Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

diisohexyl phthalate

EC Number: 276-090-2
CAS Number: 71850-09-4

CLH-O-0000001412-86-158/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

9 June 2017

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: 1,2-Benzenedicarboxylic acid, diisohexyl ester

EC Number: **276-090-2**

CAS Number: **71850-09-4**

Index Number:

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	1,2-benzenedicarboxylic acid, diisohexyl ester
EC number:	276-090-2
EC name:	Diisohexyl phthalate
CAS number:	71850-09-4
Annex VI Index number:	-
Degree of purity:	No information available.
Impurities:	No information available

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Repr. 1B – H360FD	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B – H360FD	

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIISOHEXYL PHTHALATE

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	None		None	Not evaluated
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		None	Not evaluated
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	None		None	Not evaluated
3.7.	Reproductive toxicity	**Repr. 1B – H360FD**			
3.8.	Specific target organ toxicity –single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram: GHS08
Signal word: Danger
Hazard statements: H360FD: May damage fertility. May damage the unborn child
Precautionary statements: Not harmonized

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There is no previous harmonised classification and labelling of 1,2-benzenedicarboxylic acid, diisohexyl ester, hereafter referenced as diisohexyl phthalate in the report.

The Swedish Competent Authority submitted a proposal on harmonised classification of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS number 68515-50-4) in 2012 that was subsequently included in ATP7 to CLP Annex VI (index number 607-710-00-5) as Repr. 1B; H360FD. 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear is composed of branched and linear C6 isomers to a varying extent, and diisohexyl phthalate with CAS number 71850-09-4 is one of the branched constituents.

The intention of the Swedish Competent Authority was to have also CAS number 71850-09-4, EC number 276-090-2 included in the classification proposal and consequently also included in the same entry of Annex VI as CAS number 68515-50-4. However, since this was not clearly stated in the CLH-report, ECHA/RAC clarified that *it is not possible to add another substance with a different EC and CAS number after public consultation and since the CLH dossier submitted and published for public consultation covered only the substance with EC number 271-093-5, CAS number 68515-50-4, the opinion and the future entry in Annex VI to CLP will only cover the substance 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS nr. 68515-50-4, EC nr. 271-093-5).*

Therefore, this proposal on harmonised classification is intended for diisohexyl phthalate (CAS number 71850-09-4).

2.2 Short summary of the scientific justification for the CLH proposal

This classification proposal is based on a chemical category approach. There are no mammalian fertility or developmental toxicity studies available for diisohexyl phthalate, however, there is a convincing literature demonstrating adverse effects of structurally similar transitional (C4-C6) phthalates on these endpoints. Hence, for the purpose of filling data gaps of reproductive toxicity for harmonized classification and labelling of diisohexyl phthalate in the current report, a chemical category was established, according to OECD recommendations as adapted by REACH Guidance, including eight selected *ortho*-phthalates with side chain lengths of 3-6 carbons.

Phthalate esters are characterized by a diesterified 1,2-benzenedicarboxylic acid. Diisohexyl phthalate has carbon sidechains that are methylbranched at the 4-positions of the linear 5-carbon

chains, i.e. the straight-chain carbon backbones are C5. The substance 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS no. 68515-50-4) also consists of branched isomers but commercial blends of the substance also contain up to 25% of linear di-n-hexyl phthalate (DnHP; CAS no. 84-75-3). Thus, the branched structures (C5; including CAS no. 71850-09-4) are the predominant isomers (NICNAS, 2008c) of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear. However, there is no information available on the exact concentrations of the isomers that constitute 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear.

There is no data available on the toxic properties of diisohexyl phthalate. However, diisohexyl phthalate, 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear and DnHP belongs to a group of 'transitional' phthalates defined as those produced from alcohols with straight-chain carbon backbones of C4-6 (ACC Phthalate Esters Panel HPV Testing Group, 2001). Phthalates of this backbone length have been associated previously with reproductive and developmental toxicity (Foster et al., 1980; Oishi and Hiraga, 1980; Lamb et al., 1987; Heindel et al., 1989). Information from structurally similar phthalates, where available, was therefore used in a chemical grouping approach to confirm potential toxicity of diisohexyl phthalate. Read-across information on toxicity endpoints was obtained from 7 reference *ortho*-phthalates with ester side-chain lengths within the interval of 3-6 carbon atoms based on the transitional phthalate category (C4-6), however, in the current report the transitional category has been extended by the dossier submitter to include C3 (diisobutyl phthalate). The available studies demonstrate significant effects on the male reproductive organs and developmental effects of the selected phthalates in the category. Moreover, all included reference phthalates have a harmonised classification in Repr. 1B. Thus, the similarity of reproductive toxicity across the category of phthalates supports the notion that these effects are intrinsic properties of the reference phthalates and that diisohexyl phthalate, with C5 side chains, falls within the limits of the chemical category of phthalates (C3-6) and should have the classification Repr. 1B.

2.3 Current self-classification and labelling

2.3.1 Current self-classification and labelling based on the CLP Regulation criteria

There is no C&L notification for diisohexyl phthalate.

RAC general comment
RAC adopted an opinion for harmonised classification of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS number 68515-50-4, DHP) on 7 June 2013. The substance was classified as Repr. 1B; H360FD. 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear is composed of branched and linear C6 isomers to a varying extent, and diisohexyl phthalate (DIHP) with CAS number 71850-09-4 is one of the branched constituents. At the time, the intention of the dossier submitter for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS number 68515-50-4, DHP) was to include CAS number 71850-09-4 in the same classification proposal, however due to procedural issues this was not possible. Hence, an additional proposal for a harmonised classification of 1,2-benzenedicarboxylic acid, dihexyl ester (CAS number 71850-09-4, DIHP) was submitted to ECHA.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Diisohexyl phthalate has a CMR property (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a Community-wide action under article 36 of CLP. Repeated dose toxicity data are presented for information as they may provide relevant data for assessment of reproductive toxicity but no classification is discussed and proposed for this endpoint.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

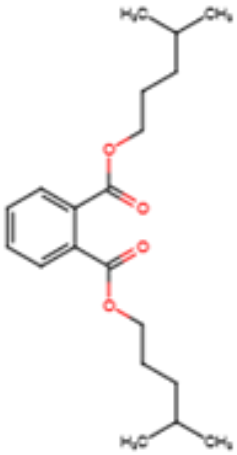
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	276-090-2
EC name:	diisohexyl phthalate
CAS number (EC inventory):	
CAS number:	71850-09-4
CAS name:	1,2-benzenedicarboxylic acid, diisohexyl ester
IUPAC name:	1,2-bis(4-methylpentyl) benzene-1,2-dicarboxylate
CLP Annex VI Index number:	none
Molecular formula:	$C_{20}H_{30}O_4$
Molecular weight range:	334 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Diisohexyl phthalate (CAS no. 71850-09-4)	No information available		

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No information available			

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No information available				

1.2.1 Composition of test material

1.3 Physico-chemical properties

No data on diisohexyl phthalate (CAS no. 71850-09-4) is available. Therefore, data on physico-chemical properties from CAS no. 68515-50-4, consisting of predominantly branched C6 isomers (including CAS no. 71850-09-4) and also linear isomers (<25%), was used to fill the data gap.

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	liquid	ExxonMobil Biomedical Sciences Inc., 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Not available
Melting/freezing point	-27.4°C	Staples et al., 1997; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Not available (2, valid with restriction; ACC Phthalate Esters Panel HPV Testing Group, 2006)
Boiling point	373°C at 1013 hPa	U.S. EPA, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Estimated (2, valid with restriction; ACC Phthalate Esters Panel HPV Testing Group, 2006)
Relative density	1.01	ExxonMobil Chemical Co, 2000; U.S. OSHA, 2001	Test substance: CAS no. 68515-50-4 Not available
Vapour pressure	0.344×10^{-5} hPa at 25°C	Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Measured, calculated data also considered in determining recommended values (2, valid with restriction; ACC Phthalate Esters Panel HPV Testing Group, 2006)
Surface tension	No data		
Water solubility	0.159 mg/L at 25°C	Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Measured, calculated data also considered in determining recommended values (2, valid with restriction; ACC Phthalate Esters Panel HPV Testing Group, 2006)

Partition coefficient n-octanol/water	6 at 25°C	Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006	Test substance: CAS no. 68515-50-4 Measured, calculated data also considered in determining recommended values (2, valid with restriction; ACC Phthalate Esters Panel HPV Testing Group, 2006)
Flash point	192°C	ExxonMobil Chemical Co, 2000; U.S. OSHA, 2001	Test substance: CAS no. 68515-50-4 Not available
Flammability	No data		
Explosive properties	No data		
Self-ignition temperature	>500°C	ExxonMobil Chemical Co, 2000; U.S. OSHA, 2001	Test substance: CAS no. 68515-50-4 Not available
Oxidising properties	No data		
Granulometry	Not relevant (liquid)		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity	37 cSt at 20°C	Scientific Polymer Inc. 1996; Flick, 2002	Test substance: CAS no. 68515-50-4 Not available

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

No relevant information is available for diisohexyl phthalate. 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including the branched isomer CAS no. 71850-09-4) have been used as lubricant in steering fluid and as plasticizer.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

No toxicokinetic data is available for diisohexyl phthalate. However, the substance is closely structurally related with the linear C6 isomer DnHP. Based on data for DnHP and other transitional phthalates, diisohexyl phthalate is likely to be rapidly absorbed as the monoester from the gut and excreted via the urine (Elsisi et al., 1989; NICNAS, 2008c). The rate of absorption and excretion of [^{14}C] phthalate esters applied dermally, including DnHP, with varying length of the alkyl side chain was investigated (Elsisi et al., 1989). It was concluded that as the length of the alkyl side chain increased, the amount of ^{14}C excreted in the first 24 hours decreased significantly. The cumulative percentage dose excreted in 7 days was greatest for diethyl (DEP), dibutyl (DBP), and diisobutyl phthalate (DIBP) (about 50-60% of the applied ^{14}C); and intermediate (20-40%) for dimethyl (DMP), benzyl butyl (BBP), and DnHP. 18% of the applied dose of DnHP was absorbed and excreted after 24 hr. Urine was the major route of excretion of all phthalate diesters except for diisodecyl phthalate. This compound was poorly absorbed and showed almost no urinary excretion. After 7 days, the percentage dose for each phthalate that remained in the body was minimal and showed no specific tissue distribution. Most of the unexcreted dose remained in the area of application. These data show that the structure of the phthalate diester determines the degree of dermal absorption, and thus supports the assumption that the fate of diisohexyl phthalate is similar to that of DnHP.

4.1.2 Human information

None.

4.1.3 Summary and discussion on toxicokinetics

No toxicokinetic data is available for diisohexyl phthalate. No information is available on biotransformation of diisohexyl phthalate. However, as other phthalates are converted to monoesters and alcohol and rapidly excreted, it is anticipated that diisohexyl phthalate would behave in the same way (NTP-CERHR, 2003) resulting in monohexyl phthalate and isohexanol.

4.2 Acute toxicity

Not evaluated in this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

No data on diisohexyl phthalate (CAS no. 71850-09-4) is available. Data on 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS no. 68515-50-4), consisting of predominantly branched C6 isomers (including 71850-09-4) and also linear isomers (<25%), was included here to support the classification of reproductive toxicity.

Table 17: Summary table of relevant repeated dose toxicity studies for diisohexyl phthalate

Method	Results	Remarks	Reference
Sprague-Dawley rats (10/sex/group) fed with 0, 0.05, 0.1 and 0.5% (corresponding approx. to 0, 38.3, 76.6, 383 mg/kg/day respectively) daily for 13 weeks (90 days). The 0.05% group was adjusted to 1.0% (766 mg/kg/day) at 7 weeks and 3.0% (2298 mg/kg/day) at 12 weeks.	Rats in the 0.05% group (increased to 1.0 % at 7 weeks and 3.0% at 12 weeks) displayed signs of respiratory distress, stiff gait, and rigidly or arched tail over the last three weeks of the study. Body weight gains and food consumption were decreased. Total leukocyte counts were significantly increased for the 3.0% females at 90 days. Blood chemistry, hematology and urinalysis values were comparable for all other groups and intervals. Heart-body weight ratios for males in all three dose groups and thyroid weights for females in the 0.1% group were significantly increased. No effects other than heart-body-weight ratios were observed at 0.5%. Rats of both sexes in the 3.0% group showed significantly increased liver weights and decreased weights of spleen, kidneys, and adrenals. Significantly decreased weight of gonads in both sexes at 3.0%. The quantification of the effect is not stated in the report. Males in the 3.0% group displayed atrophy of the spermatogenic epithelium in the testes. LOAEL for general effects (heart/body weight ratios in both sexes, and thyroid weights in females) was 0.1% (76.6 mg/kg/day) LOAEL for testicular effects was 1-3% (766-2298 mg/kg/day)	Test substance: CAS no. 68515-50-4, with ≤ 15% diheptyl phthalate. Reliability 2 (valid with restrictions), as the protocol was not standardized and validated internationally (ACC Phthalate Esters Panel HPV Testing Group, 2006).	Esso Research and Engineering Company, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006

Beagle dogs (unknown number of animals/sex/group) fed 0, 0.1, 0.5, or 1.0% (corresponding to approx. 0, 18, 90, 180 mg/kg/day respectively) for 13 weeks (90 days) daily. Low dose was adjusted to 5.0% (900 mg/kg/day) during weeks 9-13.	No significant variations in weight, clinical blood chemistry, hematology, and urinalysis values were observed. Increased absolute and relative liver weights in the 5.0% group. Decreased absolute and relative testes weights in the 5.0% group. Enlarged hepatic cells were observed in the males in the 5.0% group; these two males also exhibited atrophy of the seminiferous epithelium in the testes. LOAEL for testicular and general effects was 5% (approximately 900 mg/kg/day).	Test substance: CAS no. 68515-50-4 The composition (concentration of isomers) of the test substance is not known. Reliability 2, as the protocol was not standardized and validated internationally (ACC Phthalate Esters Panel HPV Testing Group, 2006).	Esso Research and Engineering Company, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Two studies on repeated dose toxicity are available for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including the branched isomer CAS no. 71850-09-4). The studies were performed by Esso Research and Engineering Company in 1962 and are therefore not complying with international standardized and validated test guidelines. The studies have been compiled and summarized for the U.S. EPA by the American Chemical Council Phthalate Ester Panel HPV Testing Group (the full report, including both testing in rat and dog, is not available to the dossier submitter). According to their assessment the studies are not completely satisfactory reported and do not provide complete information (i.e. information on number of animals (dogs) is missing; it is unclear whether a dose-response relationship was established; and quantification of the findings and the statistics are not presented). However, as these studies are the only available *in vivo* studies on 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear, and no studies are available for diisohexyl phthalate alone (not in mixture), they are included because the findings are in line with reported effects of transitional (C4-6) phthalates and thus support the category approach described in section 4.11.3.

A repeated dose toxicity study was performed in male and female Sprague-Dawley rats (Esso Research and Engineering Company, 1962). The rats were fed daily with 0, 0.05%, 0.1% or 0.5% (approx. 0, 38.3, 76.6, 383 mg/kg/day, respectively) 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear for 13 weeks (90 days). The 0.05% group was adjusted to 1.0% (766 mg/kg/day) at 7 weeks and 3.0% (2298 mg/kg/day) at 12 weeks. There was no post exposure period. Clinical observations, body weights and food consumption were recorded weekly. Clinical blood chemistry, hematology, and urinalysis were performed on 5 rats/sex/group at 30 and 90 days. A complete necropsy was performed after 13 weeks and organ weights were recorded. Tissues from the control, 0.5% and 3.0% groups were examined microscopically.

Rats in the 0.05% group, which was increased to 1.0 % (776 mg/kg/day) at 7 weeks and 3.0% (2298 mg/kg/day) at 12 weeks displayed effects on male reproductive organs including decreased testis weights and atrophy of the spermatogenic epithelium. This dose group also displayed signs of respiratory distress, stiff gait, and rigidly or arched tail over the last three weeks of the study. In addition, body weight gains and food consumption were decreased in the animals during that period. Rats of both sexes in the 3.0% group showed significantly decreased gonads, spleen, kidneys, and adrenals weights. Liver weights were increased and microscopic examination of tissues from rats in the 3.0% group revealed slight changes in the liver characterized as eosinophilic areas in the cytoplasm and variation in the size of the nuclei. Total leukocyte counts were significantly increased for the 3.0% females at 90 days; blood chemistry, hematology and urinalysis values were comparable for all other groups and intervals. Heart/body weight ratios for males in all three dose groups and thyroid weights for females in the 0.1% group were significantly increased over the controls. It should be noted that the dose-level 0.05% (lowest dose tested) is not relevant as LOAEL since this dose was changed (as described above) 7 weeks into the study, and consequently the 0.05% dose level was only effective for 6 weeks. Correspondingly, the toxicity of the 3% dose is probably underestimated since this dose was only administered during the last week of the study; during weeks 7-12 the rats were given 1% 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear. Thus, the LOAEL was set to 0.1 % (approximately 76.6 mg/kg/day) based on increased heart/body weight ratios in males and increased thyroid weights in females. No NOAEL could be established.

The systemic effects of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear were also examined in Beagle dogs by Esso (Esso Research and Engineering Company, 1962). Male and female dogs were administered by oral feeding with 0, 0.1, 0.5, or 1.0% 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear for 13 weeks (90 days) daily. Low dose was adjusted to 5.0% during weeks 9-13. The animals were observed daily; body weights and food consumption were recorded weekly. Hematology, blood chemistry, and urinalysis were performed initially and at 30 and 90 days. A complete necropsy was performed after 13 weeks. Organ weights were recorded and the tissues from the control and high dose groups were examined microscopically.

As in rats, 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear also affected male reproductive organs in Beagle dogs in the 5% dose group. The males exhibited decreased absolute and relative testes weights, and two males exhibited atrophy of the seminiferous epithelium in the testes. All animals displayed normal appearance and behaviour throughout the study. No significant variations in weight were attributed to the test substance. Clinical blood chemistry, hematology, and urinalysis values were within normal limits and comparable to the controls. Absolute and relative mean liver weights for the dogs in the 5.0% group increased. Enlarged hepatic cells were also observed in the males in the 5.0% group. Similarly to the rat study (discussed above), it is likely that the toxicity of 5% 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear is underestimated when taking into account that this dose was only administered during 4 weeks at the end of the study. Nonetheless, LOAEL was set to 5% (approximately 900 mg/kg/day) and the NOAEL was 1.0% (approximately 180 mg/kg/day).

