Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

15-Nov-2016

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF DIBUTYL PHTHALATE

Series on Testing & Assessment
No. 249

The corresponding annex is available in the following cote : ENV/JM/MONO(2016)46/ANN1

JT03405433

Complete document available on OLIS in its original format

ENV/JM/MONO(2016)46
Unclassified

English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing & Assessment

No. 249

REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF DIBUTYL PHTHALATE

Joint Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris, 2016

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations.

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This publication is available electronically, at no charge.

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FOREWARD

In 2014, the OECD Task Force on Hazard Assessment (TFHA) and the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (JM) agreed to provide a coordination role for a pilot classification project upon invitation from the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals (UNSCEGHS). A report of the Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals detailing the process of the pilot project and learnings is published along with this report. (Report on the Pilot Project on Assessing the Potential Development of a Global List of Classified Chemicals. ENV/JM/MONO(2016)16/REV1, Series on Testing & Assessment No. 246). It also contains a template for Proposals for Classification and Labelling (Annex 1 to ENV/JM/MONO(2016)16/REV1/ANN1/PART1 & PART2).

Accompanying the report are three case study chemicals where non-binding agreement on their classification have been reached. The results of this pilot project will be submitted to the UNSCEGHS for consideration in their deliberations on the potential development of a global list of classified chemicals.

This report on the Proposal for Classification and Labelling (C&L) of Dibutyl Phthalate was prepared by the United States, with review and input from the project team established for this pilot project under the OECD Task Force for Hazard Assessment. It contains a C&L report as well as an Annex with additional background information.

The following two reports on the Proposal for Classification and Labelling (C&L) are published with this report:

1. Report on the Proposal for Classification and Labelling (C&L) of Dimethyltin Dichloride ENV/JM/MONO(2016)44, Series on Testing & Assessment No. 247.

2. Report on the Proposal for Classification and Labelling (C&L) of Dicyclopentadiene ENV/JM/MONO(2016)45, Series on Testing & Assessment No.248.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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Proposal for Classification and Labelling (C&L)

Based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

International Chemical Identification: Dibutyl Phthalate

CAS Number: 84-74-2

Contact details for dossier submitter:

U. S. Department of Labor
Occupational Safety and Health Administration
200 Constitution Ave, NW
Washington, DC 20210

Version number: 5 Date: 30 August 2016

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information.

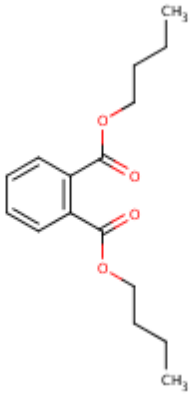
Disclaimer: This proposal for classification and labelling, is for a candidate substance as identified by the UN Sub-Committee on GHS and contributes to the Joint GHS Sub-Committee/OECD Pilot classification exercise (work plan in UN/SCEGHS/28/INF.22, Annex I). It is not intended for any other purposes. The proposal does not represent an official position of the U. S. Occupational Safety and Health Administration

1. IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

International Chemical Identification - **Name(s) in the IUPAC nomenclature or other international chemical name(s)**	Dibutyl phthalate *IUPAC guidance on polymer nomenclature:* http://iupac.org/polyedu/resources/140-Brief-Guide-to-Polymer-Nomenclature-Web-Final-d.pdf - *US EPA guidance on new substances:* http://www.epa.gov/oppt/newchems/pubs/genericnames.pdf - *EU Guidance for identification and naming of substances under REACH and CLP: http://echa.europa.eu/guidance-documents/guidance-on-reach]*
Other names (usual name, trade name, abbreviation)	Dibutyl phthalate DBP Di-n-butyl phthalate 1,2-Benzenedicarboxylic acid, dibutyl ester Benzene-o-dicarboxylic acid di-n-butyl ester Dibutyl 1,2-benzenedicarboxylate Butyl phthalate n-Butyl phthalate 1,2-Benzenedicarboxylic acid, 1,2-dibutyl ester 1,2-Benzenedicarboxylic acid, dibutyl ester o-Benzenedicarboxylic acid, dibutyl ester Phthalic acid, dibutyl ester ortho-Dibutyl phthalate Phthalic acid, dibutyl ester Dibutyl-o-phthalate 4-09-00-03175 (Beilstein Handbook Reference) AI-3-00283 BRN 1914064 Caswell No. 292 CCRIS 2676 Celluflex DPB Di-n-butylester kyseliny ftalove Di-n-butylester kyseliny ftalove [Czech] Elaol EPA Pesticide Chemical Code 028001 Ergoplast FDB Ersoplast FDA Genoplast B Hatcol DBP Hexaplas M/B HSDB 922 Kodaflex DBP NSC 6370 Palatinol C Polycizer DBP PX 104 RC Plasticizer DBP RCRA waste number U069 Staflex DBP Uniflex DBP UNII-2286E5R2KE

	Unimoll db Witcizer 300 RCRA waste no. U069
ISO common name (if available and appropriate)	N/A
CAS number (if available)	84-74-2
Other identifier(s) (if available)	EC 201-557-4 EINECS 201-557-4
In case the substance is already included in a classification list - identifier of the entry	
Molecular formula	C_{16}-H_{22}-O_4
Structural formula (from http://chem.sis.nlm.nih.gov/chemidplus/rn/84-74-2 on 5-13-15**)**	
SMILES notation (if available)	CCCCOC(=O c1ccccc1C(=O)OCCCC)
Molecular weight or molecular weight range	278.34 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not known
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the classification proposal)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum
Not Applicable	

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	The impurity contributes significantly to the classification and labelling [yes/no][1]
Butal-1-ol (CAS 71-36-3)	0.01% (w/w)	No
Butyl benzoate (CAS 136-60-7)	0.01% (w/w)	No

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	The additive contributes significantly to the classification and labelling (yes/no)
None			

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Not applicable				

2. PROPOSED CLASSIFICATION AND LABELLING

2.1 Proposed classification and labelling according to the GHS criteria

The proposed classification in Table 6 is based on information in subsequent tables in this document and its addendums. This information was compiled for the purpose of aiding decisions on classification and does not represent a comprehensive review of all aspects of DBP.

Table 6: Proposed classification and reason for not proposing a classification for a hazard class

GHS chapter ref.	Hazard class or differentiation	Proposed classification - Hazard Class and Category Code(s); Hazard statement Code(s)	Proposed SCL(s) and M-factor(s)	Reason for no proposed classification*
2.1	**Explosives**	No classification		Hazard class not applicable
2.2	**Flammable gases**	No classification		Hazard class not applicable
2.3	**Aerosols**	No classification		Hazard class not applicable
2.4	**Oxidising gases**	No classification		Hazard class not applicable
2.5	**Gases under pressure**	No classification		Hazard class not applicable
2.6	**Flammable liquids**	No classification		Hazard class not applicable
2.7	**Flammable solids**	No classification		Hazard class not applicable
2.8	**Self-reactive substances**	No classification		Hazard class not applicable
2.9	**Pyrophoric liquids**	No classification		Hazard class not applicable
2.10	**Pyrophoric solids**	No classification		Hazard class not applicable
2.11	**Self-heating substances**	No classification		Hazard class not applicable
2.12	**Substances which in contact with water emit flammable gases**	No classification		Hazard class not applicable
2.13	**Oxidising liquids**	No classification		Hazard class not applicable
2.14	**Oxidising solids**	No classification		Hazard class not applicable
2.15	**Organic peroxides**	No classification		Hazard class not applicable
2.16	**Corrosive to metals**	No classification		Hazard class not applicable
2.17	**Desensitized explosives**	No classification		Hazard class not applicable
3.1	**Acute toxicity - via oral route**	No classification		Data conclusive but not sufficient for classification.
	- via dermal route	No classification		Data inconclusive
	- via inhalation route	No classification		Data conclusive but not sufficient for classification.
3.2	**Skin corrosion/irritation**	No classification		Data conclusive but not sufficient for classification

3.3	**Serious eye damage/eye irritation**	No classification		Data inconclusive
3.4	**Respiratory sensitisation**	No classification		Data lacking
	Skin sensitisation	No classification		Data inconclusive
3.5	**Germ cell mutagenicity**	No classification		Data inconclusive
3.6	**Carcinogenicity**	No classification		Data inconclusive
3.7	**Reproductive toxicity**	1B: H360 May damage fertility and unborn child		
3.8	**Specific target organ toxicity-single exposure**	No classification		Data inconclusive
3.9	**Specific target organ toxicity-repeated exposure**	No classification		Data conclusive but not sufficient for classification
3.10	**Aspiration hazard**	No classification		Data lacking
4.1	**Hazardous to the aquatic environment**	Acute 1: H400 Chronic 1 H410	M=1 M=1	
4.2	**Hazardous to the ozone layer**	No classification		Data conclusive but not sufficient for classification

Proposed labelling

Pictogram Code(s):

Health hazard Environment

Signal Word Code(s): Danger

Hazard statement Code(s): H360, H400, H410

Supplemental information*:*

3. IDENTIFIED USES

According to the National Library of Medicine's Hazardous Substance Data Bank (HSDB), dibutyl phthalate is used as a plasticizer; a solvent for oil-soluble dyes, insecticides and other organics; an antifoam agent; a textile fiber lubricant; a fragrance fixative; and an insect repellent. Additional uses identified by EPA (2012) include paints, wood varnishes and lacquers, use in cosmetics, medical supplies, textiles, propellants, food packaging, dental materials, and paper.

4. DATA SOURCES

National Institute of Health, National Library of Medicine: PubMed, ToxNet, ToxLine, Hazardous Substance Data Bank. Google Scholar, Environmental Protection Agency, National Academies of Science/National Research Council, Agency for Toxic Substances and Disease Registry, EU/REACH, OECD ChemPortal, Health Canada, Environment Canada

5. PHYSICOCHEMICAL PROPERTIES

According to the U. S. Environmental Protection Agency dibutyl phthalate is an odorless and colorless to faint yellow oily liquid with a chemical formula of $C_{16}H_{22}O_4$, and molecular weight of 278.35 g/mol. The vapor pressure for dibutyl phthalate is 1.0×10^{-5} mm of Hg at 25 °C., and it has a log octanol/water partition coefficient (log K_{ow}) of approximately 4.5-4.6.

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	Colorless to faint yellow oily liquid	HSDB, 2015	Primary source not available for review
Melting/freezing point	-35°C	http://chem.sis.nlm.nih.gov/chemidplus/rn/84-74-2 accessed 5-13-15	Reliable data source
Boiling point	340°C	http://chem.sis.nlm.nih.gov/chemidplus/rn/84-74-2 accessed 5-13-15	Reliable data source
Relative density	1.049 g/cm^3 (20°C)	ECHA, 2015	Reliable data source
Vapour pressure	2.01E-05 mm Hg at 25 °C	http://chem.sis.nlm.nih.gov/chemidplus/rn/84-74-2 accessed 5-13-15	Reliable data source
Surface tension	34 DYNES/CM= 0.034 N/M at 20°C	HSDB, 2015	Reliable data source
Water solubility	11.2 mg/L at 25°C	http://chem.sis.nlm.nih.gov/chemidplus/rn/84-74-2 accessed 5-13-15	Reliable data source
Partition coefficient n-octanol/water	Log Kow = 4.57 at 20˚C, tested at ≥98% purity Log Kow = 4.46 at 30˚C, tested at >99% purity	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/4/8 http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/4/8	OECD Guideline 107, GLP compliant (Key Study, Klimisch score 2 per REACH dossier) EU Method A.8, not GLP compliant (Key Study, Klimisch score 2 per REACH dossier)
Flash point	157°C	CPSC, 2010; NIOSH Pocket Guide; ICSC Card 0036	Primary source not available for review
Flammability	1	NFPA	Primary source not available for review
Explosive properties	Explosive limits, vol% in air: 0.5 (at 235°C) to about 2.5	ICSC Card 0036 ECHA, 2015	DBP contains no chemical groups associated with explosive properties
Self-ignition temperature	402° C	ICSC Card 0036 ECHA, 2015	Primary source not available for review
Oxidising properties	N/A	ICSC Card 0036 ECHA, 2015	DBP contains no chemical groups associated with oxidizing properties
Granulometry	N/A	ICSC Card 0036 ECHA, 2015	Primary source not available for review
Stability in organic solvents and identity of relevant degradation products	N/A	ICSC Card 0036 ECHA, 2015	Primary source not available for review
Dissociation constant	3.21	ECHA, 2008 http://echa.europa.eu/documents/10162/13638/svhc_supdoc_dibutylphthalate_publication_en.pdf	Primary source not available for review
Viscosity	0.203 poise at 20°C	HSDB, 2015	Primary source not available for review

Supplemental Information: According to the U. S. Environmental Protection Agency dibutyl phthalate is an odorless and colorless to faint yellow oily liquid with a chemical formula of $C_{16}H_{22}O_4$, and molecular weight of 278.35 g/mol. The vapor pressure for dibutyl phthalate is 1.0×10^{-5} mm of Hg at 25 °C, and it has a log octanol/water partition coefficient (log K_{ow}) of 4.5-4.6.

6. EVALUATION OF PHYSICAL HAZARDS

6.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on explosive properties

No studies are available.

According to the registrant it can be concluded from the structural formula that the substance is not explosive as it does not have functional groups associated with explosivity.

Comparison with the GHS criteria

Not applicable – Based on information obtained from ECHA, 2015 the substance contains no chemical groups with explosive properties

Conclusion on classification and labelling for explosive properties

No classification

6.2 Flammable gases

Table 9: Summary table of studies on flammable gases

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on flammable gases

Not applicable – Physical property of DBP is a liquid

Comparison with the GHS criteria

A substance is not classified as explosive if there are no chemical groups associated with explosive properties present in the molecule (examples of groups which may indicate explosive properties are given in table A6.1 in Appendix 6 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria).

Conclusion on classification and labelling for flammable gases

From the structural formula it can be concluded that the substance is not explosive as it does not have functional groups associated with explosivity.

6.3 Aerosols

Table 10: Summary table of studies on aerosols

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on aerosols

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for aerosols

No classification

6.4 Oxidising gases

Table 11: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on oxidising gases

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for oxidising gases

No classification

6.5 Gases under pressure

Table 12: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on gases under pressure

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for gases under pressure

No classification

6.6 Flammable liquids

Table 13: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on flammable liquids

Not applicable – NFPA rating 1; FP=157°C

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for flammable liquids

No classification

6.7 Flammable solids

Table 14: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on flammable solids

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for flammable solids

No classification

6.8 Self-reactive substances

Table 15: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on self-reactive substances

Not applicable – DBP contains no chemical groups with explosive or self-reactive properties

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for self-reactive substances

No classification

6.9 Pyrophoric liquids

Table 16: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on pyrophoric liquids

Not applicable – According to ECHA (2015) ignition on contact with air does not occur; substance is not classified as pyrophoric

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for pyrophoric liquids

Not applicable

6.10 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on pyrophoric solids

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for pyrophoric solids

Not applicable

6.11 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on self-heating substances

Not applicable - DBP does not meet the criteria of a self-heating substance

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for self-heating substances

Not applicable

6.12 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not applicable

6.13 Oxidising liquids

Table 20: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on oxidising liquids

Not applicable – According to ECHA (2015) DBP contains oxygen bound only to carbon; no test necessary, not classified

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for oxidising liquids

Not applicable

6.14 Oxidising solids

Table 21: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on oxidising solids

Not applicable - Physical property of DBP is a liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for oxidising solids

Not applicable

6.15 Organic peroxides

Table 22: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on organic peroxides

Not applicable – DBP does not contain a bivalent structure –O-O-; not a peroxide

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for organic peroxides

Not applicable

6.16 Corrosive to metals

Table 23: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on the hazard class corrosive to metals

Not applicable – ICSC 0036 non-corrosive liquid

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for corrosive to metals

Not applicable

6.17 Desensitized explosives

Table 24: Summary table of studies on desensitized explosive properties

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on desensitized explosive properties

Not applicable – DBP does not meet the criteria for desensitized explosives

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for desensitized explosive properties

Not applicable

7. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 25: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Oral Rat	In the rat, orally administered DBP is rapidly absorbed from the gut and metabolized by hydrolysis of one ester bond and oxidation of the remaining alkyl chain.		Albro and Moore, 1974; EC, 2004
	Intestinal esterases might be important in the absorption and metabolism of phthalate diesters such as DBP.		White et al, 1980
Rodent	Excretion after oral administration is rapid; 63 - >90% of orally administered radioactivity from ^{14}C-DBP given to rats or hamsters was excreted in urine within 24-48h. Fecal excretion is low (1.0-8.2%).	Radioactive tracer	EC, 2004.
Rat	DBP or its metabolites was not significantly retained in any organ in male rats.		Tanaka et al, 1978; Williams and Blanchfield, 1975; (both as cited in EC, 2004)
Rat and human	The major part of DBP is hydrolysed to MBP and the corresponding alcohol prior to absorption by the small intestines, but hydrolysis can also occur in liver and kidneys. The metabolites that occur in urine are MBP, MBP-glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones, carboxylates) and a small amount of free phthalic acid. Species differences in the excretion of MBP and its glucuronide were observed; rats excreted a larger proportion unconjugated MBP in urine than hamsters (EC, 2003; Fennell et al, 2004). As in rats and other species, MBP is the major metabolite in humans (Koch et al, 2012) and Silva et al (2007) suggested that MBP, the major DBP metabolite, is an optimal biomarker of exposure to DBP in rats and humans. Peak concentrations of MBP and other metabolites were at 2-4 hours after an oral dose, followed by a monotonic decline (Koch et al, 2012). Chang et al (2013) also reported rapid degradation of DBP within 2 hours in the rat.		EC, 2003; Fennell et al, 2004; Koch et al, 2012; Silva et al 2007; Chang et al 2013
Human	Oral absorption of DBP can occur in humans who eat food that has been in contact with plastic that contains DBP (Tomita et al, as cited in EC, 2003) and levels of DBP and its metabolites in humans have been reported in several studies such as Wittassek et al (2007), Seckin et al (2009), Han et al (2009), Göen et al (2011), and Saravanabhavan et al (2013).		Tomita et al, as cited in EC, 2004; Wittassek et al 2007; Seckin et al 2009; Han et al 2009; Göen et al 201); Saravanabhavan et al 2013

Rat	In pregnant rats dosed orally with ^{14}C-DBP, unchanged DBP and its metabolites MBP and MBP-glucuronide were rapidly transferred to the embryonic tissues, where their levels were consistently lower than those in maternal plasma. (Saillenfait et al., 1998, as cited in EC, 2003). Clewell et al (2009) reported similar findings in pregnant rats. Fennell et al (2004) found fetal plasma levels of radioactivity from 14C-DBP to be approximately half maternal plasma levels. Levels of several metabolites were reported, plasma MBP being the major metabolite; no parent DBP was detected. The half-life for the maternal plasma MBP was similar in all doses (2.75-2.94 hours). Kremer et al (2005) also found that maternal serum levels of MBP (DBP metabolite) in pregnant rats decreased by 80% within 2 hours after an i.v. dose.		Saillenfait et al., 1998, as cited in EC, 2004; Clewell et al 2009; Kremer et al 2005
Dermal study Multiple species	DBP can be absorbed dermally.		Elsisi et al, 1989; Pan et al, 2014
Inhalation study	Data on absorption after exposure by inhalation are not available.		
Review	Summary findings with overarching review of the toxicity and risk assessment for dibutyl phthalate – expert panel report		NRC, 2008
PBPK model	Alternate models describing enterohepatic circulation, diffusion-limitation, tissue pH gradients (pH trapping), and a simpler, flow-limited model were evaluated. A combined diffusion-limited and pH trapping model was also tested. The combined diffusion-limited and pH trapping model was the best overall, having the highest log-likelihood function value. This result is consistent with a previous finding that the pH trapping model was the best model for describing DEHP and MEHP (DBP metabolites) blood dosimetry, though it was necessary to extend the model to include diffusion-limitation.		Keys et al., 2000
Pharmacokinetic Study in Fish	The absorption half-life (t1/2Ka) of DMP was 3.01 h and 0.020 h at 18°C and 28°C respectively The elimination half-life (t1/2β) of the chemical was 57.62 h at 18°C and 40.16 h at 28°C		REACH Dossier, 2015

Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Animal and human data suggests that DBP has an absorption half-life between 2-3 hours via oral route and the elimination half-life of DBP is between 48-54 hours. Dermal absorption studies indicated 10-12% of the administered dose of 43.7 mg/kg bw per day was excreted in urine for a total of ca. 60% within 7 days. In feces ca.1% of the dose was excreted in 24 hours (totally ca. 12% within 7 days. DBP is widely distributed to most organs including brain and reproductive organs.

Although detailed information on physiologically based pharmacokinetic models is not necessary for decisions on classification and labeling in the present document, it is important to note that PBPK "models have been developed for the two better studied phthalates, DBP and DEHP. Keys et al. (1999, 2000) first developed PBPK models to evaluate the role of various transport processes in the clearance of the metabolites MBP and MEHP in the adult male rat. The models accurately describe plasma MBP and MEHP kinetics after administration of the phthalates. More recently, a PBPK model was developed for disposition of DBP in the adult, pregnant, and fetal rat (Clewell et al. 2009)" (NRC, 2008). "The DBP model has also been extrapolated for use in the human by adjusting the physiologic parameters and scaling chemical-specific parameters allometrically. Preliminary results reported in an abstract (Campbell et al. 2007) indicated that the model was able to predict MBP concentrations in the urine of human adults given controlled doses of DBP without changing chemical-specific parameters; this suggested that the metabolism of DBP to MBP and of MBP to MBP-glucuronide is similar in the rat and human at human-relevant doses. In particular, the kinetics of free MBP and MBP-glucuronide are well described by the allometric scaling" (NRC, 2008).

A pharmacokinetic study submitted as part of a REACH dossier (2015) states that "at both temperatures, more DMP was found in tilapia brain than in other tissues. This showed that DMP was prone to accumulate in brain and hurt the brain. At each temperature, the elimination half-life in skin was longer than in other organs. That indicated that tilapia skin may be regarded as the marker organ of DMP residue. On the other hand, the elimination half-life differed significantly between two temperature groups. In cold water, the value was higher than in warm water. So in cold water the longer half-life of DMP in tissues means a longer withdrawal period." (REACH, 2015)

8. EVALUATION OF HEALTH HAZARDS

Information on the toxicity of DBP is voluminous. For example, searches on the CAS number yielded 2,540 hits in TOXLINE and 729 in PubMed. Therefore a limited number of relevant references were selected and summarized; a comprehensive summary of all available information was not attempted. Summary tables of information selected for several toxicology endpoints were too lengthy to include in the main body of the classification document, and including them would make the document difficult to read. Therefore the tables for several of the sections on toxicology are presented in Appendix 1 rather in the main body of this document. Abbreviated versions of those tables are in the following respective sections together with summaries of the information.

8.1 Acute toxicity

Acute toxicity - oral route

Table 26a: Summary table of animal studies on acute oral toxicity

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
No methodological information. Route of administration: oral. GLP compliance not reported.	Rat	DBP	Oral – dose range not given	8000 mg/kg	Smith, 1953 as cited in EC (2004)
No methodological information. Route of administration: oral. GLP compliance not reported.	Rat	DBP	Oral – dose range not given	6300 mg/kg	BASF, 1961 as cited in EC (2004)
No methodological information. Route of administration: oral. GLP compliance not reported.	Mouse	DBP	Oral – dose range not given	5289 mg/kg	Antonyuk, 1963 as cited by NIOSH (1994)
No methodological information. Route of administration: oral. GLP compliance not reported.	Rat, Sprague-Dawley	DBP	Oral dose -100 and 200 mg/kg	6 out of 10 died within 7 hours at 200 mg/kg; 3 out of 10 died within 2 days at 100 mg/kg	Sajiki et al, 1979, as cited in HSDB (2015)

No methodological information. Route of administration: oral. GLP compliance not reported.	Guinea pig	DBP	Oral – dose range not given	10000mg/kg	Timofeevskaia et al., 1980 as cited by NIOSH (1994)
No methodological information. Route of administration: oral. GLP compliance not reported.	Rat	DBP	Oral – dose range not given	8000mg/kg	Sine, 1993 as cited by NIOSH (1994)
No methodological information. Route of administration: oral. GLP compliance not reported.	Mouse	DBP	Oral – dose range not given	4840 mg/kg	BIBRA, 1987 as cited in EC (2004)
No methodological information. Route of administration: oral. GLP compliance not reported.	Rat	DBP	Oral dose - 4, 8, and 16 g/kg bw	8-10 g/kg bw (4 out of 9 deaths)	Lefaux, 1968 as cited in HSDB (2015)
No methodological information. Route of administration: oral. GLP compliance not reported.	Pregnant female rats	DBP	Oral – dose range not given	0.305 ml/kg (concentration not given)	US EPA, 1980, as cited in HSDB (2015)

Short summary and overall relevance of the provided information on acute oral toxicity

Review of the existing information obtained from HSDB indicated that DBP orally administered in rats caused LD_{60} in rats at 200 mg/kg after 7 hours observation time but no LD_{50} was found (Sajiki et al, 1979). Other studies cited in HSDB and NIOSH indicated a LD_{50} to range between 4.8-10 g/kg in various species (rat, mouse, guinea pig) (Lefaux, 1968; Antanyuk, 1963; Timofeevskaia et al 1980; Sine, 1993; BIBRA, 1987; BASF, 1961; Smith 1953)). None of these studies could be independently analyzed for reliability.

