SUBSTANCE EVALUATION REPORT

Public Name: **Diethyl phthalate (DEP)**

EC Number(s): **201-550-6**

CAS Number(s): **84-66-2**

Submitting Member State Competent Authority:

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Year of evaluation (as given in the CoRAP): 2014

VERSION NUMBER: 0.1 **DATE: 23 September 2015**

Conclusions of the most recent evaluation step*	Tick relevant box(es)
Concern not clarified; Need to request further information from the Registrant(s) with the draft decision	
Concern clarified; No need of further risk management measures	X
Concern clarified; Need for risk management measures; RMO analysis to be performed	
Other: [*please specify*]	

DISCLAIMER

The Substance evaluation report has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Executive summary

Grounds for concern

- Suspected CMR
- Suspected endocrine disruptor
- Consumer use (Wide dispersive use)

Procedure

Human Health

The evaluation of the toxicity of Diethyl phthalate (DEP) has been based on the registration dossiers as well as on reviews by a variety of international bodies/regulatory programs and original publications. Data available up to June 2014 for all endpoints have been assessed. The potential for DEP to act as an endocrine disruptor and the non-classification of DEP as provided by the lead registrant was reviewed.

Consumer Exposure

The Substance Evaluation is targeted towards consumers. DEP is neither considered as harmonised classified according Regulation (EC) No 1272/2008 (CLP), nor is it self-classified by the registrants. According to Article 14(4) of the REACH Regulation, exposure assessment (including the generation of exposure scenarios and exposure estimations) and risk characterization have to be provided by the registrants when they concluded that the substance fulfils the criteria for specific classes or categories set out in Annex I of the Regulation (EC) No 1272/2008. The registrants have determined that DEP did not fulfil these criteria. The exposure is therefore not recorded in their Registration dossiers. However, to identify possible consumer risks, public product information and national monitoring data were collected and evaluated with regard to potential consumer exposure. There are similarities between exposure situations of identified consumer uses under REACH and cosmetic products. Therefore product information as well as evaluation reports of cosmetics were considered.

Conclusions

Human Health

Evaluation of the existing information on the toxicity of DEP does not confirm the disruption of endocrine functions. Furthermore, following the requirements set down in Annex I of Regulation (EC) No 1272/2008 (CLP) and the data available, DEP does not appear to fulfil the criteria for classification.

Consumer Exposure

The assessed data do not indicate a concern based on consumer exposure to DEP via consumer uses.

Statement of reasons

Human Health

The existing information on DEP is sufficient to conclude that classification of DEP is not justified. In agreement with the CLP Regulation the changes in one out of eleven sperm parameters alone as seen in animals were not considered to warrant classification for fertility effects. According to 3.7.2.3.3 of Annex I, CLP Regulation effects of low or minimal toxicological significance (including small changes in semen parameters) should not lead to classification. The developmental findings such as reduced pup weight at weaning and reduced litter size occurred at doses above the limit dose of 1000 mg/kg bw/day. According to and 3.7.2.5.8 and 3.7.2.5.9, Annex I of the CLP Regulation, effects at such high doses would normally not lead to classification unless expected human response indicate the need for a higher dose level.

Finally, the low molecular weight phthalate DEP and the shorter side chain (C2) do not support that DEP could act as a potent testicular toxin and could induce developmental changes in the male reproductive system as observed after prenatal exposure to mid molecular weight (so-called ‘transitional’) phthalates with critical lengths of carbon side chains (C4-C6).

Overall, by means of a weight of evidence approach the eMSCA considers the effects observed on male fertility and the observed developmental effects as not sufficient for classification as Repr. 2 according to Annex I, Part 3 of Regulation (EC) No 1272/2008 (CLP).

The existing information on DEP is sufficient to conclude that DEP does not exhibit endocrine disrupting effects similar to those observed with other phthalate diesters. Predominantly negative results on the oestrogenic or anti-androgenic potency of DEP are reported and an endocrine disrupting mechanism cannot be attributed to the DEP effects on the male reproductive system.

Consumer Exposure

DEP is mainly used as a carrier for fragrances and therefore common as an ingredient in scented mixtures and articles, such as air care products or washing and cleaning products. Although DEP is in widespread use, the literature survey generally indicates only small concentrations of DEP, usually beneath 3 %. The results of exposure assessments derived from cosmetics can be adopted to those of consumers uses under REACH.

CONTENTS

TABLES

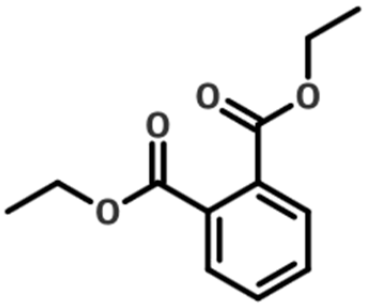
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

Public Name:	Diethyl phthalate
EC number:	201-550-6
EC name:	Diethyl phthalate
CAS number (in the EC inventory):	84-66-2
CAS number:	84-66-2
CAS name:	1,2-Benzenedicarboxylic acid, 1,2-diethyl ester
IUPAC name:	Diethyl benzene-1,2-dicarboxylate
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{12}H_{14}O_4$
Molecular weight range:	222.24 g/mol
Synonyms:	Diethyl 1,2-benzenedicarboxylate Phthalic acid, diethyl ester DEP

Structural formula:



1.2 Composition of the substance

Name: Diethyl phthalate

Description: mono-constituent substance

Degree of purity: confidential

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
Diethyl phthalate 201-550-6	confidential	confidential	For further information, please refer to the confidential Annex or rather IUCLID File.

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks
confidential	confidential	confidential	For further information, please refer to the confidential Annex or rather IUCLID File.

Table 4: Additives

Additives	Typical concentration	Concentration range	Remarks
confidential	confidential	confidential	For further information, please refer to the confidential Annex or rather IUCLID File.

1.3 Physico-chemical properties

Table 5: Overview of physicochemical properties1

Property	Value	Remarks
Physical state at 20°C and 101.3 kPa	organic, colourless to pale yellow liquid with faint odour	Handbook data/Experimental data (Test Guideline: EPA OPPTS 830.6303 (Physical State), EPA OPPTS 830.6302 (Colour), EPA OPPTS 830.6304 (Odour))
Melting/freezing point	-60 °C	Experimental data (Test Guideline: ASTM D 97-02)
Boiling point	297.3 °C	Experimental data (Test Guideline: EU Method A.2 (Boiling Temperature); OECD Guideline 103 (Boiling point/boiling range); EPA OPPTS 830.7220 (Boiling Point / Boiling Range); ASTM E 537-07)
Density	1.1181 g/cm^3 (20°C)	Experimental data (Test Guideline: EU Method A.3 (Relative Density); OECD Guideline 109 (Density of Liquids and Solids); EPA OPPTS 830.7300 (Density / Relative Density / Bulk Density); ASTM D 4052-96)
Vapour pressure	0.28 Pa (25°C)	Experimental data (Test Guideline: EU Method A.4 (Vapour Pressure))
Surface tension	-	In accordance with column 2 of REACH Annex VII section 7.6. the study only needs to be conducted if - based on structure, surface activity is expected or can be predicted, or - surface activity is a desired property of the material. The chemical structure of diethyl phthalate does not suggest that it may possess surface activity. Furthermore, surface activity is not a desired property of the substance.
Water solubility	932 mg/L (20°C and pH 7.2)	Experimental data (Test Guideline: EU Method A.6 (Water Solubility); OECD Guideline 105 (Water Solubility); EPA OPPTS 830.7840 (Water Solubility))
Partition coefficient n-octanol/water (log value)	Log Pow = 2.2 (40 ± 1°C)	Experimental data (Test Guideline: EU Method A.8 (Partition Coefficient); OECD Guideline 117 (Partition Coefficient (n-octanol / water), HPLC Method); EPA OPPTS 830.7570 (Partition Coefficient, n-octanol / H_2O, Estimation by Liquid Chromatography))

Flash point	idem	idem
Flammability	idem	idem
Explosive properties	idem	idem
Self ignition temperature	idem	idem
Oxidising properties	idem	idem
Granulometry	-	In accordance with column 2 of REACH Annex VII section 7.14., the study does not need to be conducted if the substance is marketed or used in a non-solid or granular form. Diethyl phthalate is a liquid under ambient conditions. Therefore, granulometry is not applicable to diethyl phthalate.
Stability in organic solvents and identity of relevant degradation products	-	In accordance with column 1 of REACH Annex IX section 7.15., a study is only required if stability of the substance is considered to be critical. The stability of the substance diethyl phthalate in organic solvents is not considered to be critical.
Dissociation constant	-	The substance diethyl phthalate does not contain any functional groups that dissociate and therefore testing does not appear scientifically necessary.
Viscosity	Kinematic viscosity: 11.53 mm^2/s (20.0°C) 5.73 mm^2/s (40.0°C)	Experimental data (Test Guideline: ASTM D 445-06; OECD Test Guideline 114 (Viscosity of Liquids); EPA OPPTS 830.7100 (Viscosity))
Auto flammability	idem	idem
Reactivity towards container material	idem	idem
Thermal stability	idem	idem

2 MANUFACTURE AND USES

2.1 Quantities

Table 6: Aggregated tonnage (per year)

1 – 10 t	10 – 100 t	100 – 1000 t	1000- 10,000 t	10,000-50,000 t
-	-	-	**x**	-

2.2 Identified uses

2.2.1 Uses by consumers

DEP is used as a solvent and softener. It is mainly used as a carrier for fragrances and therefore common as an ingredient in scented mixtures and articles.

According to information provided on the dissemination website within "Chemical Substance Search" on 2014-10-21 by ECHA (aggregated registration dossiers), consumer uses of DEP have been identified within the following chemical product categories (PC):

- PC 3: Air care products
- PC 9a: Coatings and paints, thinners, paint removes
- PC 9b: Fillers, putties, plasters, modelling clay
- PC 21: Laboratory chemicals
- PC 28: Perfumes, fragrances
- PC 29: Pharmaceuticals
- PC 31: Polishes and wax blends
- PC 32: Polymer preparations and compounds
- PC 35: Washing and cleaning products (including solvent based products)
- PC 39: Cosmetics, personal care products

Furthermore it is registered as AC 13: Plastic articles.

According to Article 14(4) of the REACH Regulation, exposure assessment (including the generation of exposure scenarios and exposure estimations) and risk characterization have to be provided by the registrants when they concluded that the substance fulfils the criteria for specific classes or categories set out in Annex I of the Regulation (EC) No 1272/2008. The registrants have determined that DEP did not fulfil these criteria. The exposure is therefore not recorded in their Registration dossiers.

However, to identify possible consumer risks, public product information and national monitoring data were collected and evaluated with regard to potential consumer exposure. There are similarities between exposure situations of identified consumer uses under REACH and cosmetic products. Therefore product information as well as evaluation reports of cosmetics were considered.

The product information is derived mainly from the Federal Office of Consumer Protection and Food Safety (BVL)[2], national product databases, German monitoring programs, the SPIN database[3] and public available literature. Although some product categories are outside the scope of REACH, there were still considered to determine in general the concentration range of DEP in consumer products.

[2] written communication

[3] http://195.215.202.233/DotNetNuke/default.aspx

The BVL has provided 231 measured samples: 151 samples of toys and joke articles, 50 articles of daily use with skin contact, 28 food contact materials, and two samples of personal care products. The detection of DEP was positive in seven samples. But the maximal concentration does not exceed 0.03 %.

The German GIFAS Database[4] has listed DEP mainly as an ingredient in air care products, washing and cleaning agents, cosmetics, de-icers and biocides. In the majority of cases the concentration was below 1 %. A concentration above 5 % was only indicated in two single products.

The Swedish Commodity Guide[5] systematizes the general knowledge available on the typical composition of commodities and materials providing an overview of commodities and material used in Sweden. DEP is likely to occur in several commodity groups, but it can be assumed that the content is below 1 %.

The entries of 2012 (latest version) in the SPIN database indicate a "very probable exposure" for consumers with a "very wide range of applications" and a "probable use in article productions". The SPIN database has listed the following use categories: softeners, cleaning/washing agents, odour agents, cosmetics, adsorbents, paints, lacquers and varnishes, surface treatments, impregnation materials, pharmaceuticals.

The Bavarian Health and Food Safety Authority (LGL) has measured a total of 51 alcohol-water based cosmetics (e.g. after shave, eau de toilette, eau de perfume) coming from the German market in 2003 and 2006 (LGL, 2012). In both series DEP was present in 50 % of the samples in a concentration of 0.1 to 0.5 %. Also, levels of > 1 % were rarely found in either study series, values above 5 % did not occur.

Dodson et al. (2012) analyzed 213 consumer products in a range of cosmetics, personal care products, cleaners, sunscreens, and vinyl products coming from the U.S. market. The highest contents of DEP were detected in fragrance/perfumes (1.4 %) and car air fresheners (0.8 %). Cohen et al. (2007) measured 14 samples of air fresheners. DEP was not detectable in two of the samples. The highest content was 0.73 %.

Hubinger and Havery (2006) also analyzed 48 consumer cosmetics products of the U.S. market (body lotion, hairspray, deodorant, fragrance, skin lotion, hair gel, hair mousse, body wash, shampoo, hand cream, nail enamel). Once again the highest concentration was found in fragrances: 38,663 ppm (3.9 %).

Koniecki et al. (2011) determined DEP levels in cosmetic and personal care products obtained from the Canadian market. Overall 252 products including 98 baby care products were collected in 2007. Here too, the fragrances have the highest contents of DEP: 1679 µg/g as median and 25542 µg/g (2.6 %) as maximum. The concentrations of all other cosmetic categories are about 100-fold smaller.

Babich and Carlson (2014) recorded data on personal care products, household products, and environmental media (indoor/outdoor air, dust). The presence of DEP in indoor air or house dust gives an indication of available exposure sources in households. The highest DEP content was found in perfume/fragrance with 2.7 %. The DEP concentration of 8 aerosol air fresheners was between 1 and 1100 µg/g with a mean of 294 µg/g (0.3%). The mean (and the 95th percentile) of the environmental media come from several publications: 0.57 (1.4) µg/m³ for indoor air, 0.06 (0.16) µg/m³ for outdoor air, 8.5 (11.0) µg/g for dust.

In comparison to mixtures, the content of DEP in articles is much lower. Ionas et al. (2014) reported a maximum of 250 µg/g (0.025 %) of 50 measured toys. The Danish Environmental Protection Agency carried out a survey for "mapping of perfume in toys and children's articles" (Glensvig & Ports, 2006). In doing so, DEP was detected in one of the ten selected samples with a content of 310 mg/kg (0.031 %).

In conclusion, there is a good match between the registered identified consumer uses by the registrants and information coming from the national product data bases. It seems that small concentration of DEP already fulfills the function as carrier for fragrances. At the moment there is no evidence that concentrations above 3 % are common.

[4] Giftinformations- und Erfassungssystem GIFAS

[5] http://webapps.kemi.se/varuguiden/AmneVarugrupp.aspx

3 CLASSIFICATION AND LABELLING

3.1 Harmonised Classification in Annex VI of the CLP Regulation

Diethyl phthalate is not listed in Annex VI of the CLP Regulation.

3.2 Self classification

No self classification is proposed by the lead registrant.

Deviating self classification of diethyl phthalate as notified to the "Classification and Labelling Inventory" of ECHA comprises the following endpoints and hazard categories from 21 notifications:

Table 7: Classification and Labelling according to CLP criteria notified to the C&L Inventory

EC Name	Classification		Labelling	
	Hazard Category	**Hazard statement**	**Signal Words Hazard Pictogram Codes**	**Hazard statements**
diethyl phthalate	Acute Tox. 1	H311	Danger	H311: Toxic in contact with skin.
		H331	Warning	H331: Toxic if inhaled.
		H302		H302: Harmful if swallowed.
	STOT RE 2	H373	GHS02: Flame	H373: May cause damage to organs through prolonged or repeated exposure.
			GHS03: Flame over circle	
	Eye Irrit. 2	H319		H319: Causes serious eye irritation.
	Skin Irrit. 2	H315	GHS05: Corrosion	H315: Causes skin irritation.
	Skin Sens. 1	H317	GHS06: Skull and crossbones	H317: May cause an allergic skin reaction.
	STOT SE 3	H335	GHS07: Exclamation mark	H335: May cause respiratory irritation.
		H336	GHS08: Health hazard	H336: May cause drowsiness and dizziness
	Repr. 2	H361		H361: Suspected of damaging fertility or the unborn child.
	Aquatic	H400	GHS09: Environment	H400: Very toxic to aquatic life
	Chronic 1	H410		H410: Very toxic to aquatic life with long lasting effects.

Different notifications can be found in the inventory for DEP. The above listed notifications comprise all endpoints for which notifications have been made.

4 ENVIRONMENTAL FATE PROPERTIES

Not evaluated.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The toxicokinetics of DEP have been studied in experimental animals following oral and dermal exposure. No data are available for inhalation exposure. A limited number of studies have also examined the toxicokinetics of DEP in humans.

