European Chemicals Agency (EChA), Helsinki

To support the DNEL setting for diisopentylphthalate on its toxicity for reproduction related to the use in Application for Authorisation

Service Request
under the Multiple Framework Contract with re-opening of competition
for scientific services for ECHA

CONTRACT NUMBER ECHA/2015/368 – SC-30 (LOT 2)

FINAL REPORT

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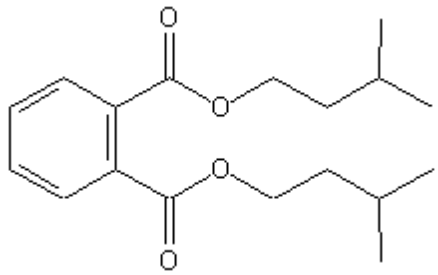
Consortium ETeSS
Expert Team providing scientific support for ECHA

CONTENTS

1. INTRODUCTION

Diisopentylphthalate (DIPP) has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The purpose of this review is to evaluate the available information relevant to deriving DNELs for the reproductive toxicity of DIPP.



The identification of DIPP as a Substance of Very High Concern (SVHC) was on the basis of its classification with H360FD (Repr Cat 1B).

The harmonised classification of DIPP is in a group entry of pentyl phthalates:

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling	
				Hazard class + category	Hazard statement	Pictogram + signal word	Hazard statement
607-426-00-1	1,2-benzenedicarboxylic acid dipentylester, branched and linear [1] n-pentyl isopentylphthalate [2] di-n-pentylphthalate (DPP) [3] diisopentylphthalate (DIPP) [4]	284-032-2 [1] [2] 205-017-9 [3] 210-088-4 [4]	84777-06-0 [1] [2] 131-18-0 [3] 605-50-5 [4]	Repr. 1B Aquatic Acute 1	H360FD H400	GHS08 GHS09 Dgr	H360FD H400

The DIPP SVHC support document (ECHA, 2012a), refers to a single developmental toxicity study conducted with a mixture of DIPP and DPP (Hellwig et al., 1997), but mention is made also of a possible read-across from the better characterised reproductive toxicity of di-n-pentylphthalate (DPP) and dibutylphthalate (DBP).

The contractor has performed an extensive literature search (see chapter 11) for DIPP and identified only one publication – the developmental toxicity study referred to in the preceding paragraph. The contractor has also reviewed registration dossiers for DIPP. Only one 10 tpa registration was identified, and this did not include any reproductive toxicity data.

The one identified reproductive toxicity study on DIPP/DPP is reviewed below; however, it is noted that this study alone may not be sufficient to establish DNELs for fertility and development for DIPP and that read-across from other structurally-related low molecular weight phthalates might be required.

Substance identity and physical-chemical properties of DIPP

Table 1: Substance identity for DIPP

EC number	210-088-4
EC name	diisopentylphthalate
CAS number	605-50-5
CAS name	1,2-benzenedicarboxylic acid, 1,2-bis(3-methylbutyl) ester
IUPAC name	Bis(3-methylbutyl) phthalate
Index number in Annex XI of the CLP Regulation	607-426-00-1
Molecular formula	$C_{18}H_{26}O_4$
Molecular weight	306,40 g/mol

Table 2: Overview of physico-chemical properties of DIPP

Physical state at 20°C and 101.3 kPa	Clear liquid, slightly yellow
Melting/freezing point	Freezing point less than -25°C
Boiling point	339°C
Vapour pressure	0.025Pa at 25°C
Water solubility	1.1mg/l at 20°C
Partition coefficient (logPow)	log P_{OW} =5.6
Flashpoint	166°C
Auto flammability at 1013hPa	>=400°C
Oxidising property	No oxidising properties.
Density	1.02 g/cm³

Toxicokinetics of DIPP

No specific information on the kinetics of DIPP has been located. However, there is significant information on the kinetic behaviour of medium-chain phthalate (4-10 carbon side chain length) in general. These phthalates are in general rapidly absorbed and excreted (mainly in urine) after oral administration Faecal excretion is generally low. Dermal absorption also occurs up to a certain extent. Inhalation absorption tends to be significant. Excretion in bile with subsequent entero-hepatic recirculation has also been observed for some of these phthalates. No significant accumulation in tissues is generally observed.

A significant proportion of these phthalates is hydrolysed to the respective monoester and the corresponding alcohol prior to absorption by the small intestines, but hydrolysis can also occur in liver and kidneys. The monoesters are generally excreted in urine as glucuronide conjugates.

Transplacental transfer of medium-chain phthalates and its metabolites is generally observed in rats. Levels of radioactivity in placenta and embryo tend to be lower than those in maternal plasma; the monoesters tend to account for most of the radioactivity in maternal plasma, placenta and embryo. However,

generally, there is no accumulation of radioactivity in maternal or embryonic tissues.

2. REPRODUCTIVE TOXICITY DATA ON DIPP

In a prenatal developmental toxicity study in Wistar rats broadly consistent with OECD guideline 414 (with the main exception that exposure did not continue until gestation day 19), a number of different phthalates including DIPP and DEHP were tested (Hellwig et al., 1997). Although the publication refers to the test substance as DIPP, in fact the substance with CAS No. 84777-06-0 is a mixture of pentyl phthalates consisting of:

- 42-45% Di-n-pentylphthalate (CAS No. 131-18-0)
- 44-47% mixed ester n-pentyl-isopentylphthalate (no CAS No or EINECS No.) and
- 8-12% Diisopentylphthalate (CAS No. 605-50-5).

The test substance (in olive oil) was administered by gavage to groups of 10 pregnant females at 0, 40, 200 or 1000 mg/kg bw/d from day 6 to day 15 of gestation.

At the top dose, maternal food consumption, body weight gain and terminal body weight (↓26%) were significantly reduced. Six dams showed vaginal haemorrhage during treatment or post-treatment. The absolute and relative kidney weights and relative liver weights were significantly increased.

At this 1000 mg/kg bw/d dose, there was 100% post-implantation loss due to total embryolethality (predominantly early resorptions). It is unclear whether such a severe effect was the secondary, unspecific consequence of the maternal toxicity observed. There were neither maternal effects nor developmental effects at the next lower dose level of 200 mg/kg bw/d. Therefore, a NOAEL of 200 mg/kg bw/d can be identified for maternal and developmental toxicity from this study. However, it should be noted that since prenatal exposure did not occur during gestation days 15-19, which is the key window for sexual differentiation in rats and hence the key window of susceptibility to the reproductive toxicity of phthalates, the NOAEL derived from this study is not reliable.

In the same study, DEHP was teratogenic (producing external, skeletal and visceral malformations) at the top dose of 1000 mg/kg bw/d. At this dose, it also caused pronounced (40% vs 10% in controls) post-implantation loss, reduced numbers of live foetuses per dam and decreased foetal body weights. Maternal toxicity (decreased food consumption on the first days of treatment, slightly reduced body weights and significantly impaired body weight gain) was also observed at the top dose of 1000 mg/kg bw/d DEHP. There were neither maternal effects nor developmental effects at the next lower dose level of 200 mg/kg bw/d DEHP. Therefore, a NOAEL of 200 mg/kg bw/d can also be identified for DEHP for maternal and developmental toxicity from this study. However, again, since prenatal exposure did not occur during

gestation days 15-19, which is the key window for sexual differentiation in rats and hence the key window of susceptibility to the reproductive toxicity of phthalates, the NOAEL derived from this study for DEHP is also not reliable.

Overall, this study shows that prenatal exposure to a mixture of DIPP and DPP during gestation days 6-15 caused total embryolethality at the top dose of 1000 mg/kg bw/d in the presence of significant maternal toxicity. A NOAEL of 200 mg/kg bw/d was identified for maternal and developmental toxicity. However, it should be noted that since prenatal exposure did not occur during gestation days 15-19, which is the key window for sexual differentiation in rats and hence the key window of susceptibility to the reproductive toxicity of phthalates, the NOAEL derived from this study is not reliable.

3. CONSIDERATION OF READ-ACROSS FROM THREE PHTHALATES STRUCTURALLY SIMILAR TO DIPP ON THE BASIS OF CHEMICAL STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES

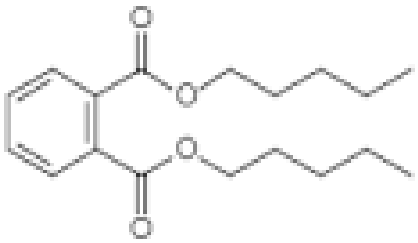
The contractor considered a possible read-across from the better characterised reproductive toxicity of three phthalates structurally similar to DIPP: di-n-pentylphthalate (DPP), diisobutylphthalate (DIBP) and dibutylphthalate (DBP). These three phthalates are all of high structural similarity to DIPP and all share a common Mode-of-Action (MoA) for their reprotoxic potential. Therefore, they are all very good candidates for read-across to DIPP. These are considered below, one by one. In addition to structural similarity and information on physico-chemical properties, dose-response information on developmental effects on male reproduction in rodents, typical of the “phthalate syndrome”, for each of these three candidates are taken into account to determine which of these three phthalates is the most appropriate/relevant candidate for read-across to DIPP.

For DPP, the contractor has been unable to identify recent reviews of its reproductive toxicity; therefore, the primary literature has been examined (see chapter 4) and DNELs for the most sensitive effect have been derived (see chapter 5). For DBP and DIBP, recent reviews of their reproductive toxicity including derivation of DNELs for the most critical effects have already been considered by RAC (Risk Assessment Committee) or published for consultation; therefore only extracts from the most recent draft review on these phthalates (ECHA, 2016) have been reproduced in this document.

Potency information on the three possible candidates for read-across to DIPP would also be very informative in selecting the most appropriate one. However, it should be considered that determining the relative potency of these three phthalates accurately and in relation to DIPP depends significantly on the size of the database, the quality and design of the available studies and the sensitivity of the parameters investigated. Unfortunately, these elements are not equal for the three phthalates of interest, and, for DIPP, reproductive toxicity data are extremely limited.

