2 kg = 89 cm^2/kg (EPA, 20 8; 2011)	German KiGGS survey (Mekel et al., 2007; RKI, 2011); calculated from median values for height and body w ight (see Appendix A-2): Boys and girls combined, 6-12months: 501 cm^2/kg Boys and girls combined, 2-3 years: 450 cm^2/kg EPA, 2008; 2011; Phillips et al., 1993*: US boys and girls combined, 0-2 years: Mean: 641 cm^2/kg Median: 617 cm^2/kg 95th percentile: 846 cm^2/kg Range: 421-1142 cm^2/kg Bremmer et al. (2006) for: 1.5-years: 4,800 cm^2/9.85 kg = 487 cm^2/kg 2.5-years: 5,750 cm^2/12.5 kg = 460 cm^2/kg Tønning et al. (2009) for 2 years: 6,000 cm^2/15.2 kg = 395 cm^2/kg
Children (2-3 years)	440 cm^2/kg	Means for US children aged 2 3 ye rs (recommended values): 6 100 cm^2/13 8 kg = 442 cm^2/kg (EPA, 2008; 2011)	

* The data in EPA (2011) were rounded, but the original data from Phillips et al. (1993) are shown here.

The body weights and total skin surface areas used in the calculation of SA/BW ratios above are also used –

where required – in other exposure estimates and are summarised in Table 108.

Table 108 Key values for body weight and skin surface area

	Body weight	**Skin surface area**
Adults and pregnant women	70 kg (men), 60 kg (women)	19,400 cm^2 (men), 16,900 cm^2 (women)
Children (6-12 months)	9.2 kg	4,500 cm^2
Children (2-3 years)	13. 8 kg	6,100 cm^2

Note that the values for adults are the ones proposed in the ECHA Guidance on Consumer Exposure E timation and the ones for children are "recommended values" of the U.S. Environmental Protection Agency for the specific age groups (EPA, 2008; 2011).

DEHP migration from articles to the skin

Another key value in dermal exposure assessment is the migration of DEHP from articl to he skin surface, which is usually measured using artificial sweat as a surrogate for real-life c ndition Many migration analyses for DEHP have been performed in the context of the "Surveys of Chemical Substances in Consumer Products" by the Danish EPA (Andersen et al., 2012; Svendsen et al., 2007; Tønning t al., 2010a; Tønning et al., 2009; Tønning et al., 2010b).

Migration data from these surveys were extracted and analysed. The procedure for this analysis is described in Appendix A-1. Briefly, all values in the original reports were conv rted into a unit of µg/cm^2 x h on the basis of information provided in the reports. In some cases such a c nversion was possible because the data were already expressed in mg/cm^2 and conversion only had to consider the extra tion times. In these cases, both values from duplicate analyses of the same sample entered the evalu tion. In other cases, conversions had to consider sample area and other parameters. In most cases, these latter parameters were only given as the mean of duplicate samples so that only one value per sample could be gen rated Full details of this evaluation and a discussion of important issues in relation to migration rates ar provid in Appendix A-1.

The analysis showed that some of the duplicate values were highly variable. For example, coefficients of variance of 50% or above (i.e. one very high and ne very low value for the same sample) were found for some of the duplicate analyses of plastic sandal in Survey No. 107 (Tønning et al., 2010b). In some, but not all of these cases, these values were btained from samples with very low DEHP concentration (< 0.03%; see Appendix A-1 for a full discussion).

The approach chosen here is based on the statistical evaluation of all available migration data from these Surveys, rather than using article-specific migration values. This approach has the following advantages:

- Plastic sandals are th only article group for which many different analyses have been performed. As show bove (and described in more detail in Appendix A-1), however, the results from these analyses are conside ed highly uncertain.
- For all o her article groups analysed in the surveys, e.g. swimming equipment, school supplies, balance bal s and similar items, only few migration values (usually 1-2, sometimes 4) are available and must be considered uncertain. The lower number of values available per product group also reflects the fact that a) n t all samples analysed were made of PVC and b) not all PVC articles contained DEHP.
- The ECHA Guidance on Consumer Exposure Estimation (ECHA, 2010b) specifically mentions migration rates as one of the types of measured data that can be used in consumer exposure estimation. Like any other measured data, however, such measurements must be "*reliable and representative for the situation that needs to be assessed*" (ECHA, 2010b). The authors do not provide any interpretation of the term "reliable". Nonetheless, the ECHA Guidance on Occupational Exposure Estimation propose for data with high variation and uncertainty (e.g. GSD > 3.5, which applies here, see Table 109 below) to have at least 20-30 measurements for RCRs < 0.1 and more than 50 measurements for RCRs of 0.5-1 (ECHA, 2010a). It is clear that article-specific migration values (i.e. if specific articles are analysed separately) are not sufficient to fulfil these requirements.
- It must be stressed in this context, that migration rates could also show some variation due to real differences in migration rates, e.g. due to the different matrices. The data presented in Appendix A-1do

not point to pronounced differences in this respect. However, large differences are observed within the article group for which most analyses are available (plastic sandals), even under identical test conditions (see all static tests, 16 h extraction in Appendix A-1).

- In this context, the statistical approach chosen here is based on a sufficiently large number of values (n=51) and thus overcomes the implicit uncertainties associated with use of article-specific migration values based on (usually) one or two values only. It is considered to be still representative of the situation to be assessed since all migration rates relate to the dermal exposure to articles.
- Article-specific migration values are, in any case, only available for a limited number of article groups. For example, no migration values exist for PVC artificial leather used in car seats, PVC waterbeds and many other articles. The approach chosen here allows estimating exposure resulting from DEHP use in these articles using a migration value derived from the statistical analysis of many different products.
- The data presented in Appendix A-1 also show that there is no clear association between the DEHP concentration in the article and the migration rate measured. In fact, if the migration rate is p otted o r the DEHP concentration in the article, the coefficient of determination from the regression analy is (R^2) is less than 0.1.

The descriptive statistics of the data presented in Appendix A-1 are shown in Table 109. Two d fferent approaches are shown. In the initial evaluation, all analyses showing migration values below the limit of detection entered the calculation with half the limit of detection. These latter cases wer lmost exclusively encountered in Survey No. 117, which, however, had a comparatively high limit of detecti n of 4 µg/cm^2. These values therefore contributed a substantial number of high migration values (8/11 values were bove 0.5 µg/cm^2 x h due to the exceptionally high limit of detection). In the second analyses, these no-detects of Survey No. 117 were removed, reducing the number of values to 51. This results in a clear d line f the mean, upper percentile and maximum values.

Table 109 Descriptive statistics for DEHP migrati n into ar ificial sweat

Parameter	**DEHP migration into artificial sweat [µg/cm^2 x h]**	
	All*	**Without no-detects in Survey 117****
n	62	51
MIN	0.0011	0.0011
AM	0.17	0.077
GM	0.048	0.029
GSD	6.0	4.5
Median	0.060	0.040
75th perc ntile	0.19	0.085
90th percentile	0.57	0.18
95th percentile	**0.82**	**0.27**
MAX	1.0	0.83

All values rounded to two significant figures; see Appendix A-1 for details
* No-detects entered the calculation with half the limit of detection.
** No-detects from Survey 117 were excluded due to the exceptionally high limit of detection.

Based on the principle of using the 95th percentile in the exposure estimation, a migration rate of
0.27 µg/cm^2 x h
is chosen as the key value for DEHP migration rate from articles in contact with the skin. It represents the 95th percentile in the second evaluation, thus excluding artificially high values from a high limit of detection in Survey No. 117. Even after exclusion of the Survey No. 117 values, the migration rate derived is still based on

51 measurements, a number considered sufficiently large for datasets with high variation (see above). The data are considered to be broadly representative, since many different articles were included in the analysis (e.g. pencil cases, plastic sandals, shower curtains, oil cloths and balance balls).
While this value is largely based on static migration tests, the discussion in Appendix A-1 suggests that this value also covers dynamic test conditions, the only exception being simultaneous application of sun lotion under dynamic conditions. The latter will be discussed separately in the context of the relevant use/exposure scenario.

The EU Risk Assessment Report (ECB, 2008) uses two different models to assess dermal exposure to PVC articles. One relies on a combined migration/absorption rate of 0.24 µg/cm^2 x h from an in vivo rat study (actually two experiments with 4 rats each), in which a PVC film containing 40% DEHP was applied over 24 hours to the clipped skin using a cover made of aluminium foil and an elastic bandage to ensure tight continuous contact (Deisinger et al., 1998). The rate given is based on the total amount migrated (about 0.73 µg/cm^2 x h) multiplied with the bioavailable fraction (ca. 33%), i.e. subtracting the substantial amount retrieved from the bandage material and skin washes.
The other model relies on a migration rate of 0.11 µg/cm^2 x min, taken as 6.6 µg/cm^2 x h in ECB (2008 These authors describe the underlying experiment, in which a DEHP-containing "flat vinyl product" was s rubbed with a lanolin-impregnated cotton cloth, allegedly simulating human exposure (oils present on human skin t be represented by lanolin). No information on the actual test duration, the number of trials replicat s, standard deviations etc. is provided. The source of this information is not properly identified in th list of references and the data cannot be evaluated. A similar experiment ("scrubbing the PVC consum p oducts on a metal tray, using a cotton T-shirt cloth coated with 50 mg of lanolin for a 2-min period, selected to simulate dermal contact") served as the basis for another evaluation, resulting in a similar migrati n rat of 7.4 µg/cm^2 x h (DG SANCO, 2006). Obviously, this value was deduced from the 2-minute exp rimental period and then applied to the assumed exposure period of 2 hours. It is questionable whether su h an extrapolation is appropriate and migrations rates obtained over a period close to the exposure time are certa nly more valid. Again, the original document (a document of the Swedish Chemicals Agency, KEMI) was not publicly available, preventing further analysis of the data. The experimental design remains somewha obscure, ince it is unclear whether the lanolin-treated cloth was in between the PVC article and the metal tray or erved to hold the article.
Overall, it is unclear if the experimental design used in hese xperiments is relevant to dermal DEHP exposure from PVC articles. They either used tight occlusion f the est material in a study mostly cited in relation to dermal absorption rather than migration, or involved ex ensiv scrubbing of a PVC product, sometimes with unclear experimental design.
A recent publication (Pfaff et al., 2012), published by German Authorities (Federal Institute for Risk Assessment (BfR) and Federal Environment Agency (UBA) reported higher migration rates than derived here. Only a limited number of samples were analys d for mig ation into artificial sweat and a method developed for migration into saliva was used. These and other issues relating to this recent report are fully discussed in Appendix A-1.

As shown in Table 109 nd in more detail in Appendix A-1, the values used in ECB (2008) are above all migration rates dete mined in Danish EPA surveys with artificial sweat. The reason for these discrepancies cannot be determin d due to cking information on the RAR data. However, from the available data a likely explanation is that ve y high migration values determined over a short (minute) period were used in ECB (2008) and DG SANCO (2006), disregarding a decline in the migration rate with time. In contrast, the Danish EPA surveys used migration values determined over prolonged periods, generally the times also used as exposure duration in the xposure estimation (one to several hours, e.g. 16 hours for plastic sandals). The migration rate det rmined in su h a way levels off a potentially higher migration at the beginning of contact and a lower migra i n towards the end. Basically, this approach averages the migration from the article over the assumed exposure duration, an approach believed to be more realistic.

The calculation flow for estimating dermal exposure is presented in the following table.

Table 110 Calculation flow for dermal exposure scenarios

Calculation	Parameter	Value	Unit	Source
	Migration rate	0.27	µg/cm^2 x h	Derived above
multiplied by	Total skin surface area/body weight	280 (adults, pregnant women) 440 (children, 2-3years) 490 (children, 6-12 months)	cm^2/kg b.w.	Derived above
multiplied by	Fraction of total skin surface area in contact		dimensionless	ES-specific
multiplied by	Contact time		h/d	ES-specific
multiplied by	Contact frequency		/d	ES-specific
results in	External exposure		µg/kg b.w. x d	

Clothing penetration factor

In some exposure situations, direct contact between the bare skin and the PVC article is very unlikely. For example, waterbeds are draped with a mattress cover and hence even people sleeping naked in such a bed are not in direct skin contact with the PVC material. Other situations include wearing special clothing, such as rainwear, where it can be assumed that direct contact with bare skin is limited to very small areas, because other clothes are worn underneath the PVC article.

No data are available in relation to the degree of protection offered by such additional clothing or fabric between the PVC article and the skin of consumers. However, data from biocide/pesticide penetration through clothing can be used for this purpose.

Generally, the "Technical Notes for Guidance" (TNsG) of human exposure to biocidal products (EC, 2007; 2010) assume a clothing penetration of 50% for non-professionals wearing a long-sleeved shirt and trousers or skirt with shoes . This factor should not be applied for liquid products potentially wetting the clothes, but can be assumed in the context of the exposure estimates relevant in this dossier. However, this value is very conservative, as shown by detailed evaluations. On the basis of 2,129 individual inner/outer paired measurement samples (2,029 patch samples and 100 whole-body dosimeter samples) from the Pesticide Handlers Exposure Database (PHED), Driver et al. (2007) derived single-layer clothing penetration values. It must be noted that this analysis excluded all data points obtained with impermeable or double layer clothing. The values differentiated by sampling technique were:

- whole body dosimeters: 8.21% (AM) 3.64% (median)
- patch samples: 12.12% (AM) 5.93% (median).

For the combined analysis of the 2,129 samples, the median was 5.84% with a 95th confidence interval of 5.35-6.38% and the 75th and 97.5th percentiles were 16.67% and 50%, respectively. Mean values for liquid products were higher than means for solid products in this evaluation.

The analysis by Driver et al. (2007) with a mean clothing penetration of 8-12% is supported by a review of Ross et al. (2008), who found pesticide clothing penetration to range between 0.082 and 10% and noted the conservatism of an average value of 10% as employed in several exposure assessments.

Driver et al. (2007) also predicted clothing penetration as a function of outer loading from the available paired measurements. The authors observed a clear inverse relationship between outer loading and the percentage of clothing penetration. The following figure illustrates the decrease in clothing penetration predicted in dependence of the outer loading. The squares included in the chart indicate the loadings assumed in the exposure assessments of this dossier. The migration rate of 0.27 µg/cm^2 x h (see above) multiplied with the exposure duration for specific scenarios is considered to represent the loading in the scenario. For example, with an

exposure duration of 9 hours for adults sleeping, the loading is calculated to be (0.27 µg/cm^2 x h * 9 h =) 2.43 µg/cm^2 (right squares in the chart). A lower loading of (0.27 µg/cm^2 x h * 1.3 h =) 0.35 µg/cm^2 (left squares in the chart) is calculated for an exposure duration of 1.3 hours for 2-year old children wearing rainwear, resulting in a higher predicted clothing penetration.

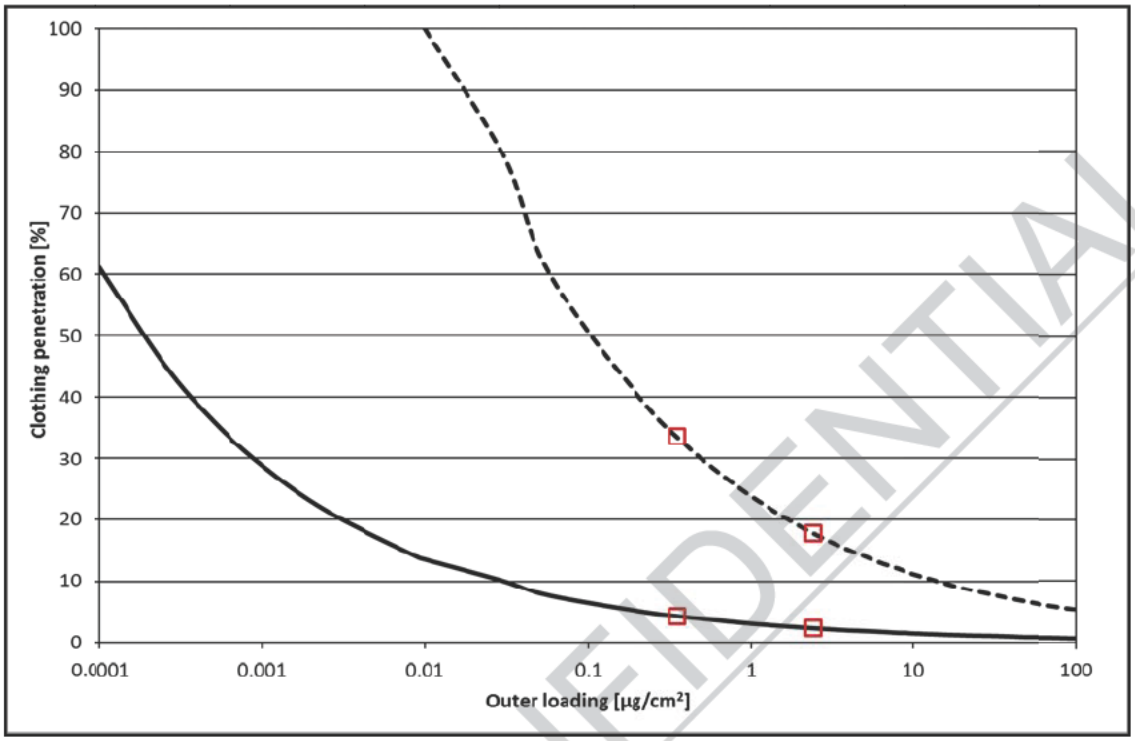

Figure 9 Percentage of clothing penetra ion (med n (solid line) and its upper 90th percentile (dashed line)) in dependence of outer loading (data from (D iver et al., 2007))

These data show that a median clothing penetrati n of less than 10% results at the loadings assumed in this exposure assessment. The respective upper 90th percentiles of the median are below 40% for the loadings assumed here, with the maximum of almost 35% obtained for the comparatively low loading due to short exposure duration for children wearing r inwear.

On the basis of these data,
a clothing penetra ion of 35%
is derived for the exposure as essment in this dossier. It is considered conservative for the following reasons:

- The val e derived represents the upper 90th percentile of the median (rather than the median) to account for the diff ren substances/conditions considered.
- The val e is twice as high as the 75th percentile (16.67%) and only somewhat lower than the 97.5th percentile (50%; equivalent to the TNsG value, see above) derived in the original analysis (Driver et al., 007), probably representing a value close to the 95th percentile.
- It i higher than the value of 10% considered conservative in other sources (Ross et al., 2008)[3],
- It considers high clothing penetrations associated with the application of liquid products and specific application types (e.g. aerial spraying and rights-of-way sprayers) considered to be less relevant here.

It could be argued, though, that the data in Figure 9 suggest considerably higher clothing penetration at lower loadings. However, it is also clear from the data in Figure 9 that full penetration (at the upper 90th percentile of the median) is only achieved at a loading at least one order of magnitude below the loadings assumed here. The approach taken here – i.e. a comparatively high loading derived from the 95th percentile of the migration rate, multiplied with a clothing penetration factor of 35% – therefore leads to an overall higher exposure estimate

[3] The California Department of Pesticide Regulation uses default values of 10% for applicators and 25% for harvesters; see e.g. http://www.cdpr.ca.gov/docs/whs/pdf/hs1455.pdf

than a low loading with complete clothing penetration.

The calculation flow for estimating dermal exposure for these specific scenarios is presented in the following table.

Table 111 Calculation flow for dermal exposure scenarios (with clothing penetration factor)

Calculation	**Parameter**	**Value**	**Unit**	**Source**
	Migration rate	0.27	µg/cm^2 x h	Derived above
multiplied by	Total skin surface area/body weight	280 (adults, pregnant women) 440 (children, 2-3years) 490 (children, 6-12 months)	cm^2/kg b.w.	Derived above
multiplied by	Fraction of total skin surface area in contact		dimensionless	ES-specific
multiplied by	Contact time		h/d	ES-specific
multiplied by	Contact frequency		/d	ES-specific
multiplied by	Clothing penetration factor	0.35	dimensionless	Derived above
results in	External exposure		µg/kg b.w. x d	

Key values for oral exposure assessment

Mouthing behaviour of children

In the case of oral exposure, the mouthing behaviour of young children is critically important. This not only refers to the mouthing duration (e.g. in min t /day), but also to the type of object actually mouthed by children and the different types of mouthing behaviour (e.g. licking, sucking and chewing/biting). Mouthing – as a form of oral exploration – is a natura part of early childhood development and thus both the extent and pattern of such behaviour changes with age (Cohen Hubal et al., 2000; van Engelen et al., 2008; van Engelen and Prud'homme de Lodder, 2007). F r example, access to objects other than fingers and child articles increases as the child becomes more mob le. The age group considered is therefore decisive for the input values derived.