Comparison with the GHS criteria

The range of doses for LD_{50} was 4.8 to 10 g/kg after oral administration of DBP. The GHS criterion indicates that Category 4 cutoff is 2 g/kg. Therefore DBP is not classifiable for acute oral toxicity.

Conclusion on classification and labelling for acute oral toxicity

No classification

Acute toxicity - dermal route

Table 27a: Summary table of animal studies on acute dermal toxicity

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
No methodological information. Route of administration: dermal (unspecified). GLP compliance not reported.	Rabbit	DBP	Dermal – dose range not given	LD_{50} > 20,000 mg/kg bw	Clayton and Clayton, 1994 as cited in EC (2004)

Short summary and overall relevance of the provided information on acute dermal toxicity

Only one study could be identified for this endpoint. A study by Clayton and Clayton (1994) as cited in a report by the European Chemicals Bureau (2004) indicated that DBP produced 50 percent lethality above 20,000 mg/kg body weight. The study could not be independently verified for reliability.

Comparison with the GHS criteria

A GHS criterion for classification due to dermal exposure is 2000 mg/kg. The only study identified examining this endpoint indicates an LD50 at 20000 mg/kg, 10 times higher than category 4 cutoff. Therefore, no classification is necessary for the dermal endpoint.

Conclusion on classification and labelling for acute dermal toxicity

No classification

Acute toxicity - inhalation route

Table 28a: Summary table of animal studies on acute inhalation toxicity

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**

No methodological information. Route of administration: inhalation GLP compliance not reported	Rat	DBP (unknown particle size)	Not indicated	4250 mg/m^3 (4.25 mg/L)	Antonyuk and Aldyreva (1973) as cited by NIOSH
No methodological information. Route of administration: inhalation GLP compliance not reported	Not indicated	DBP (unknown particle size)	Not indicated	25,000 mg/m^3 (25mg/L) with derived value at 4000 mg/m^3 (4 mg/L)	Izmerov et al 1982 as cited by NIOSH
No methodological information. Route of administration: inhalation GLP compliance not reported	Cat	DBP (unknown particle size)	1 mg/L for 5.5 hr	>1 mg/L (nasal irritation observed, but no deaths)	Clayton and Clayton, 1993-1994 , as cited in HSDB, 2015
No methodological information. Route of administration: inhalation). GLP compliance not reported	Mouse	DBP (unknown particle size)	0.25 mg/L for 2 hr	Not provided. Labored breathing, incoordination, paralysis, and convulsions were seen. Deaths 'in some animals".	ACGIH, 2007, as cited in HSDB, 2015
No methodological information. Route of administration: inhalation GLP compliance not reported	Rat – Sprague Dawley Male and female	DBP 4.7 µm MMAD	4 hr; 15.68 mg/L: 1 hr 12.45 and 16.27 mg/L	LC50 ≥ 15.86 mg/L	Greenough et al., 1981 as cited in EC (2004)

Short summary and overall relevance of the provided information on acute inhalation toxicity

Information obtained from toxicity studies indicates some nasal irritation in cats at levels greater than 1mg/L, labored breathing convulsions, paralysis and deaths in some mice (not quantified) at 0.25mg/L. The REACH dossier indicated a rat study provided inconsistent data for extrapolating to dose-rate with the mid-dose range showing deaths at 4 hours (15.68 mg/L) and, no deaths observed at higher concentrations at 1 hour (16.27 mg/L for 1 hour, converted to 4.1 mg/L according to GHS criteria A.1.2.c). Studies cited by NIOSH indicated the LC_{50} ranged from 4.25 to 25 mg/L with the notation that due to the low volatility of DBP the concentrations could only be reached at elevated temperatures or if the liquid droplets became

airborne as in a mist (NIOSH, 1994). Study quality for all those cited could not be verified since primary studies could not be located.

Comparison with the GHS criteria

Study data indicates a range for LC_{50} of 4.25 mg/L to 25 mg/L of DBP (mists). No deaths were observed at 4.1 mg/L in the study showing an LC_{50} of 15.86 mg/L (Greenough, 1981). GHS criteria indicates that Category 4 classification is warranted for concentrations >1.0 and ≤ 5.0. The Greenough study is the only study with reliable data on particle size and is therefore considered primary for classification purposes. The weight of evidence suggests the data indicates no classification is warranted.

Conclusion on classification and labelling for acute inhalation toxicity

Not classified.

8.2 Skin corrosion/irritation

Table 29a: Summary table of animal studies on skin corrosion/irritation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results/Observations	Reference
No methodological information. Route of administration: dermal (unspecified). GLP compliance not reported	Rabbit, 3/sex	Vestinol C (trade name for DBP)	0.5 ml to intact and abraded skin on each animal (2.5 x 2.5 cm)	According to the ECB (2004) mild reaction seen at 24 hr and no reaction by 72 hr. Irritation index was given as 0.54/8. According to ECHA after 4 and 24 hours very slight (grade 1) erythema were observed for 2/3 animals. They were completely reversible within 48 hours	Greenough et al., 1981 as cited in European Chemicals Bureau, 2004 and ECHA
No methodological information. Route of administration: dermal (intradermal and epicutaneous). GLP compliance not reported	Rabbit, 10 in the control groups, 20 in the test group	DBP	5% at intradermal induction, 75% at epicutaneous induction and 50% at challenge 0.5 ml to intact and abraded skin on each animal for 24 hr under occluded patches	Very slight irritation observed.	BASF, 1990 as cited in European Chemicals Bureau, 2004
Subchronic dermal study GLP compliance not reported	Rabbit	DBP	0.5, 1.0, 2.0, 4.0 mL/kg per day for 9-0 days – clipped, intact skin (applied to 10% body surface	Slight skin irritation observed	CIR Expert Panel, 1985

Short summary and overall relevance of the provided information on skin corrosion/irritation

Three rabbit studies were identified that examined skin irritation. The available information suggests that DBP is only very slightly irritating and completely reversible, however, no concentration levels were supplied. Only one study provided an irritation index (0.54/8) (Greenough, 1981). Study quality for all those cited could not be independently verified.

Comparison with the GHS criteria

Only slight or mild irritation was observed at 24 hours but completely reversed by 72 hours. According to the data presented DBP does not meet GHS criteria for classification because the irritant scores are below 2.3 and skin reactions were completely reversed within 72 hours. In addition, DBP does not meet GHS criteria for classification using pH.

Conclusion on classification and labelling for skin corrosion/irritation

No classification

8.3 Serious eye damage/eye irritation

Table 30a: Summary table of animal studies on serious eye damage/eye irritation

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results/Observations**	**Reference**
OECD guideline 405	Rabbit,	DBP	Not indicated	Well defined conjunctival redness observed in all animals at 1 and 24 hours; slight to well-defined redness in all animals at 48 hours; all redness resolved by 72 hours	BASF, 1990 as cited in ECB (2004)
FDA recommended method under GLP conditions	Rabbit, 3/sex	Vestinol C (trade name for DBP)	0.1 ml	1 hr: mild/very mild redness in 6/6 and very mild swelling in 3/6 at24 hr: very mild redness in 2/6 rabbits By 48 hr: All eye appeared normal. Irritation index was reported to be 0.11/110 and was not irritating.	European Chemicals Bureau, 2004

Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Two studies were found that indicate mild reversible eye reaction, both studies are found to be reliable due to use of OECD and FDA test guidelines under GLP conditions. Irritation index was listed as 0.11/110.

Comparison with the GHS criteria

Data from the 2 identified studies indicate the effects observed were completely reversed by 72 hours. However, because scoring information was either not given or was not given as a standardized index no classification can be determined.

Conclusion on classification and labelling for serious eye damage/eye irritation

No classification due to insufficient data

8.4 Respiratory or skin sensitisation

Respiratory sensitisation

Table 31a: Summary table of animal studies on respiratory sensitisation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available.					

Table 31b: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 31c: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on respiratory sensitisation

No relevant studies available

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for respiratory sensitisation

No classification

Skin sensitisation

Table 32a: Summary table of animal studies on skin sensitisation

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results/Observations**	**Reference**
Guinea pig maximization tests; OECD Test 406	Guinea pig	DBP	No information on concentration reported	No sensitization reactions were observed.	BASF, 1990 as cited in European Chemicals Bureau, 2004
Guinea pig maximization tests; FDA recommended method under GLP conditions	Guinea pig	DBP	No information on concentration reported	No sensitization reactions were observed.	Greenough et al., 1981 as cited in ECB, 2004
Repeat patch test	Rabbit	DBP	No information on concentration reported	Not sensitizing to rabbits in a patch test	BASF, 1957 as cited by European Chemicals Bureau, 2004

Short summary and overall relevance of the provided information on skin sensitisation

No observed effects indicated in available studies

Comparison with the GHS criteria

Insufficient information regarding concentration of DBP available to compare to GHS criteria

Conclusion on classification and labelling for skin sensitisation

No classification due to insufficient data

8.5 Germ cell mutagenicity

Table 33a: Summary table of mutagenicity/genotoxicity tests in vitro

Method, test guideline, and deviation(s) if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Results/Observations**	**Reference**

Mouse Lymphoma L5178Y cells GLP compliance not reported	12.5 – 150nl DBP/ml	With and without metabolic activation	DBP induced gene-mutations in the presence of a metabolic activation system; in the absence of a metabolic activation assay negative results were seen.	REACH dossier, 2015
GLP compliance not reported	DBP	Balb/3T3 cells – in vitro assay	DBP did not increase the frequency of transformations in Balb/3T3 cells	Barber et al, 2000
OECD Test Guideline No. 489 Comet assay GLP compliance not reported	DBP	Human cells	DBP induced single-strand breaks in DNA in Comet assay of human epithelial cells (from oropharynx) and human mucosal cells (from inferior nasal turbinate) (Kleinsasser et al, 2000). DBP and DiBP were also positive for genotoxicity in a Comet assay using in human mucosal cells from the oropharynx and in lymphocytes	Kleinsasser et al, 2000
OECD Test No. 473 Chromosomal aberrrations, Sister chromatid exchange GLP compliance not reported	DBP	Chinese hamster cells	DBP had a marginal response in assay for sister chromatid exchange in Chinese hamster cell line, but no chromosomal aberrations	Abe and Sasaki, 1977
OECD test no. 490 GLP compliant	DBP	Mouse – in vitro assay	induced mutations in L5178Y mouse lymphoma cells treated without metabolic activation	NTP, 1995
OECD test no. 490 GLP compliance not reported	DBP	Mouse – in vitro assay	DBP produced significant increases in the frequency of mutations in the mouse lymphoma assay using L5178Y cells in the presence but not in the absence of a metabolic activation system	Barber et al, 2000
OECD 471 GLP compliance not reported	DBP	Bacterial assay - *Saccharomyces cerevisiae* and *Salmonella*	DBP was not mutagenic in *Saccharomyces cerevisiae* or *Salmonella* strains	Shahin and VonBorstel, 1977 Zeiger et al, 1985

Table 33b: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, test guideline, and deviation(s) if any	Test substance,	Relevant information about the study (as applicable)	Results/Observations	Reference
OECD Test No. 474 Micronucleus assay GLP compliance not reported	DBP	Mouse – B6C3F1 0, 0.125, 0.25, 0.5, 1.0, 2.0% in diet	DBP was administered to mice in a diet (equal to 163-4278 mg/kg bw) for 13 weeks-no chromosomal aberrations observed	REACh Dossier, 2015 (indicated study results from 2004)
NTP study GLP compliant	DBP	Mouse – 13 week study	In peripheral blood samples obtained from male and female mice at the end of NTP's 13-week study, frequencies of micronucleated normochromatic erythrocytes were similar between exposed and control mice	(NTP, 1995)

Short summary and overall relevance of the provided information on germ cell mutagenicity

Results of in vitro tests for genotoxicity of DBP were inconclusive, namely being positive (Comet assay for DNA breaks in human cells, mutations in mammalian cell line), negative (transformation of mammalian cells, mutagenicity in yeast and bacteria), and marginal (sister chromatid exchange in mammalian cell line). A micronucleus assay in vivo was negative.

The most robust studies were determined to be from the NTP. The NTP study found that dibutyl phthalate was not mutagenic in Salmonella typhimurium strain TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation but did induce mutations in L5178Y mouse lymphoma cells treated without metabolic activation. In peripheral blood samples obtained from male and female mice at the end of the 13-week study, frequencies of micronucleated normochromatic erythrocytes were similar between exposed and control mice.No germ cell studies were available.

Comparison with the GHS criteria

There was no evidence found from human epidemiological studies as indicated for GHS Category 1A and no studies found to support heritable mutations in humans to support 1B. As the somatic in vivo studies were negative and the in vitro studies indicated both positive and negative results, there is insufficient evidence to conclude that Category 2 is warranted.

Conclusion on classification and labelling for germ cell mutagenicity

No classification due to inconclusive data

8.6 Carcinogenicity

Table 34a: Summary table of animal studies on carcinogenicity

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results and Reference
Research/peer-reviewed No GLP study	Rat	DBP	1800 mg/kg/day; 1, 3, 14 days	The relationship between DBP-induced hypomethylation of the c-Myc promoter region and the expression of c-Myc and DNMT1 genes (at messenger RNA and protein level) was evaluated in the liver of Wistar rats given daily doses of DBP (1800 mg/kg/day) for 1, 3, or 14 days. Conclusions were (1) DBP exerted biological activity through epigenetic modulation of c-Myc gene expression; (2) it seems possible that DBP-induced active demethylation of c-Myc gene through mechanism(s) linked to generation of reactive oxygen species by activated c-Myc; and (3) control of DNA replication was not directly dependent on c-Myc transcriptional activity and we attribute this finding to DNMT1gene expression which was tightly coordinated with DNA synthesis (Urbanek-Olejnik et al, 2013).
Peroxisome proliferation test Research/peer-reviewed No GLP	Human cell line	DBP	In vitro assay	Effects of phthalates might be mediated in part by peroxisome proliferator-activated receptors (PPARs). Evaluations of the monoester metabolites of phthalates as ligands toward PPARs have been investigated. This study evaluated other metabolites, including oxidized derivatives. Results might imply indirect PPAR-mediated mechanisms that lead to observed biological effects such as peroxisome proliferation (Kusu et al, 2008).
Subchronic Oral - dietary	F344 rats Male and Female	DBP	0, 600, 1,200, and 2,100 mg/kg/bw	A NOAEL for increased activities of peroxisome associated enzymes with dietary DBP was less than ~ 600 mg/kg bw, the lowest dose tested (Barber et al, 1987).
Subacute (2 week) Dietary	Wistar rats	DBP	0, 20, 60, 200, 600 and 2,000 mg DBP/kg of diet (equal to 0, 1.1, 5.2, 19.9, 60.6 and 212.5 mg/kg bw	The NOAEL for the induction of peroxisomal associated enzymes in a 2-week dietary study in rats was ~19.9 mg/kg (Jansen et al, 1993).
Subchronic - dietary	Wistar rats 3 male, 3 female /group	DBP	400, 2,000, or 10,000 mg DBP/kg of diet (~ ca. 30, 152 and 752 mg/kg bw)	A NOAEL for hepatic peroxisome proliferation in a 3-month dietary study in rats was ~152 mg/kg (Kaufmann, 1992).
Cancer bioassay				An adequate carcinogenicity study on DBP was not located.

Table 34b: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 34c: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Results/Observations and Reference
Multiple studies were found that suggested possible influences of DBP on proliferation of tumors through hormone-related mechanisms.			
In vitro assay Research study/peer-reviewed No GLP	DBP	Ovarian cell line Gene expression	The estrogenic effects of DBP and HBCD were examined in an ovarian cancer cell line. Results suggest that DBP and HBCD have sufficient potency to disrupt the endocrine system and to stimulate cell growth in estrogen receptor-positive cancer cells (Park et al, 2011).
Research study/peer-reviewed No GLP	DBP	Gene expression study Human breast cell lines Colony formation assays	An investigation of the role of phthalates in the etiology of hormone-independent cancer yielded data to support a possible novel oncogenic mechanism of phthalates in breast cancer that is independent from their estrogenic activities and based on phthalate-induced AhR promoted tumorigenesis of estrogen receptor-negative breast cancer (Hsieh et al, 2011).
In vitro assay Research study/peer-reviewed No GLP	DBP	MCF-7 cell line	In a study of the effect and pathway of phthalates on the growth of MCF-7 breast cancer cells, the results demonstrated that, even at a very low concentration, BBP, DBP, and DEHP were not only still capable of inducing a proliferative effect through the PI3K/AKT signaling pathway but also displaying estrogenic activity (Chen and Chien, 2014).
Research study/peer-reviewed No GLP	DBP	Human prostate carcinoma cell line	In a study on the impact of DEHP and DBP on the proliferation of androgen-sensitive human prostate carcinoma LNCaP cells, data indicated that phthalates may exert long-term negative effects on the proliferation of prostate epithelial cells derived from the carcinoma model (Hrubá et al, 2014).
In vitro assay Research study/peer-reviewed No GLP	DBP	Human prostate carcinoma cell line	Results in LNCaP prostate cancer cells indicate that DBP may induce the growth of LNCaP prostate cancer by acting on the crosstalk between TGF-β and ER signaling pathways (Lee et al, 2014).

Short summary and overall relevance of the provided information on carcinogenicity

There was some evidence that DBP might affect methylation of DNA. Some studies, mainly in cultured cell lines, have been reported on mechanisms by which DBP might influence proliferation of tumors by hormone-related mechanisms, but no demonstration was found to support in vivo activity with tumors. Although DBP might increase peroxisome proliferation, no adequate in vivo studies were found to indicate that DBP is a carcinogen by that or any other mechanisms.

Comparison with the GHS criteria

Criteria for GHS classification in category 1A is largely based on human data with epidemiological data being the main supporting information. Currently no epidemiological studies could be found to support classification under category 1A. Category 1B can be based on information from animal studies with some human evidence. Data from animal studies is not conclusive as most published studies were mechanistic with no bioassay available for review. Category 2 requires that data from a combination of animal and human data be suggestive but not sufficient for category 1. While some of the mechanistic data was suggestive, the data as a whole was inconclusive and therforee insufficient to meet criteria for classification of DBP as a carcinogen.

Conclusion on classification and labelling for carcinogenicity

No classification due to inconclusive data.

8.7 Reproductive toxicity

Adverse effects on sexual function and fertility

Table 35a: Summary table of animal studies on adverse effects on sexual function and fertility

Method, test guideline, and deviation(s) if any	**Species Strain Sex no/group**	**Test substance**	**Dose levels duration of exposure**	**Results and Reference**
Continuous breeding protocol GLP compliance not reported	Rat	DBP	50 mg/kg bw, 500 mg/kg bw;	Fertility was not affected in males or female rats treated with DBP prior to and during mating. Females were treated also during gestation and lactation. The NOAEL for male fertility and embryotoxicity in this study is 500 mg/kg bw, the highest dose tested. The NOAEL in the female fertility study is 50 mg/kg bw study based on maternal toxicity (reduced weight gain) and embryotoxicity (reduced pup weight and, in male pups, testicular lesions and reduced testicular weight) at 500 mg/kg bw (IRDC, 1984).
NTP continuous breeding study GLP compliant	CD-1 mice	DBP	0.03, 0.3, or 1.0% DBP in their diet	In a continuous breeding protocol at NTP, CD-1 mice received 0.03, 0.3, or 1.0% DBP in their diet. During a 105 day dosing period, decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive at 1.0% but not at lower doses. Similar effects were seen in treated females mated with untreated males (Lamb et al, 1987).

NTP continuous breeding study GLP compliant	Sprague-Dawley rat	DBP	0.1, 0.5, and 1.0% DBP on their diet	In another continuous breeding study at NTP, Sprague-Dawley rats received 0.1, 0.5, and 1.0% DBP on their diet. F0 rats had dose-dependent reductions in total number of live pups/litter (all treated groups) and live pup weights (two highest doses). Dose-dependent reductions of postnatal dam weights were seen in all groups. In crossover mating tests to determine the affected sex, the number of offspring was unchanged, but the weights of pups from treated females were significantly decreased. At necropsy, high-dose F0 females had a 14% reduction in body weight. When F1 animals were mated, indices of mating, pregnancy, and fertility at 1.0% were all sharply decreased dam body weight was decreased. Live F2 pup weights were 6-8% lower in all dose groups. F1 necropsy results revealed that epididymal sperm counts and testicular spermatid head counts were significantly decreased at 1.0%. F1 males at 1.0% DBP had degenerated seminiferous tubules and half had underdeveloped or otherwise defective epididymides. No ovarian or uterine lesions were observed (Wine et al, 1997).
Multi-generational study GLP compliance not reported	Long Evans hooded rat	DBP	250, 500, or 1000 mg/kg bw	In a multigenerational study in LE hooded rats, puberty was delayed in males in all groups (250, 500, or 1000 mg/kg bw by gavage from weaning through mating and lactation. Fertility was reduced at 500 and 1000 mg/kg bw and males had testicular atrophy and reduced sperm production. F1 offspring (exposed in utero and lactational) had a low incidence of urogenital abnormalities in both sexes, reduced sperm counts, and reduced fecundity. The LOAEL was 250 mg/kg bw based on delayed puberty and urogenital abnormalities, reduced sperm count, reduced fecundity, and other effects in F1 offspring (Gray et al, 1999).
Oral gavage study GLP compliance not reported	Sprague-Dawley rat	DBP	50 mg/kg/day	This study was performed to determine if exposure in utero to low doses of DBP result in these cellular responses in the fetal testis in Sprague-Dawley rats. Dams were given DBP by oral gavage on GD 12-20. Fetal testes were taken on GD 21 for histological evaluation. The effect of DBP on the size, total cell number, and cordial cross-section number was significant at 50 mg/kg/day. Although there was a trend indicating that the 50 mg/kg/day dose-level increases the incidence of MNG, statistical significance was achieved only at the 100 mg/kg/day dose-level (Kleymenova et al, 2005).

Hershberger assay GLP compliance not reported	Sprague-Dawley rat	DBP	250, 500, or 1000 mg/kg/day	DBP, DEHP, and BBP were tested in the Hershberger assay using Sprague-Dawley rats. DBP was administered to immature males by oral gavage at 250, 500, or 1000 mg/kg/day after dosing with testosterone propionate. DBP did not affect accessory sex organ weights at any dose. These results suggested DBP did not act as an androgen antagonist in this assay (Kang et al, 2005).
Oral dose; generational study GLP compliance not reported	Long Evans rat	DBP	500 and 1000 mg/kg/day	Effects of long-term oral dosing of DBP to female Long Evans hooded rats on reproductive performance was investigated to determine if DBP might have a significant effect on female reproduction. In a two-part study, the authors concluded that DBP can cause a negative effect on female fertility at doses of 500 and 1000 mg/kg/day. Also, the F1 generation is more sensitive to phthalate reproductive toxicity than the F0 generation. The authors concluded that the effect of phthalate exposure on female reproduction was previously over shadowed by phthalate effects on male reproduction because an effect on pregnancy is not seen with shorter term studies. In addition, in standard testing treated females are mated with treated males. As a result of no obvious changes in females, it may have been assumed that infertility was due to the altered male reproductive tract development induced by phthalate exposure (Gray et al, 2006).
Oral dose fetal study GLP compliance not reported	Cynomolgus macaque	DBP	500 mg/kg/day 6 weeks during fetal development	The purpose of this study was to determine if maternal DBP exposure during early pregnancy in female cynomolgus macaques would result in lower maternal estrogen excretion, indicating that the fetal adrenal is a target for DBP. Results supported the concept that DBP treatment (500 mg/kg bw daily orally) for 6 weeks during the time of fetal adrenal formation suppressed fetal adrenal androgen production and that the normal increase of estrogen production during early pregnancy is reduced by DBP exposure (Gee et al, 2007).
Oral dose GLP compliance not reported	Wistar rat	DBP	500, 1000 and 1500 mg/kg bw for 7 days	DBP was given orally at a dose of 500, 1000 and 1500 mg/kg bw for 7 days to Wistar rats. Histological and fertility parameters were assessed. DBP exposure caused dose-dependent testicular toxicity (morphological changes, reduced caudal sperm density and viability, and reduced serum testosterone) (Nair et al, 2008).

