SCOEL/REC/143
Di-n-butyl phthalate

Recommendation from the
Scientific Committee on Occupational Exposure Limits

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Adopted 14 December 2016

EUROPEAN COMMISSION

Directorate-General for Employment, Social Affairs and Inclusion
Directorate B — Employment
Unit B.3 — Health and Safety

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Luxembourg: Publications Office of the European Union, 2017

ISBN: 978-92-79-66632-2
doi: 10.2767/972673

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RECOMMENDATION FROM THE SCIENTIFIC COMMITTEE ON OCCUPATIONAL EXPOSURE LIMITS FOR DI-N-BUTYL PHTHALATE

8-hour TWA:	0.05 ppm (0.58 mg/m^3)
STEL:	None
BLV:	None
BGV	70 µg mono-*n*-butyl phthalate/l urine
Additional categorisation:	No
Notation:	No

The present Recommendation was adopted by SCOEL on 2016-12-14.

This evaluation is based on ACGIH (2001), ATSDR (2001), ECB (2000), ECB (2003), NTP (2000), WHO (1997), Hartwig (2010) and the references cited in these reviews. Further literature update (covering 2009-2013) was performed in June 2013.

RECOMMENDATION EXECUTIVE SUMMARY

Critical endpoints for occupational exposure to DBP are irritation and reproductive toxicity. There were no adequate human data on DBP to derive a recommended OEL.

Local effects

In an animal subacute study with aerosol inhalation exposure according to OECD guidelines, DBP was irritating to the upper respiratory tract of rats (LOAEC 1.2 mg/m^3, no NOAEC; Gamer *et al* 2000).

Systemic effects including reproductive toxicity

The study by Gamer *et al* (2000) reveals a reliable NOAEC of 509 mg/m^3 for systemic effects after repeated inhalation exposure.

After repeated oral exposure, DBP produced effects on liver, kidney and blood in rats and mice. The LOAEL and NOAEL in the most valid study by Schilling *et al* (1992) were 752 and 152 mg/kg bw/day for rats, respectively.

DBP like diethyl hexyl phthalate induces effects in the liver and the reproductive organs (testes), which may result from the same mechanisms of action. These mechanisms, which are associated with proliferative effects in the liver and the testes and which induce reproductive toxicity are disturbances in intercellular communication or signal transduction, changes in the activities of enzymes involved in steroid metabolism (which probably leads to a reduction in the testosterone level), increased expression of nuclear factor-kappa B (NF-κB) (can lead to the activation of Kupffer's cells), and reduced gene expression (PPAR induced or PPAR independent) (Hartwig 2010).

On the basis of the available data, the effects on the testes are regarded as relevant for humans. Swan *et al* (2005) provided indications that the development of the reproductive organs in humans is influenced by DBP. Also, Schultz *et al* (1999) suggested that the presence of PPAR α in human germ cells has a greater influence on human fertility as compared to rats.

The lowest relevant LOAEL (sperm abnormalities) from studies with repeated administration (4 weeks) in rats is 31.25 mg/kg bw and day. A NOAEL for the effects on the testes cannot be derived (Mitsuhashi *et al* 2004). A benchmark calculation resulted in a lower confidence limit (BMDL) of 1.5 mg/kg bw/day. The findings by Mitsuhashi *et al* (2004) are supported by the occurrence of testicular effects at higher doses in numerous studies.

Concerning developmental effects, the relevant exposure situation at the workplace is prenatal exposure combined with postnatal investigation of the offspring. Such studies resulted in a NOAEL of 50 mg/kg bw and day (Mylchreest *et al* 2000). At the LOAEL of 100 mg/kg bw and day, retention of the areola or nipple (Barlow *et al* 2004, Mylchreest *et al* 2000) and effects on the testes (Mahood *et al* 2007) in rats were observed. Applying route-to-route extrapolation and allometric scaling the NOAEL corresponds to an air NOAEC of 87.5 mg/m^3 (assuming a human body weight of 70 kg and a respiratory volume of 10 m^3, the species-specific correction factor for rats of 4, an assumed oral and inhalation absorption of 100 %).

In summary, with respect to systemic and reproductive toxicity, the BMDL for the most sensitive endpoint, i.e. for sperm abnormalities in rats, is 1.5 mg/kg bw/day, resembling after allometric scaling as described above and additionally the consideration of 5 day exposure of humans as apposed to 7 day weekly exposure in animals an air

concentration of 3.6 mg/m^3. This is about 6-fold higher than the recommended OEL (see below).

Genotoxicity and carcinogenicity

DBP was considered a non-genotoxic substance, as the only clear positive *in vitro* result (mouse lymphoma assay; Hazleton 1986) and a weak positive result *in vivo* (Comet Assay) (Dobrzyńska *et al* 2010) were not confirmed in (negative) *in vivo* micronucleus tests (BASF 1990, NTP 1995).

Adequate long-term studies on carcinogenic effects were not available.

Published studies in rodents (Krauskopf 1973, Nikonorow *et al* 1973, Smith 1953, Kim and Cho 2009a,b), which were all inadequate in methods or conduction, provide no indication for increased tumour incidences.

In developmental toxicity studies by Mylchreest *et al* (1999, 2000), the incidence of Leydig cell adenomas was slightly increased in 30-day old rats and the incidence of Leydig cell hyperplasia was increased in around 100 to 110-day-old rats, which were exposed prenatally (gestation day 12 – 21) to 500 mg/kg bw/day (dams). No carcinomas were observed.

Other phthalate esters have been shown to produce hepatic cancers in rodents, which are attributed to an increased sensitivity of rodents to the induction of peroxisome proliferation compared to monkeys or humans (ECB 2003). Several peroxisome proliferators produce tumours also outside the liver, shown for Leydig cells or pancreatic acinar cells in rats (Biegel *et al* 2001).

DBP leads to the same effects as diethylhexyl phthalate (liver peroxisome proliferation, induction of lipid metabolism enzymes, liver enlargement, malformation of the reproductive organs after prenatal exposure, reduced testosterone concentrations, Leydig cell hyperplasia and multinuclear germ cells), which are the result of proliferative effects on the liver and testes.

Based on mechanistic data, there is therefore some remaining suspicion for non-genotoxic formation of tumours. However, the recommended OEL of 0.05 ppm (0.58 mg/m^3) is far below the NOAEC for systemic effects.

Overall assessment

The recommended OEL is based on the data showing irritating of the upper respiratory tract of rats at 1.2 mg/m^3 of DBP aerosol (LOAEC; no NOAEC) (Gamer *et al* 2000). Within this study, epithelial-like metaplasia in the larynx and hyperplasia of the goblet cells in the nasal cavity were observed at 1.2 mg/m^3. These effects were hardly more pronounced after 500 mg/m^3, an aerosol concentration that is more than two orders of magnitude higher. Also, the local deposition in the nose and larynx is probably higher with exposure in aerosol form, which is not present below concentrations of 1 mg/m^3 DBP. Therefore at concentrations below 1 mg/m^3, an increase in severity of the observed effects are not expected. Based on the comparatively weak effects at the LOAEC of 1.2 mg/m^3 and a flat dose-response-relationship, *an OEL of 0.05 ppm (0.58 mg/m^3) is proposed*.

No STEL is proposed to limit short-term exposure as irritation was only observed after subacute exposure duration, and developmental effects are observed only at higher exposure concentrations compared to the recommended OEL.

Other assignments

A "skin" notation is not recommended. Dermal absorption was shown to be relevant and toxic effects were seen after dermal exposure, but compared to the calculated systemic no effect level of 1.5 mg/kg bw and day (BMDL of the benchmark calculation, see above), the contribution of absorption through the skin is considered to be low (Hartwig 2010).

There are few cases of sensitisation to DBP. With regard to the limited quality of the studies and conflicting results, the human data are not appropriate for a definite conclusion (ECB 2000 and 2003). As a result of its widespread use, the few positive human case-reports and the probably only minimal sensitisation potential in animals, there is no concern (Hartwig 2010).

Biological monitoring

MBP, the major metabolite of DBP, is a marker for biological monitoring. It has to be considered, however, that MBP is also a minor metabolite of benzylbutyl phthalate, which may be relevant in case of simultaneous exposure towards both compounds. However, there are no studies available which quantitatively link biological values and external inhalation exposure. Nevertheless, there are some data published on background exposure of adults in Germany and in the US. Recent 95^{th} percentile values of 68.9 µg MBP per litre urine, respectively, were derived–(CDC 2013). Therefore, urinary levels exceeding 70 µg/l urine may indicate occupational exposure, and this value is regarded as a biological guidance value (BGV). It should be noted that urinary concentrations in women can be higher than those in men.

Sampling, analysis and measurements

Analytical measurement systems exist to determine the recommended levels with an appropriate level of precision and accuracy.

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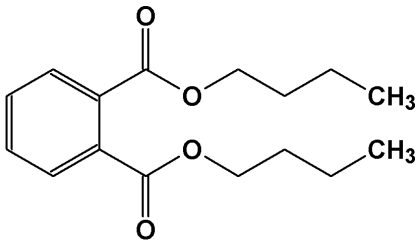
RECOMMENDATION REPORT

1. CHEMICAL AGENT IDENTIFICATION AND PHYSICO-CHEMICAL PROPERTIES

Name:	Di-*n*-butyl phthalate
Synonyms:	DBP; 1,2-benzenedicarboxylic acid, dibutyl ester; phthalic acid, di-*n*-butyl ester; phthalic acid, dibutyl ester; bis-*n*-butylphthalate; butyl phthalate; *n*-butyl phthalate; dibutyl-*o*-phthalate
Molecular formula:	$C_{16}H_{22}O_4$
Structural formula:	
EC No.:	201-557-4
CAS No.:	84-74-2
Molecular weight:	278.34 g/mol
Boiling point:	340 °C
Melting point:	–69°C °C
Vapour pressure (25 °C):	9.7 x 10^{-5} hPa
Conversion factors:	1 ppm = 11.58 mg/m^3;
(20 °C, 101.3kPa)	1 mg/m^3 = 0.086 ppm

Di-*n*-butyl phthalate (DBP) is a colourless oily liquid with a slight ester-like odour. The water solubility of DBP is 10–11 mg/l at 20 °C and the log P_{OW} is 4.6–5.38. The substance has a flash point of 157 °C (closed cup) and a density of 1.05 g/cm^3 (ACGIH 2001, ATSDR 2001, ECB 2000, 2003).

2. EU HARMONISED CLASSIFICATION AND LABELLING

Information about the EU harmonised classification and labelling for Di-n-butyl-phthalate is provided by ECHA, as summarised in Tables XX and YY.