In addition, information from the available literature (Health Canada, 2015) indicates that no clear trend can be established for the medium-chain phthalates (main backbone side chain length of 3 to 7 carbons) between the length of the side chains and their reproductive potency. Usually phthalates with main backbone side chains of 4 to 6 carbons are more potent than phthalates with main backbone side chains of 7-10 carbons. It is also unclear whether phthalates with branched side chains are more potent than phthalates with linear side chains. For some phthalates, the branched structure is more potent than the respective linear structure; for others, it is vice versa. Usually, it depends on a combination of the chain length with the size and position of the branching.

Dipentylphthalate (DPP)



DPP (CAS No. 131-18-0) appears a good candidate for read-across to DIPP as it is the isomer of DIPP. DPP has the same molecular weight as DIPP and has two side chains of 5 carbons in total as does DIPP. The only difference is that in DPP the alcohol chains are linear, while in DIPP they have a one-carbon branch at the end. In addition, both DPP and DIPP are liquid at room temperature and have very similar physico-chemical properties (see table 9), including vapour pressure (0.025 Pa for DIPP and 0.02 Pa for DPP), water solubility (1.1 mg/l for DIPP and 0.8 mg/l for DPP) and in particular the partition coefficient (5.6 for both).

Reproductive hazard information and dose-response data on developmental effects on male reproduction in rodents, typical of the “phthalate syndrome”, for DPP are considered in chapter 4; data on the most sensitive reproductive effects of DPP are presented in chapter 5 and a comparison of this information with that for DIBP and DBP is presented in chapter 7.

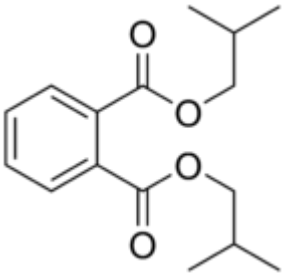
Table 3: Substance identity for DPP

EC number	205-017-9
EC name	dipentylphthalate
CAS number	131-18-0
CAS name	1,2-Benzenedicarboxylic acid, 1,2-dipentyl ester
IUPAC name	Dipentyl phthalate
Index number in Annex XI of the CLP Regulation	607-426-00-1
Molecular formula	$C_{18}H_{26}O_4$
Molecular weight	306,40 g/mol

Table 4: Overview of physico-chemical properties of DPP

Physical state at 20°C and 101.3 kPa	Colourless, oily liquid
Melting/freezing point	-55°C
Boiling point	342°C
Vapour pressure	0.02 Pa at 25°C
Water solubility	0.8 mg/L at 25°C
Partition coefficient (logPow)	log Pow 5.62 at 20°C
Flashpoint	118-180^{0}C
Density	1.03 g/cm^{3}

Diisobutylphthalate (DIBP)



DIBP can also be considered a good candidate for read-across to DIPP. Although DIBP has a smaller molecular weight (278 g/mol) than DIPP (306 g/mol) as a consequence of having side chains with 4 carbons in total compared to DIPP's total 5 carbon side chains, the two substances have both branched side chains (one carbon branch). In addition, although the partition coefficient and water solubility are slightly different (log Pow 5.6 for DIPP and 4.2 for DIBP; water solubility 1.1 mg/l for DIPP and 20 mg/l for DIBP), the other physico-chemical properties of DIBP (see table 9) are very similar to those of DIPP (e.g. vapour pressure is 0.025 Pa for DIPP and 0.01 Pa for DIBP; relative density is 1.02 g/m^3 for DIPP and 1.04 g/m^3 for DIBP; and they are both liquids at room temperature).

Dose-response information on developmental effects on male reproduction in rodents, typical of the "phthalate syndrome", for DIBP is considered in chapter 6 and a comparison of this information with that for DPP and DBP is presented in chapter 7.

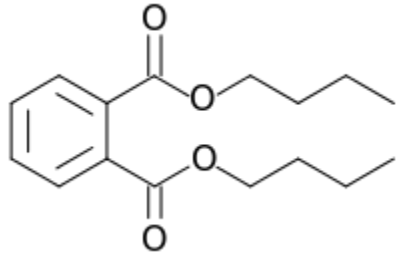
Table 5: Substance identity for DIBP

EC number	201-553-2
EC name	diisobutylphthalate
CAS number	84-69-5
IUPAC name	Bis(2-methylpropyl) benzene-1,2-dicarboxylate
Molecular formula	$C_{16}H_{22}O_4$
Molecular weight	278.34 g/mol

Table 6: Overview of physico-chemical properties of DIBP

Physical state at 20°C and 101.3 kPa	Colourless liquid
Melting/freezing point	-37°C
Boiling point	320°C
Vapour pressure	0.01 Pa at 20°C
Water solubility	20 mg/L at 20°C
Partition coefficient (logPow)	log Pow 4.2 at 20°C
Density	1.04 g/m^3

Dibutylphthalate (DBP)



DBP can also be considered a good candidate for read-across to DIPP. Although DBP has a smaller molecular weight (278 g/mol) than DIPP (306 g/mol) as a consequence of having side chains with 4 carbons in total compared to DIPP's total 5 carbon side chains, and although in DBP the alcohol chains are linear, while in DIPP they have a one-carbon branch at the end, the main backbone of the side chains for both DIPP and DBP is made of 4 carbons. According to Health Canada (2015), the main carbon backbone length of the side chains is more important in determining similarity in toxicological properties than the total number of carbons in these side chains. Also, although the partition coefficient and water solubility are slightly different (log Pow 5.6 for DIPP and 4.57 for DBP; water solubility 1.1 mg/l for DIPP and 10 mg/l for DBP), the other physico-chemical properties of DBP (see table 9) are very similar to those of DIPP (e.g. vapour pressure is 0.025 Pa for DIPP and 0.01 Pa for DBP; density is 1.02 g/m^3 for DIPP and 1.045 g/m^3 for DBP; and they are both liquids at room temperature).

Dose-response information on developmental effects on male reproduction in rodents, typical of the "phthalate syndrome", for DBP is considered in chapter 6 and a comparison of this information with that for DPP and DIBP is presented in chapter 7.

Table 7: Substance identity for DBP

EC number	201-557-4
EC name	dibutylphthalate
CAS number	84-74-2
IUPAC name	dibutylphthalate
Molecular formula	$C_{16}H_{22}O_4$
Molecular weight	278.34 g/mol

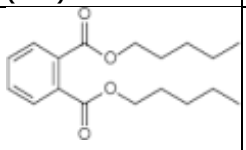
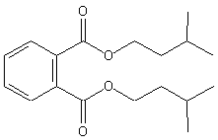
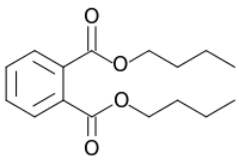
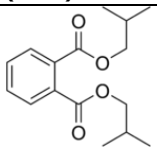
Table 8: Overview of physico-chemical properties of DBP

Physical state at 20°C and 101.3 kPa	Oily liquid
Melting/freezing point	-69°C
Boiling point	340°C
Vapour pressure	0.01 Pa at 20°C
Water solubility	10 mg/L at 20°C
Partition coefficient (logPow)	log Pow 4.57 at 20°C
Density	1.045 g/m^3

DIPP, DPP, DIBP and DBP: a comparison of physico-chemical properties

A comparative table of the structures and physico-chemical properties of these 4 phthalates is provided below:

Table 9: A comparison of the structures and physico-chemical properties of DPP, DIPP, DBP and DIBP

Properties	**Dipentylphthalate (DPP)**	**diisopentylphthalate (DIPP)**	**Dibutyl phthalate (DBP)**	**Diisobutyl phthalate (DIBP)**
Structure				
MW	306.40 g/mol	306.40 g/mol	278.34 g/mol	278.34 g/mol
Physical state	Colourless, oily liquid	Clear, slightly yellow liquid	Oily liquid	Colourless liquid
Melting/freezing point	-55 °C	< -25 °C	-69 °C	-37 °C
Boiling point	342 °C	339 °C	340 °C	320 °C
Relative density	1.03 g/m^3	1.02 g/m^3	1.045 g/m^3	1.04 g/m^3
Vapour pressure	0.02 Pa at 25 °C	0.025 Pa at 25 °C	0.01 Pa at 25°C	0.01 Pa at 20 °C
Water solubility	0.8 mg/l at 25 °C	1.1 mg/l at 20 °C	10 mg/l at 20 °C	20 mg/l at 20 °C
Partition coefficient (logPow)	5.6	5.6	4.6	4.2
Reference	DPP SVHC Support Doc (ECHA, 2013a)	DIPP SVHC Support Doc (ECHA, 2012a)	EU RAR (2004)	Opinion on Annex XV restriction dossier on 4 phthalates (ECHA, 2012b)

Overall, based on structural similarity and physico-chemical properties, DPP, DIBP and DBP appear all good candidates for read-across to DIPP. In addition, they all share a common anti-androgenic mode of action. They inhibit foetal testosterone production; reduce male anogenital distance; decrease gene expression related to steroid biosynthesis; increase nipple retention in male offspring; increase the incidence of genital malformations

(hypospadias and cryptorchidism); induce delayed puberty onset; reduce semen quality; and induce testicular changes including testicular atrophy in rats (ECHA, 2016). It is considered that structural similarity and similarity in physico-chemical properties are insufficient to determine which one of these three phthalates is the most suitable for read-across to DIPP. Therefore, it is proposed that dose-response information on developmental effects on male reproduction in rodents, typical of the "phthalate syndrome", for each of these three candidates should be considered as it may provide further insight which could help in determining which one of these three phthalates is the most appropriate for read-across to DIPP.