Many studie n the mo thing behaviour of children are available, but most of them relate to mouthing frequency rather than to mouthing duration (for reviews, see EPA, 2008; EPA, 2011). In addition, most of the studie on mou hing duration were conducted with North American (mostly US) children, while there are only two studie from Europe, one from the Netherlands (Groot et al., 1998; Könemann, 1998; Steenbekkers, 2001) and another one from the United Kingdom (DTI, 2002; Smith and Norris, 2003).

In he context of the exposure estimation performed here, only uses in articles to be authorised are considered. Specifically, the use of DEHP in toys and childcare articles (pacifiers/soothers/dummies and teethers) is r st icted under REACH Annex XVII (entry 51) and is not considered here. The exclusion of toys and childcare articles has an important impact on the mouthing duration. The following figure shows the daily mean mouthing times for 236 UK children, differentiated by age group and type of object mouthed (note that the solid line is the one also presented in
Figure 8).

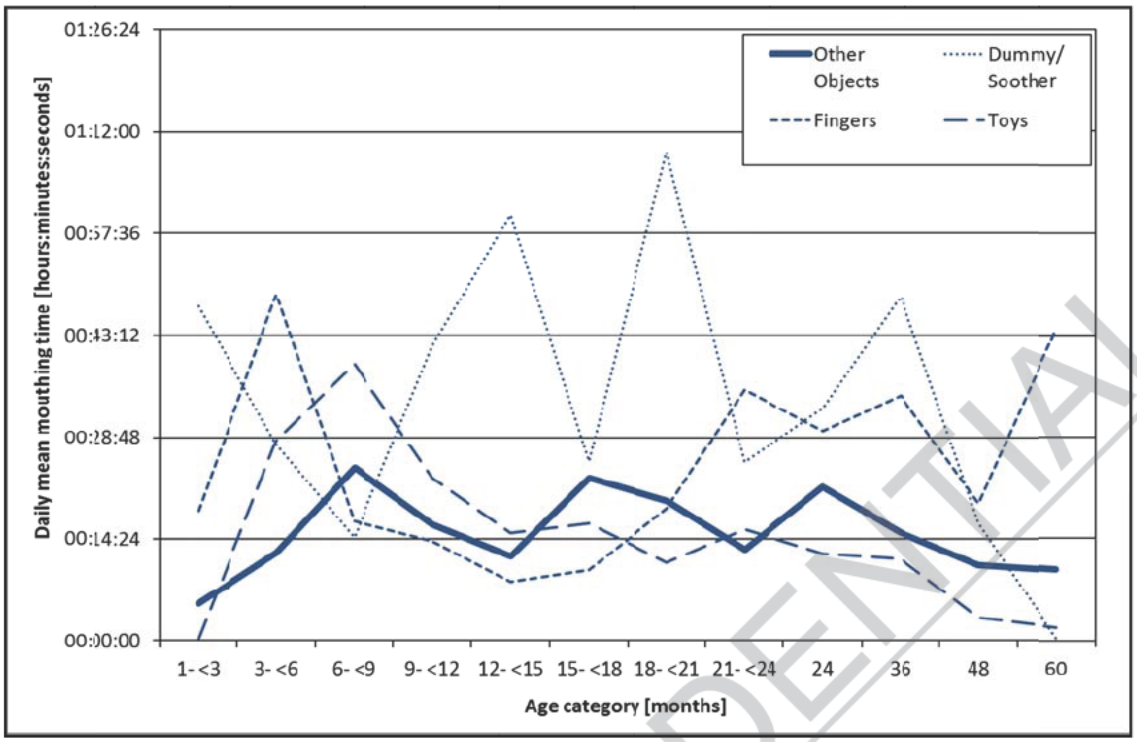

Figure 10 Daily mean mouthing duration, differentiated by obj ct mouthed, in UK children (data from: DTI, 2002; Smith and Norris, 2003)

It is evident from these data that objects other than dummie /soothers, fingers and toys are mouthed only for relatively brief periods. These "other objects" are relevant in the exposure estimation below since they potentially represent – among many other items PVC ar cles of the different exposure scenarios (see below for details).

The highest daily mean mouthing durat on for "o her objects" was observed for children 6-9 months old (about 24 minutes), For this age group, the rank order in terms of mouthing times is toys > other objects > fingers > dummies. A roughly similar pattern was f und for Dutch children of the 6-12 months age group (Groot et al., 1998; Könemann, 1998; Steenbekkers, 2001), although they had a higher value for dummies. The latter finding may not necessarily contradict he UK data, since the mouthing duration for dummies increases in the 9-12 months old group again.

From these data, it is clear that fingers, dummies/soothers etc. and toys account for most of mouthing duration. In addition, these two studies allow the derivation of a daily mouthing duration for PVC articles. They will therefore be discuss d in deta l and discussed in the context of additional (North American) studies. An overview of the Dutch nd the UK study is provided in the following table.

Table 112 Overview of Dutch and UK studies on mouthing behaviour of children

Parameter		**Dutch study**	**UK study**
Number of children	Total	42	236
	Per age group	5-14	9-39
Number of age groups		4	12
Observation periods		10 observation periods of 15 minutes each per day on 2 days (= 5 hours total observation) during awake times	20 observation periods of 15 minutes each over 2 weeks (= 5 hours total observation) during awake times
Observation by		Parent	Parent
Validation by		Shadow observations by researcher (no significant difference found) and (limited) video-taping (results not sufficient for statistical analysis	Shadow observations by trained observers nd video-taping (no significan diff rence found)
Mean total mouthing duration (excluding dummy) [minutes/day]*	0-18 months	3-6 months: 36.9 6-12 months: 44.0 12-18 months 16.4	1-3 mon h : 25 3-6 months: 90 6 months: 81 9-12 months: 54 12-15 months: 36 15-18 months: 50
	18-36 months	18-36 mon hs: 9.3	18-21 months: 50 21-24 months: 78 24 months: 66 36 months: 62 48 months: 33 60 months: 59
Differentiation by objects		Dummy Fingers Toys for mouthing Other toys Non-toys	Dummy Fingers Toys Other objects
Differentia ion by type of mou hing		Licking Sucking/biting	Licking Sucking Biting
References		Groot et al., 1998; Könemann, 1998; Steenbekkers, 2001	DTI, 2002; Smith and Norris, 2003

* The mouthing duration refers to the time that the child is awake, since children were not observed at night. For the UK study, the mouthing duration without dummies was calculated from the original data.

The data show that the UK children displayed a considerably longer mouthing duration than the Dutch children. This is also noted by the authors, who also included data from a US study (Juberg et al., 2001) in their comparison of mouthing duration for aggregated age groups (fingers, toys and other objects combined). For the

age group 0-18 months, they noted a higher mean mouthing duration (55 minutes) in their subjects compared to both the Dutch and US studies (32 minutes in each). The difference was even more pronounced for the 18-36 months age group that showed a mean mouthing duration of 65 minutes in the UK study, while it was 4 minutes (US study) and 9 minutes (Dutch study). The authors suggested the high level of thumb sucking in their sample as a possible explanation (Smith and Norris, 2003).

For the exposure estimation required here, the mouthing duration for “non-toys” (Dutch study) and “other objects” (UK study) is required. An analysis of the two European studies is presented in the following table. The table also shows the results for the type of mouthing activity, but these are reported in a very detailed manner in the original studies.

Table 113 Mouthing duration for objects other than toys, childcare articles and fingers (values used for exposure assessment in bold)

Parameter		**Dutch study**	**UK study**
Total mouthing duration [minutes/day] for non-toys/other objects*	Mean values	3-6 months: 2.8 ± 2.8 6-12 months: 9.4 ± 8.4 12-18 months: 7.2 ± 14.2 18-36 months: 2.0 ± 3.4	1-3 months: 5 3-6 months: 12 **6-9 months: 25** 9-12 months: 16 12-15 months: 12 15-18 months: 23 18-21 months: 20 21-24 months: 13 24 months: 22 **36 months 15** 48 m nths: 11 60 months: 10
	Minimum-maximum values (Dutch study) and maximum values (UK study)	3-6 months: 0.0-6.9 6-12 months: 0 2-25.7 12-18 month : 0.0-50.3[§] 18 36 months: 0.0-11.6	1-3 months: 28 3-6 months: 37 6-9 months: 70 9-12 months: 91 12-15 months: 63 15-18 months: 98 18-21 months: 66 21-24 months: 40 24 months: 178 36 months: 85 48 months: 76 60 months: 53
Type of mouthing	Licking	3-6 months: 42% 6-12 months: 34% 12-18 months: 17% 18-36 months: 23%	Across all age groups[&]: Licking: <30% Sucking: >35% Biting: <20%
	Sucking/biting	3-6 months: 58% 6-12 months: 66% 12-18 months: 83% 18-36 months: 77%	
References		Groot et al., 1998; Könemann, 1998; Steenbekkers, 2001	DTI, 2002; Smith and Norris, 2003

***** The mouthing duration in the Dutch study refers to the time that the child is awake, since children were not observed at night; values from the UK study were rounded to the nearest minute.

[§] This value refers to an outlier as is evidenced by the total mouthing duration (excluding dummy) in this age category of 53.2 minutes, which is an outlier (see text for details).

[&] Figures were derived from a graphical presentation in DTI (2002), which contains detailed data for all age groups and combinations of the different types of mouthing (see text for details).

The data summarised above again show higher values for UK children than for Dutch children. These differences notwithstanding, both studies found the highest mean mouthing duration for non-toys/other objects in the same age group: 6-12 months (Dutch study) and 6-9 months (UK study). Children of the 2-3 years age group also show comparatively high means in the UK, but not in the Dutch study.

Percentile distributions are not provided in the UK study, precluding an analysis of maximum values in relation to outliers. Percentile distributions, in the form of box plots, are given in the Dutch study only for total mouthing duration (excluding dummy), but not for object-differentiated data. The Dutch study identified individual values as outliers in all age groups for the total mouthing time (excluding dummy). The authors defined outliers as values at least 1.5-times larger than the height of the box (i.e. the interquartile range) in their box blo s For children 12-18 months old, the maximum mouthing duration for other objects of 50.3 minutes/day is identi ied as an outlier, since the maximum total mouthing time (excluding dummy) of 53.2 minutes/day is identified as an outlier in the box plot. Similarly, the maximum total mouthing time (excluding dummy) of 171.5 minute /day for 6-12 months old children – which served as the basis for an assumed mouthing time o 3 hours for CSTEE (1998) – is identified as an outlier by the authors (Groot et al., 1998).
In the light of the difficulties, the following approach is chosen to derive a key value for mouthing time for objects other than dummies, toys and fingers (see table below):

- The age category 6-12 months is taken as the most critical population, since these children had the highest mean mouthing time (for objects other than dummies, toys and fin ers) in the two European studies.
- The mean value from the UK study for children 6-9 months old (25 min/day) is taken as the key value for 6-12 months old children, which is a conservative assumption ince the value for 9-12 months old children is considerably lower (16 min/day).
- The value is substantially higher than the mean fr m the Dutc tudy (9.4 min/day) and corresponds to the maximum of the Dutch study.
- This mean from the UK study is also higher than s pporting values from US studies (Babich et al., 2004; Greene, 2002; Juberg et al., 2001; Kiss, 2002) although the latter differ by age boundaries and/or the types of objects covered.

Recently, data on the mouthing duration were as essed in relation to the entry 52 of Annex XVII to REACH review for DIDP and DINP (ECHA, 20 2; RAC, 2013). ECHA (2012) derived mouthing durations of 79 (typical) and 126 minutes/day (rea nabl worst case) for children 6-12 months old (values for children 12-18 months old were assumed to be much lower: 4.9 and 13.2 minutes/day, respectively). RAC (2013), on the basis of the data in the ECHA report used a re sonable worst case value of 2 h/d (only slightly different from the 126 min/d derived by ECHA), but did no derive a typical (i.e. mean) estimate. The difference between the means (25 minutes/day assumed here vs. 79 minutes/day in ECHA (2012)) is entirely due to the fact that ECHA (2012) – and also RAC (2013) – derived a duration for the mouthing of toys, childcare articles and other articles (only excluding pacifiers while th values derived here refer to the mouthing of "other objects" (excluding toys, childcare articles and pacifiers, for which the use of DEHP is prohibited and which are not subject to this application).
For children 2 3 years old, the mean for children 3 years of age of the UK study (15 min/day) is chosen as the key value. The igher mean for children 2 years old (22 min/day) is considered to be too conservative for the foll wing reason

- t is about 2-times higher than the maximum found in the Dutch study and about 12-times the mean from this study.
- There is some evidence to suggest that the mean for the 2-years old children is unduly affected by a high maximum value (or several of these), since this is the only age group in which the maximum is more than 8-times the mean (typically, this factor is about 4-5, see Appendix A-3 for a graphical presentation).

The US values more clearly support the lower of the two values in question.

The US studies chosen here to support the key value are largely the ones that the EPA (2008; 2011) used to derive their recommended values, although the latter are expressed in minutes/hour in contrast to the approach chosen here (minutes/day) and only refer to total mouthing times.

Table 114 Key values for mouthing duration for objects other than toys, childcare articles and fingers

Daily mouthing time for objects other than dummies, toys and fingers	**Key value**	**Rationale**	**Supporting values**
Children (6-12 months)	25 minutes/ day	UK study: 25 minutes/day (rounded mean, 6-9 months, n=15) 16 minutes/day (rounded mean, 9-12 months, n=17) Dutch study: 9.4 minutes/day (mean, 6-12 months, n=14) 25.7 minutes/day (maximum, 6-12 months, n=14)	9 minutes/day (mean, US children, 0-18 months, n=107, objects other than pacifier, teether or toy, including zero values) 22 minutes/day (mean, US children, 0-18 months, n=46, objects other than pacifier, teether or toy, excluding zero values) Juberg et al. (2001) 4.4 minutes/day (me n, US ch ldren, 3-12 months, n=54 soft plas ic items*) 17.5 minutes/day (95th percentile, US children, 3-12 month , n=54, soft plastic items*) Babich et a (2004); Greene (2002); Kiss (2002)
Children (2-3 years)	15 minuets/ day	UK study: 22 minutes/d y (ounded mean 2 y ars, n=39) 15 minu es/day (rounded mean, 3 years, n=31) Dutch study: 2.0 minutes/day (maximum, 18-36 months, n=11) 11.6 minutes/day (maximum, 18-36 months, n=11)	2 minutes/day (mean, US children, 19-36 months, n=110, objects other than pacifier, teether or toy, including zero values) 15 minutes/day (mean, US children, 19-36 months, n=18, objects other than pacifier, teether or toy, excluding zero values) Juberg et al. (2001) 3.8 minutes/day (mean, US children, 12-24 months, n=66, soft plastic items, includes soft plastic toys) 13.0 minutes/day (95th percentile, US children, 12-24 months, n=66, soft plastic items*) 4.2 minutes/day (mean, US children, 24-36 months, n=49, soft plastic items*) 18.5 minutes/day (95th percentile, US children, 24-36 months, n=49, soft plastic items*) Babich et al. (2004); Greene (2002); Kiss (2002)

* includes soft plastic toys

While the non-toy category in the Dutch study is not fully explained, the full report of the UK study contains a complete list of all "other objects" noted by parents to be mouthed by their children, differentiated by object category, individual items and with figures for the times mouthed provided. The most prominent individual items

mouthed were (with the number of times mouthed among the 1,665 observations for 236 children, see Table 111 above):

- Spoons: 45 times
- Pen: 33 times
- Beakers): 31 times
- Cups: 25 times
- Toothbrush: 29 times
- Flannel: 29 times
- Remote control: 29 times
- Jumpers: 27 times
- T-shirts: 25 times
- Socks: 24 times
- Cushions: 22 times
- Bottle: 21 times
- Pencil: 21 times
- Blankets: 21 times

The UK study is more detailed in the types of objects mouthed, because its main focus is the identification of choking hazards and not exposure assessment. The Dutch study, in contrast, is directly related to an exposure assessment and risk characterisation for phthalates in toys (Könemann, 1998).

The UK study also provides some evidence in relation to the type of material mouthed (glass wood cer mics, plastic etc.), although no differentiation in relation to "plastic" is made (in any case it woul be virtually impossible for parents to reliably differentiate between different types of plastic). Of all items (including toys) mouthed by children aged between 6 and 12 months, 50% were made of plastic, a valu that rem ins stable at higher ages. This figure includes items intended to be mouthed. For example, 16-18% of all plastic items mouthed by children consisted of items intended to be mouthed (e.g. bottles, cups, beakers d cutlery).

It is clear from these data that the mouthing time of 25 minutes/day deriv d above includes mouthing of objects that do not (or only in exceptional circumstances) contain PVC and are not PVC arti les covered by this CSR. The UK study also indicates that some articles potentially consisting of PVC (nd therefore covered by this CSR) were only rarely mouthed by UK children compared to the objects m ntioned above. For example "Packaging (prob. soft plastic)" was mouthed 3 times, "handb g" 2 imes and "red vinyl purse" 2 times among the 1,665 observations (0.1-0.2%) of the study. This sugge ts tha many children of the 236 children do not mouth PVC articles at all. This is also suggested by the indings from the supporting data provided above. For example, Juberg et al. (2001) found that only 43% (46/1 7) of children up to 18 months old mouthed objects other than pacifiers, teethers or toys , a value declining to 16% (18/110) in the children 19-36 months old. Similar values have been observed in other studies of US children. For example, mouthing prevalence of "blanket/cloth" declined from 29% (1 year old children) t 11% (2 years old) and 5% (6 years old). The respective values for "plastic/plastic wrap" we e 7% 4% and 0 (EPA, 2008; 2011).

Taken together, the mouthing duration f 25 and 5 minutes/day derived above therefore represent conservative mean values for the mouthing of PVC arti les This is also suggested by the supporting values for "soft plastic items" taken from a US study (means: ca 4 minutes/day, 95th percentiles: 13-18.5 minutes/day, depending on the age group).

Mouthing of PVC articles is assessed in three exposure scenarios below. Since the values from these exposure scenarios will be combined to assess aggregated exposure, the overall mouthing duration of 25 and 15 minutes cannot be assumed in every e posure scenario (which would result in an overall mouthing duration of 75 and 45 minutes, resp ctively). A fraction of one third in each exposure scenario is therefore used to divide the overall mouthing dur tion qually between the exposure scenarios.

As a last is ue, the type of mouthing needs to be addressed. Both European studies contain information on the different types of mouthing, such as licking, sucking and biting, although the differentiation differs between the studies For the age groups of interest here, Groot et al. (1998) noted that 34% (6-12 months old) and 23% (18-36 months old) of the total mouthing time was spent licking on objects, while the remainder was spent sucking/biting. No further differentiation of the latter category is provided, but a differentiation between sucking and biting/chewing is considered crucial due to different migration rates (see below).

More details are provided for the UK study (DTI, 2002), noting different behaviours and combinations thereof even for specific object categories. The data in the following table show the mouthing times for the relevant category ("other objects") according to the mouthing behaviour.

Table 115 Mouthing times according to type of mouthing behaviour by UK children: other objects

Type of mouthing	**Mean (hh:mm:ss)/day**
6-9 months old	
Bite	00:05:29
Lick	00:03:27
Lick and bite	00:00:43
Lick and suck	00:02:13
Lick, suck and bite	00:00:24
Not recorded	00:00:29
Suck	00:10:44
Suck and bite	00:01:00
Sum	00:24:30
All biting categories	**00:07:36**
% of total	**31%**
9-12 months old	
Bite	00:03:20
Lick	00:02:17
Lick and bite	-
Lick and suck	00:00:39
Lick, suck and bite	00:00:04
Not recorded	00:00:08
Suck	00:09:37
Suck and bite	00:00:20
Sum	00:16:25
All biting categories	**00:03:44**
% of total	**23%**
3 years old	
Bite	00:03:52
Lick	00:06:14
Lick and bite	00:00:06
Lick and suck	00:00:13
Lick, suck and bite	00:00:00
Not recorded	00:00:35
Suck	00:04:11
Suck and bite	00:00:04
Sum	00:15:15
All biti g categories	**00:04:02**
% of otal	**26%**

These data show that – depending on the age group – 23-31% of the mean mouthing time is spent biting on the object. These figures are slight overestimates since they count the aggregated categories ("lick and bite" etc.) completely as pure biting. However, these aggregated categories only contribute a small fraction to the overall time spent biting.

The exposure estimate will be based on migration rates largely derived from biting/chewing experiments (see below), another very conservative element considering the figures for the relevant age group of children presented above.