Table 1: Di-n-butyl-phthalate: Classification according to part 3 of Annex VI, table 3.1 (list of harmonised classification and labelling of hazardous substances of Regulation (EC) No1272/2008 ECHA (2015)

Index no.	Internat. Chemical Identification	EC no.	CAS no.	Classification		Labelling			Spec. Conc. Limits, M-factors
				Hazard Class & Category Code (s)	Hazard statement code (s)	Pictogram Signal Word Code (s)	Hazard statement code (s)	Suppl. Hazard statement code (s)	
607-318-00-4	dibutyl phthalate DBP	201-557-4	84-74-2	Repr. 1B Aquatic Acute 1	H360Df H400	GHS09 GHS08 Dgr	H360Df H400		

Table 2: Di-n-butyl-phthalate: Classification according to part 3 of Annex VI, table 3.2 (list of harmonised classification and labelling of hazardous substances from Annex I of Council Directive 67/548/EEC of Regulation (EC) No1272/2008 ECHA (2015)

Classification	Risk Phrases	Safety Phrases	Indication of danger	Concentration Limits	
				Concentration	Classification
Repr. Cat. 2; R61 **Repr. Cat. 3; R62** **N; R50**	61 50 62	53 45 61	T N		

3. CHEMICAL AGENT AND SCOPE OF LEGISLATION

DBP is a hazardous chemical agent in accordance with Article 2 (b) of Directive 98/24/EC and falls within the scope of this legislation.

DBP is <u>not</u> a carcinogen or mutagen for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC.

4. EXISTING OCCUPATIONAL EXPOSURE LIMITS

Occupational exposure limits for di-n-butyl-phthalate exist in a number of countries, as shown in the table below. The values presented below represent examples and are not an exhaustive listing of all limit values within the EU and other countries.

Table 3: Existing OELs for Di-n-butyl-phthalate; adapted from the GESTIS database (GESTIS, 2015)

EU-countries	TWA (8 hrs)		STEL (15 min)		
	ppm	mg/m³	ppm	mg/m³	References
Austria		5			GKV (2011)
Belgium		5			RD (2014)
Denmark		3		6	BEK (2011)
France		5			INRS (2012)
Germany (AGS)	0,05 (1)	0,58 (1)	0,1 (1)(2)	1,16 (1)(2)	BAUA (2006)**
Germany (DFG)	0,05 (1)	0,58 (1)	0,1 (1)(2)	1,16 (1)(2)	DFG (2014)
Ireland		5			HSA (2011)
Latvia		0,5			GESTIS (2015)
Poland		5			MLSP (2002)**
Spain		5			INSHT(2011)
Sweden		3			SWEA (2011)
United Kingdom		5		10	HSE (2011)
Non EU-countries	ppm	mg/m³	ppm	mg/m³	
Australia		5			Safe work Australia (2011)
Canada (Ontario)		5			Ontario Ministry of Labour (2013)
Canada(Québec)		5			IRSST(2010)

China		2,5			GESTIS (2015)
Japan		5			JSOH (2015)
New Zealand		5			HS (2013)
Norway		3			NLIA (2011)
Singapore		5			GESTIS (2015)
South Korea		5			GESTIS (2015)
Switzerland	0,05	0,8	0,1	1,16	SUVA (2015)
USA(NIOSH)		5			NIOSH (2007)
USA(OSHA)		5			OSHA (2006)

Remarks:
Germany (AGS) (1) Inhalable aerosol and vapour (2) 15 minutes reference period
Germany (DFG) (1) Inhalable fraction and vapour (2) 15 minutes reference period
Ireland (1) 15 minutes reference period
Sweden (1) Short-term value, 15 minutes average value

In addition to the above OELs, (SCOEL 2013) established a biological guidance value of 150 µg/l urine.

5. OCCURRENCE, USE AND OCCUPATIONAL EXPOSURE

5.1. Occurrence and use

DBP does not occur naturally in the environment. However, it is found in all environmental media in various concentrations as a result of release from industrial and residential sources. DBP ends up in a large number of consumer products. DBP is mainly used as a plasticiser in polyvinyl chloride polymers (PVC). It is also used as a plasticiser for papers, sealants, paints, printing inks, adhesives, film coatings and glass fibres. It is also used as an antifoaming agent, a plasticiser in nail polish or as a solvent and fixative in cosmetics and synthetic fragrances (ECB 2004) as well as an additive e.g. in lubricants, carpets, tapestry, clothing treatment, concrete, propellants and explosives (ATSDR 2001).

Numerous indirect sources exist in water, air and foodstuffs due to the pervasiveness of DBP in the environment and by contamination through leachable products. In this respect, the wide-spread presence and release of DNP from consumer products has been of concern recently. According to (Kopelovich et al. 2015) in 2012, the California Environmental Protection Agency evaluated 25 nail products and found that 14% of the products that claimed to be DBP-free contained DBP at concentrations ranging up to 88,000 ppm.

The most important entry routes of DBP into the environment during production may be emissions to water and air. Monitoring data from a PVC imitation leather production site in January 1999 gave concentrations in soil (150 m from stack emissions) of 0.02 and 0.09 mg/kg dry weight and an air level (100 m from the emission source) of 0.18 µg/m^3. There was no discharge of process water (ECB 2004).

DBP may be present in food, either due to migration from food contact materials containing DBP or due to its widespread presence as an environmental contaminant (EFSA 2005). In the UK, intakes of DBP from dietary sources were estimated to be up to 0.5 µg/kg bw/day kg in adults (97.5th percentile). In two Danish studies, dietary exposures from 1.6 to 4.1 µg/kg bw/day were reported for adults (EFSA 2005).

After released in the environment DBP slowly volatilize from surface waters, i.e. virtually all of the DBP will remain in the water phase at equilibrium. Soil and sediment thus appear to be important sinks for DBP. Resuspension of DBP from the sediment to the water column may occur. Although DBP is only poorly soluble in water, it may be transported in water following the adsorption of DBP to humic substances. Despite its low volatility, DBP has been reported as particulate and as a vapour in the atmosphere. In the air DBP is transported and removed by both wet and dry deposition. DBP has not a high potential for bioaccumulation (ECB 2004).

5.2. Production and use information

DBP is produced by the reaction of phthalic anhydride with n-butanol in the presence of concentrated sulphuric acid as a catalyst. Excess alcohol is recovered and recycled and the di-n-butyl phthalate is purified by vacuum distillation and/or activated charcoal (ECHA 2009, ECB 2004, ATSDR 2001).

In the EU, there have been 3 production sites located in Belgium, Poland and in the Czech Republic, one of which ceased production in 2006 (ECHA 2009). In 2007 the EU production capacity was less than 10 kt. A significant part of the manufactured tonnage is exported to countries outside the EU. According to ECPI (2008), in Western Europe about one million tonnes of phthalates are produced each year, of which approximately 900,000 tonnes are used to plasticise PVC (polyvinyl chloride). DBP seems to represent less than 1% of the production. The market for DBP has been decreasing over the last decade, mainly because:

- DBP is not permitted for use in toys and childcare articles (Directive 2005/84/EC) or in cosmetics;

- The classification of DBP as toxic to reproduction (Repr. Cat. 2) (ECHA 2009).

In 1998 the production volume of DBP in the EU was estimated at 26,000 tonnes, of which 8,000 tonnes was thought to be exported outside the (ECB 2004). Already at that time there was a clear decreasing trend in the production of DBP from a level of 49,000t/y in 1994. No data has been available for estimating the global production of DBP.

A number of authors have given estimates of the quantitative usage distribution of DBP (Industry report, 1995; BUA, 1987; RIVM, 1991; Canadian EPA, 1994; Cadogan et al., 1994). Based on 1997 data, (ECB 2004) estimated:

- 76% of DBP is used as a plasticizer in polymers,
- 14% in adhesives,
- 7% in printing inks,
- and the remaining 3% of DBP is used in miscellaneous other applications.

The current uses of DBP have been listed by (ECHA 2009) as follows:

- Gelling aid in combination with other plasticisers in plastics. DBP is used in PVC: floor coverings (BASF 2008a), automotive uses and garden hoses (ECB 2004). The European Plastic Converters (EuPC) assumes that DBP today is used by relatively few companies for different niche purposes. In Germany for 1995 it is mentioned that polymer applications are mainly PVC (Leisewitz and Schwarz 1997).

- Rubbers: According to (ECB 2004), DBP is used in some polychloroprene rubber and nitrile rubber, but not in all polychloroprene (neoprene) or nitrile rubbers.

- In the adhesives industry to plasticize polyvinyl acetate (PVA) emulsions (ECPI 2008b). The low viscosity and compatibility of DBP make it suited for PVA-based adhesives for bonding cellulosic materials. The most important uses of the adhesives are for paper and packaging, wood building and automobile industry (ECB 2003);

- Epoxy resins (Feldman et al. 1983);
- In the coatings industry as a primary plasticiser-solvent for nitrocellulose lacquers (ECPI 2008b);
- As grouting agents, used to reduce water leakages in tunnels, sewer systems, buildings etc (ECB 2004);
- Solvent for many oil-soluble dyes, insecticides, peroxides and other organic compounds (ECPI 2008b);
- Antifoam agent and as a fiber lubricant in textile manufacturing (ECPI 2008b);
- In compounding flavours (ZAK 2008);
- Printing inks, polishing agents, corrosion inhibitor materials;
- Use in PP (polypropylene) catalytic systems.

5.3. Routes of Exposure and uptake

According to (ECHA 2009) the production of DBP usually takes place in closed systems. However, both inhalation and dermal exposure may occur during the production of DBP. Such exposures may occur from "breathing" of the system at elevated temperatures, during system leaks, filling of road and rail tankers, drumming, cleaning of tanks, during service and maintenance, transfer, and process sampling.

Because of its many uses, di-n-butyl phthalate is widespread in the environment and has been identified at low levels in all environmental media. Therefore, humans may be exposed to di-n-butyl phthalate by inhalation, by ingestion of water or food containing di-n-butyl phthalate, and by dermal contact with plastics, cosmetics, or other materials containing di-n-butyl phthalate. In air, di-n-butyl phthalate may be adsorbed to particulate matter or occur as a vapour (ECHA 2009).

6. MONITORING EXPOSURE

Dibutyl phthalate can be monitored in the air of the workplace by applying the following methods:

- OSHA 104
- NIOSH method 5020

In the two methods dibutyl phthalate (DBP) is sampled from the air in the workplace by adsorption onto a solid sorbent, followed by extraction of DBP with organic solvents. The DBP-containing extract can then be analysed by gas chromatography (GC), using flame ionisation detection (FID) as shown in Table XXX.

Table 3: Overview of sampling and analytical methods for monitoring Dibutyl phthalate in the workplace.

Method	Sorbent	Desorption solution	Analysis	Recovery (%)	LOQ	Concentration range	References
OSHA 104	OVS-Tenax sampling tube	Toluene	GC-FID	99.1	2.4 ug/m^3	From 0.5 to 2 times the OSHA PEL and ACGIH TLV (5mg/m^3 TWA)	OSHA 1994
NIOSH method 5020	Cellulose ester membrane	Carbon disulfite	GC-FID	n.a.	0.01 mg/sample	0.05-0.5 mg/sample	NIOSH 1994

n.a. not available

The OSHA 104 (OSHA 1994) and the NIOSH 5020 (NIOSH 1994) are completely evaluated methods. The OSHA 104 has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch. NIOSH 5020 is the method that combines and replaces the previous methods S33 and S40.