4. DATA ON DPP

Toxicokinetics of DPP

No specific information on the kinetics of DPP has been located. However, information on the kinetic behaviour of medium-chain phthalate (4-10 carbon side chain length) in general has already been presented above under the heading "Toxicokinetics of DIPP".

Reproductive toxicity data on DPP

The contractor has identified 14 studies, including 2 reviews on the reproductive toxicity of DPP as specified below:

Foster et al., 1980;
Gangolli, 1982;
Creasy et al., 1983;
Foster et al., 1983;
Gray and Gangolli, 1986;
Heindel et al., 1989;
Liu et al., 2005;
Howdeshell et al., 2008;
Benson, 2009;
Hannas et al., 2011;
Hannas et al., 2012;
Beverly et al., 2014;
US CHAP on phthalates, 2014
Gray et al., 2016;

These studies are summarised below and tabulated in Table 5.

Studies specifically investigating toxicity towards male reproductive organs in rats

Foster et al (1980)
Foster et al (1980) administered DPP in corn oil by oral gavage to groups of 12 young male Sprague-Dawley rats at 0 or 1800 mg/kg bw/d for 4 days. On

the day following the last dose, the animals were sacrificed and the testes removed for histopathological examination.

There were no significant effects on food intake or body weight gain. However, relative testis weight was markedly reduced. Histological examination of the testes showed severe atrophy of the seminiferous tubules, the majority of which presented a complete loss of spermatocytes and spermatids. Only a few spermatogonia and Sertoli cells remained attached to the basement membrane of the tubule.

Overall, this study showed that treatment of rats for 4 days with the high dose of 1800 mg/kg bw/d DPP caused severe testis atrophy. As only one dose level was used, a LOAEL of 1800 mg/kg bw/d was identified from this study.

Gangolli (1982)

In a similar study, DPP (in corn oil) was administered by oral gavage to groups of young male Sprague-Dawley rats at 0 or 2200 mg/kg bw/d for up to 4 days (Gangolli, 1982). Animals were sacrificed at different time-points during treatment and at the end of treatment. The testes were removed for histopathological examination

Relative testis weight was markedly reduced (by 44%). Histological examination of the testes showed severe atrophy of the seminiferous tubules. Within 3 hours of treatment, there was already evidence of testicular injury characterised by the dissociation of germ cells from the basal membrane of the seminiferous tubules. At 24 hours, vacuolation of the Sertoli cells was evident.

In addition, similar effects on the seminiferous tubules (detachment of the germinal cells from the Sertoli cells) were seen in ex vivo preparations obtained from untreated rat testes treated with the monoester of DPP (from 30 to 1000 µM).

Overall, this study showed that treatment of rats for up to 4 days with the high dose of 2200 mg/kg bw/d DPP caused severe testis atrophy. As only one dose level was used, a LOAEL of 2200 mg/kg bw/d was identified from this study.

Creasy et al. (1983)

In another similar study, groups of young prepubertal (3-4 weeks of age) male Sprague-Dawley rats were treated by oral gavage with DPP in corn oil at 0 or 2200 mg/kg bw (Creasy et al., 1983). Animals were treated with a single dose or with repeated doses for up to 4 days. Animals (3 per treatment group) receiving a single dose were sacrificed at 1, 3, 6, and 24 hr after dosing. Animals (3 per treatment group) receiving repeated doses were sacrificed 2, 3, and 4 days after the start of dosing. The testes were removed and examined histologically. Mitochondrial succinic dehydrogenase activity (a marker of cell injury) was also measured in the testes.

At 3 hr, in a proportion of the seminiferous tubules, the Sertoli cells 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. Early degenerative changes were also apparent in spermatocytes and spermatids and were accompanied by an acute interstitial inflammatory infiltrate. By 24 hr, germinal cell degeneration was excessive with desquamation and general disorganisation of cell layers within the epithelium. Mitochondrial succinic dehydrogenase activity in Sertoli cells was reduced a 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.

Two, three and four days of daily DPP treatment resulted in a gradual depletion of germinal cells from all tubules, leaving a Sertoli cell matrix containing a few necrotic spermatocytes and occasional normal spermatogonia.

Overall, this study showed that treatment of prepubertal male rats for up to 4 days with the high dose of 2200 mg/kg bw DPP caused severe testis injury. The study also showed that the Sertoli cells are the target of the DPP toxicity. As only one dose level was used, a LOAEL of 2200 mg/kg bw/d was identified from this study.

Foster et al. (1983)

Foster et al (1983) treated groups of young (4 weeks old) male Sprague-Dawley rats by oral gavage with DPP in corn oil at 0 or 2200 mg/kg bw for up to 4 days. Animals were sacrificed after single or multiple doses. At necropsy, liver and testes were excised and microsomal suspensions prepared to measure the cytochrome P-450 content and to perform enzyme assays for the activities of 3 steroidogenic enzymes: 17-α-hydroxylase, 17-20 lyase and 17-β- dehydrogenase.

Testicular atrophy was present in all treated animals even after a single dose. Treatment with DPP produced significant decreases in testicular cytochrome P-450 content, cytochrome P-450 dependent microsomal steroidogenic enzymes (17-α-hydroxylase, 17-20 lyase) and in the maximal binding of progesterone to testis microsomes. There was no effect of DPP on hepatic cytochrome P-450 content.

Overall, this study showed that treatment of young male rats for up to 4 days with the high dose of 2200 mg/kg bw DPP caused severe testis injury which was associated with significant decreases in the activity of steroidogenic enzymes involved in androgen production.

Gray and Gangolli (1986)

Gray and Gangolli (1986) conducted a series of experiments on DPP in rats. In one experiment, groups of young immature male Sprague – Dawley rats (4 – 5 weeks of age) and groups of mature male rats (15-week old) were given DPP (in corn oil) by gavage at 0 or 2200 mg/kg bw/d for up to 10 days. Tubular atrophy was evident in both the mature and immature rats, but was more severe and developed more quickly in the immature rats.

In a second experiment, groups of young immature male Sprague – Dawley rats (4 – 5 weeks of age) were given single doses of DPP (in corn oil) by gavage at 0, 220, 440 and 2200 mg/kg bw/ to measure the secretion from the testes of seminiferous tubule fluid and androgen-binding-protein (ABP), two specific markers of Sertoli cell function. At the top dose, such secretion was completely suppressed and the effect was still marked at 440 mg/kg bw, but was absent at 220 mg/kg bw. However, after 3 daily doses of 220 mg/kg bw DPP, one out of 5 rats was partially affected. Administration of single doses of DPP at 2200 mg/kg bw to mature male rats produced only a 60% reduction compared to controls in the secretion of seminiferous tubule fluid and ABP.

Overall, this study showed that DPP administered at the high dose of 2200 mg/kg bw for up to 10 days caused more severe testicular damage in immature rats compared to mature rats. This study also showed that DPP affected Sertoli cell function in immature rats from a dose of 220 mg/kg bw/ for 3 days. Therefore, a LOAEL of 220 mg/kg bw/d can be identified from this study for effects on the testes in immature rats.

Fertility study in mice

Heindel et al. (1989)
DPP was tested by continuous breeding in Swiss CD-1 mice by the NTP (Heindel et al., 1989). Based on the results of a dose-range-finding study, DPP was given to both male and female mice (20 pairs per treatment group, 40 pairs of control animals) in the diet at 0, 0.5, 1.25 or 2.5% (equivalent to 0, 760, 2160 and 4800 mg/kg bw/d). The animals were dosed for 7 days prior to and during a 14-week cohabitation period. At the end of the 14 weeks, the pairs were separated and housed one animal/cage with continued dosing. Any litters born after the continuous breeding phase were reared by the dams until weaning, after which treated feed was provided at the same concentrations.

During the 14-week continuous breeding phase, male mice of the mid- and high-dose groups had reduced body weight gain compared to controls. All control pairs had at least 1 litter (with an average of 4.8 litters/pair), while only 4 of 19 low dose pairs delivered 1 litter, and no middle or high dose pairs delivered any litter. Overall, DPP at 2160 and 4800 mg/kg bw/d caused complete infertility and at 760 mg/kg bw/d caused reduced fertility in mice. In the litters delivered at the low dose, the number of live pups/litter was reduced (by 90%; 1.1 at 760 mg/kg bw/d vs 11.1 in controls) compared to controls; there were insufficient live pups at this dose to calculate pup weights.

At the end of the continuous breeding phase, the control and high dose mice were cross-mated. The groups that contained either treated males or treated females gave birth to no live young, while 61% of cohabited control pairs bore live young. This finding indicates that the effect on fertility caused by DPP had both a strong male and female component.

The F0 control and high dose mice were necropsied after the cross-over mating trial. The treated high dose females weighed 9% less than their controls. The treated high dose males weighed 10% less than controls. At the high dose, absolute testis weight was decreased by 78% and there was no detectable epididymal sperm.

A second generation evaluation was not performed.

Overall, this study showed that DPP at a dose of 760 mg/kg bw/d and above produced impaired fertility, reduced litter size, decreased number of litters/pair and reduced number of live pups/litter in mice. Therefore, a LOAEL for fertility of 760 mg/kg bw/d can be identified from this study.

Developmental toxicity studies in rats

Liu et al. (2005)
In a mechanistic study aimed at identifying which testicular genes may be involved in the genesis of male reproductive abnormalities associated with some phthalates, groups of pregnant Sprague-Dawley rats were treated with DPP (in corn oil) by gavage at 0 or 500 mg/kg bw/d from gestational day (GD) 12 to GD 19 (Liu et al., 2005). On GD 19, dams were sacrificed and testes removed from the male foetuses to analyse gene expression. Ano-genital distance (AGD) was also measured in male foetuses.