DEHP migration from articles for oral exposure

The second key value in oral exposure assessment is the migration of DEHP from articles into the oral cavity, thus contributing to oral exposure. This type of migration is usually measured using artificial saliva as a surrogate for real-life conditions. Many in vitro migration analyses for DEHP have been performed in the context of the "Surveys of Chemical Substances in Consumer Products" by the Danish EPA (Andersen et al., 2012; Svendsen et al., 2007; Tønning et al., 2010a; Tønning et al., 2009; Tønning et al., 2010b) as well as in other studies (Fiala et al., 2000; Niino et al., 2003; Niino et al., 2002; Steiner et al., 1998).

In contrast to migration rates for dermal exposure (see above), however, in vivo data from studies with humans are available for DEHP migration into saliva. These will be used as a basis for the derivation of a migration rate, since these in vivo data have also been used by the CSTEE (1998) for DINP. In fact, the DINP data will be discussed here in parallel, since CSTEE (1998) suggested using the DINP migration rate for DEHP as well. The use of in vivo data is also indicated by the fact that it is very difficult to simulate real-world conditions in vitro and the results of in vitro studies very much depend on the method of extraction and agitation used in vitro. For example, both lower (Fiala et al., 2000; Steiner et al., 1998) and higher (Niino et al., 2003; Niino et al., 2002) migration values have been found in vitro than in vivo. If in vivo data are available (as is the case for migration into saliva), they are therefore given preference over in vitro data.

In addition, some in vitro studies employed artificial saliva adjusted to a pH value of 4.5-5 (Fiala et al., 2000; Steiner et al., 1998; Svendsen et al., 2007). As human saliva has a pH value of 7-8, such pH values must be considered non-physiological. In fact, more acidic saliva would inactivate important enzymes, such as α-amylase (Löffler et al., 2007; Silbernagl and Despopoulos, 1988). In this context, it is important to note that the high migration rate of 1,000 mg/kg observed for one eraser in a Danish EPA Survey (Svendsen et al., 2007), which also served as the basis for the exposure assessment in the Phthalate Restriction Proposal Assessment, was obtained at pH 5. While the Phthalate Restriction Proposal Assessment adjusted this value to a final migration rate of 83.3 mg/kg for several reasons, this value is still one to two orders of magnitude higher than in vitro migration rates observed in other Danish EPA Surveys (Tønning et al., 2010a; Tønning et al., 2009) using artificial saliva at a physiological pH of 6.8.

The CSTEE (1998) primarily relied on the human volunteer study of the "Dutch Consensus Group" (Könemann, 1998), with additional evidence from Austrian in vivo migration studies (Fiala et al., 2000; Steiner et al., 1998). These studies are therefore discussed here in detail with additional data from more recent Japanese (Niino et al., 2003; Niino et al., 2002) and Mexican (Corea-Tellez et al., 2008) studies.

In the Austrian studies, it is somewhat unclear how many subjects actually performed the tests and how they were recruited (Fiala et al., 2000; Steiner et al. 1998). In the more of the two comprehensive study "*tests were performed by one of the authors and some students*" (Fiala et al., 2000), potentially introducing a bias. It is obvious from the data reported in the article that the same subjects took part in all experiments, but neither the sequence nor the interval between experiments is given.

In contrast, the Dutch (Könemann, 1998), Japanese (Niino et al., 2003; Niino et al., 2002) and Mexican (Corea-Tellez et al., 2008) studies were performed with human volunteers. With the exception of the Mexican study (only one object, three volunteers) all subjects appear to have been involved in mouthing several objects and the interval between mouthing the different objects is not given. In addition, the sequence of mouthing the various objects is not provided in the Japanese study, but clearly described in the Dutch study. In the latter, all 20 volunteers first mouthed item no. 1 and were afterwards divided into groups of 10 subjects each, who then mouthed item 2 and 3 respectively. The time between the last session with item no. 1 and the first session with item no. 2 or 3 is not given. It is noted here that DINP migration values (means and minimum values) are higher for items no. 2 and 3 than for item no. 1. While this could suggest some residual DEHP in saliva, such a suggestion is speculative in the absence of more detailed data.

None of the studies reports whether appropriate measures (e.g. testing "blank saliva" between test items) were taken and those studies using control objects not containing phthalates, did not report the results for the control objects. Lacking information on pre-test and control values is a problem, since DEHP metabolites may be present in unstimulated saliva samples of the general population. In one US study, 18/39 (46%) of the volunteers had detectable MEHP levels (LoD: 1 µg/L) in saliva (Silva et al., 2005), while in another study the DEHP metabolites 5OH-MEHP and 5oxo-MEHP were below the limit of quantification of 1.07 and 0.80 µg/L in saliva samples from fasting breast-feeding US mother (Hines et al., 2009). However, MEHP was not analysed in the latter study.

The following table summarises the results from these in vivo migration studies. All results reported in the original documents were converted to µg/10 cm^2 x min, since this is the unit used by CSTEE (1998). Note that this unit is assumed because the contact area for small children is assumed to be 10 cm^2. This assumption is also made here for both age groups.

Table 116 Summary of human volunteer studies on DEHP, DINP and DBP migration into saliva

Sub-stance	Test item	N	Agitation, duration	Migration rate [µg/10 cm² x min]		Reference	Remarks
				Mean	Range		
DINP	1) Standard PVC disk*, 38.5% DINP	20	Biting and sucking, 1 h	1.38	0.3-8.3	Kö em nn 1998	4 x 15 minutes mouthing sessions with 5 minutes break between each session
	2) Commercially available teething ring, finger part	10	Biting and sucking, 1 h	2.44	0.9-8.9§		Test item no. 3 was punched from the same teething ring test as item no. 2; DINP concentration for this object was not provided.
	3) Commercially available teething ring, flat part	10	Biting and sucking, 1 h	1.63	0 9-5.7		
	4) Control (PTFE disk)	20	Biting and sucking, 1 h	-			Values for the control item are not provided.
DEHP	PVC film, 27.3% DEHP	3/2	Sucking, no biting, 3 h (A) and 6 h (B)	A: 0 62 ± 0.17 B: 0 043 ± 0.006		Steiner et al., 1998	Experiments for 3 hours (n=3) and 6 hours (n=2) with continuous sucking (probably one volunteer in all experiments)
DEHP	Commercial plasticised PVC sample, 60% DEHP	3	Chewing	1 0		Corea-Téllez et al., 2008	Experiment for 30 minutes; 3 male volunteers; the nature of the value given is unclear and is taken here as mean
DEHP	PVC sheet, 32% DEHP	9(?)$	Sucking, no biting, 3 h	0.44		Fiala et al., 2000	An extreme value of 9.73 µg/10 cm² x min for chewing on the yellow teether (DINP) was excluded by the authors in calculating the mean. This extreme value is the one CSTEE (1998) interpreted as confirmation of the maximum Dutch value.
DINP	Yellow teether, 36% DINP	9	Sucking, 1 h	1.39 ± 0.66	0.50-2.42		
		9	Chewing, 1 h	2.21 ± 0.86	1.28-3.59		
		9 ( )$	Chewing, 3 h	1.46			
		9 (?)$	Sucking, 3 h	0.50			
	Red teether, 36% DINP	9 (?)$	Chewing, 3 h	0.73			

Sub-stance	Test item	N	Agitation, duration	Migration rate [µg/10 cm^2 x min]		Reference	Remark
				Mean	Range		
		9 (?)[$]	Sucking, 3 h	0.49			
DEHP	Ball A (toy), 18.5% DEHP	4	Chewing (gentle), 1 h	0.73		Niino et al., 200 ; 2033	4 x 15 minutes mouthing sessions with 5 minutes reak between each session; standard deviations not given for DEHP and DBP experiments
DBP	Ball A (toy), 10% DBP	4	Chewing (gentle), 1 h	0.20			
DINP	Ball C (toy), 25.5% DINP	4	Chewing (gentle), 1 h	1.30 ± 0.48			
	Rattle, 38% DINP	3	Chewing (gentle), 1 h	3.65 ± 0.43			Values for the control item are not provided.
	Teether, 38.9% DINP	3	Chewing (gentle), 1 h	2.08 ± 0.32			
	Pacifier, 58.3% DINP	3	Chewing (gentle), 1 h	3.33 ± 1.00			
	Soft doll, 16% DINP	4	Chewing (gentle), 1 h	0.63 ± 0.15			
	PVC plate, 46.2% DINP	4	Chewing (gentle), 1 h	5.43 0.43			
	Control (PP disk)	4	Chewing (gentle), 1 h	-			

[*] Produced under controlled conditions; [$] This maximum value forms the basis for he CSTEE (1998) evaluations of phthalates in toys (rounded to 9 µg/10 cm^2 x min or 1600 µg/10 cm^2 x 3 h)
[$] The number of subjects is not explicitly stated for these summary result and no stand rd deviations or ranges are given.

The data from this evaluation shows the following:

- Chewing/biting leads to higher mean migration rates than sucking. However, this only applies to the mean values observed and individual differences are substantial. For example, chewing resulted in lower values than sucking in some subjects (Fiala et al., 2000).
- There are substantial differences even when the same protocol and objects with identical DINP concentration were used. For example, chewing for 3 hours on a yellow teether led to 2-times higher values than chewing on a red teether with an identical DINP concentration (Fiala et al., 2000). Also, the mean obtained with item no. 2 was considerably higher than the one obtained with item no. 3 from the same object (Könemann, 1998).
- These authors noted that saliva production during exposure to item no. 2 was significantly reduced (10.9 g) compared to items no. 1 and no. 3 (16.3 and 16.7 g, respectively).
- The range of mean migration rates is 0.043-1.0 µg/10 cm^2 x min for DEHP and 0.49-5.43 µg/10 cm^2 x min for DINP, with 2 of the 5 values for DEHP being below the minimum for DINP.
- This suggests a lower DEHP migration from PVC articles compared to DINP, bu this interpretation is limited by:
  - the small number of DEHP values (n=5)
  - three of these values being obtained without biting/chewing
  - these three values were obtained over relatively long times (3 and 6 h urs), p t ntially attenuating a potentially higher rate of release during the initial phase; in fact, the limited study by Corea-Téllez et al. (2008) with a short duration (30 minutes) and chewing gave th high st migration rate for DEHP (but still 5.5-times lower than the maximum for DINP)
  - potential formation of MEHP in the saliva (Niino et al., 2001), i . reduc d DEHP, although probably to a small extent.

In the light of the uncertainties mentioned above and because none of t e studies provides percentile distributions, the key value for DEHP migration rate for saliva is de ived from descriptive statistics of all the means shown in Table 116 above. The following table shows this evaluation for all phthalates (15 values for DINP, 5 for DEHP and 1 for DBP) as well as for DINP and DEHP only (although percentile values for the latter are not very meaningful due to the small number of valu s).

Table 117 Descriptive statistics or in vivo migration into saliva

	Migration rate [µg/10 cm^2 x min]		
Parameter	**All phthalates**	**DINP only**	**DEHP only**
n	21	15	5
MIN	0.043	0.49	0.043
AM	1.51	1.91	0.57
GM	0.99	1.51	0.39
GSD	2.95	2.06	3.53
Median	1 30	1.46	0.62
75th percent le	2.08	2.33	0.73
90th p centil	3.33	3.52	0.89
95th percentile	**3.65**	**4.18**	**0.95**
MAX	5.43	5.43	1.0

On he basis of this evaluation, the 95th percentile of all phthalates of
3.65 µg/10 cm^2 x min
is taken as the key value for DEHP migration into saliva.

The key value derived here is considered to represent a conservative approach for DEHP migration from PVC articles into saliva for the following reasons:

- It represents the 95th percentile from a comparatively large number of in vivo migration data. While it is based on an evaluation of the mean migration values, a detailed evaluation of the study reporting individual values (Fiala et al., 2000) supports the conservative nature. Thus, the 95th percentiles for DINP are 2.4 for sucking and 3.5 µg/10 cm^2 x min for chewing (9 subjects each; see Appendix A-4 for details). Only the Dutch study reports higher maximum values (Table 116). However, due to lacking individual

data, calculation of percentiles from this study is impossible.

- The underlying basis comprises primarily data for DINP. There is some evidence to suggest that the migration rate from PVC articles is lower for DEHP than for DINP. In fact, the key value derived here is almost 4-times higher than the maximum migration rate measured for DEHP in any in vivo test. The latter was obtained with chewing and over a comparatively short duration of 30 minutes, which is very similar to the mouthing duration assumed (25 and 15 minutes/d see above).
- The key value to a large extent reflects migration under conditions facilitating phthalate release. Overall, 15/21 values (71%) used in its derivation were obtained with biting/chewing on the object. Together with the relative small fraction of time a child spends biting (23-31%, see above), this adds another conservative element in the overall exposure estimate.
- The GSD in Table 117 above points to medium variation in the dataset (ECHA, 2010a). Up to RCRs f <0.5, about 20-30 measurements are therefore required according to this Guidance and the num r of measurements in our dataset (N=21) fulfils this criterion (see Risk Characterisation p rt for he RCRs derived). Note that each of these 21 values represents the mean from 3-20 individual samples. The data are also considered to be broadly representative, since both standardised PVC materials and commercial PVC articles and different mouthing behaviours were included in the analysis (s e above). These differences may in fact be responsible for some of the variation observed since in the only study reporting individual values, differentiated by sucking and chewing (Fiala et al., 2000) the v riation in the datasets was very small (GSD < 2, see Appendix A-4).

It should be noted that the value chosen as a "worst case" by CSTEE (1998) is a maximum value from the Dutch study (8.9 µg/10 cm^2 x min), supported by a maximum value from the Austr an study. In relation to the Dutch study, this maximum value was not reached when a different part f the same te hing ring was tested as item no. 3 (maximum 5.7 µg/10 cm^2 x min). In the Austrian study, the high maximum was in fact excluded by the authors from calculation of the mean (see Table 116 above), since the subjec bit off pieces of the article, which were extracted together with the saliva. Together with the issue discus ed ab ve, these maximum values are therefore considered highly uncertain and the 95th percentile of 3 65 µg/10 cm^2 x min represents a conservative estimate.

In a recent report in relation to the entry 52 of Annex XVII t REACH review for DIDP and DINP, ECHA (2012) derived migrations rates of 14 (typical) d 45 µg/cm^2 x h (reasonable worst case) for both DINP and DIDP in toys, childcare articles and other articles (excluding sex toys). The reasonable worst case estimate was also used by RAC (2013) for DINP and DIDP. I is higher than the one used here (3.65 µg/10 cm^2 x min = 21.9 µg/cm^2 x h), primarily for the followin reason:
The key value used here is entirely based on vivo data, while the reasonable worst case used by ECHA (2012) and RAC (2013) is based on a s ngle value f und in an in vitro study. In fact, the in vivo data reported in ECHA (2012) suggest a lower in vivo migration rate than in vitro.

The key value used here applies to DEHP, for which a lower migration rate is suggested by the data discussed above.
Exposure due to mouthing is onsidered in three different exposure scenarios (ES). Therefore, only part of the total mouthing time is applic ble to each ES and the total mouthing time is equally divided between the three ES (fraction 0.3 3 ach).

The calculation low for estimating oral exposure from mouthing is presented in the following table.

Table 118 Calculation flow for oral exposure from mouthing

Calculation	Parameter	Value*	Unit	Source
	Migration rate	3.65	µg/10 cm^2 x min	Derived above
multiplied by	Mouthing duration	25 / 15	minutes	Derived above
multiplied by	Fraction for ES	0.333	dimensionless	Derived above
divided by	Body weight	9.2 / 13.8	kg	Derived above
results in	External exposure		µg/kg b.w. x d	

* When two values are given, these refer to children 6-12 months old / children 2-3 years old

Dust ingestion by children and adults

Oral DEHP exposure of children via house dust is assessed in the indoor scenario below. One of the input values for this exposure pathway is the daily dust ingestion assumed. The ECHA Guidance on Consumer Exposure Estimation (ECHA, 2010b) mentions a conservative estimate of 100 mg/d for ho e dust int ke by children (no differentiation by age) on the basis of a report by Oomen et al. (2008). The latter au hors derived dust ingestion values of 100 mg/day for children and 50 mg/day for adults as a "*conservative but realistic estimate*" on the basis of estimates from several studies, which were not discussed to any ex ent and were often cited from other sources. In fact, it appears the some of the studies cited did not investig te so l or dust ingestion *per se*. Oomen et al. (2008) recognise that many of the underlying studie investig ted soil (rather than dust) ingestion and that soil loading of the child's hands (outdoors) is higher than dust load ng (indoors). They nonetheless propose to use the values from soil ingestion studies, since children spend more time indoors than outdoors. Again, no differentiation for children of different age groups is provided.

In contrast to this rather limited data basis, EPA (20 8; 20 1) provides a detailed discussion of available studies, including an identification of key studies along specific criteria. These authors derived "recommended values" both for soil and dust ingestion, as well as com ned oil and dust ingestion, with some differentiation by age. These "recommended values" are central tendency (CT) and upper percentile (UP) estimates (EPA, 2008; 2011) and are summarised in the following table. Note hat n upper percentile estimate could only be derived for children 3-<6 years old.

Table 119 Recommended values for soil and dust ingestion data (adapted from EPA, 2008; 2011)

Age group	Soil* ingestion [mg/d]		Dust** ingestion [mg/d]		Soil and dust ingestion [mg/d]	
	CT	UP	CT	UP	CT	UP
6 weeks- <1 year	30	200 (3-<6 years)	30	100 (3-<6 years)	60	200 (3-<6 years)
1- <21 yea s	50		60		100	
Adults	20		30		50	

*Soil and utdoor settled dust; ** indoor settled dust only

In th exposure assessment performed here, dust ingestion indoors is considered to be the only relevant pathway and the specific value for dust ingestion is therefore used. The age differentiation shown in the table allows establishing values for children 6-12 months old (30 mg/d), children aged 2-3 years (60 mg/d) as well as adults (30 mg/d). The central tendency values are used because exposure will be estimated on the basis of an upper percentile of DEHP concentrations in house dust (see below). In addition, age-differentiated values are only available as central tendencies, while upper percentiles were only derived by EPA (2008; 2011) for children 3-<6 years old.

The data in Table 119 also show that

- the value of 100 mg/d dust ingestion – as proposed in ECHA (2010b) for children – represents an upper percentile estimate for children 3-<6 years old
- the value of 50 mg/d for adults represents a central tendency estimate for soil and dust ingestion, while an upper percentile has not been derived for adults.

DEHP concentration in house dust

Over the past two decades, several studies investigating DEHP concentrations in (house) dust have been performed and values from many of these studies were included in the Phthalate Restriction Proposal Assessment, although mostly cited from Bornehag et al. (2005). The European studies served the Phthalate Restriction Proposal Assessment as a basis for assessing oral exposure via ingestion of dust.

The following assessment is also based only on studies conducted in Europe, thus excluding studies f om countries such as the USA and China. Studies evaluated in the Phthalate Restriction Proposal Assessment w re consulted in original form to identify additional information (e.g. sampling details and maximum value) whenever possible.

In addition, several studies not considered in the Phthalate Restriction Proposal Assessment were eval ated below, including results from the German Environmental Survey for Children (GerES 2003-2006), designed to be representative of the houses/apartments of children 3-14 years old, involving 600 samples (Nagorka et al., 2010; Nagorka et al., 2011).

While most of these studies were conducted in residential settings, they often differ in several aspects such as

- method of dust collection,
- dust fraction analysed,
- date of sampling,
- sample size,
- representativeness of the samples and the
- level of statistical analysis and results presented
-

Overall, 19 different studies were included in the asses ment and an overview is provided in the following table.