5.1.1 Non-human information

Table 8: Basic toxicokinetics according to registration dossier

Method	Results	Remarks	Reference
***in vivo* studies** Species: rats and mice Exposure route: oral gavage Doses/concentration: not stated Guidelines not specified Test material: ^{14}C-DEP	Oral absorption: Following oral administration of 14C-DEP to rats and mice (doses not stated), much of the radioactivity from the administered dose (90%) was excreted in the urine within 48 h, with the majority (82%) being eliminated during the first 24 h. Approximately 3% of the radioactivity was found in the faeces over the same period of time. Distribution: the radioactivity was widely distributed with the highest concentrations observed in kidney and liver, followed by blood, spleen and adipose tissue. Highest levels were noted within 20 minutes, followed by a rapid decrease to only trace amounts after 24 h. Metabolism: following oral dosing of rats and mice, MEP was the major urinary metabolite with phthalic acid as a minor secondary metabolite (Ioku et al., 1976; Api, 2001). Elimination& Excretion: DEP is rapidly eliminated and does not accumulate in tissues. The urine appears to be the major route of DEP excretion. Most (90%) of the oral dose administered to rats and mice was excreted in the urine within 48 h post-dosing, with the majority (82%)	RL 2 (with restrictions) Key study	Ioku T Mukaide A Kitanaka H Sakagami Y Kameevama T (1976)[6] Api AM (2000a)[7]

[6] Ioku T Mukaide A Kitanaka H Sakagami Y Kameevama T (1976). In vivo distribution of drugs. Labelled compounds. Yakuri To Chiryo 4 510-514

[7] Api AM (2000a). Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. Food and chemical toxicology 39: 97-108 2001

Species: rat Exposure route: stomach intubation Exposure regime: Single dose Doses/concentration: 100 mg/kg and 10 mg/kg Test material: ^{14}C-DEP	being eliminated during the first 24 h. Oral absorption: following administration of DEP (10 or 100 mg) by stomach intubation in rats, 85%-93% of the administered dose was excreted in the urine within 7 d. For both dose levels, approximately 78% of the administered dose was excreted in urine within 24 h as monoethyl phthalate (MEP) (~70%), phthalic acid (~9%) and parent compound (0.1%-0.4%). The overall results indicate that the oral absorption of DEP is extensive and rapid based on measurement of urinary and faecal excretion.		Kawano M (1980)[8] ;IPCS, 2003 Api AM (2000d)[7]
Dermal route Species: rat (Fischer 344) male Coverage (dermal absorption study): semiocclusive Exposure regime: 7 days Doses/concentration: 30 - 40 mg/kg bw , approximating to 5 - 8 mg/cm2 (157 umol/kg), giving a radiolabel dose of 40 microcuries/kg bw Test material: ^{14}C-DEP	Dermal absorption: Urine and faeces were collected every 24 hours, and the amount of [14C] excreted was taken as an index of the percutaneous absorption. 24% and 1% of the applied dose to rat skin was excreted in the urine and faeces respectively, within 24 h; the metabolites were not characterized. Distribution: after a single dose of DEP was applied to rat skin, very little radioactivity was found in the tissues after 7 d of exposure. The amounts of radioactivity in the adipose tissue, muscle, skin, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose.	RL 2 Key study	Elsisi et al., 1989; IPCS, 2003
Species: rabbit (female) Exposure route: dermal under occlusion Dose: not stated	Dermal absorption: 49% and 1% of the dose applied was excreted in the urine and faeces respectively, after 4 days; the metabolites were not characterized. Distribution: when 14C-DEP was	RL 2 Key study	RIFM, 1973; Api, 2001

[8] Kawano M (1980). Toxicological studies on phthalate esters 2: Metabolism accumulation and excretion of phthalate esters in rats. Japanese Journal for Hygiene 35: 693-701 1980

Test material: ^{14}C-DEP	applied to the female rabbit skin, very little radioactivity was found in tissues 4 d after exposure with the amounts as follows: liver (0.004% of dose), kidney (0.003% of dose) and blood (less than 1% of dose) (Api, 2001; RIFM, 1973*).		
Intraperitoneal route Species: rats Exposure route: ip Dose: not stated Test material: ^{14}C-DEP	Distribution: Following intraperitoneal (ip) injection of ^{14}C-DEP in pregnant rats on gestational day (GD) 5 or 10, radioactivity was detected in amniotic fluid, as well as in maternal, placental, and foetal tissues, indicating that the compound can pass through the placenta to the developing foetus. The half-life of the compound in foetal tissue was approximately 2.2 d .	RL 3 Supportive information	Singh et al., 1975*; IPCS, 2003
***in vitro* studies** Dermal absorption study: a flow through diffusion cell system was performed to evaluate differences in percutaneous absorption between rats and humans.Species: rat (Fischer 344) male/dorsal skin and human/breast skin Exposure conditions: occlusive (4 replicates) and non-occlusive (3 replicates) Duration: 72 hours Doses/concentration: Nominal doses: 0.5 uL applied over a 0.32 cm2 area(16.3–20.6 mg/cm2) Solubility of test substance in receptor fluid: No data Flow-through system: Yes - flow rate 1.5 mL/hour	The receptor fluid was collected for up to 72hours and analyzed for radioactivity by liquid scintillation spectrometry. Results showed that dermal absorption of 14C-DEP through male rat dorsal skin was approximately 35.9%, while average absorption in human breast skin in vitro was approximately 3.9% after 72 h under occlusive conditions. Metabolites identification in rat skin in vitro study: DEP was completely hydrolyzed and was not detected in the receptor fluid. DEP was mainly metabolized to monoethyl phthalate (~13.6 %) at 24h, with phthalic acid (~0.5 %) as a minor metabolite. Metabolism: hydrolysis to the monoester by skin was demonstrated in vitro for both rats and humans (Hotchkiss and Mint, 1994*; Api, 2001).	RL 2 Key studies	Hotchkis SAM Mint A (1994)[9]; IPCS, 2003 Api AM (2000b) [7]
	Another *in vitro* study performed under similar conditions (Scott et al, 1987) reported that the *in vitro*	Supportive information (Scott et al, 1987)	Scott et al, 1987; Scott et al,

[9] Hotchkis SAM Mint A (1994). Metabolism of phthalic acid during percutaneous absorption through rat and human skin in vitro. Journal of Investigative Dermatology, 102(4): 647, 1994

	absorption of DEP through rat skin was more than 30 times higher than through human skin with the steady state absorption rate of 413.7vs. 12.8 µg/cm2/h for rat and human skin respectively.		1989[10]
Species: mouse (ddN) male Exposure regime: Not specified Doses/concentration: Not specified	The major metabolic pathway of phthalate esters is the hydrolysis of diester to monoester. Hydrolytic activity for phthalate esters has been detected in pancreas, liver, kidney lung and the rat mucosa. The esterases or lipases involved have not been identified.	Supportive information	Kayano Y Watanabe K Matsunaga T Yamamoto I & Yoshimura H (1997)[11] Api AM (2000c)[7]
(Q)SAR method Test material: DEP		Supporting information	AIHA Exposure Assessment Strategies Committee (EASC) - Dermal Project (2011)[12], (2013h)[13]

[10] Scott RC et al, 1987 – In vitro absorption of some phthalate diesters through human and rat skins, Environmental Health Perspectives, 74:223-227; Scott RC et al, 1989 Errata, In vitro absorption of some phthalate diesters through human and rat skins, Environmental Health Perspectives, 79:323

[11] Kayano Y Watanabe K Matsunaga T Yamamoto I & Yoshimura H (1997). Involvement of novel mouse hepatic microsomal esterase, ES46.5k, in the hydrolysis of phthalate esters. Biological and Pharmaceutical Bulletin 20(7): 749-751, 1997

[12] AIHA Exposure Assessment Strategies Committee (EASC) - Dermal Project Team (DPT) in collaboration with Wil ten Berg (2011). IH SkinPerm v1.15. IH SkinPerm READ ME Help Manual - Tibaldi R. S., ten Berg W., Drolet D. - http: //www. aiha. org/insideaiha/volunteergroups/EASC/Projectteams/Pages/DermalProjectTeam. aspx. Owner company: American Industrial Hygiene Association, 3141 Fairview Park Drive, Suite 777, Falls Church, VA

[13] 2013h: skinPerm model; unpublished study record, confidential

5.1.2 Human information

Method	Results	Remarks	Reference
Dermal absorption study in human males N: 26 Coverage: non-occlusive Exposure duration: 1 week (5 d/week) Doses/concentration (nominal doses): 34 - 48 g of cream formulation applied over whole body surface, corresponding to 2 mg/cm2 of cream in accordance with FDA and COLIPA guidance. Test material: DEP concentration in cream formulation = 2% w/w, nominal DEP dose = 0.04 mg/cm2.	The purpose of this blind study was to investigate whether diethyl phthalate (DEP), dibutyl phthalate (DBP), and butyl paraben (BP) were systemically absorbed and influenced endogenous reproductive and thyroid hormone levels in humans after topical application. Twenty six healthy young male volunteers took part in a two week single-blinded study. Volunteers were assigned to daily whole-body topical application of 2 mg/cm2 basic cream formulation each without (first week) and with (second week) the three 2% (w/w) compounds, diethyl phthalate (DEP), dibutyl phthalate (DBP), and butyl paraben (BP). The concentrations of BP and the main phthalate metabolites monoethyl (MEP) and monobutyl phthalate (MBP) were measured in serum. Two hours after the first cream application containing approximately 800 mg DEP, serum concentrations of MEP peaked at 1000 µg/L (corresponding to 6.9 mg or ~10% of absorbed DEP) and decreased to 23 µg/L after 24 h just before the second application, but did not reach the baseline levels observed in the first week. Average daily recovery of DEP excreted in urine as MEP was 5.8%, ranging between 0.3%-13.9%, indicating large intra-individual variability	RL 2 (reliable with restrictions) Supporting evidence	Janjua NR, Mortensen GK, Andersson A-M, Skakkebaek NE and Wulf HC (2007)[14] Janjua NR, Frederiksen H, Skakkebaek NE, Wulf HC and Andersson A-M (2008)[15]

[14] Janjua NR, Mortensen GK, Andersson A-M, Skakkebaek NE and Wulf HC (2007). Systemic Uptake of Diethyl Phthalate, Dibutyl Phthalate, and Butyl Paraben Following Whole-Body Topical Application and Reproductive and Thyroid Hormone Levels in Humans. Environ. Sci. Technol. 41: 5564-5570, 2007

[15] Janjua NR, Frederiksen H, Skakkebaek NE, Wulf HC and Andersson A-M (2008). Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. International Journal of Andrology 31: 118-130, 2008

5.1.3 Summary and discussion on toxicokinetics

Absorption via the oral route

Available data indicate that the oral absorption of DEP is extensive and rapid based on measurement of urinary and faecal excretion. Following oral administration of ^{14}C-DEP to rats and mice (doses not stated), much of the radioactivity from the administered dose (90%) was excreted in the urine within 48 h, with the majority (82%) being eliminated during the first 24 h. Approximately 3% of the radioactivity was found in the faeces over the same period of time (Ioku et al., 1976*; Api, 2001).

Following administration of DEP (10 or 100 mg) by stomach intubation in rats, 85%-93% of the administered dose was excreted in the urine within 7 d as measured by gas chromatography - mass spectroscopy (Kawano, 1980*; IPCS, 2003). For both dose levels, approximately 78% of the administered dose was excreted in urine within 24 h as monoethyl phthalate (MEP) (~70%), phthalic acid (~9%) and parent compound (0.1%-0.4%).

No information is available concerning differences in absorption and bioavailability of orally administered DEP between adult and immature animals or between animals and humans. However the oral bioavailability of diethylhexyl phthalate (DEHP) appears to be higher in young rats (Sjöberg et al., 1985). The higher proportion of intestinal tissue in relation to body weight (Younoszai & Ranshaw, 1973), and the relatively higher blood flow through the gastro-intestinal (GI) tract (Varga & Csaky, 1976) have been suggested as the likely factors causing an increased absorption in young animals. Therefore, for the purposes of this assessment, bioavailability of DEP via the oral route is assumed to be 100% (children and adults).

Absorption via the dermal route

When ^{14}C-DEP was applied to male rat skin at 5-8 mg/cm^2 under occlusion, 24% and 1% of the applied dose was excreted in the urine and faeces respectively, within 24 h (Elsisi et al., 1989; IPCS, 2003). In a similar experiment where ^{14}C-DEP (dose not stated) was applied to female rabbit skin, around 49% and 1% of the dose was excreted in the urine and faeces respectively, after 4 d (RIFM, 1973*; Api, 2001). The metabolites were not characterised for these studies.

In an in vitro study, the comparative percutaneous absorption of DEP between human and rat skin was evaluated in flow-through diffusion cells. Results showed that dermal absorption of ^{14}C-DEP through male rat dorsal skin was approximately 35.9%, while average absorption in human breast skin in vitro was approximately 3.9% after 72 h under occlusive conditions (Mint et al., 1994*; IPCS, 2003). Scott et al. (1987; 1989 Errata) using a similar experimental system reported that the in vitro absorption of DEP through rat skin was more than 30 times higher than through human skin with the steady state absorption rate of 413.7 vs. 12.8 µg/cm^2/h for rat and human skin respectively.

In a 2-week single-blinded study, 26 healthy male Caucasians were given a whole body topical application (5 d/week) of 2 mg/cm^2 basic cream without (week 1–control week) and with (week 2) DEP, dibutyl phthalate (DBP), and butyl paraben at 2% w/w each. Two hours after the first cream application containing approximately 800 mg DEP, serum concentrations of MEP peaked at 1000 µg/L (corresponding to 6.9 mg or ~10% of absorbed DEP) and decreased to 23 µg/L after 24 h just before the second application, but did not reach the baseline levels observed in the first week. Average daily recovery of DEP excreted in urine as MEP was 5.8% (Janjua et al., 2007; 2008).

In conclusion, based on the use of urinary and faecal excretion as an index of absorption, DEP appears to be well absorbed via the skin with around 25% to 50% of administered doses excreted within 24 h and 4 d respectively in rats and rabbits. Recent human studies indicated a lower dermal absorption than that seen in rats, with approximately 10% and 5.8% of dermally applied DEP found in serum and urine, respectively within 24 h. The difference in dermal absorption between rats and humans may reflect species differences,

differences in vehicle (alcohol vs. skin cream), and/or differences in application (occlusive vs. non-occlusive) (Janjua et al., 2008). On a weight of evidence basis, a dermal bioavailability for DEP of 10% in humans is assumed for the purposes of this risk assessment.

Distribution

Following oral administration of ^{14}C-DEP (doses not stated) to rats and mice, the radioactivity was widely distributed with the highest concentrations observed in kidney and liver, followed by blood, spleen and adipose tissue. Little radioactivity was found in the tissues 7 days following application of a single dose of DEP to the skin of the male rat. The amounts of radioactivity in the adipose tissue, muscle, skin, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose. Highest levels were noted within 20 minutes, followed by a rapid decrease to only trace amounts after 24 h (Ioku et al., 1976; Api, 2001).

In female rabbits, when ^{14}C-DEP (dose not stated) was applied to the skin, very little radioactivity was found in tissues 4 d after exposure with the amounts as follows: liver (0.004% of dose), kidney (0.003% of dose) and blood (less than 1% of dose) (Api, 2001; RIFM, 1973*). When a single dose of DEP was applied to male rat skin, very little radioactivity was found in the tissues after 7 d of exposure. The amounts of radioactivity in the adipose tissue, muscle, skin, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose (Elsisi et al., 1989; IPCS, 2003).

Following intraperitoneal (ip) injection of ^{14}C-DEP in pregnant rats on gestational day (GD) 5 or 10, radioactivity was detected in amniotic fluid, as well as in maternal, placental, and foetal tissues, indicating that the compound can pass through the placenta to the developing foetus. The half-life of the compound in foetal tissue was approximately 2.2 d (Singh et al., 1975*; IPCS, 2003).

Ioku et. al[6] report that, following oral administration of ^{14}C-DEP to rats and mice, the radioactivity was widely distributed with the highest concentrations observed in kidney and liver, followed by blood, spleen and adipose tissue. Highest levels were noted within 20 minutes, followed by a rapid decrease to only trace amounts after 24 hours.
Little radioactivity was found in the tissues 7 days following application of a single dose of DEP to the skin of the male rat. The amounts of radioactivity in the adipose tissue, muscle, skin, brain, lung, liver, spleen, small intestine, kidney, testis, spinal cord and blood were each less than 0.5% of the dose[14].

Metabolism

Metabolism following oral administration of diethyl phthalate to rats was mainly as a result of hydrolysis, with the principal urinary metabolite being monoethyl phthalate and phthalic acid as a minor secondary urinary metabolite[16]. After systemic absorption hydrolysis to monoethyl phthalate, the monoester, can occur in the lumen of the gastrointestinal tract or in intestinal mucosal cells following oral administration

[16] Chambon, P., Riotte, M., Daudon, M., Chambon-Mougenot, R., Bringuier, J. Etude du métabolisme des phtalates de dibutyle et du diéthyle chez le rat. Comptes Rendus des Seances de L'Académie des Sciences 273: 2165-2168, 1971.

inaddition to the liver, kidney and lung [9] [11] [17] [18] [19]. Hydrolysis to the monoester by skin has also been demonstrated during *in-vitro* percutaneous absorption studies using rat and adult human skin[20] [21].
It has been demonstrated that human plasma-derived arylesterase did not hydrolyse diethyl phthalate[22] but the substance can be hydrolysed by purified carboxylesterase obtained from human liver and rat liver[23].
There is limited evidence for the induction of enzymes by diethyl phthalate. Preincubation in microsomal pellets and supernatant isolated from Sprague Dawley male rats treated with phenobarbital intraperitoneally for 3 days, had no effect on cytochrome P450 or on N-acetyl transferase activity in rat liver microsomal suspensions, but the activity of UDP glucuronyl transferase was reduced[24]. Increased activity of peroxisomal enzyme carnitine acetyl transferase has been observed in rat primary hepatocyte cultures in the presence of diethyl phthalate[25]

The mono-ester, MEP, was the major urinary metabolite identified following oral dosing of rats and mice [6]. Phthalic acid was identified as a minor secondary metabolite.
In another study, approximately 70% of the dose administered by stomach intubation in rats was excreted in urine within 24 hours as the mono-ester, MEP[11]. Hydrolysis of the di-ester to the monoester in the skin has also demonstrated *in vitro* for both rats and humans by Hotchkiss and Mint[9].