Occupational exposures to dibutyl phthalate have been quantified using biomonitoring methods (Kwapniewski et al. 2008, Hines et al. 2011). Although it appears that standard methods have not been established, most studies refer to analytical procedures to monitor urinary metabolites of DPB that are described by (Koch et al. 2003, Silva et al. 2004a, Kato et al. 2005, Preuss et al. 2005, Itoh et al. 2005, and Silva et al. 2007). These analytical procedures, involve enzymatic hydrolysis of phthalate metabolite conjugates, preconcentration with solid phase extraction, high performance liquid chromatography (HPLC) separation, and detection by isotope-dilution tandem mass spectrometry. Limits of detection (LODs) were ca.1 ng/mL or less for all phthalate metabolites. Metabolites that have been monitored include MBP, the main metabolite of DBP, and mono-3-carboxypropyl phthalate (MCPP), an oxidative metabolite of DBP, and MiBP, a metabolite of di-iso-butyl phthalate, a structural isomer of DBP.

7. HEALTH EFFECTS

7.1. Toxicokinetics and Absporption, Distribution, Metabolism, Excretion (ADME)

7.1.1. Human data

No human data on the uptake after inhalation exposure were available. DBP is absorbed after ingestion (ECB 2003). A dermal study with 12 volunteers resulted in a maximum flux of 10 µg/cm^2 (Hagedorn-Leweke and Lippold 1995). An *in vitro* study revealed a lower absorption by human skin compared to rat skin (2.4 µg/cm^2/hour vs. 93.4 µg/cm^2/hour) (ECB 2003). A more recent *in vitro* study resulted in a dermal flux of 0.59 µg/cm^2/hour for human skin and 48.9 µg/cm^2/hour for hairless rats (Beydon *et al* 2010).

The metabolite mono-*n*-butyl phthalate (MBP) and its glucuronide have been identified in human urine (n = 149) and blood (n = 283) (ω- or ω-1 oxidation products were not examined). In human serum, the major part of MBP is present as glucuronide, 25-30 % as free MBP (Silva *et al* 2003).

One capsule containing 3 600 µg of DBP was given orally to 17 volunteers. Within 24 hours, 78 % of the administered dose was found in urine as free or glucuronidated MBP. After 24 hours, the MBP level in urine returned to normal showing a fast elimination. Free MBP was observed with a median of 4 % of total MBP (Seckin *et al* 2009).

In a study in Spanish pregnant women (n = 118), the MBP concentration was 27.5 ng/ml with a limit of detection (LOD) for MBP of 0.6 ng/ml urine (Casas *et al* 2011). In Danish men (n = 60), the concentration of MBP was 42.49 ng/ml urine, 0.43 ng/ml serum and 0.77 ng/ml seminal plasma (Frederiksen *et al* 2011).

In 99 healthy volunteers (age 20–25 years), the concentrations in semen samples were 0.39 µg/ml for phthalic acid, 0.06 µg/ml for MBP, and 0.003 µg/ml for DBP (Han *et al* 2009).

7.1.2. Animal data

Quantitative data on absorption by inhalation exposure were not available. The presence of DBP in the organs of rats and the occurrence of systemic effects after inhalation exposure (Kawano 1980) indicate uptake by this route. The gastrointestinal absorption of DBP in rodents was 60 to > 90 % and dermal absorption in rats *in vivo* was about 60 %. The *in vitro* absorption of DBP by rat skin was 93.4 µg/cm^2/hour (ECB 2003).

DBP is widely distributed throughout the body and transplacental transfer has been demonstrated. A relevant amount of DBP (up to 60% within 3 days in rats) is initially excreted in the bile and subsequently enters enterohepatic circulation. Most DBP is cleaved prior to absorption in the small intestine, yielding the monoester (MBP) and butanol. Hydrolysis can also occur in liver and kidneys. The main metabolites in urine are MBP (unbound or as glucuronide), ω- or ω-1 oxidation products of MBP (hydroxyl and keto compounds) and traces of free phthalic acid. The glucuronidation of MBP in hamsters is more efficient than in rats (ECB 2003).

Most of the absorbed compound (independent of the route) is excreted in urine, whereas faecal elimination is low (ATSDR 2001, ECB 2003, WHO 1997). DBP has been identified in human breast milk in concentrations of 10–51 µg/kg (ECB 2003).

7.1.3. In vitro data

7.1.1. Toxicokinetic modelling

7.1.2. Biological Monitoring

In principle, MBP excretion can be used to determine exposure to DBP, but there are uncertainties with regard to the relationship between external and internal exposure. Marsee *et al* (2006) compared two methods estimating the DBP uptake on the basis of human urinary MBP concentrations and found a difference of about 20 % between these methods. The medians of 16.2 µg/l urine and 20.6 µg/g creatinine (MBP) of the collective was correlated with exposure estimates of 0.67 or 0.84 µg/kg/day of DBP, depending of the exposure model used.

Cahill *et al* (2005) used a physiologically based pharmacokinetic (PBPK) model to estimate the amount of MBP retained in the body of rats 24 hours after oral exposure to 100 mg DBP/kg (unconjugated: 0.1–4.9 %, conjugated: 0.1–5.1 %, each stated as 5–95 percentile range). The total urinary elimination was estimated to be 86–99 % of the administered dose, but the amount of MBP was not specified.

Some authors discussed the possibility of measuring DBP metabolites in mother's milk or amniotic fluid (Calafat *et al* 2006), but a relationship between external and internal exposure is not yet established.

According to Aylward *et al* (2009), urinary excretion of MBP as a marker of DBP exposure should be used with some caution, because this metabolite can be produced both as the major metabolite of DBP and as a minor metabolite of benzylbutyl phthalate. The amount of MBP potentially attributable to benzylbutyl phthalate should therefore be considered in the evaluation of the MBP concentration as a marker of DBP exposure.

The elimination half-life time for MBP was reported to be 2.6 hours in one subject (Koch *et al* 2012).

With regard to background exposure, urine samples of 19 adults of the general German population were analysed for MBP excretion. Values ranged from 35.1 to 184 µg/l urine, with a median of 91.8 and a 95th percentile of 166 µg/l urine. Based on creatinine, the range was 48.1–149 µg/g creatinine, with a median of 79.3 (Koch *et al* 2005). Thus, based on 1 461 participants, Silva *et al* (2004b) reported a median of 23 µg/l and a 95th percentile of 142 µg/l urine. Nevertheless, the values are based on exposure levels around the year 2000, and may have changed since then. Accordingly lower values were reported for the US general population (20 years and over) within the National Health and Nutrition Examination Survey (NHANES): In 1914 samples, derived within survey years 2009–2010, 95th percentile values of 68.9 µg MBP/l urine and 50.9 µg MBP/g creatinine were found (CDC 2013). The data reported in this survey further show that MBP concentration in urine vary significantly based on gender and age. Therefore other background data available from Sweden (Jönsson *et al* 2005) and Germany (Wittassek *et al* 2007 and Göen *et* al 2011) cannot be used as a basis for a BLV, as these studies involve specific populations such as students only or men only.

Altogether, exposure levels above 70 µg MBP/l urine may be indicative of occupational exposure. It should be noted that urinary concentrations in women can be higher than those in men.

7.2. Acute toxicity

7.2.1. Human data

Accidental ingestion of 10 g DBP by a man caused nausea, vomiting and dizziness, followed by headache, lacrimation, photophobia and severe cornea damage. Urine analysis showed microhaematuria, pathological leukocyte counts and oxalate crystals. The subject recovered completely after one month (Cagianut 1954).

7.2.2. Animal data

The 2-hour inhalation LC_{50} in mice was 25 000 mg/m^3. Symptoms after these high exposures were irritation of the eyes and respiratory tract, slowed respiration, ataxia, pareses and paralysis of the hind limbs. The 4-hour LC_{50} in rats was > 15 680 mg/m^3. Several animals showed red/dark foci in the lung at macroscopic evaluation. Two animals had white foci in all lung lobes (ECB 2003). Cats exposed for 5.5 hours to 110 mg/m^3 showed salivation, restlessness and languor. At concentrations of 1 000 mg/m^3, cats showed irritation of nasal mucous membranes, as did mice exposed for 2 hours to 25 000 mg/m^3 (ECB 2003).

The oral LD_{50} values were 6 300 to > 20 000 mg/kg in rats, 4 840 to > 20 000 mg/kg in mice and 10 000 mg/kg in guinea pig. Signs of toxicity included depressed activity, laboured breathing and lack of coordination. The dermal LD_{50} in rabbits was > 22 000 mg/kg (ATSDR 2001, ECB 2003, WHO 1997).

7.2.3. In vitro data

In vitro data was not available.

7.3. Specific Target Organ Toxicity/Repeated Exposure

7.3.1. Human data

Milkov *et al* (1973) examined 147 workers with employment in the artificial leather manufacture. They were exposed to DBP and other phthalates (1.7–60 mg/m^3) as well as adipates, sebacates and tricresyl phosphate. Polyneuritis was diagnosed in 47 workers, 22 had functional disturbances of the nervous system. The workers complained frequently of pain in the extremities, accompanied by spasms and numbness (increasing from 57 % to 80 % with increasing exposure duration). No control group was included in this study.

Gilioli *et al* (1978) performed a cross-sectional study on 23 workers employed in phthalate ester production who were exposed to DBP and other phthalates at concentrations of 1–5 mg/m^3 (mean). Workers frequently complained of paraesthesia of the limbs and perspiration. Twelve of the workers were diagnosed to have polyneuropathy. In 7 workers, bilateral painful decreased sensitivity of skin or senses of the hands and feet were noted. Three had a decreased sense of vibrations.

Due to the restricted quality of these studies (co-exposure with other substances, conceptional flaws) both studies may not be used for risk assessment.

7.3.2. Animal data

7.3.2.1. Inhalation

Gamer et al (2000) exposed Wistar rats (5 per sex and group) to nominal concentrations of 0, 1, 5, 50 and 500 mg/m^3 (actual: 0, 1.2, 5.6, 49.3, 509 mg/m^3, mass median aerodynamic diameter (MMAD): 1.5–1.9 µm; GSD: ≈ 2.0, purity: 99.8 %) liquid aerosol of DBP via the head/nose on 6 hours/day, 5 days/week for 4 weeks. This study was performed according to OECD guidelines 412 and 407. Absolute lung weights were increased and absolute testes weights were decreased, but both effects were not dose-related and the effects were observed with the relative organ weights. At the high concentration from days 13 to 27, formation of red scabs around the snout was observed after the end of the daily exposure in a maximum of 4 of the 10 animals. The effect was reversible within 18 hours.

The incidence of squamous metaplasia (grade 1) in section I of the larynx was increased at all concentrations and the effect was dose-dependent (Table 1). In sections II–IV of the nasal cavity, evaluated together, minimal to slight goblet cell hyperplasia occurred in all exposed animals with a dose-dependent increase in incidence (Table 2). The severity increased with dose from grade 1 (minimal) to grade 2 (slight). No exposure-related systemic (including neurotoxic) effects were observed in the exposed animals. According to the authors of the study, the effects on the larynx and on the nasal cavity are unspecific adaptive effects resulting from the exposure of the epithelium of the upper respiratory tract to the aerosol. They concluded that the NOAEC of the study is 500 mg/m^3 (Gamer et al 2000). The authors of the EU Risk Assessment Report (ECB 2003) described the effects as an adaptive response, but nevertheless "adverse in nature".