AGD was significantly reduced (by 31%) in male foetuses compared to controls. Of the approximately 30000 genes queried, expression of 391 genes was significantly altered. Gene pathways disrupted included gene for cholesterol transport and steroidogenesis, as well as newly identified pathways involved in intracellular lipid and cholesterol homeostasis, insulin signalling, transcriptional regulation and oxidative stress. Additional gene targets included alpha inhibin, which is essential for normal Sertoli cell development and genes involved with communication between Sertoli cells and gonocytes.

Overall, this study showed that prenatal exposure of rats to DPP on GD 19 at 500 mg/kg bw/d altered the expression of a number of genes which may play a role in the molecular mechanisms of DPP-induced male reproductive tract abnormalities.

Howdeshell et al. (2008)
A study was conducted mainly to characterise the dose-response relationship of six phthalates, including DPP, on foetal testicular testosterone production in Sprague-Dawley rats during prenatal development (Howdeshell et al., 2008). This summary will focus mainly on the DPP investigations and findings. Groups (2 to 5) of pregnant Sprague-Dawley rats were treated by oral gavage with DPP from GD 8 to GD 18 at 0, 25, 33, 50, 100, 200, 300, 600 or 900 mg/kg bw/d. On GD 18, the dams were sacrificed and the foetuses removed. A number of maternal and reproductive parameters were examined. In addition, the testes were removed to measure testosterone

production/secretion. This was done incubating the testes (from 3 male foetuses per litter) *ex vivo* for 3 hours in an appropriate medium and then measuring testosterone levels in the medium using a radioimmunoassay.

Complete litter loss was observed at 300 mg/kg bw/d DPP and above. Maternal terminal body weight was significantly reduced (by 19%) at 900 mg/kg bw/d DPP. Maternal body weight gain was significantly decreased at 300 (by 72%) and 600 mg/kg bw/d DPP (by 87%). At 900 mg/kg bw/d DPP, there was actual body weight loss (-20 g vs +72 g in controls). Total resorptions leading to no live foetuses (100% foetal mortality) occurred at 300 mg/kg bw/d DPP and above. Foetal testicular testosterone production was significantly decreased from 100 mg/kg bw/d DPP (by 56%). At 200 mg/kg bw/d DPP, there was a 62% reduction. At higher doses of DPP, testosterone levels could not be examined because of the 100% foetal mortality observed at these higher doses. There were no effects on foetal testicular testosterone production at 50 mg/kg bw/d DPP.

Five other phthalates were also investigated in this study: benzylbutyl phthalate (BBP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), diisobutyl phthalate (DIBP) and diethyl phthalate (DEP). In relation to the key endpoint of testosterone production, BBP, DBP, DEHP and DIBP were equivalent (Effective dose causing 50% reduction -ED50 = 440 mg/kg bw/d), but DPP was about threefold more potent (ED50 = 130 mg/kg bw/d). DEP had no effects on testosterone production up to the highest dose tested of 900 mg/kg bw/d.

Overall, in this study, prenatal exposure of rats to DPP on GD 8-18 caused major fetotoxicity (complete litter loss) from a dose of 300 mg/kg bw/d. DPP also caused decreased foetal testicular testosterone production from a dose of 100 mg/kg bw/d. A developmental NOAEL of 50 mg/kg bw/d can be identified from this study for testosterone production. DPP was about threefold more potent in reducing testosterone levels compared to other medium-chain phthalates (BBP, DBP, DEHP and DIBP) following prenatal exposure from GD 8-18.

Benson (2009)

A review article derived oral chronic reference doses (RfD) according to the US EPA methodology for a number of phthalates (DPP, DBP, DIBP, BBP, DEHP, DINP –diisononyl phthalate) containing side chains of 4 to 10 carbon in the di-ortho positions, including DPP on the basis of their reproductive toxicity (Benson, 2009). The paper also derived relative potency factors for these phthalates. A factor of 1 was assigned to DEHP. Values for the other phthalates were calculated by dividing the RfD of DEHP expressed in mmol/kg bw/d by the RfD of the other phthalates also expressed in mmol/kg bw/d.

For DPP, the author noted that the toxicological data available at that time (2009) were extremely limited. The author referred to 3 publications (Foster et al., 1980; 1983 and Howdeshell et al., 2008). From these studies, the author selected the Howdeshell et al (2008) publication as it provided the most

sensitive effect (foetal testicular testosterone production) in the most sensitive life stage – the reproductive tract of the developing male foetus. As described above (where the Howdeshell (2008) paper is summarised), a NOAEL of 50 mg/kg bw/d was identified in this study with a LOAEL of 100 mg/kg bw/d. Rather than using the NOAEL, the author applied the benchmark methodology (US EPA BMS, version 1.4.1c) to derive a BMD_{1SD} (the dose at which the mean response in the treated group is one standard deviation lower than the mean response in controls) of 26 mg/kg bw/d and a $BMDL_{1SD}$ of 17 mg/kg bw/d. Dividing the $BMDL_{1SD}$ by a total uncertainty factor of 100, a RfD of 0.2 mg/kg bw/d (rounded from 0.17 mg/kg bw/d) was obtained. This was equivalent to 0.000548 mmol/kg bw/d using a molecular weight of 306 g/mol.

For the other phthalates considered in the review, a RfD of 0.3 mg/kg bw/d was derived for DBP; a RfD of 0.8 mg/kg bw/d was obtained for DIBP; a RfD of 1 mg/kg bw/d was calculated for BBP; a RfD of 0.3 mg/kg bw/d was derived for DEHP; and a RfD of 0.8 was estimated for DINP. The RfDs for these other phthalates were not necessarily based on reductions in foetal testicular testosterone production but on the most sensitive endpoint identified for each of these phthalates at the time (2009). The following relative potency factors were derived: 1.26 for DPP; 1.00 for DEHP; 0.64 for DBP; 0.39 for DINP; 0.24 for DIBP and 0.21 for BBP. These data indicate that DPP is more potent in its toxic action on the development of the male reproductive tract compared to DEHP, DBP, DINP, DIBP and BBP.

It is noted that the relative potency factors derived in this review are slightly different from those determined by Howdeshell et al. (2008), where following prenatal exposure during GD 8-18, DPP was about threefold more potent in reducing testosterone levels compared to BBP, DBP, DEHP and DIBP, all of which being of equivalent potency. These differences might be explained by variations in the endpoint chosen for the comparison, but also by inconsistencies in the dose descriptor (ED50, NOAEL or BMDL) selected for the comparison.

Hannas et al. (2011)

A study was conducted to establish a more comprehensive data set for DPP, focussing on dose-response and potency information for foetal and postnatal male reproductive endpoints (Hannas et al., 2011). A number of different experiments were conducted. In the first experiment, pregnant Sprague-Dawley rats (6/group) were given a single gavage dose of DPP at 0 or 500 mg/kg bw on GD 17 to measure foetal testicular testosterone production (from 3 males per litter). GD 17 was selected because GD 16.5 is the earliest foetal age at which a significant level of testosterone production can be detected. In a second experiment, pregnant Sprague-Dawley rats (3/group) were given single gavage doses of DPP at 0, 300, 600, 900 or 1200 mg/kg bw on GD 17. Five hr post-dosing, foetal testes were excised (3 males per litter) to measure foetal testicular testosterone production and to establish a dose-response relationship. In a third experiment, pregnant Sprague-Dawley rats (3/group) were given by gavage DPP at 0, 11, 33, 100 or 300 mg/kg bw/d on GD 14-18. On GD 18, dams were sacrificed and foetal testes removed. One testis from three males per litter was used to measure testosterone production while the

remaining testes within the litter were homogenised for gene expression analysis. The following three genes involved in steroidogenesis and male reproductive development were examined: insl3, StAR and Cyp11a. In a fourth experiment, pregnant Sprague-Dawley rats (5/group) were gavaged daily on GD 8-18 with 0, 11, 33, 100 or 300 mg/kg bw/d DPP. Dams were allowed to deliver the pups naturally. Male offspring were weighed and AGD appropriately measured on post-natal day (PND) 2. On PND 13, male offspring were appropriately assessed for areola or nipple retention. Malformations of the reproductive tract were not investigated. Male offspring were weaned on PND 24 and housed in groups of 2 to 3 per cage. Following weaning, dams were sacrificed and the number of uterine implantation sites recorded in order to determine post-implantation loss.

In the first experiment, there was no overt maternal toxicity after a single dose of 500 mg/kg bw DPP on GD 17. Foetal testicular testosterone production was significantly reduced (by 57%) at this dose 5 hr after dosing. In the second experiment, even the high single dose of 1200 mg/kg bw on GD 17 did not cause any maternal toxicity or foetal toxicity 5 hr after dosing. However, a dose-related decrease in foetal testicular testosterone production was observed from a dose of 100 mg/kg bw (this was statistically significantly different from controls at 300 mg/kg bw and above). In the third experiment, repeated administration of DPP over the period of sexual differentiation (GD 14-18) did not cause any maternal toxicity up to the highest tested dose of 300 mg/kg bw/d. Foetal testicular testosterone production was reduced in a dose-dependent manner from a dose of 33 mg/kg bw/d. There were no effects on testosterone production at the lower dose of 11 mg/kg bw/d after 5-day dosing during GD 14-18. Comparison of these data with equivalent data generated for DEHP indicated that DPP is eightfold more potent than DEHP in reducing foetal testosterone production following prenatal exposure during GD 14-18, the critical window of sexual differentiation. It is noted that this result differs from that obtained by Howdeshell et al. (2008), where following prenatal exposure during GD 8-18, DPP was about threefold more potent in reducing testosterone levels compared to DEHP. The difference could be due to the different window of prenatal exposure employed in the two studies (GD 14-18 in Hannas et al., 2011 and GD 8-18 in Howdeshell et al., 2008). It would appear that prenatal exposure during GD 14-18 is more critical in reducing foetal testicular testosterone levels than prenatal exposure during GD 8-18.