Oral dose Pre-pubertal GLP compliance not reported	Sprague-Dawley rat - male	DBP	0.1, 1.0, 10, 100 and 500 mg/kg/day for 30 days	To evaluate effects of low doses of DBP on reproductive parameters and expression of proteins, pubertal male Sprague-Dawley rats were given DBP orally at 0.1, 1.0, 10, 100 and 500 mg/kg/day for 30 days. Endpoints included reproductive organ weights, testicular histopathology and serum hormonal levels, and proteomic analysis. High doses of DBP led to testicular toxicity, and low doses of DBP led to changes in the expression of proteins involved in spermatogenesis as well as changes in the number and function of Sertoli and Leydig cells, although no obvious morphological changes appeared (Bao et al, 2011).
Subcutaneous dose study on pre-pubertal male rat GLP compliance not reported	Wistar rat - male	DBP	20, 200 μg/daily 16 days	In a study to assess the impact of di(n-butyl) phthalate (DBP) on the rat's prepubertal testis, male Wistar rats were injected (s.c.) daily with DBP (20 or 200 μg) from PND 5-15. On PND 16, the rats were euthanized, and the testes were collected for a series of histological endpoints. Also, an estrogenicity *in vitro* test was performed by means of a transgenic yeast strain expressing human estrogen receptor alpha. No effects on testicular development were seen histologically. The in vitro yeast screen showed that DBP was a weak estrogenic compound, approximately six to seven orders of magnitude less potent than 17β-estradiol (Filipiak et al, 2011). [Assuming a body weight of 125 g, doses of DBP were on the order of only 0.16 and 1.6 mg/kg. Thus results seem consistent with other studies.]
Multi-generational study GLP compliance not reported	Mouse	DBP	500 or 2000 mg/kg; 8 weeks	A multigenerational study was performed in mice to investigate the effects of paternal DBP exposure pre- and postnatally on F1 generation offspring, and prenatally on F2 generation offspring. Male mice were exposed to DBP (500 or 2000 mg/kg) for 8 weeks and mated with untreated females. Paternal DBP exposure disturbed the sex ratio of the offspring, delayed female sexual maturation, and deteriorated the sperm quality of F1 generation males (Dobrzyriska et al, 2011).
Oral dose study GLP compliance not reported	Mouse	DBP	1 to 500 mg/kg day;	To determine the impact during prepuberty, the consequences of oral administration of 1 to 500 mg DBP/kg/day to male mice from 4 to 14 days of age was assessed. Among several effects, growth of testes was, affected and proliferation of Sertoli cells was reduced. Also, long-term effects were evident, with smaller anogenital distance and indications of disrupted spermatogenesis in adult mice that had been exposed prepubertally to doses from 1 mg DBP/kg/day (Moody et al, 2013).
Oral gavage study	Rat	DBP	500 mg/kg/day for 30 days	Rats receiving 500 mg DBP/kg/day by gavage for 30 days led to histological structural degeneration in the ductus epididymis and deferens (Sahin et al, 2014).

In vitro assay	Leydig cells	DBP		In a study on Leydig cells, DBP was used to reduce intratesticular testosterone in rats, probably by reduced testicular steroidogenic acute regulatory protein expression, which is associated with increased histone methylation (H3K27me3) in the proximal promoter. Subsequent findings included reduced adult Leydig cell (ALC) stem cell number by ~40% at birth to adulthood and induced compensated ALC failure (low/normal testosterone and elevated luteinizing hormone) (Kilcoyne et al, 2014).
Oral dose GLP compliance not reported	Rat	DBP	0, 200, 400, or 600 mg/kg/day for 15 days	Adult male rats were treated orally with DBP at doses of 0, 200, 400, or 600 mg/kg/day for 15 days. Testicular weight, sperm count, and motility were significantly decreased. Treatment with DBP decreased serum follicle-stimulating hormone, testosterone levels, and testicular lactate dehydrogenase activity. DBP treatment also provoked degeneration with absence of spermatogenesis and sperms and necrosis in some of seminiferous tubules (Aly et al, 2015).
Oral administration GLP compliance not reported	Rat, mouse, hamster, guinea pig	DBP		Species (rat, mouse, hamster, and guinea pig) differed widely in their sensitivity to the testicular toxicity of DBP with oral administration (Gray et al, 1982).
Repeat oral dose GLP compliance not reported	Male rat	DBP	0 or 2400 mg/kg/day for 7 days	In an investigation of testicular effects of short-term dosing with DBP in rats, males received 0 or 2400 mg/kg/day for 7 days. Animals were killed up to 96 hours after the last dose. DBP caused sloughing of germ cells from seminiferous tubules leaving only Sertoli cells. Observed decreases in glucose and fructose concentration in testicular homogenates suggested that DBP might disturb an interaction between Sertoli cells and germ cells (Fukuoka et al, 1989).
Gavage study GLP compliance not reported	Male rat	DBP	250, 500 and 1,000 mg/kg body weight/day for 15 days	To evaluate effects of DBP on testis during early life, DBP was administered to young male rats by gavage at the doses of 250, 500 and 1,000 mg/kg body weight/day for 15 days. Testes weight was decreased with 500 and 1,000 mg/kg. Marked degeneration of seminiferous tubules was noted and the activities of testicular enzymes associated with postmeiotic spermatogenic cells were decreased significantly. Activities of enzymes associated with premeiotic spermatogenic cells, Sertoli cells or interstitial cells were significantly increased. The alterations in activity of testicular cell specific enzymes suggest that DBP exposure during early life could affect the testicular functions. The LOAEL was 250 mg/kg/day, the lowest dose tested (Srivastava et al, 1990).

Oral gavage GLP compliance not reported	Rat	DBP	750 mg/kg/day for 30 days Gene expression assay	The aim of this study was to identify the DBP-induced differentially regulated genes (DEGs) in the testes of male rats using a novel annealing control primer (ACP) system. Rats received DBP by oral gavage for 30 days. Of 59 genes, 31 genes were altered significantly after exposing the rats to high dose of DBP (750 mg/kg/day). Significant differences in the expression levels of selected genes (LDH, lactate dehydrogenase; spag4, a spermatid specific gene and BPR, benzodiazepine receptor) were observed between the DBP-treated and control groups. These results suggest that the spermatogenesis-related genes identified in this study will provide insights into the molecular mechanisms of DBP on the testicular development and dysgenesis. (Ahn et al, 2006).
Oral dose	Pre-pubertal male rat	DBP	0, 250, 500, 1000, 2000 md/kg/day for 30 days	In an investigation of whether the inhibitory effect of DBP on testosterone (T) biosynthesis was mediated by the glucocorticoid (GC) pathway in prepubertal male rats and T production after the exposure to DBP ceased, the resulting data suggested that DBP inhibits testosterone production through a GC-mediated pathway in prepubertal male rats, and after exposure to DBP ceases, testosterone biosynthesis returns (Xiao-feng et al, 2009).

Table 35b: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations and Reference
Case study	DBP, MBP (metabolite)	Human - male	In a study of 168 men who were part of subfertile couples, semen parameters were compared to reference values and concentrations of 8 phthalate monoesters in urine spot samples were measured. A significant relation between monobutyl phthalate (MBP) and decreased sperm motility was found and a suggestive relation between MBP and sperm concentration was seen (Duty et al, 2003).
Epidemiology– case study	DBP, MBP (metabolite)	WHO reference value for sperm concentration and motility	A subsequent study in 463 men from subfertile couples also showed a relationship of altered semen quality (low sperm concentration) with exposure to MBP at general population levels (Hauser et al, 2006).
Cross-sectional study	DBP		A possible relation between DBP concentration in semen and sperm motility was seen in samples from men at reproductive institute in Shanghai (Zhang et al, 2006).
Case study	DBP	Human - female	In a survey of women with and without endometriosis, serum DBP concentrations were significantly higher in infertile women with endometriosis compared to infertile women without endometriosis and fertile women . Serum DBP might be associated with increased endometriosis in women (Reddy et al, 2006).
Case control	DBP, DEHP	Occupational exposure (74 male DBP; 63 control)	Associations between hazard index (HI) of cumulative DBP and DEHP exposures and serum concentrations of free testosterone (fT), estradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was evaluated in 74 men occupationally exposed to high levels of DBP and DEHP. Both T production and hypothalamo-pituitary-testis (HPT) axis function were damaged in workers with high HI of phthalate exposures. HPT feedback function was activated in workers with both high and low HI, and plays an important role in preventing fT level from further decreasing with a rise in HI (Pan et al, 2011). These results suggest effects of exposure of adult men to phthalates, including DBP, and alterations in hormonal control.
Human in vitro study	DBP	Luteal cells isolated from mid-luteal menstruating patients – DBP and examined for changes in gene expression	In a study on the influence of phthalates (including DBP) on the function of human luteal cells, phthalates affected luteal steroidogenesis as well as the balance between luteotrophic and luteolytic factors, suggesting an interference of phthalates in human luteal function. These data may contribute to clarify the classically known impaired reproductive health observed after phthalates exposure (Romani et al, 2014).
Case study	DBP	Male	In an epidemiological study on the relation between urinary phthalate metabolites and reduced testosterone, suggestive relationships were found, including an association in men who were 40-60 years old (Meeker and Ferguson, 2014).

Case study	DBP	Pregnant women	In samples taken to screen for gestational diabetes mellitus, women with the highest urinary concentrations of MiBP and MBzP had lower blood glucose levels. The authors urged care in interpreting these results due to variation in glucose levels, but the findings might have implications on fetal health (Robledo et al, 2015).

Table 35c: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations and Reference
Oral study –	DBP	Prepubertal rat	Testicular toxicity from phthalates in the pubertal-rat model is related to the side chain on the phthalate. The ester side-chain length of linear-chain phthalates needed to be four to six carbon atoms to produce testicular toxicity (Foster et al, 1980).
Investigational in vitro study	DBP	Human and rat testis microsomes	3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) are involved in synthesis of androgens in Leydig cells. In this study, inhibitory activities on these enzymes of 14 different phthalates with various carbon numbers in the ethanol moiety were tested. Clear structure-activity responses for phthalates were found, particularly for the length of carbon chains in the ethanol moieties of phthalates. DBP had one the lowest half maximal inhibitory concentrations (Yuan et al, 2012).
Investigational in vitro study	DBP, MBP	Leydig tumor cell line	In an evaluation of the effects of DBP/MBP on steroidogenesis in the murine Leydig tumor cell line MLTC-1 in vitro, it appeared that alterations of the steroidogenic enzymes and INSL3 in MLTC-1 cells may be involved in the biphasic effects of DBP/MBP on androgen production (Chen X et al, 2013).
Fruit fly - male	DBP	Metabolized to MBP (same as human, rat, mouse, other mammals)	Effects of DBP on the male reproductive system in the fruit fly were comparable to those in mammals (Misra et al, 2014). These results indicate similar effects across a range of species.

Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Numerous studies have been identified evaluating the reproductive effects of DBP, the most reliable animal studies were those conducted by the National Toxicology Program (NTP) (Lamb et al., 1987; Wine et al., 1997) as well as the human studies (Zhang et al., 2006; Hauser et al., 2006). While these could be identified as key studies, the classification for reproductive toxicity was evaluated using a weight of evidence approach.

DBP has been shown to have effects on reproduction in adult animals, however, offspring exposed to DBP in utero have even more pronounced effects. For example, although effects of DBP varied somewhat among multigenerational studies in rats (Wine et al, 1997; Gray et al, 1999), they included reduced pup weight and reduced live pups/litter. Puberty in F1 males was delayed. The F1generation had reduced sperm

counts, decreased epididymal sperm counts, testicular spermatid head counts, degenerated seminiferous tubules, and underdeveloped or defective epididymides, and reduced fecundity

Mice treated in a similar study by NTP had decreased percent of fertile pairs and number of litters/pair (Lamb et al, 1987). Mice were also used in a multigenerational study in which males were exposed to DBP for 8 weeks and mated with untreated females. Effects of exposure pre- and postnatally in the F1 generation offspring and prenatally on F2 generation offspring were measured. Paternal DBP exposure disturbed the sex ratio of the offspring, delayed female sexual maturation, and deteriorated the sperm quality of F1 generation males (Dobrzyriska et al, 2011).

Oral exposure of male rats has resulted in morphological changes, reduced caudal sperm density and viability, and reduced serum testosterone (Nair et al, 2008).Also reported were histological structural degeneration in the ductus epididymis and deferens (Sahin et al, 2014), decreased testicular weight, sperm count, sperm motility, serum follicle-stimulating hormone, testosterone, and testicular lactate dehydrogenase activity (Aly et al, 2015), degeneration with absence of spermatogenesis and sperms and necrosis in some of seminiferous tubules (Aly et al, 2015), sloughing of germ cells from seminiferous tubules leaving only Sertoli cells (Fukuoka et al, 1989), and degeneration of seminiferous tubules, decreased activities of testicular enzymes associated with postmeiotic spermatogenic cells, and increased activities of enzymes associated with premeiotic spermatogenic cells, Sertoli cells or interstitial cells (Srivastava et al, 1990). Reduced testosterone probably results from reduced testicular steroidogenesis (Kilcoyne et al, 2014). In an early study (Foster et al, 1980), four to six carbons were found to be needed to produce testicular toxicity in rats.

Many of these effects, but not all, occur are relatively high doses on the order of 500 mg/kg/day. As an example of effects at lower doses, pubertal male rats given DBP orally at 0.1, 1.0, 10, 100 and 500 mg/kg/day for 30 days, high doses (100-500 mg/kg/day) led to testicular toxicity and low doses (~1 mg/kg/day) altered expression of proteins involved in spermatogenesis as well as changes in the number and function of Sertoli and Leydig cells, even though no obvious morphological changes appeared (Bao et al, 2011). No effects of DBP on testicular development were seen histologically on PND 16 in male rats given ~0.16 to 1.6 mg/kg/day on PND 5-15 (Filipiak et al, 2011), findings that are consistent with other studies at similar doses.

Also notable was a study in prepubertal mice in which oral doses of 1 to 500 mg DBP/kg/day were given on PND 4-14. Reduced growth of testes and proliferation of Sertoli cells occurred. Also, smaller anogenital distance and indications of disrupted spermatogenesis were seen in adults exposed prepubertally to doses as low as 1 mg DBP/kg/day (Moody et al, 2013).

Effects of DBP are not limited to males. In a two-generation study, Gray et al (2006) concluded that DBP can cause a negative effect on female fertility at doses of 500 and 1000 mg/kg/day. Also, the F1 generation is more sensitive to DBP reproductive toxicity than the F0 generation..

Relatively recent studies have investigated molecular mechanisms that might be involved with the effects of DBP. DBP can alter gene expression in rats, such as spermatogenesis-related genes (Ahn et al, 2006). DBP might inhibit testosterone production through a glucocorticoid-mediated pathway in prepubertal male rats, and after exposure to DBP ceases, testosterone biosynthesis returns (Xiao-feng et al, 2009). Among a series of phthalates, DBP had one the lowest half maximal inhibitory concentrations for specific enzymes involved in synthesis of androgens in Leydig cells (Yuan et al, 2012). Alterations of the steroidogenic enzymes and INSL3 in murine Leydig tumor cell line MLTC-1 in vitro may be involved in the biphasic effects of DBP/MBP on androgen production (Chen X et al, 2013). An in vitro yeast screen showed that DBP was a weak estrogenic compound (Filipiak et al, 2011).

Two studies were found in which DBP did not have effects on reproductive endpoints. No effect of DBP prior to mating was seen on fertility in one study in rats (IRDC, 1984) and no effects of DBP were seen in a Hershberger assay in rats, suggesting that DBP did not act as an androgen antagonist in this assay (Kang et al, 2005).

DBP affects reproductive organs in other species, although the rat, mouse, hamster, and guinea pig differed widely in their sensitivity to the testicular toxicity of DBP with oral administration (Gray et al, 1982). Effects of DBP on reproduction are not limited to rodents. Oral dosing of pregnant cynomolgus macaques with DBP (500 mg/kg/day) for 6 weeks during fetal adrenal formation suppressed fetal adrenal androgen production and a reduction in the normal increase of estrogen production during early pregnancy (Gee et al, 2007). And adult male Japanese quails exposed prepubertally to 0, 1, 10, 50, 200 and 400 mg DBP /kg/day in their diet had dose-related poorly developed or mis-shaped testes and reduced spermatogenesis due to tubular degeneration and atrophy of seminiferous tubules. Several key enzymes involved in testicular steroidogenesis were altered (Bello et al, 2014). Effects of DBP on the male reproductive system in the fruit fly were comparable to those in mammals (Misra et al, 2014).

Among studies performed in people, suggestive evidence of effects of DBP has been found in men. A significant relation between monobutyl phthalate (MBP) and decreased sperm motility was found in 168 men from subfertile couples (Duty et al, 2003) and a subsequent study in 463 men also showed a relationship of low sperm concentration and exposure to MBP (Hauser et al, 2006). A possible relation was seen between DBP in semen and sperm motility in men at a reproductive institute (Zhang et al, 2006). Suggestive relationships were seen between urinary phthalate metabolites and reduced testosterone (Meeker and Ferguson, 2014). Also, exposure of human sperm to phthalates for 0.5 to 96 hours resulted in decreased motility (Pant et al, 2011). Results in occupationally exposed men suggest a relation between exposure to phthalates, including DBP, and alterations in hormonal control (Pan et al, 2011). In contrast, no association between MBP and sperm or semen endpoints was found in a general survey during military medical examinations (Jönsson et al, 2005).

Regarding studies in women, a survey of women with and without endometriosis, serum DBP appeared to be possibly associated with increased endometriosis (Reddy et al, 2006). Phthalates (including DBP) affected luteal steroidogenesis as well as the balance between luteotrophic and luteolytic factors (Romani et al, 2014).

Comparison with the GHS criteria

Under GHS, adverse effects on sexual function can include “alterations to the male and female reproductive systems, adverse effects on the onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.”

DBP has produced as series of effects related to reproductive health. Effects are more prominent in males, but not limited to them. The rat has been the most commonly used model and postnatal exposure to DBP (typically via the oral route) of male rats has resulted in structural degeneration in the ductus epididymis and deferens, decreases in testicular weight, sperm count, sperm motility, serum follicle-stimulating hormone, and testosterone, and several other changes in the male reproductive organs, particularly the testis. Many of these effects were reported with relatively high doses of DBP, on the order of 300 to 500 mg/kg/day. However, more subtle related effects have been reported at much lower doses.

As discussed in the section on developmental toxicity, DBP also adversely affects male offspring following in utero exposure. Dams exposed to DBP had male pups with reduced sperm counts, degenerated seminiferous tubules, underdeveloped or defective epididymides, and reduced fecundity, among the many observed changes. Thus the F1 generation in multigenerational studies was more affected than the F0 generation.

Suggestive evidence of similar adverse effects on the reproductive system in men has been reported, with decreases in sperm mobility and testosterone. Concern for effects in humans based on adverse effects in rats and other animals is supported by data on pharmacokinetics. As discussed previously, phthalates with one alkyl moiety, including DBP, are rapidly removed after phthalates are ingested, leaving the monoester (MBP in the case of DBP). Further oxidation of MBP occurs, but MBP is the predominant metabolite and it is eliminated primarily in the urine. This pattern occurs across species. MBP is often considered to be an active metabolite toxicologically. Among the studies summarized here, effects of DBP on reproductive parameters have been reported in rats, mice, hamsters, guinea pigs, rabbits, monkeys, quail, frogs, fish, and fruit flies. These observations support the use of data from animals in the classification of DBP for health effects in people. A number of mechanistic and molecular studies also support that extrapolation.

Therefore, data from animal studies on DBP provide clear evidence of an adverse effect on sexual function and fertility. Pharmacokinetic and mechanistic data support this conclusion.

Table 36a: Summary table of animal studies on adverse effects on development of the offspring

Method, test guideline, and deviation(s) if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results**
Oral, dietary study Developmental toxicity study	ICR-JCL mouse	DBP	100 mg/kg bw, 400 mg/kg bw for	In a dietary developmental toxicity study in ICR-JCL mice, increased maternal kidney weights, lower number of live offspring, and increased incidence of external anomalies in pups were seen at 0.5% DBP (~400 mg/kg bw). The NOAEL was 0.05% DBP (~100 mg/kg bw) (Hamano et al, 1977).
Oral dose – diet Developmental toxicity study	CD rat	DBP	660 mg/kg bw, 2100 mg/kg/bw	In a dietary developmental toxicity study in mice, a high dose of DBP (~2100 mg/kg bw) had apparent embryotoxic and teratogenic effects, but a lower dose (0.4%, ~660 mg/kg bw) did not.
Oral gavage Developmental toxicity study	Rat	DBP	0, 500, 750, 1000 mg/kg/bw on days 7-9, 10-12, 13-15 days of pregnancy	The LOAEL for maternal toxicity (lower weight gain) and embryotoxicity (lower fetal weight, number of resorptions, dead fetuses/litter, and postimplantation loss) was 500 mg DBP/kg in a developmental toxicity study in rats, the lowest dose tested. The NOAEL for teratogenic effects was 500 mg/kg. Doses were given b gavage on GD 7-15 (Ema et al, 1993).

Developmental toxicity study, oral dose	Wistar rat	DBP	750, 1000, or 1500 mg DBP/kg on days 7-9, 10-12, 13-15 days of pregnancy	Susceptibility to the teratogenicity of DBP varied with the developmental stage at dosing in Wistar rats. With gavage doses of 750, 1000, or 1500 mg DBP/kg on GD 7-9, 10-12, or 13-15, the highest incidence of malformed fetuses occurred after treatment with DBP on days 13-15 (Ema et al, 1994).
NTP reproductive study Oral study – diet GLP compliant	Rat	DBP	0.125, 0.25, 0.5, 0.75, 1.0, or 2.0% during gestation + 4 weeks post weaning	In a study to determine the maximum perinatal exposure for F344 rats, dams were given 0.125, 0.25, 0.5, 0.75, 1.0, or 2.0% DBP in their diet during gestation and lactation. Pups received DBP for 4 additional weeks postweaning. Although other effects were noted at higher doses, epididymal hypospermia was noted in pups starting at a dose of 0.5% (NTP 1995). In a parallel study in mice at the same doses, no treatment-related gross lesions were identified at necropsy, and no histopathologic lesions definitively associated with treatment were observed in male or female mice in the 7,500 ppm groups. However, the gestation period was longer than controls at 0.25% and the number of live pups/litter was low at this dose (NTP, 1995).
Oral gavage Developmental toxicity study	Rat	DBP	0, 250, 500 or 750 mg DBP/kg/day; GD 3 throughout pregnancy and lactation until the offspring were at postnatal day (PND) 20	In a developmental toxicity study in CD rats, pregnant dams were dosed by gavage at 0, 250, 500 or 750 mg DBP/kg/day from GD 3 throughout pregnancy and lactation until the offspring were at postnatal day (PND) 20. Undescended testes, decreased testicular size, and poorly developed or absent epididymis were observed in all treated groups. Anogenital distance on PND 2 was significantly less in males from dams at 500 and 750 mg/kg/day and female pups in those groups had lack of patent vagina and malformed or absent uteri and ovaries (Mylchreest and Foster, 1997).
Dietary Developmental toxicity study	Rat	DBP	0, 0.5, 1.0, 2.0 % on days 11-21 of pregnancy	Different responses were noted in pups from female rats that were treated once with DBP on different days during pregnancy (Ema et al, 1998a).