Table 120 Overview of studies on DEHP concentrations in house dust (in µg/g)

Country (source)	Design	Surveyed in	Sampling	Location	Fraction	N	Median§	Upper (95th) percentile	Maximum
Norway (Oie et al., 1997)	Residential (children)	1992-1993	Filter samples from vacuum cleaner	Surface dust, pooled from child's bed floor in child's bedroom, floor in central living room and on top f shelves in central living room	not gi en	38	640		1610
Germany (AnBUS, 1997)	Residential, interested private persons had their dust analysed and paid for the analysis (potentially samples suspected of contamination)	1995-1996	Vacuum cleaner bags	N/A	"fine dust"	272	450	2000	8600
Germany (Becker et al., 2004a) (GerES 1998)	Residential, houses/apartments of adults 18-69 years old, representative	1997-1999	Vacuum cleaner bags	N/A	<2 mm	199	416	1190	7530
Germany (Butte et al., 2001)	Residential	1998-1999	Vacuum cleaner bags	N/A	<63 µm	286	740	2600	12000
Germany (Kersten and Reich, 2003)	Residential	1998-2000	Vacuum cleaner bags	N/A	<63 µm	65	600	1600	2700
Germany (Fromme et al., 2004)	Residential	2000-2001	Vacuum cle er bags	N/A	"fine dust"	30	703	1542	1763
Germany (Lange and Eis, 2004) (Pretest GerES 2003-06)**	Residential (children), 4 samples points selected for pretest (Berlin, Brandenburg and Lower Saxony)	2001-2002	V m cleaner bags	N/A	<2 mm	503	78	456	1460
Germany (Becker et al., 2004b) (Pilot GerES 2003-06)	Residential (children), 4 samples points selected for pretest (Berlin, Brandenb rg and Lower Saxony)	2001-2002	Vacuum cleaner bags	N/A	<63 µm	252	515	1840	5330
Sweden (Bornehag et al., 2005)	Residential	2001-2002	Filter samples from vacuum cleaner	Above floor	N/A	346	770	4069	40459

Denmark (Clausen et al., 2003)***	Residential	not given	Filter samples from vacuum cleaner		N/A	23	858	2595	
Denmark (Clausen et al., 2003)**	Schools	not given	Special vacuum cleaner	Floor dust	N/A	15	*3214*	7063	
Germany (Volland et al., 2010)**	Boarding schools	2006	Wipes	Not given	N/A	40	*1880*		2600
Germany (Nagorka et al., 2010; Nagorka et al., 2011) (GerES 2003-06)**	Residential (children), houses/apartments of children 3-14 years old, representative	2003-2006	Vacuum cleaner bags	N/A	<63 µm	600	500	1700	3500
Bulgaria (Kolarik et al., 2008)**	Residential (children)	2004-2005	Filter samples from vacuum cleaner	Above floor	N/A	177	1050		
Denmark (Langer et al., 2010)	Residential (children)	2008	Filter samples from vacuum cleaner	No -floor urfaces	N/A	497	210	980	
	Day-care centres	2008	Filter samples f m vacuum cleaner	Non floor surfaces	N/A	151	500	1900	
Germany (Abb et al., 2009)	Residential	not given	Vacuum cleaner bags	N/A	<63 µm	30	604	1600	2800
Germany (Personal communication UBA, 2011)***		not given				10	310	1680	
Sweden (Bergh et al., 2011)**	Homes, day-care centres and workplaces (mostly offices), 10 samples each, surface dust	not given	Filter samples from vacuum cleaner	Above floor (at least 80 cm)	N/A	30	1100	3520	5800

§ Values in italics represent mean rather than median values.
* Identical to "Pohner, 1997" in the Phthalate Restriction P posal Assessment
** not included in the Phthalate Restriction Proposal Ass sment
*** cited from the Phthalate Restriction Proposal Asses men
t

These data show that the studies on DEHP concentrations in house dust differ substantially in sample size and – to a lesser extent – in the design of the study, the method of sampling and type of dust fraction analysed. More than half of the studies were conducted in Germany (including two Surveys designed to generate representative data), with the remainder coming from Scandinavian countries and one from Bulgaria. Most of the studies have been conducted in residential settings, sometimes focussing on children's rooms.

In terms of sampling, basically two different approaches are represented:

- Sampling of dust from vacuum cleaner bags, generally < 2mm or < 63 µm fractions
- Sampling of dust on (often quite elaborated) filtering devices attached to vacuum cleaners.

The data are suggestive of higher median values and 95th percentiles in samples of <63 µm fractions compar d to <2 mm fractions. This is also plausible since smaller particles have a higher specific surface and may therefore bind more DEHP. Within the German Environmental Survey (GerES) 1998, this issue was addressed y analysing a subset of the dust samples differentiated by different fractions. The results of this an lysis are shown in the following table. Like many other chemicals, DEHP showed a higher median and 95th percentile in the <63 µm fraction than in the <2 mm fraction. The difference was statistically significant (Beck r t al., 2004a).

Table 121 DEHP concentration in house dust from vacuum cleaner bags: differentia ion by fraction*

Dust fraction	**DEHP concentration [µg/g]**	
	Median	**95th p rcentile**
<2 mm	415	1130
<63 µm	521	1330
<32 µm	507	1340

* n=100 per fraction, randomly selected subset of GerES 1998 (dat from Becker et al., 2004a)

The differences must be considered when comparing results from different studies.

It must be stressed in this context that the studies using filter samples mostly collected dust above floor surfaces, e.g. to establish the relationship with DEHP concentrations in air (Bergh et al., 2011). This also applies to the study by Bornehag et al. (2005) While resul from these studies might not be representative for dust intake for a crawling child, they might non theless b considered for a hand-to-mouth pathway in the children, especially for the older children (2-3 years old) evaluat d in this assessment.

Due to the declining trend of DEHP consumption in Europe, a differentiation by sampling date would be interesting. Howev r, such an analysis is limited by the fact that many studies actually do not report the date of sampling, which can often be several years prior to the publication of the results. In addition, differences in study design and methodology could well mask any possible time trends. Nonetheless, Nagorka et al. (2010; 2011) noted a time trend of decreasing concentrations of "traditional phthalates" in house dust in Germany.

It i clear f om the data in Table 120 that very high DEHP concentrations of more than 5,000 µg/g in house dust are o ca ionally found. In studies with small sample sizes, some very high values can also lead to very high 95th percentiles (and very high arithmetic means). For example, the highest 95th percentile is reported by Clausen et al (2003) on the basis of only 15 samples, which means that the 95 percentile values is based on one sample only, the latter being the maximum value found in this study.

Therefore, a differentiation of studies by sample size was performed. As the results in the following table show, studies with a larger sample size lead to substantially lower DEHP concentrations in dust (in terms of the descriptive statistics shown) than studies with a smaller sample size. For example, the 95th percentile of all median values is 2013 µg/g. If only median values from studies with a large sample size are considered, the 95th percentile is more than 2-times lower, since studies with a small sample size contribute many high median values.

Table 122 Descriptive statistics for DEHP in European house dusts with differentiation of studies by

sample size

	DEHP concentration in dust [µg/g]		
	All studies	**Studies with n > 50**	**Studies with n <=50**
Evaluation based on median values of studies			
Number of medians	19	11	8
Mean of medians	797	530	1,164
Median of medians	604	447	781
90th percentile of medians	1,256	**770**	2,280
95th percentile of medians	2,013	**910**	2 747
Evaluation based on upper percentiles of studies			
Number of upper percentiles*	16	10	6
Mean of upper percentiles	2,271	**1,834**	3,000
Median of upper percentiles	1,770	**1,770**	2,138
90th percentile of upper percentiles	3,795	2 747	5,292
95th percentile of upper percentiles	24,807	3 408	6,177

* Upper percentiles usually refer to 95th percentiles

The studies with a sample size of more than 50 are considered the most reliable and representative ones. On this basis, a DEHP concentration in house dust of (r unded)
1800 µg/g as a 95th percentile
is derived and used in this assessment. This valu

- is based on the median of upper percenti es (usually 95th percentiles) from studies with a large sample size (1,770 µg/g),
- is supported by an almo t identical mean of upper percentiles from studies with a large sample size (1,834 µg/g),
- is supported by an identical median of upper percentiles from all studies (1,770 µg/g) and
- is higher than the 95th percentiles from GerES 1998 and GerES 2003/2006, the only two studies that have been designed to be re resentative (although only of the German population),
- is only v ry slightly below the 95th percentile observed in Danish day-care centres (1,900 µg/g), but almost twi e th 95th percentile in Danish homes (980 µg/g) from the same study (Langer et al., 2010), wh ch represents the one with the most recent, ascertained sampling data (2008)
- is lower than the 95th percentile of approximately 2260 µg/g implicit in the Phthalate Restriction Proposal Assessment, if the weighted body doses are converted to DEHP concentrations in dust. The re son for this difference lies in the fact, that the value derived here is based on studies with a sufficiently large sample size (the mean of upper percentiles from all studies (2,271 µg/g; Table 122) is in fact almost identical to the value used in the Phthalate Restriction Proposal Assessment).
- primarily represents dust samples obtained prior to the ban of DEHP in toys and childcare articles, suggesting the DEHP concentrations in house dust in 2013 may be considerably lower.

In addition, the data base includes the only study not performed in Germany or one of the Scandinavian countries (Kolarik et al., 2008), as well as the study with the second highest 95th percentile of all studies (Bornehag et al., 2005).

Overall, the calculation flow for estimating oral exposure from dust ingestion is presented in the following table.

Calculation	Parameter	Value	Unit	Source
	DEHP concentration in house dust	1800	µg//g	Derived above
multiplied by	Dust ingestion	0.03 / 0.06 / 0.03	g/d	Derived above
divided by	Body weight	9.2 / 13.8 / 70	kg	Derived above
results in	Oral intake		µg/kg b.w. x d	

* When three values are given, these refer to children 6-12 months old / children 2-3 years old / adults

Key values for inhalation exposure assessment

The inhalation exposure assessment is based on a single figure, namely the DEHP concentr tion in indoor air. The DEHP concentration in the gas phase is limited by its comparatively low saturated vapour concentration (SVC) of 5.3 µg/m^3 and particle-bound DEHP may significantly add to DEHP concentr tions in indoor air leading to much higher total DEHP concentrations (ECB, 2008; Phthalate Restriction Prop sal Assessment). However, a word of caution is warranted here since some doubtful reports on very high DEHP concentrations in air appear to have persisted in the literature. For example, both ECB (2008) and Fromme et al. (2004) cite values of 200-300 µg/m^3 for homes with new PVC flooring/wall coverings on th basi of the paper by Wams (1987). This review gives a paper published more than a decade earlier (Peakall 1975) as a ource for this value. Peakall (1975) in turn is a review and the underlying article was published in 1970 in Ru sian. It is quite astonishing that such evidence served – at least partly – ECB (2008) to justify the derivation of a DEHP concentration in the EU Risk Assessment Report.

The Phthalate Restriction Proposal Assessment used three diff rent pproaches for assessing DEHP concentrations in indoor air:

- simulations with two different scenarios of real stic r oms (children's playroom and bathroom) furnished with furniture/materials marketed in D nm rk (DEHP in PVC flooring and wall paper based on 2001 ("old") and 2010 ("new") data,
- calculations according to the me hod in th EU Risk Assessment Report (ECB, 2008) and
- an evaluation of DEHP concent ations m asured in indoor air based on a literature survey published in 2007. The means and maximum va ues from these sources were average in the evaluation.

The results from these various pproaches – as reported in the Phthalate Restriction Proposal Assessment are presented in the following table

Table 123 Summary of DEHP concentrations in air derived in the Phthalate Restriction Proposal Assessment

Approach	Scenario/Method	DEHP concentration [µg/m^3]
Simulations, ga phase only	Children's playroom, new PVC	0.16
	Children's playroom, old PVC	0.81
	Bathroom, new PVC	0.26
	Bathroom, old PVC	0.80
Calculations, gas phase only	Children's playroom	9.4
	SVC	5.3
Literature values, mostly gas phase and particle-bound	Average of means	0.23
	Average of maximum values	1

The authors noted that the method according to ECB (2008) is "rough", since it assumes a fixed emission rate and also recognised that it is above the SVC. The simulated values for children's playrooms (0.16 and 0.81 µg/m^3) are multiplied with a factor of 5 to account for particle-bound DEHP, resulting in "reasonable realistic" and "reasonable worst case" concentrations of 0.8 and 5 µg/m^3, which are taken to risk characterisation in the Phthalate Restriction Proposal Assessment.

It is unclear, given that the literature data are not discussed to any extent and no justification is provided, why the simulation data with an added factor for particle-bound DEHP was suggested to be more reliable than actual measured data which mostly reflects gas phase and particle-bound DEHP according to the Phthalate Restriction Proposal Assessment.

It should be noted that the "reasonable worst case" value derived on this basis (5 µg/m^3) is 5-times higher than the average of the maximum values reported in the literature data evaluated in the Phthalate Restricti n Proposal Assessment. In fact, it is higher than any of the maximum values evaluated (3.13 µg/m^3, see Table 124 bel w) This proposed "reasonable worst case" value is also about 10-times the maximum value reported for pri ate homes (0.53), offices (0.32) or day-care centres (0.48 µg/m^3, all values gas phase and particle-bound DEHP, n=30) in Sweden in a more recent study (Bergh et al., 2011), the corresponding arithmetic m ans being 0.21, 0.27 and 0.12 µg/m^3.

Overall, the contribution of DEHP in indoor air to the total DEHP exposure is ve all (P thalate Restriction Proposal Assessment), even with the comparatively high value taken to risk charact risation. Therefore, a detailed evaluation of all available information is not considered necessary here Rather the literature data reported in the Phthalate Restriction Proposal Assessment are evaluated using he approach applied above for other pathways. All mean and maximum values from the literature su y pu lished in 2007 (Table 24 in the Phthalate Restriction Proposal Assessment) were included and analysed sta istically. The results of this evaluation are presented in the following table.

Table 124 Descriptive statistics for DEHP concentrations in indoor air (based on data in Table 24 in RAC/SEAC (2012))

	DEHP concentration in indoor air [µg/m^3]	
	Mean	**Maximum**
n	12	12
Minimum	0.060	0.110
AM	0.229	0.982
GM	0.183	0.676
Median	0.180	0.810
75th percentile	0.323	1.100
90th percentile	0.420	2.135
95th per entile	**0.507**	**2.646**
Maximum	0.600	3.130

As noted in the Phthalate Restriction Proposal Assessment, the underlying data primarily reflect both gas phase and particle-bound DEHP. Therefore, an additional factor – as applied to simulation data – is not required. Since some of the underlying data may, however, only represent gas phase concentrations, the 95th percentile of the maximum values is taken as a conservative estimate for exposure to DEHP in indoor air.

On this basis, a DEHP concentration (gas phase and particle-bound) in indoor air of (rounded)
2.6 µg/m^3 as a 95th percentile
is derived and used in this assessment.

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AppendixA-1: Evaluation of DEHP migration from PVC articles into artificial sweat

Source documents:
Survey 84 (Svendsen et al., 2007)
Survey 102 (Tønning et al., 2009)
Survey 107 (Tønning et al., 2010b)
Survey 109 (Tønning et al., 2010a)
Survey 117 (Andersen et al., 2012)

Materials and methods:
Details on the migration analyses performed can be found in the source document, although some of the su veys lack descriptions of the articles and/or samples. From the information available, it appears that all surveys used artificial sweat simulant as described in DS/EN ISO 105-E04, consisting of l-histidine-monohydroch orid-1-hydrate, sodium chloride, sodium dihydrogen phosphate and sodium hydroxide for adjustment of pH t pH 5.5
The only exception is Survey 84, which employed artificial sweat according to DS/EN 1811:2000
Unless indicated otherwise, all migration analyses were performed under static conditions.

Evaluation and results:
The following notes help in reading the data in the table:

- The sample ID in column 1 refers to the code assigned in the original Surv y.
- Weight and area of the sample in columns 3 and 4 are means in the ase of duplicate analyses.
- For the migration data,
  - values in *italics* are nominal values based on one half f the limit o detection for reported 'non-detects' and
  - values in **bold** values identify those showing a coefficient of var ance (CV) above 50%.
  - n.d. (not detected) in the table was assigned when the val e was below the limit of detection, but the latter was not stated in the report, precluding calculation of one half of the limit of detection.

Calculation details:
When migration was not expressed in mg/cm^2, the migr tion in mg/kg sample was multiplied with the sample weight and divided by the sample area and the migrati n p riod. For example, DEHP migration for a training ball (sample ID 1-24) was 2.5 mg/kg sampl , mult plied with 0.55 g = 1.375 µg, divided by 17.3 cm^2 and by 1 h = 0.079 µg/cm^2 x h. Since sample weight and area are only given as the means for the duplicates, only one value could be calculated in these cases.

For Survey 84, areas were not iven for the sample. However, the weight of the product on the skin (in g/cm^2) is given on p. 88 of the report and was used in the calculation of the migration rate (in µg/cm^2 x h): migration in µg/g product / 4h * g product/cm^2 = µg/cm^2 x h.

For Survey 102, areas were not given for the sample and the migration rate (in µg/cm^2 x h) could not be calculated. This als applies t sample 7-1, which showed the highest migration (in mg/kg sample) of all articles investigated Exposur was alculated in the original report and from the data provided migration from the entire product can be estimated to be 2530 µg/h. Under the assumption of an area of 3025 cm^2 (54 cm x 54 cm, as found in a brief internet survey), this results in 0.84 µg/cm^2 x h, which is about the maximum value found in our observatio (se descriptive statistics at the end of the table above). However, due to lack of data in the original rep rt, thi calculation can only serve as an indication.
It need to be stressed that DEHP migration from most articles investigated in this survey was below the limit of detection which was not stated.

In Su vey 107, migration from sample 8.1 was measured using a variety of different conditions, including dynamic conditions. For all other samples, migration was measured under static conditions.

Survey 117 used dynamic conditions (stirring), but had a very high limit of detection of 4 µg/cm^2 for DEHP migration into artificial sweat. Using one half of this limit of detection led to very high estimated migration values (8/11 values were above 0.5 µg/cm^2 x h) due to the exceptionally high limit of detection. Therefore, a second analysis was performed with exclusion of these 11 values from Survey 117. This led to a clear reduction in mean and upper percentile values as well as the maximum.

The results of this analysis are shown in the following table and descriptive statistics for the migration rate can

be found at the end of the table.

Sample ID	Article type, migration period	Sample characteristics					Migration int artificia we t								
		Weight [mg]	Area [cm^2]	DEHP content [mg/kg]			mg/kg		CV (%)	mg/cm^2		Calculated [$\mu g/cm^2 * h$]			CV (%)
				a	b	Mean	a	b			b	a	b	Mean	
Survey 117															
M11	Cell phone cover, 3.5 h			80000						*0.002*		*0.571429*			
M14	Cell phone cover, 3.5 h			60000						*0.002*		*0.571429*			
M16	Cell phone cover, 3.5 h			130000						*0.002*		*0.571429*			
M19	Cell phone cover, 3.5 h			90000						*0.002*		*0.571429*			
M20	Cell phone cover, 3.5 h			1200						*0.002*		*0.571429*			
H9	Work gloves, 6 h			n.d.						*0.002*		*0.333333*			
H11	Work gloves, 6 h			260000						*0.002*		*0.333333*			
L5	Sleeping mat, 2 h			140000						*0.002*		*1.000000*			
HT7	Handbags, 2 h			80000						*0.002*		*1.000000*			
HT9	Handbags, 2 h			700						*0.002*		*1.000000*			
G7	Sneakers, 10 h			00						*0.002*		*0.200000*			
Survey 109															
1-65	Sponge bag, 1 h	330	16	220000	132000	176000	2.5	3.6	26%	0.00005	0.00007	0.05	0.07	0.06	11%
1-69	Rucksack, 1 h	3 0	15.8	188000	216000	202000	9.8	10.5	5%	0.0002	0.00022	0.2	0.22	0.21	3%
1-31	Shower curtain, 1 h	170	19.8	246000	256000	251000	8.5	5.6	29%	0.00007	0.00005	0.07	0.05	0.06	13%
1-39	Shower curtain, 1 h	260	16.8	288000	304000	296000	6	4.6	19%	0.00009	0.00007	0.09	0.07	0.08	9%
1-6	Oilcloth, 1 h	170	19.8	256000	251000	253500	4.7	6.9	27%	0.00004	0.00006	0.04	0.06	0.05	13%

Sample ID	Article type, migration period	Sample characteristics					Migration int artificia we t								
		Weight [mg]	Area [cm^2]	DEHP content [mg/kg]			mg/kg		CV (%)	mg/cm^2		Calculated [$\mu g/cm^2 * h$]			CV (%)
				a	b	Mean	a	b			b	a	b	Mean	
1-8	Oilcloth, 1 h	310	16.8	124000	136000	130000	4.7	5.1	6%	00009	0.00009	0.09	0.09	0.09	0%
1-73	Water wings, 1 h	330	17.2	331000	335000	333000	4.3	4.2	2%	0.00008	0.00008	0.08	0.08	0.08	0%
1-18	Swimming pool, 1 h	390	14	248000	267000	257500	4.3	3 2	21%	0.00012	0.00009	0.12	0.09	0.105	11%
1-14	Balance ball, 1 h	1130	16.8	426000	458000	442000	6.6	4 7	24%	0.00045	0.00031	0.45	0.31	0.38	14%
1-24	Training ball, 1 h	550	17.3	4.4	13.9	9.15	*2 5*	*2.5*		n.d.	n.d.	*0.079*			
Survey 107															
1.1	Sandals, sole, 16 h	3810	16	22100	22300	22200	0.8	0.5	33%			0.010			
5.2	Sandal, strap, 16 h	2390	12	29	18	23.5	**0.1**	**0.4**	**85%**			0.003			
6.1	Sandal, sole, 16 h	5070	23	302000	302 00	302000	**1.1**	**0.5**	**53%**			0.011			
8.1	Sandal, sole, static, 16 h	5130	22	151000	145000	148000	0.33	0.33	0%			0.005			
8.1	Sandal, sole, static, 16 h (replacement of simulant after 8 h)	5390	22	151000	145000	148000	**12**	**0.3**	**135%**			0.094			
8.1	Sandal, sole, static, 8 h	5390	22	151000	145000	148000	0.16	0.2	16%			0.006			
8.1	Sandal, sole, dynamic, 16 h	6530	19	151000	145000	148000	**15**	**1.5**	**116%**			0.177			
8.1	Sandal, sole, static, 16 h, sun lotion	6960	21	151000	145000	148000	**7.6**	**1.7**	**90%**			0.096			
8.1	Sandal, sole, dynamic, 1 h, sun lotion	4560	17	151000	145000	148000	**25**	**74**	**70%**			0.830			
8.2	Sandal, strap, 16	2010	9.6	168000	174000	171000	0.5	0.3	35%			0.005			