4.7.1.2 Repeated dose toxicity: inhalation

4.7.1.3 Repeated dose toxicity: dermal

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

Repeated dose toxicity data are presented for information as they may provide relevant data for assessment of reproductive toxicity and no classification is discussed and proposed for this endpoint. There are two repeated dose toxicity studies available for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including branched isomer CAS no. 71850-09-4). Based on the literature for phthalates with a backbone of 4-6 carbon atoms, liver and kidney effects from repeated doses studies is expected, particularly at high doses. Indeed, 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear induced hepatic effects typical of structurally similar phthalates (see Table 19) including significantly increased liver weights of both sexes in rats exposed to 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (3% or 2298 mg/kg/day) through the diet for up to 90 days. Weights of spleen, kidneys and adrenals were also reduced in these animals. Moreover, weights of gonads of both males and females at 1-3% (776-2298 mg/kg/day) were decreased, and the males displayed atrophy of the spermatogenic epithelium in testes. Similar manifestations were observed in dogs in a comparable 90 day study. Increased absolute and relative mean liver weights, decreased absolute and relative testes weight, and testicular effects, including atrophy of seminiferous epithelium, in the 5.0% (900 mg/kg/day) group were noted.

The data presented in this section is the only available *in vivo* data on 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including the branched isomer CAS no. 71850-09-4), and is not sufficient to confirm testis toxicity for classification of diisohexyl phthalate as such, however the findings supports classification in reproductive toxicity based on a weight of evidence approach including a category approach of structural similar phthalates (see section 4.11.3.2) below).

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

No information available.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

No information available.

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

4.11.3.1 Mode of action/ Endocrine disruptor property

The estrogenic activity of dihexyl phthalate isomers have been examined using a series of short-term *in vitro* and *in vivo* assays. Diisohexyl phthalate (information on CAS number not available; purity 97-99%) was negative for estrogenic activity in a recombinant yeast assay ($4.8x10^{-7}$ - 10^{-3} M) and did not induce cell proliferation in the estrogen responsive human breast cancer cell line ZR-75 (10^{-7} – 10^{-5} M) (Harris et al., 1997). Furthermore, an isomeric mixture of dihexyl phthalate (information on CAS number not available; purity 99.6%) (10^{-5} M) did not have an effect on estrogen-inducible growth of yeast, and was not able to induce estrogenic responses *in vivo* in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats at any of the concentrations tested (20, 200, and 2000 mg/kg) for the duration of the five day long study (Zacharewski et al., 1998). In contrast, other studies reveal estrogenic responses of dihexyl phthalate isomers. In an *in vitro* rat uterine competitive ligand-binding assay an isomeric mixture of dihexyl phthalate was a weak competitive agonist at the estrogen receptor (ER) and weakly induced ER-mediated gene expression in MCF-7 cells at 10^{-5} M (Zacharewski et al., 1998). Moreover, an isomeric mixture of dihexyl phthalate (information on CAS number not available; purity >98.5%) (10^{-5} M) demonstrated estrogenic activities in a human ERα (but not β) reporter gene assay in CHO-K1 cells transfected with expression vectors for human ERα, ERβ and androgen receptor (AR). Dihexyl phthalate isomers demonstrated anti-estrogenic activity via ERβ in the presence of 17β-estradiol and anti-androgenic activity in the hAR-transactivation assay (Takeuchi et al., 2005).

In summary, some *in vitro* studies suggest that an isomeric mixture of dihexyl phthalate was able to induce human estrogen receptor α-agonistic activity and androgen receptor-antagonistic activities, but did not induce vaginal cornification response or an increase in uterine weight *in vivo*.

4.11.3.2 Category approach - Chemical grouping

There are no mammalian fertility or developmental toxicity studies available for diisohexyl phthalate, however, there is a convincing literature demonstrating adverse effects of structurally similar transitional (C4-C6) phthalates on these endpoints. To generate information on the potential reproductive and developmental toxicity of diisohexyl phthalate for the purpose of harmonized classification a chemical grouping approach was utilized. The method of chemical categories or grouping is supported in REACH Article 13 - *Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across).*

The REACH Guidance document on grouping of chemicals (Chapter R.6) is complying with the OECD principles for the validation of Chemical grouping (2007) and recommends a stepwise procedure to the formation of chemical categories. The reporting format is described below.

Identification of a structure-based category and its members

Phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. Three isomeric forms of benzenedicarboxylic acid esters exist: *ortho*-phthalates, *meta*-phthalates and *para*-phthalates, also known as phthalates, isophthalates and terephthalates, respectively. The most common *ortho*-phthalates possess ester side chains ranging from C1 to C13. Side chains may be linear, branched or a combination of linear, branched and ringed structures. Commonly, both side chains are structurally identical, but for some phthalates they differ. The structural characteristics of the ester side chains affect both the physical/chemical and biological properties of phthalate esters. The phthalate esters are divided into three subcategories based on their physicochemical and toxicological properties (ACC Phthalate Esters Panel HPV Testing Group, 2001; U.S. EPA, 2010):

(1) low molecular weight phthalates,

(2) transitional phthalates, and

(3) high molecular weight phthalates.

The transitional phthalates group includes phthalates containing >10 percent molecules derived from alcohols with alkyl chains of four, five, or six carbons and tend to have higher water solubility, volatility, propensity to migrate, and dermal absorption compared to high molecular weight phthalates (Elsisi et al., 1989; ACC Phthalate Esters Panel HPV Testing Group, 2001).

According to Fabjan et al 2006 a chemical category approach of *ortho*-phthalate esters with different side chain lengths and substance structure can be readily applied for predicting adverse reproductive effects in experimental animals. The authors tested the commonly accepted assumption introduced by Foster et al 1980 stating that phthalates with the alkyl side-chain length from C4 to C6 produce similar severe reproductive effects in experimental animals. The main effects are reported to be on male sexual development and the effects related to anti androgenic activity appeared to be the most critical. Hence, for the purpose of filling data gaps in reproductive toxicity for harmonized classification and labelling of diisohexyl phthalate in the current report, a chemical category was established, according to OECD recommendations as adapted by REACH Guidance, including eight selected *ortho*-phthalates with side chain lengths of 3-6 carbons (Table 19). Diisohexyl phthalate,

with a side chain length of 5 carbons is already indirectly a member of several published phthalate categories for hazard characterization screening carried out by international associations and public bodies (ACC Phthalate Esters Panel HPV Testing Group, 2001; NICNAS, 2008; U.S. EPA, 2010; U.S. CPSC, 2010) as being one of the constituents of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear.

Reporting format for the transitional ortho-phthalate category

1. Category definition and its members

1.1. Category definition

The category is defined as *ortho*-phthalates with carbon side chains in the length interval 3-6 and is based on already published phthalate categories (Phthalate Esters Panel HPV Testing Group, 2001; OECD 2004; Fabjan et al., 2006; NAS, 2008; U.S. EPA, 2010).

1.1.a. Category Hypothesis

The selected *ortho*-phthalates with the alkyl side-chain length from C3 to C6 have similar physicochemical, biological, and toxicological properties that would be expected to behave in a predictably similar manner across the defined category spectrum. Thus, reproductive toxicity is an intrinsic hazard of all the selected phthalates in the category and read-across can be performed to fill data gaps of reproductive toxicity where data of diisohexyl phthalate is lacking.

1.1.b. Applicability domain (AD) of the category

The category applies to di-alkyl *ortho*-phthalate esters. Criterion for selection of the *ortho*-phthalates was primarily the length of the alkyl chain (3-6 carbon atoms) and the total number of carbon atoms in the side chain: at least four carbons, and maximum eight carbons in total in the side chain. Secondly, the structural similarities and similarity in linearity and branching of the alkyl chains, and thirdly, the availability of documented effects on reproductive toxicity was decisive for the inclusion in the category.

The eight members of this category consist of linear and/or branched dibutyl, dipentyl, and dihexyl phthalate esters. The branched alkyl chains are composed of varying mixed isomers. The length of the alkyl chains varies by substance, the backbones range from C3 to C6. The backbones in all but one category member contain methyl branching, only the bis(2-ethyl hexyl) (DEHP) phthalate backbone contains ethyl branching.

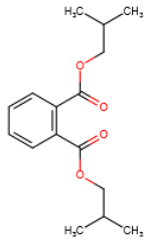
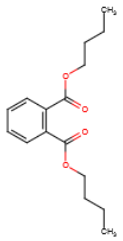
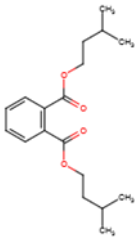
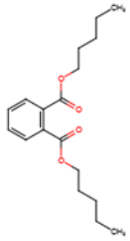
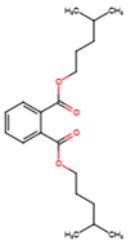
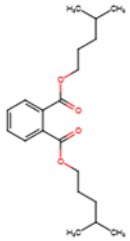
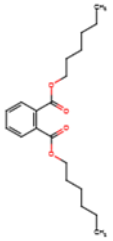
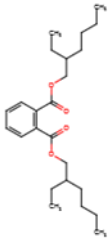
Seven out of the eight phthalates in the category belong within the already recognized transitional subcategory (C4-C6). One of the phthalates in the category of the current dossier, diisobutyl phthalate (DIBP), has a side chain length of 3 carbons with a methyl-branching and thus has a total of 4 carbons in the side chain and has the same molecular weight as DBP (4C). DIBP is included in the current chemical grouping as a sentinel chemical.

1.1.1c. List of endpoints covered

For the purpose of harmonized classification and labelling the category approach was applied to the endpoint reproductive toxicity.

1.2. Category Members

Category members are eight *ortho*-phthalates with carbon side chains in the interval of 3-6 carbon atoms. Numbers indicates the length of the carbon side chain, and in parentheses the total number of carbon atoms in the side chain is indicated. Diisohexyl phthalate, the substance subject to read-across, is indicated in bold text.

Diisobutyl phthalate (DIBP) CAS 84-69-5  3C (4C)	Di-n-butyl phthalate (DBP) CAS 84-74-2  4C
Diisopentyl phthalate (DIPP) CAS 605-50-5  4C (5C)	Di-n-pentyl phthalate (DPP) CAS 131-18-0  5C
Diisohexyl phthalate (DIHP) CAS 71850-09-4  5C (6C)	1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (DHP) CAS 68515-50-4  5C* (6C) (*predominant length, representative structure))
Di-n-hexyl phthalate (DnHP) CAS 84-75-3  6C	Diethylhexyl phthalate (DEHP) CAS 117-81-7  6C (8C)

1.3. Purity / Impurities

DBP: Degree of purity ≥ 99% (w/w); impurities ca. 0.01% (w/w) butal-1-ol (CAS 71-36-3), ca. 0.01% (w/w) butyl benzoate (CAS 136-60-7)

DEHP: Degree of purity ≥ 99.6%; impurities CAS. 84-77-2, 5444-75-7, 10143-60-9.

No information on the other members of the category regarding impurities was reported in the CLH or SVHC dossiers, or by the registrants.

2. Category justification

The category includes eight *ortho*-phthalates ordered according to increasing side chain length and molecular weight. The ordering of the members by increasing side chain length also reflects a trend across the category for decreasing water solubility and increasing n-octanol/water partition coefficient ($LogK_{OW}$), i.e. longer side chain gives lower water solubility. Lower molecular weight phthalates with shorter side chains exhibit slight to moderate water solubility, whereas, higher molecular weight phthalates with longer side chains are insoluble.

However, no observed trend in increasing/decreasing toxicity for higher/lower molecular weight across the category is observed, but the LOAELs may depend on the degree of testing and dose-spacing. The category members exhibit low acute oral, dermal and inhalation toxicity and they are not classified for acute toxicity. According to available information none of the selected phthalates are classified for irritation, or skin sensitization. Moreover, the selected phthalates are not classified as mutagenic or as carcinogenic.

Furthermore, there is no apparent difference in effect associated with side chain length in repeated dose toxicity studies. Adverse effects on a variety of tissues have been reported and for the majority of phthalates toxicity was noted at doses at or above 100 mg/kg bw/day. The most common target organs (not including the reproductive organs) were the liver and kidney.

When ranking the toxicity of the selected reference phthalates DBP appears as the most potent reproductive toxicant, and DPP as the least potent phthalate. The available data on reproductive and developmental toxicity have been evaluated previously and DIBP, DBP, DIPP, DPP, 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear, DnHP and DEHP have harmonized classification according to the CLP Regulation as Repr. 1B. Consequently, the available data on reproductive toxicity is considered sufficient and valid for the reference phthalates and may be appropriately used for read-across and data gap filling purposes for diisohexyl phthalate.

3. Data matrix

The data matrix is constructed with category endpoints versus members. The members are ordered according to increasing chain length and molecular weight (Table 19). Data for physicochemical properties are included in the matrix, and experimental results of repeated dose toxicity studies are presented to indicate similar adverse effects and potencies of the category members. For read-across purposes, experimental data on reproductive toxicity are listed. To fill the data gaps on reproductive toxicity of diisohexyl phthalate interpolation from measured values of reference members of the category from both sides of diisohexyl phthalate in the data matrix was used to estimate missing data points. A table with summaries of fertility and developmental toxicity studies of the supporting members are found in Appendix I.

4. Conclusions per endpoint for C&L and dose descriptor

The data from the reference phthalates cover the majority of the carbon numbers and molecular types found within this category. Thus, it is reasonable to assume that the data from the extensively tested members of this category can be used to fairly predict the toxicological properties of the less well studied member diisohexyl phthalate. All reference substances included in the category have a harmonized classification as reproductive toxicants, Repr. 1B, have been identified as Substances of Very High Concern and subsequently included in the Candidate List pursuant to REACH article 57c (CAS no. 84-69-5, ED/68/2009; CAS no. 84-74-2, ED/67/2008; CAS no. 605-50-5, ED/169/2012; CAS no. 131-18-0, ED/69/2013; CAS no. 68515-50-4, ED/49/2014; CAS no. 84-75-3, ED/121/2013; CAS no. 117-81-7, ED/67/2008). The phthalates for which most data is available are DEHP and DBP. Less information is available for DIBP,

DnHP and DPP. Only very limited data on toxicity of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear is available. No mammalian toxicity data is available for DIPP (see Table 18 and 19). DIPP has, however, been grouped and has a harmonized classification as Repr. 1B together with dipentyl phthalate esters: 1,2-benzenedicarboxylic acid, dipentylester, branched and linear (CAS no. 84777-06-0), n-pentyl-isopentylphthalate, di-n-pentyl phthalate (CAS no. 131-18-0) (Annex VI; Index No. 607-426-00-1).
The available data permit an assessment of the reproductive toxicity of this category of phthalates. Reproductive toxicity is concluded to be an intrinsic hazard of the phthalates in the current chemical group and consequently diisohexyl phthalate is anticipated to behave in a similar way as the reference chemicals. Therefore, classification of diisohexyl phthalate as Repr. 1B is warranted.

Table 18. A summary of the available data on selected endpoints of mammalian toxicity for the *ortho*-phthalate category

	Repeat dose toxicity	**Fertility**	**Developmental toxicity**
DIBP (3C) (CAS 84-69-5)	A	A	A
DBP (4C) (CAS 84-74-2)	A	A	A
DIPP (4C) (CAS 605-50-5)	No data	No data	No data
DPP (5C) (CAS 131-18-0)	A	A	A
DIHP (5C) (CAS 71850-09-4)	R	R	R
DHP (5-6 C) (CAS 68515-50-4)	S	No data	No data
DnHP (6C) (CAS 84-75-3)	A	A	A
DEHP (6C) (CAS 117-81-7)	A	A	A

A= acceptable data
S = some data, but not sufficient to draw conclusion directly
R= subject to read-across in this proposal

Table 19. Data matrix for the phthalate category: Physicochemical properties and mammalian toxicity

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
CHEMICAL FORMULA	$C_{16}H_{22}O_4$	$C_{16}H_{22}O_4$	$C_{18}H_{26}O_4$	$C_{18}H_{26}O_4$	$C_{20}H_{30}O_4$	$C_{20}H_{30}O_4$	$C_{20}H_{30}O_4$	$C_{24}H_{38}O_4$
SIDE CHAIN LENGTH	3C	4C	4C	5C	5C	5C* (6C) (* predominant length)	6C	6C
	PHYSICO-CHEMICAL DATA							
Molecular weight	278.35	278.35	306.41	306.41	334.46	334.46	334.46	390.57
Physical state	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid
Melting Point (C°)	-37 (Woodward, 1988; cited in ECHA Annex XV-dossier, 2009)	-69 (DIN-ISO 3016 BASF AG Ludwigshafen; Huels AG Marl Sicherheitsdaten blatt Palatinol C 25.4.1994; cited in ECB RAR, 2004)	< -25 (ECHA CHEM, IUCLID)	< -55 (U.S. CPSC, 2010)	Read-across to CAS no. 68515-50-4	-27.4 (Staples et al., 1997; ACC Phthalate Esters Panel HPV Testing Group, 2006)	-27.4 (NICNAS, 2008d; cited in ECHA Annex VI-dossier, 2010)	-55 or -50 (ECHA Annex XV-dossier, 2008)
Boiling Point (C°)	320 (Härtel, 1985; cited in ECHA Annex XV-dossier, 2009)	340 (BASF AG Ludwigshafen/Kirk-Othmer 1982; Huels AG Marl/i.a. Kemppinen & Gogcen 1956;	339 (ECHA CHEM, IUCLID)	342 (U.S. CPSC, 2010)	Read-across to CAS no. 68515-50-4	373 (U.S. EPA, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006)	350 (NICNAS, 2008d; cited in ECHA Annex VI-dossier, 2010)	385 230°C at 5 mm Hg (ECHA Annex XV-dossier, 2008)