Dietary Developmental toxicity study	Rat	DBP	0, 0.5, 1.0, 2.0 % on days 11-21 of pregnancy	Pregnant rats received a DBP in their diet at equivalent doses of ~0, 331, 555 or 661 mg/kg bw during GD 11-21. No effects on postimplantation loss, number of live fetuses, or number of resorptions were seen. Among the effects observed on pups, males at 555 and 661 mg/kg/day had an increased incidence of undescended testes and decreased anogenital distance. Anogenital distance was not affected in female pups. Maternal body weight gain and food consumption were decreased at the two higher doses (Ema et al, 1998b).
Oral gavage Developmental toxicity study	Rat	DBP	100, 250, 500 mg/kg on gestational days 1-21	Given that (1) gestational and lactational exposure of rats to DBP at $\geq$250 mg/kg/day causes reproductive tract malformations and testicular toxicity in the adult male offspring, (2) this disruption of androgen-regulated sexual differentiation indicates an antiandrogenic mechanism, and (3) DBP and MBP do not bind to the androgen receptor (AR) in vitro, this study was designed to compare the activity in vivo of DBP and a known androgen receptor antagonist, flutamide (FLU). Pregnant rats received by gavage either the FLU at 100 mg/kg/day (n = 5) or DBP at 0, 100, 250, or 500 mg/kg/day (n = 10) on GD 12-21. The epididymis was absent in 10 and 50% of males at 250 and 500 mg DBP/kg/day, respectively, and no vas deferens was found at these dose levels in 2 and 27% of DBP-exposed males. DBP produced abdominal testes at 2 and 10% of males at 250 and 500 mg/kg/day, respectively No malformations were observed at 100 mg DBP/kg/day, but preputial separation was delayed at all DBP dose levels. Given the differences observed between these effects and those from FLU, the authors concluded that DBP is not a classical androgen receptor antagonist like FLU (Mylchreest et al, 1998a).

Oral gavage Developmental toxicity study	Sprague-Dawley rat	DBP	250, 500, 750 mg/kg on gestational days 1-21	In a developmental toxicity study in rats with maternal dosing throughout gestation to postnatal day 20, DBP produced the same spectrum of effects elicited by the antiandrogen flutamide, suggesting that DBP is not estrogenic but antiandrogenic in the rat at high dose levels. Effects in offspring included (1) at 750 mg DBP/kg, reduced number of live pups/litter, (2) at 500 and 750 mg DBP/kg, decreased anogenital distance in males and absence of prostate glands and seminal vesicles, and (3) at 250, 500, and 750 mg DBP/kg, absent or undeveloped epididymis, testicular atrophy and loss of germ cells, hypospadias, ectopic of absent testes. Incidence of effects was dose-related. Malformations were seen also in female reproductive tracts with 500 and 750 mg DBP/kg (Mylchreest et al, 1998b).
Oral gavage Developmental toxicity study	CD rat	DBP	0.5 to 500 mg/kg/day on GD 1-21	A developmental toxicity study was performed to establish a NOAEL for male reproductive and developmental toxicity. Pregnant CD rats were given dose of DBP by gavage ranging from 0.5 to 500 mg/kg/day on GD 1-21. The NOAEL was 50 mg/kg/day, the lowest NOAEL established at the time (Mylchreest et al, 1999a)
Oral gavage Developmental toxicity study	CD rats	DBP	0, 100, 250, or 500 mg/kg/day orally on GD 12-21	A developmental toxicity study was performed in rats to compare the effects of DBP and the antiandrogen flutamide. The study was performed because, although the disruption of male rat reproductive development and function by DBP given during gestation and lactation indicates an antiandrogenic mechanism, DBP and its biologically active metabolite do not interact with the androgen receptor (AR) in vitro. Pregnant CD rats received DBP at 0, 100, 250, or 500 mg/kg/day orally on GD 12-21. The expected effects on the male reproductive system were seen at the higher doses; the only effect seen at 100 mg DBP/kg/day was delayed preputial separation. A NOAEL was not established for DBP and the LOAEL was 100 mg/kg/day. Authors concluded that flutamide and DBP disrupted the androgen signaling necessary for male sexual differentiation but with a different pattern of antiandrogenic effects (Mylchreest et al, 1999b).

Dietary study Developmental toxicity study	CD rat, male	DBP, DEHP, and BBP	0.5, 1.0, or 2.0% DBP in the diet on GD 11-21	A dietary developmental toxicity study was performed in CD rats with 0.5, 1.0, or 2.0% DBP in the diet on GD 11-21. Reduced fetal weight, fused sternebrae, and cleft palate occurred at 2%. Undescended testes and decreased anogenital distance in males were noted at 1 and 2%. Authors concluded that DBP given during the second half of pregnancy produces adverse effects on reproductive development in male fetuses (Ema et al, 1999)
Dietary study Developmental toxicity study	CD rat	DBP	0, 50, 100, 250, 500 mg/kg bw on gestational days 12-21	A developmental toxicity study was performed to establish as NOAEL for alterations in male reproductive development and function in CD rats with maternal exposure on GD 12-21 (as opposed to GD 1-21 in their 1999 paper). The NOAEL and LOAEL were 50 and 100 mg/kg/day, respectively (Mylchreest et al, 2000).
Developmental toxicity study Oral dose	Rat	DBP	0, 250, 500, 1000, 1250, 1500 mg/kg on days 0-8 of pregnancy	A developmental toxicity study was performed in rats with oral DBP at maternal doses up to 1,500 mg/kg on GD 0-8. Based in part on parallel work with DBP-dosed pseudopregnant rats, the authors concluded that early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function (Ema et al, 2000a)
Developmental toxicity study	Rat	DBP	0, 250, 500, 1000, 1250, 1500 mg/kg on gestational days 15-17	A developmental toxicity study was performed in rats to determine the susceptible days for the adverse effects of DBP on the development of the male reproductive system during late pregnancy. GD 15-17 was the most susceptible time for DBP-induced undescended testes and decreased AGD (Ema et al, 2000b).
Developmental toxicity study Oral dose	Rat	DBP	0, 250, 500, 1000, 1250, 1500 mg/kg on days 0-8 of pregnancy	The effects of DBP on reproductive function were investigated in a developmental toxicity study using both pregnant and pseudopregnant rats. The results findings suggested that early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function (Ema et al, 2000c)

Developmental toxicity study Oral dose	Rat	DBP (MBP metabolite of DBP)	250, 500, 750 mg/kg days 15-17 of pregnancy	A developmental toxicity study was performed to determine the susceptible days for the adverse effects of DBP on the development of reproductive system in male offspring during late pregnancy. GD 15-17 appeared to be the most susceptible for DBP-induced decreased AGD and undescended testes in male offspring (Ema and Miyawaki, 2001).
Oral dose Developmental toxicity study	Rat	DBP	500 mg/kg/day on GD 12-21 with examination thru PND 70	A developmental toxicity study in rats with 500 mg/kg/day on GD 12-21 included examination of reproductive tracts of male offspring at intervals to postnatal day 70. DBP initiated fetal testicular and epididymal changes that may not manifest as clear malformations until adulthood, e.g., progressive degeneration of seminiferous epithelium and progression of malformed epididymides (Barlow and Foster, 2003).
Developmental toxicity study Dietary	Rat	DBP	20 – 10000 ppm Gestational Day 15 to Post Natal Day 21	In a dietary developmental toxicity with rats, dams received 20 to 10,000 ppm DBP from GD 15 to PND 21. Developmental exposure to DBP affected female sexual development involving pituitary function, while in males testicular toxicity was mostly reversible but mammary gland toxicity (degeneration and atrophy of mammary gland alveoli) was persistent at a dose level as low as 20 ppm (1.5-3.0 mg/kg/d) (Lee et al, 2004).
Developmental toxicity study Gavage	Rat	DBP	100 or 500 mg DBP/kg/day on Gestational Day 12-21 and the male offspring matured to 6, 12, or 18 months of age	In a developmental toxicity study in rats, dams were gavaged with doses of 100 or 500 mg DBP/kg/day on GD 12-21 and the male offspring matured to 6, 12, or 18 months of age. Gross malformations in the male reproductive tract and histologic lesions in the testes were similar to those previously described. However, testicular dysgenesis, a lesion of proliferating LCs and aberrant tubules that has not been previously described in DBP-exposed testes, was diagnosed. Decreased AGD was a sensitive predictor of lesions in the male reproductive tract, relatively small changes in AGD were associated with a significant incidence of male reproductive malformations. Also, lesions similar to Leydig cell adenomas were observed, although they differed somewhat from the traditional adenomas (Barlow et al, 2004).

Developmental toxicity study Gavage	Rat	DBP	0, 50, 100, 250, 500 mg/kg bw	Expected effects were reported in a developmental toxicity study of DBP in rats with doses being given by gavage. The NOAEL for developmental toxicity was based on pup body weight and male reproductive lesions at 50 mg/kg/day (Zhang et al, 2004).
Developmental toxicity study S.C. injections (in corn oil) of neonates	Sprague-Dawley rat	DBP	0, 5, 10, 20 mg/animal from days 5 to 14 after birth	A study was conducted to evaluate male reproductive organ development in early postnatal male Sprague-Dawley rats following neonatal exposure to DBP (no *in utero* exposure, s.c. injections on PND 5-14, sacrifice on PNDs 31 and 42). The results demonstrated that neonatal exposure to DBP causes permanent changes in the endocrine system and results in abnormal male reproductive tract development up to puberty. The data suggest that DBP is likely to exert its antiandrogenic actions through the disruption of AR or ERbeta expression during the early neonatal stage (Kim et al, 2004).
Developmental toxicity study Oral dose	Sprague-Dawley rat Female	DBP	500 mg/kg GD 14-20	In a developmental toxicity study in Sprague-Dawley rats, dams received 500 mg/kg/day for only two successive days during the period of GD 14 to 20. Effects on male offspring were evaluated. Two-day DBP exposure was highly detrimental to the developing reproductive tract of the male fetus and the critical window for abnormal development appeared to be GD 16-18 (Carruthers and Foster, 2005a) A similar study was conducted with emphasis on the testis and epididymis. Results were similar (Carruthers and Foster, 2005b).
Developmental toxicity study Oral dose	Rat	DBP	0, 4, 20, 100, 500 mg/kg/day	A developmental toxicity study was performed in rats to evaluate end points affected by DBP action in rats in fetal and adult life that are relevant to human TDS. A NOAEL of 20 mg/kg/d was established with multiple effects on male reproductive organs at 100 mg/kg/day (Mahood et al, 2007).

Developmental toxicity study Oral dose	Sprague Dawley rat	DBP	0, 250, 500, 750, 1000 mg/kg/day from GD 14-18 (10 rats/group)	Developmental toxicity study in rats was performed that included a comparison of hypospadic (a malformation where the urethral opening is not at the top of the penis) and non-hypospadic male Sprague Dawley rats. Males showing hypospadia were more severely affected by DBP exposure than those rats not showing hypospadia from the same litter (Jiang et al, 2007).
Developmental toxicity study	Rat	DBP		In a study of the testicular dysgenesis-like syndrome induced in rats by fetal exposure to DBP, investigators focused on formation of focal dysgenetic areas comprising malformed seminiferous cords/tubules and intratubular Leydig cells. Differentiation of the fetal Leydig cells is drastically delayed after DBP exposure, which may be indicative of a wider delay in testis cell development and organisation (Hutchison et al, 2008).
Developmental toxicity study Oral dose (in olive oil)	Sprague-Dawley rat	DBP, DIBP	0 500 DBP mg/kg/day from GD 12-21	This developmental toxicity study was performed in rats to determine whether in utero exposure to DIBP would induce permanent and dose-responsive alterations of male reproductive development. Groups of dams also received DBP for comparison. DIBP caused severe and specific adverse effects on the male rat reproductive development, with a pattern similar to that of DBP. However, DIBP appeared slightly less potent than DBP in inducing malformations (Saillenfait et al, 2008).
Developmental toxicity study Dietary	Rat	DBP	0, 112, 582 mg/kg/day form GD 12-10	Results from a dietary developmental toxicity study in rats were compared to those from previous gavage studies with DBP. Approximately equal doses of oral DBP exposure of pregnant rats, from diet or gavage, resulted in similar responses in male offspring (Struve et al, 2009).
Developmental toxicity study Oral dose	Male rats	DBP	0, 100 mg/kg/day from GD 12 to PND 21	To evaluate the effects of DBP exposure during fetal and lactational periods on the male adult rat prostate, pregnant dams received DBP (100 mg/kg, by gavage) from GD 12 to PND 21. Animals were killed at 90 days of age. Endpoints included levels of serum and testicular testosterone, weight of prostate, histopathology, histochemistry, and immunohistochemistry. Results showed that DBP could play a role in proliferative and inflammatory disorders of the rat prostate (Scarano et al, 2009).

Developmental toxicity study Oral dose	Rats – male offspring	DBP	0,100 mg/kg/day	In an investigation of effects of in utero and lactational exposure to 100 mg DBP/kg/d in adult male rat offspring, with emphasis on the epididymis, fetal testes were affected by DBP as evidenced by testicular histopathologic alterations. However, reproductive parameters and epididymal structure/function were not significantly altered in the adult animals exposed to 100 mg/kg DBP in utero and during lactation (Scarano et al, 2010).
Developmental toxicity study Oral dose	Rat	DBP	0, 500 mg/kg/day fetal exposure	Given that androgens may be important regulators of Sertoli cell (SC) proliferation perinatally, with implications for the testicular dysgenesis syndrome (TDS) hypothesis. Exposure of pregnant rats to 500 mg DBP/kg reduces fetal testosterone production and SC number at birth of male pups, but SC number recovers to normal by PND 25. Among other findings, results in this study suggested that postnatal compensatory increase in SC proliferation after prenatal DBP exposure is androgen dependent (Auharek at al, 2010).
Developmental toxicity study Oral dose	Male rats	DBP		A developmental toxicity was performed to investigate the dysplasia, histological malformations, and genetic abnormalities in male rats induced by maternal exposure to DBP. The results demonstrated that in utero exposure to DBP leads to an increased likelihood for the development of anorectal malformations (ARMs) and subsequent complicating megacolon in male rat offspring. Serum testosterone in male rats with ARMs was lower than controls, along with additional testosterone-related endpoints (Jiang et al, 2011).

Developmental toxicity study Oral dose	Rats	DBP	0, 500 mg/kg/day from GD 12 to birth	A developmental toxicity study used p53-deficient mice due to their ability to display greater resistance to apoptosis during development. This model was chosen to determine whether multinucleated germ cells (MNG) induced by gestational DBP exposure could survive postnatally and evolve into testicular germ cell cancer. Pregnant dams were dosed with 500 mg DBP/kg/day on GD 12 to birth. DBP exposure induced MNGs, with greater numbers found in p53-null mice. Histologic examination of adult mice exposed in utero to DBP revealed persistence of abnormal germ cells only in DBP-treated p53-null mice, not in p53-heterozygous or wild-type mice (Saffarini et al, 2012).
Developmental toxicity study Oral dose	Rats	DBP, MBP	0, 10^{-3}, 10^{-4}, 10^{-5}M DBP and MMP (as DBP metabolite)	The effect of DBP's metabolite, MBP, on development of preimplantation embryos was investigated. Treatment of embryos with 10^{-3} M MBP impaired developmental competency, whereas exposure to 10^{-4} M MBP delayed the progression of preimplantation embryos to the blastocyst stage. Results indicated a possible relationship between MBP exposure and developmental failure in preimplantation embryos (Chu et al, 2013).
Developmental toxicity study Oral	Sprague Dawley rats	DBP	0, 0.5, 5, 50 mg/kg/day PND 1-5 and 26-30	A study in Sprague-Dawley rats designed to determine: (1) the difference between the effects of neonatal and prepubertal DBP exposure on female pubertal timing; (2) whether kisspeptin/GPR54 expression in hypothalamus would respond to neonatal and prepubertal DBP exposure differently. Female rats were exposed by s.c. injection of 0.5, 5 and 50 mg DBP/kg during PND 1-5 (neonatal) or PND 26-30 (prepubertal). Exposure-period-related difference was found significant with prepubertal exposure groups having longer estrous cycle duration, heavier at vaginal opening and having higher serum estradiol level compared with neonatal exposure groups. Results demonstrated that small dose of DBP could induce earlier pubertal timing in females and both neonatal and prepubertal periods were critical windows for DBP exposure (Hu et al, 2013).

Developmental toxicity study Oral dose	Sprague-Dawley rats	DBP	Exposure GD 14.5 to PND 6	To investigate whether such early gestational and/or lactational exposure can influence the later adult-type Leydig cell phenotype, female Sprague-Dawley rats were exposed to DBP from GD 14.5 to PND 6 and male offspring were subsequently analysed for various postnatal testicular parameters. Maternal treatment appeared to modify specific Leydig cell gene expression in male offspring, particularly during the dynamic phase of mid-puberty, with a modest acceleration of the pubertal trajectory. Maternal exposure can influence the development of the adult-type Leydig cell population (Ivell et al, 2013).
Developmental toxicity study Oral dose	Sprague-Dawley rats	DBP	0. 100 mg/kg/day from GD 12 to 21	Pregnant Sprague-Dawley rats received 100 mg DBP/kg/day on GD 12 to 21 and male offspring were evaluated for effects on Leydig cells (LCs). Atypical LC hyperplasia was seen in 20-week-old male offspring with low testosterone and high luteinizing hormone levels (Wakui et al, 2013).
Developmental toxicity study Oral dose (corn oil)	Rat	DBP	0, 750 mg/kg/day from GD 14-19	Pregnant rats were daily treated by gavage with 750 mg DBP/kg from GD14 to GD18. We used the technique of proteomic analysis to compare the testis protein patterns obtained by two-dimensional gel electrophoresis from fetal rats of gestation day 19. Several differentially regulated proteins and demonstrated the differential expression of Prdx6, AnxA5 and UchL1 in fetal rat testis after maternal exposure to DBP, when compared with controls. Combining the results on the cellular location of these proteins and their function in other tissues, this study indicated that oxidative injury and abnormal apoptotic regulation may have participated in the formation of testicular dysgenesis in fetuses of dams exposed to DBP (Shen H et al, 2013).

Developmental toxicity study	Rat	DBP	Exposures from GD 8-14	DBP was part of an experimental mixture with BPA and DEHP. Gestating F0 generation females were exposed to the mixture during GD 8-14 of gonadal sex determination and the incidence of adult onset disease was evaluated in F1 and F3 generation rats. Among the findings were increases in the F3 generation in pubertal abnormalities, testis disease, obesity, and ovarian disease (primary ovarian insufficiency and polycystic ovaries) (Manikkam et al, 2013). This study is not restricted to DBP, but it is of relevance to concerns about DBP.
Developmental toxicity study	Rat	DBP		Previous analysis of in utero DBP-exposed fetal rat testes indicated that DBP's antiandrogenic effects were mediated, in part, by indirect inhibition of steroidogenic factor 1 (SF1), suggesting that peroxisome proliferator-activated receptor alpha (PPARα) might be involved through coactivator (CREB-binding protein [CBP]) sequestration. Pathway analysis of expression array data in fetal rat testes examined at gestational day (GD) 15, 17, or 19 indicated that lipid metabolism genes regulated by SF1 and PPARα, respectively, were overrepresented, and the time dependency of changes to PPARα-regulated lipid metabolism genes correlated with DBP-mediated repression of SF1-regulated steroidogenesis genes. The data indicate that PPARα may act as an indirect transrepressor of SF1 on steroidogenic genes in fetal rat testes in response to DBP treatment (Plummer et al, 2013).
Developmental toxicity study Oral dose	Rat	DBP	0, 2, 10, 50 mg/kg/day from GD 14 to parturition	Pregnant rats were treated orally with DBP (2, 10, 50 mg/kg) from GD14 to parturition. A significant reduction in dams' body weight on GD21 was seen. Decreased weight of male pups was significant at PND 75 and the weight of most of the reproductive organs and sperm quality parameters was impaired significantly with 50 mg DBP/kg. DBP exposure during late gestation might have adverse effects on offspring's development, spermatogenesis, and steroidogenesis in adult rats (Ahmad et al, 2014).
Developmental toxicity study I. P.	Rats	DBP		Transplacental exposure to DBP impaired male reproductive performance by decreasing steroidogenesis and spermatogenesis (Giribabu et al, 2014).

Developmental toxicity study examining gene expression	Rat	DBP		Mounting evidence has indicated the crucial role of Wnt5a in the embryonic development including guts. However, the Wnt5a involvement in the process of anorectal malformations (ARMs) remains unclear. In this study the expression of Wnt5a during ARMs development in the offspring of DBP-treated pregnant rats was evaluated. The results demonstrated the aberrant expression of Wnt5a during anorectal development, which suggests that Wnt5a might be involved in DBP-induced ARMs (Li EH et al, 2014).
Developmental toxicity study examining gene expression	Mouse	DBP, BPA		This study was designed to explore the effect of environmental endocrine disruptors (EEDs, namely DBP and BPA) on sexual differentiation in androgen receptor (AR)-/-, AR+/- and AR+/+ male mice by using a Cre-loxP conditional knockout strategy to generate AR knockout mice. Exposure to EEDs induced hypospadias in heterozygous and wild-type male mice offspring during sexual differentiation, but has no effect on homozygous offspring. Therefore, EEDs play an important role during the third stage of sexual differentiation (Liu D et al, 2015).
Developmental toxicity study	Mouse	DBP		Given that DBP causes masculinization disorders in rats, the authors investigated whether DBP exposure impairs steroidogenesis by the human fetal testis, more specifically whether DBP affected testosterone production by normally growing human fetal testis xenografts. No effect was noted in their study while effects were seen in rat fetal xenografts. The authors concluded that exposure of human fetal testes to DBP is unlikely to impair testosterone production as it does in rats (Mitchell et al, 2011).

Table 36b: Summary table of human data on adverse effects on development of the offspring

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations and Reference
Case study	MBP, MEHP (as metabolites for DBP)	Occupational exposure to DBP, urine analysis	In a study to assess the effect of occupational exposure to high levels of phthalate esters on the balance of gonadotropin and gonadal hormones (luteinizing hormone, follicle-stimulating hormone, free testosterone (fT), and estradiol), a modest and significant reduction of serum fT was observed in workers with higher levels of urinary MBP and MEHP compared with unexposed workers (Pan et al, 2006).
Case study	DBP	Pregnant women Urine analysis	To assess play behaviour in relation to phthalate metabolite concentration in prenatal urine samples, authors contacted previous participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples. Metabolites of DBP were associated with a decreased composite score for play. Although based on a small sample, the results suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys (Swan et al, 2010).
Case control study	DBP, DEHP	460 mother-infant pairs Urine analysis	A study was performed to explore the association between prenatal exposure to DEHP and DBP and the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) of the Bayley Scales of Infant Development at 6 months. The results suggested that prenatal exposure to phthalates, including DBP, may be inversely associated with the MDI and PDI of infants, particularly males, at 6 months (Kim Y et al, 2011).
Case study	DBP		Hypospadias is a birth defect found in boys in which the urinary tract opening is not at the tip of the penis. The etiology of hypospadias is still unidentified, but endocrine disruptors are considered as one possible cause. In this study, levels of specific endocrine disruptors, including DBP, were measured in blood and urine of mothers. No relation between the levels of endocrine disrupters and hypospadias was found (Choi et al, 2012). [Sample size and timing of the sampling was not in the abstract used for this summary.]
Case study	DBP, DEHP	122 mother-infant pairs Urine analysis	In a study to assess the relationship between prenatal exposure to phthalate esters and behavior syndromes in 122 mother-child pairs in Taiwan, positive associations between maternal DEHP and DBP exposure (urine samples collected during the 3rd trimester of pregnancy) and externalizing domain behavior problems in 8-year-old children (Delinquent Behavior and Aggressive Behavior scores) (Lien et al, 2015).

Table 36c: Summary table of other studies relevant for adverse effects on development of the offspring

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations and Reference
Developmental toxicity study using rats	MBuP, BBP, DBP	0, 500, 625 or 750 mg/kg on days 7-9, days 10-12 or days 13-15 of pregnancy	A study was conducted to determine the phase specificity of the developmental toxicity of mono-n-butyl phthalate (MBuP) and to assess the role of MBuP in the developmental toxicity of BBP and DBP in rats. Pregnant rats were given MBuP by gastric intubation at a dose of 500, 625 or 750 mg/kg on days 7-9, days 10-12 or days 13-15 of pregnancy. The dependence of gestational days of treatment on the manifestation of the developmental toxicity and the spectrum of fetal malformations induced by MBuP were consistent with those induced by BBP and DBP. Therefore MBuP and/or its further metabolites may be responsible for the production of the developmental toxicity of BBP and DBP (Ema et al, 1995),
Developmental toxicity study using rats	MBuP, BBP, DBP	0, 500, 625 or 750 mg/kg on GD 7-9, 10-12, or 13-15	To further characterize the developmental toxicity of mono-n-butyl phthalate (MBuP), pregnant rats were given MBuP by gastric intubation at a dose of 500, 625 or 750 mg/kg on GD 7-9, 10-12, or 13-15. Increased postimplantation loss was noted with dosing on GD 7-9 and 10-12 at doses $\geq$625 mg/kg and on GD 13-15 at $\geq$500 mg/kg. No evidence of teratogenicity was found when MBuP was given on GD 10-12. External and skeletal malformations were also observed (Ema et al, 1996).
Developmental toxicity study using rats	DBP, MBP	0, 1.8, 3.6, 5.4, or 7.2 mmol DBP or MBP/kg on GD 10	Embryotoxic profiles of DBP and MBP were compared at midgestation in pregnant Sprague-Dawley rats a single oral dose of 1.8, 3.6, 5.4, or 7.2 mmol DBP or MBP/kg on GD 10. Embryos were examined on GD 12. Results provided strong evidence that DBP-induced embryotoxicity is mediated through its main metabolite MBP (Langonne et al, 1998).
In vitro assay	DBP		Estrogenic activity of DBP was weak in in vitro assays and not observed in an in vivo assay (Zacharewski et al, 1998).
Developmental toxicity, oral dose	DBP, DEHP	0, 250, 500, 1000 mg/kg bw of P_0 generation only	In a description of the reproductive effects of 10 known or suspected anti-androgens, the in vivo data suggested that the chemicals alter male sexual differentiation via different mechanisms. The anti-androgens V, P, and p,p'-DDE produce flutamide-like profiles that are distinct from those seen with DBP, DEHP, and L (Gray et al, 1999).