Gene expression of insl3, StAR and Cyp11a in the testes was also significantly reduced from a dose of 100 mg/kg bw/d after 5-day dosing during GD 14-18, indicating that testosterone production was a more sensitive effect of DPP compared to the expression of three testis genes (insl3, StAR and Cyp11a).

In the fourth experiment, repeated administration of DPP during GD 8-18 caused a slight reduction (by 12.5%) in maternal body weight gain at the top dose of 300 mg/kg bw/d. However, at this dose there was a significant increase (51% vs 5% in controls) in foetal/pup mortality and a significant decrease in the mean number of live pups on PND 2 (6.6 v 13 in controls).

AGD was significantly reduced in male offspring on PND 2 from a dose of 100 mg/kg bw/d (by 15% at 100 mg/kg bw/d and by 30% at 300 mg/kg bw/d) and nipple retention on PND13 was observed at the top dose of 300 mg/kg bw/d. No effect on AGD was observed at 33 mg/kg bw/d. No other foetal or offspring parameters were investigated in this experiment. Comparison of these data with equivalent investigations generated for DEHP showed that DPP is approximately twofold more potent than DEHP in reducing AGD and 4.5-fold more potent than DEHP in inducing male nipple retention.

Overall, this study showed that prenatal exposure of rats to DPP caused abnormalities of the developing male reproductive system. The most sensitive effect was reduction of testicular testosterone production (from 33 mg/kg bw/d for 5-day exposure during GD 14-18) followed by testis gene expression (from 100 mg/kg bw/d for 5-day exposure during GD 14-18), reduction in AGD (from 100 mg/kg bw/d for 10-day exposure during GD 8-18) and at higher dose levels, induction of male nipple retention (at 300 mg/kg bw/d for 10-day exposure during GD 8-18). An overall NOAEL of 11 mg/kg bw/d (over 5-day exposure during GD 14-18) can be identified from this study on the basis of decreases in foetal testicular testosterone production. This study also showed that DPP is more potent than DEHP in causing disruption of male reproductive development.

Hannas et al. (2012)

In a similar study performed by the same authors (Hannas et al., 2012), pregnant Sprague-Dawley rats (3-4/group) were given by gavage DPP at 0, 11, 33, 100 or 300 mg/kg bw/d on GD 14-18. On GD 18, dams were sacrificed and foetal testes removed. One testis from three males per litter was used to measure ex vivo testosterone production while the remaining testes within the litter were homogenised for gene expression analysis. 89 candidate target genes involved in sexual determination and differentiation, steroidogenesis, gubernaculum development and androgen signalling pathways were examined.

Foetal testicular testosterone production was reduced in a dose-dependent manner from a dose of 33 mg/kg bw/d. Testis gene expression levels of Cyp11b1, Scarb1, Star, Cyp11a1, Cyp17a1, Insl3 and Hsd3b were consistently reduced in a dose-dependent manner. Some levels were reduced from a dose of 33 mg/kg bw/d; some from a dose of 100 mg/kg bw/d and some at the top dose of 300 mg/kg bw/d. Star, Cyp11a1, Hsd3b and Scarb1 were down-regulated from the lowest dose of 11 mg/kg bw/d. Genes in the PPARα pathway were not induced by DPP exposure, suggesting that this pathway is not involved in DPP-induced foetal testis toxicity.

Overall, this study showed that prenatal exposure of rats to DPP during GD 14-18 caused reductions in foetal testicular testosterone production and down-regulation of a number of testis genes involved in male reproductive development. Although some genes were down-regulated from a dose of 11 mg/kg bw/d, this down-regulation is not considered to be adverse because at this dose level the reduced expression of these genes did not lead to any biochemical, functional or morphological adverse changes. Hence, it is more

appropriate to set the study NOAEL on the reduction of testosterone production. Overall, therefore, a NOAEL of 11 mg/kg bw/d can be established from this study on the basis of decreases in foetal testicular testosterone production at the next dose level of 33 mg/kg bw/d.

Beverley et al. (2014)
In a more recent study (Beverley et al., 2014), pregnant Sprague-Dawley rats (3-4/group) were given by gavage DPP (in corn oil) at 0 or 50 mg/kg bw/d on GD 14-18. On GD 18, dams were sacrificed and foetal testes removed. One testis from three males per litter was used to measure testosterone production *ex vivo* while the remaining testes within the litter were homogenised for gene expression analysis. Genes investigated were those involved in cholesterol transport and synthesis, sex differentiation and steroidogenesis.

DPP at 50 mg/kg bw/d did not affect maternal weight gain or foetal survival. At this dose DPP produced a 37% decrease in foetal testicular testosterone production. It also significantly down-regulated the following testis genes: Cyp11a1, NrOb1, Star, Cyp11b2, Hsd3b, Cyp17a1, Scarb1, Insl3, Dhcr7, Cyp11b1 and Inha (genes involved in cholesterol transport and synthesis, sex differentiation and steroidogenesis).

Overall, this study showed that prenatal exposure of rats to 50 mg/kg bw/d DPP during GD 14-18 caused reductions in foetal testosterone production and downregulation of a number of testis genes involved in cholesterol transport and synthesis, sex differentiation and steroidogenesis. As only one dose level was employed, a LOAEL of 50 mg/kg bw/d can be identified from this study based on decreased testosterone production and down-regulation of key testis genes involved in cholesterol transport and synthesis, sex differentiation and steroidogenesis.

US CHAP on phthalates (2014)
In a report on the hazards of phthalates and phthalate alternatives produced for the US Consumer Product Safety Commission (US CHAP on phthalate, 2014), the reproductive hazard characterisation of DPP was briefly presented. A NOAEL of 11 mg/kg bw/d was proposed based on reductions in foetal testosterone production at the next dose level of 33 mg/kg bw/d following prenatal exposure during GD 14-18 from the Hannas et al (2011) study.

Gray et al. (2016)
In a comprehensive recent study (Gray et al., 2016) conducted by the same group as in Hannas et al., 2011; 2012, various aspects of the toxicity of DPP to male reproductive development were investigated. In a prenatal exposure experiment, pregnant Sprague-Dawley rats (11-16/group) were given by gavage DPP (in corn oil) at 0, 11, 33, 100 or 300 mg/kg bw/d on GD 14-18. On GD 18, the dams were sacrificed and the foetuses removed. Foetal blood was collected and testes removed. The foetal blood (from 7-9 litters/dose) was used to measure levels of testosterone in plasma. One testis (from 3 males per litter) was used to measure ex vivo testosterone production. Testes from other males (up to 3 per litter) were used to determine testis weight (5-6 litters/dose group) and to measure extracted testicular testosterone levels (7-8

litters/dose group). In addition, remaining testes were homogenised and used for gene expression analysis.

In a postnatal experimental design, pregnant Sprague-Dawley rats (5/group) were given by gavage DPP (in corn oil) at 0, 11, 33, 100 or 300 mg/kg bw/d on GD 8-18. Dams were allowed to deliver the pups. Male and female offspring were weaned on PND 24 and housed in groups of 2-3 per cage by sex until necropsy. Male offspring were checked daily from 37 to 55 days of age to determine the age and weight at puberty (preputial separation) and both male and female offspring were necropsied at 120 days of age. F1 females were examined for gross malformations. In addition, the weight of pituitary, uterus, ovaries, kidney and liver were recorded (from 2 females per litter). In F1 males, the weight of the reproductive organs, liver and kidney were recorded. Males were also checked for gross malformations and for any gubernacular cord (organ involved in the descent of the testes from the renal area to the inguinal area) abnormalities. Testis and epididymal tissues were also examined histologically.

In the prenatal foetal study, DPP administered during GD 14-18 did not induce any signs of overt maternal toxicity up to the top dose of 300 mg/kg bw/d, at which there was only a slight reduction in maternal body weight gain. In addition, the total number of foetuses, the number of resorbed foetuses or the number of dead foetuses was also not affected up to the top dose. However, at necropsy, the descent of the testes from the renal area to the inguinal region was significantly delayed in males from most litters and testis weight was significantly reduced at the highest dose. Ex vivo testicular testosterone production was significantly reduced in a dose-dependent manner from 33 mg/kg bw/d. Testosterone extracted from the testis of GD 18 male foetuses was also significantly decreased in a dose-dependent manner from 33 mg/kg bw/d, as it was foetal plasma testosterone. Down-regulation of a number of testis genes involved in sex differentiation and steroidogenesis was also observed in a dose-dependent manner generally from 33 mg/kg bw/d; however, some genes were affected from the lowest dose of 11 mg/kg bw/d.

In the postnatal study, DPP administered during GD 8-18 did not induce any signs of overt maternal toxicity up to the top dose of 300 mg/kg bw/d. However, at this dose there was a significant increase in foetal/pup mortality and a significant decrease in the mean number of live pups on PND 2. In the high dose group, preputial separation (PPS) was delayed. Ten out of 13 males had not attained full PPS by 55 days of age, and in 8 out of 10 males, PPS had not begun at all by 55 days of age. These results were due to the fact that these animals had hypospadias rather than a true delay in male puberty. Male necropsy data collected at 6-7 months of age showed a number of abnormalities (agenesis of vas deferens, small ventral prostrate, agenesis or abnormal seminal vescicle, testis atrophy, agenesis of epididymis, agenesis of gubernaculum, undescended testes) in the reproductive and urogenital tract of male offspring. The majority of these malformations occurred at 100 and 300 mg/kg bw/d, with the exception of mild testis atrophy which was observed from 33 mg/kg bw/d. Female rat necropsy data collected at 3-4 months of age showed that at the top dose of 300 mg/kg bw/d 3 out of

10 females displayed malocclusions and skull malformations, with 1 out of 10 females displaying reproductive tract malformations (e.g. uterus unicornis).