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
		cited in ECB RAR, 2004)						
Density ***(kg/m^3)***	1038 at 25°C (NICNAS, 2008)	1045 at 20°C (NICNAS, 2008)	1020 at 20°C (ECHA CHEM, IUCLID)	Not available	Read-across to CAS no. 68515-50-4	1010 (ExxonMobil Chemical Co, 2000; U.S. OSHA, 2001)	1011 at 25°C (NICNAS, 2008d; cited in ECHA Annex VI-dossier, 2010)	984 at 20°C (NICNAS, 2008a)
Vapour Pressure ***(Pa)***	0.01 Pa at 20°C (Potin-Gautier et al., 1982; cited in ECHA Annex XV-dossier, 2009)	0.0097 Pa at 25°C (BASF AG Ludwigshafen; Huels AG Marl Banerjee & Howard, 1984; cited in ECB RAR, 2004)	0.025 Pa at 25°C (ECHA CHEM, IUCLID)	0.026 Pa at 25°C (U.S. CPSC, 2010)	Read-across to CAS no. 68515-50-4	0.000344 Pa at 25°C (Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006)	0.000667 Pa at 25°C (NICNAS, 2008d; cited in ECHA Annex VI-dossier, 2010)	0.000034 Pa at 20°C (ECHA Annex XV-dossier, 2008)
Partition Coefficient ***(log Kow)***	4.11 (Leyder and Boulanger, 1983; cited in ECHA Annex XV-dossier, 2009)	4.57 (Huels AG Marl/Leyder & Boulanger, 1983; cited in ECB RAR, 2004)	5.45 (ECHA CHEM, IUCLID)	5.62 (Ellington and Floyd, 1996)	Read-across to CAS no. 68515-50-4	6 at 25°C (Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006)	6.30 (NICNAS, 2008d; cited in ECHA Annex VI-dossier, 2010)	7.5 (ECHA Annex XV-dossier, 2008)
Water Solubility (mg/L)	20 mg/L at 20°C (Leyder and Boulanger, 1983; cited in ECHA Annex XV-dossier, 2009)	10 mg/L at 20°C (ECB RAR, 2004)	1.1 mg/L at 20°C (ECHA CHEM, IUCLID)	0.8 mg/L at 25°C (U.S. CPSC, 2010)	Read-across to CAS no. 68515-50-4	0.159 mg/L at 25°C (Cousins and Mackay, 2000; ACC Phthalate Esters Panel HPV Testing Group, 2006)	0.05 mg/L at 25°C (NICNAS, 2008d, cited in ECHA Annex VI-dossier, 2010)	0.003 mg/L at 20°C (ECHA Annex XV-dossier, 2008)

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
	MAMMALIAN TOXICITY							
Repeated Dose Toxicity	LOAEL liver = 3500 mg/kg bw/day based on increased liver weights (male and females). Rat, oral (diet), 4 months, pre-GLP study. (Hodge 1954; cited in ECHA Annex XV dossier, 2009)	LOAEL liver = 752 mg/kg bw/day based on increased liver weight; reduced hepatocellular lipid deposition; increased palmitoyl-CoA oxidase activity. Rat, oral (diet), 90 days, OECD TG 408. (Schilling 1992; cited in ECB RAR, 2004) LOAEL liver = 359 mg/kg/day based on increased liver weights (males) and increased peroxisomal enzyme activity in livers (male and females). Rat, oral, 13-weeks, other. (NTP, 1995; Wine et al., 1997; cited in	No repeated dose toxicity studies available for DIPP	LOAEL liver = 4790 mg/kg bw/day based on increased liver weights Mouse, oral (diet), 14-weeks, continuous breeding protocol. (Heindel et al., 1989)	Read-across	LOAEL liver = 776-2298 mg/kg bw/day based on increased liver weights. Rat, oral (diet), 90 days, pre-GLP study. (Esso, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006) LOAEL liver = 900 mg/kg/day based on increases in absolute and relative (to body weight) liver weights, hepatic cell enlargement. Dog, oral (diet), 90 days, pre-GLP study. (Esso, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006)	LOAEL liver = 1824 mg/kg bw/day (only dose tested) based on hepatocellular necrosis, fat accumulation, loss of glycogen; increased liver enzymes. Rat, oral (diet), 21 days, modified OECD TG 407 (1 dose instead of 3, few animals/time point). (Mann et al., 1985; cited in ECHA Annex VI-dossier, 2010)	LOAEL liver = 24 mg/kg bw/day based on increased relative liver weight. Rat (only males), oral (diet), 28 days, other (GLP). (BIBRA, 1990; cited in ECB RAR, 2008) LOAEL liver = 63 mg/kg/day (males) and 73 mg/kg/day (females) based on increased liver weight. Rat, oral (diet), 13 weeks, US-EPA standard, GLP. (Eastman Kodak, 1992; cited in ECB RAR, 2008)

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
		ECB RAR, 2004).						
	LOAEL kidney = 2000 mg/kg bw/day based on reduced kidney weight. Rats, oral (diet), 1 week, other. (Oishi and Hiraga 1980b)	LOAEL kidney = 752 mg/kg bw/day based on increased kidney weight. Rat, oral (diet), 90 days, OECD TG 408. (Schilling 1992; cited in ECB RAR, 2004) LOAEL kidney = 359 mg/kg/day based on increased kidney weights (males). Rat, oral, 13-weeks, other. (NTP, 1995; Wine et al., 1997; cited in ECB RAR, 2004).		LOAEL kidney = 4790 mg/kg bw/day based on reduced kidney weights. Mouse, oral (diet), 14-weeks, continuous breeding protocol. (Heindel et al., 1989)	Read-across	LOAEL kidney = 776-2298 mg/kg bw/day, based on decreased kidney weights. Rat, oral (diet), 90 days, pre-GLP study. (Esso, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006)	LOAEL kidney = 1670-1870 mg/kg bw/day, based on decreased relative kidney/adrenal weights. Mice, oral (diet), 105 days continuous breeding protocol (2 generations). (Lamb et al., 1987; cited in ECHA Annex VI-dossier, 2010)	LOAEL kidney = 147 mg/kg bw/day, based on increased kidney weight. Rat, oral (diet), 104 weeks, comparable to guideline study. (Moore 1996; cited in ECB RAR, 2008)
	LOAEL general = 3500 mg/kg bw/day based on decreased body	LOAEL general = 359 mg/kg bw/day based on decreased		LOAEL general = 4790 mg/kg bw/day based on	Read-across	LOAEL general = 75 mg/kg bw/day, based on increased	LOAEL general = 2000 mg/kg bw/day, based on increased	LOAEL general = 70 mg/kg bw/day based on

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
	weights (male) based on retarded growth Rat, oral (diet), 4-months, pre-GLP study. (Hodge 1954; cited in ECHA Annex XV dossier, 2009)	haemoglobin values and erythrocyte counts (males); increased numbers of blood platelets (males). Rat, oral, 13-weeks, other. (NTP, 1995; Wine et al 1997; cited in ECB RAR, 2004).		reduced body weights. Mouse, oral (diet), 14-weeks, continuous breeding protocol. (Heindel et al., 1989)		heart weights in males Rat, oral (diet), 90 days, pre-GLP study. (Esso 1962a; EPA 2010)	activity in the thyroid gland and microscopic changes. Rat, oral (diet), 21 days, modified OECD TG 407 (1 dose instead of 3, few animals/time point). (Hinton et al., 1985; cited in ECHA Annex VI-dossier, 2010)	decreased body weight. Rat, oral (diet), 102 weeks, comparable to guideline study (only males) (Ganning et al., 1987, 1990; cited in ECB RAR, 2008)
Reproductive Toxicity ***- Harmonised Classification*** ***-Fertility*** ***-Developmental Toxicity*** ***- Effects on reproductive system in Repeated Dose Toxicity studies***	**Repr. 1B** H360Df	**Repr. 1B** H360Df	**Repr. 1B** H360FD	**Repr. 1B** H360FD	*Proposed harmonised classification:* **Repr. 1B** H360FD	**Repr. 1B** H360FD	**Repr. 1B** H360FD	**Repr. 1B** H360FD
	Fertility: LOAEL = 125 mg/kg bw/day based testicular damage, with degeneration of seminiferous tubules.	*Fertility:* LOAEL = 1.5 - 3.0 mg/kg bw/day based on reduced number of spermatocytes in adult male offspring.	Data not available.	*Fertility:* LOAEL = 1000 mg/kg bw/day based on histopathological lesions in testicular tissue.	Read-across	Data not available	*Fertility:* LOAEL= 125 mg/kg bw/day based on severe malformations of the reproductive tract observed in	*Fertility:* LOAEL = 14 mg/kg bw/day based on testicular toxicity. LOAEL = 359 mg/kg bw/day based on impaired

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
	Rat, oral (gavage), GD 12-21, guideline study. (Saillenfait et al., 2008, cited in ECHA Annex XV dossier, 2009)	Rat, oral (diet), GD 15 to PND 21, other. (Lee et al., 2004) LOAEL = 52-80 mg/kg bw/day (male-female) based on reduced number of live born pups. Rat, oral (diet), 119 days, continuous breeding protocol (2-generations). (NTP, 1995; Wine et al., 1997; cited in ECB RAR, 2004)		Rat, oral (gavage, single dose), 10 weeks, other. (Lindström et al., 1988; cited in ECBI PM, 2000) LOAEL = 760 mg/kg bw/day based on significantly decreased fertility, number of litters and number of pups per litter. Mice, oral (diet), 14-weeks continuous breeding. (Heindel et al., 1989)			young adult males. Rat, oral gavage, GD 12-20, other. (Saillenfait, Sabaté and Gallissot 2009; cited in ECHA Annex VI-dossier, 2010) LOAEL = 380-430 mg/kg bw/day based on reduced number of litters/pair, live pups/litter and proportion of pups born alive. Mice, oral (diet), 105 days continuous breeding protocol (2 generations). (Lamb et al., 1987; cited in ECHA Annex VI-dossier, 2010)	fertility and litter parameters Rat, oral (diet), 2 years, guideline study (3-gen.) (Wolfe et al., 2003; cited in ECB RAR, 2008)

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
	Developmental study: LOAEL = 125 mg/kg bw/day based on testicular damage, with degeneration of seminiferous tubules. Rat, oral gavage, GD 12-21, guideline study. (Saillenfait et al., 2008; cited in ECHA Annex XV dossier, 2009)	*Developmental study:* LOAEL = 1.5 - 3.0 mg/kg bw/day based on persistent mammary gland toxicity and reduced number of spermatocytes. Rat, oral (diet), GD 15 to PND 21, other. (Lee et al., 2004) *Two generation study:* Developmental LOAEL = 256-385 mg/kg bw/day (male-female) based on testicular atrophy and seminiferous tubule degeneration in F1. Rat, oral (diet), 119 days, continuous breeding protocol (2-generations).	Data not available.	*Developmental study:* Only dose tested: 500 mg/kg bw/day resulted in decreased AGD (male) Rat, oral gavage, GD 12-19, other. (Liu et al., 2005; U.S. CPSC, 2010) LOAEL = 300 mg/kg bw/day based on nipple retention in male offspring. Rat, oral gavage GD 8-18, other. (Hannas et al., 2011)	Read-across	Data not available	*Developmental study:* LOAEL= 125 mg/kg bw/day based on degeneration of seminiferous tubules in young adult males and reduced AGD on PND 1 males. Rat, oral gavage, GD 12-20, other. (Saillenfait, Sabaté and Gallissot 2009; cited in ECHA Annex VI-dossier, 2010)	*Developmental study:* LOAEL = 14 mg/kg bw/day based on increased incidences of small or aplastic testes and epididymis, seminiferous tubule atrophy. Rat, oral (diet), 2 years, guideline study (3-gen.) (Wolfe et al., 2003; ECB, 2008)

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
		(NTP, 1995; Wine et al., 1997; cited in ECB RAR, 2004)						
	Repeated dose toxicity study: LOAEL = 1500 mg/kg bw/day (only one dose tested) based on decreased testes weight, histological changes. Rats, oral (diet), 1 week, other. (Oishi and Hiraga 1980a; cited in ECHA Annex XV dossier, 2009) LOAEL reproductive system = 3500 mg/kg bw/day based on decreased testes weights. Rat, oral (diet), 4-months, pre-GLP study.	*Repeated dose toxicity study:* LOAEL reproductive system = 250 mg/kg bw/day based on testicular degeneration in tubules, and changes in testicular enzymes associated with degeneration of spermatogenic cells. Rat, oral, 15 days, other (limited). (Srivastava et al., 1990; cited in ECB RAR, 2004) LOAEL reproductive system = 712 mg/kg bw based on degeneration	Data not available	*Repeated dose toxicity study:* LOAEL reproductive system = 4790 mg/kg bw/day based on decreased testis, seminal vesicles, epididymis weights, histopathological lesions in testes and epididymis. Mouse, oral (diet), 14-weeks, continuous breeding protocol. (Heindel et al., 1989)	Read-across	*Repeated dose toxicity study:* LOAEL reproductive system = 2298 mg/kg bw/day based on decreased testes weight and atrophy of the spermatogenic epithelium. Rat, oral (diet), 90 days, pre-GLP study. (Esso, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006) LOAEL reproductive system = 900 mg/kg/day based on testicular changes.	*Repeated dose toxicity study:* LOAEL reproductive system = no effect on testes were observed (in available repeated dose toxicity studies). Rat, oral (diet), 21 days, modified OECD TG 407 (1 dose instead of 3, few animals/time point). (Mann et al., 1985; Hinton et al., 1985; cited in ECHA Annex VI-dossier, 2010)	*Repeated dose toxicity study:* LOAEL reproductive system = 7 mg/kg bw/day based on atrophy and inhibition of spermatogenesis Rat, oral (diet), 102 weeks, comparable to guideline study (only males) (Ganning et al., 1987, 1990; cited in ECB RAR, 2008) LOAEL reproductive system = 789 mg/kg bw/day based on decreased absolute and relative testis weight with associated increased incidence of

CAS NO.	84-69-5	84-74-2	605-50-5	131-18-0	71850-09-4	68515-50-4	84-75-3	117-81-7
CHEMICAL NAME	DIBP	DBP	DIPP	DPP	DIHP	DHP	DnHP	DEHP
	(Hodge 1954; cited in ECHA Annex XV dossier, 2009)	of germinal epithelium. Rat, oral, 13-weeks, other. (NTP, 1995; Wine et al., 1997; cited in ECB RAR, 2004).				Dog, oral (diet), 90 days, pre-GLP study. (Esso, 1962; ACC Phthalate Esters Panel HPV Testing Group, 2006)		bilateral aspermatogenesis. Rat, oral (diet), 104 weeks (chronic toxicity), comparable to guideline study. (Moore 1996; cited in ECB RAR, 2008).

Note: The existing data for the mammalian toxicology endpoints were reviewed using literature searches to identify the most relevant studies for each chemical in the group. Published studies from the general literature, as well as a number of unpublished company reports, were obtained and summarized. Some of the individual members in the category had no relevant studies identified in the searches. Studies that were chosen represent the best available data for a particular endpoint, and/or the lowest LOAEL. Some endpoints include more than one study in order to present a more complete data set.

4.11.4 Summary and discussion of reproductive toxicity

There are no mammalian reproductive toxicity studies available for diisohexyl phthalate. Available *in vitro* studies have yielded conflicting results as to the antagonistic activity of dihexyl phthalate to human androgen receptors. An isomeric mixture of dihexyl phthalates demonstrated human estrogen receptor α-agonistic activity and androgen receptor-antagonistic activities in some studies *in vitro* but did not induce vaginal cornification response or an increase in uterine weight *in vivo*. Therefore, a chemical grouping based on the structural similarity of eigth *ortho*-phthalates, including diisohexyl, with a carbon backbone length of 3-6 carbon atoms was constructed to fill in data gaps on reproductive toxicity of diisohexyl phthalate. The reference substances and diisohexyl phthalates are considered to belong to the defined subcategory of transitional phthalates with straight-chain carbon backbones of C4-6 that have been included in several published chemical categories for hazard screening purposes previously. In the current report, the category has been extended by the dossier submitter to include C3 (diisobutyl phthalate). A vast body of data has demonstrated that the transitional phthalates have similar reproductive and developmental adverse effects. The most well studied phthalates in the current chemical grouping are DEHP and DBP. Less information is available for DIBP, DnHP and DPP. No mammalian toxicity data is available for DIPP (see Table 18 and 19). It should be considered that the available data and LOAEL:s may depend on the degree of testing and dose spacing utilized.

Adverse effects on sexual function and fertility

The most sensitive reproductive endpoint of phthalate esters in animals is effects on the male such as decreased fertility and testes weight, and adverse effects on male accessory organs (NICNAS, 2008a). Repeatedly reported histopathological lesions include atrophy of seminiferous tubule, degeneration of germinal cells and vacuolation of Sertoli cells (Foster et al., 1980; NTP, 1982; Creasy et al., 1983; Lamb et al., 1987; Heindel et al., 1989; Moore et al., 1997; Poon et al., 1997). Moreover, changes of the zinc distribution and levels in testes have also been demonstrated (Cater et al., 1977; Foster et al., 1980; Oishi and Hiraga 1980a,b). The observation of reproductive effects associated with C4-6 backbone lengths has been noted in several studies e.g. Foster et al. (1980), Oishi and Hiraga, (1980a,b), Lamb et al. (1987), Heindel et al. (1989) and in reviews such as by Fabjan et al., 2006; NICNAS 2008a; US EPA, 2010; OECD, 2004. In comparative studies on fertility it was demonstrated that the potency of a number of phthalates to affect fertility increased as the length of the side chain increased from 3 up to 6 carbons (Heindel et al., 1989; Lamb et al., 1987). The reference phthalates, included in the category in the current dossier, with this range of side chain length (DBP, DPP, DnHP, and DEHP) all have demonstrated effects on male reproductive organs, most notably decreased testes weight. The sentinel chemical in the chemical grouping of the current dossier, DIBP, has a side chain length of 3 carbons with a methyl-branching and a total number of 4 carbons in the side chains, and the same molecular weight as DBP (a defined member of the transitional phthalate category with side chain backbone of 4-6 carbon atoms in length). In spite of the fact that the side chain length of DIBP is < 4C, DIBP induce adverse effects on fertility comparable to transitional phthalates (Borch et al., 2006; NICNAS, 2008a).

Phthalates with backbones of 8 carbons in length (Di-n-octyl phthalate, DnOP) or 2 carbons (Di-ethyl phthalate, DEP) have been reported to have no effect on sensitive reproductive endpoints. For example, in short-term studies in pubertal male rats administered via oral gavage with a range of phthalates (Foster et al., 1980; Oishi & Hiraga, 1980a,b) relative testis weights were reduced in animals treated with phthalates with backbones of 4-6 carbons in length (DBP, DPP, DnHP, DEHP),

but not in animals administered phthalates with backbones of < 3 or > 7 carbon atoms. However, the high molecular phthalate diisononyl phthalate (DINP) with 9 carbons in total in side chains (consisting of 45-55% dimethylheptyl phthalate (7 carbons in side chain length), 5-20% methyloctyl phthalate (8 carbons in side chain length), and 15-25% isodecyl phthalate (9 carbons in side chain length); ECHA, 2012) was reported to decrease testicular weight in mouse at 742 mg/kg bw/day in a chronic dietary study (Aristech 1995). Moreover, DINP caused decreased live birth and survival indices in rat at 966 mg/kg bw/day in a one-generation reproductive toxicity study (Exxon 1996, Waterman et al., 2000).