Sample ID	Article type, migration period	Sample characteristics					Migration int artificia we t								
		Weight [mg]	Area [cm^2]	DEHP content [mg/kg]			mg/kg		CV (%)	mg/cm^2		Calculated [$\mu g/cm^2 * h$]			CV (%)
				a	b	Mean	a	b			b	a	b	Mean	
12.1	Sandal, sole, 16 h	3800	16	451000	470000	460500	1.1	1.5	22%			0.019			
13.2	Sandal, strap, 16 h	2750	12	280	250	265	**8.2**	**0.2**	**135%**			0.060			
17.1	Sandal, sole, 16 h	5370	16	237000	253000	245000	**0.47**	**0 21**	**4%**			0.007			
18.1	Sandal, middle of sole, 16 h	290	16	112000	109000	110500	7.7	8 9	10%			0.009			
18.2	Sandal, outer part of sole, 16 h	5780	18	213000	206000	209500	**0.41**	**0.17**	**59%**			0.006			
20.2	Sandal, strap, 16 h	2890	12	10100	10300	10200	**0.1**	**7.2**	**138%**			0.055			
26.1	Sandal, sole, 16 h	4740	20	335000	354000	344500	0.55	0.3	42%			0.006			
29.2	Sandal, outer part of sole, 16 h	2750	23	5	5	5	0.38	0.21	41%			0.002			
37.2	Sandal, strap, 16 h	1480	9.6	209000	209 00	209000	**4.2**	**0.4**	**117%**			0.022			
38.2	Sandal, strap, 16 h	1800	10	184	92	138	0.1	0.1	0%			0.001			
39.2	Sandal, strap, 16 h	1390	20	250000	266000	258000	**1.5**	**0.7**	**51%**			0.005			
43.1	Sandal, sole, 16 h	4610	23	30000	325000	327500	0.35	0.51	26%			0.005			
44.1	Sandal, sole, 16 h	3160	16	445000	478000	461500	**0.6**	**1.4**	**57%**			0.012			
46.1	Sandal, middle of sole, 16 h	750	24	108000	102000	105000	**30**	**12**	**61%**			0.041			
46.2	Sandal, outer part of sole, 16 h	2 80	19	151000	157000	154000	1.5	2.1	24%			0.014			
54.2	Sandal, strap, 16 h	2370	12	3570	3850	3710	**0.1**	**0.26**	**63%**			0.002			
55.1	Sandal, sole, 16 h	480	24	150	105	127.5	**8.1**	**1.7**	**92%**			0.006			
Survey 102															

Sample ID	Article type, migration period	Sample characteristics					Migration int artificia we t								
		Weight [mg]	Area [cm^2]	DEHP content [mg/kg]			mg/kg		CV (%)	mg/cm^2		Calculated [$\mu g/cm^2 * h$]			CV (%)
				a	b	Mean	a	b			b	a	b	Mean	
3-1	Rubber clogs, upper, 6 h	2500		15658			n.d.								
3-3	Rubber clogs, 6 h	2500		137			n.d.								
5-3	Pacifier, coverage, 7.75 h	2500		275			n.d.								
6-1	Soap packaging (PVC), 0.5 h	2500		133			n.d.								
6-2	Soap packaging (PVC), 0.5 h	2500		206			n.d.								
6-5	Soap packaging (PVC), 0.5 h	2500		80130			2	n.d.							
7-1	Shower mat (PVC), 0.5 h	2500		128625			25								
Survey 84															
35B	Pencil case (PVC with phthalate), 4 h	2000		not given			4					0.026			
35C	Pencil case (PVC with phthalate and chalk), 4 h	2000		n given			6					0.039			
38A	Toy bag (polyamide textile) , 4 h	2000		ot given			2.4					0.025			
39C	School bag (PVC with phthalate) , 4 h	2000		not given			1					0.010			
40C	School bag (Polyester textile with PET)) , 4 h	2000		not given			trace								
41A	School bag (PVC with phthalate and chalk) , 4 h	2000		not given			1					0.011			
Evaluation													**All**	**Without no-detects from**	

Sample ID	Article type, migration period	Sample characteristics					Migration int artificia we t								
		Weight [mg]	Area [cm^2]	DEHP content [mg/kg]			mg/kg		CV (%)	mg/cm^2		Calculated [$\mu g/cm^2 * h$]			CV (%)
				a	b	Mean	a	b			b	a	b	Mean	
														Survey 117	
												n	62	51	
												MIN	0.0011	0.0011	
												AM	0.17	0.077	
												GM	0.048	0.029	
												GSD	6.0	4.5	
												Median	0.060	0.040	
												75^{th} perc.	0.19	0.085	
												90^{th} perc.	0.57	0.18	
												95^{th} perc.	0.82	0.27	
												MAX	1.0	0.83	

This analysis shows that the 95th percentile (see Ch. 9.0. for the rationale of using the 95th percentile) is 0.82 µg/cm^2 x h in the overall evaluation and 0.27 µg/cm^2 x h when all data from Survey 117 are excluded due to the exceptionally high limit of detection.

The data in the table show that the DEHP concentrations in some samples were low, sometimes even below 0.1%. Therefore, an analysis was performed using only samples that contained more than 10% (100,000 mg/kg) DEHP and excluding the no-detects from Survey 117. As shown in the following table, there is only a slight increase in upper percentiles. This finding is due to the fact that there is no clear correlation between the DEHP concentration in the sample and the migration rate.

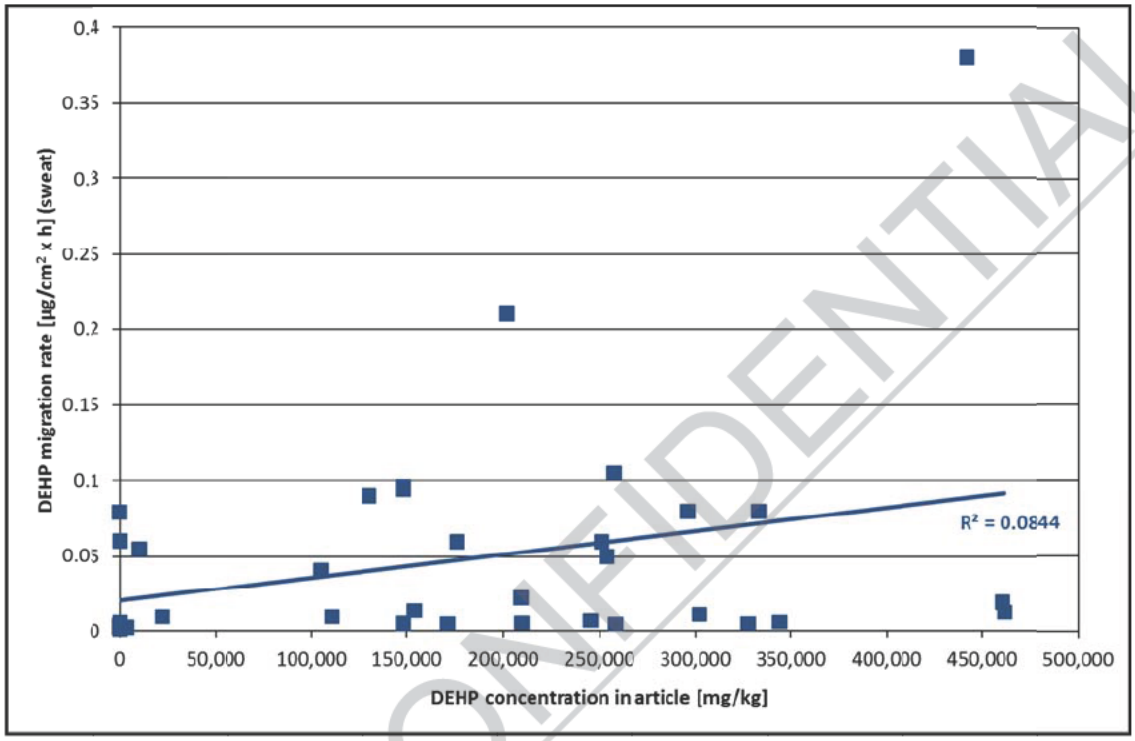
	All	**Without no-detects from Survey 117**	
		All	**Only samples containing >100,000 ppm**
n	62	51	37
MIN	0.0011	0.0011	0.0048
AM	0.17	0.077	0.10
GM	0.048	0.029	0.040
Median	0.060	0.040	0.060
75th perc.	0.19	0.085	0.090
90th perc.	0.57	0.18	0.21
95th perc.	**0.82**	**0.27**	**0.34**
MAX	1.0	0.83	0 83

It is also evident from the overall evaluation tha very high CVs (>50%) were observed for some samples in Survey 107 (sandals), while such high CVs were never observed in Survey 109, the other survey with several samples subjected to duplicate analysis. This finding relates to almost all samples with very low DEHP concentrations (<1,000 ppm). In fact, a consistent low migration was only observed for sample 38.2, while all other samples with DEHP concentra ions below 1,000 ppm produced inconsistent results, sometimes yielding very high migration values in one of the duplicates (see e.g. samples 13.2a and 55.1a). It is worth noting that these latter samples contained only about 0.01-0.03% DEHP, but gave a very high migration of about 8 mg/kg, otherwise only obs rved for samples containing DEHP in concentrations above 10%.
Highly variable migration va ues were also obtained for both measurements under dynamic conditions and the ones using sun lotion This was also acknowledged by the authors, who stated in relation to the additional tests on sample 8.1 tha "*the analysis uncertainty on the results of the analyses in duplicate for the additional tests is much higher t an on the other migration analyses. During the tests with sun lotion the reason might be that it was not possible to ensure that the sun lotion was homogenously applied on the sample before the migration analyses Comparative tests were carried out between the dynamic and static migration and the result shows that th analysis uncertainty is larger for dynamic migration. Pattern in the material can also contribute to the variation in the analyses in duplicate.*" (Tønning et al., 2010b). However, largely diverging values were also found for some samples when tested under standard (static) conditions, e.g. for sample 37.2.
Overall, the migration analyses from Survey 107 appear to be very uncertain.

In the context of static and dynamic test conditions, the latter are primarily discussed in the context of oral exposure, i.e. a migration into saliva, where dynamic test conditions are employed in in vitro tests to mimic chewing as well as peristaltic movements in the gastrointestinal tract (van Engelen et al., 2008).
For dermal exposure to phthalates from plastic sandals, it has been argued that dynamic conditions mimic situations when persons are walking or placing weight on the products (Tønning et al., 2010b). It is, however, entirely unknown whether the dynamic conditions chosen in this study (shaking the sample in artificial sweat with 155 directional changes/minute, i.e. 2.6/second) have any relationship to the realistic conditions of use. In addition, dermal contact with some PVC articles, such as packaging and office supplies, may be better

represented by static conditions. Even sleeping in a waterbed or lying on an air mattress may be considered static rather than dynamic. Available data, however, do not allow a differentiation of scenarios and their respective migration rates.

The following chart shows the regression analysis on the basis of mean values (for DEHP concentration and migration from each sample) from static tests only (to exclude an undue impact due to test design). The data clearly show that DEHP concentrations in the article do not predict the migration (coefficient of determination < 0.1).



For the exposure estimation in Chapter 9 the 95th percentile from the second evaluation of 0.27 µg/cm^2 x h is chosen as the DEHP migration rate for dermal exposure. As indicated, this value is used because the initial evaluation resulted in high upper percentiles solely due to an exceptionally high limit of detection in one of the surveys.

The data analysed were largely derived from static migration tests and do not allow a detailed discussion of potentially higher values obtained under dynamic test conditions (see also above for the applicability of static vs. dynamic conditions). However, the limited additional tests conducted on sample 8.1 in Survey 107 (means from duplicate analyses) indicate that the value chosen here of 0.27 µg/cm^2 x h is:

- higher than the mean, when the simulant was replaced after 8 hours (0.094 µg/cm^2 x h)
- higher than the mean under dynamic conditions without sun lotion (0.177 µg/cm^2 x h)
- higher than the mean under static conditions with sun lotion (0.096 µg/cm^2 x h)
- only lower than the mean under dynamic conditions with sun lotion (0.830 µg/cm^2 x h)

As indicated above, all these values show CVs of 70% and above and are therefore considered uncertain. Overall, the value chosen can therefore be considered a conservative input value in the context of exposure estimation and risk characterisation.

Comment on recent DEHP migration data provided by German BfR/UBA

In a recent publication (Pfaff et al., 2012), published by German Authorities (Federal Institute for Risk Assessment (BfR) and Federal Environment Agency (UBA), some additional migration rates were reported. The following tables show the values derived above and the new data from the BfR/UBA report.

Summary of migration data used in the exposure estimation (95th percentiles)

	Dermal (sweat simulant, n=51)	Oral (in vivo basis*, n=21)
Migration rate	0.27 µg/cm^2 x h	3.65 µg/10 cm^2 x min
Normalised migration rate	0.27 µg/cm^2 x h	21.9 µg/cm^2 x h

* Including many data involving chewing/biting on the objects

Summary of migration data from BfR/UBA report (median and maximum)

	Dermal (sweat simulant)		Oral (saliva simul nt)	
	Median	Maximum	Median	Maximum
Migration rate (Berlin data, n=8/5)	2.4 µg/cm^2 x h	6.0 µg/cm^2 x h	2.3 µg/cm^2 x h	3.0 µg/cm^2 x h
Migration rate (Federal data, n=22)			.6 µg/ m^2 x h	27.4 µg/cm^2 x h

The comparison shows that

- the migration rate for oral exposure (mouthing) derived for the exposure estimation is
considerably higher than the maximum of the Berlin data (n=5)
only slightly lower than the maximum of the Federal da a (n 22)
Also note the difference between the Berlin and F deral data (factor 3.3 in the median and factor 9.1 in the maximum values).

- Large differences exist in the case of dermal exposure, where the exposure estimation applied a lower migration rate than given in the Berlin d ta of the BfR/UBA report (no Federal data reported).

The following discussion therefore focu ses on the migration into sweat simulant relevant for dermal exposure. Several issues need to be addre sed:

- The BfR/UBA data re ate to only 8 measurements, while the migration rate used in the exposure estimation was derived from 51 measurements.

- The BfR/UBA data were obtained using the "head over heels" method, a fact that raises several important is ues:

The method employed is ba ed on the one described in an EU report (Simoneau et al., 2001). This method was developed f r he migration of phthalates from toys and childcare articles into saliva simulant to estimate exposure from m uthing. More specifically, the method employs dynamic agitation to simulate chewing and biting on artic s that is relevant for mouthing activities. The conditions (e.g. the type of agitation) for this me hod w re set so that a migration rate similar to the one found in vivo was achieved.
It is questionable whether these dynamic agitation conditions (e.g. 60 rotations/minute in the BfR/UBA measurements) – set to simulate chewing/biting on articles – are representative of the dermal exposure pathway. In fac , the underlying method (Simoneau et al., 2001) does not make any reference to migration into sweat simulant. The Phthalate Restriction Proposal Assessment acknowledged that the "head over heels" method reveals even higher migration rates from sandals than dynamic shaking conditions", as already observed during method development (Simoneau et al., 2001).
The data underlying the migration rate used in the exposure estimation are largely based on static migration tests (since these are the kind of tests primarily performed by the Danish EPA with sweat simulant) with some additional data from dynamic shaking tests. This appears to be in agreement with a recent opinion of the EU Scientific Committee on Health and Environmental Risks (SCHER, 2010), which proposes the head over heels method to account for additional migration from mechanical stressing action (licking, chewing, sucking), i.e. in the context of migration into saliva (oral exposure). After discussing this point, the SCHER opinion states that "the migration of chemicals in sweat **under static conditions** should be determined using artificial sweat…"

(our emphasis). Although the SCHER opinion is concerned with toys only, the authors do not appear to see dermal exposure from chemicals in articles as being related to similar mechanical stressing than for oral exposure pathways.
Since the method employed by BfR/UBA for the dermal pathway was developed for the oral pathway, the authors selected a test duration of 30 minutes for the actual migration measurements, which may be appropriate for mouthing activities, but not so much for the dermal exposure pathway. A short duration may lead to higher values based on an initial high release from the article, but may not be the appropriate value for long-term contact. In fact, two high migration rates similar to the maximum value determined by BfR/UBA were reported in ECB (2008) as well as in a training exercise in the context of RAPEX risk assessments (DG SANCO, 2006). Both values were obviously obtained under drastic conditions (scrubbing of PVC articles) over very short durations of a few minutes. The latter source, obviously extrapolated the exposure for a 2-hour period from a migration experiment of only 2 minutes duration.
The Danish EPA Surveys, on which the migration rate employed in the dermal exposure estimation is based, used different durations depending on the article investigated, trying to be representative of "in-use" durations (e.g. migration from plastic sandals over 16 h, since this duration was assumed in the Danish EPA exposure assessment). We believe that such an approach is more representative of the actual use situation to be assessed.

The lowest migration rate of the 8 BfR/UBA measurements for the dermal pathway was 0.5 µg/cm^2 x h, i.e. almost twice the value applied in the exposure estimation (and above almost all migration rates applied in the Phthalate Restriction Proposal Assessment). This migration rate was obtained for a mask containing 1.9% DEHP, i.e. a low concentration unrepresentative of DEHP concentrations in articles when the substance is added as a plasticiser. Note that DEHP migration from the same article into saliva simulant was below the LoD.

Finally, application of basically the same method (in terms of agitation) results in very similar migration rates for the dermal and oral pathway in the BfR experiments (even slightly lower for the latter). This is in contrast to the considerably lower migration rate applied for dermal exposure (about a factor of 80 lower than for mouthing) in the exposure estimation within this application. We therefore propose that this higher migration during mouthing (as derived from in vivo data) reflects the real (physical) situation, i.e. that biting/chewing on PVC articles leads to a higher DEHP release than, for example, walking in sandals or sitting in a car seat.

Overall, it is considered that the migration rate applied in the dermal exposure estimation in this application for authorisation represents a conservative estimate based on sufficiently large number of samples representing different article groups. In contrast, the BfR/UBA data relate to a very restricted data set of measurements, mostly with a single article type (sandals) and using "head over heels" method that is clearly of questionable relevance to the dermal pathway.
The BfR/UBA report acknowledged that the measured migration rates for the dermal pathway were considerably higher than those of the Danish EPA Surveys (used in the exposure estimation in this application for authorisation, but also forming the basis of the values used in the Phthalate Restriction Proposal Assessment). However, the authors suggested that these differences result from the different analytical methods for determining DEHP and do not address the factors discussed above.

AppendixA-2: Dermal exposure estimation: input values

Total skin surface area per kg body weight for German children

The total skin surface area (SA) is rarely measured, but can be approximated from the height (in cm) and the body weight (in kg) with three constants (for a discussion, see EPA, 2007). The data for SA as well as SA/BW in the following table were calculated from height and body weight data (medians each) from the German KiGGS, a representative survey of German children conducted in 2003-2006 ((RKI, 2011; Stolzenberg et al., 2007)).
Different values for the three constants are available, but the ones referring to children <5 years of age from EPA (2008) were used for the calculations shown below.