In this study, it was not shown whether the severity of laryngeal metaplasia changes over time. As the tested aerosol concentration of 500 mg/m^3 is very high and the dose–response curve is flat, an intensification of the effects is not to be expected, but there are no data available to date which confirm this. Therefore, based on the available data, the described laryngeal metaplasia and goblet cell hyperplasia (1) has to be seen as a reaction to the inhalation of DBP, and (2) it is not clear from the database whether the effects observed after 4 weeks develop into goblet cell hyperplasia in severity grades higher than 2 or laryngeal hyperplasia after longer periods of exposure. In summary, the LOAEC for local toxicity was 1.2 mg/m^3, no NOAEC can be derived from this study (Hartwig 2010).

Table 1. Incidence of Squamous metaplasia of the larynx in the rat after a 28-day exposure (Gamer et al 2000)

	Di-n-butyl phthalate levels (mg/m^3)				
	0	1.18	5.57	49.3	509
Males [a]	0	1	3	4	5
Females [a]	0	1	3	5	4

[a] n = 5

Table 2. Incidence of goblet cell hyperplasia in the rat after 28-day exposure (Gamer et al 2000)

		Di-n-butyl phthalate levels (mg/m^3)									
		0	1.18	5.57	49.3	509	0	1.18	5.57	49.3	509
		Males [a]					Females [a]				
Total incidences (sections II–IV [b])		0	2	6	11	15	0	7	13	14	15
Section I [b]	grade 1	1	1	1	1	1	1	2	3	1	3
	grade 2	4	4	4	4	4	4	3	2	4	2
Section II	grade 1	0	2	1	3	0	0	3	2	3	0
	grade 2	0	0	2	2	5	0	0	3	2	5
Section III	grade 1	0	0	2	4	1	0	2	3	5	2
	grade 2	0	0	0	0	4	0	0	1	0	3
Section IV	grade 1	0	0	1	2	2	0	2	3	4	3
	grade 2	0	0	0	0	3	0	0	1	0	2

[a)] n = 5
[b)] as no effects were observed in section I, this was not included in the further evaluations

In a study by Kawano (1980), Wistar rats (11–14 males per group) were exposed by inhalation to 0, 0.5 and 50 mg/m^3 DBP mist on 6 hours/day, 6 days/week for 6 months. At the higher concentration, there was a reduced body weight gain and subsequent increases in relative organ weights. Some clinical chemical and haematological parameters were altered at 50 mg/m^3. The NOAEC of this study was 0.5 mg/m^3. This study by Kawano (1980) and other older inhalation studies have a limited design and flaws of data presentation and may therefore not be used for risk assessment (ECB 2003).

7.3.2.2. Oral exposure

Numerous studies examined the effects of repeated exposure of rats and mice (overviews e.g. in ATSDR 2001, ECB 2000 and 2003, WHO 1997). Only the most relevant studies are described below.

In an unpublished study by Schilling et al (1992), Wistar rats (10 per sex and group) were exposed to 0, 0.04, 0.2 and 1 % in feed (mean body doses of 30, 152 and 752 mg/kg/day) for 90 days, according to OECD guideline 408. Effects were only observed at the highest concentration. Liver and kidney weights were increased and there were alterations of haematological parameters and clinical chemical parameters. In addition, hepatic lipid peroxidation (indicated by an increase in the activity of the cyanide-insensitive palmitoyl-CoA oxidase) and a decrease in triiodothyronine (T_3) were observed. The only histopathological finding was a decreased or missing lipid deposition in hepatocytes. Neurofunctional examination revealed no abnormalities related to exposure. The NOAEL of this study was 152 mg/kg/day.

NTP (1995) exposed F344 rats and B6C3F1 mice (10 per sex and group) for 13 weeks to DBP in the diet. The feed concentrations were 0, 0.25, 0.5, 1, 2 and 4 % DBP (176–178, 356–359, 712–720, 1 413–1 540 and 2 943–2 964 mg/kg/day) for rats and 0, 0.125, 0.25, 0.5, 1 and 2 % DBP (163–238, 353–486, 812–971, 1 601–2 137 and 3 689–4 278 mg/kg/day) for mice. In the rat study, no effects could be observed at 0.25 % DBP in feed (177 mg/kg/day, NOAEL). At ≥ 0.5 %, haematological alterations, increased liver and kidney weights and indications of lipid peroxidation were evident. At higher doses, there were alterations in clinical chemical parameters, histopathological liver changes and decreased body weight gain. In the mice study, the kidney weights were increased in all exposed females, but this effect was not dose-related. At 0.5 % and above (812–971 mg/kg/day), there was a decrease in body weight gain and an increase in liver weights. Higher doses produced haematological alterations and histopathological liver changes. ECB (2003) considered the (not dose-related) effects on female kidney weights as adverse (LOAEL of 238 mg/kg/day). According to WHO (1997), however, the NOAEL of this study was 353 mg/kg/day. Alterations in reproductive parameters, starting at feed concentrations of 1 % in rats and 0.5 % in mice, are described in Section 7.8.

Jansen et al (1993) performed a special study on peroxisome proliferation in rats. A 2-week exposure to 20, 60, 200, 600 and 2 000 mg DBP/kg diet increased the activities of the 11- and 12-lauric acid hydroxylase at feed concentrations of 600 mg/kg (60.6 mg/kg/day; NOAEL 200 mg/kg feed, 19.9 mg/kg/day) and the activity of the cyanide-insensitive palmitoyl-CoA oxidase at 2 000 mg/kg feed (212.5 mg/kg/day). These effects indicate peroxisome proliferation due to DBP.

However, PPAR α, which is the relevant receptor for induction of peroxisome proliferation in the liver of rodents, is considerably lower concentrated in the human than in the rodent liver. Peroxisome proliferation in humans is therefore not regarded as relevant for the evaluation (Swan et al 2005, Schultz et al 1999).

Metabolite profiles (metabolomics) were determined of plasma samples of Wistar rats dosed with 150, 1 000 and 7 000 mg/kg diet (approx. 15, 100, 700 mg/kg bw/day) for 28 days. The metabolite changes at the high dose were more pronounced in males than in females. The NOAEL for toxicity and for metabolomic changes was 150 mg/kg diet. At 1 000 mg/kg diet and higher, relative liver weights in males were increased. A part of the profile was consistent with a pattern of changes indicative of peroxisome proliferation, confirmed by increased cyanide-insensitive palmitoyl-CoA oxidation at 7 000 mg/kg feed (significant in males). The NOAEL of this study is 15 mg/kg bw, the LOAEL 100 mg/kg bw (van Ravenzwaay 2010).

7.3.2.3. Dermal exposure

In a limited 90-day rabbit study by Lehman et al (1955), 50 % mortality was reported at a dose of 4 200 mg/kg/day. The exposure also produced skin irritation, dermatitis (no effect doses given) and kidney damage (LOAEL 4 200 mg/kg/day, NOAEL 2 100 mg/kg/day). This study may not be used for risk assessment (ATSDR 2001).

7.3.3. In vitro data

In vitro data was not available.

7.4. Irritancy and corrosivity

7.4.1. Human data

When cosmetic products (deodorants with 4.5 % DBP or nail polish with 9 % DBP) were tested in different patch tests on 13–159 subjects, there were sporadic slight irritations (ECB 2003).

Phthalate particles irritated the eyes of exposed workers (no further details) (ECB 2000).

7.4.2. Animal data

7.4.2.1. Skin

In a study according to OECD test guideline 404, undiluted DBP was slightly irritating to rabbit skin (ECB 2000 and 2003).

7.4.2.2. Eyes

Inhalation exposure of rats to high concentrations of DBP caused irritation of the mucous membranes of the eyes. The instillation of undiluted DBP into the eyes of rabbits produced no or only slight irritation in a study according to OECD test guideline 405 (ECB 2000 and 2003).

Respiratory tract

Acute inhalation exposure of rats to high, unspecified concentrations of DBP caused irritation of the mucous membranes of the respiratory tract in rats, mice and cats (ECB 2003). Repeated inhalation exposure of rats to concentrations of $\geq$ 1.2 mg/m^3 produced irritation of the upper respiratory tract (Gamer et al 2000; see also Section 7.3).

7.4.3. In vitro data

In vitro data was not available.

7.5. Sensitisation

7.5.1. Human data

There are few case reports of sensitisation to DBP following dermal contact to DBP-containing plastic watch strips, hearing aids, glass frames and antiperspirant sprays. When cosmetic products (deodorants with 4.5 % DBP or nail polish with 9 % DBP) or 5 % DBP in petrolatum were tested with different patch tests on 13–159 subjects, no sensitisation was observed. In a routine patch test with a mixture of phthalates (dimethyl, diethyl and dibutyl phthalate, 2 % each, in petrolatum) on 1 532 persons, there was one subject with a positive reaction. Workers who handled PVC granulate were patch tested with DBP. Three out of 30 workers with dermatitis and 5 out of 30 without dermatitis reacted positively. DBP was also positive in a patch test in 8 out of 52 workers, who were exposed to dioctyl phthalate in PVC production (ATSDR 2001, ECB 2000 and 2003, WHO 1997). Out of 199 workers, exposed to metalworking fluids, only two questionable reactions to DBP were observed. There is one case report of an anaphylactic reaction to DBP with pruritus, urticaria and symptoms of the respiratory tract (Geier *et al* 2004, Gall *et al* 1999).

7.5.2. Animal data

No sensitisation was observed in two maximisation tests on guinea pigs (one according to OECD guideline 406 with intradermal induction with 5 % DBP, percutaneous induction with 75 % and challenge with 50 % DBP). A repeated patch test in rabbits with 50 % DBP solution revealed no sensitising reactions (ECB 2000 and 2003). A Buehler test was reported to be positive, but data on possible impurities of the test substance are missing (NTIS 1982).

7.5.3. In vitro data

In vitro data was not available.

7.6. Genotoxicity

7.6.1. Human data

The urinary concentration of MBP, the main metabolite of DBP, was not significantly correlated with the extent of DNA damage in human sperm (measured by the Comet assay) in a study on 141 urine and semen samples from males of the general population in the US (Duty *et al* 2003b).

7.6.2. Animal data

A micronucleus test in NMRI mice (according to OECD guideline 474) revealed a negative result after a single oral administration of doses up to 3 000 mg/kg (BASF AG 1990). DBP did not induce micronuclei in B6C3F1 mice after oral exposure to 2 % in diet (about 4 300 mg/kg/day) for 13 weeks (NTP 1995).

Kleymenova *et al* (2005) exposed pregnant Sprague-Dawley rats orally on gestation days 12–20 to 500 mg/kg bw/day of DBP. Abnormal germ cells (multinucleated gonocytes) in the testes of male foetuses were significantly increased compared to controls. These abnormal gonocytes exhibited aberrant mitoses, but were not apoptotic. Similar results were obtained with male mice (Gaido *et al* 2007).