Thus, it is noted that there may be exceptions from the general hypothesis on toxicological properties of phthalates based on molecular weight since some low and high molecular weight phthalates do show effects at higher dose levels. Furthermore, it appears that a backbone length of C4-6 is not the sole determinant of e.g. testicular toxicity of phthalates; linearity and branching of the side chains also seem to be involved (Foster et al., 1980; Gray et al., 2000; NAS, 2008).

The phthalates included in the current category all have similar linearity and branching, with linear backbone or with methyl branching; with DEHP as the least similar phthalate (ethyl branching). Diisohexyl phthalate has a backbone length of 5 carbons and is constituted of branched C6 isomers with methyl branching. It is predicted that diisohexyl phthalate has similar effects as the supporting members with analogous structures. Effects of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including CAS no. 71850-09-4) on the reproductive system was indicated in repeat dose toxicity studies where rats exposed to 1-3.0% (776-2298 mg/kg bw/d) of the substance for 90 days displayed atrophy of the spermatogenic epithelium in the testes (Esso, 1962). Similarly, male Beagle dogs fed 5% (900 mg/kg bw/d) 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear had exhibited decreased absolute and relative testes weight (Esso, 1962). Two males in the high dose group also showed atrophy of the seminiferous epithelium in the testes. These findings are in line with the adverse effects reported for the phthalates included in the category. Moreover, the doses used in the studies of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear are high, but they are within the concentration range of reported effects of the reference phthalates.

Adverse effects on development of the offspring

Developmental toxicity of phthalates has been extensively reviewed in the literature, and some of the most studied phthalates include DBP, butyl benzyl phthalate (BBP), and DEHP (NTP-CERHR, 2006). In analogy to fertility effects, there is also a structure-activity relationship for developmental toxicity described, i.e., that phthalate esters with side chains of the length C4-C6 carbons (transitional phthalates) produce more severe effects than either shorter or longer molecules. Phthalates with various side chain lengths have been tested in comparative studies in CD-1 mice using a continuous breeding protocol and cross-over trial (Lamb et al., 1987; Heindel et al., 1989). The results from the cross-over study with treated females and untreated males demonstrated that none of the dams in the high-dose groups of DEHP, DnHP, DPP or Di-n-propyl phthalate (DiPrP; C3) produced a viable litter. In contrast, DEP (2C) or DnOP (8C) did not affect litter size, pup viability or weight in these studies. Moreover, in utero exposure of DBP, DEHP, BBP or DPP by gavage to pregnant rats during GD 12-19 induced decreased anogenital distance (AGD) in male fetuses, but not after exposure to DMP or DEP (Liu et al., 2005).

Consequently, in view of this recognized relationship, a number of authors, including Gray et al. (2000) and ACC Phthalate Esters Panel HPV Testing Group (2001) have suggested grouping phthalates on the basis of developmental effects. Observed developmental effects can be clustered into male reproductive effects, skeletal variations and lactational effects. Some phthalates induce all three types of effects (OECD, 2004). Transitional phthalates cause a number of malformations,

variations and developmental effects in animal studies of prenatal exposure. These include decreased pup weight at birth and through weaning, malformations of the male reproductive system and feminisation of male sexual differentiation as typified by decreased AGD, delayed preputial separation and retained thoracic nipples (OECD, 2004). Additional effects include decreased testes weight, decreased sperm production (Moore et al., 1997; Wine et al., 1997; Mylchreest et al., 1998; Gary et al., 1999; Wolfe et al., 2003; Saillenfait et al., 2008) and decreased testosterone levels (Borch et al., 2006; Howdeshell et al., 2008). Higher doses induce hypospadias and cryptorchidism (Mylchreest et al., 1998; Gray et al., 1999; Saillenfait et al., 2008; Saillenfait, Sabaté, Gallissot, 2009), and skeletal variations particularly increased frequency in lumbar ribs (Singh et al., 1972; Tyl et al., 1988; Saillenfait et al., 2006; Saillenfait, Gallissot, Sabaté, 2009). Reproductive effects in the developing male pup appear to be the most sensitive developmental endpoint. There are indications for more potent effects of phthalates on testes when exposure begins early during prenatal life. For example, in a two-generation study with BBP, testes weight was reduced in the offspring (F1) but not in the parental generation at 750 mg/kg bw/day (Tyl et al., 2004). Similar effects were also noted for diisoheptyl phthalate (DiHepP; principal constituents are dimethylpentyl and >80% methylhexyl isomers; McKee et al., 2006), DBP (Gray et al., 1999) and DEHP (Wolfe and Layton, 2003).

Among the low molecular weight phthalates, DMP (1C) and DEP (2C) are the lowest molecular weight phthalates with simple linear side chains. These two phthalates have no demonstrated developmental effects. However, the conclusions are less clear for other low molecular weight phthalates. For example, low incidences of cryptorchidism was observed for di-n-propyl phthalate at 1500 mg/kg bw/day and the mean percentage of fetuses per litter with cervical and thoracic rudimentary ribs was significantly increased from 1000 mg/kg bw/day (Saillenfait et al, 2011b).

The high molecular weight phthalates have less significant effects on male sexual differentiation that appear at lower incidences and higher doses compared to the transitional phthalates. For example, di-n-heptyl phthalate (DnHepP; 7C) caused a decrease in AGD at highest dose tested 1000 mg/kg/day in rats exposed prenatally (Saillenfait et al., 2011a). Recently, prenatal developmental toxicity rat studies of diisooctyl phthalate (DIOP, 7 carbons in sidechain length and methyl branching, i.e. 8 carbons in total in the sidechain) have shown, in contrast to its linear isomer DnOP (linear with 8 carbon in length and in total in side chains), that male offspring had abnormalities of reproductive system (e.g. hypospadias, non-scrotal testes, and hypospermatogenesis) starting at 500 mg/kg/day, and with high incidence at 1000 mg/kg/day. DIOP was also reported to cause malpositioned testes in fetus at 1000 mg/kg/day and to reduce fetal testosterone at 100 mg/kg/day and above (Saillenfait et al., 2013). In addition to effects on male development significant increase in resorptions at 1000 mg/kg/day and reduction in fetal weights at 500 and 1000 mg/kg/day were demonstrated. DINP (9 carbons in total in side chains; consisting of 45-55% dimethylheptyl phthalate (7 carbons in side chain length), 5-20% methyloctyl phthalate (8 carbons in side chain length), and 15-25% isodecyl phthalate (9 carbons in side chain length); ECHA, 2012)) have also been reported to notably decrease foetal testicular testosterone in rats exposed prenatally and during lactation at 250 mg/kg bw/day (Clewell et al., 2011a and b). Moreover, 966 mg/kg bw/day caused decreased live birth and survival indices (Exxon 1996, Waterman et al., 2000). Effects on male development have not been reported for diisodecyl phthalate (DIDP; 10 carbons in total in side chains; 70-80% dimethyloctyl phthalate (8 carbon in side chain length), 0-10% trimethyl heptyl phthalate (7 carbons in side chain length) and 0-10% methyl nonyl phthalate (9 carbons in side chain length)). The most sensitive effect of DIDP was reduced survival of F2 pups observed in two-generation reproductive toxicity studies in rat (Hushka et al., 2001). Commonly, the high molecular weight phthalates, including DINP and DIDP, cause skeletal and visceral variations observed at high doses (NICNAS, 2008a; Hellwig et al., 1997, Waterman et al., 1999). Saillenfait et al (2011a) reported on significant increase in rudimentary lumbar ribs at all doses tested of DnHepP (7C) and DnOP (8C) starting from 250 mg/kg/day.

The reference phthalates in the current chemical grouping all have developmental toxicity data available, except for DIPP and 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear (summarized in Table 19 and Appendix I). There is no developmental toxicity data for diisohexyl phthalate. The developmental toxicity of phthalates with no or very little data is assumed to be predictable on the basis of the backbone length categorizations i.e. < 3 carbons; 4-6 carbons; or > 7 carbons. Moreover, phthalates with the backbone length 4-6 carbons are suggested to have a common anti-androgenic mode of action in the induction of developmental toxicity. The existing data in the current chemical grouping thus permit an assessment of the developmental toxicity of diisohexyl phthalate. In conclusion, based on read-across from structurally similar phthalates, there are strong reasons to assume that diisohexyl phthalate also have the potential to cause adverse effects on the development of the offspring.

Conclusion
The coherent data on adverse effects on sexual function and fertility and developmental toxicity of the transitional phthalates in the category (supported with C3, diisobutyl phthalate) allow for read-across to fill data gaps of diisohexyl phthalate and supports the conclusion of diisohexyl as a reproductive toxicant. Furthermore, the similarity of the adverse effects of 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear (including the branched isomer CAS no. 71850-09-4) in two repeated dose toxicity studies in two species, including decreased weights of testes and ovaries and degeneration of reproductive accessory organs, with those described for the reference phthalates also supports the conclusion that reproductive toxicity is an intrinsic property of diisohexyl phthalate.

4.11.5 Comparison with criteria

Rationale for classification in Repr. 1B:

The CLP criteria for classification in Repr. 1B are as follows: *"The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Regarding identification and examination of available information on substances it is stated in CLP Regulation (EC) No 1272/2008, Article 5.1: *Manufacturers, importers and downstream users of a substance shall identify the relevant available information for the purposes of determining whether the substance entails a physical, health or environmental hazard as set out in Annex I, and, in particular, the following: (c) any other information generated in accordance with section 1 of Annex XI to Regulation (EC) No 1907/2006.* Classification based on grouping of substances and read-across approach is supported in REACH regulation (EC) No 1907/2006, Annex XI, section 1.5: *Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or "category" of substances. Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). […]*

The similarities may be based on:

1) a common functional group;

2) the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals; or

3) a constant pattern in the changing of the potency of the properties across the category.

If the group concept is applied, substances shall be classified and labelled on this basis.

Overall, based on a chemical grouping approach of eight structurally similar *ortho*-phthalates it is concluded that the data provided in the report provide clear evidence of reproductive toxicity as an intrinsic and hazardous property of the transitional phthalates included in the chemical category. Furthermore, the similarity of structure and effects between the phthalates in the category support that this effect is also an intrinsic property of diisohexyl phthalate. The available data provide clear evidence of an adverse effect on male sexual function and fertility, and adverse effects on the development of the offspring. Effects on development were clearly identified for the majority of the reference phthalates included in the category and are not considered to be secondary to other maternal toxic effects. Moreover, there is no mechanistic evidence to indicate that the observed effects on reproduction and development are not relevant for human.

A classification **Repr. 1B – H360FD** is therefore warranted. It is proposed not to specify route of exposure in the hazard statement.

Classification in Repr 1A is not appropriate as it should be based on human data and no human data specific of diisohexyl phthalate is available. Moreover, human data on reproductive and developmental toxicity is not sufficient for the majority of the phthalates included in the category, and thus read-across for data gap filling is not applicable.

Classification in Repr 2 is not appropriate as all the existing experimental data on reproduction and development available for the reference substances in the category are considered reliable based on existing classifications. Moreover, the chemical grouping is considered robust and appropriate for the endpoint and applicable for diisohexyl phthalate. Finally, considering the whole literature of the transitional phthalate class in a weight of evidence approach, the level of evidence is considered as clear evidence and not as some evidence.

4.11.6 Conclusions on classification and labelling

A classification **Repr. 1B – H360FD** is proposed with no specific route of exposure added.

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal There are no mammalian reproductive toxicity studies available for DIHP and the proposal is therefore based on read-across, using a chemical category approach. DIHP has a

branched structure and is one of the isomers in DHP, which consist of a mixture of branched and linear isomers.

The estrogenic activity of an isomeric mixture of DHP (including DIHP) has been examined using a series of short-term *in vitro* and *in vivo* assays. Results from some *in vitro* studies suggest that an isomeric mixture of DHP was able to induce human estrogen receptor α-agonistic activity as well as androgen receptor-antagonistic activities, but did not induce a vaginal cornification response or an increase in uterine weight *in vivo*.

The dossier submitter performed an extensive read-across analysis based on the existing data on reproductive and developmental toxicity of the transitional phthalates with high structural similarity to DIHP, which includes DIBP, DBP, DIPP, DPP, DnHP and DEHP (the full names and chemical structures for each of these are presented in the figure below).

DIPP was included in the group, but it should be noted that there are no data available for this substance and the classification of this substance was based on read-across using data from other phthalates.

DHP, which to a large extent consists of branched isomers including DIHP, was also included in the category. The harmonised classification for reproductive toxicity of DHP is largely based on read-across using data from other phthalates.

These phthalates constitute a clear structural category that allows for read-across to fill data gaps for DIHP and supports the conclusion that DIHP is a reproductive toxicant. Adverse effects in the developing male pup, including malformations of the male reproductive system and feminisation of male sexual differentiation, appear to be the most sensitive developmental endpoints. Other relevant effects are decreased testes weight, decreased sperm production, and decreased testosterone levels.

Comments received during public consultation

Comments were received from three MSCAs, all of which supported the classification proposal of the dossier submitter. One MSCA pointed out that not all C4-C6 phthalates in the group have a harmonised classification as Repr. 1B for fertility. The DS in their response indicated that H360FD would be appropriate, but noted that not all of the substances used in the category approach have this hazard statement (DEHP, DIPP, DPP, DHP and DnHP are classified as H360FD, while DIBP and DBP are classified as H360Df).

One MSCA briefly mentioned studies questioning the mode of action and human relevance of the anti-androgenic effects of phthalates, but no new references were included in the comment. The DS in their response referred to studies indicating that there might be some differences in the response to phthalates between humans and rats, and that humans might not be more sensitive to phthalates than rats. However, the results were not considered to be conclusive.

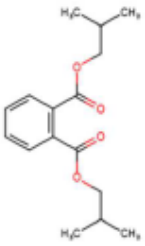
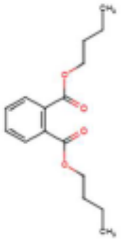
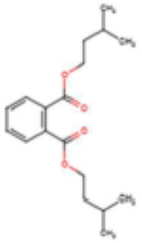
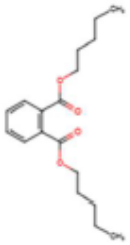
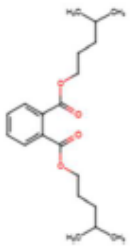
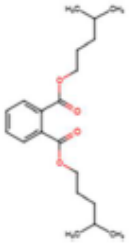
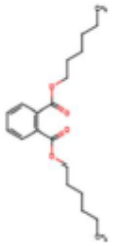
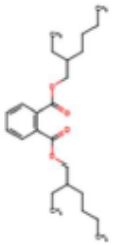
One commenting MSCA suggested removing DHP from the read-across justification, since the classification for this substance already is based on a read-across. The DS responded that DHP is included in the group as a supporting member since DIHP is only one of the branced constituents of DHP.

Assessment and comparison with the classification criteria

The CLP criteria for classification as Repr. 1B requires data from animal studies, with evidence of effects on the reproductive system in the absence of major general toxic effects, and with a mode of action relevant to humans. There are no such data for DIHP, and the proposed classification is based on read-across from other phthalates with similar chemical structures, for which there are consistent data for adverse reproductive effects.

To enable such read-across, the CLP Regulation requires that a group of substances are identified which have similar physicochemical, toxicological and ecotoxicological properties, based on their structural similarities, common functional group(s), common precursors and/or a consistent pattern of variation of the relevant biological potency across the category. These conditions are met in the case of DIHP, where a category was built consisting of eight structurally similar ortho-phthalates (DIBP, DBP, DIPP, DPP, DIHP, DHP, DnHP and DEHP) with increasing alkyl side-chain length (C3(C4), C4, C4(C5), C5, C5(C6), C5(C6), C6, C6(C8)), respectively (see figure below).

Figure: Category members are eight ortho-phthalates with carbon side chains in the interval of 3-6 carbon atoms.

Diisobutyl phthalate (DIBP) CAS 84-69-5  3C (4C)	Di-n-butyl phthalate (DBP) CAS 84-74-2  4C
Diisopentyl phthalate (DIPP) CAS 605-50-5  4C (5C)	Di-n-pentyl phthalate (DPP) CAS 131-18-0  5C
Diisohexyl phthalate (DIHP) CAS 71850-09-4  5C (6C)	1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (DHP) CAS 68515-50-4  5C* (6C) (*predominant length, representative structure))
Di-n-hexyl phthalate (DnHP) CAS 84-75-3  6C	Diethylhexyl phthalate (DEHP) CAS 117-81-7  6C (8C)

RAC considers the justification given for this chemical category by the dossier submitter well-explained and well-argued. RAC supports the conclusion of the dossier submitter that there is clear evidence of reproductive toxicity (both fertility and developmental toxicity) as an intrinsic and hazardous property of the transitional phthalates in the category, all of which are already classified (in Annex VI to CLP) as Repr. 1B.

There are no relevant toxicity data for DIHP. Reduced fertility and number of viable offspring and effects on male reproductive organs (testicular lesions) were seen following

treatment with DPP, DnHP and DEHP (all of which have harmonised classifications as Repr. 1B for fertility). In addition, there are data from two 90-day (oral) repeated dose toxicity studies available for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (including branched isomer CAS no. 71850-09-4), in which decreased testes weight and atrophy of the spermatogenic epithelium were seen in rats and testicular changes were seen in dogs. Developmental toxicity findings in those members of the category for which data were available (DIBP, DBP, DPP, DnHP and DEHP, all of which have harmonised classifications as Repr. 1B for development) included decreased anogenital distance, degeneration of seminiferous tubules and testicular damage.

It is noted that for fertility the classification for the various phthalates in the category varies between Repr. 2 and Repr. 1B. However, classification as Repr. 2 for fertility is considered inappropriate, as the read-across is based on data where reproductive effects relevant for classification as Repr. 1B have been seen in at least two species (rat and mouse) and the proposed mechanism of action is considered relevant to humans. Furthermore, the read-across data include endpoints for both fertility and developmental toxicity and the substances in the category include both phthalates with shorter and longer chain lengths compared to DIHP, which are classified as Repr. 1B H360FD (based on alkyl side-chain length; DIPP and DPP having shorter alkyl side-chains, and DnHP and DEHP having longer alkyl side chains). The proposed read-across from these phthalates to DIHP is therefore considered justified, and **RAC agrees with the DS that classification of DIHP as Repr. 1B; H360FD is warranted.**

4.12 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

Information considered in this report was collected by a literature search last updated on November 2015. Reports, toxicological reviews and robust summaries from internationally recognized associations including the Australian Government (National Industrial Chemicals Notification and Assessment Scheme, NICNAS), Center for the Evaluation of Research on Human Reproduction (CERHR), European Chemicals Agency (ECHA), European Chemicals Bureau (ECB), U.S. Environmental Protection Agency (EPA) High Production Volume (HPV) program, U.S. official federal agency Consumer Product Safety Commission(CPSC), and Organization of Economic Co-operation and Development (OECD), are referenced throughout this report.