Developmental toxicity, oral dose Sprague Dawley rats	DBP	Pregnant rat dosed once orally on GD 10 at 1.8, 3.6, 5.4, or 7.2 mmol/kg	DBP and mono-n-butyl phthalate were each given separately once orally to pregnant Sprague-Dawley rats on GD 10 at 1.8, 3.6, 5.4, or 7.2 mmol/kg. Fetal growth and development evaluated on GD 12. Types of effects and potency were approximately equivalent between DBP and its major metabolite (Saillenfait, et al, 2001).
Developmental toxicity, oral dose Sprague Dawley rats	DBP	0, 500 mg DBP/kg/day from GD 12 to 21	To determine the chronology of lesion development by assessing the male reproductive tracts of rats exposed to DBP in utero, pregnant Sprague-Dawley rats were dosed by gavage on GD 12 to 21 with 500 mg DBP/kg/day. Fetuses were examined on GD 18 to 21 and male pups were necropsied on PND 3, 7, 16, 21, 45 and 70. Results supported the conclusion that DBP has primary effects on the testes, which are further compounded by increased testicular intratubular pressure resulting from malformations of the epididymides (Barlow and Foster, 2001).
In vitro assay	DBP		In an in vitro study, authors analyzed the cell cycle and examined the effects of changes in cell cycle regulators on DBP-induced cytotoxicity and inhibition of differentiation in limb bud cells. Results demonstrated that DBP or MBuP induces cytotoxicity and inhibition of differentiation in rat embryonic limb bud cells by accumulating cells in the G1 phase and inducing apoptosis (Choi et al, 2002).
Developmental toxicity	DBP	250, 500, 750, 1000, and 1250 mg/kg of DBP or MBP on GD 7-15	Pregnant Wistar rats were dosed orally on GD 7-15 with 250, 500, 750, 1000, and 1250 mg/kg of DBP or MBP. The spectrum of fetal malformations, dependence on gestational days of treatment on the manifestation of teratogenicity, and decreased AGD and increased incidence of fetuses with undescended testes in male fetuses observed with DBP were in good agreement with those observed with MBP. These findings suggest that MBP may be responsible for the developmental effects of DBP (Ema, 2002).
Ex vivo reperfusion assay	MBP (as a metabolite of DBP)		To examine whether testicular toxicity in rats is caused by a direct effect of MBP or by a secondary effect attributed to a hypoxic condition due to the MBP-induced hemoglobin deprivation, testes were perfused with a solution of MBP or the solvent with/without oxygen, and the activities of testicular enzymes were measured. Results support the idea that the toxicity might be caused by hypoxia and a coincident depletion of SUDH activity, followed by an apoptotic testicular cell death (Watanabe et al, 2002).

Fetal tissue explants (ex vivo) from Sprague Dawley rats	DBP	0, 500 mg/kg DBP pregnant rats via oral gavage from GD 12 to 19	Previous studies have shown that several genes involved in cholesterol transport and steroidogenesis are downregulated at the mRNA level following in utero exposure to DBP. The purpose of this study was to make a functional determination of the points in the cholesterol transport and steroidogenesis pathways affected by DBP. In cultured fetal testis explants derived from fetuses whose dams were exposed to DBP at 500 mg/kg/day on GD 12-19, data indicated that the toxic effects of DBP on the fetal testis are mediated at the level of cholesterol cleavage by P450 scc and possibly at the level of cholesterol transport into the mitochondria (Thompson et al, 2003).
Developmental toxicity study Rabbit Oral dose study	DBP	0 or 400 mg DBP/kg/day on GD 15-29 or during PND 4-12 male offspring were examined at 6, 12, and 25 weeks of age	Rabbits were exposed to 0 or 400 mg DBP/kg/day on GD 15-29 or during PND 4-12, and male offspring were examined at 6, 12, and 25 weeks of age. Another group was exposed after puberty (for 12 weeks) and examined at the conclusion of exposure. The most pronounced reproductive effects were in male rabbits exposed in utero, with reduction in numbers of ejaculated sperm and lower weights of testes and accessory sex glands. Serum testosterone levels were down; a slight increase in histological alterations of the testis and a doubling in the percentage of abnormal sperm were seen; and 1/17 males manifested hypospadias, hypoplastic prostate, and cryptorchid testes with carcinoma in situ-like cells. In the group exposed to DBP during adolescence, basal serum testosterone levels were reduced at 6 weeks while at 12 weeks, testosterone production in vivo failed to respond normally to a GnRH challenge. In addition, weight of accessory sex glands was reduced at 12 weeks but not at 25 weeks after a recovery period; there was a slight increase in the percentage of abnormal sperm in the ejaculate; and 1/11 males was unilaterally cryptorchid. In short, DBP induces lesions in the reproductive system of the rabbit, with the intrauterine period being the most sensitive stage (Higuchi et al, 2003).

Developmental toxicity study Rats Gene expression Oral dose - gavage	DBP	0, 500 mg/kg/day from GD 12 to 21	Given that in utero exposure to 500 mg/kg/day DBP on GD 12-21 inhibits androgen biosynthesis, resulting in decreased fetal testicular testosterone (T) levels. Reduced fetal T levels may be responsible for malformed epididymides since T is required for Wolffian duct stabilization and their development into epididymides. The objective of this study was to identify changes in gene expression associated with altered morphology of the proximal Wolffian duct following in utero exposure to DBP. Pregnant rats were gavaged with 500 mg DBP/kg/day on GD 12-19 or 21. On GD 21, 89% of male fetuses in the DBP dose group showed marked underdevelopment of Wolffian ducts characterized by decreased coiling. RNA was isolated from Wolffian ducts on GD 19 and 21 and gene expression was examined using cDNA microarrays. Results were suggestive of altered paracrine interactions between ductal epithelial cells and the surrounding mesenchyme during Wolffian duct differentiation due to lowered T production (Bowman et al, 2004).
Developmental toxicity study Oral dose - gavage Sprague Dawley rats	DBP	0, 0.1, 1.0, 10, 50, 100, or 500 mg/kg/day for GD 12 to 19	The objective here was to determine the dose-response relationship for the effect of DBP on steroidogenesis in fetal rat testes. Pregnant Sprague-Dawley rats received corn oil (vehicle control) or DBP (0.1, 1.0, 10, 50, 100, or 500 mg/kg/day) by gavage daily from gestation day (GD) 12 to 19. Testes were isolated on GD 19, and changes in gene and protein expression were quantified by RT-PCR and Western analysis. Results demonstrated a coordinate, dose-dependent reduction in the expression of key genes and proteins involved in cholesterol transport and steroidogenesis and a corresponding reduction in testosterone in fetal testes at dose levels below which adverse effects are detected in the developing male reproductive tract (Lehmann et al, 2004).
Developmental toxicity study Oral dose - gavage Sprague Dawley rats	DBP	10, 50, or 500 mg/kg/day from GD 12-19	In a study of effects of DBP on fetal liver, pregnant Sprague-Dawley rats were orally dosed with DBP at levels of 10, 50, or 500 mg/kg/day from GD 12-19; maternal and fetal liver samples were collected on GD 19 for analyses. The results indicated that hepatic steroid- and xenobiotic-metabolizing enzymes are susceptible to DBP induction at the fetal stage; such effects on enzyme expression are likely mediated by xenobiotic-responsive transcriptional factors, including CAR and PXR. DBP is broadly reactive with multiple pathways involved in maintaining steroid and lipid homeostasis (Wyde et al, 2005).

Developmental toxicity study Oral dose - gavage Wistar rats Genomic analysis	DBP	0, 500 mg/kg/day	To identify signalling pathways associated with DBP-induced testicular dysgenesis and to determine the region-specificity of the gene expression alterations, transcriptional profiling of RNA isolated from laser capture microdissected interstitial (INT) and tubular (TUB) regions of foetal testes of Wistar rats exposed in utero to 500 mg DBP/kg was performed. Results indicated that DBP-induced testicular dysgenesis involves region- and cell- type-specific effects on a number of genes many of which are regulated by nuclear hormone receptors (Plummer et al, 2006).
Development toxicity study Oral dose Sprague Dawley rats	DBP	0, 250, 500, or 700 mg/kg/day from GD 10-19	The goal of this study was to compare the effects of in utero exposure of chemicals (DBP and flutamide) which have antiandrogenic characteristics on the development of reproductive organs and to investigate the specific mechanisms related to the abnormalities observed in the male reproductive system. During GD 10-19, pregnant Sprague-Dawley (SD) female rats were given orally flutamide (1, 12.5, or 25 mg/kg/day) or DBP (250, 500, or 700 mg/kg/day). At 31 days of age, At 31 of age, the SD male rats reproductive tract abnormalities (hypospadias, cryptorchidism) were dose-dependently increased in the DBP or flutamide treated groups. At 31 days of age, abnormalities in the reproductive tract of males (hypospadias, cryptorchidism) were dose-dependently increased in the DBP or flutamide treated groups. Histopathology, hormonal levels, and microarray analysis demonstrated that exposure to antiandrogen during gestation days 10-19 causes changes in the endocrine system resulting in abnormal development of male reproductive organs (Kang et al, 2006).
Development toxicity study Oral dose Rats Gene expression	DBP	0, 500 mg/kg/day from GD 14-18	The authors hypothesized that (1) co-administered DBP and DEHP would act in a cumulative fashion to induce reproductive malformations, and (2) cumulative changes in fetal steroid hormones and expression of genes responsible for insl3 and steroid production would enhance the incidence of reproductive malformations in adulthood. Pregnant rats were gavaged on GD 14-18 with 500 mg/kg DBP and/or DEHP. In experiment one, adult male offspring were necropsied, and reproductive malformations and androgen-dependent organ weights were recorded. In experiment two, GD18 fetal testes were incubated for T production, and processed for gene expression by qrt-PCR. Results indicated that individual anti-androgenic phthalates with a similar mode of action can elicit cumulative effects on fetal testis hormone production and reproductive tract differentiation when administered as a mixture (Howdeshell et al, 2006).

Oral dose Adult male rats Gene expression	DBP	250, 500, or 750 mg DBP/kg/day for 30 days	To investigate the gene expression profiles in testes, male rats were given DBP orally 250, 500, or 750 mg DBP/kg/day for 30 days. Testes weights in the 500 and 750 mg/kg/day rats were reduced. Using GeneFishing PCR on total RNA that was isolated from these males, 56 differentially expressed genes were seen in the 750 mg/kg/day dosed rat testes. The known genes were involved in xenobiotic metabolism, testis development, sperm maturation, steroidogenesis, and immune response, as well as the up regulation of peroxisome proliferation and lipid homeostasis genes. Using these and additional results, the authors concluded that DBP can significantly affect the testicular gene expression profiles involved in steroidogenesis and spermatogenesis affecting testicular growth and morphogenesis (Ryu et al, 2007)
In vitro study – human cells Gene expression	DBP		The goal of this study was to elucidate mechanisms of phthalate toxicity in normal human cells to provide information concerning interindividual variation and gene-environment interactions. Only 57 genes were found to be altered in all four cell strains following exposure to DBP. These included genes involved in fertility (inhibin, placental growth factor), immune response (tumor necrosis factor induced protein), and antioxidant status (glutathione peroxidase) (Gwinn et al, 2007).
Male Sprague Dawley Rats Oral dose	DBP	Exposure times from 1, 7, 14, or 28 days	The time-response effects of di(n-butyl) phthalate (DBP) on the expression patterns of the testicular genes in male Sprague-Dawley rats were examined for different periods of exposure (1, 7, 14, or 28 d). Results suggested that the acute and chronic effects of DBP on the steroidogenic pathways in the testes show mechanistically distinct patterns. Data thus provide some insights into the molecular mechanisms underlying DBP-induced testicular dysgenesis (Ryu et al, 2008).
Developmental toxicity study rats	DBP	750 mg/kg body weight (bw)/day from GD 14-18	In a study to evaluate the developmental abnormalities and carry out the molecular analysis of external genitalia in newborn hypospadiac male rats induced by maternal exposure to DBP, pregnant rats were given DBP by gastric intubation at dose of 750 mg/kg body weight (bw)/day from GD 14-18 to establish a hypospadiac rat model. Autopsy on PND 7 revealed development of reproductive organs (testes, genital tubercle (GT)), hollow organs (stomach, bladder), and solid organs (brain, heart, liver, spleen, lung, kidney, pancreas) in hypospadiac male rats (46.7% of male pups) to be affected by DBP. Also, significantly decreased gene expression of important signaling molecules necessary for GT formation were observed in the GT of newborn hypospadias induced by DBP (Zhu et al, 2009).

In vitro assays (cellular system examining gene expression and competitive binding assay)	DBP		No strong evidence of species-specific binding was found in an assessment of whether binding of several chemicals differs significantly between full-length recombinant estrogen receptors from fathead minnows (fhERalpha) and those from humans (hERalpha) (Rider et al, 2009
Developmental toxicity study Dietary study Wistar rats	DBP	0, 0.037, 0.111, 0.333 and 1% in the diet) from GD 6 to PND 28	To investigate the neurobehavioral effects of DBP on rodent offspring following in utero and lactational exposure, Wistar rats were treated with DBP (0, 0.037, 0.111, 0.333 and 1% in the diet) from GD 6 to PND 28 and selected developmental and neurobehavioral parameters of the offspring were measured. Some differences were noted with exposure to DBP. For example, shortened forepaw grip time (PND 10), inhibited spatial learning, and inhibited reference memory in male pups were noted with exposure to 0.037% DBP. Authors concluded that produced a few adverse effects on the neurobehavioral parameters, and it may alter cognitive abilities of the male rodent (Li Y et al, 2009).
Developmental toxicity study Oral dose – gavage Wistar rats	DBP	Pregnant rats exposed to 0, 25, 75, 225 and 675mg DBP/kg/day from GD 6 to PND 21	Pregnant Wistar rats were treated orally by gavage with 0, 25, 75, 225 and 675mg DBP/kg/day from GD 6 to PND 21, and then the weaned offspring continued receiving the same treatment till PND 28. Effects of DBP on maze performance in male offspring were evaluated by spatial learning tasks; the effects of DBP on the expression of brain-derived neurotrophic factor (BDNF) were also analyzed in both mRNA and mature protein levels in the hippocampus. Results suggested that developmental treatment with high-dose DBP improves spatial memory in male rats, and this effect may be related to an increase in BDNF expression in the hippocampus in a p-CREB independent route (Li Y et al, 2010).
Development toxicity study Sprague Dawley rats Oral dose	DBP	0, 250, 500 mg/kg/day orally on GD 10-19	In a study to determine the effects of DBP on male reproductive organ development in F1 Sprague-Dawley rats following in utero exposure, pregnant rats received DBP at 250, 500 mg/kg/day orally on GD 10-19. On PND 31 male offspring had reduced weights of testes and accessory sex organs, reduced AGD, and reduced testosterone. Expression of various genes was affected, such as reduced expression of androgen receptor (AR) and 5α-reductase type 2 in the proximal penis. Authors concluded that several abnormal responses in male reproductive organs might be due to disruption of the stage-specific expression of genes related to androgen-dependent organs development (Kim et al, 2010).

Adult male rats Reproductive toxicity Oral dose	DBP	0, 100, 250, and 500 mg/kg/d for 2 consecutive weeks.	The present study was designed to investigate the potential male reproductive toxicity of DBP; oxidative stress was assessed in rat testes as an underlying mechanism. DBP was administered to adult rats by oral gavage at doses of 0, 100, 250, and 500 mg/kg/d for 2 consecutive weeks. Dose-dependent effects were seen in males at the two higher doses and, at the same doses, levels of superoxide dismutase, glutathione peroxidase, glutathione, and malondialdehyde were altered. Authors concluded that DBP alters the testicular structure and function, at least partly, by inducing oxidative stress in testes of adult rats (Zhou et al, 2010). Similar results were presented in Zhou et al (2011).
Developmental toxicity study Gene expression Rats	DBP		Germ cell (GC) number, proliferation, apoptosis, differentiation (loss of OCT4, DMRT1 expression, DMRT1 re-expression, GC migration) and aggregation were evaluated at various foetal and postnatal ages after in utero exposure to DBP. DBP differentially affects foetal GC in rats according to stage of gestation, effects that may be relevant to the human because of their nature (OCT4, DMRT1 effects) or because similar effects are demonstrable in vitro on human foetal testes (GC number) (Jobling et al, 2011).
	DBP		Pregnant rats were given DBP by gastric intubation at 750 mg/kg/day on GD 14-18 to establish a rat model of hypospadias. Wnt/β-catenin pathway in the fetal rat genital tubercle (GT) was assessed on GD 19. Results indicated that DBP may affect the development of GT by down-regulating the Wnt/β-catenin pathway in fetal male rats (Zhang et al, 2011).
Developmental toxicity study Genomic analysis Rats and mice	DBP		By bioinformatically examining fetal testis expression microarray data sets from susceptible (rat) and resistant (mouse) species after DBP exposure, we identified decreased expression of several metabolic pathways in both species. The results suggest that phthalate-induced inhibition of fetal testis steroidogenesis is closely associated with reduced activity of several lipid metabolism pathways and SREBP2-dependent cholesterologenesis in Leydig cells (Johnson et al, 2011).
Male rats Prenatal exposure	DBP		Fibroblast growth factor 8 (FGF8) is an androgen-induced growth factor (AIGF) that is crucial for embryonic development. This study was developed to investigate the role of FGF8 in developmental abnormalities of the genital tubercle (GT) in hypospadiac male rats when prenatally exposed to DBP. The results demonstrated an interaction between androgen and FGF8, which might play an important role in the occurrence of hypospadias and abnormal organ development induced by DBP (Liu et al, 2012).

Mouse and rat Gene expression	DBP		Exposure of rat fetuses to DBP, which induces masculinization disorders, dose-dependently prevented the age-related decrease in LC COUP-TFII expression and the normal increases in LC size and ITT. Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII) is involved in Leydig cell (LC) steroidogenesis and lifting of repression by COUP-TFII may be an important mechanism that promotes increased testosterone production by fetal LC to drive masculinization (van den Driesche et al, 2012).
Case study – gap analysis of existing studies	DBP		A case study was conducted, using DBP, to explore an approach to using toxicogenomic data in risk assessment. The toxicity and toxicogenomic data sets relative to DBP-related male reproductive developmental outcomes were considered conjointly to derive information about mode and mechanism of action. This case study serves as an example of the steps that can be taken to develop a toxicological data source for a risk assessment, both in general and especially for risk assessments that include toxicogenomic data (Makris et al, 2013).
In vitro assay – ovarian follicle culture system isolated from adult CD-1 mice	DBP	0, 1, 10, 100, and 1000 µg/ml for 24 or 168 h	An ovarian follicle culture system was used to evaluate the effects of DBP on antral follicle growth, cell cycle and apoptosis gene expression, cell cycle staging, atresia, and 17β-estradiol (E(2)) production. The results suggest that DBP targets antral follicles and alters the expression of cell cycle and apoptosis factors, causes cell cycle arrest, decreases E(2), and triggers atresia, depending on dose (Craig et al, 2013).
Developmental toxicity study Rats Oral dose - intragastric	DBP	0, 500 mg/kg/day from GD 6 to PND 21	To investigate the neurotoxicity of perinatal exposure of DBP on rodent offspring. Pregnant rats received intragastric DBP (500 mg/kg/day) from GD 6 to PND 21. Brain sections or tissues from offspring rats on PND5, PND21 and PND60 were collected for analysis. Histological examination demonstrated that perinatal exposure of DBP resulted in hippocampal neuron loss and structural alternation in neonatal and immature offspring rats (PND5 and PND21), while no significant change was found in mature rats (PND60) (Li et al, 2013).
Developmental toxicity study Rats Oral dose - intragastric	DBP	0, 500 mg/kg/day from GD 6 to PND 21	In a study on neurotoxicity induced by perinatal exposure to DBP on the immature and mature offspring, pregnant rats received intragastric DBP (500 mg/kg/day) from GD 6 to PND 21. Authors concluded that perinatal exposure of DBP could induce neurotoxicity in immature offspring through regulation of AROM, ER-β, BDNF and p-CREB expression, while it has no influence on mature offspring animals (Li et al, 2014).

Developmental toxicity study Rat Oral dose - intragastric	DBP	0, 100 mg/kg/day form GD 12 to 21	Seminiferous tubule degeneration and atypical hyperplasia of LCs during adulthood in rats exposed in utero to DBP was associated with an increase in expression of estrogen receptor α (ERα) and a decrease of estrogen receptor β (ERβ) and androgen receptor (AR) in the testis (Wakui et al, 2014).
Developmental toxicity study Sprague Dawley rats Oral dose Genomic analysis	DBP	0, 100, 500 mg/kg/day from GD 16-20	Changes in gene expression following in utero exposure to DBP were investigated in rat foreskin. Dams were exposed on GD 16-20 and foreskin was taken on GD 20 and PND 5. Changes in expression of Marcks, Pum1, Nupr1, and Penk caused by in utero exposure were maintained after birth (Pike et al, 2014).
Castrated adult male athymic nude mice with human festal testis xenograph	DBP	14 d, 75 mg/kg/d oral	DBP did not affect androgenic endpoints in a human fetal testis xenograft (Spade et al, 2014).
Rat testis – in vivo dosing, human testis – in vitro cultures Protein expression via immunohisto-chemistry	DBP		Findings in this study provide the first comparison of DBP effects on germ cell number, differentiation, and aggregation in human testis xenografts and in vivo in rats. Authors observed comparable effects on germ cells in both species, but the effects in the human were muted compared with those in the rat. Nevertheless, phthalate effects on germ cells have potential implications for the next generation, which merits further study. The results indicate that the rat is a human-relevant model in which to explore the mechanisms for germ cell effects (van den Driesche et al, 2015).
	DBP, MBP		DBP and MBP are known to change steroid biosynthesis and impair male reproductive function, but this study was done to help determine the regulatory mechanism underlying the steroid biosynthesis disruption by MBP. The resulting data revealed an important and novel mechanism whereby SF-1 and GATA-4 may regulate StAR during MBP-induced steroidogenesis disruption (Hu et al, 2015).

Short summary and overall relevance of the provided information on adverse effects on development of the offspring

In a dietary developmental toxicity study in mice, increased maternal kidney weights, lower number of live offspring, and increased incidence of external anomalies in pups were seen at 0.5% DBP (~400 mg/kg) (Hamano et al, 1977). Similar effects were found in mice by Shiota and Nishimura (1982). In contrast, no consistent treatment-related were noted at DBP doses up to 7,500 ppm in a dietary developmental study in mice (NTP, 1995).

Gavage doses to rats of 500 mg/kg on GD 7-15 led to maternal toxicity (lower weight gain) and embryotoxicity (lower fetal weight, number of resorptions, dead fetuses/litter, and post-implantation loss) (Ema et al, 1993). Susceptibility to the teratogenicity of DBP varied with the developmental stage at dosing in rats with the highest incidence of malformed fetuses occurring after treatment on GD 13-15 (Ema et al, 1994). Although other effects were noted at higher doses in a dietary study in pregnant rats, epididymal hypospermia was noted in pups starting at a dose of 0.5% (NTP 1995).