In male Pzh:Sfis mice, DNA damage in liver and bone marrow cells were evaluated by Comet assay following prolonged exposure to DBP (8 weeks, 3 days per week *per os*, 500 mg/kg bw (1/16 LD_{50}) and 2 000 mg/kg bw (1/4 LD_{50}). Mice were analysed 4 and 8 weeks after the start of exposure and 4 weeks after the end of exposure. Following 8 weeks of exposure to 2 000 mg/kg bw of DBP, decreased body weight of mice and statistically significant decreased absolute and relative liver weights were observed. In addition, higher, however not statistically significant, levels of DNA damage in liver cells were noted. In bone marrow cells, DBP did not induce enhanced frequency of DNA damage. According to the authors, DBP acts as a weak mutagen for DNA of somatic cells (Dobrzyńska *et al* 2010; only abstract available).

7.6.3. In vitro

DNA strand breaks were induced by DBP in human mucosa cells and peripheral lymphocytes *in vitro* without metabolic activation (Kleinsasser *et al* 2001). In human leukocytes *in vitro*, there was no induction of chromosomal aberrations (Tsuchiya and Hattori 1977), but the results cannot be used for risk assessment due to the lack of documentation (ECB 2003).

Mutagenicity tests with DBP in *Salmonella typhimurium* or *Escherichia coli* mostly yielded negative results. A slight increase in mutation frequency was observed in two tests with *Salmonella* strain TA100 and in one test with TA1535, but only without metabolic activation at cytotoxic concentrations. Other authors could not confirm these results. A test on mutagenic effects in yeast was negative (ATSDR 2001, ECB 2003, WHO 1997).

Tests on gene mutations in mouse lymphoma assays (TK-locus in L5178Y cells) gave conflicting results (Hazleton 1986, NTP 1995). Two tests were positive with metabolic activation (but not without S9-mix), another revealed a weakly positive response only without metabolic activation. The effects in the latter test were obtained only at cytotoxic concentrations.

In studies on the induction of chromosomal aberrations or sister chromatid exchanges in Chinese hamster lung (CHL) and ovary (CHO) cells, there were equivocal results (weak positive responses or effects not dose-related). There was no induction of DNA repair in

E. coli or *Bacillus subtilis* (performed without metabolic activation) and no indication of DNA damage in *B. subtilis* (ATSDR 2001, ECB 2003, WHO 1997).

7.7. Carcinogenicity

7.7.1. Human data

Human data on carcinogenic effects were not available.

7.7.2. Animal data

Adequate long-term studies on carcinogenic effects were not available. Krauskopf (1973) did not observe increased tumour incidences in rats exposed to doses of 100–500 mg/kg/day for 15–21 months, but details are lacking. No tumours were reported in rat studies by Nikonorow *et al* (1973) and Smith (1953). In these investigations, the animals were exposed to DBP up to 1.25 % in feed (625 mg/kg/day) for 1 year.

The carcinogenic potential of DBP was evaluated in the framework of a co-exposure study. B6C3F1-mice (20 male, 20 female) were exposed through diet to 5 000 mg/kg of DBP, for 16 and 32 weeks (approx. 750 mg/kg bw/day assuming a body weight of 20 g and a daily feed uptake of 3 g). No treatment-related deaths were seen. Significant differences in body and organ weights between control and treated mice were observed during the study (not clear, if this is a result of co-exposure or exposure to DBP alone). In female mice, an increased incidence (10 %) of oviductal carcinomas was observed after 16 weeks of exposure, but not at the end of the study. There was no incidence of tumour formation in the liver (not clear, if this is a result of co-exposure or exposure to DBP alone). An increase in absolute liver and testes weight, an increase in absolute and relative lung weight and a decrease in relative testes weight were observed in male mice. In females, there was an increase in absolute and relative liver weight, in relative kidney weight, and in absolute lung weight (Kim und Cho 2009a,b).

The study shows some deficiencies for evaluation of carcinogenic potential of DBP: only one dose tested, tumour incidences not reported, detailed data on body weight are missing, wrong header in Table 4. The oviductal carcinomas were observed after 16 weeks of exposure, but not at the end of the study after 32 weeks. According to the authors, a few carcinomas were reported in the oviduct of B6C3F1 mouse after treatment with DBP in the NTP study (NTP 1995) (Section, which could not be ascertained upon a subsequent verification).

7.8. Reproductive toxicity

Several *in vitro* and *in vivo* tests show that DBP interfere with the endocrine system (endocrine-disrupting potential), including the oestrogenic and the thyroid hormone systems (Ghisari and Bonefeld-Jorgensen 2009, Hartwig 2010, Johnson *et al* 2012)

According to Johnson *et al* (2012), the mouse appears to be resistant to *in utero* phthalate-induced foetal testis endocrine disruption, and the human foetal testis responds more like a mouse than the rat. However, all three species, human, rat and mouse exhibited foetal testis mononucleated germ cells (MNG) as a result of DBP application (Klemenova *et al* 2005, Bokelheide 2009, Hallmark *et al* 2007, Lehmann *et al* 2004, Struve *et al* 2009, Heger *et al* 2012).

7.8.1. Human data

Aldyreva *et al* (1975) performed a cross-sectional study on 189 women working in processes involving exposure to DBP (> 0.5 mg/m^3, not further specified) and to other substances. There were indications of hormonal disturbances, reflected in decreases in the frequency of pregnancy and alterations in the vaginal cycle. Due to the flaws of this study, it may not be used for risk assessment (ECB 2003).

Duty *et al* (2003a) reported a dose-response relationship between the urinary concentration of MBP (range: not detectable to 434 µg/l, geometric mean: 15.7 µg/l), the main metabolite of DBP, and the decrease of sperm concentration and sperm motility in a study on 143 urine and semen samples from males with fertility problems of the general population in the US (the authors acknowledged the uncertainty of determining the phthalate burden by a single urine sample). Others confirmed these results (Latini *et al* 2006).

Free testosterone concentrations (but not levels of other hormones) were lower and urinary MBP concentrations (and also mono-ethylhexylphthalate (MEHP) and total phthalates) were higher in 74 workers of a PVC plant in China, compared to 63 unexposed controls. The exposed workers had urine concentrations of 644.3 µg MBP/g creatinine and 565.7 µg MEHP/g creatinine (geometric mean), the controls 129.6 µg MBP/g creatinine and 5.7 µg MEHP/g creatinine (Pan *et al* 2006). Because of the clearly higher exposure of the worker to diethylhexyl phthalate (DEHP) compared to DBP, this study may not be used for risk assessment (Hartwig 2010).

In contrast to these findings, Jönsson *et al* (2005) could not observe associations between MBP concentration in urine and reproductive function in males (semen volume, sperm concentration and motility, epidiymal and prostatic function, reproductive hormones) at urine concentrations of 24 µg/l (50th percentile) in men of the general population of Sweden. In a study by Duty *et al* (2005), only a marginal, but not significant, correlation between urinary concentrations of MBP and the levels of inhibin B, a reproductive hormone, in 295 males of the US general population could be detected. Other reproductive hormones were not affected. The geometric mean of urinary MBP of this collective was 16.8 µg/l (5th percentile: 3.2 µg/l, 95th percentile: 69.9 µg/l).

A recent study on 118 men who were suspected of infertility showed that serum prolactin levels were positively associated with serum DBP. Furthermore, an inverse correlation between semen DBP and serum testosterone was established. Prolactin exerts effects on the hypothalamic-pituitary-testis-axis, where prolactin can inhibit the secretion of gonadotropin-releasing hormone and alter the activity of certain steroidogenic enzymes. In men, excessive prolactin secretion causes decreased testosterone and sperm production (Li *et al* 2011). Pant *et al* (2011) detected in an infertile category DBP in > 80 % of the semen, with a maximum DBP concentration of 13.47 µg/ml in oligoasthenospermic men in comparison to 4.11 µg/ml in asthenospermic and 0.80 mg/ml in fertile subjects. However, in both studies there was co-exposure to DEHP.

Taken together, the data show an association between DBP exposure and male reproductive functions, but a final evaluation is not possible because of inconsistences and co-exposure to other phthalates, especially DEHP. No valid data were available to evaluate the influence of DBP on female fertility. The relation between prenatal phthalate exposure and genital effects was studied by Swan *et al* (2005) and Swan (2006). The authors examined the urinary concentrations of nine phthalate metabolites of 85 US-American pregnant women as predictors of an age-adjusted anogenital index (AGI, anogenital distance (AGD) divided by the body weight) in male offspring. Regression analysis revealed a significant correlation between an increased urinary MBP burden (5th, 50th and 75th percentile: 7.2, 13.5 and 30.9 µg/l, respectively) and a decrease in AGI (p-value 0.031). The decrease in age-adjusted AGI was also significantly correlated with increasing phthalate burden, based on a summary phthalate score (low, medium and high burden of MBP, mono-benzyl, -ethyl and -isobutyl phthalate; p-value 0.009). In

addition, there was a significant relationship between decreased AGI and completeness of testes descent as well as smaller penis size in these boys. Marsee *et al* (2006) estimated the external exposure to DBP (see Section 7.1) to 0.67–0.84 µg/kg/day (median, range 0–5.86 µg/kg/day), depending of the exposure model used. Data about the AGD was available from the historical controls and was found to correlate better with body size than with body weight (Salazar-Martinez *et al* 2004). Other authors (McEwen and Renner 2006) criticise that the AGI was calculated but no other information, such as parental phenotypes, was included in the study by Swan *et al* (2005). McEwen and Renner (2006) criticise that only one sample of urine was available in each case and that no data are available on the gestation day at the time of sampling. The MBP concentration in urine was not standardised to the urine volume. Also, the phthalate concentration seems very low compared to the effective doses in experiments with animals and the AGI cannot be used as the methods are not validated and other influences on foetal development are possible.

According to Sharpe (2005), the study of Swan *et al* (2005) was well carried out but must be verified by further investigations.

In a nested-case-control study in Shanghai during 2005–2006 with 201 newborn-mother pairs (88 low birth weight cases and 113 controls), prenatal DBP exposure was associated with low birth weight (Zhang *et al* 2009).

Main *et al* (2006) investigated a possible correlation between phthalate monoester burden of human breast milk in a Danish-Finnish cohort of 130 mothers (62 with chryptorchid and 68 with healthy boys) and developmental effects in the boys. The median value for MBP was 9.6 µg/l. No association was found between phthalate monoester levels and cryptorchidism. However, monoethyl phthalate and MBP showed significant positive correlations with sex-hormone binding globulin levels, monomethyl phthalate, monoethyl phthalate and MBP with the luteinising hormone/free testosterone ratio and monoisononyl phthalate with luteinising hormone. MBP was negatively correlated with free testosterone. Other phthalate monoesters revealed similar but non-significant tendencies. These data agree with rodent data (see below).

LC/MS-MS analyses of amniotic fluid and urine samples from pregnant women for five phthalate monoesters and the relationships between these and the birth weight, gestational age and AGD of the offspring were compared. There was a significant positive correlation between the creatinine-related MBP concentration in urine and that in amniotic fluid. There was also a significant positive correlation, but only in female offspring, between the MBP concentration in amniotic fluid and the AGD and the birth weight-related AGD (Huang *et al* 2009).