Girls, Medians					**Boys, Medians**				
Age [Months]	**BW [kg]**	**Height [cm]**	**SA [m^2]**	**SA/BW [cm^2/kg]**	**Age [Months]**	**BW [kg]**	**Height [cm]**	**SA [m^2]**	**SA/BW [cm^2/kg]**
4	6.25	60.6	0.34396	550	4	6.84	64.04	0.36881	539
5	6.82	62.83	0.36555	536	5	7.45	66 37	0.39151	526
6	7.3	64.78	0.38366	526	6	7.96	68.37	0.41037	516
7	7.72	66.49	0.39937	517	7	8 4	70.1	0.42651	508
8	8.09	68.03	0.41319	511	8	8.79	71.63	0.44070	501
9	8.43	69.46	0.42584	505	9	9.15	73.01	0.45364	496
10	8.75	70.82	0.43771	500	10	9.47	74.28	0.46519	491
11	9.06	72.11	0.44910	496	1	9.76	75.48	0.47572	487
12	9.34	73.35	0.45951	492	12	10.03	76.63	0.48557	484
15	10.1	76.73	0 48764	483	15	10.75	79.88	0.51214	476
18	10.76	79.69	0.51193	476	18	11.41	82.88	0.53637	470
21	11.35	82.28	0.53336	470	21	12.05	85.66	0.55940	464
24	11.95	84.65	0.55438	464	24	12.68	88.21	0.58147	459
30	13.18	89.1	0.59601	452	30	13.87	92.88	0.62245	449
36	14.42	93.35	0.63687	442	36	15.03	97.14	0.66125	440
42	15.54	99.79	0.68022	438	42	16.14	101	0.69746	432
48	16.6	103.51	0.71479	431	48	17.15	104.56	0.73028	426
54	17.69	107.15	0.74957	424	54	18.07	107.94	0.76034	421
60	18.84	110.82	0.78552	417	60	19.05	111.23	0.79134	415
66	20.06	114.31	0.82224	410	66	20.19	114.51	0.82566	409
72	21.35	117.59	0.85959	403	72	21.5	117.78	0.86338	402
Boys and girls combined, median									

6-12	8.8		0.44	501					
24-36	13.5		0.61	450					

Percentage of total skin surface area in contact with the article: X-1

One half of the hands and legs (50% each) is assumed to be in contact with the article (gym mats as sentinel article).

	Head	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Head**	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Sum**
	Mean Percent of Total Surface Area*						Percentage in contact with rticle						
Birth to 1 month	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0 0	2 7	10.3	0.0	13.0
1 to <3 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	10.3	0.0	13.0
3 to <6 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	10.3	0.0	13.0
6 to <12 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0 0	2.7	10.3	0.0	13.0
1 to <2 years	16.5	35.5	13.0	5.7	23.1	6.3	0.0	0.0	0.0	2.9	11.6	0.0	14.4
2 to <3 years	8.4	41.0	14.4	4.7	25.3	6	0.0	0.0	0.0	2.4	12.7	0.0	15.0
3 to <6 years	8.0	41.2	14.0	4.9	25.7	6.4	0.0	0.0	0.0	2.5	12.9	0.0	15.3
6 to <11 years	6.1	39.6	14.0	4.7	8.8	6 8	0.0	0.0	0.0	2.4	14.4	0.0	16.8
11 to <16 years	4.6	39.6	14.3	4.5	30.4	6.6	0.0	0.0	0.0	2.3	15.2	0.0	17.5
16 to <21 years	4.1	41.2	14.6	4.5	29.5	6.1	0.0	0.0	0.0	2.3	14.8	0.0	17.0
Adult Males >21	6.6	40.1	15.2	5.2	33.1	6.7	0.0	0.0	0.0	2.6	16.6	0.0	19.2
Adult Females >21	6.2	35.4	12 8	4.8	32.3	6.6	0.0	0.0	0.0	2.4	16.2	0.0	18.6

* Recommended values rom EPA, 2011

Percentage of total skin surface area in contact with the article: X-2

One half of th hands (50%) is assumed to be in contact with the article (handles as sentinel article):

	Head	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Head**	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Sum**
	Mean Percent of Total Surface Area*						Percentage in contact with article						
Bir h to 1 month	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	0.0	0.0	2.7
1 to <3 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	0.0	0.0	2.7
3 to <6 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	0.0	0.0	2.7
6 to <12 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	2.7	0.0	0.0	2.7
1 to <2 years	16.5	35.5	13.0	5.7	23.1	6.3	0.0	0.0	0.0	2.9	0.0	0.0	2.9
2 to <3 years	8.4	41.0	14.4	4.7	25.3	6.3	0.0	0.0	0.0	2.4	0.0	0.0	2.4

3 to <6 years	8.0	41.2	14.0	4.9	25.7	6.4	0.0	0.0	0.0	2.5	0.0	0.0	2.5
6 to <11 years	6.1	39.6	14.0	4.7	28.8	6.8	0.0	0.0	0.0	2.4	0.0	0.0	2.4
11 to <16 years	4.6	39.6	14.3	4.5	30.4	6.6	0.0	0.0	0.0	2.3	0.0	0.0	2.3
16 to <21 years	4.1	41.2	14.6	4.5	29.5	6.1	0.0	0.0	0.0	2.3	0.0	0.0	2.3
Adult Males >21	6.6	40.1	15.2	5.2	33.1	6.7	0.0	0.0	0.0	2.6	0.0	0.0	2.6
Adult Females >21	6.2	35.4	12.8	4.8	32.3	6.6	0.0	0.0	0.0	2.4	0.0	0.0	.4

* Recommended values from EPA, 2011

Percentage of total skin surface area in contact with the article: X-3

The soles of both feet are assumed to be in contact with the article (sandals as sentinel articl), taken a 50% of the feet surface.

	Head	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Head**	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Sum**
	Mean Percent of Total Surface Area*						Pe entage in contact with article						
Birth to 1 month	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0 0	0 0	0.0	0.0	3.3	3.3
1 to <3 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	0.0	0.0	3.3	3.3
3 to <6 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	.0	0.0	0.0	0.0	3.3	3.3
6 to <12 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	0.0	0.0	0.0	0.0	3.3	3.3
1 to <2 years	16.5	35.5	13.0	5.7	23 1	6.3	0.0	0.0	0.0	0.0	0.0	3.2	3.2
2 to <3 years	8.4	41.0	14.4	4 7	25	6.3	0.0	0.0	0.0	0.0	0.0	3.2	3.2
3 to <6 years	8.0	41.2	14.0	4.9	2 .7	6.4	0.0	0.0	0.0	0.0	0.0	3.2	3.2
6 to <11 years	6.1	39.6	14.0	4.7	28.8	6.8	0.0	0.0	0.0	0.0	0.0	3.4	3.4
11 to <16 years	4.6	39.6	14.3	.5	30.4	6.6	0.0	0.0	0.0	0.0	0.0	3.3	3.3
16 to <21 years	4 1	41.2	14.6	4.5	29.5	6.1	0.0	0.0	0.0	0.0	0.0	3.1	3.1
Adult Males >21	6	40.1	15.2	5.2	33.1	6.7	0.0	0.0	0.0	0.0	0.0	3.4	3.4
Adult Females >21	6 2	35.4	12.8	4.8	32.3	6.6	0.0	0.0	0.0	0.0	0.0	3.3	3.3

* Recommende v lues from EPA, 2011

Perce tage of total skin surface area in contact with the article: X-4

One f urth of trunk, arms and legs is assumed to be in contact with the article (outdoor seating as sentinel articl)

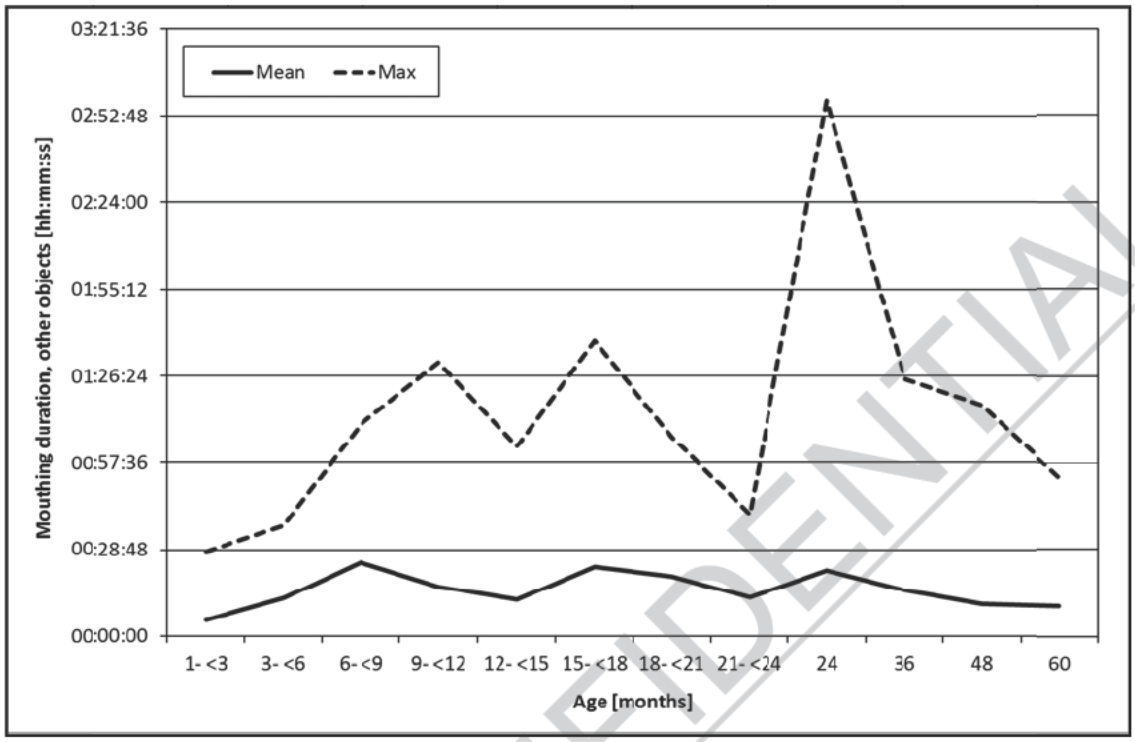
	Head	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Head**	**Trunk**	**Arms**	**Hands**	**Legs**	**Feet**	**Sum**
	Mean Percent of Total Surface Area*						Percentage in contact with article						
Birth to 1 month	18.2	35.7	13.7	5.3	20.6	6.5	0.0	8.9	3.4	0.0	5.2	0.0	17.5
1 to <3 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	8.9	3.4	0.0	5.2	0.0	17.5

3 to <6 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	8.9	3.4	0.0	5.2	0.0	17.5
6 to <12 months	18.2	35.7	13.7	5.3	20.6	6.5	0.0	8.9	3.4	0.0	5.2	0.0	17.5
1 to <2 years	16.5	35.5	13.0	5.7	23.1	6.3	0.0	8.9	3.3	0.0	5.8	0.0	17.9
2 to <3 years	8.4	41.0	14.4	4.7	25.3	6.3	0.0	10.3	3.6	0.0	6.3	0.0	20.2
3 to <6 years	8.0	41.2	14.0	4.9	25.7	6.4	0.0	10.3	3.5	0.0	6.4	0.0	20.2
6 to <11 years	6.1	39.6	14.0	4.7	28.8	6.8	0.0	9.9	3.5	0.0	7.2	0.0	0.6
11 to <16 years	4.6	39.6	14.3	4.5	30.4	6.6	0.0	9.9	3.6	0.0	7.6	0 0	21.1
16 to <21 years	4.1	41.2	14.6	4.5	29.5	6.1	0.0	10.3	3.7	0.0	7.4	0.0	21.3
Adult Males >21	6.6	40.1	15.2	5.2	33.1	6.7	0.0	10.0	3.8	0.0	8.3	0 0	22.1
Adult Females >21	6.2	35.4	12.8	4.8	32.3	6.6	0.0	8.9	3.2	0.0	8.1	0.0	20.1

* Recommended values from EPA, 2011

AppendixA-3: Mouthing other objects: mean and maximum duration for different age groups

Figure based on data in Smith and Norris (2003) and DTI (2002)



AppendixA-4: Evaluation of selected in vivo data for DINP

Source: Fiala et al (2000)

Rationale for selection: This is th only study that provides individual values.

Values excluded from analyses: Che ing (1 h): 5839 µg/dm^2 (9.7 µg/10 cm^2 * min)

Rationale for exclusion: This value was excluded by the authors in the calculation of the mean, since the subject bit off pieces of the article, which were extracted together with the saliva. The value is 6.5-times (5839 µg/dm^2/900 µg/dm^2) higher than the IQR, while all other values (sucking and chewing) differ from the IQR by less than a factor of 3.

	Sucking (1 h)		Chewing (1 h)	
	µg/dm^2	µg/10 cm^2 * min	µg/dm^2	µg/10 cm^2 * min
n	9	9	9	9
MIN	297	0.495	768	1.28
AM	833	1.39	1330	2.22
GM	735	1.22	1234	2.06
GSD	1.73	1.73	1.50	1.50
25th percentile	563	0.94	882	1.47
Median	715	1.19	1069	1.78
75th percentile	1104	1.84	1782	2.97

90th percentile	1434	2.39	2054	3.42
95th percentile	1443	2.41	2103	3.51
MAX	1452	2.42	2152	3.59
IQR	541	0.90	900	1.50

Annex #2# Biomonitoring data for workers

Formulation and processing

The following three tables summarise biomonitoring data of workers engaged in compounding and processing (Gaudin et al., 2011; Hines et al. 2009; Dirven et al., 1993). Values in bold were used for calculation of the daily DEHP intakes.

DEHP metabolites in urine	**MEHP**		**5cx-MEPP**		**Sum**	**Sum**	**Ratio**
Sampling	**pre-shift**	**post-shift**	**pre-shift**	**post-shift**	**pre-shift**	**post-shift**	**pre/post**
Reference, location	**Gaudin et al. (2011), France**						
Sampling period	**Not given (probably 2005-2010)**						
Process name in source	**Plastisol coating**						
N workers	5	5	5	5			
N samples	25	25	25	25			
Unit	**µg/L**						
Mean	25,2	68,2	73,7	149 7	98,9	217,9	0,45
Median	16,1	55,9	37,6	103,7	53 7	159,6	0,34
GM	15,3	56,4	42,9	82 7	58,2	139,1	0,42
Min	1,7	5,3	6,2	18,8			
Max	98	303	581	61			
Unit	**µg/g crea inine**						
Mean	12,7	51,1	36	98,4	48,7	149,5	0,33
Median	7,7	41,1	21,4	63	29,1	104,1	0,28
GM	7,4	34,8	20,9	56,6	28,3	**91,4**	0,31
Min	0,6	2 1	4,1	11,1			
Max	46,6	187,6	277	533			
Process name in source	**PVC 1 compounding**						
N workers	9	9	9	9			
N samples	41	41	41	41			
Unit	**µg/L**						
Mean	29,6	83,1	105	237,4	134,3	320,5	0,42
Median	18,9	52	57,5	166,4	76,4	218,4	0,35
GM	18,2	57,4	56,6	156,2	74,8	213,6	0,35
Min	2	10,9	6,4	18,6			
Max	134,4	467,1	618	1320			
Unit	**µg/g creatinine**						
Mean	19,2	44,4	61,9	125,8	81,1	170,2	0,48
Median	14,5	39,5	44,2	105,1	58,7	144,6	0,41
GM	16,1	35,9	50,2	97,6	66,3	**133,5**	0,50
Min	5,3	7,9	18,8	15,2			
Max	59,6	131,6	224	371,8			
Process name in source	**PVC 2 compounding**						
N workers	5	5	5	5			
N samples	32	31	32	31			
Unit	**µg/L**						
Mean	9,6	23,5	33	74,6	42,6	98,1	0,43
Median	7,3	17,3	27	57,6	34,3	74,9	0,46

DEHP metabolites in urine	MEHP		5cx-MEPP		Sum	Sum	Ratio
Sampling	**pre-shift**	**post-shift**	**pre-shift**	**post-shift**	**pre-shift**	**post-shift**	**pre/post**
GM	5,1	14	16,1	37,3	21,2	51,3	0,41
Min	0,5	0,6	0,5	1			
Max	45	89,8	1229,9	487,9			
Unit	**µg/g creatinine**						
Mean	7,7	20,5	23,8	58,2	31,5	78,7	0,40
Median	6,3	9,6	21	26,9	27,3	36,5	0,75
GM	5,4	10,6	16,2	27,1	21,6	**37,7**	0 57
Min	0,4	1,6	1,6	2,6			
Max	32,6	213,1	87,2	578,8			
Process name in source	**Polymers moulding**						
N workers	9	9	9	9			
N samples	41	41	41	41			
Unit	**µg/L**						
Mean	12,3	34	31,9	76,3	44 2	110,3	0,40
Median	5,4	18,3	15,4	34 3	20,8	52,6	0,40
GM	5	14,7	17	38,6	22	53,3	0,41
Min	0,3	0,3	0,6	,1			
Max	100,6	247,7	122 6	529			
Unit	**µg/g creatinine**						
Mean	7,2	16,1	19,6	38,1	26,8	54,2	0,49
Median	3,6	12,4	12,1	27,7	15,7	40,1	0,39
GM	4	10,7	13,7	28	17,7	**38,7**	0,46
Min	0,5	,2	2,5	3,5			
Max	53,8	90,1	68,1	177			
Process name in source	**Wall coverings products**						
N workers	18	18	18	18			
N samples	79	78	79	78			
Unit	**µg/L**						
Mean	41,7	77,7	128,4	232,4	170,1	310,1	0,55
Median	22,4	41,9	68,6	134,6	91	176,5	0,52
GM	18,5	42,3	63,1	133,3	81,6	175,6	0,46
Min	0,3	2,3	2,7	17			
Max	328,2	481	636,9	1410			
Unit	**µg/g creatinine**						
Mean	21,1	47,5	64,3	119,2	85,4	166,7	0,51
Median	15,5	25,9	50,8	78,6	66,3	104,5	0,63
GM	13,2	24,9	45,2	78,5	58,4	**103,4**	0,56
Min	1,3	1,6	3	6,4			
Max	111,4	942,2	280,4	924,4			

DEHP metabolites in urine	MEHP		5OH-MEHP		5oxo-MEHP		5cx-MEHP		Sum	Sum	Ratio
Sampling	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	post-shift	mid/end
Reference, location	Hines et al. (2009), USA										
Sampling period	2003-2005 (recruitment)										
Process name in source	PVC film production										
N workers	25	25	25	25	25	25	25	25			
N samples	25	25	25	25	25	25	25	25			
Unit	µg/L										
Median	26,7	37,3	224	282	111	148	222	283	583,7	75 3	0,78
GM	24,7	31,4	220	283	125	159	235	298	604 7	771,4	0,78
GSD	2,61	3,55	2,3	3,22	2,21	3,14	2,31	2,93	9,4	12,8	0,73
Min	2	<LOD	45,8	11,8	26,6	7	47 9	23			
Max	186	257	1230	3090	648	1490	1430	2030			
Unit	µg/g creatinine										
Median	12	20	93,4	138	54,8	74	102	142	262,2	374,0	0,70
GM	11,8	16,7	105	151	60	84,6	112	158	288,8	**410,3**	0,70
GSD	2,37	3,08	1,85	2,31	1,78	2 21	1,93	2,09	7,9	9,7	0,82
Min	1,77	< LOD	40,5	12	24,9	7,14	34	23,5			
Max	74,6	83,3	456	703	212	348	574	625			
Process name in source	Vehicl filter manufacturing										
N workers	18	18	18	18	18	18	18	18			
N samples	18	18	18	18	18	18	18	18			
Unit	µg/L										
Median	10,1	11,1	35,8	45,4	36,6	131	nm	nm	82,5	187,5	0,44
GM	11,1	13	35	44	34,4	65,2	nm	nm	80,5	122,2	0,66
GSD	2,49	2,32	2,45	2,53	2,39	4,81	nm	nm	7,3	9,7	0,76
Min	3,3	2,9	6,5	11,4	8,3	4,7	nm	nm			
Max	96	66,7	427	294	179	327	nm	nm			
Unit	µg/g creatinine										
Median	10	9	27,4	32,4	23,5	27,8	nm	nm	60,9	69,2	0,88
GM	9,33	10,2	29,6	34,6	23,1	27,1	nm	nm	62,0	**71,9**	0,86
GSD	2,24	2,13	2,33	2,29	2	2,12	nm	nm	6,6	6,5	1,00
Min	3,63	3,58	7,5	11,4	6,8	9,38	nm	nm			
Max	30	60,6	283	267	148	163	nm	nm			
Process name in source	PVC compounding										
N workers	12	12	12	12	12	12	12	12			
N samples	12	12	12	12	12	12	12	12			
Unit	µg/L										
Median	22,6	29,2	190	289	131	200	286	391	629,6	909,2	0,69
GM	13	24	103	203	65,2	121	140	246	321,2	594,0	0,54