In a developmental toxicity study in rats, pregnant dams were dosed by gavage at 0, 250, 500 or 750 mg DBP/kg/day from GD 3 to PND 20. Undescended testes, decreased testicular size, and poorly developed or absent epididymis were observed in all treated groups. Anogenital distance on PND 2 was less in male pups at 500 and 750 mg/kg/day, and female pups in those groups had lack of patent vagina and malformed or absent uteri and ovaries (Mylchreest and Foster, 1997). Generally similar results were found in other studies (IRDC, 1984; Ema et al, 1998a, b; Mylchreest et al, 1998b; Mylchreest et al, 1999a, b; Ema et al, 1999; Mylchreest et al, 2000; Ema et al, 2000a, b, c; Ema and Miyawaki, 2001; Barlow and Foster, 2001; Zhang et al, 2004; Carruthers and Foster, 2005a,b; Jiang et al, 2007; Hutchison et al, 2008; Saillenfait et al, 2008; Struve et al, 2009; Auharek at al, 2010; Jiang et al, 2011), with additional finding including absence of prostate glands and seminal vesicles, testicular atrophy and loss of germ cells, hypospadias, ectopic of absent testes. Maternal exposure to DBP can influence the development of the adult-type Leydig cell population in rats (Ivell et al, 2013; Wakui et al, 2013).

Many of the developmental studies showing pronounced effects in males used doses in the range to 250 to 750 mg/kg/day, but effects of DBP have been reported at lower doses. Some authors, such as Mahood et al (2007) reported NOAELs of ~100 mg/kg/day, but DBP significantly affected the size, total cell number, and cordial cross-section number in testes at 50 mg/kg/day. (Kleymenova et al, 2005). An investigation of changes in gene and protein expression in testes of DBP-exposed fetal males demonstrated a coordinate, dose-dependent reduction in the expression of key genes and proteins involved in cholesterol transport and steroidogenesis and a corresponding reduction in testosterone in fetal testes at dose levels below which adverse effects are detected in the developing male reproductive tract (Lehmann et al, 2004).

Studies that evaluated male offspring into adulthood included one with in utero exposure and examination of male offspring at intervals to PND 70. DBP initiated fetal testicular and epididymal changes that may not be apparent until adulthood, e.g., progressive degeneration of seminiferous epithelium and progression of malformed epididymides (Barlow and Foster, 2003). Lee et al (2004) reported that testicular toxicity in males was mostly reversible but mammary gland toxicity (degeneration and atrophy of mammary gland alveoli) was persistent at a maternal dietary dose as low as 20 ppm (1.5-3.0 mg/kg/d). In another study in rats, dams were gavaged with 100 or 500 mg DBP/kg/day on GD 12-21 and the male offspring matured to 6, 12, or 18 months of age. Gross and histologic in males were similar to those previously described, but testicular dysgenesis involving proliferating Leydig cells (LCs) and aberrant tubules was diagnosed. Decreased AGD was a sensitive predictor of lesions in males (Barlow et al, 2004). Pregnant rats were treated orally with DBP (2, 10, 50 mg/kg) from GD14 to parturition. A

significant reduction in dams' body weight on GD21 was seen. Decreased weight of male pups was significant at PND 75 and the weight of most of the reproductive organs and sperm quality parameters was impaired significantly with 50 mg DBP/kg. DBP exposure during late gestation might have adverse effects on offspring's development, spermatogenesis, and steroidogenesis in adult rats (Ahmad et al, 2014). Giribabu et al (2014) reached a similar conclusion that in utero exposure to DBP impaired male reproductive performance in F1 offspring (reduced fertility) by decreasing steroidogenesis and spermatogenesis.

In the same vein, DBP was part of an experimental mixture with BPA and DEHP. Gestating F0 generation females were exposed to the mixture during GD 8-14 of gonadal sex determination and the incidence of adult onset disease was evaluated in F1 and F3 generation rats. Among the findings were increases in the F3 generation in pubertal abnormalities, testis disease, obesity, and ovarian disease (primary ovarian insufficiency and polycystic ovaries) (Manikkam et al, 2013). This study is not restricted to DBP, but it is of relevance to concerns about DBP.

Several references delved into the mode of action of DBP. for example, Mylchreest et al (1998a) stated that, given that (1) gestational and lactational exposure of rats to DBP at $\geq$250 mg/kg/day causes reproductive tract malformations and testicular toxicity in the adult male offspring, (2) this disruption of androgen-regulated sexual differentiation indicates an antiandrogenic mechanism, and (3) DBP and MBP do not bind to the androgen receptor (AR) in vitro, a study was designed to compare the activity in vivo of DBP and a known androgen receptor antagonist, flutamide (FLU). The differences observed between effects from DBP and FLU led to the conclusion that DBP is not a classical androgen receptor antagonist like FLU. Kim et al (2004) determined that DBP is likely to exert its antiandrogenic actions through the disruption of AR or ERbeta expression during the early neonatal stage.

Effects of DBP in utero were not limited to the reproductive tract of male offspring. Females were also affected by in utero and lactational exposure to DBP, including effects on female sexual development involving pituitary function (Lee et al, 2004). DBP also induced earlier pubertal timing in rats and both neonatal and prepubertal periods were critical windows for DBP exposure (Hu et al, 2013). Also, perinatal exposure of DBP resulted in hippocampal neuron loss and structural alternation in neonatal and immature offspring rats (PND5 and PND21), while no significant change was found in mature rats (PND60) (Li et al, 2013). The induction of neurotoxicity in immature offspring was thought to be through regulation of AROM, ER-β, BDNF and p-CREB expression, while the DBP had no remaining influence on mature offspring animals (Li et al, 2014).

Several recent studies focused on molecular aspects of the effects of DBP. For example, gestational DBP exposure induced multinucleated germ cells (MNG), with greater numbers found in p53-null mice. Persistence of abnormal germ cells into adulthood occurred only in p53-null mice, not in p53-heterozygous or wild-type mice (Saffarini et al, 2012). In another study, proteomic analysis was used to evaluate testis protein patterns in rats exposed in utero. Differential expression of Prdx6, AnxA5 and UchL1 was seen, indicating that oxidative injury and abnormal apoptotic regulation might be involved in the testicular dysgenesis in male fetuses (Shen H et al, 2013). Pathway analysis of expression array data in fetal rat testes indicated that PPARα may act as an indirect transrepressor of SF1 on steroidogenic genes in fetal rat testes in response to DBP treatment (Plummer et al, 2013). Aberrant expression of Wnt5a during anorectal development might be involved in DBP-induced anorectal malformations (Li EH et al, 2014). Lastly, information of the effects of DBP were sufficient for an evaluation of the toxicogenomic data set for DBP and male reproductive developmental effects to be performed as part of a larger case study to test an approach for incorporating genomic data in risk assessment (Euling et al, 2013a, b; Makris et al, 2013).

Data on developmental effects in humans are limited and mixed. Prenatal exposure to antiandrogenic phthalates (urinary DBP metabolites measured in mid-pregnancy) may be associated with less male-typical

play behaviour in a small sample of boys (Swan et al, 2010). In a similar survey, prenatal exposure to phthalates, including DBP, may be inversely associated with the Mental and Psychomotor Developmental Indices of infants, particularly males, at 6 months (Kim Y et al, 2011). Similarly, a positive association between maternal DEHP and DBP exposure (urine samples collected during the 3rd trimester) and externalizing domain behavior problems in 8-year-old children was found in 122 mother-child pairs (Lien et al, 2015).

On the other hand, measuring testosterone production by normally growing human fetal testis xenografts, investigators concluded that exposure of human fetal testes to DBP is unlikely to impair testosterone production as it does in rats (Mitchell et al, 2011). Using levels of specific endocrine disruptors, including DBP, in blood and urine of mothers, no relation between the levels of endocrine disrupters and hypospadias was found (Choi et al, 2012). DBP did not affect androgenic endpoints in a human fetal testis xenograft (Spade et al, 2014). However, van den Driesche et al (2015) reported comparable effects on germ cells in human testis xenografts and in vivo in rats, but the effects in the human were muted compared with those in the rat. The authors noted that effects of phthalates (DBP) on germ cells have potential implications for the next generation.

In other studies related to mode of action of DBP, monobutyl phthalate (MBP) and/or its further metabolites may be responsible for the production of the developmental toxicity of DBP (Ema et al, 1995; Ema et al, 1996; Langonne et al, 1998; Saillenfait, et al, 2001; Choi et al, 2002; Ema, 2002; Watanabe et al, 2002).

References related to mode of action of DBP present a multitude of effects. Estrogenic activity of DBP was weak in in vitro assays and not observed in an in vivo assay (Zacharewski et al, 1998). DBP is considered to have antiandrogenic activity (Gray et al, 1999; Kang et al, 2006; Howdeshell et al, 2006; Toxic effects of DBP on the fetal testis are mediated at the level of cholesterol cleavage by P450 scc and possibly at the level of cholesterol transport into the mitochondria (Thompson et al, 2003). Bowman et al (2004) used microarrays to identify changes in gene expression associated with altered morphology of the proximal Wolffian duct following in utero exposure to DBP. An interaction between androgen and fibroblast growth factor (FGF8), which might play an important role in the occurrence of hypospadias and abnormal organ development induced by DBP (Liu et al, 2012). DBP was reported to alter the testicular structure and function, at least partly, by inducing oxidative stress in testes of adult rats (Zhou et al, 2010). Similar results were presented in Zhou et al (2011).

DBP can significantly affect the testicular gene expression profiles involved in steroidogenesis and spermatogenesis affecting testicular growth and morphogenesis (Ryu et al, 2007). DBP-induced inhibition of fetal testis steroidogenesis is closely associated with reduced activity of several lipid metabolism pathways and SREBP2-dependent cholesterologenesis in Leydig cells (Johnson et al, 2011). DBP and MBP are known to change steroid biosynthesis and a mechanism has been described whereby SF-1 and GATA-4 may regulate StAR during MBP-induced steroidogenesis disruption (Hu et al, 2015). Aside from reproductive organs, Wyde et al (2005) determined that hepatic steroid- and xenobiotic-metabolizing enzymes are susceptible to DBP induction at the fetal stage.

Gene expression altered by DBP in human cell lines included genes involved in fertility (inhibin, placental growth factor), immune response (tumor necrosis factor induced protein), and antioxidant status (glutathione peroxidase) (Gwinn et al, 2007). Acute and chronic effects of DBP on the steroidogenic pathways in the testes show mechanistically distinct patterns of testicular genes in rats (Ryu et al, 2008). DBP-induced testicular dysgenesis involves region- and cell- type-specific effects on a number of genes many of which are regulated by nuclear hormone receptors (Plummer et al, 2006). Significantly decreased gene expression of important signaling molecules necessary for genital tubercle (GT) formation were observed in the GT of newborn rats with hypospadias induced by DBP (Zhu et al, 2009). DBP may affect

the development of GT by down-regulating the Wnt/β-catenin pathway in fetal male rats (Zhang et al, 2011).

Several studies were reported on various effects of DBP on gene expression. DBP was reported to differentially affect foetal germ cells (GC) in rats according to stage of gestation, effects that may be relevant to the human because of their nature (OCT4, DMRT1 effects) or because similar effects are demonstrable in vitro on human foetal testes (GC number) (Jobling et al, 2011). Several abnormal responses in male reproductive organs might be due to disruption of the stage-specific expression of genes related to androgen-dependent organs development (Kim et al, 2010). Exposure of rat fetuses to DBP dose-dependently prevented the age-related decrease in LC COUP-TFII expression and the normal increases in Leydig cell (LC) size and ITT. Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII) is involved in LC steroidogenesis and lifting of repression by COUP-TFII may be an important mechanism that promotes increased testosterone production by fetal LC to drive masculinization (van den Driesche et al, 2012). Seminiferous tubule degeneration and atypical hyperplasia of LCs during adulthood in rats exposed in utero to DBP was associated with an increase in expression of estrogen receptor α (ERα) and a decrease of estrogen receptor β (ERβ) and androgen receptor (AR) in the testis (Wakui et al, 2014). Changes in expression of Marcks, Pum1, Nupr1, and Penk in rat foreskin caused by in utero exposure to DBP were maintained after birth (Pike et al, 2014).

In relation to the weak estrogenic activity of DBP and species differences, no strong evidence of species-specific binding was found in an assessment of whether binding of several chemicals (including DBP) differs significantly between full-length recombinant estrogen receptors from fathead minnows (fhERalpha) and those from humans (hERalpha) (Rider et al, 2009).

Studies on mode of DBP extended to females. In an ovarian follicle culture system DBP appeared to target antral follicles and alters the expression of cell cycle and apoptosis factors, causes cell cycle arrest, decreases E(2), and triggers atresia, depending on dose (Craig et al, 2013).

Comparison with the GHS criteria

Under GHS, adverse effects on development of the offspring means "adverse effects . . . induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth and functional deficiency." (GHS 3.7.1.3)

DBP has consistently and reproducibly produced embryotoxicity and teratogenicity in a very large number of developmental toxicity studies. Both male and female offspring are affected, although effects in the male reproductive system have been the most prominent. Effects in the males have been pronounced and involve the testes, secondary sex organs, spermatogenesis, associated hormones (e.g., testosterone), and other endpoints. A large number of mechanistic and molecular studies provides additional information on the development of the changes observed with DBP, including descriptions of possible antiandrogenic and estrogenic activity of DBP. As stated in the section on sexual function and fertility, pharmacokinetic and mechanistic inforation supports extrapolation of data from animals to human health effects.

Therefore, data from animal studies on DBP provide clear evidence of an adverse effect on development. These effects are not secondary to maternal toxicity or to other toxic effects. Pharmacokinetic and mechanistic data support this conclusion.

Adverse effects on or via lactation

Table 37a: Summary table of animal studies on effects on or via lactation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available on lactation alone					

Table 37b: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations and Reference
Government Report	DBP		Based on available data, the exposure to DBP via breast milk for infants was calculated to vary between 1.2 and 6 µg DBP/kg bw/day (EC, 2003).
Peer-reviewed study/ case study	DBP	Milk collected from 21 breast-feeding mothers over 6 month period	Mean DBP concentration less than 1 µg / day / infant. (Zhu et al., 2006)
	MMP (as a metabolite for DBP)		In an analysis of maternal breast milk and serum from boys 1-3 months old who had cryptorchidism, no association between phthalate monoesters and cryptorchidism was found, but MBP was associated with SHBG and LH:free testosterone ratio and negatively associated with free testosterone. Authors considered these data and results with other monoesters to be in accordance with rodent data and suggested that human Leydig cell development and function may also be vulnerable to perinatal exposure to some phthalates (Main et al, 2006).

(Abbreviated version. Full table is in Appendix 1.)

Table 37c: Summary table of other studies relevant for effects on or via lactation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Developmental toxicology study Dietary study	Rat	DBP	0, 20, 200, 2000, 10000 ppm Exposure from gestational day 15 through postnatal day 21	At 10,000 ppm, male offspring showed a decreased neonatal anogenital distance and retention of nipples (PND 14), while females showed a slight non-significant delay in the onset of puberty. At PND 21, reduction of testicular spermatocyte development was evident from 20 ppm, as well as mammary gland changes at low incidence in both sexes. Changes in pituitary hormone-immunoreactive cells were observed at 10,000 ppm with a similar pattern of increase in the percentages of luteinizing hormone (LH)-positive and decrease in follicle-stimulating hormone (FSH) and prolactin producing cells in both sexes, effects also being evident on FSH from 200 ppm and LH from 2000 ppm in females. During postnatal week (PNW) 8-11, marginal increase of the number of cases with extended diestrus was found at 10,000 ppm. In females, relative pituitary weights were decreased after 10,000 ppm at PNW 11, and from 200 ppm at PNW 20. The proportion of FSH-positive cells in the pituitaries at PNW 11 was increased in both sexes at 10,000 ppm.	Lee et al., 2004

Short summary and overall relevance of the provided information on effects on or via lactation

Available studies in lab animals that involved exposure to DBP during lactation also included prior dosing of mothers during gestation, these studies are summarized in Appendix 1, Table 37a. No studies were found on the effects of DBP administered during lactation alone.

In humans, the exposure to DBP via breast milk for infants was calculated to vary between 1.2 and 6 µg DBP/kg bw/day (EC, 2004). A study by Zhu et al. (2006) analysed breast milk from 21 women over a 6 month period and calculated infants were exposed on average to 1 µg/day/infant. In an analysis of maternal breast milk and serum from boys 1-3 months old who had cryptorchidism, no association between phthalate monoesters and cryptorchidism was found. Nonetheless, MMP (as a metabolite of DBP) was associated with SHBG and LH:free testosterone ratio and negatively associated with free testosterone. These results and those with other monoesters were considered to be in accordance with rodent data and the authors suggested that human Leydig cell development and function may also be vulnerable to perinatal exposure to some phthalates (Main et al, 2006).

Comparison with the GHS criteria

While data from animal and human studies with DBP are insufficient to make a judgement regarding lactation; data suggests that exposure of human infants to DBP and/or its metabolites potentially occur via the mother's milk.

Conclusion on classification and labelling for reproductive toxicity

Data from animal studies on DBP provide clear evidence of adverse effects on sexual function and fertility and also on development in the absence of evidence that the effects on development might be secondary to other toxic effects. Pharmacokinetic and mechanistic data support extrapolation of data from animal studies to health effects in humans. Although there is suggestive evidence from human studies of effects in people, but the data are insufficient for classification of DBP as a category 1A reproductive toxicant. Therefore DBP is classified as a category 1B reproductive toxicant, a presumed human reproductive toxicant.

The evidence is insufficient to conclude that DBP interferes with lactation, or may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child. Therefore it is not classified as having an effect on or via lactation. However, because there is evidence that DBP is present in breast milk, it is suggested that a statement to that effect may be included in supplemental information on the label.

8.8 Specific target organ toxicity-single exposure (STOT SE)

Table 38a: Summary table of animal studies relevant for STOT SE

Method, test guideline, and deviation(s) if any	Test substance	Species, strain, sex, no/group	Route of exposure	Dose levels, duration of exposure	Results and Reference
No methodological information. Route of administration: inhalation GLP compliance not reported	DBP (unknown particle size)	cat	1 mg/L for 5.5 hr	>1 mg/L (nasal irritation observed, but no deaths)	Clayton and Clayton, 1993-1994 , as cited in HSDB, 2015
Acute study No GLP information	DiBP (as a substitute for DBP)	Sprague Dawley rats C57BL/6N mice	Oral dose	0, 1000 mg/kg/day for 7 days	Di-iso-butyl phthalate (DiBP) is used as a substitute for DBP. The effects of DiBP on testes in prepubertal rodents still remain to be obscure. Testicular toxicity of DiBP was investigated in 21-day-old Sprague-Dawley rats and C57BL/6N mice, using with in situ TUNEL method. DiBP can induce testicular atrophy in rats due to the increase of TUNEL-positive spermatogenic cells in both acute and 7-day (500 mg/kg/day) exposures. (Zhu et al, 2010)
Acute exposure	DBP	Rats	Single oral dose	0, 500 mg/kg	Morphological alterations in seminiferous tubules caused by single administration of DBP (500 mg/kg) in 3-week-old rats were investigated throughout the first wave of spermatogenesis. A single administration of DBP to prepubertal rats appeared to delay maturation of spermatogenic cells, even after completion of first wave of spermatogenesis (Alam et al, 2010a)

Acute exposure	DBP	Rats	Single oral dose	0, 500 mg/kg	A single oral administration of 500 mg/kg DBP to rats caused progressive detachment and displacement of spermatogenic cells away from the seminiferous epithelium and sloughing of them into the lumen. In vivo and in vitro experiments indicated that DBP-induced collapse of Sertoli cell vimentin filaments may lead to detachment of spermatogenic cells, and then detached cells may undergo apoptosis because of loss of the support and nurture provided by Sertoli cells (Alam et al, 2010b)

Abbreviated version. Full table is in Appendix 1

Table 38b: Summary table of human data relevant for STOT SE

No relevant studies identified

Table 38c: Summary table of other studies relevant for STOT SE

No relevant studies identified

Short summary and overall relevance of the provided information on STOT SE

Target organ toxicity of DBP is also discussed in the sections on reproduction. A few references related to acute exposure are given here. A single administration of 500 mg DBP/kg to prepubertal (3-wk old) rats appeared to delay maturation of spermatogenic cells, even after completion of first wave of spermatogenesis (Alam et al, 2010a). The same dose to rats caused progressive detachment and displacement of spermatogenic cells away from the seminiferous epithelium and sloughing of them into the lumen. (Alam et al, 2010b). DiBP (di-iso-butyl phthalate) can induce testicular atrophy in rats in both acute and 7-day (500 mg/kg/day) exposures. (Zhu et al, 2010). The Zhu et al. study (2010) indicated DiBP could be used as a substitute for DBP but provided insufficient information and did not provide a SAR analysis.

An acute inhalation study was identified which provided some information on effects to the respiratory tract. The study by Clayton and Clayton (1993) as cited in HSDB (2015) indicated a single inhalation exposure at 1 mg/L for 5.5 hr (unclear if this was in the form of vapor or mist and what effect would be observed at 4 hours) resulted in nasal irritation in a cat model (Clayton and Clayton, 1993). However, study quality could not be independently verified and important information regarding form of DBP for the inhalation study could not be located.

Comparison with the GHS criteria

Insufficient information for other types of classification (e.g. nasal or respiratory irritant) because the information reported in the study do not meet the GHS criteria for 4 hour response and form is not reported (vapor or mist).

Conclusion on classification and labelling for STOT SE

No classification due to classification under reproductive toxicity and insufficient information for inhalation.

8.9 Specific target organ toxicity-repeated exposure (STOT RE)

Table 39a: Summary table of animal studies relevant for STOT RE

Method, test guideline, and deviation(s) if any	Test substance	Species, strain, sex, no/group	Route of exposure	Dose levels, duration of exposure	Results and Reference
Subchronic toxicity study	DBP	mouse	Oral	0, 500 and 5,000 mg/kg bw) 86 or 90 days	0.25 or 2.5% DBP in diet (~ 500 and 5,000 mg/kg bw) was administered to mice for 86 or 90 days. Kidneys and liver were affected by the exposure to DBP (Ota et al, 1973; 1974).
NTP protocol GLP compliant	DBP	Sprague-Dawley rat	Oral – feeding study	0, 1000, 5000, 10,000, 15,000, or 20,000 ppm (exposures in males were 0, 70, 340, 650, 910, or 1190 mg/kg-day and in females were 0, 70, 350, 700, 930, or 1150 mg/kg-day	NTP conducted a 14-day dietary range-finding study with DBP in CD Sprague-Dawley rats. No animals died and clinical signs were normal. Food consumption and body weights were affected at the higher doses. The results were used to select the exposures of 0, 1000, 5000, or 10,000 ppm in the continuous breeding study reported by NTP in 1995 (NTP, 1991).
Subchronic study OECD Guideline 408 GLP compliant	DBP	Wistar rat	Oral – dietary (feeding) study		In a 3-month dietary study in rats (OECD Guideline 408), a dose of 152 mg/kg bw appeared to be the NOAEL. Changes at the next higher dose of 752 mg/kg bw included altered hematological and clinical chemical parameters, an increase in the activity of cyanide-insensitive palmitoyl-CoA oxidase (an indicator for peroxisomal proliferation), a decrease in T3, increases in liver and kidney weights, and decreased or missing lipid deposition in hepatocytes. No effect on the testes was observed (Schlling et al, 1992).

Subchronic study Oral dietary study NTP GLP compliant	DBP	F344 rat Male and female			A 13-week evaluation by NTP of the toxicity of DBP was performed by NTP in male and female F344 rats. DBP was given in the diet. Effects were noted in the liver (hypocholesterolemia, hypotriglyceridemia, signs of cholestasis, and altered hepatocellular cytoplasm) and testes (lower weight, degeneration of germinal epitheliumlower serum testosterone, and impaired spermatogenesis). The NOAEL for effects in the testis is 359 mg/kg/day (5000 ppm in diet), and the LOAEL is 720 mg/kg/day (10,000 ppm in diet). The NOAEL for effects in the liver is 176 mg/kg/day (2500 ppm), and the LOAEL is 359 mg/kg/day (5000 ppm). (NTP, 1995).
Subchronic study Oral dietary study NTP GLP compliant	DBP	B6C3F1 mouse			A 13-week dietary study with DBP was conducted in B6C3F1 mice by NTP. Hepatocellular cytoplasmic alterations were seen and mean body weight was lower at high doses. The NOAEL is 5000 ppm (equivalent to 812 mg/kg-day in males and 971 mg/kg-day in females) and the LOAEL is 10,000 ppm (equivalent to1601 mg/kg-day in males and 2137 mg/kg-day in females). (NTP, 1995).
Subacute inhalation study (28 day)	DBP	Wistar rats 5/sex/group 6 hr/day, 5 days/week, 4 weeks	Inhalation – head only	0, 1.18, 5.57, 49.3 or 509 mg DBP/m^3. MMAD was 1.5-1.9 µm and GSD was ~ 2.	No systemic effects were seen in a 4-wk inhalation study conducted according to OECD Test Guidelines. Head-nose exposures were 6 hours/day, 5 days/week, to measured aerosol concentrations of 0, 1.18, 5.57, 49.3 or 509 mg DBP/m^3. MMAD was 1.5-1.9 µm and GSD was ~ 2. Adverse local effects in the upper respiratory tract were seen even at the lowest dose (Gamer et al., 2000).