Excretion
In experimental animals, DEP is rapidly eliminated and does not accumulate in tissues. The urine appears to be the major route of DEP excretion.
Approximately 90% of the dose orally administered to rats and mice was excreted in the urine within 48 hours of dosing, with the majority (82%) being eliminated during the first 24 hours[7]. Administration by oral gavage to rats resulted in 85%-93% of the administered dose being excreted in the urine within 7 days[11]. Elsisi et al report that 24% and 1% of the administered ^{14}C-DEP dose was excreted in the urine and faeces respectively after 24 hours following application to the dorsal skin of rats[14].
In humans, following daily whole body dermal application of DEP over one treatment week, the mean recovery rate of DEP in the urine was 5.8% as the mono-ester, MEP, with an unconjugated (free) fraction of up to 78%. The majority of MEP was excreted within the first 8 hours of application to the skin.

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[17] Lake, B. G., Phillips, J. C., Hodgson, R. A., Severn, B. J., Gangolli, S. D., Lloyd, A. G. Studies on the hydrolysis in vitro of phthalate esters by hepatic and intestinal mucosal preparations from various species. Biochemical Society Transactions 4: 654-655, 1976.

[18] Lake, B. G., Phillips, J. C., Linnell, J. C., Gangolli, S. D. The in vitro hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. Toxicology and Applied Pharmacology 39: 239-248, 1977.

[19] Rowland, I. R., Cottrell, R. C., Phillips, J. C. Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. Food and Cosmetics Toxicology 15: 17-21, 1977.

[20] Hotchkiss, SA. M., Mint, A. Metabolism of phthalic acid esters during percutaneous absorption through rat and human skin in vitro. Journal of Investigative Dermatology 102: 647, 1994.

[21] Hotchkiss, S. A. M. Absorption of fragrance ingredients using in vitro models with human skin. In: Frosch, P. J., Johansen, J. D., White, I. R. (Eds.), Fragrances. Beneficial and Adverse Effects. Springer-Verlag, Berlin, pp. 125-135, 1998.

[22] Augustinsson, K-B., Ekedahl, G. On the specificity of arylesterases. Acta Chemica Scandinavica 16: 240-241, 1962.

[23] Mentlein, R., Butte, W. Hydrolysis of phthalate esters by purified rat and human liver carboxylesterases. Biochemical Pharmacology 38: 3126-3128, 1989.

[24] Gollamudi, R., Lawrence, W. H., Rao, R. H., Autian, J. Effects of phthalic acid esters on drug metabolizing enzymes of rat liver. Journal of Applied Toxicology 5: 368-371, 1985.

[25] Cray, T. J. B., Lake, B. C., Beamand, J. A., Foter, J. R., Peroxisomal effects of phthalate esters in primary cultures of rat hepatocytes. Toxicology 28: 167-179, 1983.

The recovery rates recorded daily were between 0.3%-13.9%, indicating large intra-individual variations[30].

5.2 Acute toxicity

5.2.1 Non-human information

5.2.1.1 Acute toxicity: oral

The results of studies on acute toxicity after oral administration are summarised in the table below.

Table 9: Overview of experimental studies on acute toxicity after oral administration according to registration dossier

Method	Results	Remarks	Reference
Species: rat (Wistar) similar to OECD Guideline 401 (Acute Oral Toxicity) Test material: Diethyl phthalate	LD50: > 5 mL/kg bw	RL 2 **key study**	Lewis CA (1978a)[26]
Species: rat No details reported Test material: Diethyl phthalate	LD50: 8.2 mL/kg bw.	RL 3 Supporting evidence	L G Krauskopf (1973)[27]
Species: rabbit No details reported Test material: Diethyl phthalate	LD50: 1000 mg/kg bw	RL 3 Supporting evidence	Patty FA editor (1963)[28] anon (1978a)[29]
Species: mouse No details reported Test material: Diethyl phthalate	LD50: 2500 mg/kg bw	RL 3 Supporting evidence	Patty FA Editor (1963)[28] 1978b: toxicity test, Mouse; unpublished study record, confidential

[26] Lewis CA (1978a). Oral LD50 Test in Rats of Diethyl Phthalate. US EPA. Testing laboratory: Consumer Product Testing Co Inc. Report no.: 78106-1 (78-032-02). Report date: 1978-03-31

[27] L G Krauskopf (1973). Studies on the toxicity of phthalates via ingestion. Environmental Health Perspectives, 3: 61-72, 1973

[28] Patty FA editor (1963). Industrial Hygeine and Toxicology 2nd revised edition Vol II 1963. Office of Toxic Substances. Testing laboratory: Unknown. Owner company: Unknown

[29] anon (1978a). Toxicity and Health hazard Summaty. Office of Toxic Substances. Testing laboratory: Unknown. Owner company: Unknown.

5.2.1.2 Acute toxicity: inhalation

The results of studies on acute toxicity after inhalation exposure are summarised in the following table.

Table 10: Overview of experimental studies on acute toxicity after inhalation exposure according to registration dossier

Method	Results	Remarks	Reference
Species: rat Test material: Diethyl phthalate	LC50 (6 h): ≥ 511 ppm LC50 (6 h): ≥ 4.64 mg/L air (nominal)	RL 2 **key study**	HSDB - http://toxnet.nlm.nih.gov/ (2008)[30] Confidental IUCLID information

5.2.1.3 Acute toxicity: dermal

The results of studies on acute toxicity after dermal administration are summarised in the following table.

Table 11: Overview of experimental studies on acute toxicity after dermal administration according to registration dossier

Method	Results	Remarks	Reference
Species: rat Coverage: occlusive Test material: Diethyl phthalate Hagan EC (1959) Acute toxicity in Appraisal of the Safety of chemicals in foods, drugs and cosmetics, pp 17-25	LD50: > 10 mL/kg bw	RL 2 **key study**	Lewis CA (1978b)[31]

5.2.1.4 Acute toxicity: other routes

The results of studies on acute toxicity (other routes) are summarised in the following table.

[30] HSDB - http: //toxnet. nlm. nih. gov/ (2008). EPA HPV Challenge Program's Robust Summaries and Test Plans. Available from the Database Query page at: http: //www. epa. gov/hpv/pubs/hpvrstp. htm on Phthalate Esters Category, Diethyl phthalate (84-66-2) p. 23 (2007) as of April 28, 2008 - peer reviewed

[31] Lewis CA (1978b). Dermal LD50 (Rat). NTIS. Testing laboratory: Consumer Product Testing Co. Inc. Report no.: 78106-2. Report date: 1978-03-31

Table 12: Overview of experimental studies on acute toxicity after intraperitoneal exposure according to registration dossier

Method	Results	Remarks	Reference
Species: mouse (ICR) male intraperitoneal Test material: Diethyl phthalate Groups of 10 male ICR mice received undiluted DEP via the intraperitoneal route and were then observed for 7 days. [A review paper presented more than 30 years ago with limited details of methodology and before the introduction of OECD guidelines & GLP]	LD50: 3.22 g/kg bw (male) LD50: 2.87 mL/kg bw (male)	RL 4 (not assignable)	Lawrence WH, Malik M, Turner JE, Singh AR and Autian J (1975a)[32]

5.2.2 Human information

This information is not available from the registration dossiers.

5.2.3 Summary and discussion of acute toxicity

Acute toxicity following administration of a single oral dose has been investigated in the rat. The LD50 was found to be in excess of 5 mL/kg body weight (5591 mg/kg when corrected for density).

A poorly documented study by the inhalation route indicates no mortality to have occurred when rats were exposed to a saturated vapour of 511 ppm (4.64 mg/L) by the inhalation route for 6 hours.

Acute toxicity following administration of a single dermal dose over a 24 hour period has been investigated in the rat. The LD50 was determined to be in excess of 10 mL/kg body weight (11181 mg/kg when corrected for density).Acute oral toxicity: LD50: 5591 mg/kg bw

Acute dermal toxicity: LD50: 11181 mg/kg bw

Acute inhalation toxicity: LC50 (6 h): ≥ 4.64 mg/L air (nominal)

In conclusion, DEP has been investigated for acute toxicant properties by the oral, dermal and inhalation routes of exposure. The outcome of the studies does not indicate a justification for classification according to the criteria of Regulation (EC) n.º 1272/2008.

[32] Lawrence WH, Malik M, Turner JE, Singh AR and Autian J (1975c). A toxicological Investigation of some acute, short-term and chronic effects of administering Di 2 Ethylhexyl phthalate (DEHP) and other phthalate esters. Environmental Research Vol 9 pp 1-11, 1975. Testing laboratory: N/A review presented at the Society of Toxicology's 13th annual meeting Washington DC USA 1974

5.3 Irritation

5.3.1 Skin

Table 13: Overview of experimental studies on skin irritation as reported in the**registration dossier**

Method/ Guideline	Species, Strain, Sex, No/group	Average score 24, 48, 72 h		Reversibility yes/no	Results	Remarks	Reference
		Erythema	Oedema				
Skin Irritation Test FHSA test methods occlusive	Rabbit albino 3 animals Strain, Sex not reported	0 throughout for all 3 rabbits		not applicable (observation period: 72 hours)	exposure period 24 h: No response on intact or abraded skin	2 (reliable with restrictions) key study 0.5 mL DEP unchanged (no vehicle)	1978: Unpublished study record, confidential
Skin irritation / corrosion Designed to comply with OECD TG 404 (Acute Dermal Irritation / Corrosion)	Rabbit 3 animals Strain, Sex not reported	primary dermal irritation index (PDII) ∅ 0.17 per animal calculated from the sum of erythema grades at 24/48/72h + the sum of oedema grades at 24/48/72h The maximum possible PII was 8.		As mean value no maximum score or reversibility indicated.	not irritating	3 (not reliable) weight of evidence 0.5 mL DEP undiluted	Bagley DM Gardener JR Holland G Lewis RW Regneri J-F Stringer DA & Walker AP Toxicology in vitro 10: 1-6, 1996

5.3.2 Eye

Table 14: Overview of experimental studies on eye irritation as reported in the**registration dossier**

Method/ Guideline	**Species, Strain, Sex, No/group**	**Average Score 24 – 72 h**		**Reversibility**	**Results**	**Remarks**	**Reference**
		Cornea, Iris, **Chemosis**	Redness **Conjunctiva**				
Acute eye irritation FHSA test methods equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion)	Rabbit albino 3 animals right eye with no further treatment untreated left eye as its own control	24 – 72- h: animal #1: 1.7 of max. 2 animal #2: 1.3 of max. 2 animal #3: 1.7 of max. 2 ∅ 1.6 Draize scale	24 – 72- h: animal #1: 2.3 of max. 3 animal #2: 2 of max. 2 animal #3: 2.3 of max. 3 ∅ 2.2 Draize scale	Chemosis 2 fully reversible within: 4 d, animal #1: not fully reversible within: 7 d Conjunctiva all not fully reversible within: 7 d	severe conjunctival **irritation** in all 3 animals day 7: slight hyper-aemia in conjunctivae	2 (reliable with restric-tions) WoE single instillation: 0.1 mL: 12.5% DEP in 95% **ethanol**	; 1963: OECD 405; Unpublished study record, confidential
Eye irritation Review paper Details on methods / results not available	Rabbit: Strain, Sex, Number not reported	0.5 – 48 h: 0	0.5 – 48 h: 0	no grossly obervable irritation seen	Basis not reported	3 (not reliable) weight of evidence DEP unchanged (no vehicle)	Lawrence WH, Malik M, Turner JE, Singh AR Autian J, Env Res9: 1-11, 1975

Table 15: Overview of experimental studies on eye irritation according to original publications and reviews (such only partly reported in registration dossiers as well as additional publications)

Method/ Guideline	**Species, Strain, Sex, No/group**	**Average Score 24 – 72 h**		**Rever-sibility**	**Results**	**Remarks**	**Re-ference**
		Cornea, Iris, **Chemosis**	Redness **Conjunctiva**				
Eye irritation Details on methods/ results not available	Rabbit: 6 animals Strain, Sex not reported, untreated eye as a control	Average eye injury: 1 h: 3.2 mean value for 6 animals 24 h: 1.5 score for lesions Draize scale		not fully reversible within: 24 h	Irritation after 1 h decreased signifi-cantly by 24 hours	3 (not reliable) weight of evidence DEP unchanged (no vehicle)	Draize JH et al. 1944
Primary Eye irritation	Rabbit: Albino	No data	slight redness of the conjunctivae	not persistent	minimal irritation	no robust study summary	RIFM 1978

Method/ Guideline	**Species, Strain, Sex, No/group**	**Average Score 24 – 72 h**		**Reversibility**	**Results**	**Remarks**	**Reference**
		Cornea, Iris, **Chemosis**	Redness **Conjunctiva**				
with or without washing						available 0.1 mL DEP undiluted	
Eye irritation unwashed eyes	Rabbit: New Zealand	No data	No data	Practically non-irritating in washed eye	minimal irritation	0.1 mL DEP	ATSDR 1995

5.3.3 Respiratory tract

No specific studies are available from the registration dossiers.

5.3.4 Summary and discussion of irritation

The evidence for skin and eye irritation of DEP was obtained from animal testing. No standard guideline tests for skin or eye irritation in rabbits were submitted for DEP. However, by means of a weight of evidence approach the information provided in the registration dossiers is sufficient to conclude that the irritation potential of DEP is very low. This is supported by reviews of international bodies/regulatory programs (ATSDR 1995, WHO 2003, NTP 2006, NICNAS 2011, HSDB 2015). No signs of irritation were noted in the skin irritation studies with undiluted DEP and only slight irritation of rabbit eyes was reported in eye irritation studies with pure DEP.

In a primary skin irritation study according to FHSA test methods (Exp. Study skin irritation,1978) which was designed to comply with OECD TG 404 1981 or 1992 no significant irritating properties were reported. DEP was not irritating even to the abraded skin of the albino rabbit, although exposure was for a 24 hour period compared to 4 hours in current OECD/EU test methods.

Study results(Exp. Study eye irritation, 1963) seem to justify classification as eye irritant category 2 because the CLP criteria are met (at least 2/3 animals tested showed conjunctival redness mean score ≥2 which was not fully reversible after 7 days). Although conducted similar to OECD Guideline 405 (Acute Eye Irritation/Corrosion) the study is not appropriate for evaluation of the eye irritating potential of DEP as ethanol is the main component (> 80 %) of the test solution used which itself causes eye irritation. All other non-reliable (Table 14) studies are not in conformity with actual test guidelines or GLP and describe only minimal eye irritation with undiluted DEP.

Based on the available data, classification of DEP as skin or eye irritant is not warranted under Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP).

5.4 Corrosivity

No signs of corrosion were seen in the skin/eye irritation tests.

5.5 Sensitisation

5.5.1 Skin

The results of studies on skin sensitisation are summarised in the following table.

Table 16: Overview of experimental studies on skin sensitisation according to registration dossier

Method	Results	Remarks	Reference
Species: mouse (CBA/Ca) female LLNA 25 µL of 25%-100% DEP in acetone-olive oil OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) Test material: Diethyl phthalate	not sensitising Stimulation index: AOO =1; 25% v/v = 1.0; 50% V/V = 1.3; 100% V/V = 1.5	RL 2 Supporting evidence	Ryan CA Gerberick GF Cruse LW Basketter DA Lea L Blaikie L Dearman (2000)[33]
Species: guinea pig male Buehler test Induction: epicutaneous, occlusive Challenge: epicutaneous, occlusive Vehicle: water Test material: Diethyl phthalate similar to OECD Guideline 406 (Skin Sensitisation)	not sensitising No. with positive reactions: 1st reading: 0 out of 11 (test group); 0 h after chall.; dose: 50% w/v aqueous solution 2nd reading: 0 out of 11 (test group); 24 h after chall.; dose: 50% w/v aqueous solution	RL 2 **key study**	Lewis CA (1978c)
Species: guinea pig (Himalayan white spotted guinea pigs) male/female Results of open epicutaneous test compared with results of the Draize (DT), Maximisation (MT) and Freund's complete aduvant (FCA) test Induction: epicutaneous, open Challenge: epicutaneous, open Vehicle: acetone, water & ethanol reported Test material: Diethyl phthalate Based on results from Magnusson & Kligman test DEP was tested in an OET, DT and MT concurrently. To confirm an OET could reliably detect skin irritation and contact hypersensitivity in guinea pigs. A dose response curve was plotted and the irritant and/or allergenic activity was	not sensitising No. with positive reactions: OET 1st reading: 0 out of 6 (test group); 24 h after chall.; dose: 100% OET 2nd reading: 0 out of 6 (test group); 48 h after chall.; dose: 100% OET 3rd reading: 0 out of 6 (test group); 72 h after chall.; dose: 100% MT: Allergenicity in guinea pigs: 0 out of 6 (test group); 48 h after chall.; dose: 5% FCAT: Allergenicity in guinea pigs: 0 out of 6 (test group); 48 h after chall.; dose: 100%	RL 2 Supporting evidence	Klecak G Geleick H & Frey JR (1977)[34]

[33] Ryan CA Gerberick GF Cruse LW Basketter DA Lea L Blaikie L Dearman RJ Warbrick EV Kimber I (2000). Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 43: 95-102, 2000

[34] Klecak G Geleick H & Frey JR (1977). Screening of fragrance materials for allergenicity in the guinea pig 1 Comparison of four testing methods. J Soc Cosmet Chem 28: 53-64, 1977

calculated as % concentration.			