DEHP metabolites in urine	MEHP		5OH-MEHP		5oxo-MEHP		5cx-MEHP		Sum	Sum	Ratio
Sampling	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	end-of-shift	mid-shift	post-shift	mid/end
GSD	5,03	4,82	4,76	3,36	4,81	3,56	4,39	3,46	19,0	15,2	1,25
Min	<LOD	>LOD	8,5	16,2	4,7	8	12,8	22,4			
Max	112	220	565	1040	327	675	998	1080			
Unit	**µg/g creatinine**										
Median	12,1	12,6	121	164	82,5	92	150	200	365,6	468,6	0,78
GM	8,09	12,1	63,8	102	40,4	60,8	86,8	124	199,1	**298 9**	0,67
GSD	4,51	4,4	4,24	3,05	4,27	3,27	3,86	3,18	16,9	13,9	1,21
Min	<LOD	<LOD	6,64	10,6	3,67	5,23	10	14,6			
Max	95	85	407	366	261	233	598	444			

nm: not measured

DEHP metabolites in urine	MEHP		5OH-MEHP		5oxo-MEHP		5cx-MEHP		Sum	Sum	Ratio
Sampling	pre-shift	end-of-shift	pre-shift	end-of-shift	pre-shift	end f-shift	pre-shift	end-of-shift	pre-shift	post-shift	pre/ end
Reference, location	**Dirven et l. (1993), The Netherlands?**										
Sampling period	**Not given (before 1993)**										
Process name in source	**PVC boot production (samples from day 1 of working week)**										
N workers	9	9	9	9	9	9	9	9			
N samples	9	9	9	9	9	9	9	9			
Unit	**µg/g creatinine**										
Median	87,22	120,17	78,52	150,54	27,3	64,74	69,05	82,92	262,1	**418,4**	0,63
Min	21,07	5 ,05	32,24	64,22	3,9	25,74	29,72	30,28			
Max	128,87	166,36	107,12	388,96	45,24	244,66	126,22	365,64			
Process name in source	**PVC boot production (samples from day 5 of working week)**										
N work rs	9	9	9	9	9	9	9	9			
N samples	9	9	9	9	9	9	9	9			
Unit	**µg/g creatinine**										
Median	80,12	119,81	115,7	140,4	44,46	62,92	69,62	107,26	309,9	**430,4**	0,72
Min	35,53	76,44	12,22	103,48	5,98	53,04	19,64	70,75			
Max	171,01	178,36	140,14	315,38	82,68	234,78	149,14	400,16			
Process name in source	**Cable production (samples from day 1 of working week)**										

DEHP metabolites in urine	**MEHP**		**5OH-MEHP**		**5oxo-MEHP**		**5cx-MEHP**		**Sum**	**Sum**	**Ratio**
Sampling	**pre-shift**	**end-of-shift**	**pre-shift**	**end-of-shift**	**pre-shift**	**end-of-shift**	**pre-shift**	**end-of-shift**	**pre-shift**	**post-shift**	**pre/ end**
N workers	6	6	6	6	6	6	6	6			
N samples	6	6	6	6	6	6	6	6			
Unit	**µg/g creatinine**										
Median	37	43,86	29,38	46,28	10,4	28,6	14,72	31,13	91,5	**149 9**	0,61
Min	12,01	8,82	18,98	18,98	7,8	16,38	8,77	12,17			
Max	62,48	150,43	43,94	282,36	13,78	119,34	30,56	183,67			
Process name in source	**Cable production (samples from day 5 of working week)**										
N workers	6	6	6	6	6	6	6	6			
N samples	6	6	6	6	6	6	6	6			
Unit	**µg/g creati ine**										
Median	39,69	84,53	36,14	67,34	9,62	43,16	17, 6	42,73	102,7	**237,8**	0,43
Min	9,8	12,01	9,62	46,54	4,68	5 72	,23	20,09			
Max	63,95	185,96	77,22	196,3	53,3	97,5	73,01	107,26			

Annex #3# Biomonitoring data for the general population

Adults

The following table summarises the biomonitoring data for adults (general population) from European studies of the last decade (in chronological order according to sampling time).

Reference Location	Sample type (spot/24 h)	MEHP	5OH-MEHP	5oxo-MEHP	5cx-MEPP	Sum of MEHP, 5-OH- and 5-oxo-MEHP	Sum of all 4 meta-bolites
Koch et al., 2003 Germany	Sampling period 2002 Age 7-64 years N = 85/both sexes Spot (1st morning urine)						
	µg/L urine						
	Mean	15,7	79,6	57,2		152,5	
	SD	21,4	116	75,4			
	50 percentile	10,3	46,8	36,5		93,6	
	95 percentile	37,9	224	156		417,9	
	Max	177	818	544			
	µg/g creatinine						
	Mean	12,4	57,2	41,7		111,3	
	SD	14,6	65	41 4			
	50 percentile	9,2	40 2	30,4		79,8	
	95 percentile	34,7	143	106		283,7	
	Max	123	449	262			
Koch et al., 2004 Germany	Sampling period 2003 Age 20-59 year N = 19/both s xes Spot (1st morn ng urine						
	µg/L urine						
	50 percentile	9	32,1	19,6		59,1	
	95 perc ntile	29	64	36,7		125,0	
	Max	43,1	103	55,1			
	µg/g creatinine						
	50 p rcentile	8,6	28,1	17,2		50,9	
	95 percentile	24,7	48	34,7		108,0	
	Max	26,6	63,6	40,9			
Wittas k et a 2007 Germany	Sampling period 2003 Age 20-28 years N = 59/both sexes 24 h urine						
	µg/L urine						
	50 percentile	4,6	13,4	12,2	17,5		47,7
	95 percentile	25,2	38,8	34,9	60,6		159,5
	Max	63,9	83,2	57,9	129		
	µg/g creatinine						
	50 percentile	5,5	15,2	12	20		52,7
	95 percentile	21,1	30,7	27,1	51,6		130,5
	Max	34,8	33,2	35,2	56,7		

Ye et al., 2008 The Netherlands	Sampling period 2004 Age 18-41 years N = 100/females Spot urine (different times)						
	µg/L urine						
	GM	6,9	14,3	15	19,4		55,6
	SD	2,7	4,7	2,6	2,8		
	50 percentile	6,9	14	14,5	18,4		53,8
	95 percentile	82,8	86,2	104	141		414,0
	Max	392	494	514	421		
	µg/g creatinine						
	GM	10,8	22,2	23,3	30,1		86 4
	SD	2,1	3,4	2	2,3		
	50 percentile	9,9	20,3	20,9	25,8		6,9
	95 percentile	88,7	99,5	126	155		469,2
	Max	321	371	386	317		
Ye et al., 2009 Norway	Sampling period 2004 Age 15-53 years N = 10 pooled samples from 110 females (pregnant) spot samples						
	µg/L urin						
	Mean	22,32	21,88	21,55	32,43		98,2
	GM	14,12	15,52	14,5	21 2		65,3
	µg/g creatinin						
	No data						
Philippat et al., 2012 France	Sampling period 2002-2006 Age 29.5 years (mean) N = 287/females (pregnant) spot samples?						
	µg/L urine						
	50 percentile	7 1	32,3	25	43,8		108,2
	95 percentile	40,7	147	112	183		482,7
	µg/g creatinine						
	No data						
Fromme et al., 2007 Germany	Samp ng period 2005 Age 14 60 year N = 50/both exes Spot urine (morning, 8 per subject)						
	µg/L urine						
	50 p rcentile	4,9	19,2	14,7	26,2		65,0
	95 percentile	21,7	81,8	56	93,6		253,1
	Max	207,3	681,8	447,1	946,7		
	µg/g creatinine						
	No data						
Bevan t al., 2012 UK	Sampling period ? (>2005) Age > 18 years N = 337/both sexes Spot urine (different times)						
	µg/L urine						
	95 percentile		42,9	67,2			
	µg/g creatinine						
	95 percentile		42,3	66,5			
Frederiksen et al., 2010 Denmark	Sampling period 2006 Age 18,2-26,2 years N = 60/males Spot urine (different times)						

	µg/L urine						
	Mean	9,18	46,56	34,4	24,7		114,8
	50 percentile	4,85	26,13	20,04	17,04		68,1
	95 percentile	40,8	207,37	159,31	102,04		509,5
	Max	59,12	423,87	264,89	126,43		
	µg/g creatinine						
	No data						
Casas et al., 2011 Spain	Sampling period 2004-2008 Age not stated N = 118/females Spot urine (different times)						
	µg/L urine						
	50 percentile	4,4	17,3	15,7	32,2		69,6
	IQR	6,8	21,6	19,3	40,7		
	µg/g creatinine						
	No data						
Göen et al., 2011 Germany	Sampling period 2008 Age 19-29 years N = 60/both sexes 24 h urine						
	µg/L urin						
	50 percentile	3,3	9,6	6,4	10,2		29,5
	95 percentile	13,1	28,3	15,3	25 1		81,8
	Max	23,9	107	61,6	97,4		
	µg/g creatinine						
	50 percentile	5,4	15	11	17		48,4
	95 percentile	17,7	35	28	35,6		116,3
	Max	32,6	60,7	32,5	54,4		
Kaspar-Sonnenberg et al., 2012 Germany	Sampling period 2007-2009 Age 39,2 ± 4,6 years N = 103/females Spot (1st morn ng urine)						
	µg/L urine						
	Mean						
	GM	4,4	16,8	12	21,2		54,4
	95% CI	3,6-5,4	14,4-19,6	10,2-14,0	18,2-24,6		
	50 percentile	4,6	17,3	12,9	20,5		55,3
	95 p rcentile	15,3	45,5	35	69,4		165,2
	Max	808	416	279	379		
	µg/g creatinine						
	GM	3,7	14,1	10	17,7		45,5
	95% CI	3,1-4,5	12,2-16,2	8,7-11,5	15,5-20,3		
Norström Joensen et al., 2012 Denmark	Sampling period 2007-2009 Age 19.5 (mean) years N = 881/male spot samples						
	µg/L urine						
	Mean	6,7	39	26	27		98,7
	50 percentile	4	23	14	15		56,0
	µg/g creatinine						
	No data						
DEMOCOPHES, 2012 17 countries	Sampling period 2011-2012 Age 20 - <45 years N = 1844/females morning urine						

	µg/L urine						
	GM					29,2	
	SD					28,1-31,3	
	95 percentile					91,0	
	SD					84 - 100	
	µg/g creatinine						
	No data						

Children

The following table summarises the biomonitoring data of children from European studies of the last decade (in chronological order according to sampling time).

Reference **Location**	**Sample type (spot/24 h)**	**MEHP**	**5OH-MEHP**	**5oxo-MEHP**	**5cx-MEPP**	**Sum of MEHP, 5-OH- and 5-oxo-MEHP**	**Sum of all 4 meta-boli es**
Becker et al., 2004 Germany	Sampling period 2001/2002 Age 3-14 years N = 254/both sexes Spot (morning)						
	µg/L urin						
	GM	7,91	52,1	39,9		99,9	
	95% CI	7,13-8,77	47,2-57,6	36 0-44,2			
	50 percentile	7,18	52 1	4 4		100,7	
	95 percentile	29,7	188	139		356,7	
	Max	226	2590	1420			
	µg/g creatinine						
	GM	18	40,7	31,2		78,1	
	95% CI	5,57-6,85	36,8-45,1	28,3-34,5			
	50 perc ntile	5,85	39,9	30,5		76,3	
	95 percentile	23,7	170	119		312,7	
	Max	223	1990	1090			
Koch t al., 2004 Germ ny	Sampling period 2002 Age 2,6-6,5 years N = 36/both sexes Spot (1st morning urine)						
	µg/L urine						
	50 percentile	6,6	49,6	33,8		90,0	

	95 percentile	14,6	107	71		196,0	
	Max	18,3	129	90,6			
	µg/g creatinine						
	50 percentile	8,7	55,8	38,3		98,8	
	95 percentile	27,5	113	75,8		206,0	
	Max	48,4	258	158			
Becker et al., 2009 Germany	Sampling period 2003-2006 Age 3-14 years N = 599/both sexes Spot (morning)						
	µg/L urin						
	GM	6,4	47,9	37	62,5		153,8
	95% CI	6,0-6,8	45 1 50,8	3 9-39,1	58,9-66,2		
	50 percentile	6,7	46	36,3	61,4		150,4
	95 percentile	25,1	164	123	209		521,1
	Max	319	3640	2490	4490		
	µg/g creatinine						
	No data						
Boas et al., 2010 Denmark	Sampling period 2006-2007 Age 4-9 years N = 503/boys Spot urine						
	µg/L urine						
	GM	4,1	33,2	17	29		83,3
	50 percentile	4,5	37	19	30		90,5
	Max	78	1718	656	676		
	µg/g creatinine						

	GM	6,9	53	27	46		132,9
	50 percentile	6,8	52	26	43		127,8
	Max	210	1818	794	1648		
Boas et al., 2010 Denmark	Sampling period 2006-2007 Age 4-9 years N = 342/girls Spot urine						
	µg/L urine						
	GM	3,6	28	15	27		73,6
	50 percentile	3,1	31	16	27		77,1
	Max	231	1672	734	1755		
	µg/g creatinin						
	GM	7,2	55	29	51		142,2
	50 percentile	6,7	52	28	49		135,7
	Max	1 6	220	536	1280		
Casas et al., 2011/2012 Spain	Sampling peri d 2004-2008 Age 19 yea s N = 4 Spot urine (different sampling times)						
	µg/L urine						
	50 percentile	6,2	57,4	44,6	115		223,2
	IQR	6,7	56,6	46,7	94		
	µg/g creatinine						
	50 percentile	8	79,7	60,3	163,6		
Koch et al., 2011 Germany	Sampling period 2007 Age 5,6-6,7 years N = 111/both sexes Spot urine (different times)						

	µg/L urine						
	Mean	8,5	28,8	24,1	43,4		104,8
	95% CI	5,3-11,8	22,0-35,6	18,9-29,3	34,3-52,6		
	GM	4,5	17,8	14,7	30,5		67,5
	95% CI						
	50 percentile	4,7	17,4	15,1	28,4		65,6
	95 percentile	21,1	86,1	71	101		279,2
	Max	172	311	214	441		
	µg/g creatinine						
	Mean	11,5	41,2	34,2	64 8		151,7
	95% CI	8,4-14,6	32,8-49,6	27 8 40 6	54,0 75,6		
	GM	7,3	29,2	24,3	50,3		111,1
	95% CI						
	50 percentile	7,8	28	24,3	46,9		107,0
	95 percentile	25,8	104	97,2	158		385,0
	Max	153	290	205	394		
Frederiksen et al., 2011 Denmark	Sampling period 2007 Age 6-21 years N = 129/both sexes 24 h urine sample						
	µg/L urine						
	50 percentile						107,0
	95 percentile						352,0
	Max						733,0
	µg/g creatinine						
	No data						

Frederiksen et al., 2012 Denmark	Sampling period 2006-2008 Age 5,6-19,1 years N = 725/girls Spot urine (1st morning urine)						
	µg/L urine						
	Mean	7,6	73	39	42		161,6
	50 percentile	4,7	47	24	28		103
	95 percentile	16	163	82	89		350,0
	Max	307	2968	1585	1462		
	µg/g creatinin						
	No data						
Mieritz et al., 2012 Denmark	Sampling period 2006-2008 Age 6,07-19,83 years N = 517/boys Spot urine (morning urine)						
	µg/L urine						
	Mean	6,87	60,57	32,3	34,42		134,2
	50 percentile	5,05	47,36	25,06	27,39		104,9
	95 percentile	18,02	158	81,83	79,54		337,4
	Max	58,29	893	385,6	462,5		
	µg/g creatinine						
	No data						
Kaspar-Sonnenberg t al., 2012 G rmany	Sampling period 2007-2009 Age 6,8 ± 0,6 years N = 104/both sexes Spot (1st morning urine)						
	µg/L urine						
	GM	3,9	29,3	26,2	41,8		101,2
	95% CI	3,3-4,7	25,2-34,0	22,7-30,3	36,4-		

					48,0		
	50 percentile	4	31	26,4	42,1		103,5
	95 percentile	17,1	88,2	88,8	127		321,1
	Max	27,7	163	135	259		
	µg/g creatinine						
	GM	3,8	28,6	25,6	40,8		98,8
	95% CI	3,3-4,4	25,2-32,4	22,8-28,8	36,4-45,7		
	50 percentile						
	95 percentile						
	Max						
Carlstedt et al. 2012 Sweden	Sampling period ? (>2009) Age 0,17-0,5 years (2-6 months) N = 83/both sexes Overnight diaper sample						
	µg/L urine						
	GM		5,1	7,5			
	IQR		7,6	10,9			
	50 percentile		5	8,8			
	95 percentile						
	Max		82,4	76,7			
	µg/g creatinine						
	Creatinine values in ng/mol creatinine, calculation into µg/g yields unrealistic high values (e.g. 5,1 µg/L urine paired with 4 ng/mol creatinine, corresponding to 36,3 µg/g (x 1000/113,1).Therefore, these values are not reported here						
DEMOCOPHES, 2012 17 countries	Sampling period 2011-2012 Age 6 - 11 years N = 1844/both sexes morning urine						
	µg/L urine						

	GM					47,6	
	SD					46 - 49,3	
	90 percentile					137,0	
	SD					126 - 150	
	µg/g creatinine						
	No data						

Annex 4 ART modelling protocol

ART REPORT – dust exposure PROC8a coarse dust – 26-Jun-13

transfer of dusty product - non-dedicated facilities - coarse dust, emptying small bags 25 kg

Chemical details	
Chemical	DEHP in soft PVC recyclate
CAS No.	117-81-7
Scenario details	
Number of activities	1
Total duration (mins)	480
Nonexposure period (mins)	0
Metadata	
ART version	1.5

Details for Activity PROC 8a - transfer of coarse dust - non-dedicated facilities, small bags

Emission sources: Near field ✓ Far field

Duration (mins): 480

Near-field exposure

Operational Conditions

Substance emission potential	
Substance product type	Powders, granules or pelletised material
Dustiness	Coarse dust
Moisture content	Dry product (< 5 % moisture content)
Powder weight fraction	0.2
Activity emission potential	
Activity class	Falling powders
Situation	Transferring 10 – 100 kg/minute
Handling type	Routine transfer
Drop height	Drop height > 0.5 m
Containment level	Open process
Surface contamination	
Process fully enclosed?	No
Effective housekeeping practices in place?	Yes
Dispersion	
Work area	Indoors
Room size	300 m³

Risk Management Measures

Localised controls	
Primary	Fixed capturing hood (90.00 % reduction)
Secondary	No localized controls (0.00 % reduction)
Dispersion	
Ventilation rate	3 air changes per hour (ACH)

Predicted exposure levels

ART predicts air concentrations in a worker's personal breathing zone outside of any Respiratory Protection Equipment (RPE). The use of RPE must be considered separately.

Mechanistic model results

The predicted 90th percentile full-shift exposure is 1.3 mg/m³.

The inter-quartile confidence interval is 0.65 mg/m³ to 2.7 mg/m³.

ART REPORT – dust exposure PROC 8b coarse dust – 16-Jul-13

transfer of product - coarse dust - big bags (1000 kg)

Chemical details	
Chemical	DEHP in soft PVC recyclate
CAS No.	117-81-7

Scenario details	
Number of activ ties	1
Total durat on (mins)	480
Nonexposure period (mins)	0

Metadata	
ART version	1.5

Details for Activity PROC 8b - transfer of coarse dust - dedicated facillities, big bags up to 1000 kg

Emission sources: Near field — Duration (mins): 480

Far field ✓

Far-field exposure

Operational Conditions

Substance emission potential	
Substance product type	Powders, granules or pelletised material
Dustiness	Coarse dust
Moisture content	Dry product (< 5 % moisture content)
Powder weight fraction	0.2
Activity emission potential	
Activity class	Falling powders
Situation	Transferring 100 – 1000 kg/minute
Handling type	Routine transfer
Drop height	Drop height > 0.5 m
Containment level	Open process
Surface contamination	
Process fully enclosed?	No
Effective housekeeping practices in place?	Yes
Dispersion	
Work area	Indoors
Room size	300 m³

Risk Management Measures

Localised controls	
Primary	No localized controls (0.00 % reduction)
Secondary	No localized controls (0.00 % reduction)
Segregation	No segregation (0.00 % reduction)
Personal enclosure	No personal enclosure (0.00 % reduction)
Dispersion	
Ventilation rate	3 air changes per hour (ACH)

Predicted exposure levels

ART predic air oncentrations in a worker's personal breathing zone outside of any Respiratory Protection Equipment (R E). The use of RPE must be considered separately.