Abbreviated version. Full table is in Appendix 1

Table 39b: Summary table of human data relevant for STOT RE

Type of data/report	**Test substance, reference to table 5**	**Route of exposure**	**Relevant information about the study (as applicable)**	**Observations and Reference**
Case study 261 children	DBP metabolites – MEHP, MEOP, DEHP, MNBP	Environmental exposures	Urine analysis in children aged 8-11	In a study to investigate the impact of phthalates on symptoms of ADHD in school-age children. A strong positive association between phthalate metabolites in urine and symptoms of ADHD among school-age children was found (Kim BN et al, 2009).

Case study 118 adult men with fertility issues	DBP, DEHP	Environmental exposures	Serum and semen analysis	To investigate the associations of hormone circulation with phthalate exposure in adult men, DBP and DEHP in serum and semen were measured and compared to serum levels of follicle stimulating hormone, luteinizing hormone, testosterone, estradiol and prolactin. Serum prolactin appeared to be positively associated with both DBP and DEHP and an inverse relation between semen DBP and serum testosterone was seen (Li S et al, 2011).
Case study Neurobehavior in infants (5 weeks old) 350 mother/infant pairs	DBP	Environmental prenatal exposures	Maternal urine analysis at 16weeks and 26 weeks	The association of prenatal exposure to bisphenol A and select common phthalates with infant neurobehavior was evaluated at 5 weeks of age. The association between prenatal phthalate exposure and infant neurobehavior differed by type of phthalate and was evident only with exposure measured at week 26 of pregnancy. Prenatal exposure to DBP was associated with improved behavioral organization in 5-week-old infants. (Yolton et al, 2011).
Cross-sectional study 1346 adults (20 years of age or older) vs 329 adolescents (ages 12-19)	DBP, DEHP	Environmental exposures NHANES data from 2007-2008	Evaluate thyroid hormones in urine and DBP, DEHP concentrations in urine	This study explored the cross-sectional relationship between urinary concentrations of metabolites of di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and BPA with a panel of serum thyroid measures among a representative sample of U.S. adults and adolescents. The results supported previous reports of associations between phthalates-and possibly BPA--and altered thyroid hormones (Meeker and Ferguson, 2011).
Cross-sectional study Children 6-15 years of age	DBP and other phthalates	Environmental exposures	Urine analysis for DBP and other phthalates Behavior analysis	This study investigated the association between urinary phthalate metabolite levels and attention deficit disorder (ADD), learning disability (LD), and co-occurrence of ADD and LD in 6-15-year-old children. Cross-sectional evidence indicated that certain phthalates are associated with increased odds of ADD and both ADD and LD (Chopra et al, 2013).
Case control study 104 girls (Prepubescent)	MBP (as a metabolite of DBP)	Environmental exposure	Urine analysis for LH, FSH, other hormones	In a case-control study of 104 girls, it was found that kisspeptin may promote the onset of puberty in girls who have high levels of urinary phthalates, especially MBP. The study suggests that the early onset of puberty is related to increased kisspeptin secretion (Chen et al, 2013).

Case control study Females with endometriosis	DBP metabolites	Environmental exposure	Urine analysis for metabolites	In an investigation of the relationship between urinary phthalate metabolite concentrations and the risk of a hormonally-driven disease, endometriosis, in reproductive-age women, the results suggested that phthalates may alter the risk of a hormonally-mediated disease among reproductive-age women (Upson et a, 2013).

Abbreviated version. Full table is in Appendix 1

Table 39c: Summary table of other studies relevant for STOT RE

No relevant references were found other than those related to reproductive effects.

Short summary and overall relevance of the provided information on STOT RE

No systemic effects were seen in rats during a 4-wk inhalation study at concentrations up to 509 mg DBP/m^3, but adverse local effects in the upper respiratory tract were seen even at the lowest dose, 1.18 mg DBP/m^3 (Gamer et al., 2000).

Effects of DBP have been reported in dietary studies in addition to those in the reproductive tract. Kidneys and liver of mice were affected by dietary doses of 0.25 and 2.5% DBP for 90 days (Ota et al, 1973; 1974). Effects limited to altered food consumption and body weights in a 14-day dietary study in rats were used to select the exposures of 0, 1000, 5000, or 10,000 ppm in the continuous breeding study reported by NTP in 1995 (NTP, 1991). The NOAEL in a 3-month dietary study in rats was 152 mg/kg. Effects at 752 mg/kg included altered hematological and clinical chemical parameters, an increase in the activity of cyanide-insensitive palmitoyl-CoA oxidase (an indicator for peroxisomal proliferation), a decrease in T3, increases in liver and kidney weights, and decreased or missing lipid deposition in hepatocytes. No effect on the testes was observed (Schlling et al, 1992). In a 13-week dietary study in rats, Effects were noted in the liver (at $\geq$359 mg/kg/day) and testes (at $\geq$720 mg/kg/day). In a parallel study in mice, hepatocellular cytoplasmic alterations were seen and mean body weight was lower at high doses (NTP, 1995).

Studies in humans include investigations on behavior. A strong positive association between phthalate metabolites in urine and symptoms of ADHD among school-age children was found (Kim BN et al, 2009). This study investigated the association between urinary phthalate metabolite levels and attention deficit disorder (ADD), learning disability (LD), and co-occurrence of ADD and LD in 6-15-year-old children. Cross-sectional evidence indicated that certain phthalates are associated with increased odds of ADD and both ADD and LD (Chopra et al, 2013). Prenatal exposure to DBP has been associated with improved behavioral organization in 5-week-old infants. (Yolton et al, 2011).

In hormonal studies in men, serum prolactin appeared to be positively associated with both DBP and DEHP and an inverse relation between semen DBP and serum testosterone was seen (Li S et al, 2011). In a cross-sectional study of urinary of BPA and other compounds compared to a panel of serum thyroid measures, an associations between phthalates-and possibly BPA--and altered thyroid hormones was reported (Meeker and Ferguson, 2011).

In hormonal studies in girls, it was found that kisspeptin may promote the onset of puberty in girls who have high levels of urinary phthalates, especially MBP (Chen et al, 2013). Results of another study suggested that these six substances (MBP, t-OP, n-NP, daidzein, equol, and genistein) have an effect on precocious puberty (Yum et al, 2013).

Table 39d: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Not applicable

Comparison with the GHS criteria

Adverse local effects in the upper respiratory tract were observed at the lowest dose, 1.18 mg DBP/m^3 in rats during a 4-wk inhalation study at concentrations ranging from 1.18 mg DBP/m^3 to 509 mg DBP/m^3 (Gamer et al., 2000). No systemic effects were observed at any dose. GHS cutoff value for inhalation study to be considered category 1 is 0.02 mg/L/6hr/day in a 90 day study with category 2 being $0.02<C\leq0.2$. DBP exhibits effects via inhalation far above these ranges in a 28 day study. No 90 day study could be located to determine effect via inhalation.

Liver and kidney have been reported to be affected in animal studies with repeated doses, but the effects (weight gain, altered hepatocellular cytoplasm) do not meet the criteria of "significant organ damage" under GHS. Disruption of the thyroid was reported in a few references, such as in the study in humans cited in this section and a few studies in frogs (Xenopus sp.) cited in the ecotoxicity sections. However, the evidence for effects on the thyroid by DBP alone is insufficient to meet the GHS criteria for STOT. Therefore no effects were identified that meet the GHS criteria for STOT RE.

Conclusion on classification and labelling for STOT RE

No classification

8.10 Aspiration hazard

Table 40: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on aspiration hazard

No relevant data found

Comparison with the GHS criteria

Not applicable

Conclusion on classification and labelling for aspiration hazard

No classification

9. EVALUATION OF ENVIRONMENTAL HAZARDS

9.1 HAZARDOUS TO THE AQUATIC ENVIRONMENT

9.1.1 Rapid degradability of organic substances

Table 41: Summary of relevant information on rapid degradability

Method, test guideline, and deviation(s) if any	**Results**	**Remarks**	**Reference**
Hydrolysis			
Secondary source with no methodological information	pH 7, 25˚C: DT_{50} = 22years	No study details available Supporting Study, Klimisch score 4	Wolfe et al. 1980, as cited in Staples et al. 1997
Secondary source with no methodological information	pH 4.0 and 7.0, temperature not noted: <10% hydrolysis after 5 days; pH 9, 50˚C: DT_{50} = 65.8 hours	No study details available Supporting Study, Klimisch score 4	ECB, 2004
EU Method C.7	pH 4, 50˚C: DT_{50} = 218 days pH 7, 50˚C: DT_{50} = 103 days pH 9, 50˚C: DT_{50} =2.7 days pH 9, 39˚C: DT_{50} = 8.2 days	Minimal study details provided, purity of test substance not available, temperature not environmentally relevant Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/2/3 http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/5/2/3
Photochemical degradation			
Secondary source with no methodological information	Photodegradation (air) DT_{50} = 21.4 hours	Experimental, no details available, Supporting Study, Klimisch score 4	ECB, 2004
Secondary source with no methodological information	Photodegradation (air) DT_{50} = 7.4 hours to 3.1 days	Estimated, no details available Supporting Study, Klimisch score 4	Howard et al. (1991), as cited in ECB, 2004
Secondary source with no methodological information	Photodegradation (air) DT_{50} = 0.6 to 6 days	Estimated, no details available Supporting Study, Klimisch score 4	Atkinson 1988, as cited in Staples et al. 1997
Secondary source with no methodological information	Aqueous photodegradation DT_{50} = 2.4 to 12 years	Estimated, no details available Supporting Study, Klimisch score 4	Howard 1991, as cited in Staples et al. 1997
Ready biodegradability			

Method, test guideline, and deviation(s) if any	**Results**	**Remarks**	**Reference**
EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test), GLP compliant	Percent degradation, measured as O2 consumption: Day 1: 0 Day 4: 42 Day 8: 56 Day 13: 69 Day 22: 76 readily biodegradable	activated sludge, non-adapted used as inoculum, 28-day test duration, test substance purity not provided, pH not noted, initial concentration of DBP 21.7 mg/L Supporting Study, Klimisch score 1 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/2/?documentUUID=0614b8a1-1e8c-455b-8d23-7d1eded1982b
OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test), GLP compliance not indicated	Percent degradation, measured as CO2 evolution: Day 1: 0 Day 4: 42 Day 8: 56 Day 13: 69 Day 18: 73 Day 22: 76 Day 28: 81 readily biodegradable	sewage, domestic used as inoculum, 28-day test duration, test substance purity not provided, pH not noted, initial concentration of DBP not noted, reference substance used Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/2/?documentUUID=0d57d82a-cb7f-4641-be25-5a3ca5603196
OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I)), not GLP compliant	14-d biodegradation: 69% per O2 consumption; 100% per test substance analysis readily biodegradable, per REACH dossier	Biodegradation not reported at standard 10-day window, non-standard media used (mixture of sewage, soil and natural water), test substance purity not provided, 28-day test duration, pH 7, initial concentration 100 mg/L DBP Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/5/3/2
BOD_5/COD			
Secondary source with no methodological information	BOD5:COD ratio of 0.63 readily biodegradable	non-adapted inoculum Supporting Study, Klimisch score 4	ECB, 2004
Aquatic simulation tests			

Method, test guideline, and deviation(s) if any	**Results**	**Remarks**	**Reference**
No guideline followed, shake-flask method	Half-lives for active sediment (AS) ranged from 0.6 d to 10.8 d (average of half-life for AS was 2.96 d). Half-lives for active water (AW) ranged from 3.4 d to 17 d (average of half-life for AW was 7.01 d).	Four test systems: active (natural) sediment (AS), sterile sediment (SS), active (natural) water (AW), sterile water (SW); initial concentration of DBP 500 µg/L; disappearance of DBP was determined by periodic sampling and analysis by HPLC or GC, pH not noted, reference substance used Supporting Study, Klimisch score 1 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=4e37f505-599c-4f8c-87f7-1576cf0b47cc
Primary degradation in surface river water monitored by GC-ECD, aerobic conditions (assumed)	10-d biodegradation at 20˚C = ca. 100%, per test material analysis Primary DT50 at 20˚C = 3 days	Initial concentration of DBP 3.3 µg/L, conducted in surface water of Rhine river, recovery of the method of analysis was 83 – 97% Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=76f861bc-3b5f-4a9c-85d2-97e7892887a6
Inherent and Enhanced Ready Biodegradability tests			
EPA OTS 796.3340 (Inherent Biodegradability: Modified SCAS Test)	Percent degradation, measured as DOC removal: Week 1 of Acclimation (1 mg/L) 69 Week 2 of Acclimation (3 mg/L) 71 Week 3 of Acclimation (3 mg/L) 66 DBP underwent primary degradation in excess of 90% in 24 hours. readily biodegradable, per REACH dossier	Activated sludge, adaptation not specified, used as inoculum; 19-day test duration; test substance purity not provided; pH 6.5-8; initial concentration of DBP: 1 and 3 mg/L concentration tested, reference substance used Supporting Study, Klimisch score 1 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/2/?documentUUID=2b34a27d-3ea0-44cc-b321-6ee3fb7ef937
No guideline followed,	DBP degraded by > 90% in < 8 days per test material analysis readily biodegradable, per REACH dossier	Anaerobic sludge used as inoculum, > 99% in purity, 32-day test period, initial test substance concentration 4 mg/L Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/2/?documentUUID=1ec9c264-7152-442b-b7ce-563c03f37a23
Soil and sediment degradation			

Method, test guideline, and deviation(s) if any	Results	Remarks	Reference
No guideline followed	Under aerobic conditions, DBP degradation of 85 % by mineralization at 22 °C after 5 days, per Radiochemical. meas., transformation products measured, but not reported During anaerobic conditions, only one-sixth of the rate of aerobic transformation of DBP was observed (no further details provided).	Tested in freshwater sediments under aerobic and anaerobic conditions, initial concentration of 0.018 to 10 mg/L radiolabelled DBP, no data on use of reference substance, test duration 28 days Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=4959e004-e27d-45be-a736-b1d3a46daae6
No guideline followed	Under aerobic conditions, DBP degradation of 85 % at 22 °C after 14 days, per radiochemical measurement	freshwater sediments under aerobic and anaerobic conditions, initial DBP concentration of 1 mg/L, no data on use of reference substance, test duration 30 days Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=57aba995-cb5f-48b2-8a00-79e2449fa0ec
Aerobic degradation measured in mangrove sediment	DT50s = 1.6 to 2.9 d	Limited study details available Supporting Study, Klimisch score 4	Yuan et al. 2010

Further information is in Table 41 in Appendix 1

Hydrolysis

Studies examining hydrolysis of DBP at environmentally relevant temperature and pH are limited, with more data available from studies conducted at temperatures not relevant to environmental conditions (50˚C). However, available data do not indicate that hydrolysis would be a major pathway of rapid degradation, as the half-life predicted under environmentally relevant conditions (pH 7 and 25˚C) was 22 years (Wolfe et al. 1980, as cited in Staples et al. 1997a).

Photochemical degradation

No reliable measured data are available for photodegradation of DBP in air and water; however, estimated half-lives (DT50s) are available. Estimated DT50s indicate that while photodegradation of DBP proceeds rapidly in air (DT50s= hours to a few days)(ECB, 2004; Atkinson 1988, as cited in Staples et al. 1997a) , aqueous photodegradation proceeds much more slowly (DT50s on the order of years)(Howard 1991, as cited in Staples et al. 1997a).

Ready biodegradability

Several reliable studies, conducted according to standard guidelines, investigating the ready biodegradability of DBP are available. These studies, described in Table 43, allow for a conclusion that

DBP is readily degraded under aerobic, environmentally relevant conditions, a finding that is generally well established (ECB, 2004). The findings for BOD5/COD, described below also support this finding, though few study details are available.

BOD_5/COD

According to ECB (2004) a BOD5:COD ratio of 0.63 was obtained in a study conducted with a non-adapted inoculum, indicating that DBP may be regarded as readily biodegradable; however, no other details were provided.

Aquatic simulation tests

Two reliable studies are available that investigated biodegradation of DBP in natural waters. In a study investigating degradation in active natural freshwater and sediments collected from several different sites the average DBP half-life in active water was 7.01 days (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=4e37f505-599c-4f8c-87f7-1576cf0b47cc). A similar half-life for DBP (3 days) was measured in a study conducted in surface water of the Rhine River and primary degradation of DBP of close to 100% was measured at 10 days (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=76f861bc-3b5f-4a9c-85d2-97e7892887a6).

Field investigations and monitoring data (if relevant for C&L)

Not applicable

Inherent and Enhanced Ready Biodegradability tests

Two reliable studies were available that examined the inherent biodegradability of DBP, as described in Table 41. These studies are secondary to findings of ready biodegradability discussed above and are thus not discussed here in detail.

Soil and sediment degradation data

Three reliable studies are available that investigated biodegradation of DBP in natural sediments. In a study investigating degradation in active natural freshwater and sediments collected from several different sites (see aquatic simulation test section of Table 43) (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=4e37f505-599c-4f8c-87f7-1576cf0b47cc), the average DBP half-life in active sediment was 2.96 days. The other two studies examined degradation of DBP in freshwater sediments using radiolabelled DBP. DBP was found to be degraded under aerobic conditions in these studies by 85%, as measured radiochemically, after 5 days in one study (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=4959e004-e27d-45be-a736-b1d3a46daae6) and after 14 days in the other (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/5/3/3/?documentUUID=57aba995-cb5f-48b2-8a00-79e2449fa0ec). An additional study was also identified from the publically available literature that examined aerobic biodegradation of DBP in natural sediments collected from mangrove communities, (Yuan et al. 2010). Based on the review of the abstract from this study, measured half-lives of DBP were reported to be 1.6 to 2.9 days.

9.1.2 Environmental transformation of metals or inorganic metal compounds

Summary of data/information on environmental transformation

Not relevant for organic compounds such as DBP.

9.1.3 Environmental fate and other relevant information

Not considered in this document.

9.1.4 Bioaccumulation

Table 42: Summary of relevant information on bioaccumulation

Method, test guideline, and deviation(s) if any	**Species**	**Results**	**Remarks**	**Reference**
OECD 305E, GLP compliant	Carp (*Cyprinus carpio*)	BCF of parent compound = 1.8 L/kg, based on measurements for the highest exposure DBP concentration in water	exposed to 10 and 50 µg/l for 28 days, low analytical recovery noted, as well as possible background contamination, major metabolite, i.e. the mono-ester MBP, was not analysed Key study, Klimisch score 2	Hüls, 1996, as cited in ECB, 2004
Measured ^{14}C-content	Fathead minnow (*Pimephales promelas*)	BCF = 2125	No study details available, secondary study Supporting study, Klimisch score 4	Canadian EPA, 1994, as cited in ECB, 2004
static method	Sheepshead minnow (*Cyprinidon variegatus*)	BCF = 11.7	No study details available, secondary study Supporting study, Klimisch score 4	Wofford et al., 1981, as cited in ECB, 2004
Measured ^{14}C-content	Brown shrimp (*Penaus aztecus*)	BCF = 2.9	No study details available, secondary study Supporting study, Klimisch score 4	Canadian EPA, 1994, as cited in ECB, 2004
Measured ^{14}C-content	*Daphnia magna*	BCF = 5000	No study details available, secondary study Supporting study, Klimisch score 4	Mayer and Sanders,1973, as cited in ECB, 2004
Measured ^{14}C-content	*Gammarus pseudolimnaeus*	BCF = 6700	No study details available, secondary study Supporting study, Klimisch score 4	Mayer and Sanders,1973, as cited in ECB, 2004

Further information is in Table 42 in Appendix 1

Estimated bioaccumulation

An EPIWIN estimation is available that derived a BCF value of 432.6 L/kg; however, the results are not relevant for classification purposes.

Measured partition coefficient and bioaccumulation test data

Measured logKow values for DBP are ≥ 4, ranging from 4.46 to 4.57, indicating that DBP has a potential for bioaccumulation.

Measured BCF values vary drastically depending on the methodology employed. Studies using measurement of ^{14}C-labelled material likely overestimate the BCF, as results would reflect measured ^{14}C-DBP, any ^{14}C-labelled metabolites of DBP, as well as ^{14}C built into the tissue of the organism in e.g. fatty acids. BCFs derived from these types of studies were 2125 for fish and 2.9 to 6700 for invertebrates. A GLP compliant study, conducted according to OECD guideline 305 E, found a BCF of 1.8 for DBP in Carp (*Cyprinus carpio*); however, the primary metabolite (mono-ester MBP) was not measured in this study. Therefore, this study only reflects the BCF for the parent compound. Thus, the true BCF of DBP and major metabolites in fish is likely between 1.8 and 2125. Per UN GHS 2015, Annex 9, A9.5.2.3.9.4, if only BCFs based on parent compound and on radiolabeled measurements are available, the latter should be used for classification. Thus, the BCF value of 2125 will, conservatively, be used for classification.

9.1.5 Acute aquatic hazard

It should be noted that study results cited as secondary sources were considered for inclusion for classification purposes; however, when multiple study results were available for the same species, only results reported from studies using standard test durations (96 hours for fish, 48-hours for most invertebrates and 72 or 96 hours for algae) and reporting results in measured concentrations were included when available.