5.5.2 Respiratory system

This information is not available from the registration dossiers.

5.5.3 Summary and discussion on sensitisation

Skin sensitisation

The skin sensitising potential of DEP has been investigated using a number of standardised guinea pig test methods and a local lymph node assay (mouse). Data for respiratory sensitisation are not available.

One Buehler study (Lewis CA (1978c) with DEP 50% w/v aqueous solution didn't show any skin sensitisation effect. Also, no dermal sensitisation responses were observed with undiluted DEP in an open epicutaneous test, the Draize intradermal test and the Freund's complete adjuvant test (Klecak et al., 1977).

In a local lymph node assay, DEP (25 µL of 25%-100% DEP in acetone-olive oil) did not induce significant increases in thymidine incorporation into lymph nodes (Ryan et al., 2000*; IPCS, 2003).

Overall, data indicate that DEP is not a skin sensitiser. Classification is therefore not warranted.

Respiratory sensitisation

There are no alerts for respiratory sensitisations based on the information available from a number of dermal sensitisation assays.

5.6 Repeated dose toxicity

5.6.1 Non-human information

5.6.1.1 Repeated dose toxicity: oral

Table 17: Overview of experimental studies on repeated dose toxicity after **oral exposure** as reported in the **registration dossier**

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
Sub-chronic screen-ing test assess-ment of the toxicity of di-iso-butyl	oral: feed, 4 months	rat M/F	0 (con-trol), 0.1, 1.0 & 5 % (nomi-nal in diet)	no NOAEL identi-fied	0.1 % in the diet (M/F)	↓ in wt gain, the effect was dose related and was at least the same or more marked when a dietary level of 1 % was administered,	3 (not reliable) weight of evidence Test ma-terial: no data on details Common name: diethyl	1954a: Exp. Study repeated dose toxicity,co nfidential

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
phthalate using diethyl phthalate for com-parison						the effect at 5% was very marked, mortality was similar in all groups	phthalate mortality, bw only in weeks 1-8 re-ported	
Sub-chronic screen-ing test assess-ment of the toxicity of di-isobutyl phthalate using diethyl phthalate for com-parison	oral in capsule 2 months	dog	5 %, 1 % & 2 % DEP (dog 1) dog 2: 0.1 % DEP /kg by capsule (conc. of 5 & 1 % incor-porated into the diet) then due to contin-ued inappe-tance dog 1 was given 2 %/d by capsule a 2nd dog re-ceived 0.1 % /kg by cap-sule for 8 w	0.1 %/ kg by capsule	2 %/ kg	Initially the dose administered was 5 % /kg and reduced to 1 %/kg DEP in the diet. At both levels dog 1 refused to eat diet. Subse-quently given 2 % /kg daily by capsule which resulted in a loss of ap-prox. 1 kg in body weight in approxi-mately 8 weeks. The second dog received 0.1 % /kg for 6 weeks with no loss in body weight	4 (not assign-able) weight of evidence experi-mental result Test ma-terial no data on details Common name: diethyl phthalate	1954b: Exp. Study repeated dose toxicity,co nfidential

Table 18: Overview of experimental studies on repeated dose toxicity after **oral exposure** according to **original publications** (such only partly reported in registration dossiers as well as additional publications)

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
Sub-chronic study not de-signed to meet a particu-lar guide-line	oral, feed 16 weeks additional groups for 2 and for 6 weeks	**rat**, Sprague-Dawley 5 M / 5 F 2 + 6 w expo-sures 15 M / 15 F 16 w exposure 6 M and 6 F for pair feeding study	Nomi-nal in diet: 0 (con-trol), 0.2, 1.0 and 5.0 % for stan-dard study 0, 5 % pair feed-ing study	150 mg/kg bw/d (0.2 % in diet)	750 mg/kg bw/d (1.0 % in diet)	16 weeks: ↓ bw F 1 %, M/F 5 % ↓ overall food consumption F 1 %, M/F 5 % necropsy: abs. wts of brain, heart, spleen and kidneys statistically significantly lower than controls in M/F 5%	2 (reliable with restric-tions) key study Test material: DEP (99%)	1978: Exp. Study repeated dose toxicity, confidential
Testi-cular toxicity after repeat admini-stration	oral, feed 1 week	Rat (Wistar) male n = 10 /group	0, 2 % in diet		2000 mg/kg/ d	↑ liver weight (12 %), **no** changes in body wt, kidney wt or testis wt	mean DEP-intake M: 2000 mg/ kg/d	Oishi & Hiraga, 1980
Hepatic effects and hepatic peroxi-some prolife-ration and peroxiso mal en-zymes after repeat admini-stration	oral, feed 3 weeks	Rat (F-344) male control n=13 treated n=4	0 and 2 % in diet, mean DEP-intake M: 2000 mg/ kg/d		2000 mg/kg/ d	↑ liver wt (15 %) marginally ↑ hepatic catalase activity and carnitin acetyltransfe-rase activity significantly ↓ serum triglyceride with no change in serum cho-lesterol	authors concluded on marginal response for induction of hepatic peroxi-somes	Moody & Reddy, 1978, 1982
Induc-tion of microso-mal levels of laurate hydro-xylase	oral, gavage 3 days	Rat, Sprague Dawley male n=5 /group	1200 mg/ kg/d			compared to controls: 1.6 fold ↑ in laurate hydroxylase activity 1.3 fold ↑ in peroxisomal	marginal response (signify-cantly less potent, when compared	Okita & Okita, 1992

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
(marker for per-oxisome prolifer-ation) after repeat admin-istration						palmityl-CoA oxidation	to DEHP)	
Com-parative toxicolo-gical evalua-tion after repeat admin-istration accord-ing to OECD TG 407	oral, gavage 4 weeks	Rat, Sprague Dawley male n=5 /group	500 mg/ kg /d	500 mg/kg /d		**no** change in body wt, **no** changes in relative or-gan wts (thymus, heart, spleen, liver, kidney, adrenal, testis epidi-dymidis) **no** changes in hematology, serum bio-chemistry (except CA), urinalysis		Kwack et al., 2009
Sub-acute oral toxicity Guide-line ac-cording study (OECD TG 407, en-hanced)	Oral, gavage Prelimi-nary study: 14 days full study: 28 days (at least)	Rat, Sprague Dawley 10 M / 10 F n=10/sex /group	0, 40, 200 and 1000 mg/ kg/d vehicle con-trol: corn oil	200 mg/kg	1000 mg/kg/ d	terminal bw ↓ in M of the 1000-mg group (↑ fre-quency of urination), slight ↑ in kidney wt in F of the 40-mg and 1000-mg group, and slight ↑ in adrenal wt in F of the 1000-mg group without any histopatho-logical changes in these organs, **no** changes in liver wt or	as an en-hance-ment hormone analysis, spermato-logy and oestros cycling were in-cluded, **no** indi-cations for endo-crine-mediated properties detected within this test design	Shiraishi et al., 2006

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
						liver histo-pathology		
Two-generation repro-ductive toxicity study similar to OECD TG 416	oral, feed prelimi-nary dose selection study main study	Rat, Sprague Dawley 8 M / 8 F n=8/sex/ group 24 M / 24 F n=24/ sex/ group	5000, 10000, 20000 or 40000 ppm in diet 600, 3000 or 15000 ppm in diet (40-56, 197-267, or 1016-1375 mg/ kg bw/d)	15000 ppm (1016-1375 mg/kg bw/d), deter-mined by study au-thors		bw ↓ in M & F at high-dose, rel. liver wt ↑ in M ≥ 10000 ppm, bwg of F1 pups inhib-ited ≥ 20000 ppm at 15000ppm: **F0 & F1 pa-rental ani-mals**: **no** effects on bw, no histo-pathological effects in liver abs and/or rel. liver wts ↑ in both sexes **F1 & F2 weanlings** suppression of bwg, rel. liver wts ↑ in in both sexes without any histopatho-logical abnormalities		Fujii et al., 2005
Toxicity of DEP in female Wistar rats	oral feed 150 days	**Rat** (Wistar) female n = 6/group	50 ppm in diet intake ap-proxi-mately 2.9 mg/kg bw/d			**no** effects on liver: bw ra-tio, liver lipid peroxi-dation in-creased, vacuolization & granular deposits in hepatocytes, slight ↑ liver & serum ACP and	DEP was dissolved in corn oil and mixed with diet 21 day old F1-offspring pups showed mild	Pereira & Rao, 2006, Pereira & Rao, 2007

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
						LDH liver & serum AST ↑ liver chole-sterol ↑	vacuoli-sation in hepato-cytes	
Toxicity of DEP in male/ female Wistar rats during exposure of several gene-rations	oral feed 150 days (respec-tively in the parental, F1- and the F2-genera-tion)	**Rat** (Wistar) 6 M / 6 F n = 6/sex/gro up	P-ge-nera-tion: 50 ppm, F1-gen 25 ppm, F2-gen 10 ppm in diet intake ~ 0.6, 1.4 and 2.9 mg /kg bw/ day			P- and F1-generation: **no** effects on adrenal & thyroidal wts at 150 days, P-, F1-, F2-generation: vacuoli-zation, granular deposits & fatty degen-eration in hepatocytes liver & serum ALT/AST ↑, liver & serum triglycerides ↑, serum cholesterol in P ↑ & in F2↓	DEP was dissolved in corn oil and mixed with diet	Pereira et al., 2007a Pereira et al., 2007b
chronic toxicity of DEP in male Wistar rats	oral feed 150 days	**Rat** (Wistar) male n = 6/group	10, 25 and 50 ppm in diet intake ap-proxi-mately 0.6, 1.4 and 2.9 mg/kg/ d			↑ liver: body wt ratio and ↑ serum ACP, LDH, ALT for the 10 ppm-group only, liver tri-glycerides ↑ and ↑ lipid peroxidation in liver of all treated groups, mitochon-drial prolife-ration in hepatocytes, ↑ numbers of peroxisomes	DEP was dissolved in corn oil and mixed with diet Several inconsist-encies in the data hampered the inter-pretation of the re-sults from this pub-lication	Pereira et al., 2006a
Gender-	oral,	**Rat**	50			**no** change in		Sinkar &

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
based compara tive toxicity of DEP	drinking water *ad libitum* 180 days	(Wistar) male/ female n = not reported	ppm in drinkin g water			bw, liver wt, body: liver wt ratio, **no** changes in serum ALP, AST & SDH, vacuolization and de-generative changes in hepatocytes M: liver ACP & kidney gluthathione levels ↑, serum ACP & LDH ↓, F: serum LDH ↓		Rao, 2007
toxicity of DEP in male Sprague-Dawley rats	oral, drinking water *ad libitum* 120 days	**Rat** Sprague-Dawley male n=6/ group	50 ppm			**no** change in bw, liver wt, body:liver wt ratio, ↑ serum AST,ALT, ALP levels, ↑ serum & liver ACP, ↑ liver LDH & glycogen levels, ↓ liver triglycerides and ↑ liver & serum chole-sterol levels, ↑ liver peroxidation		Sonde et al., 2000
effects on liver & hepatic peroxi-some prolifera tion and on liver enzymes & lipid	oral, feed 90 days	**mice** (Swiss) female n=5/ group	10, 25 & 50 ppm in diet intake ap-proxi-mately 1.25, 3.1 &			**no** effects on bw or on abs. or rel. liver organ wt, intracellular vacuolization in all treated groups with additional degeneration and hypertro-	DEP dissolved in corn oil mixed with the diet	Mapuskar et al., 2007

Method/ Guide-line	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Reference
metabo-lism after repeat admi-nistra-tion			6.3 mg/kg bw/d			phy of the hepatocytes at 50 ppm, evidence for proliferation of mitochon-dria and per-oxisomes, ↑ serum liver glycogen, cholesterol & triglycerides, ↑ serum levels of ACP, ALT& AST		
Con-tinuous breeding study	oral, feed 14-day doses setting (task I) con-tinuous breeding phase	mice, (Swiss CD-1) male/ female n=8/sex/ group n=20/sex /group	 0.25, 0.5, 1.0, 2.5 and 5.0 % in feed 0.25, 1.25, 2.5 % in feed (340, 1770, 3640 mg/kg bw/d)			 less body wt gain in high-dose group less body wt gain in high-dose group ↑ in liver wt in F in high-dose group		Lamb et al., 1987

5.6.1.2 Repeated dose toxicity: inhalation

This information is not available from the registration dossiers.

5.6.1.3 Repeated dose toxicity: dermal

Table 19: Overview of experimental studies on repeated dose toxicity after **dermal exposure**

Method/ Guide-line	Route of expo-sure Dura-tion	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Re-ference
Subacute study (4-week study)	dermal 4 weeks (5 d / w)	rat, Fischer 344/N 10 M / 10 F	0, 37.5, 75, 150, 300 µl (0, 46, 92, 184, 369 µg) DEP (>99%) neat	75 µl (92 mg)	150 µl (184 mg)	M/F, 300 µl, F 150 µl: ↑ rel. liver wts, M 150 µl, 300 µl, F 150 µl: ↑ rel. kidney wts **No** other adverse effects were observed	**No** derma-totoxicity, **no** adverse clinical signs, **no** effects on bwg and food con-sumption.	NTP (1995)
2-year study	dermal 103 weeks (5d/w) up to 10 animals/ group evalu-ated after 15 months	rat, Fischer 344/N male/ female n=60/ dose/sex	0, 100 or 300 µl (0; 123, or 369 µg)			15 months: Survival of dosed rats similar to controls, 2-year survival significantly reduced in all M (survival probabilities: 0 µl 8%; 100 µl 12%; 300 µl 12%), M, 300 µl: mean bw slightly less than controls throughout the study	**no** adverse clinical signs were observed, including **no** evidence of dermato-toxicity	NTP (1995)
Subacute study (4-week study)	dermal 4 weeks (5 d / w)	mouse, $B6C3F_1$ 10 M / 10 F	0, 12.5, 25, 50, 100 µl (0, 15, 31, 62, 123 µg) DEP (>99%) neat	12.5 µl (15 µg)	25 µl (31 µg)	M/F 25, 100 µl: ↑ liver wt (abs. & rel.) **No** other adverse effects were observed	**No** derma-totoxicity, **no** adverse clinical signs, **no** effects on bw and food con-sumption.	NTP (1995)
2-year study	dermal 103 weeks (5d/w) with a one week recovery period	Mouse, $B6C3F_1$ male/ female n=60/ dose/ sex	0, 7.5, 15 or 30 µl (0, 9, 18, or 37 µg)			2-year survival of dosed mice similar to controls: 43/50, 41/48, 46/50, and 43/50 (M), and 41/50, 38/51, 37/49, and 36/49 (F), mean bw of	**no** adverse clinical signs were observed in mice, including **no** gross and morpholo-gical	NTP (1995)

Method/ Guide-line	Route of expo-sure Dura-tion	Species, Strain, Sex, No /group	Dose levels	NO(A)EL	LO(A)EL	Results Main effects/ Target organs	Remarks	Re-ference
	up to 10 animals/ group evalu-ated after 15 months					dosed mice similar to con-trols throughout the study, feed consumption similar to or up to 13% greater than that by controls	evidence of dermato-toxicity	

5.6.1.4 Repeated dose toxicity: other routes

Table 20: Overview of experimental studies on repeated dose toxicity after **intraperitoneal exposure**

Method/ Guideline	Route of exposure Duration	Species, Strain, Sex, No /group	Dose levels mg/m³	NO(A)EL mg/m³	LO(A)EL mg/m³	Results Main effects/ Target organs	Remarks	Reference
Chronic, Method employed essentially that of Boyd as described in Boyd EM 100-day LD50 index of chronic toxicity Clin Toxicol 4 pp,205-213 1971	Intra-peritoneal injection 14 w (daily for 5 (Monday -Friday) d/week)	mouse (ICR) male				LD50: 2.87 ml/kg (male) based on: test mat. (Previously calculated LD 50. Apparent LD50 at end of week 7 was 1.77 ml/kg, and reached in weeks 12-14, of 1.39.)	4 (not as-signable) weight of evidence experimental result Test material (Common name): diethyl phthalate	Lawrence WH, Malik M, Turner JE, Singh AR and Autian J (1975)

5.6.2 Human information

This information is not available from the registration dossiers.

5.6.3 Summary and discussion of repeated dose toxicity

Information on possible repeated dose toxicity of DEP was obtained from studies performed in rats and mice with repeated administration via the oral and the dermal route of exposure for various periods of exposure duration. The liver appeared to be the primary target organ for DEP in short- and medium- to long-term studies. Observed effects were increased absolute and relative organ weight, vacuolisation, changes in serum and liver enzyme levels, and proliferation of mitochondria and peroxisomes. Furthermore, body weight was affected at higher exposure levels.

The studies with oral exposures and investigating several dose levels (Brown et al., 1978; Lamb et al., 1987; Fujii et al., 2005; Shiraishi et al., 2006) as well as the studies with guideline according test design (Fujii et al., 2005; Shiraishi et al., 2006; Kwack et al., 2009) are considered the most relevant for the assessment of possible repeat dose toxicity of DEP. As evidenced from these studies reduced body weights/body weight gain and increases in absolute and/or relative liver organ weight (without histopathological changes) occurred consistently and at oral exposures of 750 mg/kg bw/d (Brown et al., 1978), 1000 mg/kg bw/d (Shiraishi et al., 2006) or 1016-1375 mg/kg bw/d (Fujii et al., 2005) in rats and at 3640 mg/kg bw/d (Lamb et al., 1987) in mice after exposure periods above 4 weeks. No such effects were observed at exposures to 150 mg/kg bw/d (Brown et al., 1978), 197 – 267 mg/kg bw/d (Fujii et al., 2005), 200 mg/kg bw/d (Fujii et al., 2005) and 500 mg/kg bw/d (Kwack et al., 2009).