Overall, this study showed that prenatal exposure of rats to DPP during GD 14-18 caused reductions in foetal testicular testosterone production, testosterone extracted from the testes and foetal plasma testosterone levels from a dose of 33 mg/kg bw/d. DPP also caused down-regulation of a number of testis genes involved in steroidogenesis and sex differentiation mainly from 33 mg/kg bw/d, but, for some genes, even from the lowest dose of 11 mg/kg bw/d. In addition, at the top dose of 300 mg/kg bw/d, descent of the testes in the inguinal area was significantly delayed in males from most litters and testis weight was reduced.

Prenatal exposure of rats to DPP during GD 8-18 caused foetal/pup mortality on PND 2 at the top dose of 300 mg/kg bw/d. Delayed PPS was observed in most males at the age of 55 days at the highest dose. This was mainly due to the presence of hypospadias. In addition, a number of abnormalities in the reproductive and urogenital tract of male offspring were seen at 6-7 months of age from 100 mg/kg bw/d. However, testis atrophy was present from 33 mg/kg bw/d. Based on these findings, a NOAEL of 11 mg/kg bw/d can be identified from this study. Although some genes were down-regulated from a dose of 11 mg/kg bw/d, this down-regulation is not considered to be adverse because at this dose level the reduced expression of these genes did not lead to any biochemical, functional or morphological adverse changes.

Table 10: Reproductive toxicity studies with DPP

Endpoint	Study description	Repro findings	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Reliability	Reference
Studies investigating male reproductive organs and fertility						
Male reproductive organs	S-D rats (12 males/group); Gavage Exposure for 4 days to 1800 mg/kg bw/d;	Severe testis atrophy	« 1800	1800	Relatively old study but of reasonable quality	Foster et al., 1980
Male reproductive organs	S-D rats; Gavage Exposure for up to 4 days to 2200 mg/kg bw/d; Also ex vivo preparations of seminiferous tubules treated with monoester of DPP (30 – 100 µM)	Severe testis atrophy Same effects in ex vivo preparations	« 2200	2200	Relatively old study but of reasonable quality	Gangolli, 1982
Male reproductive organs in prepubertal rats	S-D rats; Gavage Exposure for up to 4 days to 2200 mg/kg bw/d;	Severe testis injury even following a single dose Sertoli cells appear to be the target of DPP toxicity	« 2200	2200	Relatively old study but of reasonable quality	Creasy et al., 1983
Male reproductive organs in young rats	S-D rats; Gavage Exposure for up to 4 days to 2200 mg/kg	Severe testis injury even following a single dose	« 2200	2200	Relatively old study but of reasonable quality	Foster et al., 1983

	bw/d; Measurements of activity of steroidogenesis enzymes in testicular microsomes	Decrease in steroidogenesis enzymes involved in androgen production in testes				
Male reproductive organs in immature and mature rats	S-D rats Gavage Exposure to 0, 220, 440 and 2200 mg/kg bw for up to 4 days in immature rats	Testicular damage was more severe in immature rats than mature rats at 2200 mg/kg bw for up to 10 days Sertoli cell function was affected in immature rats from the lowest dose of 220 mg/kg bw for 3 days	< 220	220	Relatively old study but of reasonable quality	Gray and Gangolli, 1986
Fertility	Swiss CD-1 mice; Diet 1-generation continuous breeding; Exposure to 760, 2160 and 4800 mg/kg bw/d	↓fertility at low dose; Complete infertility at the mid and high dose; ↓litter size from lowest dose; ↓no litters/pair from lowest dose; ↓no of live pups/litter from lowest dose	< 760	760	NTP study of reasonable quality	Heindel et al., 1989
Studies involving in utero exposure						
Mechanistic study	S-D rats	↓AGD in male	Not applicable –	Not applicable –	Good quality study	Liu et al., 2005

investigating gene expression in foetal testis	Gavage Exposure of pregnant females during GD 12-19 to 500 mg/kg bw/d Foetal livestigations performed at the end of gestation	foetuses; Alteration in expression of a number of genes in foetal testis	mechanistic study	mechanistic study		
Development and foetal testicular testosterone production	S-D rats Gavage Exposure of pregnant females during GD 8-18 to 25, 33, 50, 100, 200, 300, 600 and 900 mg/kg bw/d; Foetal investigations performed at the end of gestation	Fetotoxicity including lethality from 300 mg/kg bw/d; ↓foetal testicular testosterone levels from 100 mg/kg bw/d; DPP threefold more potent than BBP, DBP, DEHP and DIBP	50	100	Good quality study	Howdeshell et al., 2008
Review article deriving oral chronic RfD for a number of phthalates including DPP and relative potency factors	The Howdeshell et al (2008) paper was selected as the most critical study	A $BMDL_{1SD}$ of 0.17 mg/kg bw/d was estimated and used to derive a RfD of 0.2 mg/kg bw/d by applying an uncertainty factor of 100. DPP was more potent than DEHP, DBP, DINP, DIBP, BBP	Not applicable	Not applicable	Good quality review	Benson, 2009
Prenatal and postnatal development	**S-D rats Gavage Experiment 1:**	**↓foetal testosterone from 33 mg/kg bw/d;**	**11**	**33**	**Good quality study**	**Hannas et al., 2011**

	single dose of 500 mg/kg bw on GD 17; Experiment 2: single dose of 300, 600, 900 and 1200 mg/kg bw on GD 17; Experiment 3: repeated 5-d exposure to 11, 33, 100 and 300 mg/kg bw/d on GD 14-18; Experiment 4: repeated 10-d exposure to 11, 33, 100 and 300 mg/kg bw/d on GD 8-18; Investigations performed at the end of gestation and post-natally	**↓ expression of 3 specific testis genes from 100 mg/kg bw/d; ↓AGD on PND2 from 100 mg/kg bw/d; ↑nipple retention on PND 13 at 300 mg/kg bw/d**				
Prenatal development looking at gene expression and testosterone production	**S-D rats Gavage Repeated 5-d exposure to 11, 33, 100 and 300 mg/kg bw/d on GD 14-18; Foetal investigations performed at the end of gestation;**	**↓testosterone levels from 33 mg/kg bw/d; ↓ of some gene expression levels even from 11 mg/kg bw/d but of uncertain toxicological significance**	**11**	**33**	**Good quality study; mainly mechanistic**	**Hannas et al., 2012**
Prenatal development looking at gene	S-D rats Gavage Repeated 5-d	↓testosterone levels; Down-regulation of genes involved in	< 50	50	Good quality study; mainly mechanistic	Beverly et al., 2014

expression and testosterone production	exposure to 0 or 50 mg/kg bw/d on GD 14-18; Foetal investigations performed at the end of gestation;	cholesterol transport and synthesis, sex differentiation and steroidogenesis				
Review of the reproductive hazards of a number of phthalates including DPP to derive point of departure	The Hannas et al., (2011) study was selected as the critical study.	A NOAEL of 11 mg/kg bw/d was proposed based on reductions in foetal testosterone production at the next dose level of 33 mg/kg bw/d following prenatal exposure during GD 14-18	Not applicable	Not applicable	Good quality review, although rather brief.	US CHAP on phthalates, 2014
Prenatal and postnatal development	**S-D rats Gavage Repeated 5-d exposure to 11, 33, 100 and 300 mg/kg bw/d on GD 14-18; Repeated 10-d exposure to 11, 33, 100 and 300 mg/kg bw/d on GD 8-18; Investigations performed at the end of gestation and post-natally;**	**↓testosterone production, testosterone levels extracted from testes and foetal plasma testosterone levels from 33 mg/kg bw/d; Down-regulation of genes involved in, sex differentiation and steroidogenesis even from 11 mg/kg bw/d; Delayed descent of**	**11**	**33**	**Good quality study**	**Gray et al., 2016**

		testes and ↓testis weight at 300 mg/kg bw/d; Foetal/pup mortality on PND 2 at 300 mg/kg bw/d; Delayed PPS at 55 days of age due to hypospadias at 300 mg/kg bw/d; Abnormalities of a number of male reproductive organs at 6-7 months of age from 100 mg/kg bw/d; Testis atrophy from 33 mg/kg bw/d				

Summary of the reproductive toxicity of DPP and selection of starting points for DNEL derivation

The reproductive toxicity of DPP has been investigated in a rather limited number of published studies. These studies were all conducted by oral gavage in the Sprague-Dawley rat, with the exception of a fertility study in mice performed by dietary administration. Some of the studies included mechanistic investigations and some studies compared the relative potency of DPP with that of other phthalates.

A number of relatively old studies in adult rats treated for up to 4 days showed that DPP at relatively high doses (1800 – 2200 mg/kg bw/d) caused testis atrophy. In one study (Foster et al., 1983), this was associated with decreases in the activity of steroidogenic enzymes involved in androgen production. In another study (Creasy et al., 1983), it was evident that the Sertoli cells were the target of the testicular toxicity of DPP. Testis atrophy was more severe and developed more quickly in immature, prepubertal rats compared to mature, adult rats. In immature rats, testis effects were already seen from 220 mg/kg bw/d for 10 days, the lowest dose tested compared to a dose of 1800 mg/kg bw/d (the lowest dose tested) for up to 4 days in adult rats.

In a fertility study by continuous breeding in mice (Heindel et al., 1989), DPP caused complete infertility at the high doses of 2160 and 4800 mg/kg bw/d and reduced fertility at 760 mg/kg bw/d, the lowest dose tested. No sperm was detected in the epididymis of the top dose males. Cross-mating experiments showed that fertility was impaired in both sexes.