DBP elicited an oestrogenic response in different human cell lines *in vitro*. However, *in vivo* studies in rats (e.g. uterotrophic assay) revealed generally negative results (ECB 2003, Gray *et al* 2006, Hong *et al* 2005, Seidlová-Wuttke *et al* 2005).

Analysis based on a small sample of 74 boys suggests that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys (Swan *et al* 2010). Prenatal exposure to DBP may be inversely associated with the mental and psychomotor developmental indices of infants, particularly males, at 6 months (Kim *et al* 2011).

In a retrospective cohort study in the children of 71 New Zealand soldiers, increases in the incidences of hypospadias ($p < 0.05$), cryptorchidism ($p < 0.05$) and breast cancer ($p < 0.05$) were observed. The veterans were exposed 1948–1960 to DBP applied daily to their clothing as an acaricide to prevent ticktransmitted bush typhus. The authors modelled skin absorption of DBP and calculated a large theoretical absorbed dose of 64 mg/kg bw/day. Questionnaires were used to collect data on the following disorders: cryptorchidism, defects of the penis (respondents were asked to specify, e.g. hypospadias), precocious puberty (female offspring only), low sperm count, reduced

fertility, disorders of the ovary or uterus, breast cancer. Of the 71 veterans included in the study, 58 (81.7 %) had children. Of these 155 offspring, 79 (51 %) were male and 76 (49 %) were female. There were 4 cases (5.1 % of cryptorchismus, compared to 1.09 (in the year 2000) or 0.91 (2005) in the general population. Two cases of hypospadia (2.5 %) were observed; in the general population the incidences were 0.33 % (2000) and 0.3 % (2005). Breast cancer was observed in 3 cases (4 %) of the children of the veterans compared to 0.48 % in 2008 in the general population (Carran und Shaw 2012).

In comments on the study, the following points have been criticised: the study by Carran und Shaw (2012) is neither a cohort nor a case-control study, but a cross-sectional convenience sample and a cluster investigation without determination of the size of the cluster. The reported cases are without statistical power. Selection bias and confounder were present. The assumptions for the calculation of dermally administered doses are flawed (McBride und Schep 2012). Information on the attained ages of the offspring is missing. The comparison with the general population is incorrect, as this comparison rate is an annual incidence rate, while the survey assessed any breast cancer occurring up to the time of the survey. In fact, the 4 % observed cumulative incidence may not be any greater than the usual rate. For hypospadias, the correct national rate for 2000 is 0.65 % and for 2005, 0.55 %; the authors may have included both male and female livebirths in the denominator. A re-calculation indicated that the rate of hypospadias in the survey is marginally statistically significantly higher than this national rate, but not to the extent cited by Carran and Shaw (2012). The same applies to the calculation for cryptorchismus. The corrected values give a relative risk of 2.56 with a 95 % confidence interval of 0.98–6.65, not quite statistically significantly increased. For both cryptorchidism and hypospadias, the rates are based on very small case numbers and so could be due to chance. In addition, the potential for recall and selection bias is high, when using questionnaire data only from the father, and with a minority of subjects responding, (Elwood und Bormann 2012).

It is known from animal experiments that DBP, like DEHP, has anti-androgenic effects which lead to hypospadia, cryptorchism or a reduced AGD (Section 7.8.2). In humans, the evaluation of these parameters provides evidence of possible changes, but the available data do not allow any valid statement (Hartwig 2010).

7.8.2. Animal data

Numerous studies examined the effects of repeated exposure of rats and mice (overviews e.g. in ATSDR 2001, ECB 2000 and 2003, WHO 1997). Only the most relevant studies are described here, as well as more recent studies, which are not yet considered in the reviews.

7.8.2.1. Fertility

DBP was shown to produce testicular damage in rats and mice in numerous studies (Hartwig 2010, NTP 2000, WHO 1997, Chen *et al* 2011).

The lowest LOAEL for testicular damage resulted from a more recent study by Mitsuhashi *et al* (2004) who exposed male F344 rats (from week 10 of age) for 4 weeks to oral doses (intragastric injection) of 0, 31.25, 125 and 500 mg/kg/day (9 animals per group). Organ weights and histological examination of reproductive organs as well as serum testosterone levels and sperm motility showed no alterations. A reduction in sperm counts was only observed occasionally. A significant increase in the percentage of abnormal sperm was evident in all exposed animals and was dose-related (LOAEL 31.25 mg/kg/day, no NOAEL), but only 6 animals were examined. A benchmark calculation resulted in a lower confidence limit (BMDL) of 1.5 mg/kg bw/day for an increase in the frequency of abnormal sperms (Hartwig 2010).

Chen *et al* (2011) showed a significant increase in the serum testosterone level in Sprague-Dawley rats at oral exposure to 250 mg/kg bw/day for 12 weeks, but not after 4 and 8 weeks. Relative testis weight was decreased (not significantly).

A recent study showed that DBP, given orally for two weeks to adult Sprague-Dawley-rats induced oxidative damage in testes at doses of 250 mg/kg bw/day and higher. DBP significantly inhibited superoxide dismutase, glutathione peroxidase, and glutathione while the level of malondialdehyde was significantly increased. These effects were accompanied by decreases in sperm count and motility and decreases in body and testicular weight at 500 mg/kg bw (Zhou *et al* 2010). Sprague-Dawley rats (20 per sex and group) were exposed to DBP in the diet at concentrations of 0, 0.1, 0.5 and 1 % (females: 80, 385 and 794 mg/kg bw/day; males: 52, 256 and 509 mg/kg bw/day) 7 days before mating and during a 112-day cohabitation period. Thereafter, cross-over mating was performed and 2 generations of offspring were generated (NTP 1995, Wine *et al* 1997). The F_0 generation showed reduced body weight gain and increased relative liver and kidney weights at 1 % DBP in the diet and the males showed increased relative epididymis weights, but no alterations in sperm parameters. The number of live pups per litter was reduced dose-relatedly in the continuous breeding phase at all concentrations. The pregnancy and fertility indices were markedly reduced at 1 % in the F_1 mating trial and the relative weights of the reproductive organ of males were decreased, while the other organ weights were increased. Further effects in adult F_1 males of the high dose group were decreased sperm head counts, poorly developed or absent epididymis, testicular atrophy, undescended testes, poorly developed seminal vesicles and underdeveloped prepuce or penis as well as histopathological degeneration of seminal tubuli and testicular hypertrophy. These effects were partly seen also in the 0.5-% group, but to a lesser extent (NOAEL 0.1 %, 52–80 mg/kg/day). Embryotoxic effects observed in this study are described under "developmental toxicity" below. Foster (2005) considered this report to be the most valid study to show more marked adverse effects on reproduction in offspring than in the F_0 generation, suggesting the importance of *in utero* and postnatal exposure.

DBP was administered in the diet at concentrations of 1 (control group), 4, 10, 30, 100, 1 000 and 10 000 mg/kg [0.1 (control), 0.2, 1.7, 6, 60 and 600 mg/kg bw/day] to Sprague-Dawley rats (17 per sex and group) over two generations (Wolfe and Patel 2002, Price 2004). The F_0 generation was exposed for 6 weeks before mating and was bred to produce F_{1a}, F_{1b}, and F_{1c} pups. The F_{1b} adults were raised and bred to produce F_{2a}, F_{2b} and F_{2c} pups. An outbreeding cohabitation was conducted by mating 10 000-mg/kg F_1 males with untreated females and 10 000-mg/kg F_1 females with untreated males. There were no consistent treatment-related effects on body weights, feed consumption, clinical observations, incidental gross findings, mortality and F_0 reproduction, including sperm examination (NOAEL 10 000 mg/kg diet, 600 mg/kg bw). The effects on the offspring are described under "developmental toxicity" below.

In the 13-week rat study by NTP (1995) (F344 strain, for details see Section 7.3), there was a dose-related degeneration of germinal epithelium in testes at concentrations of 1 % in diet (720 mg/kg bw/day, NOAEL 0.5 %, 359 mg/kg bw/day) and above, as well as lower testicular zinc concentrations and testosterone concentrations, and decreases in sperm counts and motility at 2 % (1 540 mg/kg bw/day) and above.

In a multigeneration study performed by Gray *et al* (1999), 10–12 Long Evans hooded rats per sex and group were exposed to 0, 250, 500 and 1 000 mg/kg bw/day by gavage from weaning through adulthood, mating and lactation. Males of the F_0 generation revealed a delayed preputial separation (onset of puberty) at all dose levels. The onset of puberty in females was not affected. A reduced fertility was observed at 500 mg/kg/day and above, attributed to testicular atrophy and reduced sperm counts in males and abortions in females (NOAEL 250 mg/kg bw/day). For developmental effects, see "developmental toxicity" below.

A study by Gray *et al* (2006) with female rats (Long Evans hooded, orally exposed from weaning to adulthood to 0, 250, 500 and 1 000 mg/kg/day by gavage) did not observe delays in vaginal opening, first oestrus or mating indices, but the ovarian hormone production was decreased on gestational day 13 at ≥ 500 mg/kg/day (prior to abortions of litters, see section "developmental toxicity").

An oral dose of 250 mg/kg/day, given from day 1 to 8 of gestation to rats increased the preimplantation losses. The effect was not significant, but dose-dependent. Doses of 500 mg/kg bw/day and above produced an increase in postimplantation losses. As one of the possible causes, suppression of the decidualisation of the uterus is discussed (Ema *et al* 2000a).

Mice are considered to be less sensitive than rats with regard to testis toxicity (Foster 2006). The studies on B6C3F1 mice (NTP 1995) revealed higher testicular zinc concentrations at 0.5 % and above (812 mg/kg bw/day, NOAEL 0.25 %, 353 mg/kg bw/day) and decreased epididymal weights at the highest feed concentration of 2 % (3 689 mg/kg bw/day, NOAEL 1 %, 1 601 mg/kg bw/day). A continuous breeding study was performed by Lamb *et al* (1987) and Morrissey *et al* (1989) using CD-1 mice. The animals were exposed to 0, 0.03, 0.3 and 1 % DBP in the diet (about 39, 390 and 1 300 mg/kg bw/day), starting 7 days before mating and during a cohabitation period of 98 days. At the highest dose, F_0 males showed decreased body weight gain and females an increased liver weight. This dose caused a decrease in fertility, of the number of litters and number of live pups. A cross-over mating trial revealed the females to be affected by the exposure. The NOAEL of this study was 0.3 % (420 mg/kg bw/day). Paternal DBP exposure (Dobrzynska *et al* 2011) to 500 mg/kg bw (lowest dose tested) 8 weeks before mating with non-exposed females gave no changes in the fertility of parents and in intrauterine development of the offspring, but growth-retardation of pups and almost twice the number of males than females born in the F_1 generation. Female pups had a 2.5-day delay in vaginal opening. Paternal exposure to 2 000 mg/kg bw resulted in an increase in sperm head malformations in F_1 generation males, but no changes in the fertility and viability of foetuses in F_2 generation.