In other studies in rats and mice by Pereira et al., 2006 ff and Mapuskar et al., 2007, respectively, the reported liver effects of DEP were accompanied by evidence of peroxisome proliferation. This mechanism of toxicity is well known with the phthalate esters and has been extensively discussed in the literature including the NICNAS Phthalates Hazard Compendium (NICNAS 2008). In general, phthalate induced hepatomegaly in rodents, when related to peroxisome proliferative effects, is not considered relevant to humans.

From the available data the 16-week dietary study in rats by Brown et al., 1978 and the 28-day gavage study by Shiraishi et al., 2006 are considered the critical studies for defining the point of departure (PoD) for repeat dose toxicity risk assessment. In the study of Brown et al., 1978, relative kidney and liver weights were statistically significantly increased in both sexes at DEP concentrations of 5% in the diet. In females, increases in relative liver weights were dose-related and were statistically significantly increased across all doses. In male rats, small intestine weights were statistically significantly increased at the 5% concentration only, whereas stomach weights were increased at both the 1% and 5% dietary concentrations. There was no abnormal histopathology of the liver, kidney or digestive organs. Neither were there significant effects on haematology, serum enzyme levels or urinary parameters. A conservative **NOAEL** of 0.2 % (corresponding to **150 mg/kg bw/d**) can be established from this study based on dose-related **reductions in body weight gain** in females, dose-related **increases in relative liver weight** in females and dose-related **increases in stomach weight** in males at 1 % (**LOAEL** of **750-770 mg/kg bw/d**).

Overall, the observed organ weight changes are not sufficient for classification as STOT RE 2 according to Annex I, Part 3 of Regulation (EC) No 1272/2008 (CLH), since the observed changes have not affected the function or morphology of the organs nor have produced serious changes to the biochemistry or haematology of the organism which are toxicologically relevant.

Non-classification by the registrant is justified by the available data on effects after repeated dose administration which show a lack of significant toxicity.

5.7 Mutagenicity

5.7.1 Non-human information

5.7.1.1 In vitro data

Table 20: Overview of experimental *in vitro* genotoxicity studies

Method/ Guideline	Test system (Organism, strain)	Concentrations tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	– S9		
Genetic *toxicity in vitro* mammalian cell gene mutation assay OECD 476 GLP	mouse lymphoma L5178Y cells TK+/- (Clone 3.7.2C)	72.3l - 925 µg/ml Vehicle DMSO	Nega-tive	Nega tive	key study 1 (reliable without restriction) DEP 99.96 % Cytotoxicity: yes	2010: Exp. Study genetic toxicity 01, confidentia l
Genetic *toxicity in vitro* mammalian chromosome aberration test OECD 473 GLP	Lymphocytes : Human	69.6 - 1780 µg/ml corresponding to 0.314 - 8.02 mM Vehicle DMSO	Nega-tive	Nega tive	key study 1 (reliable without restriction) DEP 99.96 % Cytotoxicity: no	2010: Exp. Study genetic toxicity 02, confidentia l
Genetic *toxicity in vitro* bacterial reverse mutation assay (e.g. Ames test) OECD 471 GLP	S. typhimu-rium TA 1535, TA 1537, TA 98 and TA 100	0, 10, 33, 100, 333, 667, 1000, 3333, 10000 µg/plate Vehicle: no data	Nega-tive	Nega tive	supporting study 2 (reliable with restrictions) DEP > 99 % Cytotoxicity > 1000 µg/plate	NTP 1995
Genetic *toxicity in vitro* bacterial reverse mutation assay (e.g. Ames test) OECD 471 GLP	S. typhimu-rium TA 1535, TA 1537, TA 98 and TA 100 E. coli WP2 uvr A	50 – 5000 µg/plate Vehicle DMSO	Nega-tive	Nega tive	key study 1 (reliable without restriction) DEP 99.97 % Cytotoxicity > 1250 µg/plate	2014: Exp. Study genetic toxicity, confidentia l

5.7.1.2 In vivo data

This information is not available from the registration dossiers.

5.7.2 Human information

This information is not available from the registration dossiers.

5.7.3 Summary and discussion of mutagenicity

The data on the genotoxic potential of DEP were obtained from *in vitro* testing. All of the four tests were carried out in accordance with EU Regulation (EC) No 440/2008 or current OECD guidelines for the testing

of chemicals and are GLP compliant. DEP shows neither gene mutations in bacterial and mammalian cells nor chromosome aberrations in human lymphocytes. Overall, these data do not support a genotoxic potential for DEP (NICNAS 2011). This is supported by reviews of international bodies/regulatory programs (ATSDR 1995, WHO 2003, NTP 2006, HSDB 2015).

Data available for germ cell mutagenicity do not warrant classification according to Regulation (EC) No. 1272/2008 and Directive 67/548/EEC.

5.8 Carcinogenicity

5.8.1 Non-human information

5.8.1.1 Carcinogenicity: oral

This information is not available from the registration dossiers.

5.8.1.2 Carcinogenicity: inhalation

This information is not available from the registration dossiers.

5.8.1.3 Carcinogenicity: dermal

Table 21: Overview of experimental studies on carcinogenicity

Title/ Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/ group	Dose levels (mg/kg bw/d)	Results Main effects/ Target organs/ Tumours	Remarks	Reference
Carcino-genesis Study equiv. or similar to OECD 451 (Carcino-genicity Studies)	Dermal 103 w 5 d/w Post-exposure period: 1 w recovery	Mouse B6C3F1 60 M/ 60 F per dose	M: 0, 260, 520, 1050 F: 0, 290, 550, 1100	↑ incidence of hepatocellular neoplasms, primarily adenomas within historical range F: no clear dose-response-relationship no carcinogenic effect M: ≥ 1050 mg/kg bw/d F: ≥ 1100 mg/kg bw/d	supporting study 2 (reliable with restrictions) only two dose groups tested ↓ survival at end of study period DEP > 99 % Vehicle: acetone	NTP 1995
Carcino-genesis Study equiv. or similar to OECD 451 (Carcino-genicity Studies)	Dermal 103 w 5 d/w Post-exposure period: none	Rat Fischer 344/N 60 M/ 60 F per dose	M: 320, 1015 F: 520, 1050	no evidence of carcinogenic activity no carcinogenic effect M: ≥ 1015 mg/kg bw/d F: ≥ 1050 mg/kg bw/d	key study 2 (reliable with restrictions) only two dose groups tested < 50 % survival M DEP > 99 % Vehicle: unchanged (no vehicle)	NTP 1995

5.8.2 Human information

This information is not available from the registration dossiers.

5.8.3 Summary and discussion of carcinogenicity

The data on carcinogenicity of DEP were obtained from animal testing. None of the tests on carcinogenicity was carried out in accordance with EU Regulation (EC) No 440/2008 or current OECD guidelines for the testing of chemicals. However, by means of a weight of evidence approach the available information is sufficient to support the conclusion that DEP is not likely to be a carcinogen. This is supported by reviews of international bodies/regulatory programs (ATSDR 1995, WHO 2003, NTP 2006, NICNAS 2011, HSDB 2015).

In mice effects were considered equivocal evidence of carcinogenic activity due to lack of dose-response relationship in females and similar incidence of combined hepatocellular adenomas and carcinomas in males at the highest dose compared to historical controls (Table 22). In rats no evidence of increased neoplasia was found other than treatment-related epidermal acanthosis at sites of DEP application, which was considered an adaptive response to irritation. No other lesions or neoplasms were noted in these 2-year studies both in mice and rats. Overall, the available data do not indicate a carcinogenic potential for DEP (NICNAS 2011).

Data available for carcinogenicity of DEP are conclusive but not sufficient for classification according to Regulation (EC) No. 1272/2008 and Directive 67/548/EEC.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

5.9.1.1 Non-human information

Table 22: Overview of **experimental studies on fertility** as reported in the **registration dossier**

Method/ Guideline	**Route of exposure, duration**	**Species, Strain, Sex, No/ group**	**Dose levels**	**Critical effect Parental, Offspring (F1, F2)**	**LO(A) EL**	**NO(A) EL Repro-ductive toxicity**	**Remarks**	**Reference**
two generation repro-ductive toxicity study according to OECD Guideline 416 (Two-Generation Repro-duction Toxicity Study), GLP	oral: feed basal diet 15 weeks for male and 17 weeks for female parents of the F0 & F1 genera-tions (Con-tinuously via the diet)	**Rat Crj:CD (SD) IGS** 24 M/ 24 F per dose	0, 600, 3000, 1500 0 ppm nomi-nal in diet	F0 & F1 in parents: ↑ abnormal sperm & tailless sperm ↑ liver wt F0 M: ↓ absolute adrenal & epidymal wt F1 M: ↓ rel adrenal wt **F1 F:** delayed	No adverse effects on systemi c & repro-ductive para meters	15000 ppm (**1016** - 1297 mg/kg/d) no adverse effect on repro-ductive per-formanc e	2 (reliable with restric-tions) **key study** Test material: DEP (99.8%) sperm effects in F0 only at 3000 ppm suggestio	Fujii S Yabe K Furukawa M Hirata M Kigouchi, Ikka T (2005)

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/ group	Dose levels	Critical effect Parental, Offspring (F1, F2)	LO(A) EL	NO(A) EL Repro-ductive toxicity	Remarks	Reference
				vaginal opening ↓ uterine weight F1 & F2 pups: ↓ pup bwgbefore weaning	15000 ppm (**1150** - 1375 mg/kg/d)	3000 ppm (**222** - 267 mg/kg/d) develop-ment, pup growth	n effects do not arise from suppres-sion of androgen synthesis during develop-ment	
Repro-duction & Fertility Assessme nt NTP's Standard continuou s breeding design andproced ure for this type of study followed no guidline	oral: feed plain diet 15 weeks (Con-tinuously via the diet)	**mouse** CD-1 20 M/20 F per dose	F1: 0.0, 0.25, 1.25, 2.5 % equi-valen t to 0, 340, 1770, 3640 mg/k g bw/d; F2: 0.0, 2.5 %	**F1 F:** ↓ bwg ↑ liver weight F1 M: ↑ prostrate weight ↓ epididy-mal sperm conc. F1 litters: ↓ number of pups/litter **F2:** ↓ bwg ↓ liver weight	3640 mg/kg bw/day Repro-ductive effects, changes in bw, liver & prostate weights moderat e repro-ductive effects	**F0:** No effects	2 (reliable with restric-tions) weight of evidence Test material: DEP same study as described below	Api AM (2001) Lamb IV JC, Chapin RE Teague J, Lawton AD, Reel JR (1987)
one generatio n fertility NTP's Standard continuou s breeding design GLP	oral: feed plain diet 21 weeks (Con-tinuously via the diet)	**mouse** CD-1 M/ F	F1: 0.0, 0.25, 1.25, 2.5 % equi-valen t to 0, 460, 2440, 4400 mg/k g bw/d; F2: 0.0, 2.5 %	**F0:** No effects **F1:** ↓ bwg ↓ litter size F1 F: ↑ liver weight ↓ uterus weight ↓ pituitary weight F1 M: ↑ prostrate weight ↓ sperm conc.	4400 mg/kg bw/d ↓ bwg ↓ litter size when sexes combin ed but not when analyse d separate ly	> 4400 mg/kg bw/d < 4400 mg/kg bw/d	2 (reliable with restric-tions) weight of evidence Test material: DEP same study as described above	Api AM (2001) Morrisey RE, Lamb IV JC, Morris RW, Chapin RE, Dushyant KG, Heindel JJ (1987)
Testicular	oral: feed	**rat**	0, 2	Lower			4 (not	Api AM

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/ group	Dose levels	Critical effect Parental, Offspring (F1, F2)	LO(A) EL	NO(A) EL Repro-ductive toxicity	Remarks	Reference
toxicity *in vivo*	plain diet 7 d (Con-tinuously via the diet)	Wistar M	% / 0, 2000 mg/k g/d nomi-nal conc.	levels of testosteron e in testes & serum			assign-able) weight of evidence Test material: DEP	(2000e) Anon (2002b) Anon (1995) Anon (2006)
effects of diethyl phthalate on male repro-ductive function		**rat** M	up to 1600 mg/k g/d	Testicular, accessory gland weight, histo-pathology unaffected			no robust study summary available	Foster et al. 1980
Testicular toxicity				no effect on progester-one binding to testes mi-crosomes, testicular CYP con-tent, or testicular ster-oidogenic enzyme activity			no robust study summary available	Foster et al. 1983
Testicular toxicity	Acute administra tion	**rat** M	2000 mg/k g/d	Leydig cell mitochon-drial swelling, focal dilatation & vesiculatio n of smooth ER			4 (not assign-able) weight of evidence Test material: DEP	Api AM (2001)
Testicular toxicity of DEP *in vivo* of MEP *in vitro*	Oral 3 daily doses	**rat**, M Sprague-Dawley , 4-5 weeks of age	1600 mg DEP/ kg	DEP: no effect on two specific markers of Sertoli cell function			MEP: de-tachment of germ cells from a Sertoli cell mono-layer	Gray T & Gangolli S. 1986

Table 23: Overview of **experimental studies on fertility** according to original publications (**such only partly reported in registration dossiers as well as additional publications**)

Method / Guide-line	**Route of exposure duration**	**Species, Strain, Sex, No/ group**	**Dose levels (mg/ kg bw/d)**	**Critical effect Parental, Offspring (F1, F2)**	**LO(A)EL (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**Remarks**	**Re-ference**
two genera-tion repro-ductive toxicity study compli-ant with OECD TG 416 and GLP	Oral: Diet 15-17 weeks per genera-tion (**10 weeks** prior to mating till weaning)	**Rats** SD 24 M / 24 F	0, 600, 3000, 15000 ppm (0, **40**-56, **197**-267, **1016**-1375) (M-F)	*Parental* **F0, F1:** ↑ liver wt **F1 F:** ↓ ute-rus, ↑ kid-ney wt *Fertility* **F0 M:** ↓ serum tes-tosterone **F0, F1 M:** ↑ abnormal and tailless sperms *Develop-mental* **F1, F2:** ↓ pup weight **F1:** delayed **M** pinna detachment **F** vaginal opening	*Systemic:* 1016-1375 *Fertility* **F0, F1 M:** **197** **F1 M:** sperm ef-fects dose-related at mid and high dose *Develop-mental* 1016-1375	*Systemic:* **197**-267 *Fertility* **F0, F1 M:** 40 **F0, F1 F:** 1375 *Develop-mental* 197-267	Test material: DEP *Fertility* **F1 M:** no data on testo-sterone **F0 M:** Stat. sign. sperm effects only at mid dose, normal at high dose, **not dose-related**	(Fujii et al., 2005)
two-generati on con-tinuous breed-ing study	Oral: Diet 18 weeks (1 week prior to mating till weaning)	**mouse** CD-1 20 M / 20 F	0, 0.25, 1.25, 2.5 % (0, 340, 1770, 3640) F1: 0, 2.5 % only	*Parental* **F1:** ↓ bw, ↑ liver weight **F1 F:** ↓ pi-tuitary wts, ↓ bwg *Fertility* **F1 F:** 4 % uterus wt ↓ ↓ litter size **F1 M:** ↓ sperm counts ↑ prostrate weight	*Systemic:* **F1:** 3640 *Fertility* **F1 F:** non-sign.	*Systemic:* **F1:** not estab-lished **F0:** ≥3640 *Fertility* **F1 F:** 3640 F1 M: not estab-	Test material: DEP *Fertility* **F1 F:** due to ↓ bwg	Lamb et al. 1997 (Lamb Iv et al., 1987) NTP 1984

Method / Guide-line	**Route of exposure duration**	**Species, Strain, Sex, No/ group**	**Dose levels (mg/ kg bw/d)**	**Critical effect Parental, Offspring (F1, F2)**	**LO(A)EL (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**Remarks**	**Re-ference**
				Develop-mental **F2:** ↓ no. of live pups/ litter (com-bined sexes)	**F1 M:** 3640 *Develop mental* F2: 3640	lished *Develop mental* F1: 3640	F1 M: tailless sperm not determin-ed only 2.5 % tested No data on F2 pups	
Study on testes and tes-ticular function *in vivo*	Oral intubation Vehicle: corn oil 4 d	**Rat** young SD 12 M	0, 1600	no effect on testes weight or Zn content, no testicular lesions	*Fertility* not estab-lished	*Fertility* **1600**	Test material: DEP (> 99 %)	Foster et al. 1980
Study on testes and tes-ticular function *in vivo*	Oral: diet 7 d	**Rat** 5 w old Wistar 10 M	0, 2% (~ 2000)	↓ serum and testis testosterone conc. by 40 %	*Fertility* **2000**	*Fertility* not estab-lished	Test material: DEP (> 98 %)	Oishi & Hiraga, 1980
Study on testes and tes-ticular function *in vivo*	Oral gavage Vehicle: corn oil 2 d	**Rat** 6-8 w old Wistar 12 M	0, 2000	Ultra-structural changes in Leydig cells	*Fertility* **2000**	*Fertility* not estab-lished	Test material: DEP	(Jones et al., 1993)
Study on testes and tes-ticular function *in vivo*	Oral: diet **150 d**	**Rat** 7-8 w old **Wistar 6 M**	0, 10, 25, 50 ppm (0, 0.57, 1.43, 2.85)	dose-related ↓ bw (-6%, -10%, -23%) ↓ abs. testis and epidi-dymis wt, ↓ testicular antioxidant enzymes, ↓ serum testosterone & andro-stenedione	*Fertility* **0.57** rel. wts not calculated serum andro-stenedione **not dose-related**	*Fertility* not estab-lished	Test material: DEP (99.99 %) dose calcula-tion not compre-hensible no data on food consump-tion	Pereira et al., 2008

Method / Guide-line	**Route of exposure duration**	**Species, Strain, Sex, No/ group**	**Dose levels (mg/ kg bw/d)**	**Critical effect Parental, Offspring (F1, F2)**	**LO(A)EL (mg/kg bw/d)**	**NO(A)EL (mg/kg bw/d)**	**Remarks**	**Re-ference**
Study on testes and testicular function *in vivo*	Oral gavage Vehicle: corn oil 28 d	**Rat** 5 w old SD 6 M	0, 250 MEP 500 DEP	↓ sperm counts & motility sperm motion: ↓ linearity	*Fertility* **250 (MEP)** 500 (DEP)	*Fertility* not estab-lished	Test material: Mono-ethyl phthalate	Kwack et al., 2009
Testicu-lar toxi-city *in vitro* induc-tion of germ cell detach-ment	Incuba-tion tem-perature of 32 °C, 1 mM sodium pyruvate in culture medium	**Rat** M Primary cocul-tures of Sertoli & germ cells	0, 1, 3, 10 mM MEP	detached germ cells from a Sertoli cell monolayer: 210 % compared to control at 10 mM			Test material: Mono-ethyl phthalate (MEP)	Gray T & Gangolli S. 1986
Sub-acute toxicity study using a draft protocol for "en-hanced OECD Test Guide-line 407 – Re-peated dose toxicity study", GLP	Oral: gavage Vehicle: corn oil 28 d	**Rat** Crj:CD (SD) 8 w old 10 M 10 F	0, 40, 200, and 1000	**no** endocrine-mediated effects detected on any of the parameters examined, e.g. sperm count & morphology , estrous cycling, serum conc. of TSH, T4, T3, testo-sterone, FSH, LH, estradiol	**1000 M:** ↓ bw, ↓ estradiol **F**: ↑ adrenal weight, kidney weight (also at 40 mg/kg bw/d)	200	Test material: DEP (99.8 %)	Shiraishi et al. 2006

5.9.1.2 Human information

This information is not available from the registration dossiers.