More recent studies targeted the prenatal window of exposure and employed lower doses of DPP. In several of these developmental studies with exposure to DPP during GD 14-18 (period of sexual differentiation), reduced foetal testicular testosterone levels were measured from a dose of 33 mg/kg bw/d upwards and down-regulation of numerous testis genes involved in steroidogenesis and sexual differentiation was evident even from the lowest dose tested of 11 mg/kg bw/d. Although some genes were down-regulated from a dose of 11 mg/kg bw/d, this down-regulation is not considered to be adverse because at this dose level the reduced expression of these genes did not lead to any biochemical, functional or morphological adverse changes. Descent of the testes in the inguinal area was delayed at the top dose of 300 mg/kg bw/d. No other foetal parameters were measured in these prenatal developmental studies. No overt maternal toxicity was observed up to the top dose of 300 mg/kg bw/d.

Further developmental studies are available. In these studies, prenatal exposure to DPP was longer (during GD 8-18) and the dams were allowed to deliver the pups, such that postnatal assessments could be performed. In these studies, in male offspring, AGD was reduced on PND 2 from 100 mg/kg bw/d and nipple retention was observed on PND 13 at 300 mg/kg bw/d. At this dose, PPS was delayed. The delayed PPS was the consequence of hypospadias. There were also a number of other abnormalities of the reproductive and urogenital tract from 100 mg/kg bw/d and mild testis atrophy

from 33 mg/kg bw/d. The top dose of 300 mg/kg bw/d also caused a significant increase in foetal and pup mortality in the absence of overt maternal toxicity. There were no effects at 11 mg/kg bw/d, the lowest dose tested.

Overall, from these data, an overall **NOAEL of 11 mg/kg bw/d** (Hannas et al., 2011; 2012; Gray et al., 2016) can be identified in relation to the development of the male reproductive tract. This NOAEL is mainly based on reductions in foetal testicular testosterone levels, down-regulation of testis genes involved in steroidogenesis, sexual differentiation and male reproductive development, and testis atrophy in offspring at the next dose level of 33 mg/kg bw/d. This is the most sensitive and robust NOAEL for the derivation of the DNEL for developmental toxicity. It is noted that this proposed overall NOAEL of 11 mg/kg bw/d is consistent with that recommended for DPP by the review undertaken in the US CHAP on phthalates (2014) report.

In relation to fertility and effects on the testes in mature animals, no NOAEL values have been identified, as very high doses were used in these experiments. Therefore, no DNEL for fertility can be derived. However, it is noted that the NOAEL for developmental toxicity is so much lower than the LOAEL values for fertility (220, 760, 1800, and 2200 mg/kg bw/d) that the developmental toxicity DNEL derived from such NOAEL (11 mg/kg bw/d) will clearly be the leading DNEL driving the risk characterisation.

In some of the studies investigating DPP, other phthalates were also studied in order to determine their relative potency. These publications show that on the basis of the studies and investigations available at the time, DPP appeared to be more potent in causing reproductive toxicity than other medium-chain (4-10 carbons) phthalates, including DEHP, DBP, DINP, DIBP and BBP. Depending on the dose descriptor, the endpoint and the window of prenatal exposure selected for the comparison, DPP appeared to be 1.26 up to 8-fold more potent than DEHP. A review article (Benson, 2009) established oral RfDs on the basis of reproductive toxicity of 0.2 and 0.3 mg/kg bw/d for DPP and DEHP, respectively. These comparisons on relative potency should be taken with caution because relative potency is significantly influenced by the size of the database, the quality and design of the available studies and the sensitivity of the parameters investigated.

In summary, it has been clearly established that DPP expresses reproductive toxicity in rats and mice – effects on fertility in both sexes and development in males only. In this respect, DPP is one of a number of medium-chain phthalate diesters that are recognised as reproductive toxicants. Their general mode-of-action (MoA) has been well studied. It involves down-regulation of foetal testicular genes involved in steroidogenesis and sexual differentiation, leading to reduced production of foetal testosterone during development, which eventually leads to demasculinisation, characterised by testis atrophy, infertility, reduced AGD, nipple retention, hypospadias and numerous other abnormalities of the reproductive and urogenital tract. All of these key events can occur in humans. Therefore, the relevance of this MoA for the

reproductive toxicity of several medium-chain phthalate diesters in humans cannot be excluded.

5. DERIVATION OF INDICATIVE DNELs for DPP

Absorption values

For the derivation of DNELs for the oral, inhalation and dermal routes and the application of route-to-route extrapolation, route-specific absorption values for inhalation, oral and dermal exposure are required. There is no kinetic information on either DPP or DIPP. The contractor has therefore selected absorption values from a much more well-studied low molecular weight phthalate, DBP. In the DBP document on establishing DNELs for applications for authorisation (RAC/24/2013/09_rev2), oral and inhalation absorption values of 100% and a dermal absorption value of 10% were selected.

Oral: 100%
Inhalation: 100%
Dermal: 10%

DNELs will be derived for developmental toxicity (from the oral NOAEL of 11 mg/kg bw/d) for workers and the general public in accordance with the ECHA guidance on chemical safety assessment , chapter R8 (ECHA, 2010).

Workers

For workers, only inhalation and dermal DNELs will be derived.

Inhalation

Modification of the starting point

For development toxicity, the starting point is an oral NOAEL of 11 mg/kg bw/d in rats exposed during gestation (GD 8-18 or 14-18).

The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 100% oral absorption and 100% inhalation absorption, the equivalent inhalation NAEL expressed on a body weight basis would be:

11 x 100%/100% =*11 mg/kg bw/d*

Taking into account a rat ventilation rate (at rest) for 8 h of 0.38 m^3/kg bw, the following 8h-inhalation rat NAEC value would be calculated:

11/0.38 = *29 mg/m^3/d*

In accordance with the ECHA (2010) guidance, an adjustment for the higher ventilation rate (x 0.67) of a worker under light activity (compared to the

experimental rat at rest) is required resulting in the following corrected inhalation 8h-NAEC:

29 x 0.67 = *19.4 mg/m^3/d (corrected inhalation 8h-NAEC)*

Given that the critical exposure window of susceptibility for the observed developmental effects in the experimental animals was 5 days (GD 14-18) there is no need to adjust for exposure duration as workers are also assumed to be exposed for 5 days/week.

Application of default assessment factors (AFs)

For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 5 for workers will be applied. The resulting inhalation (8-hr) DNEL for workers is shown below.

There is no need to apply an AF for severity of the effect (NOAEL based on reductions in foetal testosterone levels and mild foetal testis atrophy at the next dose level of 33 mg/kg bw/d) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{\text{(worker, inhalation, development)}}$: $\frac{19.4 \text{ mg/m}^3\text{/d}}{2.5 \times 5}$ = **1.6 mg/m^3/d**

Dermal

Modification of the starting point

For development toxicity, the starting point is an oral NOAEL of 11 mg/kg bw/d in rats exposed during gestation (GD 8-18 or 14-18).

The first modification step for the derivation of the dermal DNEL is route-to-route extrapolation from oral to dermal by taking into account 100% oral absorption and 10% dermal absorption. This would results in the following equivalent dermal NAEL:

11 x 100%/10% = *110 mg/kg bw/d*

Given that the critical exposure window of susceptibility for the observed developmental effects in the experimental animals was 5 days (GD 14-18) there is no need to adjust for exposure duration as workers are also assumed to be exposed for 5 days/week.

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 5 for workers will be applied. The resulting dermal DNEL for workers is shown below.

There is no need to apply an AF for severity of the effect (NOAEL based on reductions in foetal testosterone levels and mild foetal testis atrophy at the next dose level of 33 mg/kg bw/d) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{(worker, derm, development)}$: $\frac{110 \text{ mg/kg bw/d}}{4 \times 2.5 \times 5}$ = **2.2 mg/kg bw/d**

The Table below summarises the inhalation and dermal DNELs for workers in relation to DPP-induced developmental toxicity.

Table 11: Inhalation and dermal DNELs for workers for development toxicity for DPP

Endpoint	Inhalation (8-hr)	Dermal
Developmental toxicity	**1.6 mg/m^3/d**	**2.2 mg/kg bw/d**

General public

For the general public, inhalation, dermal and oral DNELs will be derived.

Inhalation

Modification of the starting point

For development toxicity, the starting point is an oral NOAEL of 11 mg/kg bw/d in rats exposed during gestation (GD 8-18 or 14-18).

The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 100% oral absorption and 100% inhalation absorption, the equivalent inhalation NAEL expressed on a body weight basis would be:

11 x 100%/100% = *11 mg/kg bw/d*

Taking into account a rat ventilation rate for 24 h of 1.15 m^3/kg bw, the following 24h-inhalation rat NAEC value would be calculated:

11/1.15 = *10 mg/m^3/d (corrected inhalation 24-hr NAEC)*

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting inhalation (24-hr) DNEL for the general public is shown below.

There is no need to apply an AF for severity of the effect (NOAEL based on reductions in foetal testosterone levels and mild foetal testis atrophy at the next dose level of 33 mg/kg bw/d) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

$DNEL_{(public, inhalation, development)}$: $\frac{10\ mg/m^3/d}{2.5 \times 10}$ = **0.4 mg/m³/d**

Dermal

Modification of the starting point

For development toxicity, the starting point is an oral NOAEL of 11 mg/kg bw/d in rats exposed during gestation (GD 8-18 or 14-18).

The first and only modification step for the derivation of the dermal DNEL is route-to-route extrapolation from oral to dermal by taking into account 100% oral absorption and 10% dermal absorption. This would results in the following equivalent dermal NAEL:

11 x 100%/10% = *110 mg/kg bw/d*

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting dermal DNEL for the general public is shown below.

There is no need to apply an AF for severity of the effect (NOAEL based on reductions in foetal testosterone levels and mild foetal testis atrophy at the next dose level of 33 mg/kg bw/d) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{(public, dermal, development)}$: $\frac{110 \text{ mg/kg bw/d}}{4 \times 2.5 \times 10}$ = **1.1 mg/kg bw/d**

Oral

Modification of the starting point

For development toxicity, the starting point is an oral NOAEL of 11 mg/kg bw/d in rats exposed during gestation (GD 8-18 or 14-18).