Diisohexyl phtalate was planned for registration on May 31st, 2013 (4 posts at 100-1000 tonnes/year), and May 31st, 2018 (majority of posts at 1-10 tonnes/year). However, no REACH-registration by the industry to ECHA has been made yet.

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8 ANNEXES

Annex I. Summary of studies on reproductive toxicity of the reference phthalates in the category

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
Diisobutyl phthalate, DIBP (CAS #84-69-5)	Prenatal developments study. Sprague-Dawley rats (5/group) were given DIBP at single doses 0.375, 0.75 and 1.25 ml/kg bw (approx. 390, 780 and 1300 mg/kg bw) by intraperitoneal injection at 3 different days during gestation (GD 5, 10, 15) and were sacrificed on GD 20.	0.375 ml/kg bw (390 mg/kg bw)	Significantly decreased average weight of fetuses (3.6 g versus control 4.83 g, p≤0.01).	LOAEL = 0.375 ml/kg bw (390 mg/kg bw) based on decreased weight of fetuses.	Singh et al., 1972 (cited in ECHA Annex XV-dossier, 2004)
		0.75 ml/kg bw (780 mg/kg bw)	Significantly decreased average weight of fetuses (3.5 g versus control 4.83 g, p≤0.01). Gross abnormalities (not specified) observed in 2 fetuses (3.9%, not statistically significant).		
		1.25 ml/kg bw (1300 mg/kg bw)	Significantly decreased average weight of fetuses (2.0 g versus control 4.83 g, p≤0.01). Increased incidence of skeletal abnormalitites (33.3%, not statistically significant). Increase in resorption (25.8%, versus control 0%, not statistically significant).		
	Wistar young male rats (20 animals in control group, 10 animals in treated group) administered 2% DIBP via diet for 7 days.	2% (1500 mg/kg bw/day)	Slightly decreased body weight. Statistically significant increase in absolute (27%) and relative (34%) liver weight. Significantly decreased testes weight (33%), incidences of histological lesions (decrease of spermatocytes and spermatogonia). Statistically significant reduced zinc levels in testes (11%) and liver (17%). Statistically increased levels of testosterone in testes (> 2.5 fold increase).	N/A	Oishi and Hiraga 1980a (cited in ECHA Annex XV-dossier, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	JCL:ICR young male mice (10 animals/group group) administered 2% DIBP via diet for 7 days.	2% (2000 mg/kg bw/day)	Significantly decreased body weight (87.4% of control). Significantly decreased kidney weight ($p<0.05$). Significantly increased liver weight (145% of control). Significantly increased testes weight (129% of control, $p<0.05$). Significantly reduced zinc concentrations in testes and liver (76 and 91% of control respectively, $p<0.05$).	N/A	Oishi and Hiraga 1980b (cited in ECHA Annex XV-dossier, 2004)
	Chernoff-Kavlock screening assay in CD-1 mice (50 dams/group) were gavaged on GD 6-13 with 4000 mg/kg bw/day.	4000 mg/kg bw/day	No pregnant dams gave birth to a live litter and 27/50 exposed dams died.	N/A	Hardin et al., 1987 (cited in ECHA Annex XV-dossier, 2004)
	Developmental study. Mated female Wistar rats (8/group) were gavaged from GD 7-19 or until GD 20/21 with 600 mg/kg bw/day DIBP.	600 mg/kg bw/day	Statically reduced AGD in male pups ($p= 0.009$) and increased AGD in female pups ($p=0.02$) at GD 20/21. 10% reduction in bodyweights of male and female fetuses GD 19 ($p<0.05$).	N/A	Borch et al., 2006 (cited in ECHA Annex XV-dossier, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Reduction in testicular testosterone production (p=0.0003) and testicular testosterone content (p<0.0001) in the male offspring at GD 20/21. Histopathological lesions in testes: clustering of Leydig cells (GD 19: 9/9, p<0.001; GD20/21:13/15, p<0.001) and vacuolization of Sertoli cells (GD 20/21: 14/16, p<0.001).		
	Developmental study. Pregnant Sprague-Dawley rats (20-22 per group) were given daily doses of DIBP, at 250, 500, 750 and 1000 mg/kg by gastric intubation on GD 6–20.	500 mg/kg bw/d and higher	Maternal toxicity	LOAEL Maternal = 500 mg/kg Developmental = 500 mg/kg	Saillenfait et al., 2006 (cited in ECHA Annex XV-dossier, 2004)
		500 mg/kg bw/d	Developmental toxicity: decreased pup weight, increased incidence of trans-abdominal testes migration (3 of 55 male fetuses at 500 mg/kg, in 30 of the 55 male fetuses at 750 mg/kg, and in 30 of the 34 male fetuses (16 of the 17 litters) at 1000 mg/kg)		
		750-1000 mg/kg bw/d	Significantly increased incidence of resorptions (% resorptions per litter at 750 and 1000 mg/kg was 27.6 and 59.3 respectively). Significant reduction in the number of live fetuses per litter (10.1 of 21 and 6.2 of 18 at 750 and 1000 mg/kg respectively). Total number of fetuses with external malformations significantly increased (5% at 750 mg/kg; 6% at 1000 mg/kg). Total number of fetuses with visceral malformations significantly increased (13% at 750 mg/kg; 10% at 1000 mg/kg), including significantly increased incidence of ectopic testis (30 at both 750 and 1000 mg/kg).		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Total number of fetuses with skeletal malformations significantly increased (18% at 750 mg/kg; 34% at 1000 mg/kg).		
	DIBP was administered by gavage to pregnant SD rats at 0, 100, 300, 600, and 900 mg/kg bw/d on GD 8-18 and testicular testosterone determined in male foetuses using radioimmunoassay. Dams per dose group: control, n=5; 100 and 300 mg/kg/day, n=8; and 600 and 900 mg/kg/day, n=5.	300, 600 and 900 mg/kg bw/d	Foetal testosterone production statistically significantly decreased (40%, 59%, 63% reduction at 300, 600, and 900 mg/kg bw/d, respectively).	LOAEL = 300 mg/kg bw/d	Howdeshell, et al., 2008 (cited in ECHA Annex XV-dossier, 2004)
		600 and 900 mg/kg bw/d	Maternal body weight at GD 18 significantly reduced (7% at 600 mg/kg bw/d and 7.7% at 900 mg/kg bw/d). Maternal body weight gain reduced (34% and 41% at 600 and 900 mg/kg bw/d, respectively), and foetal mortality increased (17.2% and 59.9% at 600 and 900 mg/kg bw/d respectively).		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	DIBP was administered to pregnant female SD rats (11-14 animals per treatment group) by gavage at doses of 0 (olive oil), 125, 250, 500 or 625 mg/kg bw/d on GD 12-21 of pregnancy, and changes in the reproductive system of male off-spring assessed post-partum up to PND 122.	250 mg/kg bw/d and higher	AGD measured on PND 1 significantly decreased in male pups. (11% at 250 mg/kg day and 22% at 625 mg/kg day, compared to control). Incidence of males with thoracic areolas and/or nipples at 12–14 days of age, or at adult stage increased in a dose-dependent manner (the number of affected males to total examined males was 4/55, 24/44, 29/38 in 250, 500 and 625 mg/kg dose groups respectively). Markedly underdeveloped (less than 10% of control weight) or absent testis and/or epididymis were seen in 2% (testis of 1 male), 16% (7 males from 5 litters), and 13% (5 males from 4 litters) of the animals, in the 250, 500 and 625 mg/kg day dose groups, respectively.	LOAEL for developmental effects = 250 mg/kg (changes in AGD and retained areolas and/or nipples) (Note: Severe degeneration of seminiferous tubules along with azoospermia were seen in three adult males from the same litter at 125mg/kg)	Saillenfait et al., 2008 (cited in ECHA Annex XV-dossier, 2004)
		500 mg/kg bw/d and above	Onset of puberty in male offspring, as expressed by preputial separation, was delayed by approx. 4 days.		
		500 and 625 mg/kg bw/d	Mature males displayed severe malformations of the external and internal genitalia at the two high doses of DIBP. The incidences were higher at 625 mg/kg: hypospadias 22/39, exposed os penis 11/39, cleft prepuce 10/39, non-scrotal testis 30/39 number of affected males. Testes and epididymes weights, seminal vesicle and prostate weights statistically significantly decreased, at necropsy of 500 and 625 mg/kg bw/d males on both PND 76-86 and PND 111-122		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		All doses	Moderate or severe degeneration of seminiferous tubules and oligospermia or total azoospermia in all treated male off-spring.		
Di-n-butyl phthalate, DBP (CAS #84-74-2)	Sprague-Dawley rats (12/group) administered by oral intubation at 2000 mg/kg bw/day for 14 days. In additional studies DBP was administered at 500 and 1000 mg/kg/day for 6 days. Only body weight and testes weight were analysed in these animals.	2000 mg/kg bw/day (14 days)	Significantly decreased relative testis weights (day 3: 26%, day 7: 50%, day 10: 53%, day 14: 64%). At day 4, reductions of both spermatocytes and spermatogonia observed. Significantly decreased zinc-turnover rates in testes, gradual decrease from day 2, reaching about approx. 80 % of control rates at day 14. Significantly increased urinary zinc, the maximum excretion was 150-170% of control (day2) and the overall zinc-65 excretion over the whole 4-day period was increased by 34-43%.	N/A	Cater et al., 1977 (cited in ECB RAR, 2004)
		500 mg/kg/day (6 days)	Significantly decreased testis weight after 6 days: 82% ($p<0.05$) of control.		
		1000 mg/kg/day (6 days)	Significantly decreased testis weight after 4 and 6 days: 76% ($p<0.01$) and 68% ($p<0.001$) of control, respectively.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		2000 mg/kg/day (6 days)	Significantly decreased testis weight after 4 and 6 days: 66% ($p<0.001$) and 57% ($p<0.001$) of control, respectively.		
	Gestation study in ICR-JCL mice. 0, 0.005, 0.05, or 0.5% DBP was administered via the diet (based upon food intake 0.05% and 0.5% were calculated to be 100 and 400 mg/kg bw) day 1-18 of gestation	0.5% (400 mg/kg bw/day)	Maternal toxicity: increased kidney wts; and embryotoxicity: lower no. of live offspring. Teratogenic effects were induced as was demonstrated by a statistically significantly higher incidence of external anomalies (non-closing eye-lid, encephalocele, cleft palate, spina bifida). Also a higher (but not statistically significantly) incidence of skeletal anomalies, especially of sternum, was seen.	LOAEL 0.05% in diet (400 mg/kg bw) for maternal, embryotoxicity and teratogenicity	Hamano et al., 1977 (cited in ECB RAR, 2004)
	Continuous breeding protocol (RACB) (one generation). 0, 0.03, 0.3 and 1.0% corresponding approximately to 0, 40, 420 and 1410 mg/kg bw was administered via diet to CD-1 mice during 115 days (including 7 days premating and 98 days during cohabitation) DBP was given to groups of 20 male and 20 female animals for a 7-day premating period, after which the animals were grouped as mating pairs and treated during a 98-day mating period. A	1% (1410 mg/kg bw/day)	F0 parents showed a significantly decreased growth (males only) and significantly increased liver weights (females only) at 1.0% in the diet. At 1.0% in the diet statistically significant decreases in percentage of fertile pairs, no. of litters/pair, no. of live pups/litter and proportion of pups born alive were seen. In crossover mating trial between control males and 1.0% females statistically significant decreases in percentage of fertile pairs, no. of live pups/litter, proportion of pups born alive and live pup weight were observed.	LOAEL for embryotoxicity and parental toxicity is 1% in diet (~1410 mg/kg bw	Lamb et al., 1987 (cited in ECB RAR, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	control group of 40 male and 40 female mice received the basal diet. After the 98-day cohabitation period the pairs were separated and exposed during which period any final litters were delivered and kept for at least 21 days. At the end of the continuous breeding period a 7-day crossover mating trial was performed with F0 animals of control and 1% groups.				
	Continuous breeding protocol (RACB). Male and female Sprague-Dawley rats (control group 40/sex/control group, 20/sex/dose group) were given DBP via diet at 0.0, 0.1, 0.5, and 1.0% (average daily DBP intakes of 0, 52,	0.1% (52-80 mg/kg bw/day) and higher	F0 rats had significantly reduced number of live pups per litter in all treated groups (8-17% less, $p<0.05$). Decrease in dam body weight (only significant in high dose) and live F2 pup weights were lower in all dose groups (5.97 g, 5.60 g, 5.60 g and 5.00 g at 0, 0.1, 0.5, and 1.0% respectively, $p=0.02$).	LOAEL = 0.1% (52-80 mg/kg/day) based on emmbryotoxicity	NTP, 1995; Wine et al., 1997 (cited in ECB RAR, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	256, and 509 mg/kg for males and 0, 80, 385, and 794 mg/kg for females, respectively).	0.5% and 1%	Live F1 pup weights in the 0.5% and 1.0% dose groups were reduced (96 and 90% of control respectively, p<0.01). Kidney weight was significantly increased at both the middle and high dose levels in F1 males (6%, p=0.006). Histopathologic examination of selected organs was performed on 10 representative F1 males from the control, 0.5%, and 1.0% groups. 3/10 males in the 0.5% dose group showed degeneration of seminiferous tubules, compared with 1/10 control, and 8/10 males in the 1.0% group. 7/10 males examined in the 1.0% DBP treatment group demonstrated apparent interstitial cell hyperplasia.		
		1%	4 weeks after cohabitation F0 male body weights were unchanged, but high dose females weighed 14% less than controls (p<0.001). Organ-to body weight ratios for the liver (males: 115% of control; female: 114% of control) and kidneys (males: 111% of control; females: 109% of control) were statistically significant increased in both sexes in the high dose group. The weights of pups from F0 1% treated females were significantly decreased (86% of control, p<0.05). F1 high dose males and females weighed 8-14 % less than their controls (p<0.05). Significant increase in liver weight in F1 males (16%, p<0.001). Statistically significant reduced		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			weights of prostate, seminal vesicles and testis (24%, 22% and 39% decrease respectively). Epididymal sperm counts and testicular spermatid head counts were significantly decreased in the F1 1.0% dose group (51% and 46% of control, respectively). Histopathologic lesions in the high-dose F1 males included underdeveloped or otherwise defective epididymides (5/10 males). Mating, fertility, and reproductive performance of second generation breeding pairs (F1) were impaired in the 1.0% dose group. (Mating index 6/20, $p<0.001$; Pregnancy index 1/20, $p<0.001$; Fertility index 1/6, $p<0.001$). Reduced number of live pups per litter (13.0 versus control 14, $p=0.233$).		
	Developmental study. DBP was administered by oral gavage at 0, 250, 500 and 750 mg/kg bw from day 3 of gestation throughout gestation and lactation. Pups were allowed to mature	250 mg/kg bw/day and higher	At sexual maturity a dose-dependent increase in the incidence of malformations of internal and external genitalia was observed at all dose-levels. Hypospadias were observed in 3, 21 and 43% of males at 250, 500 and 750 mg/kg bw, respectively. Underdeveloped or absent epididymis, frequently bilaterally, was observed in 9, 50 and 70% of the males at 250, 500 and 750 mg/kg bw, respectively, and was associated with atrophy of seminiferous tubules (50-100% of tubules affected in all treated groups) and abnormal or reduced spermatogenesis.	LOAEL = 250 mg/kg bw Critical effect: disturbed development of male reproductive tract.	Mylchreest et al., 1998 (cited in ECB RAR, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		500 mg/kg bw/day and higher	Females at 750 mg/kg bw and one at 500 mg/kg bw were not pregnant and had no implantation sites. Uterine weight was decreased at 500 and 750 mg/kg bw, but without any dose-relationship (significant at 500 mg/kg bw only). At 500 and 750 mg/kg bw seminal vesicles were not developed or their weight was decreased by 16 and 32%, respectively. Mean weight of the prostate gland was decreased by 27% at 750 mg/kg bw. One animal from each of 500 and 750 mg/kg group had no prostate at postmortem examination. An increased incidence of dilated renal pelvis was observed in male offspring at all dose-levels. Mean kidney weight was significantly decreased at 750 mg/kg bw. In male offspring at birth anogenital distance was decreased at 500 and 750 mg/kg bw At 500 mg/kg bw 1/30 rats (1/8 litters) and at 750 mg/kg bw 2/9 rats (1/4 litters) had no vaginal opening. At necropsy the rat without a vaginal opening at 500 mg/kg bw, had no patent vagina, no uterus and no left kidney. In another rat at 500 mg/kg bw right uterine horn was half of the size of the left.		
		750 mg/kg bw/day	At 750 mg/kg bw the number of live pups per litter at birth was decreased significantly. During the second half of the pregnancy body weight gain of the dams at this dose-level was slightly lower which is consistent with the smaller litters.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Pup survival to weaning was decreased significantly at 750 mg/kg bw. In one female at 750 mg/kg bw the length of the left uterine horn was normal, but only the distal segment of the right horn near the ovary was present.		
	Developmental study in Sprague-Dawley rats. DBP was administered at 0, 100, 250 and 500 mg/kg bw from day 12-21 of gestation by oral gavage.	100 mg/kg bw/day and higher	At all dose levels (100, 250 and 500 mg/kg bw) delayed preputial separation in F1 males (killed at sexual maturity at the age of 100-105 days) was seen. At the lowest dose level of 100 mg DBP/kg bw this delay (of 2 days) was attributable at least in part, to one markedly affected litter.	LOAEL = 100 mg/kg bw. Critical effect: delayed (2-days) preputial separation (one litter)	Mylchreest et al., 1999 (cited in ECB RAR, 2004)
		250 mg/kg bw/day and higher	Malformations of the (F1) male reproductive tract were observed at 250 and 500 mg/kg bw, i.e. retained thoracic nipples and decreased anogenital distance.		
		500 mg/kg bw/day	At the highest dose-level of 500 mg/kg bw one dam showed weight loss after day 18 of pregnancy and delivered dead and moribund fetuses. At 500 mg/kg bw hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of seminiferous epithelium and interstitial cell hyperplasia (5 animals from 2 litters) of the testis of F1 males were seen.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Interstitial cell adenoma occurred at 500 mg/kg bw in 2 males (in one litter).		
	Multigeneration study in LE hooded rats. Male and female rats (10-12 animals/sex/group) of only the P0 generation received orally by gavage 0, 250 or 500 mg DBP/kg bw from weaning, through puberty, young adulthood, mating and lactation. Another group of only males received 1,000 mg/kg bw. When the P0 animals were mated, treated animals were paired with untreated controls. F1 animals were not treated. After puberty F1 animals were selected (16/sex/group) for fertility assessment under continuous mating	250 mg/kg bw/day and higher	In the P0 generation delayed puberty (preputial separation) was seen in males at all dose-levels (mean age at puberty was 39.5, 42.6, 43.40, or 44.4 days in control, 250, 500 or 1000 mg/kg bw groups respectively, $p<0.05$). In the F1 offspring which were exposed only in utero and lactational via dams (data only from F1 animals from dams treated with 0, 250 and 500 mg DBP/kg bw), urogenital malformations/abnormalities including a low incidence of agenesis of the epididymis, hypospadias, ectopic testis, renal agenesis and uterine abnormalities (partial agenesis or lack of implants in one uterine horn) were seen. A few treated animals displayed anophthalmia. F1 males from treated mothers exhibited reduced cauda epididymal sperm numbers. *Continuous breeding conditions:* The F1 offspring showed reduced fecundity (significantly fewer F2 pups; number pups/litters 179/24, 76/10, and 20/4 for 0, 250 and 500 mg/kg bw, respectively) in similarly treated pairs.	LOAEL = 250 mg/kg bw based on delayed puberty in males of P0 generation, urogenital abnormali-ties and decreased fertility of F1 males and females	Gray et al., 1999 (cited in ECB RAR, 2004)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	conditions over 11 breeding cycles.				
		500-1000 mg/kg bw/day	The P0 generation showed reduced fertility in male and female animals at 500 and 1,000 (males only) mg/kg bw. Infertility in males was related to testicular atrophy and reduced sperm production, while treated females cycled and mated sucessfully, but many treated females (500 mg/kg bw) aborted their litters around midpregnancy.		
	DBP dose-response study. Pregnant CD rats given oral gavage at 0.5, 50, 100 mg/kg/day (n=19-20) or 500 mg/kg/day (n=11) on GD 12-21.	≤50 mg/kg/day	No statistically significant adverse effects were observed in the offspring.	LOAEL= 100 mg/kg bw/day based on retained nipples pr aerolas.	Mylchreest et al., 2000
		100 and 500 mg /kg/day	Retained nipples or aerolas were present in 31 and 90% of male pups at 100 and 500 mg/kg respectively at PND 1. At PND 14 a dose-dependent increase in the incidence of thoracic areola and nipple development was observed in F1 males, only significant changes at 100 and 500 mg/kg/day (44/141 rats in 16/20 litters; and 52/58 rats in 11/11 litters respectively).		
		500 mg/kg/day	Male offspring had significantly decreased AGD (12% lower than control) PND 1.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Cleft penis (hypospadias) was observed in 5/58 rats (4/11 litters). Absent or partially developed epididymis (23/58 rats in 9/11 litters), vas deferens (16/58 animals in 9/11 litters), seminal vesicles (4/58 rats in 4/11 litters), and ventral prostate (1/58 animals). In 110-day-old F1 males, the weights of the testis, epididymis, dorsolateral and ventral prostates, seminal vesicles, and levator anibulbocavernosus muscle were decreased.		
	Developmental toxicity. Pregnant CD rats (6-8/group) were exposed to 0, 20, 200, 2000 and 10,000 ppm DPB in the diet from gestational day 15 to postnatal day (PND) 21	20 ppm (1.5–3.0 mg/kg bw/d) and higher	During GDs 15–20, body weight gain of dams was slightly statistically significant decreased in the 20 and 10,000 ppm dose groups. At PND 21 in males, reduction of spermatocyte development as manifested by decreased numbers of spermatocytes in the seminiferous tubules was evident. This change was observed from 20 ppm, with dose-dependent increased incidence and/or severity. At PND 21, mammary gland changes at low incidence in both sexes. In females, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 20 ppm with statistically significant increase in the incidence at 20, 2000 and 10,000 ppm. At PNW 11, relative pituitary weights were increased (16%, $p<0.05$) in females. Vacuolar degeneration of alveolar cells was evident in the mammary glands of males, in some cases with alveolar atrophy, appeared from 20 ppm (vacuolar degeneration with statistical difference).	A LOAEL of 20 ppm (1.5–3.0 mg/kg bw/day) in maternal diet was set based on persistent mammary gland toxicity (degeneration and atrophy of mammary-gland alveoli) in males and decreased number of spermatocytes.	Lee et al., 2004