Table 24: Overview of **human studies on fertility**-related parameters according to original publications (**not reported in registration dossiers**)

Title/Method/ Guideline	**Data generation/ collection**	**Species, Sex, No**	**Dose levels**	**Results Main effects/ Target organs**	**Remarks**	**Reference**
Human sperm motility *in vitro*	Sperm preparations with DEP in acetone	Human M healthy donors	33, 330, 3300 µmol DEP/L	↓ sperm motility (10 %) at 3300 µmol/L	Test material: DEP (≥ 99 %)	Fredricsson et al. 1993; WHO 2003
Whole-body topical application 2-week single-blind study	Dermal 1 w, daily whole body, 1 w vehicle cream	Human Caucasian 26 M aged 21-36 healthy volunteers	cream 2% w/w DEP	**no** differences in serum levels of reproductive hormones or thyroid hormones	Test material: DEP (≥ 99 %)	Janjua et al., 2007
relationship between urinary levels of phthalate metabolites and Human Semen Parameters	single spot urine sample collected on the same day as the semen sample	Human 168 M 20-54 years of age	~180 ng MEP per mL Urine (Geometric Mean)	**no** dose-response relation between MEP and serum reproductive hormone levels, sperm conc., motility or morphology	DEP metabolite : Monoethyl phthalate (MEP)	Duty et al. 2003b; 2004; 2005
Urinary Phthalate Metabolites and Biomarkers of Reproductive Function	Urine, serum, and semen samples	Human 234 M age range, 18–21 (median age 18 years) Swedish military recruits	120 ng MEP/ml urine (75^{th} percenttile)	highest quartile for MEP levels **weakly associated** with low sperm motility and low LH levels	DEP metabolite : Monoethyl phthalate (MEP)	Jönsson et al. 2005
Semen Quality in Relation to Urinary Concentrations of Phthalate Monoester	single spot urine sample collected on the same day as the semen sample	Human 84 % white 463 M 20-54 years of age	180 ng MEP/mL urine (Geo-metric Mean)	**no** relationship between urinary MEP and sperm concentration, motility or morphology	DEP metabolite : Monoethyl phthalate (MEP)	Hauser et al., 2006 (re-analysis of Duty)
Study Associating Urinary Concentrations of Phthalate Metabolites and Semen Quality	home first-morning void urine Semen specimens collected on-site by masturbation	Human 78 % Caucasian 5 % African American 5 % Other race 45 M 23 to 48 years (median 34 years)	121.9 µg MEP/L urine (Geo-metric Mean)	↓ sperm concentrations with above median concentrations of urinary MEP	DEP metabolite : Monoethyl phthalate (MEP)	Wirth et al. 2008
Study	Semen of	Human	0.64-3.11	statistically	Analysis	Pant et al.,

Title/Method/ Guideline	Data generation/ collection	Species, Sex, No	Dose levels	Results Main effects/ Target organs	Remarks	Reference
investigating the Correlation of phthalate exposures with semen quality	volunteers was collected by masturbation	300 M 21–40 years old (mean 28-29 years) healthy men from rural/urban areas of Lucknow, India	µg DEP/mL	significant negative correlation between semen DEP levels and sperm conc.	of DEP	2008
Phthalate exposure and human semen quality in Shanghai: a cross-sectional study		Human 52 M Shanghai, China	mean = 0.47 µg MEP/ mL	Stat. significant positive association between semen liquefaction time and semen conc. of MEP	DEP metabolite: Monoethyl phthalate (MEP) small study	Zhang et al., 2006* NICNAS 2011
relationship between adverse reproductive health effects in women and exposure to DEP	Interview, 137 urine specimen prior to a laparoscopic diagnosis of endometrio-sis	Human 166 F 57 cases, 80 controls	Median 21.4, 39.6 µg MEP /L	urinary MEP showed **no** significant association with endometriosis	DEP metabolite: Monoethyl phthalate (MEP)	Itoh et al. 2009
cross-sectional study US National Health and Nutrition Examination Survey		Human 1227 F aged 20-54		**no** associations between urinary MEP and endometriosis or uterine leiomyomata (fibroids)	DEP metabolite: Monoethyl phthalate (MEP)	2010: unpublished study record, confidential
Urinary phthalate monoesters concentration in couples with infertility problems	spot urine samples questionnaire	Human cases: 56 M, 56 F mean age 39.57 years controls: 56 M, 56 F mean age: 39.42 y	mean = 531.59 (cases), 203.23 (controls) µg/g creatinine	urinary MEP concentrations are significantly higher for cases with respect to controls	DEP metabolite: Monoethyl phthalate (MEP)	Tranfo et al. 2012

5.9.2 Developmental toxicity

5.9.2.1 Non-human information

Table 25: Overview of **experimental studies on developmental** toxicity as reported in the **registration dossier**

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Developmental Toxicity	Remarks	Reference
Developmental toxicity evaluation equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study), GLP	Oral: feed morning of GD6 to morning of GD15. (Continuously in the diet)	**rat** (Crl: CD (SD) BR VAF/ Plus outbred Sprague Dawley rats) 25-32 gravid F	0, 0.25, 2.5, 5.0 % nominal in diet	1) ↓ bwg at 2.5 % DEP (transient) & 5 % DEP 2) ↑ incidence of supernumery ribs	0.25 % based on: test material (equivalent to 200 mg/kg)	2.5 % based on: test material (equivalent to 1910 mg/kg bw/day)	2 (reliable with restrictions) **key study** Test material: DEP (> 99 %)	Field EA, Price CJ, Sleet RB, George JD, MarrMC, Myers CB, Schwetz BA & Morrisey RE (1993)
Developmental toxicity / teratogenicity Teratological Evaluation Following Dermal Application of DEP to Pregnant Mice	**Dermal** GD 0-17 (daily)	**mouse** Jcl:ICR 17-20 pregnant F	0, 500, 1600, 5600 mg/kg/d nominal conc.	1) ↓ thymus/ spleen weights, ↑ adrenal gland weights 2) ↓ foetal bw, ↑ rib variations	< 500 mg/kg bw/day (nominal) based on: test material	1600 mg/kg bw/day (nominal) based on: test material	2 (reliable with restrictions) weight of evidence Test material: DEP	Api AM (2001) Tanaka C, Siratori K, Ikegami K, Wakisaka Y (1987)
Developmental toxicity / teratogenicity equivalent or similar to OECD Guideline 414 (Prenatal Developm	**Dermal** GD 6-18 (daily during treatment period) Duration of test: 29 d	**rabbit** (New Zealand White) 12 F	0, 5, 15, 50 % in 0.5% CMC nominal conc. (w/w)	**No** effects 50% DEP was the highest conc. administered (treated animals received 2 ml/Kg bw)	50 % based on: test material at a dose volume of 2 mL/kg bw (equivalent to 1 mL	1000 mg/kg bw/day no adverse effects on foetal development 2/84 malformed foetuses	1 (reliable without restriction) supporting study Test material	Exp. Study developmental toxicity 1985

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Developmental Toxicity	Remarks	Reference
ental Toxicity Study), GLP					DEP/kg)		: DEP	

Table 26: Overview of **experimental studies on developmental** toxicity according to original publications (**such only partly reported in registration dossiers as well as additional publications**)

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/d)	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Developmental Toxicity	Remarks	Reference
Prenatal developmental toxicity study	Intraperitoneal GD 5, 10, 15	**Rat** SD 5 F	0, 0.51, 1.01, 1.69 mL/kg (0, 500, 1000, 1500)	1) no data 2) ↓ pup weight, ↑ skeletal abnormalities		not established LOAEL **500** mg DEP/kg bw/day	small sample size, no statistical analysis, injection route	Singh et al. 1972
Prenatal developmental toxicity study	Dermal GD 0-17	**mouse** Jcl:ICR 17-20 F	0, 500, 1600, 5600	1) ↑ adrenal & kidney weights 2) ↓ pup weight, ↑ skeletal variations	1600 mg/kg bw/day small reduction in thymus/ spleen weights not considered adverse	1600 mg/kg bw/day LOAEL 5600 mg DEP/kg bw/day slight developmental effects	Only the study's summaries available in English Test material: DEP	Tanaka et al. 1987* (reviewed by SCCNFP, 2002; WHO 2003)
Prenatal developmental toxicity study	Oral gavage Vehicle: corn oil GD 6-13	**mouse** CD-1 50 F	0, 4500	1) **no** effect on bw		4500 mg/kg bw/day	Preliminary screen test with DEP	Hardin et al., 1987
Prenatal developmental toxicity study GLP	Oral: Diet GD 6-13	**rat** SD outbred CD 27-32 F	0, 0.25, 2.5, 5% (0, 200, 1900, **3200**)	1) ↓ bw & food consumption 2) ↑ skeletal variations	200 mg/kg bw/day relation to rudimentary lumbar ribs	1900 mg/kg bw/day	Test material: DEP (> 99 %)	Field et al. 1993
Gene	Oral	**rat**	0, 500	2) **No**	Dose	500	Test	Liu et al.

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/d)	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Developmental Toxicity	Remarks	Reference
Expression Following In Utero Exposure	gavage Vehicle: corn oil GD 12-19	CD 10 F control 5 F DEP		changes in testes gene expression or on AGD	chosen not to induce maternal toxicity	mg/kg bw/day	material: DEP	2005
Fetal Testicular Testosterone Production	Oral gavage Vehicle: corn oil GD 8-18	**rat** SD 5 F	0, 100, 300, 600, 900	1) **no** effect on bwg or 2) fetal testosterone	900 mg/kg bw/day	900 mg/kg bw/day	Test material: DEP (99 %)	Howdeshell et al. 2008
Perinatal Exposure	Oral gavage Vehicle: corn oil GD 14 – PND 3	**rat** SD 5 F	0, 750	2) **no** alteration of male rat sexual differentiation	**No** maternal toxicity or reduced litter sizes	750 mg/kg bw/day	Test material: DEP (99 %)	Gray et al. 2000

5.9.2.2 Human information

This information is not available from the registration dossiers.

Table 27: Overview of **human studies on developmental** effects according to original publications (**such only partly reported in registration dossiers as well as additional publications**)

Title/Method/ Guideline	Data generation/ collection	Species, Sex, No	Dose levels	Results Main effects/ Target organs	Remarks	Reference
multicenter pregnancy cohort study, at prenatal clinics from September 1999 through August 2002	completed questionnaire blood samples, urine collection midway through the study.	Human 85 mother-son pairs baby was 2–36 months of age	53.3 – 436.9 ng MEP/ml urine	Urinary MEP concentration was statistically significantly and inversely related to AGI = AGD)/wt	maternal urinary phthalate conc. was not normalised for urine volume	Swan et al., 2005

Title/Method/ Guideline	Data generation/ collection	Species, Sex, No	Dose levels	Results Main effects/ Target organs	Remarks	Reference
multicenter pregnancy cohort study, human health endpoints in relation to environmental phthalate exposure	Estimated daily exposure using 2 different PK models and urinary MEP conc. from 214 pregnant women	Human 106 mother-son pairs	6.64 (median) and 112.3 (95th percentile) µg DEP/kg bw/d	Urinary concentrations of MEP were statistically significantly and inversely related to corrected AGD	AGDs were corrected using weight percentiles (weight for age) data from US population datasets	Swan et al., 2008 Marsee et al. 2006
Danish-Finnish cohort study Human Breast Milk Con-tamination with Phtha-lates and Al-terations of Endogenous Reproductive Hormones	Breast milk samples were analysed for MEP, serum measurements for gonado-tropins, inhibin B, sex hormone-binding globulin and testosterone	Human 130 newborn 3-month old boys (62 cryptorchid and 68 healthy boys)	Median 0.95 (minimum 0.07 – maximum 41.4) µg MEP/L	MEP showed positive, stat. sign. correlations with levels of sex hormone-binding globulin and LH:free testosterone ratio–a measure of Leydig cell function	**No** associations between MEP and cryptorchidism (testis maldescent) was found	Main et al., 2006
prospective ethnically diverse birth cohort study, prenatal phthalate exposures and birth outcomes	Maternal urinary MEP concentrations during third trimester of pregnancy, size of infants at birth	Human 352 mother-infant pairs in New York City	median of 380 µg MEP/L	Pos., stat. sign. associations of MEP with gestational age and infant head circumference, no with birth weight or length	unresolved confounding with maternal anthropometric factors	Wolff et al. 2008
Prenatal exposure to phthalate esters and birth outcomes	spot urine samples collected from pregnant women	Human 149 Japanese pregnant women and their newborns	geometric mean of 9.76 µg/g creatinine, 7.42 µg/L	relationships between urinary MEP conc. and birth outcomes **no**t significant		Suzuki et al. 2010
multiethnic longitudinal study Relationships between Urinary Bio-markers of Phyto-estrogens, Phthalates, and Phenols and Pubertal Stages in	urinary exposure biomarkers measured at visit 1 and associations with breast and pubic hair development (present or absent) assessed 1	Human multiethnic cohort 1 151 girls aged 6-8 years living in New York City, New York, greater Cincinnati, Ohio, northern		Positive trend for low molecular w (LMW) phthalates (i.e. sum of urinary metabolites MEP, MBP– monobutyl phthalate, and MIBP– monoisobutyl phthalate) with	authors noted that the peripubertal period was probably not the only critical window of exposure for pubertal development	Wolff et al. 2010

Title/Method/ Guideline	Data generation/ collection	Species, Sex, No	Dose levels	Results Main effects/ Target organs	Remarks	Reference
Girls	year later	California (2004–2007)		both breast and pubic hair development		
Association between prenatal exposure to phthalates and the health of newborns	urine samples 15–20min before amnio-centesis during 2005 and 2006 AGD of the newborns	Human 65 pregnant F 65 fetuses/ newborn (33 M, 32F)	Median maternal urinary MEP (ng/mL) : 22.8 F 19.1 M	**no** association between either AGD or AGI in male newborns and prenatal DEP exposure *in utero*	longitudinal study median levels of MEP in amniotic fluid not detectable	Huang et al. 2009
Copenhagen Puberty Study cross-sectional study 2006-2008	clinical examination on same day as blood sampling and urine collection	Human 555 boys (age 6.07–19.83 years) healthy, 514 Caucasian	9.56 – 12655 ng MEP/ mL	urinary levels of MEP **no**t associated with age at pubertal onset, serum testosterone levels or presence of gynaecomastia	Pubic hair, genital stages, Testicular volume, Pubertal onset, Gynae-comastia, Testosterone	Mieritz et al. 2012
Copenhagen Puberty Study cross-sectional study 2006-2008	full first morning urine sample on the morning of the examination	Human 725 girls (age 5.6–19.1 years) healthy, 618 Danish	2.2 – 11490 ng MEP/ mL	urinary levels of MEP **no**t associated with age at pubarche or breast development	Pubic hair, breast stages, serum FSH, LH, oestradiol and testosterone	Frederikse n et al. 2012

5.9.3 Summary and discussion of reproductive toxicity

The evidence of possible reproductive toxicity of DEP was obtained from multi-generation studies in rats and mice, specific studies on testicular function, prenatal and postnatal developmental toxicity studies and epidemiological data. In conclusion, the results on the reproductive toxicity of DEP are inconsistent.