Summary: effects on fertility

Several studies were performed to investigate the toxic effects on the male reproductive organs and fertility in male and female animals. The lowest dose that increased the percentage of abnormal sperm was 31.25 mg/kg bw and day (Mitsuhashi *et al* 2004). In female rats, an oral dose of 250 mg/kg bw and day from day 1 of gestation led to an increase in preimplantation losses (Ema *et al* 2000a).

A two-generation study with Sprague-Dawley rats resulted in a slight reduction in the number of F_1 offspring and reduced birth weights in the F_2 offspring at 50 mg/kg bw and day, but no systemic toxicity (NTP 1995, Wine *et al* 1997). A second two-generation study with Long-Evans rats, showed reduced preputial separation in the F_0 animals, at the lowest dose tested of 250 mg/kg bw and day and above, and the number of F_2 offspring was reduced (Gray *et al* 1999). Male and female fertility was reduced at doses of 500 and 794 mg/kg bw and day (NTP 1995, Gray *et al* 1999). Therefore, the effects at 50 mg/kg bw and day are thus regarded as toxic on development (see "developmental toxicity" below).

Mice are considered to be less sensitive than rats. The NOAEL in mice is 420 mg/kg bw and day and derived from a cross-over mating trial (Lamb *et al* 1987, Morrissey *et al* 1989). Paternal DBP exposure to 500 mg/kg bw may disturb the sex ratio of the offspring, delay female sexual maturation, and deteriorate the sperm quality of F_1 generation males (Dobrzynska *et al* 2011).

Combination effects

Mitsuhashi *et al* (2004) (see above) reported a decrease of the weights of the reproductive organs, of sperm number and motility in thiocetamid treated rats and even more marked after combined exposure to thiocetamid and DBP. Thiocetamid alone caused liver injury, which was confirmed by histopathological findings and elevated serum levels of liver enzymes. The effects on reproduction could not be observed in rats treated only with DBP, which suggests an enhanced sensitivity to reproductive toxicity due to thiocetamide induced liver lesions. The percent of abnormal sperm also increased following the co-exposure protocol.

7.8.2.2. Developmental toxicity

In the above mentioned multi-generation study in Sprague-Dawley rats (NTP 1995, Wine *et al* 1997), the live pup weights were reduced in the continuous breeding phase at 0.5 % DBP in feed. F_1 pup weights from the treated females of the cross-over protocol were decreased. The weights of the live F_2 offspring were decreased in all exposed groups. The lowest dietary concentration (0.1 %, 50 mg/kg/day) was a LOAEL for embryotoxicity.

In the multi-generation study (Sprague-Dawley rats) reported by Wolfe and Patel (2002) and Price (2004; see above), effects on offspring were seen at the highest dose of 10 000 mg/kg diet (600 mg/kg bw/day). The mean AGD of F_{1a} male pups decreased significantly on the first postnatal day (PND 1), while the decrease in the AGD of F_{1b} male pups was not significant. During the F_1 cohabitation, the mean AGD of high-dose F_{2a}, F_{2b} and F_{2c} male pups decreased significantly. In the outbreeding cohabitation protocol, the mean AGD of male pups born to high-dose F_1 dams was also significantly decreased. Sexual development data of 10 000-mg/kg F_{1b} males revealed significant mean delays in preputial separation and in testicular descent. The only histopathological alteration observed was a seminiferous tubular atrophy in 10 000-mg/kg F_1 males. Sperm examination revealed no changes. The NOAEL of this study was 60 mg/kg bw/day.

Zhang *et al* (2004 a,b) confirmed a NOAEL of 50 mg/kg bw/day for developmental effects in rats. They exposed pregnant Sprague-Dawley rats (20 per group) by gavage to doses of 0, 50, 250 and 500 mg/kg bw/day from gestation day 1 to the end of lactation (PND 21). Part of the offspring was examined at PNDs 4, 21 and 70. Maternal toxicity was not evident. Exposure to ≥ 250 mg/kg bw/day produced a decrease in foetal weights and a decrease in live pups per litter at 500 mg/kg bw/day. The body weight gain of the female offspring was decreased at the two higher doses, but the significance of the finding was not stated. The AGD and the AGI were reduced in male pups at ≥ 250 mg/kg bw/day at PND 4. At an age of 70 days, the males revealed dose-related decreases in the absolute epididymis weight at ≥ 250 mg/kg bw/day. The prostate weight was decreased significantly only in the mid-dose group. Marked disturbances of the reproductive system of the male offspring were observed at 250 mg/kg bw/day and above, consisting of underdeveloped or absent epididymis, undescended testes, testicular atrophy as well as reduced sperm counts and motility (NOAEL 50 mg/kg bw/day).

Salazar *et al* (2004) fed female Long Evans rats (15 per group) with a diet containing 0, 610 and 2 500 mg/kg of DBP (12 and 50 mg/kg bw/days) 2.5 months before mating with unexposed males, throughout mating, pregnancy and lactation. Part of the pups were examined at PND 14, the remaining offspring were fed the corresponding DBP-containing diets after weaning until 12 weeks of age. Both doses caused decreased maternal weight gain and the pregnancy rate was reduced at the higher dose. Pup weights of both DBP exposed groups at PND 2 were lower than controls, but this effect was only evident in the low dose group on PND 6. At 14 days of age, the testes weights of exposed offspring were markedly reduced, but there was no dose-response relationship. The preputial separation was delayed dose-dependently, reaching statistical significance at the higher dose. Female offspring showed dose-related delayed vaginal opening (about 1.3 days delay at the lower dose and 1.7 days at the higher dose) and a delay in the occurrence of

the first oestrous. The former effect was significant in both exposure groups, the latter only at 2 500 mg/kg diet. The LOAEL of this study is therefore 12 mg/kg bw/day, but is criticised due to inconsistencies in estimating the body doses; 610 and 2 500 mg/kg feed would be expected to result in body doses of 50–70 and 200–300 mg/kg bw/day, respectively (EPA 2006). Decreases in maternal body weight gain indicate maternal toxicity, which may be responsible for the observations in the offspring, were observed in other studies only at higher doses (≥ 500 mg/kg bw/day, EPA 2006). In addition, delayed preputial separation and delayed vaginal opening in offspring showed higher effect levels in other studies as well (> 500 mg/kg bw/day, EPA 2006). Therefore, the body doses estimated by Salazar *et al* (2004) may be too low.

In the multi-generation study by Gray *et al* (1999) with Long Evans hooded rats, there were increases in urogenital malformations or abnormalities and reduced sperm counts in the F_1 generation, which was exposed *in utero* and during lactation to ≥ 250 mg/kg bw/day (no NOAEL). These animals were less fertile than controls as indicated by lower numbers of F_2 offspring.

In a study by Gray *et al* (2006) on female rats (Long Evans hooded, orally exposed from weaning to adulthood to 0, 250, 500 and 1 000 mg/kg bw/day by gavage), midpregnancy abortions were observed (more than 50 % at 500 mg/kg bw/day; NOAEL 250 mg/kg bw/day), which correlated with a decreased ovarian hormone production on gestational day 13 (prior to abortions of litters).

Six to eight pregnant Sprague-Dawley rats were exposed to DBP in diet from day 15 of gestation to PND 21 at concentrations of 0, 20, 200, 2 000 and 10 000 mg/kg feed, corresponding to maternal body doses of 1.5–3, 14.4–28.5, 148.2–290.9 and 712.3–1 378.8 mg/kg bw/day (Lee *et al* 2004). The offspring were sacrificed at different ages (PND 11, postnatal week 11 and 20). At the lowest and highest dose, there was a reduced maternal body weight gain. A decrease in AGD in males of the high dose group was observed at PND 2. A dose-related trend of an increased number of males with retention of nipples/aerolea was evident across all exposed groups at PND 14, but statistical significance was achieved only at the highest dose. The prepubertal foetal relative liver and adrenal weights were increased and testes weights were decreased at 10 000 mg/kg. The onset of male puberty was not affected. In females, vaginal opening occurred slightly delayed at 10 000 mg/kg, but this delay was not significantly different from controls. Female adult offspring showed some extension of the oestrous cycle, but that was not significantly different from controls. At postnatal weeks 11 and 20, the pituitary weights of females were decreased, reaching statistical significance at 10 000 mg/kg in week 11 and at ≥ 200 mg/kg in week 20. Histopathological evaluation at day 21 revealed liver cell hypertrophy in both sexes at the highest dose. All exposed males showed a significant, dose-related reduction of spermatocyte development, but this was reversible at 11 weeks of age (sperm counts were not measured). Scattered foci of aggregated Leydig cells were observed at 200 mg/kg and above (significant at ≥ 2 000 mg/kg). Lesions in epididymis consisted of decreased ductular cross sections of the epididymal duct at dietary concentrations of 2 000 mg/kg and above. At 11 weeks of age, males revealed a loss of germ cell development (significant at ≥ 2 000 mg/kg) as well as a flattening of the surface epithelia in the prostrate at the highest dose. These effects were no longer significant at week 20 (2 000 mg/kg, 10 000 mg/kg not evaluated), and are therefore not considered to be adverse (EPA 2006). The mammary glands of male offspring were affected at examination after 11 and 20 weeks. A vacuolar degeneration of alveolar cells and alveolar atrophy was observed, achieving significance in all exposed animals at week 11 and at ≥ 200 mg/kg at week 20, but the biological significance of the effect is unclear (EPA 2006). Pituitary and testicular effects were seen at higher doses. Based on liver effects, decreases in testes weights, the loss of germ cell development, reduction of AGD and nipple retention, the LOAEL of this study is 10 000 mg/kg feed (712 mg/kg bw/day) and the NOAEL is 148 mg/kg bw/day.

A LOAEL of 100 mg/kg bw/day (no NOAEL) was reported in a Sprague-Dawley rat study by Mylchreest *et al* (1999) for delayed preputial separation in male offspring exposed *in*

utero (gavage of 100, 250 and 500 mg/kg bw/day to dams on gestation days 12–21). This alteration was mainly based on one markedly affected litter. The higher doses produced an increased incidence of malformations of the male reproductive tract at 250 and 500 mg/kg bw/day. There were two animals (from one litter) of the high-dose group with interstitial cell adenoma of the testes in 90-day old offspring.

Mylchreest *et al* (2000) performed a similar study with a wider dose range (0, 0.5, 5, 50, 100 and 500 mg/kg bw/day). The delayed preputial separation observed in the former study by these authors could not be reproduced, but there was an increased number of retained areolas or nipples and a reduced AGD in males with *in utero* exposure to ≥ 100 mg/kg/day. The occurrence of malformations or abnormalities of the male reproductive tract was confirmed in this study at the highest dose. Also, histopathological lesions were evident in testes of male offspring in this group. There was one animal of the high-dose group with interstitial cell adenoma of the testes in 90-day old offspring. The NOAEL of this study was 50 mg/kg bw/day.

Retention of aerola in male offspring of rats was also observed after doses of 100 mg/kg bw and day given at gestational days 12–21 (Barlow *et al* 2004).

In the study by Mahood *et al* (2007), a reduced level of testosterone in the testes, Leydig cell aggregation, multinucleated gonocytes, and tubules containing only Sertoli cells and dysgenetic regions were observed in male offspring after oral application of 100 mg/kg bw and day to rats from gestational days 13–20. The NOAEL was 20 mg/kg bw and day.