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		200 ppm (14.4-28.5 mg/kg bw/d)	At PND 21 DBP decreased the FSH-positive cells in the anterior pituitary from 200 ppm with a non-monotonic dose-dependent response in females. At PNW 11, relative pituitary weights were increased (19%, $p<0.05$) in females. At PWN 20 relative pituitary weights were decreased (16%, $p<0.05$) in females. At PNW 20 vacuolar degeneration of alveolar cells and alveolar atrophy were evident in the mammary gland of males (with statistical significant difference from control).		
		2000 ppm (148.2-290.9 mg/kg bw/d)	The male ratio at birth was slightly reduced (43.9 ± 15.7%, $p<0.05$ compared to control 65.6%). At PND 21 scattered foci of aggregated Leydig cells, consisting of 50–100 cells (up to two foci per cross section of the testis) was evident from 2000 ppm with statistical significance. In the epididymis, decreased ductular cross sections of the epididymal duct indicating reduced coiling were observed from 2000 ppm. At PND 21, LH-positive cells were increased at 2000 and 10,000 ppm in females. At PNW 11, loss of germ cell development appeared with significance from 2000 ppm. In severely affected cases at 10,000 ppm, Leydig cell hyperplasia was evident.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			At PNW 20, similar lesions as observed at PNW 11 were detected in DBP-exposed males. At PNW 11, relative pituitary weights were increased (22%, $p<0.01$) in females. Females at PWN 20 had reduced relative pituitary weights (16%, $p<0.05$)		
		10,000 ppm (712.3-1371.8 mg/kg bw/d)	The male ratio at birth was strongly reduced at 10,000 ppm (24.7 ± 4.5%, $p<0.01$ compared to control 65.6%). AGD measured on PND 2 was reduced in males. At PND 14, retention of nipples/areolae was apparent in all males in the dose group. At PND 21, liver cell hypertrophy was observed in animals of both sexes. At PND 221 both FSH and PRL-positive cell populations were reduced ($p<0.05$), while the proportion of LH-positive cells was increased ($p<0.01$) in males. In females, LH-positive cells were increased and the positive PRL cells were decreased at 10,000 ppm. At PNW 11 the FSH-positive cell percentage was increased in both males and females at 10,000 ppm. In males PNW 11, slight (statistically significant) reduction of the relative weight of the kidneys was detected. In females, relative pituitary weights were decreased at PNW 11 (36%, $p<0.01$).		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			At PNW 20, a sufficient number of male animals could not be obtained for the 10,000 ppm dose. Females at PWN 20 had reduced relative pituitary weights (23%, $p<0.01$).		
Diisopentyl phthalate, DIPP (CAS #605-50-5)			No data available.		
Dipentyl Phthalate, DPP (CAS #131-18-0)	Short-term study (4 days). Single-dose of DPP to pubertal male Sprague-Dawley rats (1800 mg/kg bw/day) by gavage.	1800 mg/kg bw/day	Markedly reduced relative testes weights (organ weight/100 g body wt). Histological examination of testes from animals treated with DPP showed severe atrophy of the seminiferous tubules, the majority of which showed a complete loss of spermatocytes and spermatids. A few spermatogonia and Sertoli cells remained attached to the basement membrane of the tubule; interstitial cells appeared normal. Loss of zinc in the Golgi region of spermatids was clearly seen prior to any evidence of morphological damage in these cells. Significant increase in zinc concentrations in kidney ($p < 0.001$).	N/A	Foster et al., 1980(cited in ECBI PM, 2000)
	Male Sprague-Dawley rats (4-5 weeks, 10 weeks, or 15 weeks; 6-8 animals per group) were administered orally with DPP as corn oil solution	220 mg/kg bw/day	After three daily doses of DPP at 220 mg/kg, one out of five rats was partially affected in markers of Sertoli cell function: the secretion of seminiferous tubule fluid and of androgen binding protein (ABP) (not significant).	LOAEL = 440 mg/kg bw/day based on reduced Sertoli cell function	Gray and Gangolli, 1986 (cited in U.S. CPSC, 2010)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	at doses 0, 220, 440, and 2200 mg/kg bw/day for 1 or 3 consecutive days.	440 mg/kg bw/day	After a single dose of 440 mg/kg DPP production of fluid (73% reduction) and ABP (74% reduction) was suppressed in immature rats compared to control ($p<0.01$).		
		2200 mg/kg bw/day	In immature rats, after a single dose of 2200 mg/kg DPP the production of seminiferous fluid (3 mg/testis compared to control 166 mg/testis, $p<0.001$) and ABP (0 pmole/testis compared to control 21.9 mg/testis) were almost completely suppressed. When 10-week-old rats were given a single dose of DPP seminiferous tubule fluid and ABP production were reduced to around 60% of control (only significant for ABP production, $p<0.05$).		
	Sprague-Dawley rats (male) administered DPP by oral intubation at 7.2 mmol/kg bw/day (~2200 mg/kg bw/day) 1–4 days	2200 mg/kg bw/day	Decreased testicular cytochrome P-450 (55.6, 32.1, 35.8 % of control, $p<0.001$, at 16 h, 2 days and 4 days). Decreased cytochrome P-450 dependent microsomal steroidogenic enzymes 17 alphahydroxylase (58.5 and 3.7% of control, $p<0.001$, at 16 h and 2 days) and 17-20 lyase (50.5 10.2 13.3 % of control at 16h, 2 days and 4 days respectively). 17-/I-dehydrogenase activity was significantly increased in microsomes from individuals treated with DPP for 2 and 4 days (212.7 and 280.5% of control respectively). The degree of maximal binding of progesterone to microsomes in testes was decreased.	N/A	Foster et al., 1983 (cited in U.S. CPSC, 2010)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	Young male Sprague Dawley rats administered DPP by oral gavage at 2000 mg/kg. Animals sacrificed 1, 3, 6 and 24 hr following a single dose, and after 2, 3 and 4 days of repeated daily dosing. (Number of animals not specified).	2000 mg/kg	At 3 h a subpopulation of Sertoli cells in of the seminiferous tubules showed vacuolation of the perinuclear smooth endoplasmic reticulum with an associated inward displacement of germinal cells. By 6 hr the vacuolation had extended to the apical cytoplasm and was evident in most tubules (>90%). Transient acute inflammatory response appeared at 6 hr. By 24 hr, germinal cell degeneration was extensive. Mitochondrial succinic dehydrogenase activity in Sertoli cells was reduced at 3 and 6 hr and absent by 24 hr. In germinal cells it was unaffected at 3 and 6 hr but absent by 24 hr. 2, 3 and 4 days of daily phthalate treatment resulted in a gradual depletion of germinal cells from all tubules. 3 and 4 days dosing rendered all tubules severely depleted of germinal cells including the spermatogonia.	N/A	Creasy et al., 1983 (cited in ECBI PM, 2000)
	Continous breeding protocol (RACB). Swiss CD-1 mice (male and female, 20 pairs/dose group, 40 pairs/control group) fed 0, 0.5, 1.25, 2.5% (0, 760, 2160, 4790	From 0.5% (760 mg/kg bw/d)	Significantly reduced fertile pair groups, litters/pair, live pups/litter, proportion live births. In the 0.5% dose group the number of litters and live pups/litter were significantly reduced. Only 4/ 19 delivered a first litter and 2/ 19 delivered more than one litter.	LOAEL for reproduction = 760 mg/kg/day	NTP, 1985; Heindel et al., 1989; Morrissey et al., 1989 (cited in ECBI PM, 2000)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	mg/kg/day) DPP for 7 days prior to and during a 98-day cohabitation. *Crossmating trial*: 3 combinations of control and treated mice were selected for crossover mating: control males with control females, high-dose males with control females, and control males with high-dose females	1.25% and 2.5% (2160 and 4790 mg/kg bw/d)	Mice in the 1.25 and 2.5% dose groups were infertile; none of the breeding pairs delivered any litter dose group.		
		2.5% (4790 mg/kg bw/d)	Crossover mating between control and 2.5% animals: significantly decreased mating index when treated males were crossed with control females, but not vice versa. All groups infertile. In females: significantly decreased body weight and adjusted kidney weight (9% and 19% decrease compared to control respectively). Significantly increased liver weights (45% increased compared to control). In males: significantly increased relative liver weights (49% increased compared to control), significantly reduced body weights (10% less than controls), kidney weights (30%), relative testis weights (99%), seminal vesicle weights (32%), and epididymis weights (20%). Absence of epididymal sperm. Testes were atrophic in 16/20 treated and 0/40 control males. Histopatological lesions observed in 20/20 treated male animals, mainly in testes and epididymis: severe degeneration of the seminiferous tubules (0% controls; 95% treated), interstitial cell hyperplasia (5% control; 100% treated), and reduction in sperm count and accumulation of fluid and degenerated cells in the epididymis (2.5% control; 100% treated).		
	Fischer 344 rat (10 males/group) administered a single dose DPP by oral gavage	250 mg/kg bw	Small, but significant, increase in liver weights 2 days after dosing (6% above control weights). By the second week, the weights had returned to control levels.	LOAEL for reproduction = 1000 mg/kg/day	Lindström et al., 1988 (cited in ECBI PM, 2000)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	at 0, 250, 1000, or 2000 mg/kg bw. Animals killed after 2 days and each week up to 10 weeks. *Mating trial:* Male rats given single dose by oral gavage at 0, 250, 1000, or 2000 mg/kg bw followed by mating at 3, 6, and 10 weeks. 17 days after the first day of cohabitation (14±3 days of gestation) the females were killed. *Recovery:* After completion of the fertility study, the control and high-dose males were sacrificed at weeks 14, 18, and 30 after dosing for histological evaluation.				
		1000 mg/kg bw	During the first week after exposure, animals showed slightly but significantly lower body weights than controls (97% of control weights), returning to control levels the second week. Transient decrease in serum ABP levels, up to 48% increase (≤0.05) during the first week. Epididymal and testicular weights were consistently below control weights during the 10 weeks (only statistically significant at weeks 2, 3, 7 for epididymis and weeks 7, 8 for testes). Starting from week 2, there was a dose-response relationship in sperm densities with statistically significant lower levels compared to control. Histopathological lesions in testicular tissue were apparent from day 2 and were characterized by a decreased lumen size of the seminiferous tubules, a reduced number of germ cells, vacuolation of the germinal epithelium, and large intertubular spaces with a notable increase in cell numbers. 20-40% animals at each time point showed some degeneration of seminiferous tubules. Animals showed a 20% increase in sperm abnormality during weeks 3 to 8.		
		2000 mg/kg bw	During the first week after exposure, animals showed slightly but significantly lower body weights than controls (95% of control weights), returning to control levels the second week.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Small, but significant, increase in liver weights 2 days after dosing (17% above control weights). By the second week, the weights had returned to control levels. Degeneration of seminiferous tubules; decreased epididymal sperm density; decreased testicular and epididymal weight; abnormal sperm morphology. The epididymal weights gradually decreased to 56% of control values by week 4 and then remained 50-60% of control values. The testicular weights decreased from 78% of controls at 2 days after exposure to 38% at 4 weeks, thereafter remaining 43-63% of the testis weights of the control animals. Statistically significantly reduced sperm densities starting from week 2, less than half those of the controls from week 3. Significant different ($p<0.001$) changes in serum ABP, sperm density, abnormal sperm morphology, testicular weight, and epididymal weight. High-dose animals showed significantly increased serum ABP levels 2 days after exposure; ABP remained significantly elevated for 3 weeks and then fell and stayed significantly below control level to the end of the study. Histopathological lesions in testicular tissue were apparent from day 2 as described for 1000		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			mg/kg animals. Severe disruption of the germinal epithelium; more than half of the tubules were affected in >96% of the animals at all time points. In high-dose animals, up to 96% of the sperm showed abnormal morphology at week 5, and abnormalities were seen in 50-90% of the sperm for the remaining weeks.		
		2000 mg/kg bw in mating trial and recovery trial.	Significantly decreased number of successful matings: 65% of controls at week 3, 15% at week 6, and 35% at week 10. The number of live fetuses for each pregnant female significantly decreased in females mated to high-dose males (35% of controls at week 3, 43% at week 6, and 72% at week 10). Females mated to high-dose males demonstrated a preimplantation loss about three times as high as those mated to controls. Testicular lesions in males of parental generation with no signs of recovery 14, 18, and 30 weeks after DPP exposure.		
	Male Sprague-Dawley rats given a single dose of 2200 mg/kg bw by oral gavage. DPP-treated rats were killed at 0, 3, 6, 9, 12, 18 and 24 h after dosing. (Number of animals not specified).	2200 mg/kg bw	Increased testicular LAF activity after 3h. The increase was statistically significant at 6, 9, 12 and 18 h and maximal (approximately 10-fold) after 9 h. Later the activity slowly decreased again approaching control level after 24 h. 3 hours after DPP exposure, slight morphological changes in the Sertoli cells were demonstrated with rarefaction of basal cytoplasm. At 6 h the interstitial vasculature contained marginated polymorphonuclear leukocytes (PMNs), with occasional emigration of these cells into the interstitial space. Transient infiltration of a large	N/A	Granholm et al., 1992 (cited in U.S. CPSC, 2010)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			number of PMNs and some mononuclear cells in the interstitial space after 9 and 12 h, and reduced again at 24.		
	Pregnant Sprague-Dawley rats treated with DPP by gavage daily from GD 12 to GD 19 at 500 mg/kg/day (10 animals in control group, 5 animals in treated group).	500 mg/kg bw/day	Significantly decreased anogenital distance in male fetuses (31% less than control, $p<0.001$).	N/A	Liu et al., 2005 (cited in U.S. CPSC, 2010)
	DPP was administered by gavage to pregnant SD rats at 0, 25, 50, 100, 200, 300, 600, and 900 mg/kg/ on GD 8-18 and testicular testosterone determined in male foetuses using radioimmunoassay. Dams per dose group: 0 (control), n= 6; 25 mg/kg/day, n= 5; 50 and 200 mg/kg/day, n =; 300 mg/kg/day, n = 3; and 600 and 900 mg mg/kg/day, n = 2.	100 and 200 mg/kg bw/day	Reduced fetal testosterone production (3.26 ng/testis and 2.26 ng/testis at 100 mg/kg bw/day respectively, compared to control 5.89 ng/testis, $p < 0.01$). (No live fetuses at higher concentrations).	LOAEL for fertility = 300 mg/kg bw/day based on reduced number of live fetuses, increased incidence of resorptions, increased fetal mortality (in combination with significant reduced maternal weight). LOAEL for development = 100 mg/kg bw/day based on reduced fetal testosterone production.	Howdeshell et al., 2008 (cited in U.S. CPSC, 2010)
		300 mg/kg bw/day and higher	Dams treated with DPP at 300, 600, or 900 gained little or no weight from GD 8 to GD 18, whereas controls gained about 70 g. None of the dams in these dose groups had viable fetuses. No signs of overt toxicity or maternal mortality. Midgestation pregnancy loss leading to 100% fetal mortality at doses of 300, 600, and 900 mg/kg/day. Of the dams administered 300–900 mg DPP/kg/day, four of five dams were observed to have vaginal bleeding during midpregnancy, indicative of aborted pregnancies.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	DPP was also administered by gavage to pregnant Harlan SD rats at 0, 11, 33, 100, or 300 mg/kg bw/day on GD 8-18. Evaluated early postnatal endpoints in male offspring.	100 mg/kg bw/day	Pup viability from implantation through PND 2 was significantly decreased at dose levels of 100 mg/kg/day DPP and greater ($p < 0.05$). Following in utero exposure, DPP significantly reduced PND 2 male offspring AGD at a dose of 100 mg/kg/day and greater ($p < 0.05$) (Note data is only presented as % of control, not as cube root of body weight, and actual data on AGD measurements is not presented. The authors have stated that there was no significant reduction in offspring weight on PND 2 at 300 mg/kg bw/day when AGD was measured).	LOAEL for development = 100 mg/kg bw/day based on reduced pup viability	Saillenfait et al., 2011
		300 mg/kg bw/day	Dams treated with 300 mg/kg bw/day DPP displayed a slight (not statistically significant) reduction in total maternal weight gain between GD 8 – 18. Following in utero exposure, DPP significantly induced male nipple retention by PND 13 at a dose of 300 mg/kg/day DPP ($p < 0.05$.		
Diisohexyl phthalate, DIHP (CAS #71850-09-4)			No data available.		
1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear, DHP (CAS #68515-50-4)			See table 17.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
Di-n-hexyl phthalate, DnHP (CAS #84-75-3)	Short-term study. Single-dose level of DnHP to pubertal male Sprague-Dawley rats (2400 mg/kg bw/day) by gavage.	2400 mg/kg bw/day	Marked effects on testis weights and on testicular zinc content in the absence of body weight effects. Marked seminiferous tubular atrophy with the majority of tubules showing few spermatogonia and Sertoli cells, but normal Leydig cell morphology.	N/A	Foster et al., 1980 (cited in EHCA Annex VI-dossier, 2010)
	Continuous breeding protocol (RACB). 0, 0.3, 0.6, 1.2% corresponding to 0, 430, 880, 1870 mg/kg or 0, 380, 800, 1670 mg/kg bw/d were administered via diet to CD-1 mice during 7 days + 98 days cohabitation period. 16-19 pairs of males and females. Control group with n = 40 animals of each sex; 3 dose groups with n = 20 animals of each sex. *Crossover mating:* Three combinations of control and DnHP-treated mice were selected for crossover mating: control males with control females, high-dose males with control females, and control males with high-dose females	0.3% and higher	Dose-related decrease in body weight gain by DHP in the diet at all doses (10.7%, 7%, 1%, and 0.5% weight gain for control, 0.3, 0.6, and 1.2% groups respectively).	LOAEL = 0.3% = 380 or 430 mg/kg bw/day	Lamb et al., 1987 (cited in EHCA Annex VI-dossier, 2010)
		0.3% (380 or 430 mg/kg bw/day)	At low dose, there was significant reduction in number of live pups per litter, reduced from 12.3 (control) to 3.4 (14 of 17 pairs were fertile). The proportion born alive was reduced by 14%.		
		0.6% (800 or 880 mg/kg bw/day)	1 litter of 4 pups produced (1 of 19 pairs was fertile).		
		1.2% (1670 or 1870 mg/kg bw/day)	No live pups at high dose Crossover mating study revealed a significant decrease in the proportion of detected matings for the males receiving 1.2% DHP mated with control females (56%) compared to the controls (90%) and only 1 of the 18 treated males sired a litter. None of the DHP-treated females became pregnant. The percentage of motile sperm and the sperm concentration in the cauda epididymis were significantly diminished in F0 males. The percentage of abnormal sperm was lower (only 3		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			of the 18 males in the 1.2% DHP treated group had sufficient sperm to allow determination). Significant decreases in the weights of the testis, epididymis, and seminal vesicles in the DHP-treated F0 mice. Histological evaluation revealed extensive atrophy of the seminiferous tubules. Mature sperm were markedly diminished in the epididymides. Body and kidney/adrenal weights were significantly decreased and liver weight was significantly increased in F0 male and female mice fed 1.2% DHP in the diet.		
	Chernoff-Kavlock screening assay in CD-1 mice administered DNHP at 9900 mg/kg bw/day for 7 days, GD 6-13.	9900 mg/kg bw/day	No live litters.	N/A	Hardin et al., 1987 (cited in EHCA Annex VI-dossier, 2010)
	Pregnant Sprague-Dawley rats (24-25/group) administered DnHP at 250, 500 and 750 mg/kg/day by gavage on GD 6-20. Preliminary study: Pregnant rats (8–12 per group) were given 250, 500 or 750 mg kg/day of DnHP.	250 mg/kg bw/day and higher.	Significant delay of ossification at all doses. The incidence of 14th supernumerary ribs (mostly short), was also significantly greater than control at all doses, and showed dose–response dependency (19, 61, 91 and 96% of the fetuses at 0, 250, 500 and 750 mg/kg/day, respectively). Liver weight (absolute, relative to body weight on GD 21 or relative to body weight on GD 21 minus uterine weight) was significantly increased at all doses. Slight statistically significant increase in the hepatic activity of cyanide-insensitive palmitoyl-	LOAEL = 250 mg/kg bw/day	Saillenfait, Gallissot and Sabaté, 2009 (cited in EHCA Annex VI-dossier, 2010)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			CoA oxidation (1.5–2.1 fold, compared with the control). Significant and dose-related decrease in the AGD of the male fetuses was seen at all doses. AGD was 7.6, 20.3, and 35.2% below the concurrent control value at 250, 500 and 750 mg/kg/day, respectively. Undescended testes (unilateral or bilateral) were observed in 0 males for control group, 2 males at 250 mg/kg bw/day (in 2/23 litters), 15 males (9/21 litters; ($p<0.05$ compared to control) at 500 mg/kg bw/day and in 20 males (11/13 litters; $p<0.01$ compared to control) at 750 mg/kg bw/day.		
		500 and 750 mg/kg/day	Presence of malformations and significant decreases in foetal weight. Significant increase in the incidence if male fetuses with undescended testis. Fetal body weight was significantly decreased in males and females (about 9 and 18–19% less then control at 500 and 750 mg/kg/day, respectively). Internal and skeletal malformations mainly consisted of cleft palate, eye defects and alterations of the axial skeleton. These included absence of ribs, vertebral archs and/or centra, and fusion of sternebrae, vertebral archs and/or centra. Most often, fetuses exhibited more than one malformation. Two cases of central nervous malformations were also observed		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		750 mg/kg/day	Maternal body weight gain was significantly decreased during the treatment period. Increased incidence of embryolethality (46%). Higher incidence of post-implantation loss with approximately two-thirds of resorbed implants. The number of litters with resorptions was significantly increased. The total numbers of fetuses and litters displaying external, visceral or skeletal malformations were significantly elevated.		
	Pregnant Sprague-Dawley rats (9-13/group) given oral gavage at 0, 50, 125, or 500 mg DnHP/kg/day GD 12-21. *Preliminary study:* Pregnant Sprague-Dawley rats (10-12/group) administered DnHP at 0, 500 and 625 mg/kg/day on GD 12-20.	0, 50, 125, or 500 DnHP/kg/day	No significant effect of DnHP on maternal body weight gain and pup weights during lactation. Degeneration of the seminiferous tubules was seen in all groups including controls however, the incidence and severity of the lesions were much higher in the high-dose groups.	LOAEL = 125 mg/kg/day	Saillenfait, Sabaté and Gallissot, 2009 (cited in EHCA Annex VI-dossier, 2010)
		125 mg/kg/day and above	Males offspring displayed reduced AGD on PND 1 (7% decrease, not statistically significant). At necropsy on PND 111-120, severe malformations of the reproductive tract were observed in young adult males (mainly hypospadias, underdeveloped testis, and undescended testis).		
		250 and 500 mg/kg/day	Not statistically significant effects: reduced proportion of live pups on PND 1 and number of live pups on PND 1, decreased viability of the offspring during the lactation period.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Statistically significant reduced AGD in male offspring on PND 1 (11 and 18% at 250 and 500 mg/kg/day respectively). Areola/nipple retention before weaning and at adulthood. The average numbers of thoracic areolae/nipples per affected male at adulthood were 0, 2.29, and 2.86, at 0, 250 and 500 mg DnHP/kg-d, respectively. Hypospadias was present in respectively, 9 and 21% of the males from the 250 and 500 mg/kg/d groups. Approximately 6 and 38% of the adult animals exhibited undescended testis at 250 and 500 mg/kg-d, respectively. The weight of the seminal vesicles was significantly reduced at 250 mg DnHP/kg/d (relative) and 500 mg DnHP/kg/d (absolute, relative, or with body weight as covariate). Prostate weight tended to be lower at 500mg DnHP/kg/d, with significant difference in the relative weight or when body weight was used as covariate. Severe seminiferous tubule degeneration up to complete atrophy (i.e. severity grade 4 or 5) at the two high doses was seen in 25.0 (8/32 animals; 4/10 litters), and 51.5% (17/33 animals; 6/8 litters) of the males at 250, and 500 mg/kg/d, respectively.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		500 and 625 mg DnHP/kg/day *(Preliminary study)*	In utero exposure to 500 and 625 mg/kg/day DnHP resulted in a decrease in the proportion of live pups on PND 1 (98.3, 80.3, 84.3% in control, 500 and 625 mg/kg/day respectively). High incidence of undescended testis in both DnHP groups. Seminal vesicles and/or prostate were absent in one male at 500 mg DnHP/kg-d and in three males at 625 mg DnHP/kg-d. The size of the testis and/or epididymis was highly reduced in several DnHP animals. A testis was absent in two and one animals at 500 and 625 mg DnHP/kg-d, respectively.		
		625 mg DnHP/kg/day *(Preliminary study)*	Significant increase in the incidence of post-implantation loss per litter (15%, $p<0.001$). Pup weight was slightly reduced from birth to weaning (9% decrease compared to control, $p< 0.01$ on PND 1). Incidences of severe malformations of the external genitalia in mature animals, including hypospadias, cleft phallus associated with exposed os penis, cleft prepuce, and vaginal pouch.		
Diethylhexyl phthalate, DEHP (CAS #117-81-7)	Fischer 344 rats (10 rats/sex/group) given DEHP via the *diet* at 0, 1600, 3100, 6300, 12,500 or 25000 ppm (0, 80, 160, 320, 630, or 1250 mg/kg/day) for 13 weeks	12500 ppm (630 mg/kg bw/day)	Moderate testicular atrophy.	A LOAEL of 12500 ppm (630 mg/kg/day) is derived for testicular effects.	NTP, 1982 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	prior to an oncogenicity study.	25000 ppm (1250 mg/kg bw/day)	The mean body weight gain of male rats was significantly depressed (29%) in males at 25000 ppm relative to controls. Testicular atrophy was observed in all males fed 25,000 ppm.		
	Oncogenicity study: Fischer 344 rats (50 animals/sex/group; initial body weight just above 200 mg for males and around 150 mg for	6000 ppm (322 mg/kg/day)	Seminiferous tubular degeneration, (2/44, 5%; incidence in control was 1/49, 2.0%), histologically devoid of germinal epithelium and spermatocytes.	A LOAEL of 6000 ppm (322 mg/kg/day) is derived for effects on testes.	NTP, 1982 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	females) were given 0, 6000 or 12000 ppm (0, 322 or 674 mg/kg/day for males) DEHP in the diet for 103 weeks.	12000 ppm (674 mg/kg/day)	At the end of the study, mean body weights of dosed male rats and high-dose female rats were marginally to moderately lower than those of the corresponding controls. Interstitial-cell tumours of the testis were observed in a statistically significant negative relation to dose. There was a statistically significant increase in bilateral tubular degeneration of the seminiferous tubules and atrophy in the testes. The incidences was 43/48 (90%) in the high-dose group. Histologically, the seminiferous tubules were devoid of germinal epithelium and spermatocytes. Only Sertoli cells were seen on tubular basement membranes. Interstitial cells were somewhat prominent. In males, the incidence of hypertrophy of the anterior pituitary was significantly increased (45% compared with 2% of controls.		
	Continuous breeding protocol (RACB). 0, 0.01, 016, 0.3% (corresponding to approx. 0, 14, 140, and 420 mg/kg) DEHP were administered via diet to CD-1 mice during 7 days + 98 days cohabitation period. 16-19 pairs of males and females. Control group with n = 40 animals of each sex; 3	0.1% (approx. 140 mg/kg/day)	Fertility and reproductive performance of mating pairs (F0 generation) during continuous breeding was affected: significant reduced number of litters/pair (66% of control, $p<0.01$), live pups/litter (49% of control, $p<0.01$) and proportion of pups born alive (82% of control, $p<0.01$).	LOAEL for reproduction was 140 mg/kg bw/d based on decreased fertility	Lamb et al., 1987 (cited in ECB RAR, 2008)
		0.3% (approx. 420 mg/kg/day)	No fertile pairs in F0 animals during continuous breeding.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	dose groups with n = 20 animals of each sex. Crossover mating: Three combinations of control and treated mice were selected for crossover mating: control males with control females, high-dose males with control females, and control males with high-dose females		In crossover mating: decrease in fertility for treated males and treated females compared to matings of control mice. 4 litters were born to treated males x control females, and the proportion of pups born alive was decreased. There were no litters born to the control male x treated female pairs. At necropsy in F0 males in crossover mating: significant increased liver weights (127% of control, $p<0.01$), decreased weights of testis (41% of control, $p<0.01$), epididymis (81% of control, $p<0.01$), prostate (86% of control, $p<0.05$), reduced motile sperm (40% of control, $p<0.01$), sperm concentration (21% of control, $p<0.01$), and increased incidences of abnormal sperm (765% of control, $p<0.01$). At necropsy in F0 females in crossover mating: significant increase in liver weights (150% of control) $p<0.01$, significant decrease in ovaries, oviducts, uteri (84% of control, $p<0.05$).		
	Developmental toxicity study. Pregnant Fischer 344 rats (22-25 dams/dose) DEHP was administered in the diet	0.5% (approx. 357 mg/kg bw/day)	Maternal absolute and relative liver weights were increased (relative to controls) at all DEHP levels (3.94, 4.77, 5.18, 5.61, and 6.07 g at 0, 0.5, 1.0, 1.5, and 2.0%).	LOAEL for maternal toxicity = 0.5% (approx. 357 mg/kg bw/day) based on increased liver weights.	Tyl et al., 1988 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	on GD 0 through 20 at 0.0, 0.5, 1.0, 1.5, or 2.0%. (No information on mg/kg bw/day is given). At termination (GD 20), all fetuses were examined for external, visceral, and skeletal malformations and variations.	1.0%	Significantly reduced maternal body weight on GD 4, 8, 12 and 20 at dose levels 1.0, 1.5 and 2.0%. On day 20 the weights were: 232.73, 217.76, and 184.15 g for 1.0, 1.5 and 2.0%.dose groups respectively compared to control 248.30g, $p<0.01$). Piloerection and rough coat exhibited a dose-related increased incidence in exposed dams, predominantly at 1.0-2.0% DEHP. Fetal body weight per litter (male, female or total) was significantly reduced at 1.0, 1.5, and 2.0% (for total at 0, 0.5, 1.0, 1.5 and 2.0% the weights were 3.022, 3.143, 2.852, 2.557, 2.266 g).	LOAEL for development = 1.0% (approx. 714 mg/kg bw/day) based on reduced fetal body weights.	
		1.5%	On GD 16, maternal body weight was significantly reduced at 1.5 and 2.0% DEHP.		
		2.0%	Gravid uterine weight was reduced at 2.0%. Significantly increased incidences of resorptions per litter (54.4% versus control 4.14%, $p<0.01$), nonlive (dead plus resorbed) per litter (56.78% versus control 4.14%, $p<0.01$), and affected (nonlive plus malformed) implants per litter (58.13% versus control 5.28%).		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	Developmental toxicity study. Pregnant CD-1 mice (24-30 dams/dose). DEHP was administered in the diet on GD 0 through 17 at 0.0, 0.025, 0.05, 0.10, or 0.15%. At termination (GD 17), all fetuses were examined for external, visceral, and skeletal malformations and variations.	0.05% (approx. 91 mg/kg bw/day)	Rough coat and lethargy at 0.05-0.15% DEHP in pregnant dams. The number of litters with affected implants was increased at 0.05% (23% versus control 21%, $p<0.05$). The number of litters with one or more malformed fetuses (malformations in total or external, visceral, or skeletal malformations separately) was increased at 0.05, 0.10, and 0.15% DEHP, with a general increase of incidences with increasing dose. 100% of litters in the 0.15% dose groups displayed malformations. Incidence of malformations at doses 0, 0.025, 0.05, and 0.10% were 20, 19.23, 61.54 and 88.24%. Significant increase in incidence of malformed fetuses at 0.05, 0.10, and 0.15% (13.72, 37.96% $p<0.01$ and 88%, $p<0.001$, respectively).	LOAEL for developmental toxicity was 0.05% (91 mg/kg bw/day)	Tyl et al., 1988 (cited in ECB RAR, 2008)
		0.10% and higher (approx. 190.6 mg/kg bw/day)	Decreased body weight on GD 12, 16, and 17 but not on GD 0, 4, or 8, at 0.10 and 0.15% DEHP. Maternal weight gain during gestation was depressed at 0.10 and 0.15% (70% and 46% of control, $p<0.01$). Maternal relative liver weight (but not absolute liver weight) was elevated at 0.10 and 0.15% DEHP (116 and 134% of control respectively, $p<0.01$). Gravid uterine weight at termination was reduced at 0.10 and 0.15% DEHP (61 and 28% of control respectively, $p<0.01$).		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			Resorptions, late fetal deaths, nonlive (dead plus resorbed) and affected (nonlive plus malformed) implants per litter and the number of litters with one or more resorptions, late fetal deaths, nonlive or affected implants were all increased at 0.10 and 0.15% DEHP. The number of live fetuses per litter was reduced at 0.10 and 0.15%. There was no effect of treatment on sex ratio of live pups. Female fetal body weight per litter was significantly reduced at 0.10% DEHP (92% of control, $p<0.01$).		
		0.15% (approx. 292.5 mg/kg bw/day)	Fetal body weight per litter (males, females, or total) was significantly reduced at 0.15%.		
	Male Wistar rats (25-days old), 6 per dose group were given DEHP by oral gavage at 0, 50, 100, 250, or 500 mg/kg bw for 30 days.	50 mg/kg bw/day and higher	There was an exposure-related and significant decrease of absolute and relative testicular weight at all dose levels. From 50 mg/kg a dose-dependent and significant increase in the activities of LDH and GGT was noted while that of SDH decreased.	LOAEL = 50 mg/kg bw/ for effects on absolute and relative testies weight, and reduced testicular enzyme activities.	Parmar et al., 1995 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		250 mg/kg bw/day and higher	β-glucuronidase activity increased at 250 or 500 mg DEHP/kg, while acid phosphatase decreased at the same dose levels. Marked destructive changes in the advanced germ cell layers and marked degrees of vacuolar degeneration in the testes at 250 and 500 mg/kg bw.		
	F-344 rats (70 males and females/group, about 6 weeks of age) were administered DEHP at dietary concentrations of 0, 100, 500, 2500 or 12500 ppm (0, 5.8, 28.9, 146.6, or 789.0 mg/kg bw/day in males; 0, 7.3, 36.1, 181.7 and 938.5 mg/kg/day in the females) for at least 104 weeks. An additional group was administered 12500 ppm DEHP for 78 weeks, followed by a recovery period of 26 weeks.	2500 ppm (146.6-181.7 mg/kg bw/day) and higher	Dose-related increased incidence of uterine mass at 2500 and 12500 ppm in females that died or were sacrificed in extremis during the study, significant at the highest dose level. This was also found in females from the recovery group and in surviving animals from these dose groups at study termination. An increased incidence (not significant) of aspermatogenesis was present at 2500 ppm in unscheduled deaths, at interim sacrifice, and at study termination. At 2500 ppm the mean serum albumin concentration and mean liver weights were significantly increased. At Week 79 and at study termination also absolute and relative kidney weights were increased in both sexes at 2500 ppm. The incidence of mononuclear cell leukemia (MCL) was increased in both sexes, significant in males only from 2500 ppm.	LOAEL for testicular effects is 2500 ppm (146.6 mg/kg bw/day) The LOAEL for systemic non-neoplastic effects, including the effects on the kidney is considered to be 2500 ppm DEHP in the diet (corresponding to 146.6 mg/kg bw/day in the males and 181.7 mg/kg/day in the females) based on increased absolute and relative kidney weight in both sexes.	Moore, 1996 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
		12500 ppm (789.0-938.5 mg/kg bw/day)	At 12500 ppm dose level, there was a decreased survival, increased incidence of clinical abnormalities, and decreased body weight gain in both sexes. A diffuse hepatomegaly and histopathological hepatic changes were demonstrated. Effects on the kidneys were observed, including increased absolute and relative kidney weights (both sexes), increased incidence and severity of mineralisation of the renal papilla in males, increased incidence and/or severity of tubule cell pigment in both sexes, and increased severity of chronic progressive nephropathy in the males. In the males, also absolute and relative testis weights were significantly decreased at 12500 ppm, with associated increased incidence of bilateral aspermatogenesis in all males accompanied by hypospermia in the epididymis and decreased incidence of interstitial cell neoplasms (3/10 compared to 9/10 in control group). In the pituitary, an increased number of castration cells were observed in 30/60 males compared to 1/60 of the control males. There was no indication in rats killed at study termination that DEHP-related changes in the kidney, testis, and pituitary were reversible upon cessation of DEHP-exposure.		
	Prenatal toxicity in l0 rats per group after gavage administration of DEHP	1000 mg/kg/day	At 1000 mg/kg di(2-ethylhexyl) phthalate showed clear foetotoxicity, embryolethality and	LOAEL = 1000 mg/kg/day based on foetotoxicity,	Hellwig et al., 1997 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	at 0, 40, 200 and 1000 mg/kg/day from gestation day 6 to 15.		teratogenicity. No significant effects were recorded at 40 and 200 mg/kg.	embryolethality and teratogenicity.	
	Developmental toxicity study in CD-1 mice, 15 females/dose group and 30 females/control group. Oral gavage at 0, 40, 200 or 1,000 mg/kg bw/day on gestation days 6-15.	200 mg/kg bw/day	Significantly decreased number of viable fetuses	LOAEL 1000 mg/kg bw for maternal toxicity and NOAEL 200 mg/kg bw/day for developmental	Huntingdon, 1997 (cited in ECB RAR, 2008)
		1000 mg/kg bw/day	Significantly increased number of resorptions and post-implantation losses at 1,000 mg/kg bw/day and also cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney abnormalities		
	B6C3F1 mice (70-85 of each sex/dose group, about 6 weeks of age at the initiation of the study) were administered DEHP daily in the diet at concentrations of 0, 100, 500, 1500 and 6000 ppm for 104 weeks (0, 19.2, 98.5, 292.2 or 1,266.1 mg/kg/day, respectively, for males, and 0, 23.8, 116.8, 354.2 or 1,458.2 mg/kg/day, respectively). *Recovery trial:* One additional group (55 males) were administered 6000 ppm DEHP for 78 weeks, followed by a 26-week recovery period.	1500 ppm (292.2 mg/kg/day)	Significant decrease in testicular weight, with an increased incidence and severity of bilateral hypospermia and an associated increased incidence of immature/abnormal sperm forms and hypospermia in the epididymis. Significant decrease in kidney weight in males and an increased incidence and/or severity of chronic progressive nephropathy in both sexes.	The LOAEL for testicular effects in this study is 1500 ppm corresponding to 292.2 mg/kg.	Moore, 1997 (cited in ECB RAR, 2008)
		6000 ppm (1266 mg/kg/day)	Statistically significant decrease in survival, treatment-related clinical signs and a significantly reduced body weight gain for both males and females. In both males and females, the kidney weight indices were significantly decreased at study termination. In the recovery group, the effects of DEHP in the kidney and testis were at least partially reversible following cessation of exposure.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	Young male and female Sprague-Dawley rats (10 rats/sex/group) were exposed to DEHP via the diet for 13 weeks at 0, 5, 50, 500, or 5000 ppm (0, 0.4, 3.7, 37.6, or 375.2 mg/kg bw/day for males; 0, 0.4, 4.2, 42.2, 419.3 mg/kg bw/day for females).	500 ppm (37.6 mg/kg bw/day)	High incidence of minimal to mild Sertoli cell vacuolation in testis at 500 ppm (7/10).	A LOAEL of 500 ppm DEHP in the diet (37 mg/kg bw/day) is derived from the study based on Sertoli cell vacuolation.	Poon et al., 1997 (cited in ECB RAR, 2008)
		5000 ppm (376 mg/kg bw/day)	Absolute and relative testis weights were significantly reduced. Microscopic examination revealed a mild to moderate, bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules in 9 out of 10 rats. Rats of both sexes had significantly increased absolute and relative liver weights and relative kidney weight and mild histological changes of the thyroid at 5000 ppm.		
	Two-generation study Wistar rats (25 animals/group) were administered 0, 1000,	1000 ppm (113 mg/kg/day)	Minimal focal tubular atrophy occurred at 1000 ppm (113 mg/kg and day)	LOAEL = 1000 ppm (or 113 mg/kg bw/day)	Schilling et al., 2001 (cited in ECB RAR, 2008)