Fertility

A GLP compliant 2-generation dietary study in rats according to OECD Guideline 416 is available in the registration dossiers as the key study for assessment of effects on fertility and developmental toxicity. Dietary exposure of up to 15000 ppm DEP (1016-1375 mg /kg bw/d) did not reveal any impairment of reproductive performance or fertility outcome. No significant differences in sperm counts or motility were observed in F0 and F1 males between the control and DEP-treated groups. Reduced testosterone levels were observed in F0 males from 3000 ppm (197 mg/kg bw/d). However, the extent of reduction in testosterone levels was not dose-related and the observed reduction did not affect the reproductive capacity to produce the progeny. Data on testosterone levels were not available for F1 males. There was a slight but statistically significant and dose-related increase in the frequency of abnormal (mainly tailless) sperm in the F1 generation at doses of 3000 ppm (222 mg/kg bw/d) which did also not affect fertility outcomes. No consistent or dose-related effect was observed in F0 males as a significant increase in the abnormal sperm rate was only seen at the mid dose level of 3000 ppm (197 mg/kg bw/d) in F0 males. A **NOAEL of 600 ppm**

(46 mg/kg bw/d) was established for **fertility-related parameters** based on the increased incidence of abnormal sperms at 222 mg/kg bw/d for F1 males (Fujii et al., 2005).

In a non-guideline experiment to examine the long-term effects of nominal doses up to 2.85 mg/kg bw/d DEP in the diet on the rat testicular antioxidant system, a small number of 6 male Wistar rats were fed a diet containing DEP for 150 d. Body weight, absolute testis weight, absolute epididymis weight and the serum testosterone and androstenedione levels were significantly decreased in all treated groups. However, data on the relative weights of testis and epididymis were not available. The final body weights of high dose males were 23 % lower compared to control values. Since no information on the food consumption was given, lower body weights could be related to the test substance or to lower food consumption. Testicular lipid peroxidation showed a significant dose-dependent increase and was observed in parallel with a dose-dependent decrease in testicular antioxidant enzymes (such as superoxide dismutase, glutathione peroxidase and reductase). This suggests an impairment of the testicular defence system following chronic exposure to DEP (Pereira et al. 2008). A NOAEL for this study could not be determined as effects were seen at the lowest doses tested. This study is considered to be of limited reliability since no actual doses were estimated and an impact of reduced food consumption on the growth and indirectly on the organ weights could not be excluded.

In conclusion, there are some weak indications on fertility related effects such as lower testosterone levels or increased rates of tailless sperms from animal studies. These effects were either not dose-related (testosterone) or not consistent across generations (tailless sperms). In addition, these findings were not accompanied by other morphological lesions in the male reproductive organs and did not affect the fertility outcomes. No effect on testis weight at doses up to 1016 mg/kg bw/d in the available two-generation study (Fujii et al. 2005, Table 22 and Table 23) was seen.

No consistent effects were observed in **human** studies on **fertility-related parameters** (Table 24). Some studies reported adverse effects associated with DEP exposures (reflected by MEP levels) on particular adult human sperm parameters, whilst other studies failed to find such effects. The results in the human male are limited and remained questionable due to limitations of the study design and to the fact that effects could also result from exposure to other phthalates than DEP. Findings were not consistent among human studies (either no effect was estimated, or inconsistent effects (on sperm motility or sperm concentration) were observed) and were not consistent with rat studies which demonstrated increased rates of tailless sperms. With regard to the potential to cause female fertility effects, levels of DEP or MEP in plasma or urine were not associated with risk of endometriosis or uterine leiomyomata (fibroids) in other also limited human studies (Itoh et al. 2009, Weuve et al. 2010).

Developmental

Concerning developmental toxicity, the numbers of implants, pups delivered and pup weights were unaffected at birth from the F0 and F1 parents as well as pup survival and viability. The only developmental effects of DEP were significantly reduced pup weight (up to 19 %) in F1 and F2 pups of both sexes on postnatal day (PND) 21 associated with delayed onset of pinna detachment and vaginal opening in the high dose rats (1016-1375 M-F mg/kg bw/d). The effects on the body weight in F1 female pups already started at PND 4. These effects could not be interpreted as related to reduced body weights in dams. The **developmental NOAEL** was determined to be 3000 ppm (**197-267 mg/kg bw/d)** and the LOAEL was 15000 ppm (1016-1375 mg/kg bw/d) based on decreased pup weight and developmental delay. It remains unclear whether the retarded development at the end of the lactation period was a direct effect or mediated via lactation. The observation in F1 female pups that lower body weights occurred from PND 4 onwards supports the conclusion on a lactation-related effect. However, a classification is not deemed to be warranted due to the high dose level above the limit dose for testing of 1000 mg/kg bw/day (Fujii et al. 2005).

Evaluations of potential DEP toxicity to the **developing male** rat **reproductive system** have consistently found no effect on testis weight or testis integrity (testes atrophy) at doses up to 1016 mg/kg bw/d in the available two-generation study (Fujii et al. 2005, Table 22 and Table 23). There was also no **foetal or neonatal toxicity** (e.g. epididymal malformations or absence of the epididymis, increased incidence of cryptorchidism, hypospadias, decreased AGD, delayed preputial separation, and retained areolas/nipples as commonly noted with the transitional phthalates of C4-6 backbone) after pre-/perinatal exposure to DEP at

oral doses up to 3200 mg/kg bw/d (Field et al. 1993, Gray et al. 2000, Howdeshell et al. 2008, Table 25 and Table 26).

After prenatal exposure in rats and mice at higher doses (3200 mg/kg bw/d orally and 5600 mg/kg bw/d dermally, respectively), an increased frequency of skeletal variations such as rudimentary cervical and/or lumbar ribs was reported but no dose response was evident and these effects generally occurred at or above maternally toxic doses (Tanaka et al. 1987; Field et al. 1993). The increase in supernumerary ribs (either cervical or lumbar) is one of the common anomalies seen in developmental toxicity studies in rodents (Chernoff & Rogers 2004; Daston & Seed 2007; NICNAS 2008). In view of the lack of conclusive evidence to assign the skeletal defects to maternal toxicity, these skeletal variations in rodents could be interpreted as indicative of slight developmental effects at doses well above 3000 mg/kg bw/d.

Singh et al. (1972) reported some skeletal malformations (not skeletal variations) such as incomplete skull bones from gestational exposure at a lower dose of 500 mg/kg bw/d administered intraperitoneally in rats (Table 26). However, the effects were considered inconsistent with findings in the above studies that used a larger sample size and more relevant administration routes (oral and dermal).

In a two-generation continuous breeding study in mice a significantly lower number of live pups per litter (14% lower than the control value) were seen in F1 females that received 2.5 % DEP in the diet (3640 mg/kg bw/day). The NTP abstract for RACB83092 (NTP 1984, corresponding to the data of Lamb et al.) clarified that the control values for pups per litter were abnormally low (about 25 % lower) for the F0 dams. Taking the historical control data into account the litter size in high dose F0 dams would also be decreased (by 21 %). As the effects on litter size were seen at extremely high doses of 3640 mg/kg bw/day, these effects should not be relevant for classification purposes.

Several studies in **humans** have explored the association between DEP exposures and **developmental outcomes** (Table 27). These human findings are limited by certain aspects, such as the reliability of spot urine/breast milk samples as indicators of DEP exposures, other confounding factors, e.g. the measured presence of other phthalate metabolites indicating a co-exposure to other (possibly reprotoxic) phthalates. NICNAS (2011) concluded that the current human data provide contradictory evidence of developmental effects from DEP exposure. The available epidemiological studies do not provide sufficient evidence for a causal relationship between exposure to DEP (measured as urinary MEP) and possible health effects and the relevance is limited by the presence of metabolites from toxicologically more potent phthalates. Therefore, no consistent picture of effects was observed in humans, and uncertainties with regards to DEP as the source of exposure do not allow a firm conclusion on the potential for developmental toxicity.

In agreement with the CLP Regulation the changes in one out of eleven sperm parameters seen in animals were not considered to warrant classification for fertility effects. According to 3.7.2.3.3 of Annex I, CLP Regulation effects of low or minimal toxicological significance (including small changes in semen parameters) should not lead to classification. The developmental findings such as reduced pup weight at the end of weaning and reduced litter size occurred at doses above the limit dose of 1000 mg/kg bw/day. According to and 3.7.2.5.8 and 3.7.2.5.9, Annex I of the CLP Regulation, effects at such high doses would normally not lead to classification unless expected human response indicate the need for a higher dose level.

Finally, the low molecular weight phthalate DEP and the shorter side chain (C2) do not support that DEP could act as a potent testicular toxin and could induce developmental changes in the male reproductive system as observed after prenatal exposure to mid molecular weight (so-called ‘transitional’) phthalates with critical lengths of carbon side chains (C4-C6).

Overall, by means of a weight of evidence approach the eMSCA considers the effects observed on male fertility and the observed developmental effects as not sufficient for classification as Repr. 2 according to Annex I, Part 3 of Regulation (EC) No 1272/2008 (CLP).

5.10 Endocrine disrupting properties

Table 28: Overview of studies on **endocrine effects** as reported in the **registration dossier**

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentrations tested	Results, Critical effect	Remarks	Reference
Oestrogen receptor-binding characteristics	*in vitro* displacing [^{3}H]17β-estradiol	recombinant human estrogen receptor expressed on Sf9 vaculovirus	DEP	**no** binding to human oestrogenic receptor (ER)	no robust study summary available	Nakai M, Tabira Y, 1999
Oestrogen receptor-binding affinity	*in vitro* ability to inhibit 17β-estradiol (E2) binding	Ligand Screening Assay	DEP 10-3 to 10-8 M	**no** detectable binding to either ERα or ERβ	no robust study summary available	Toda C, 2004
oestrogenic activity	yeast two-hybrid assay	Human	DEP	**negative** for oestrogenic activity	no robust study summary	Nishihara T, 2000
oestrogenic activity	recombinant yeast assay estrogenic responses in other estrogen assays	**human** estrogen receptor gene **mammalian** breast cancer cell lines	DEP 10-3 to 5 × 10-7 M 10-5, 10-6, 10-7 M	**weak** oestrogenic activity little activity in MCF-7 cells, no proliferation of ZR-75 cells	no robust study summary Potency 5 × 10^{-7} and 30 % of E2	Harris CA, 1997
oestrogenic activity	Human ERα and ERβ reporter gene assay	CHO-K1 cells transfected with expression vectors for **human** ERα, ERβ receptor	DEP various concen-trations in DMSO	**no** oestrogenic activity	no robust study summary available	Takeuchi S, 2005
stably transfected transcriptional activation (STTA) assay OECD test guideline 455	*in vitro* ability to function as an ERα ligand and activate agonistic response	hERα-HeLa-9903 cell line (HeLa9903) which is a stably trans-fected human ERα gene	DEP up to 10-5 M	no estrogenic activity relative binding affinity examined using the ER binding assay: **negative**	no robust study summary available mentioned only in ED assessment	Lee & Kim, 2012(Lee et al., 2012)
standardised estrogen receptor (ER) competitive-binding assay	*in vitro* ER affinity in competition with [3H]-estradiol	Uteri from ovariectomised Sprague-Dawley rats were the ER source		authors were unable to determine IC50 values for any of the phthalates	no robust study summary available	Blair et al. 2000
Anti-oestrogenic	*in vitro* effect on	MCF-7 cells	various concen-	DEP: **no** cell proliferative	Test material: DEP,	Okubo et al. 2003

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentrations tested	Results, Critical effect	Remarks	Reference
activity by inhibition of cell proliferation assay	cell proliferation in the presence of 17β-estradiol		trations of DEP or MEP	effects MEP: **inactive**	Monoethyl phthalate (MEP)	
oestrogenic activity in in vitro model	*in vitro* incubation of 6 d: DEP in 0.1 % DMSO	MCF-7 cells	DEP	**no** significant increase of cell growth was observed	no robust study summary available	Hong EJ 2005
Effects of DEP on laurate hydroxylation in rat liver microsomes		**Rat** liver microsomes	0, administration of DEP	**increased** specific activity of laurate hydroxylase by 1.6 times more than in control	laurate hydroxylase: (marker for CYP 4 responsible for testosterone metabolism)	Okita R & Okita JR 1992
Oestrogenic effects *in vivo*	oral Vehicle: peanut oil 3 d daily Rats were killed 24 h after 3rd dose	**Rat** Crl (WI) BR 10 F per dose	0, 50, 150, 500 mg/kg/d (nominal conc.)	**no** effects on clinical observations, bw or uterus weight NOAEL > 500 mg/kg/d	4 (not assignable) weight of evidence Test material: DEP	Api AM 2001 SCCNFP/0411/01
oestrogenic activity in *in vivo* model related to expression of Calbindin-D9k	oral treatment for 3 days	**Rat** 7 days old SD F	600 mg DEP/kg	Expression of CaBP-9k mRNA, a gene highly regulated by 17β-oestradiol, **no**t increased	no robust study summary available	Hong EJ, 2005
Prenatally altered sexual differentiation	Oral GD 14 to PND 3	**rodent**	0.75 g/kg DEP	**no** impaired sexual function and no shortened AGD	no robust study summary available	Gray et al. 2000
global gene expression in the fetal testis following in utero exposure	Oral gavage Vehicle: corn oil, daily GD 12-19	**rat** Sprague Dawley F	0, 500 DEP mg/kg per day	**No** significant changes in gene expression, no significant effect on AGD	no robust study summary available	Liu et al. 2005
dose-response effects on test. testosterone production	Oral gavage Vehicle: corn oil GD 8-18	**rat** SD F	0, 100, 300, 600, 900 mg/kg/d	DEP did **no**t affect GD 18 testosterone production	no robust study summary available	Howdeshell et al. 2008

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentrations tested	Results, Critical effect	Remarks	Reference
Subacute toxicity study based on the draft Enhanced OECD Test Guideline 407	Oral: gavage Vehicle: corn oil 28 d	**Rat** Crj:CD (SD) 8 w of age 10 M/10 F	0, 40, 200, and 1000 mg DEP/kg /day	1,000 mg/kg M: ↓ estradiol **no** abnormal spermatological findings, no abnormalities in estrous cycles.	1,000 mg/kg F: ↑ adrenal weight NOEL 200 mg/kg/day no endocrine-mediated properties	Shiraishi et al. 2006
links between adverse health effects in humans and phthalate exposure	prenatal urine samples (n = 85)	**Human** 134 boys 2–36 months of age	Environmental levels	Urinary MEP concentration inversely related to AGI = AGD/wt	no robust study summary available mentioned only in ED assessment	Swan et al. 2005
Association between urinary levels of MEP and Human Serum Testosterone levels		**Human**	DEP metabolite: Monoethyl phthalate (MEP)	positive association between increased urinary MEP levels and increased serum levels of testosterone, possible antiandrogen effect	no robust study summary available mentioned only in ED assessment	Duty et al. 2005
Prospective Danish-Finnish cohort study on cryptorchidism from 1997 to 2001	breast milk samples 1–3 months postnatally (n = 130) Serum samples (74% of all boys - cryptorchid, n = 50; normal boys, n = 46)	**Human** 130 M (62 cryptorchid/ 68 healthy boys)	Median 0.95 (minimum 0.07 – maximum 41.4) µg MEP/L	MEP showed positive correlation with levels of sex hormone-binding globulin (r = 0.323, p = 0.002) and with LH:free testosterone ratio	**No** association between MEP levels and cryptorchidism no robust study summary available mentioned only in ED assessment	Main et al. 2006
Semen Parameters of sub-fertile couples between January 2000 and May 2004	phthalate metabolites measured in single spot urine sample from each man	**Human** 463 M	DEP metabolite: Monoethyl phthalate (MEP)	**no** relationship of MEP and sperm concentration, motility or morphology	no robust study summary available mentioned only in ED assessment	Hauser et al. 2006
association between hu-	urinary metabolites	**Human** 168 M	DEP metabo-	**No** correlations	no robust study	Duty et al. 2003a

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentrations tested	Results, Critical effect	Remarks	Reference
man exposure to DEP at environmental levels and male reproductive effects	Sperm parameters adjusting for age, abstinence time, and smoking		lite: Monoethyl phthalate (MEP)	were found for monoethyl phthalate (MEP).	summary available mentioned only in ED assessment	
urinary levels of phthalate metabolites association with DNA strand breaks in sperm cells	urinary levels of MEP neutral comet assay adjusting for age and smoking status	**Human**	DEP metabolite: Monoethyl phthalate (MEP)	statistically significant positive association between urinary levels of MEP and mean comet extent (DNA migration) in sperm	no robust study summary available mentioned only in ED assessment	Duty et al. 2003b

Table 29: Overview of studies on **endocrine effects** according to original publications (**such only partly reported in registration dossiers as well as additional publications**)