Mech stic model results

The pre cted 90th percentile full-shift exposure is 9.6 mg/m³.

T ter-quartile confidence interval is 4.8 mg/m³ to 20 mg/m³.

Notes/Comments/Justifications

Efficiency of containment or LEV: 95% (Default of ECETOC TRA for PROC 8b), applied outside ART since none of the control measures in ART corresponds to 95% Efficiency.
Final 90th percentile estimate: 9.6 mg/m³ x 0.05 = **0.48 mg/m³**

Annex #5# Discussion of DNELs for DEHP as proposed by RAC (, 2013)

A.5.1 Comparison of DNELs with recent reference DNELs proposed by RAC

In its 24th meeting the Committee for Risk Assessment (RAC) presented reference DNELs for DEHP for use in the discussion of applications for authorisation (ECHA, 2013). These proposed DNELs are not legally binding, but present RACs current view on the hazard assessment of DEHP.

The following table compares the DNELs as used in this CSR with RAC's proposals and indicates the reasoning underlying these differences. Overall however it is noted that all the DNELs derived in this CSR and hos proposed by RAC are based on the same datasets derived from the multi-generation study of Wolfe an Layton (2004), and have the same NOAEL of 4.8 mg/kg bw/day as the starting point.

Table 125 Comparison of DNELs as used in this CSR and proposed by RAC

DNEL (all systemic, long-term)	this CSR	RAC (ECHA, 2013)	Rem rk
DNEL workers-inhalation	1.6 mg/m³	0.88 mg/m³	Difference: 1. I traspecies factor of 3 used in his CSR compared to 5 as used by RAC; see discussion on points below; 2. absorption in rats: 70% (RAC) versus 75% (this assessment)
DNEL workers-dermal	3.4 mg/kg bw/day	1.88 mg/kg bw/day	Difference: 1. Intraspecies factor of 3 used in this CSR compared to 5 as used by RAC; see discussion on points below; 2. absorption in rats: 70% (RAC) versus 75% (this assessment)
DNEL gen.pop-adu ts-oral	0.0 8 mg/kg bw/day	0.034 mg/kg bw/day	Difference: 1. 75% oral absorption in adult humans assumed in this CSR compared to 100% as used by RAC, see discussion below; 2. absorption in rats: 70% (RAC) versus 75% (this assessment)
DNEL gen.pop-children-oral	0.036 mg/kg bw/day	0.034 mg/kg bw/day	minor difference, due to use of 70% oral absorption in rat studies instead of 75% in this CSR
DNEL gen.pop-adults-dermal	0.72 mg/kg bw/day	0.672 mg/kg bw/day	minor difference, due to use of 70% oral absorption in rat studies instead of 75% in this CSR
DNEL gen.pop-children-dermal	0.72 mg/kg bw/day	0.672 mg/kg bw/day	minor difference, due to use of 70% oral absorption in rat studies instead

			of 75% in this CSR
DNEL gen.pop-adult-inhalation	0.17 mg/m³	0.16 mg/m³	minor difference, due to use of 70% oral absorption in rat studies instead of 75% in this CSR
DNEL gen.pop-children-inhalation	0.13 mg/m³	0.12 mg/m³	minor difference, due to use of 70% oral absorption in rat studies instead of 75% in this CSR

For assessing exposure as measured in biomonitoring studies, a surrogate DNEL for external exposure of workers was derived at 230 µg/kg bw/day (for more details see chapter 9.0.). This value was derived to compare with biomonitoring data; it represents the oral dose which would lead to the same internal exposure as the inhalative DNEL for workers, using the same basis and assessment factors (assuming equal absorption rate for oral and inhalation exposure, 10 m^3 respiratory volume during a work shift for adult workers and 70 kg body weight - see CSR section 5.11).
With the RAC DNEL, workers-inhalation and a difference in absorption assumed (75% for inhalation and 100% for oral exposure) as proposed by RAC (see below) a surrogate DNEL for external exposure of workers of 94 µg/kg bw/day would result.

A.5.2 Discussion of intraspecies factor

The legal text in REACH(REGULATION (EC) No 1907/2006, Annex I, 0.5) state : "*The chemical safety assessment shall be based on the information on the substance contained in the technical dossier and on other available and relevant information. …. Available information from assessments carried out under other international and national programmes shall be included. Where available and appropriate, an assessment carried out under Community legislation (e.g. risk assesments completed under Regulation (EEC) No 793/93) shall be taken into account in the development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified*."
In chapter 4.1.3.1.6 of the EU Risk Assessment Report (EU RAR) (ECB, 2008) it is reported that "Assessment factors of 3 and 10 are used for potential intraspecies differences for the worker and general population, respectively" in the assessment.
As there is no new information available on potential inter-individual variability of susceptibility in humans, there is no justification to deviate from the EU RAR. Therefore, we consider the legal text as binding and (in deviation from the standard assesment factor proposed in ECHA Guidance on Information Requirements and CSA, R.8) a factor of 3 was used in the assessment of workers.
Importantly, from a scientific point of view this factor is fully justified because, when basing the assessment on the multi-generation study of Wolfe and Layton (2004), the assessment focusses on pregnant women at late stages of pregnancy. According to EU Directive 92/85/EEC on pregnant workers and those who have recently given birth or are breastfeeding, this group has to be protected from risks due to exposure to (reprotoxic) chemicals which could jeopardize their health and the health of the unborn. Therefore, in compliance with this Directive, worker exposure to substances classified as reproductive toxins (Cat 1A or 1B) should be completely avoided and should not occur, if EU regulations are to be complied with.

Whilst for the trial exercise in ECHA (2013) an assessment factor of 5 was adopted for intraspecies extrapolation, the comprehensive review undertaken here of the latest scientific literature during the derivation of the CSR has identified no new data or scientific knowledge that would justify deviating from the legally binding EU Risk Assessment Report in this respect and hence the previously established value of 3 was utilised.

A.5.3 Discussion of data on human absorption

In the following table, absorption rates as used in this CSR are compared to those used by RAC to derive their reference DNEL.

Table 126 Absorption rates for DEHP in humans as used for risk assessment in this CSR compared to absorption rates as proposed by RAC (ECHA, 2013)

	oral	dermal	inhalation
This CSR (adults/children)	75%/100%	5%	75%/100%
RAC proposal (adults/children)	100%/100%	5%	75%/100%

The only difference in these assumptions is in relation to the extent of absorption of DEHP by adults after oral exposure. The following reasons are given by RAC for this assumption:

RAC (ECHA, 2013) noted that in its previous opinion on the Restriction Proposal Assessment (RAC/SEAC, 2012) it was considered that oral absorption in adults is 70%, but states that "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."

For oral exposure of DEHP in experimental studies with rats (i.e. the key study Wolfe and Layton, 2004) a minor difference exists between this CSR and RAC's proposal: absorption of 75% is assumed in this CSR compared to 70% by RAC. This difference is well within the uncertainty of determination (see also below).

In the following the knowledge about absorption and metabolism of DEHP in humans is summarised.

The metabolism of DEHP in humans is well investigated (Koch et al., 2003; 2005). Cleavage of the first ester bond leads to 2-ethylhexanol and mono(2-ethylhexyl)-phthalate (MEHP). MEHP is further metabolised by side-chain oxidation, leading to various oxidative metabolites, which are excreted in urine, partly in their glucuronised form.
Using DEHP carrying a deuterium label at position 4 of the aromatic ring, Koch et al. (2003; 2005),among the first using this technique in the case of DEHP, investigated the metabolisms of DEHP in humans. This study with one male volunteer was followed by more extensive investigations (Anderson et al., 2011; Kessler et al., 2012). Three to five metabolites were analysed in these investigations, which cover, besides MEHP, oxidative metabolites arising from oxidation of

- carbon atom 5 of the ethylhexyl chain (5OH-MEHP, 5oxo-MEHP)
- carbon atom 6 of the ethylhexyl chain (5cx-MEPP)
- and carbon atom 6 of the ethylhexyl chain (5cx-MEPP)
- carbon atom 2 of the ethyl side chain of the ethylhexyl group (2cx-MMHP)

In principle, only few possibilities for oxidation reactions at other positions of the carbon chain remain: at carbon atom 2 of the ethyl side chain of the ethylhexyl group, which is probably sterically hindered, and at carbon atom 4 of the ethylhexyl chain (ATSDR, 2002; Koch et al., 2005).

When analysing urine samples after application of D4-DEHP to a volunteer by high pressure liquid chromatography (HPLC), coupled with tandem mass spectrometry detection, Koch et al. (2005) could not see any major unidentified peaks in the chromatograms. The authors could only detect "some minor peaks in the chromatograms", which may be attributed to DEHP metabolites apart from the 5 major metabolites quantified. So, as relevant polar metabolite would have been detected chromatographic analysis, it is highly unlikely that a metabolite, which could markedly add to overall absorption, went undetected in these investigations.

Table 127 Comparison of urinary excretion rates of DEHP metabolites from three different studies with human volunteers

% molar urinary elimination	Koch et al. (2005) n=1 (m), 44 h	Anderson et al. (2011) n=20 (m/f), 48 h, low dose, used in this CSR	Kessler et al. (2012) n=4 (m), 46 h
MEHP	7.34	6.94	2.6
5OH-MEHP	24.7	16.33	13.3
5oxo-MEHP	14.9	12.53	15.0
5cx-MEPP	21.9	15.9	
2cx-MMHP	5.4		
Sum of 3 metabolites	46.94	35.8	3 9
Sum of 4 metabolites	68.84	51.7	
Sum of 5 metabolites	74.24		

The table shows that excretion rates observed by Koch et al. (2005) with one v lunteer are substantially higher than average rates found by other investigators with larger groups of human volunteers. Considering the relevant intra-individual variability in these data, it could well be by ch nce that the one volunteer investigated had higher than average excretion rates. Therefore, to assess average absorption th tudy of Anderson et al. (2011) with 10 volunteers of each sex is considered more reliable to as ess absorpti n than the Koch et al. (2005) study. The Anderson et al. (2011) study, together with the discussion on undected metabolites above, point out that absorption in humans after single oral application of DEHP is in the range of 50 to 60% and similar to oral absorption in rats after single administration (f data see chapter 5.1). In conclusion, similar absorption can be assumed in humans and in rats.
As shown by the studies of Laignelet and Lhugueno (2000c; d, for details see chapter 5.1) after repeated exposure, urinary excretion rates of rad oactivity re higher than after single exposure and reach up to >70% in non-pregnant and pregnant rats (biliary xcretion and radioactivity remaining in the carcass not accounted for). Therefore, based on urinary exc etion rates, n absorption of 75% for rats and humans is well justified and would be higher for both, if biliary ex retion is onsidered in addition. Indeed, when, according to ATSDR (2002),excretion with bile can a count to more than 20%, then for both absorption in rats and humans nearly complete absorption can be assumed
For children, following RAC/SEAC (2012), an oral absorption of 100% is assumed in this CSR. It should be noted that this is no based on data which point out a higher absorption in the younger organism, but is done here on precautionary grounds only. There is only one study investigating the age-dependency of absorption of DEHP: The xperimental data of Sjöberg et al. (1985) with a urinary excretion of 44% in 25 days old rats (immature) compa ed to 26% in 60 days old rats (mature) suggest a higher rate of oral absorption after a single applic tion in t e younger animals. However, this rate of urinary excretion for young animals lies within the normal range of young animals in other studies (Kurata, 2004a; Lake et al., 1975) and the value for 60 days old rats obs rved in the Sjöberg et al. study is the lowest one reported in any of the other studies with adult rats (Laigne et and Lhuguenot, 2000b; Lake et al., 1984).

It should be noted that also in the EU Risk Assessment Report (ECB, 2008) no correction for differences in b rption between rats and humans was considered necessary, when assessing risks based on the Wolfe and Layton study: "*However, for the NOAELs coming from the 3-generation study (testicular toxicity and developmental toxicity), no recalculation to a systemic NOAEL is needed as a major part of the exposure occurs during life stages with an assumed oral absorption of 100%."* This is in line with assuming equal absorption in adult rats and humans, as done in this CSR for rats and adult humans. Our assessment for children is even more conservative than the approach taken in the EU RAR, as 100% in children has been assumed compared to 75% absorption in adult rats in the key study Wolfe and Layton et al. (2004).

Based on the above critical analysis, the assumption of RAC of a significant difference in oral absorption between rats and humans seems not to be scientifically justified given that:

- absorption after single oral exposure of DEHP in adult humans is estimated to be in the range of 50 to 60 % (Anderson et al., Kessler et al.), which is similar to the absorption found in rats (see chapter 5.1)
- major metabolites of DEHP in humans which were not accounted for in the calculation of absorption based on 5 metabolites, could not be detected in investigations with D4-labelled DEHP
- therefore, based on data on urinary excretion of metabolites for humans after repeated exposure and in analogy to experimental animals, an estimated absorption of about 75% is reasonable
- the argument that, in humans, unaccounted amounts of metabolised DEHP are possibly excreted via bile could be equally applied to the data on experimental animals. Actually, based on this argument, one would possibly deduce that there would be a near complete absorption of DEHP after repeated exposure in both rats and humans

- finally, were the Koch et al. (2005) study with one volunteer to be considered to be more reliable than the findings from both the Anderson et al. and Kessler et al. studies (a highly questioned argument due to the fact that the Koch estimates relates to a single individual only), then when converting urinary metabolite levels into intake doses it would be appropriate to use the higher UEF value of 0.688 (compared to UEF = 0.517, based on Anderson et al., 2011, see 9.0.4); this would have the consequence that all exposure levels derived from biomonitoring studies (for both workers and general population) would be lower by a factor of 0.688/0.517 = 1.33 which represents the same difference as arises from use of an absorption of 100 rather than 75% (1.33). Hence whilst the overall outcome would be similar, the underlying scientific argument would be less robust.

A.5.4 Numerical consequences for RCRs when using RAC DNELs

The assessment performed in this CSR is considered to be conservative in various ways:

- a conservative starting point was used for the assessment, using an animal model, the rat, for which there is some evidence that this species is more susceptible than primates including humans to the effects of DEHP
- conversion of urinary metabolite concentrations into external doses was done in a conservative manner
- mean and upper percentile values of urinary metabolites are used for exposure assessment, although it is generally acknowledged that means of biomonitoring studies are better indicators of long-term exposure than upper percentile values of metabolite concentrations measured on a few days for workers, the presence of pregnant female workers at the workplace has been assumed, although this is unlikely to be the case given that DEHP's reproductive toxicity is assumed to affect the later gestational stages and European workers legislation prohibits exposure of pregnant women to classified reprotoxic substances
- importantly since all exposure assessments are based on biomonitoring data that implicitly include all background contributions such as may arise from exposure from food, which at least partly comes from DEHP use in food contact material, which is not subject to authorisation, is already covered by EU law and therefore should be subtracted.

Based the arguments given above we feel that the DNELs and exposure estimates adopted in this CSR are highly conservative in nature and that further lowering of the DNEL values (as proposed in the recent non-legally binding DEHP reference DNELs publication) would be considered excessive, and difficult to justify scientifically, and using such an approach could result in the introduction of extreme levels of conservatism rather than objective scientific assessment. Nevertheless, in order to illustrate that even were these extreme assumptions to be used, then the resultant RCRs would still fall below 1; to this end, table 178 ## presents RCRs for worker and general population groups derived based on the DNELs adopted in the recent trial exercise.

Workers

Table 128 Workers RCRs recalculated with DNELs as proposed by RAC

Manufacture	**Geometric mean**	**90th percentile**
Formulation / processing		
Intake based on urinary DEHP metabolites [µg/kg bw/d]	23	92
DNEL used in this CSR [µg/kg bw/d]	230	230
resulting RCR [µg/kg bw/d]	**0.1**	**0.4**
RAC DNEL [µg/kg bw/d]	94	94
resulting RCR with RAC DNEL [µg/kg bw/d]	**0.24**	**0.98**

The alternative recalculation of RCRs for workers show that even under these overly conserv tive assumptions, adequate control is still demonstrated.

Service life exposure in the professional scenario is much lower and is not further con idered here.

General population - Assessment based on biomonitoring data

Table 129 RCRs for the general population based on biomonitoring d ta, r cal ulated with DNELs as proposed by RAC

	Children	**Adults**
Intake based on GM of sum of urinary DEHP metabolites		
Intake[µg/kg bw/d]	3.57	2.81
DNEL used in this CSR [µg/kg bw/d]	36	48
RCR based on GM intake	**0.099**	**0.059**
RAC DNEL [µg/kg bw/d]	34	34
resulting RCR with RAC DNEL [µg/kg bw/d]	**0.105**	**0.083**
Intake based on 90th percentile of urinary DEHP metabolites		
Intake[µg/kg bw/d]	10.29	8.75
DNEL used in this CSR [µg/ g bw/d]	36	48
RCR based on GM intake	**0.29**	**0.18**
RAC DNEL [µg/kg bw/d]	34	34
resulting RCR with RAC DNEL [µg/kg bw/d]	**0.30**	**0.26**

Apart from a slight increase in RCRs for adults, the outcomes remain virtually unchanged, when using the DNELs proposed by RAC for the assessment, with the highest RCR for the general population (children, 90th perc ntile) rising from 0.29 to only 0.3 providing a high level of confidence regarding the extent of risk posed.

General population – supportive assessment of exposure from articles by modelling

As outlined in chapter 9.0, exposure from food is the single most important source of exposure of the general population for DEHP, accounting for more than 90% of total exposure. Nevertheless, to address any unlikely situation, exposure from articles was assessed by modelling, using conservative assumptions and simultaneous exposure to sentinel articles from 4 different article groups and, in addition, exposure from inhalation and house dust ingestion (for more details see chapter 9.0 and Annex #1#). The comparison between the biomonitoring results and the modelled exposure from articles, taking into account that in fact most of DEHP is coming from food, clearly demonstrates the very conservative nature of the exposure modelling.

The RCRs resulting from this modelling exercise are also recalculated using the DNELs proposed by RAC. For sake of clarity, only the total RCRs, not the pathway specific RCRs obtained with the DNELs used in this CSR are included in the following table (to compare pathway specific RCRs see Table 150 # in chapter 10 3.)

Table 130 RCRs for the general population based on modelling of exposure from articles, recalculated with DNELs as proposed by RAC*

Pathway	X-1 – X-4	IND**	SUM
Adults			
Inhalation exposure [µg/m3]		2.6	2.6
RAC DNEL [µg/m3]		160	160
RCR		0.016	0.016
Dermal exposure [µg/kg bw/d]	79.0		79.0
RAC DNEL [µg/kg bw/d]	672		672
RCR	0.12		0 12
Oral exposure [µg/kg bw/d]		0.771	0 771
DNEL [µg/kg bw/d]		34	34
RCR		0.023	0.023
Total RCR based on RAC DNELs	**0.12**	**0.039**	**0.16**
Total RCR as given in chapter 10	**0.11**	**0.031**	**0.14**
Children, 6-12 months			
Inhalation exposure [µg/m3]		2.6	2.6
RAC DNEL [µg/m3]		120	120
RCR		0.022	0.022
Dermal exposure [µg/kg bw/d]	50.0		50.0
RAC DNEL [µg/kg bw/d]	672		672
RCR	0.074		0.074
Oral exposure [µg/kg bw/d]	3.27	5.87	9.18
RAC DNEL [µg/kg bw/d]	34	34	34
RCR	0.096	0.17	0.27
Total RCR based on RAC DNEL	**0.17**	**0.19**	**0.37**
Total RCR as given in chapter 10	**0.16**	**0.18**	**0.34**
Children, 2-3 years			
Inhalation exposur [µg/m3]		2.6	2.6
RAC DNEL [µ /m3]		120	120
RCR		0.022	0.022
Dermal exposure [µg/kg bw/d]	69		69
RAC DNEL [µg/kg bw/d]	672		672
RCR	0.10		0.10
Oral exposure [µg/kg bw/d]	1.31	7.83	9.15
RAC DNEL [µg/kg bw/d]	34	34	34
RCR	0.039	0.23	0.27
Total RCR based on RAC DNELs	**0.14**	**0.25**	**0.39**
Total RCR as given in chapter 10	**0.13**	**0.24**	**0.37**

* All interim calculation results were rounded to three significant figures and RCRs are given with two significant figures (slight differences due to rounding).

* Exposure from DEHP in house dust and air in the indoor environment, for details see chapter 10.

Resulting RCRs adopting the reference DNELs are only very modestly higher, rise by 0.02-0.03 for each age group. These differences are far lower than the general level for uncertainty associated with such a modelling exercise. Overall, even under the conservative assumptions, adequate control of exposure from articles is demonstrated.