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	3000 or 9000 ppm DEHP via the diet (corresponding to approximately 0, 113, 340 or 1,088 mg/kg/day)	3000 ppm (340 mg/kg/day)	Reduced testis weight in F2, focal tubular atrophy and a feminisation of 49% of the male offspring.		
	Three-generation study Sprague-Dawley rats (17/males/group) were administered DEHP via diet at 1.5 (control), 10, 30, 100, 300, 1000, 7500 and 10,000 ppm (corresponding to 0.12, 0.78, 2.4, 7.9, 23, 77, 592, and 775 mg/kg/day in the F0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391, and 543 mg/kg/day in F1 animals; 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg/day in the F2 animals). Animals in the F0 generation began exposure as adults and were bred to produce the F1 generation (F1a, 1b, 1c), the F1 adults were	1000 ppm	Treatment-related histopathological abnormalities in the liver, kidneys, and adrenal glands of F1 animals and in the liver of F2 animals.	LOAEL for fertility = 23 mg/kg bw/day based on testicular toxicity. LOAEL for development = 14 mg/kg bw/day based on increased incidences of small or aplastic testes and epididymis, seminiferous tubule atrophy.	Wolfe et al., 2003 (cited in ECB RAR, 2008)
		7500 ppm	Male AGD decreased at 7500 ppm in the F1a and F1b pups. Male and female pup weights, unadjusted and adjusted for litter size, were decreased at 7500 ppm in the F2c litter and combined F2a, b, c litters. Male and female pup weights were decreased at 7500 ppm throughout the lactation period (PND 1 -21) of the F2c pups. AGD was decreased at 7500 ppm in the F2a and F2c pups. There was also a decrease in the pregnancy index for the F2 mating pairs (45%) at 7500 ppm. Male AGD was decreased at 7500 ppm in the F3a pups. Testes descent, vaginal opening, and preputial separation were delayed at 7500 ppm in the F3c pups. Retained nipples were observed in the F3c male pups at 7500 ppm.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	bred to produce the F2 generation (F2a, 2b, 2c), and the F2 adults were bred to produce the F3 generation (F3a, 3b, 3c). Additional non-mating males (up to three per litter) were selected from the F1c, F2c, F3c litters, and were maintained following similar procedures as those for mating males, except they were not cohabited with females. The 10000 ppm animals only completed the F1 generation and were terminated due to the inability to produce any F2 generation animals.	7500 and 10000 ppm	Testes descent, vaginal opening, and preputial separation were delayed at 10000 ppm and 7500 ppm in the F1c pups. Reductions in terminal body weights were noted at 7500 ppm in the F1 and F2 males (10% and 14%, respectively), and at 10,000 ppm in the F0 and F1 males (6% and 21%, respectively) and females (12% and 19%, respectively). Reproductive effects were noted in the 7500 ppm and 10000 ppm groups: total number of males per litter was decreased at 10000 ppm in the F1a litter and at 7500 ppm across all F1 litters combined (F1a + F1b + F1c). The total number of F1a pups per litter was decreased at 7500 and 10000 ppm. The total number of pups per litter across all F1 (F1a + F1b + F1c) litters combined was also decreased at 7500 ppm. At 10000 ppm, male and female pup weights, unadjusted and/or adjusted for litter size, were decreased in the F1a and F1b litters on PND 1 and in the F1c litters on PND 1, 4, 7, 14, and 21. At terminal necropsies, various sperm end-points were found to be decreased at 7500 ppm in the F1, F2, and F3 males and at 10000 ppm in the F0 and F, males. Density (sperm/mg cauda) (F2, and F3 males only), sperm/cauda, spermatids/testis, and spermatids/mg testes were decreased at 7500 ppm in the F1, F2, and F3 males. Treatment-related histopathological abnormalities were noted at 7500 and 10000 ppm in the testes, epididymis, liver, adrenal glands, and kidney in the F0, F1, and F2 animals.		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			The absolute and/or relative liver weights were increased at 1000 ppm in the F1 males at 7500 ppm in the F0, F1, F2 and F3 males and at 10000 ppm in the F0 males. Absolute and relative liver weights were also increased at 7500 ppm in the females in all generations. Absolute and/or relative kidney weights were increased at 7500 ppm in the F0, F1, and F2 males, and F0 females and at 10000 ppm in the F0 males and F0 females. The absolute kidney weight at 10000 ppm in the F1 females was increased. The absolute and/or relative cauda, epididymis, and testis weights were decreased at 7500 ppm in the F1, F2, and F3 males and at 10000 ppm in the F0 and/or F1 males In the testes, minimal to marked atrophy of the seminiferous tubules characterized by loss of germ cells and the presence of Sertoli cell-only tubules, as well as occasional failure of sperm release, were noted at 10000 ppm in the F1 males and at 7500 ppm in the F1 and F2 males. Crossover matings were conducted using the 7500 and 10000 ppm males and females. At 7500 and 10000 ppm, when treated males were crossed with nulliparous naive females, there were decreased numbers of implantation sites, and decreased indices of mating, pregnancy, and fertility. At 7500 and 10000 ppm, when treated females were crossed with naive males there was a decrease in AGD in the male pups. Also at 7500		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			ppm, male, female, and combined pup weights were decreased, both when unadjusted and adjusted for litter size.		
		10000 ppm	AGD was decreased at 10000 ppm in the F1a, F1b, and F1c pups. None of the F1 mating pairs produced offspring at 10000 ppm. Spermatids/testis was decreased at 10000 ppm in the F0 males and no sperm or spermatids were noted in the F1 males.		
	Developmental toxicity study. Female Wistar rats (11-16 dams per group) were treated daily with DEHP by gavage from GD 6 to lactation day 21 in two wide ranges of doses: the low-doses were 0.015, 0.045, 0.135,	0.045 mg/kg bw/day and higher	Serum testosterone concentration was significantly increased at 0.045, 0.405 and 405 mg DEHP/kg/day. A significant reduction in daily sperm production of 19–25% in relation to concurrent control was observed in animals exposed to 0.045, 0.135, 0.405, 1.215, 15, 45, 135 and 405 mg/kg/day (87, 91, 88, 84, 86, 75, 81, 78, 75 % of control	LOAELs for reduced daily sperm production and a low incidence of cryptorchidism were 15 and 5 mg/kg/day, respectively	Andrade et al., 2006