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentration tested	Results, Critical effect	Remarks	Reference
Oestrogen receptor binding assay	*in vitro* incubation of 1 h at 25 °C: receptor with DEP solution and [^{3}H]17β-estradiol	recombinant human oestrogen receptor expressed on Sf9 vaculovirus	DEP	DEP was **unable** to bind to human oestrogenic receptor (hER)	Solvent 1 % DMSO (dimethyl sulfoxide) in aqueous buffer	Nakai M, Tabira Y, 1999
Oestrogen receptor binding assay	*in vitro* incubation of 1 h at 4 °C: ERα or β with DEP and 17β-estradiol	human ERs	up to 1 mM DEP	**no** detectable binding to either ERα or β	Low binding activity for 4-OH-DEP (10,000 x less than DES)	Toda C, 2004
Screening of oestrogenic activity by yeast two-hybrid assay	*in vitro* incubation of 4 h at 30 °C: ERα with DMSO solution of DEP	Yeast (*Saccharomyces cerevisiae* Y190) with ERα	up to 1 mM DEP	**negative** for oestrogenic activity	Test material: DEP	Nishihara T, 2000
Screening of oestrogenic activity by recombinant yeast assay	*in vitro* incubation of 6 d at 32 °C: hER with DEP	Gene for the hER integrated into the main yeast genome	up to 1 mM DEP	extremely **weak** oestrogenic activity, maximum response 30 % relative to	Test material: DEP (> 99.7 %) slight mitogenic activity in MCF-7	Harris CA, 1997

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentration tested	Results, Critical effect	Remarks	Reference
				17β-estradiol	assay	
Human estrogen receptors α and β, and androgen receptor reporter gene assay	*in vitro* incubation of 24 h: hERα, hERβ, and hAR with DEP in 0.1 % DMSO	CHO-K1 cells transfected with expression vectors for ERα, ERβ or **androgen** receptor	< 10 µM DEP	**no** hERα- and hERβ-mediated oestrogenic activities nor **antiandrogenic activity** demonstrated	Test material: DEP (> 99.5 %)	Takeuchi S, 2005
oestrogenic effects by MCF-7 cell proliferation assay	*in vitro* incubation of 6 d: ERα mediating a mitotic effect	MCF-7 human breast adeno-carcinoma cells	up to 10 mM DEP	increased proliferation of MCF-7 cells (**10^{-7}** times relative to estradiol)	EC_{50} max. 22 µM (no more than 75 % of cell proliferat. of estradiol)	van Meeuwen et al. 2008
oestrogenic activity in *in vitro* model	*in vitro* incubation of 6 d: DEP in 0.1 % DMSO	MCF-7 human breast cancer cell line	up to 100 µM DEP	**no** significant increase of cell growth was observed	Test material: DEP (99.5 %)	Hong EJ, 2005
oestrogenic activity by MCF-7 cell proliferation assay	*in vitro* incubation of 6 d: vehicle (0.1% ethanol)	Human breast cancer estrogen-sensitive MCF-7 cells	up to 1 mM DEP or MEP	**no** cell proliferative effects	Test material: DEP, Monoethyl phthalate (MEP)	Okubo et al. 2003
oestrogenic activity by gene expression profiling by DNA microarray containing estrogen responsive genes	*in vitro* Proliferation of cells by sulforhodamine B assay incubation of 3 d at 37 °C: DEP in DMSO	Estrogen receptor–positive human breast cancer MCF-7 cells	up to 100 µM DEP	**moderate** correlation between DEP and 17β-oestradiol (endogenous oestrogen) in gene expression profiles of MCF-7 cells	Test material: DEP maximal activity at 100 µM no enhancement of cell proliferation with 10 µM	Parveen et al. 2008
H295R steroidogenesis assay (OECD 456)	*in vitro* 48 h DEP exposure 17β-oestradiol (E_2), testosterone (T) conc. determined	human H295R adrenocarcinoma cell line	0.01, 0.1, 1, 10 mg DEP/L	10mg/L: 2.3-fold greater conc. of E_2 compared to solvent control	Test material: DEP 0.1 mg/L: 60% lesser conc. of testosterone	Mankidy 2013
Subacute toxicity study using a draft protocol for "enhanced OECD Test Guideline	Oral: gavage Vehicle: corn oil 28 d	**Rat** Crj:CD (SD) 8 w old 10 M/10 F	0, 40, 200, and 1000 mg/kg bw/d	**no** endocrine-mediated effects detected on any of the parameters examined, e.g. sperm count &	Test material: DEP (99.8 %) **1000 mg/kg bw/d M:** ↓ bw, ↓ estradiol	Shiraishi et al. 2006

Method/ Guideline	Route of exposure, duration	Test system (Organism, strain)	Concentration tested	Results, Critical effect	Remarks	Reference
407 – Repeated dose toxicity study", GLP				morphology, estrous cycling, serum conc. of TSH, T4, T3, testosterone, FSH, LH, estradiol	**F**: ↑ adrenal wt, kidney wt (also at 40 mg/kg bw/d) NOAEL 200 mg/kg bw/d	

The evidence of possible endocrine disrupting properties of DEP was obtained from *in vitro* studies, animal testing and epidemiological data. The extent to which the mode of action for transitional phthalates is reflective of the mode of action for low molecular weight phthalates such as DEP is not certain. Compared to certain transitional phthalates, there is a paucity of information to examine the mode of action of DEP with respect to reproductive effects.

DEP did not bind to human oestrogen receptor (hER) ***in vitro*** (Nakai et al., 1999; Toda et al. 2004) and showed extremely weak oestrogenic activity in both recombinant and two-hybrid yeast assays (Harris et al. 1997; Nishihara et al. 2000). DEP also did not demonstrate hERα- and hERβ-mediated oestrogenic activities, nor antiandrogenic activity in reporter gene assays using CHO-K1 cells transfected with respective expression vectors (Takeuchi et al. 2005). DEP increased proliferation of human breast cancer MCF-7 cells in one assay (van Meeuwen et al. 2008) but not in others (Okubo et al. 2003; Hong et al. 2005). There was a moderate correlation between DEP and 17β-oestradiol (endogenous oestrogen) in gene expression profiles of MCF-7 cells using a DNA microarray assay (EstrArray) (Parveen et al. 2008). MEP was shown to induce detachment of germ cells from a Sertoli cell monolayer *in vitro*, but was 10 000-fold less potent than mono-2-ethylhexyl phthalate (MEHP – a metabolite of DEHP) (Gray & Gangolli, 1986).

In vivo, expression of CaBP-9k mRNA (a gene highly regulated by 17β-oestradiol) was not increased in immature female Sprague-Dawley rats following oral treatment with 600 mg DEP/kg bw/d for 3 d (Hong et al. 2005). In OECD compliant toxicity tests (i.e. in the OECD two-generation reproductive toxicity study), DEP was positive for endocrine-mediated effects (such as reduced testosterone, abnormal sperm, and delayed physical and sexual post-natal development) in rats exposed to DEP continuously for 15 weeks at 197 and 1016 mg/kg bw/d (Fujii et al. 2005), but negative in rats dosed up to 200 mg/kg bw/d for 28 days using a draft protocol for "enhanced OECD Test Guideline 407 – Repeated dose toxicity study" (Shiraishi et al. 2006).

The above literature reporting potential links between adverse health effects in humans and phthalate exposure have been considered for assessment of endocrine disrupting effects of DEP, but the **epidemiological studies** do not to provide sufficient information to decide whether the observed associations are true causal relationships or whether they were fortuitous. Lifestyle practices that are both associated with DEP exposure and the effect are possible confounders. In particular smoking is associated both with several reproductive effects in humans, i.e. reduced sperm count in males exposed to smoking *in utero* and estrogenic effects (Jensen 2004, Storgaard 2003) and apparently also with urinary MEP (Duty 2005a,b). The median urinary concentration of MEP was higher in current smokers (236 ng/ml) and former smokers (231 ng/ml) than in never smokers (135 ng/ml) (VKM 2005).

In conclusion, the results on the oestrogenic or anti-androgenic potency of DEP are inconsistent and limited, and hence the exact mechanism of DEP effects on the male reproductive system such as reduced testosterone, sperm concentration and sperm quality cannot be determined although it appears to interfere with endocrine function (NICNAS 2011).

5.11 Other effects

5.11.1 Non-human information

5.11.1.1 Neurotoxicity

This information is not available from the registration dossiers.

5.11.1.2 Immunotoxicity

This information is not available from the registration dossiers.

5.11.1.3 Specific investigations: other studies

This information is not available from the registration dossiers.

5.11.2 Human information

This information is not available from the registration dossiers.

5.11.3 Summary and discussion of specific investigations

There are no specific investigations on other effects available from the registration dossiers.

5.12 Combined effects

This information is not available from the registration dossiers.

5.13 Derivation of DNEL(s) / DMEL(s)

5.13.1 Overview of typical dose descriptors for all endpoints

The lead registrant has given an overview of available dose-descriptors per endpoint. The dose-descriptors have been gathered from the available and relevant experimental animal studies in the registration dossier. Out of this database together with the information published in reviews of international bodies/regulatory programs (ATSDR 1995, WHO 2003, VKM 2005, NICNAS 2011) suitable studies and typical dose descriptors for derivation of DNEL values are selected.

In agreement with the assessment of NICNAS (2011) the critical toxicity endpoints for DEP in animal studies are repeated dose toxicity (dose-dependent increase in liver and stomach weights, Brown et al., 1978) and reproductive and developmental toxicity (reduced testosterone, increased abnormal and tailless sperm, decreased pup weight and developmental delays, Fujii et al., 2005)) observed in rodents. The NOAELs identified for risk characterisation are 150 mg/kg bw/d (repeated dose toxicity), 40 mg/kg bw/d (reproductive toxicity) and 197 mg/kg bw/d (developmental toxicity) as listed in Table 30.

Table 30: Overview of studies and dose descriptors per endpoint for DNEL derivation

Endpoint	Study used	Dose descriptor	Remarks on study
Repeated dose toxicity: sub-acute / sub-chronic / chronic (organ weights)	A non-guideline oral toxicity study in SD rats exposed via diet for 16 weeks (**Brown et al. 1978**)	NOAEL: 150 mg/kg bw/d	LOAEL: 750 mg/kg bw/d ↑ relative weights of liver (f) & stomach (m),
Reproductive toxicity: male fertility (effects on testosterone and sperm)	Two-Generation Reproduction Toxicity Study in SD rats (**Fujii et al. 2005**)	NOAEL: 46 mg/kg bw/d	LOAEL: 222 mg/kg bw/d ↑ abnormal and tailless sperms (F1)
Reproductive toxicity: developmental toxicity (post-natal development effects)	Two-Generation Reproduction Toxicity Study in SD rats (**Fujii et al. 2005**)	NOAEL: 197-267 mg/kg bw/d	LOAEL: 1016-1375 mg/kg bw/d ↓ pup weight on PND 21 (m-f, F1, F2) and PND 4-21 (f, F1), delayed pinna detachment (m, F1) and vaginal opening (f, F1)

Although some studies reported the association between liver toxicity and peroxisome proliferation, there is no microscopical or biochemical evidence to explain the mechanism of digestive organs enlargement seen in the critical study following repeated DEP dietary exposure. On this basis, these organ effects could not be excluded and therefore are considered relevant to humans for this risk assessment.

Reduced testosterone production and altered Leydig cell ultra structure by DEP has been reported. In addition, the frequency of abnormal and tailless sperms in the F0 and F1 generations (although that did not alter reproductive performance or affect sperm count and sperm motility) was dose-related slightly but statistically significantly increased at exposures to 197-267 and 1016-1375 mg/kg bw/day (Fujii et al. 2005). Decreased pup weight at weaning and developmental delay (delayed onset of vaginal opening and pinna detachment) were also observed in the high dose group at exposures to 1016-1375 mg/kg bw/day (Fujii et al. 2005). The effects on testosterone and sperm levels and sperm quality observed in several rodent studies are regarded as relevant to human risk assessment.

Overall, the epidemiological studies available do not provide sufficient evidence for a causal relationship between exposure to DEP and adverse health effects in humans. However, elements of the plausible mode of action for DEP effects on the developing male reproductive system are considered likely to be parallel in rats and humans if the exposure level to DEP is high and within a critical window of development. Therefore, in agreement with the assessment of NICNAS (2011) the effects on developmental toxicity observed in animal studies such as decreased pup weight, delayed onset of vaginal opening and pinna detachment are regarded as relevant to human risk assessment.

5.13.2 Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptor for critical health effects

5.14 Conclusions of the human health hazard assessment and related classification and labelling

Based on the submitted data the lead registrant concluded on the non-classification of DEP.

Evaluation of the existing information on the toxicity of DEP indicated that the non-classification of DEP is justified. Following the requirements set down in Annex I of Regulation (EC) No 1272/2008 (CLP) and the data available, DEP does not appear to fulfil the criteria for classification.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO CHEMICAL PROPERTIES

Not evaluated.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Note evaluated.

8 PBT AND VPVB ASSESSMENT

Not evaluated.

9 EXPOSURE ASSESSMENT

9.1 Human Health

9.1.1 Exposure assessment for worker

9.1.2 Exposure assessment for consumers

Several product categories were addressed in the registration dossiers (see 2.2.1). However, it can be assumed that the proportion of exposure derives mainly from scented mixtures, especially air care products or washing and cleaning products, where DEP functions as carrier for fragrances. These consumer products are widely used in households. For example, the German Federal Statistical Office reported a production of 511,000 t of universal cleaning agents and 358,000 t in dish washing agents in 2013 (Statistisches Bundesamt, 2014).

Although the concentration of DEP is low, the widespread use of such consumer products could lead to exposure predominantly by inhalation and dermal contact. The exposure situation should be similar to that of cosmetics. Therefore cosmetic exposure assessment is also considered in this chapter.

Modelled exposure, measured internal exposure via human biomonitoring and correlations are described in various publications. Koo and Lee (2004) have estimated the exposure due to the use of cosmetics with different approaches, considering the use of several products during a day. Even for high frequency cosmetic users, and using a conservative approach, the 90 percentile of the exposure did not exceed the reference value.

The Scientific Committee on Cosmetic and Non-Food Products Intended for Consumers (SCCNFP) is of the opinion “that the safety profile of Diethyl-phthalate supports its use in cosmetic products at current levels. At present the SCCNFP does not recommend any specific warnings or restrictions under the currently proposed conditions of use” (SCCNFP, 2002).

DEP was also measured in indoor air (Adibi et al., 2008; Fromme et al., 2013; Hofmann & Plieninger, 2008). Although the concentration is low, it indicates possible sources and potential exposure. All reported values are below the DNEL for long-term-systemic effects recorded by the registrants. Adibi et al. (2008) addressed the associations between DEP in indoor and personal air and its metabolite MEP, but DEP metabolites averaged has a low specificity.

The U.S. Environmental Protection Agency (U.S. EPA) has published a report about the assessment of several phthalates (CHAP, 2014). For DEP they concluded that no Commission action is currently needed, but they recommended further studies.

Under the assumption of similar use conditions for cosmetics and REACH regulated products like air care products and cleaning agents, as well as the missing need for classification, the eMSCA does not see a concern and the need for requesting further information.

10 RISK CHARACTERISATION

Not evaluated.

11 OTHER INFORMATION

The evaluation of the toxicity of DEP has been based on the registration dossiers as well as on reviews by a variety of international bodies/regulatory programs and original publications. Available data for all endpoints have been assessed. DEP has been evaluated by the U.S. Department of Health and Human Services (ATSDR 1995), the International Programme on Chemical Safety (WHO 2003), the Norwegian Scientific Committee for Food Safety (VKM 2005), and the Australian Department of Health and Aging (NICNAS 2011). Where relevant, the original publications were reviewed and evaluated as indicated in the text. In addition literature was searched in the on-line databases DIMDI, ToxNet (HSDB, Toxline incl. PubMed), ISI Web of Knowledge, and Scopus, latest search June 2014.

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TL 4 (1978b): Dermal LD50, Rat; unpublished study record, confidential

TL 5 (1993): Toxicology and Carcinogenesis Studies of Diethylphthalate, Rats and Mice; unpublished study record, confidential

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2014: Exp. Study genetic toxicity, confidential

2013h: skinPerm model; unpublished study record, confidential

2010: Exp. Study genetic toxicity 01, confidential

2010: Exp. Study genetic toxicity 02, confidential

2010: unpublished study record, confidential

2006: Confidental IUCLID information

1978: subcronic study, rat; unpublished study record, confidential

1978b: toxicity test , Mouse; unpublished study record, confidential

1963: eye irritation, rabbit; unpublished study record, confidential

1962: unpublished study record, confidential

1954a: sub-chronic screening test, rat; unpublished study record, confidential

1954b: sub-chronic screening test, dog; unpublished study record, confidential

13 ABBREVIATIONS

Table 31: List of abbreviations

4-OH-DEP	4-Hydroxy diethyl phthalate
AGD	Anogenital distance
ATE	Acute toxicity estimate
Bw(g)	Body weight (gain)
C&L	Classification and Labelling
CHO	Chinese hamster ovary
CMC	carboxymethyl cellulose
conc.	concentration
d	day(s)
DEP	diethyl phthalate
DES	Diethylstilbestrol
DMSO	dimethyl sulfoxide
EC	Effective Concentration
ED	Endocrine disruptor
ER	endoplasmic reticulum
F	Female
FSH	follicle-stimulating hormone
GD	Gestation Day
GLP	Good laboratory praxis
h	hour(s)
HPLC	High Performance Liquid Chromatography
i.v.	intravenous
LH	luteinising hormone
LTW(A)	Lifetime-weighted average
M	Male
MBP	monobutyl phthalate
MEP	Monoethyl phthalate
MIBP	monoisobutyl phthalate
NO(A)EL	no-observed (adverse) effect level
PK	pharmacokinetic
PND	postnatal day
PROC	Process category
RCR	Risk characterization ratio
RMO	Risk management options
SD	Sprague Dawley
SMR	Standardized mortality ratio
SVHC	Substances of very high concern
TSH	thyroid-stimulating hormone
T4	thyroxin
T3	triiodothy-ronine
w	week
WHO	World Health Organization