In a study by Lehmann *et al* (2004), Sprague-Dawley rats were exposed to 0.1–500 mg/kg bw/day by gavage on days 12–19 of gestation in order to examine effects on foetal testosterone levels and foetal testicular gene and protein expression. The exposure reduced foetal testosterone levels in testes in a dose-related manner (61 % reduction at 50 mg/kg bw/day), yielding a no effect level of 30 mg/kg bw/day (only 3–4 foetuses per dam were examined). Alterations of the expression of genes and proteins involved in cholesterol transport and steroidogenesis were observed at 0.1 mg/kg bw/day and above, but without a clear dose-response relationship (Lehmann *et al* 2004).

Feeding of DBP to pregnant females with approximately equal doses of oral DBP exposure compared to gavage, resulted in similar responses in male offspring (Struve *et al* 2009).

In the rat, perinatal Sertoli cell proliferation is androgen dependent and, importantly, shows that similar exposure of mothers to antiandrogenic chemicals before birth and during lactation reduces final Sertoli cell number, with implications for the origin of low sperm counts in testicular dysgenesis syndrome in F_1 rats (Auharek *et al* 2010).

Saillenfait *et al* (1998) exposed Sprague-Dawley rats by gavage with a single dose of 0, 500, 1 000, 1 500 and 2 000 mg/kg on day 14 of gestation. Decreased maternal weight gain was observed at 1 500 and 2 000 mg/kg. There were also increased incidences in resorptions and reduced foetal weights at these dose levels and a decreased number of live pups at 2 000 mg/kg. At doses ≥ 1 000 mg/kg, an increased incidence in skeletal variations was observed. The NOAEL of this study was 500 mg/kg/day.

In utero, the dose level required to induce multinucleated gonocyte (MNG) formation and to decrease steroidogenesis in the foetal testis is similar at 50–100 mg/kg bw/day DBP (Boekelheide *et al* 2009, Hallmark *et al* 2007, Lehmann *et al* 2004, Struve *et al* 2009).

Kleymenova *et al* (2005) exposed pregnant Sprague-Dawley rats on gestation days 12–20 orally to 500 mg/kg bw/day DBP. Abnormal germ cells (MNG) occurring also in the normal foetal rat testis at low frequency) in testes were significantly increased compared to controls. These abnormal gonocytes exhibited aberrant mitoses, but were not apoptotic. The Sertoli cells had retracted apical processes, altered organisation of vimentin cytoskeleton and abnormal cell-cell contact with gonocytes. The effects on

Sertoli cells were reversible. Other authors (Alam *et al* 2010) showed that oral administration of 500 mg/kg bw of DBP caused collapse of Sertoli cell vimentin filaments and progressive detachment of spermatogenic cells. Then, detached cells may undergo apoptosis because of loss of the support and nurture provided by Sertoli cells.

Prenatal investigations of after *in utero* exposure of rats to DBP resulted in foetomortality (from gestation day 7), skeletal malformations such as deformed vertebrae and ribs, 14th ribs, dilation of the renal pelvis (from gestation day 7 to 9), external malformations such as cleft palates (from gestation day 13 to 15) and anorectal malformations (from gestation day 12 to 18), reduced AGDs (from gestation day 12 to 14), undescended testes (from gestation day 15 to 17), and Leydig cell hyperplasia (from gestation day 16) (Ema *et al* 1993, 1994, 1997, 1998 and 2000b, Jiang *et al* 2011, Mylchreest *et al* 2002, Saillenfait *et al* 1998). The overall LOAEL was 500 mg/kg bw and day (Ema *et al* 2000b, Mylchreest *et al* 2002).

Further alterations in the rat foetal testis have been reported, e.g. the decreases of insulin-like factor 3 mRNA levels, as well as postnatal development of focal dysgenetic areas, comprising malformed seminiferous cords/tubuli with intracordal/intatubular Leydig cells (ITLCs) and immature Sertoli cells. The ITLCs interfere with Sertoli cell development and cell-cell interactions, leading to the development of Sertoli-cell-only tubules in adulthood. ITLCs were also detected in humans with cryptorchidism and infertility (Mahood *et al* 2006). These mechanisms are probably of concern in humans (Grandjean and Toppari 2006).

By bioinformatically examining foetal testis expression microarray data sets from susceptible (rat) and resistant (mouse) species after DBP exposure, lipid metabolism pathways transcriptionally regulated by sterol regulatory element binding protein (SREBP) were inhibited in the rat but induced in the mouse, and this differential species response corresponded with the repression of the steroidogenic pathway in rats (Johnson *et al* 2011 and 2012).

Human foetal testes were xenografted into castrate male nude mice, rat foetal testis xenografts were exposed as a positive control (Heger *et al* 2012, Mitchell *et al* 2012, to examine the response to phthalates. No suppression of steroidogenesis with phthalate treatment in human and mouse foetal testis was observed in contrast to the rat foetal testis. Consistent with the *in utero* response, phthalate exposure induced MNG formation in rat and mouse xenografts, but only rats exhibited suppressed steroidogenesis (Heger *et al* 2012, Mitchell *et al* 2012). Across a range of doses (100–500 mg/kg bw per day), human foetal testis xenografts exhibited MNG induction but were resistant to suppression of steroidogenic gene expression (Heger *et al* 2012). No inhibition of testosterone or decreased seminal vesicle weight by human testis xenografts following exposure to 500 mg/kg bw per day were observed (Mitchell *et al* 2012).

The results of xenografting suggests that the human foetal testis responds, regarding the steridogenesis, like the mouse foetal testis, in contrast to that of the rat where alteration in steridogenesis is observed (Johnson *et al* 2012).

Studies on rabbits confirmed the anti-androgen effects of DBP exposure *in utero*. Male offspring of dams exposed to 400 mg/kg bw/day on gestation days 15–29 exhibited a marked decrease in testosterone levels, ejaculated sperm, testes weights and accessory sexual gland weights. There were also histological alterations of the testes and a doubling of abnormal sperms. One animal revealed genital malformations (hypospadias), hypoplastic prostate and cryptorchid testes. Similar effects were seen with exposure during adolescence, but to a lesser degree (Higuchi *et al* 2003).

Shiota *et al* (1980) and Shiota and Nishimura (1982) exposed ICR mice on days 0–18 of gestation to 500, 1 000, 2 000, 4 000 and 10 000 mg/kg diet (80, 180, 370, 660 and 2 100 mg/kg bw/day). At the highest dose, maternal weight gain was reduced and the number of resorptions was increased. Some malformations (neural tube defects)

occurred in the high-dose group, but this effect was not statistically significant. Offspring of all exposed animals revealed delayed ossification (some inconsistencies in the dose-response relationship), and foetal weights were decreased at 660 and 2 100 mg bw/kg/day. The authors stated a NOAEL of 370 mg/kg bw/day for embryotoxicity, but NTP (2000) differed from that in considering the lowest dose of 80 mg/kg bw/day as a LOAEL (delayed ossification). However, the sample size was small and the effects are not yet confirmed by others (NTP 2000).

In another study in ICR mice (only summary available), no effects on developmental and maternal toxicity were observed up to 100 mg/kg bw and day. Doses of 250 mg/kg bw and day affected the testes of the offspring (ECB 2003).

In the study by Gaido *et al* (2007), DBP led to morphological changes in the germinal epithelium of mice at a dose of 250 mg/kg bw/day from gestation day 16 to 18.

In neurobehavioral development studies, only the highest feeding dose of DBP (797 mg/kg bw per day during gestation; 1 483 or 1 283 mg/kg bw per day on PNDs 0–15 or 16–28) produced a few effects on the neurobehavioral parameters and may alter the cognitive abilities of male rat offspring (Li *et al* 2009). In a gavage study, the highest dose of 25–675 mg/kg bw per day per gavage from gestation day 6 to postnatal day 28 improved the spatial memory in male rat offspring and this effect may be related to an increase in brain-derived neurotrophic factor (BDNF) expression in the hippocampus in a p-CREB (cyclic adenosinemonophosphate (cAMP) response element binding protein (CREB) independent route (Li *et al* 2010).

Summary: developmental toxicity

As a consequence of the reduced testosterone level, antiandrogenic effects occur in the offspring. Other disturbances in development can occur as a result of changes in the gene and protein expression involved in the development and differentiation of the foetuses (Hartwig 2010).

The NOAEL for effects on foetal testosterone levels in testes was 30 mg DBP/kg bw per day after prenatal doses given to rats on gestation days 12–19. However, even though doses of 50 mg/kg bw and day led to reduced foetal testosterone levels, they were interpreted still not to be adverse, since at this level they were not accompanied by developmental toxicity in pups (Lehmann *et al* 2004); thus, with respect to this endpoint, the NOAEL is 50 mg/kg bw and day.

Studies with prenatal exposition and prenatal investigations in rats revealed foetal mortality, skeletal malformations and external malformations. The LOAEL was 500 mg/kg bw and day (Ema *et al* 2000b, Mylchreest *et al* 2002), the NOAEL for prenatal development was 331 mg/kg bw and day (Ema *et al* 1998).

In mice, no effects on developmental and maternal toxicity were observed up to 100 mg/kg bw and day (Hamano *et al* 1977). Doses of 250 mg/kg bw and day affect the testes of the offspring (Gaido *et al* 2007).

In rabbits, 400 mg/kg bw and day and above caused effects on the testes of the offspring (Higuchi *et al* 2003). A NOAEL cannot be derived for rabbits.

For prenatal and postnatal exposures starting before day 15 of gestation, the NOAEL for alterations of the AGD or retention of the areola or nipple in male animals was 50–60 mg/kg bw/day (Wolfe and Patel 2002, Price 2004, Mylchreest *et al* 2000, Zhang *et al* 2004a,b). Lee *et al* (2004) observed a higher NOAEL for AGD decreases (250 mg/kg bw /day), but the animals were exposed only from day 15 of pregnancy. Because all these studies were performed with Sprague-Dawley rats, strain differences are not responsible for the differences observed. Perhaps there are other causes for this discrepancy, as Gray *et al* (2006) considered gestation days 16–19 as most susceptible to alterations of sexual

differentiation. Out of the two-generation study with continuous mating of Sprague-Dawley rats (NTP 1995, Wine *et al* 1997), a LOAEL of 50 mg/kg bw and day is derived (no NOAEL). Slight effects on the offspring were observed at the lowest dose. Nevertheless, more relevant with respect to workplace exposure are studies with only prenatal exposure and postnatal investigation; in this case, the NOAEL is 50 mg/kg bw and day, which is derived from the study by Mylchreest *et al* (2000).

7.8.3. In vitro data

No data were available

7.9. Mode of Action and adverse outcome pathway considerations

No data were available

7.10. Lack of specific scientific information

There is no specific lack of information.

8. GROUPS AT EXTRA RISK

There is no specific information regarding groups at extra risk.

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KE-02-17-270-EN-N

Publications Office

ISBN: 978-92-79-66632-2

doi: 10.2767/972673