Table 43: Summary of relevant information on acute aquatic toxicity

Method, test guideline, and deviation(s) if any	Species	Test material	Results	Remarks	Reference
Fish					
US EPA-660/3-75-009 (equivalent to OECD Guideline 203), GLP compliant	*Pimephales promelas*, Fathead minnow *Lepomis macrochirus*, bluegill *Cyprinodon variegatus*, sheepshead minnow (marine species) *Oncorhynchus mykiss*, rainbow trout	DBP	Static 96h LC50, mg/l = 1.54 Flow through 96h LC50, mg/l =0.92 **Static 96h LC50, mg/l = 0.48** Static 96h LC50, mg/l > 0.6 Flow through 96h LC50, mg/l = 1.6	Purity ≥95%, 22°C +/- 1°C., 7.6 to 7.9 No. of organisms per vessel: 10 No. of vessels per concentration: 2 No. of vessels per control : 2, analytical verification performed and results based on mean measured concentrations Key Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/2/?documentUUID=3d142a11-4d22-4269-b3fa-6a50ee909646
Equivalent to methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (US EPA, 1975), GLP compliant	*Lepomis macrochirus* bluegill	DBP	96h LC50, mg/l = 0.85 (0.70 - 1.0)	Purity not available, static conditions, total hardness and alkalinity ranges as $CaCO_3$ of 42-48 mg/L and 30-34 mg/L respectively, pH 6.7 - 7.8, specific conductance 150-160 microhos/cm, 20-23°C, DO below optimal range (0.-4.2mg/L at 96 hours), analytical verification performed and results based on mean measured concentrations Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/2/?documentUUID=dd7bf151-8369-40e4-9bcb-2f8226e36738

Equivalent to OECD Guideline 203 (Fish, Acute Toxicity Test), GLP compliant	*Oncorhynchus mykiss*, rainbow trout	DBP	96h LC50, mg/l = 1.6 (1.2-2.2)	"100% active material", flow-through conditions, temperature 11°C, pH 6.9 -7.6, DO 9.1 - 9.5 mg/L, Ten organisms per tank, duplicates included, analytical verification performed and results based on mean measured concentrations Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/2/?documentUUID=6b68e7bd-b05a-4287-9061-f87bf54abe73
flow through (EG&G Bionomics, 1981)	*Pimephales promelas* Fathead minnow	DBP	96h LC50, mg/l (95% C.I.) = 0.92 (0.71-1.2)	Measured concentrations; no further study details available Supporting study, Klimisch score 4	CMA (1984), as cited in ECB, 2004; Staples et al. 1997b
static	*Pimephales promelas* Fathead minnow	DBP	96h LC50, mg/l (95% C.I.) = 1.1 (1.0-1.2)	Measured concentrations; No other study details available Supporting study, Klimisch score 4	Geiger et al. (1985), as cited in ECB, 2004; Staples et al. 1997b
Flow-through	*Pimephales promelas* Fathead minnow	DBP	96h LC50, mg/l (95% C.I.) = 0.85 (0.72-1.0)	Measured concentrations; No other study details available Supporting study, Klimisch score 4	Defoe et al. 1990, as cited in Staples et al. 1997b
Flow-through	*Pimephales promelas* Fathead minnow	DBP	96h LC50, mg/l (95% C.I.) = 0.61(0.54-0.70)	Measured concentrations; No other study details available Supporting study, Klimisch score 4	Defoe et al. 1990, as cited in Staples et al. 1997b
Flow-through	*Pimephales promelas* Fathead minnow	DBP	96h LC50, mg/l (95% C.I.) = 0.90 (0.73-1.10)	Measured concentrations; No other study details available Supporting study, Klimisch score 4	Defoe et al. 1990, as cited in Staples et al. 1997b

Static renewal, conducted according to Kenaga 1981	*Cyprinus carpio* carp	DBP	96h LC50, mg/l (95% C.I.) = 16.3 (16.21-16.39)	analytic grade test substance, 20 ± 2 °C, blank (solvent) controls used, 30 fish per aquarium, 30 L water, duplicates per treatment level, not noted whether analytical verification was performed Supporting Study, Klimisch score 2	Zhao et al. 2014
semi static (EEC 92/69 C1)	*Brachydanio rerio* zebra fish	DBP	96h LC50, mg/l (95% C.I.) = 2.2 (1.3-2.5)	Measured concentrations; no further study details available Supporting study, Klimisch score 4	Hüls (1994a), as cited in ECB, 2004; Staples et al. 1997b
flow through	*Ictalurus punctatus*	DBP	96h LC50, mg/l (95% C.I.) = 0.46 (0.40-0.53)	No study details available Supporting study, Klimisch score 4	Mayer and Ellersieck (1986), as cited in ECB, 2004; Staples et al. 1997b
static (APHA, 1971)	*Ictalurus punctatus*	DBP	96h LC50, mg/l (95% C.I.) = 2.91 (1.38-6.13)	No study details available Supporting study, Klimisch score 4	Mayer and Sanders (1973), as cited in ECB, 2004; Staples et al. 1997b
flow through	*Perca flavescens*	DBP	96h LC50, mg/l (95% C.I.) = 0.35 (0.28-0.44)	No study details available Supporting study, Klimisch score 4	Mayer and Ellersieck (1986), as cited in ECB, 2004; Staples et al. 1997b
static (DIN 38 412, 1982)	*Leuciscus idus*	DBP	96h LC50, mg/l (95% C.I.) = 7.3 (4.6-10)	No study details available Supporting study, Klimisch score 4	CMA (1984), as cited in ECB, 2004

Invertebrates					
Equivalent to EPA OPPTS 850.1020 (Gammarid Acute Toxicity Test) and 850.1035 (Mysid Acute Toxicity Test), GLP compliant	*Daphnia magna* Water flea *Mysidopsis Bahia* Mysid shrimp *Paratanytarsus parthenogenica Midge*	DBP	48-hr EC50= 2.99 mg/L **96-hr LC50 = 0.5 mg/L** 96-hr LC50 = 6.29 mg/L	Purity ≥95%, static, analytical verification - results reported based on measured (arithmetic mean) concentrations, hardness 25 to 50 mg/L as $CaCO_3$, 20°C ± 1°C, pH 7.6 to 7.9, Key Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/4/?documentUUID=76cb4585-6ab1-41a9-a892-7a5d2472fab8; Adams et al. 1995 as cited in Staples et al. 1997b
ISO 6341 15 (equivalent to OECD Guideline 202), GLP compliance not available	*Daphnia magna* Water flea	DBP	6.78 (5.30 - 8.22) mg/L (48 h EC50)	Purity >99%, static, analytical performed and measured confirmed nominal concentrations, pH 7.8±0.2, 4 replicates per concentration, 5-8 test concentration per test, control group with 8 replicates Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/4/?documentUUID=85c3c81d-432b-47be-8d72-1f659c3b13f8
OECD Guideline 202, GLP compliance not available	*Daphnia magna* Water flea	DBP	4.8 mg/L (48 h EC50)	No study details available Supporting Study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/6/2/4
Equivalent to U.S. EPA, 1975: Methods for Acute toxicity Tests with Fish Macroinvertebrates, and Amphibians, GLP compliant	*Daphnia magna* Water flea	DBP	48 h EC50 (95% CI) = 3.4 (3.1-3.8) mg/L	Static, 23°C, pH 8.1-8.4, DO 7.6-8.7, analytical verification performed and results based on measured concentration Supporting study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/4/?documentUUID=4e12c65d-8c82-44e6-93dd-105a63d1c0ea
Static	*Daphnia magna* Water flea	DBP	48 h EC50 = 3.0 mg/L	Measured concentrations, no other details available Supporting study, Klimisch score 4	Springborn Bionomics, 1984, as cited in Staples 1997b

No data	*Daphnia magna* Water flea	DBP	48 h EC50 = 3.7 mg/L	Measured concentrations, no other details available Supporting study, Klimisch score 4	Call et al. 1983, as cited in Staples 1997b
Other	*Chironomus plumosus*	DBP	0.76 – 5.46 mg/L (48 h EC50)	No study details available Supporting study, Klimisch score 4	results of various studies cited in Staples et al. 1997b
Other; brackish water	*Nitocra spinipes*	DBP	1.7 mg/L (1.3-2.2) - (96 h LC(I)50)	No study details available Supporting study, Klimisch score 4	Lindén et al. (1979), as cited in ECB 2004
APHA (1971)	*Gammarus pseudolimnaeus*	DBP	2.1 mg/L (96 h LC50)	No study details available Supporting study, Klimisch score 4	Mayer and Sanders (1973), as cited in ECB 2004
Other	*Paratanytarsus parthenogenetica*	DBP	5.8 mg/L (96 h EC50)	No study details available Supporting study, Klimisch score 4	EG&G Bionomics (1984b), as cited in ECB 2004
Other; seawater	*Artemia salina*	DBP	8 mg/L (24 h LC50)	No study details available Supporting study, Klimisch score 4	Hudson et al. (1981), as cited in ECB 2004

Algae and aquatic plants					
EU Method C.3 (Algal Inhibition test), GLP compliant	*Desmodesmus subspicatus*	DBP	8.38 (7.15-10.28) mg/L (72 h EC50) based on growth rate **2.12 (1.85-2.40) mg/L (72 h EC50) based on biomass**	Purity not noted, no analytical performed, 24-25°C, pH 7.19-9.21, continuous light at 6 000 - 10000 lux, control group with 6 replicates Supporting study, Klimisch score 1 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=54c24fb2-8344-45fb-b545-83c57a81aea9
92/69/EEC	*Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)	DBP	1.2-2.0 mg/L (72 h EC50)	Measured concentrations. No study details available Supporting study, Klimisch score 4	Scholz, 1995; as cited in Staples et al. 1997b
ISO 8692 (equivalent to OECD 201), GLP compliance not available	*Pseudokirchnerella subcapitata*	DBP	2.52 mg/L (2.10 to 3.12) (72 h EC50)	Purity >99%, analytical performed and confirmed nominal concentrations, pH 8.0±0.3, 21±2˚C continuous light at 90-100 microE/m2/s, control group with 6 replicates Supporting study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=702fe575-b13f-45dc-a3e8-79c8436b71f5
OECD Guideline 201	*Pseudokirchnerella subcapitata*	DBP	2.7 (72 h EC50; growth rate)	Static, no study details available Supporting study, Klimisch score 4	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/6/2/6/?documentUUID=4648086d-d39f-40cd-b6a8-d5be8f964b16
Unclear	*Pseudokirchnerella subcapitata*	DBP	0.75 (96 h EC50),	No details available Supporting study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/registration-dossier/-/registered-dossier/1805/6/2/6
Other	*Gymnodium breve* (marine dinoflagellate species)	DBP	0.0034 - 0.2 (96 h EC50), based on growth rate	No study details available Supporting study, Klimisch score 4	Wilson et al. (1978), as cited in ECB 2004; Staples 1997b

The study results that were the basis for the proposed classification are highlighted in bold in the table above.

Also see Appendix 1.

Acute (short-term) toxicity to fish

LC50 values for mortality in fish in acute toxicity tests ranged from 0.35 to 16.3 mg/L. Three studies conducted with methods equivalent to standard test protocols were available. The lowest 96-hr LC50 resulting from these studies was deemed relevant for classification purposes. This study (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/2/?documentUUID=3d142a11-4d22-4269-b3fa-6a50ee909646), which was conducted according to standard EPA methods, was GLP compliant, included analytical verification, and reported 96-hr LC50s from four species. The most sensitive species tested in this study was bluegill (*Lepomis macrochirus*), with a 96-hr LC50 of 0.48 mg/L. This value will be used for the purposes of classification.

Acute (short-term) toxicity to aquatic invertebrates

Reported EC50 or LC50 values for invertebrates based on mortality or immobilization, respectively, ranged from 0.5 to 6.8 mg/L. Four studies conducted with methods equivalent to standard test protocols were available. The lowest EC50 resulting from these studies was deemed relevant for classification purposes. This study (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/4/?documentUUID=76cb4585-6ab1-41a9-a892-7a5d2472fab8), which was conducted with methods equivalent to standard EPA methods, was GLP compliant, included analytical verification, and reported 96-hr EC/LC50s from three species. The most sensitive species tested in this study was mysid shrimp (*M. bahia*), with a 96-hr LC50 of 0.5 mg/L. This value will be used for the purposes of classification.

Acute (short-term) toxicity to algae or aquatic plants

EC_{50} values for algae ranged from 0.0034 to 8.38 mg/L. The lowest EC50 (0.0034 mg/L) was reported from a study conducted with a marine dinoflagellate species (*Gymnodium breve).* This is a non-standard species and no details are available to assess the reliability of this study. Further, this study was noted to have very poor reproducibility (ECB, 2004); thus, it is not sufficient for the purposes of classification. Three studies conducted with methods equivalent to standard test protocols were available. The lowest EC50 resulting from these studies was deemed relevant for classification purposes. This study (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=54c24fb2-8344-45fb-b545-83c57a81aea9), which was conducted with methods equivalent to standard EU methods and was GLP compliant, reported a 72-hr EC50 of 2.12 mg/L for *Desmodesmus subspicatus*, based on biomass. Though this study did not perform analytical verification, this EC50 is in the same range as other studies performed with this species that reported a measured 72-EC50 of 1.2 -2.0 (Scholz, 1995; Staples et al. 1997b). Thus, the EC50 value of 2.12 mg/L will be used for the purposes of classification.

Acute (short-term) toxicity to other aquatic organisms

No data relevant to classification available.

9.1.6 Long-term aquatic hazard

Table 44: Summary of relevant information on chronic aquatic toxicity

Method, test guideline, and deviation (s) if any	**Species**	**Test material**	**Results Key or Supportive study Remarks Reference**	**Remarks**	**References**
Fish					
US. EPA-TSCA, 40 CFR, Part 791.1600	*Oncorhynchus mykiss*	DBP	99-day NOEC, based on growth (weight) = 0.1 mg/L (100 µg/L) Flow-through, measured concentrations (arithmetic. mean)	Analytical verification performed (measured values 71 to 85% of nominal values), eggs were placed in the exposure chambers approximately 4.5 hours after fertilization, 100 eggs were placed in dilution water control exposure chambers (50 per duplicate chamber), harness 164 to 180 mg/L as CaCO3, alkalinity 119 to 132 mg/L as CaCO3, pH 7.0 to 8.2, conductivity 380 to 990 umhos/cm, DO remained at >75% saturation, temperature 10 to 12°C 1.5 °C. Mean measured concentrations were 0.10, 0.19, 0.40, 0.84 and 1.7 mg/L (71-85% of nominal). Key study, Klimisch score 2 per REACH dossier	Ward and Boeri, 1991, as cited in http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/3/?documentUUID=5b4a92fe-50c3-4173-bb36-f5af0d65ee91
Non-guideline, static renewal test study	Murray rainbow fish (*Melanotaenia fluviatilis*) (non-standard species)	DBP	90-day LOEC, based on growth = 0.005 mg/L (5µg/L); **NOEC, based on growth ≤ 0.005 mg/L (5µg/L) (inferred)**	Only three test concentrations used. Effects examined at 30, 60, and 90 days. Four beakers containing four fish in each were used (16 fish per treatment per time interval with 240 total fish). Temperature 23 ± 0.1 °C, conductivity 1231–1241 µS/cm, pH 6.8–7.1, DO above 80%. Analytical verification performed (measured concentrations were 70-80% of nominal for the 15 and 50µg/L test levels; 120% for the 5µg/L level). Negative and	Bhatia et al, 2014b

				solvent controls present. Control fish showed a normal growth pattern over 90 days Histological evaluation using system described in detail (Bhatia et al., 2014) as well as concentration of sex hormones E2 and 11-KT were evaluated. Results indicated . The lowest observed effective concentration to affect the condition factor after 90 days was 5 µg/L . Complete feminization of the gonad was noted in fish exposed to 5 µg l(-1) for 90 days and to 15 and 50 µg /L of DnBP for 30 or 60 days. After 90 days of exposure to DnBP, the ovaries were regressed and immature as opposed to the control fish which were in early-vitellogenic stage. Testes, present only in fish exposed to 5 µg/L of DnBP for 30 or 60 days, were immature in comparison to the control fish that contained testes in the mid-spermatogenic phase. The E2/11-KT ratio was significantly higher only after exposures to 5 µg/L DnBP for 90 days and 50 µg/L DnBP for 30 days. Key study, Klimisch score 2	
Aquatic invertebrates					
OECD Guideline 211	*Daphnia magna* Water flea	DBP	**21-day NOEC = 0.33 mg/L based on reproduction**	Semi-static, no further details provided Supporting study, Klimisch score 4	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/6/2/5
Not available	*Daphnia magna* Water flea	DBP	21-day NOEC = 0.11 to 1.05 mg/L based on survival and reproduction	Measured concentrations, no further details provided Supporting study, Klimisch score 4	Defoe et al., 1990, as cited in Staples 1997b
Non-guideline study measuring	*Gammarus pulex*	DBP	**25-day NOEC = 0.1 mg/L, based on locomotion**	Test concentrations of 100, 500 µg/L, 10-12 °C, No. of organisms per vessel:25	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/5

locomotor activity, flow-through				No. reps per concentration: 3 No. of vessels per control (replicates): 2 Supporting study, Klimisch score 2 per REACH dossier	/?documentUUID=dfd0d0fa-7c9b-41ed-a9cb-29bb83f0e015
Algae and aquatic plants					
EU Method C.3 (Algal Inhibition test), GLP compliant	*Desmodesmus subspicatus*	DBP	1.88 (1.27-2.43) mg/L (72 h EC10) based on growth rate 5 mg/L (72 h NOEC) based on growth rate **0.13 (0.08-0.20) mg/L (72 h EC10) based on biomass**	Purity not noted, static, no analytical performed, 24-25°C, pH 7.19-9.21, continuous light at 6 000 - 10 000 lux, control group with 6 replicates Supporting study, Klimisch score 1 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=54c24fb2-8344-45fb-b545-83c57a81aea9
92/69/EEC	*Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)	DBP	0.5 (72-h NOEC), based on growth rate, cell growth	Measured concentrations. No study details available Supporting study, Klimisch score 4	Scholz, 1995; Staples et al. 1997b
ISO 8692 (equivalent to OECD 201), GLP compliance not available	*Pseudokirchneriella subcapitata*	DBP	1.49 (1.08 - 2.06) mg/L (72 h EC10)	Purity >99%, static, analytical performed, results based on measured concentrations, pH 8.0±0.3, 21±2˚C continuous light at 90-100 microE/m2/s, control group with 6 replicates Supporting study, Klimisch score 2 per REACH dossier	http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=702fe575-b13f-45dc-a3e8-79c8436b71f5
OECD Guideline 201	*Pseudokirchneriella subcapitata*	DBP	0.3 mg/L (72 h NOEC; growth rate)	Static, no study details available Supporting study, Klimisch score 4	http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/6/2/6/?documentUUID=4648086d-d39f-40cd-b6a8-d5be8f964b16

Other	*Selenastrum capricornutum**	DBP	NOEC = 2.8 mg/L (7 d)	No study details available Supporting study, Klimisch score 4	Melin and Egnéus (1983), as cited in ECB, 2004
Other	*Selenastrum capricornutum*	DBP	NOEC = 0.8 mg/L (10 d)	No study details available Supporting study, Klimisch score 4	CMA (1984), as cited in ECB, 2004
Other (marine)	*Dunaliella parva*	DBP	NOEC = 0.28 mg/L (7 d), based on cell aggregation	No study details available Supporting study, Klimisch score 4	Acey et al. (1987), as cited in ECB, 2004; Staples et al. 1997b
Other (marine)	*Thalassiosira pseudomona*	DBP	NOEC = 2.0 mg/L (4 d)	No study details available Supporting study, Klimisch score 4	Acey et al. (1987), as cited in ECB, 2004
Other (marine)	*Synecchococcus lividus*	DBP	0.002 (14 d LOEC) DBP caused a decrease only in the number of non-aggregated *S. lividus*. Nevertheless, very low concentrations of DBP seem to affect the growth behaviour of these blue-green algae. (ECB, 2004)	No study details available Supporting study, Klimisch score 4	Acey et al. (1987), as cited in ECB, 2004

* *now known as Pseudokirchneriella subcapitata*

The study results that were the basis for the proposed classification are highlighted in bold in the Table above.

Also see Appendix 1

Chronic toxicity to fish

Two studies are available that examine chronic toxicity in fish. *Oncorhynchus mykiss* showed decreased growth at concentrations above 0.1 mg/L (99-day NOEC of 0.1 mg/L) in a 99-day flow-through

test (Ward and Boeri, 1991, as cited in http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/3). In a non-standard, well documented test, conducted with Murray rainbow fish (*Melanotaenia fluviatilis*), reduction in growth was observed at 0.005 mg/L after 90 days (90-day LOEC of 0.005 mg/L reported, 90-day NOEC of ≤ 0.005 mg/L inferred (Bhatia et al, 2014b). Conservatively, the NOEC value of ≤ 0.005 mg/L was used for classification. Note that this study also examined sex steroid hormone concentrations and gonadal development; however, these endpoints are not currently used in GHS classification.

Chronic toxicity to aquatic invertebrates

NOEC values from long-term toxicity tests conducted with aquatic invertebrates ranged from 0.1 to 1.05 mg/L. The lowest NOEC (25-day NOEC of 0.1 mg/L) was reported from a study examining locomotor activity of *Gammarus pulex* under flow-through conditions (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/5/?documentUUID=dfd0d0fa-7c9b-41ed-a9cb-29bb83f0e015). A study, reportedly conducted according to standard test guideline OECD 211, resulted in a 21-day NOEC of 0.33 mg/L for *Daphnia magna* (http://echa.europa.eu/registration-dossier/-/registered-dossier/1676/6/2/5). Both of these studies, as well as the remaining data reported for aquatic invertebrates in Table 41, demonstrated NOECs between 0.1 and 1.0 mg/L and will be considered for classification.

Chronic toxicity to algae or aquatic plants

NOEC/EC10 values for algae ranged from ≤0.002 to 5 mg/L. The lowest NOEC (≤0.002 mg/L) was reported from a study conducted with a marine, blue-green algae (*Synecchococcus lividus).* This is a non-standard species and no details are available to assess the reliability of this study. Furthermore, it was noted by ECB (2004) that "DBP caused a decrease only in the number of non-aggregated *S. lividus*, and when counting the total number …a significant increase was found at all test concentrations." Thus, this study result is not considered for the purposes of classification. Three studies conducted with methods equivalent to standard test protocols were available. The lowest NOEC/EC10 resulting from these studies was deemed relevant for classification purposes. This study (http://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14862/6/2/6/?documentUUID=54c24fb2-8344-45fb-b545-83c57a81aea9), which was conducted with methods equivalent to standard EU methods and was GLP compliant, reported a 72-hr EC10 of 0.13 mg/L for *Desmodesmus subspicatus*, based on biomass. Though this study did not perform analytical verification, this EC10 is in the same range as another study performed with this species that reported a measured 72-h NOEC of 0.5 (Scholz, 1995; as cited in Staples et al. 1997b). Thus, the EC10 value of 0.13 mg/L (based on biomass analysis, ECHA report) will be used for the purposes of classification.

Chronic toxicity to other aquatic organisms

No data relevant for classification are available.

9.1.7 Comparison with the GHS criteria for hazardous to the aquatic environment

Acute aquatic hazard

There are adequate data from acute toxicity studies available for all trophic levels (see section 9.1.5 and Table 43 for more details).

A study with several species of fish that was conducted according to standard guidelines, under GLP and with analytical verification was considered relevant for classification purposes. The 96-hr LC50 of 0.48 mg/L for bluegill (*Lepomis macrochirus*), which was the most sensitive species, was selected for classification purposes.

A study with several species of aquatic invertebrates that was conducted according to standard guidelines, under GLP and with analytical verification was considered relevant for classification purposes. The 96-hr LC50 of 0.5 mg/L for mysid shrimp (*Mysidopsis bahia*, currently known as *Americamysis bahia*), which was the most sensitive species, was selected for classification purposes.

In algae, three studies were performed according to OECD TG 201 or similar methodology. The lowest reported72-hr EC50 from these studies (2.12 mg/L for *Desmodesmus subspicatus*, based on biomass) was used for classification purposes.

Comparing the information from the most sensitive trophic group (fish with a 96-hr LC50 of 0.48 mg/L), derived from the most reliable and fully documented acute aquatic toxicity studies available with the GHS criteria (Table 4.1.1.(a)), results in a classification of Category Acute 1, M factor = 1 [acute aquatic toxicity value above 0.1 but below (or equal to) 1 mg/L].

Long-term aquatic hazard (including bioaccumulation and degradation)

Bioaccumulation

Measured logKow values for DBP are ≥ 4, ranging from 4.46 to 4.57, indicating that DBP has a potential for bioaccumulation. Measured BCF values vary drastically depending on the methodology employed. Studies using measurement of ^{14}C-labelled material likely overestimate the BCF, as results would reflect measured ^{14}C-DBP, any ^{14}C-labelled metabolites of DBP, as well as ^{14}C built into the tissue of the organism in e.g. fatty acids. BCFs derived from these types of studies were 2125 for fish and 2.9 to 6700 for invertebrates. A GLP compliant study, conducted according to OECD guideline 305 E found a BCF of 1.8 for DBP in Carp (*Cyprinus carpio*); however, the primary metabolite (mono-ester MBP) was not measured in this study. Therefore, this study only reflects the BCF for the parent compound. Thus, the true BCF of DBP and major metabolites in fish is likely between 1.8 and 2125. Per UN GHS 2015, Annex 9, A9.5.2.3.9.4, if only BCFs based on parent compound and on radiolabeled measurements are available, the latter should be used for classification. Thus, conservatively, based on the measured BCF value derived in fish of 2125, DBP has the potential for bioaccumulation.

Rapid degradation

DBP is considered to be readily degradable based on the data available.

Chronic aquatic toxicity

As shown in section 9.1.6, chronic aquatic toxicity of DBP is available for all three trophic levels, and included studies deemed relevant for classification purposes.

In fish, the NOEC derived from a non-standard, well documented test, conducted with Murray rainbow fish (*Melanotaenia fluviatilis*) of 0.005 mg/L was, conservatively, deemed relevant to be used for classification purposes.

In aquatic invertebrates, all NOECs reported were ≥0.1 and ≤1.0 mg/L, including the two studies deemed relevant to be used for classification purposes.

In algae, three studies were performed according to OECD TG 201 or similar methodology. The lowest reported 72-hr EC10 from these studies (0.13 mg/L for *Desmodesmus subspicatus*, based on biomass) was used for classification purposes.

Comparing the information from the most sensitive trophic group (fish with a NOEC of 0.005 mg/L), derived from the most reliable and fully documented acute aquatic toxicity studies available with the GHS criteria (Table 4.1.1.(bii)), results in a classification of Category Chronic 1, M factor = 1 [rapidly degradable, chronic aquatic toxicity value above 0.001 but below (or equal to) 0.01 mg/L; Table 4.1.5].

9.1.8 Conclusion on classification and labelling for hazardous to the aquatic environment

Aquatic Acute 1, M factor = 1; Aquatic Chronic 1, M factor 1

Potential to bioaccumulate

9.2 HAZARDOUS TO THE OZONE LAYER

9.2.1 Conclusion on classification and labelling for hazardous to the ozone layer

DBP is not listed as a controlled substance in the annexes to the Montreal Protocol, and thus is not classified as hazardous to the ozone layer.

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