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	0.405 and 1.215 mg DEHP/kg bw/day, and the high-doses were 5, 15, 45, 135 and 405 mg DEHP/kg bw/day.		respectively; $p<0.05$). Compared to historical control and using a cut off value of 20% reduction daily sperm production was significantly reduced only at 15,135 and 405 mg/kg/day. A significantly higher number of animals with more than 10% abnormal sperm was observed in the group exposed to 0.045 mg/kg/day when compared to the concurrent control. In relation to the historical control, a significant increase in the number of animals with abnormal sperm was seen at 0.045 and 0.135 mg/kg/day.		
		5 mg/kg bw/day and higher	Undescended (ectopic) testes were observed in three animals, exposed to either 5, 135 and 405 mg DEHP/kg/day (one case in each dose).		
		405 mg/kg bw/day	The weight of seminal vesicle with coagulating glands was significantly reduced at 405 mg/kg/day.		
	Developmental toxicity study. Study 1: Time-mated Wistar rats (16 dams/control group, 8 dams/dose group) were gavaged daily with DEHP from GD 7 to PND 16 with DEHP at doses at 3, 10, 30, 100, 300, 600, 900 mg/kg bw/day.	3 mg/kg bw/day and higher	The incidence of mild external genitalia dysgenesis in male offspring combined in study 1 and 2 was significantly increased at all doses except at 30 mg/kg (12, 11, 6, 16, 17, 17 and 50% at 3, 10, 30, 100, 300, 600 and 900 mg/kg bw/day respectively).	LOAEL = 10 mg/kg bw/day based on reduced AGD and nipple retention in males.	Christiansen et al., 2010
		10 mg/kg bw/day and higher	Analysis of the combined data (study 1 + 2) showed significantly decreased AGD in male offspring at all dose levels of DEHP above 3 mg/kg bw/day (AGD < 3.4 mm compared to		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
	Study 2: Time-mated Wistar rats were gavaged daily from GD 7 to PND 16 with DEHP at doses 3, 10, 30, 100, mg/kg bw/day. (Control group: 16 dams; 3 mg/kg bw/day dose group: 16 dams; 10, 30, 100 mg/kg bw/day dose groups: 8 dams).		control in study 1: 3.4 mm and control in study 2: 3.68 mm) Nipple retention was significantly induced in male offspring at all dose levels in study 1. However, no dose–response relationship was observed. The number of nipples at 10, 30, 100, 300, 600, 900 mg/kg bw/day was 3.14; 1.81; 1.23; 5.21; 4.63; 5.0, respectively, compared to control 0.22. In study 2, there appeared to be a higher number of nipples at 10 and 100 mg/kg compared to controls (1.13 and 0.86 compared to 0.38), although the difference was not statistically significant. Weights of levator ani/bulbocavernosus muscles and prostate was significantly reduced from 10 mg/kg (combined data study 1 + 2) (89% and 77% of control).		
		30 mg/kg bw/day and higher	In study 2, both the androgen-regulated genes PBP C3 and ODC in ventral prostate were significantly reduced at 30 and 100 mg/kg in males PND 16 ($p<0.01$).		
		300 mg/kg bw/day and higher	Significant decreases in the male birth weights were seen at 300, 600 and 900 mg/kg (5.91, 5.61, 5.7 respectively, versus control 6.47 g) and in the females at 900 mg/kg (5.29 g versus control 6.12 g, $p<0.001$). Expression of the androgen-regulated genes PBP C3 and ODC in ventral prostate was significantly reduced in PND 16 males after exposure to 300		

Phthalate	Species, strain, experimental regimen	Dose	Effects	LOAEL	Reference
			and 900 mg/kg DEHP ($p<0.05$ and $p<0.01$ respectively).		
		600 mg/kg bw/day and higher	Higher doses of DEHP induced histopathological effects on the testes and significant reduced testis weight (86-93% of control).		

Note: This is not a complete list of experimental data on reproduction toxicity for the reference substances in the category. The presented studies include selected key studies as indicated in previous reports and evaluations.