Modification of the starting point to derive the oral DNEL is not necessary as the starting point is an oral dose.

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting oral DNEL for the general public is shown below.

There is no need to apply an AF for severity of the effect (NOAEL based on reductions in foetal testosterone levels and mild foetal testis atrophy at the next dose level of 33 mg/kg bw/d) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{(public, oral, development)}$: $\frac{11 \text{ mg/kg bw/d}}{4 \times 2.5 \times 10}$ = **0.1 mg/kg bw/d**

The Table below summarises the inhalation, dermal and oral DNELs for the general public in relation to DPP-induced developmental toxicity toxicity.

Table 12: Inhalation, dermal and oral DNELs for the general public for developmental toxicity for DPP

Endpoint	Inhalation (24-hr)	Dermal	Oral
Developmental toxicity	**0.4 mg/m^3/d**	**1.1 mg/kg bw/d**	**0.1 mg/kg bw/d**

6. REPRODUCTIVE TOXICITY DATA AND DERIVATION OF ORAL DNELs FOR DIBP AND DBP

Information on the reproductive toxicity of DIBP and DBP and their DNELs is extracted from the draft Annex XV Restriction Proposal for DEHP, BBP, DBP and DIBP (ECHA, 2016).

DIBP and DBP all share a common anti-androgenic mode of action. They inhibit foetal testosterone production; reduce male anogenital distance;

decrease gene expression related to steroid biosynthesis; increase nipple retention in male offspring; increase the incidence of genital malformations (hypospadias and cryptorchidism); induce delayed puberty onset; reduce semen quality; and induce testicular changes including testicular atrophy in rats. In addition, DBP induces changes in germ cell differentiation (multinucleated germ cells) and persistent mammary gland histopathological changes in males, which are considered to be independent of foetal testosterone reduction (Borch et al. 2006, Gaido et al. 2007; Lambrot et al. 2009).

The critical N(L)OAELs for DIBP and DBP selected for DNEL derivation are based on developmental effects on male reproduction.

DBP (from ECHA, 2016)

The key study for selection of the critical N(L)OAEL for DBP is Lee et al. (2004). This study found reduced spermatocyte development in prepubertal rats and mammary gland changes in adult male rats perinatally (GD 15 to PND 21) exposed to 2 mg DBP/kg bw/d and above via the diet. No NOAEL was determined. In this study, anogenital distance was reduced and nipple retention was increased in males at 1000 mg DBP/kg bw/d with a NOAEL of 200 mg/kg bw/d.

The EU RAR (2004) on DBP from 2003 used an overall LOAEL of 52 mg/kg bw/d for embryotoxicity based on a study by Wine et al. (1997). In 2005, EFSA recommended a Tolerable Daily Intake (TDI) of 0.01 mg/kg bw/d for DBP based on delayed germ cell development and male mammary gland changes in the study by Lee et al. (2004).

The reduced spermatocyte development observed in the study by Lee et al. (2004) was statistically significant at PND 21 in the lowest dose group and severity was increasing with dose. Impairment of spermatocyte development persisted to adulthood in the higher dose groups only (see study details below). These changes can be related to an anti-androgenic effect of DBP, and although the low-dose effects appear to be reversible, they are a clear sign of developmental influences on testicular development already at these low doses, and are therefore considered relevant for NOAEL determination. It is also reasonable to regard the observed mammary gland effects as anti-androgenic.

As the observed effects of DBP on mammary gland and testes are considered anti-androgenic, and as EFSA has chosen to change the TDI in favour of the study by Lee et al., the **LOAEL of 2 mg/kg bw/d** is proposed for the derivation of the DNEL.

Description of key studies

In a recent developmental toxicity study (Lee et al., 2004) with exposure during the period from late gestation (Gestational day 15) to the end of lactation on postnatal day 21 (PND 21), maternal rats were given DBP at

dietary concentrations of 0, 20, 200, 2000 and 10000 mg/kg corresponding to doses of 0, 2, 20, 200 and 1000 mg/kg bw/d. Major results of this study are summarised below. At PND 2, anogenital distance was significantly reduced in 1000 mg/kg bw/d male offspring. At PND 14, the incidence of retained nipples/areolae was increased in all treated male offspring compared with controls but the increase was only significant at 1000 mg/kg bw/d. At PND 21, in males, reduction of spermatocyte development as manifested by a decreased number of spermatocytes was observed from 2 mg/kg bw/d with dose-dependent increased incidence or/and severity. A significant increase in scattered foci of aggregated Leydig cells was observed at 200 mg/kg bw/d and 1000 mg/kg bw/d.

In the epididymis, significantly decreased ductular cross sections, indicating reduced coiling, were observed at 200 and 1000 mg/kg bw/d. In the mammary glands, dilatation of alveolar buds and/or ducts was seen in male offspring from 2 mg/kg bw/d with low incidence and not achieving statistical significance in any group. In female offspring, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 2 mg/kg bw/d with a statistically significant increase at 2, 20, 200 and 1000 mg/kg bw/d ($P<0.05$). At postnatal week 11 (PNW 11), in males, loss of germ cell development was significant at 200 mg/kg bw/d and above. This lesion differed markedly in severity between animals. Significant increases in vacuolar degeneration in the mammary glands of males was present from 2 mg/kg bw/d but with similar incidence and qualitative gradation of change across the dose groups.

Other studies

Other studies on DBP were included in the NOAEL determination by EFSA (2005) but were not considered critical. These studies included developmental and reproductive studies described in detail in the EU RAR (2004): Lamb et al. (1987), Morrisey et al. (1989), Gray et al. (1999), Mylchreest et al. (2000), NTP (1995), Wine et al. (1997). The EU RAR also describes the following studies that were not discussed in the EFSA opinion from 2005: Nikoronow et al. (1973), IRDC (1984), Hamano et al. (1997), Shiota et al. (1980), Ema et al. (1993), Mylchreest et al. (1998), Mylchreest et al. (1999).

The registration report for DBP quotes strictly the EU RAR regarding reproductive and developmental toxicity and therefore describes the same studies. The EU RAR determines a LOAEL of 52 mg/kg bw/d based on embryotoxicity from the study by NTP (1995)/Wine et al. (1997). As embryotoxicity is not considered an anti-androgenic effect, this LOAEL is not considered for the derivation of the DNEL. The lowest LOAEL reported in the EU RAR related to anti-androgenicity is 100 mg/kg bw/d in a study by Mylchreest et al. (1999), in which delayed preputial separation was seen at the lowest dose of 100 mg/kg bw/d. The study description from the EU RAR follows below:

In a follow-up study of Mylchreest et al. (1999), DBP was shown to disrupt the androgen-regulated male sexual differentiation during prenatal exposure, without interacting directly with the androgen receptor. At the highest dose-

level of 500 mg/kg bw/d (in corn oil), given orally by gavage to pregnant rats during day 12-21 of gestation, one dam showed weight loss after day 18 of pregnancy and delivered dead and moribund foetuses.

At all dose levels (100, 250 and 500 mg/kg bw/d) 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/d this delay (of 2 days) was attributable at least in part, to one markedly affected litter. Furthermore, malformations of the (F1) male reproductive tract were observed at 250 and 500 mg/kg bw/d, i.e. retained thoracic nipples and decreased anogenital distance. In addition, at 500 mg/kg bw/d 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 were seen. Interstitial cell adenoma occurred at 500 mg/kg bw/d in 2 males (in one litter). In F1 females no abnormal uterine or vaginal development or kidney agenesis were seen. In contrast to flutamide, DBP caused a low incidence of prostate agenesis and hypospadias with no vaginal pouch.

A second follow-up study by Mylchreest et al. (2000) is mentioned by EFSA (2005), but not by the EU RAR from 2003. In this study a NOAEL of 50 and a LOAEL of 100 mg/kg bw/d were determined based on nipple retention in male pups at 100 mg/kg bw/d and above. This study examined exposure of pregnant CD rats to DBP by gavage from GD 12 to 21 at the doses of 0, 0.5, 5, 50, 100 or 500 mg/kg bw/d. Nipple retention was the only effect observed at 100 mg/kg bw/d, and at 500 mg/kg bw/d decreased anogenital distance of males, hypospadias and absence or malformations of epididymis, vas deferens, seminal vesicles and ventral prostate were seen together with decreased widths of male reproductive organs and histological changes in testes.

A number of reproductive/developmental studies have been published after the EU RAR from 2003. A study by Zhang et al. (2004) detected a NOAEL of 50 mg/kg bw/d based on decreased anogenital distance of males and effects on male reproductive organs and sperm production of rats exposed to 250 or 500 mg/kg bw/d of DBP in utero and during lactation (GD 1 to PND 21).

A number of reproductive, developmental and/or mechanistic studies applying large doses of DBP are not described here, as these were not considered relevant for NOAEL determination (Ryu et al., 2008, Jiang et al., 2007 and more). Among the mechanistic studies are dose-response studies on the inhibitory effect of DBP on foetal testosterone production: Howdeshell et al. (2008) described that DBP decreased foetal testosterone production in rats at doses from 300 mg/kg bw/d (NOAEL 100 mg/kg bw/d). In this study, pregnant Sprague-Dawley rats were exposed to 33, 50, 100, 300, or 600 mg/kg bw/d of DBP from GD 8 to 18 by gavage in corn oil (n=3 to 4 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old foetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for

BBP and the other tested phthalates (DIBP, BBP, and DEHP) from 300 mg/kg bw/d and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/d.

Lehmann et al. (2004) exposed pregnant Sprague-Dawley rats to 0, 0.1, 1, 10, 50, 100, or 500 mg/kg bw/d of DBP from GD 12 to 19 by gavage in corn oil (n=1 to 4 litters per group, analysis of testosterone in 3-4 males per litter). Testosterone concentration and testicular expression of steroidogenesis related genes were measured in testes of 19 day old foetuses. The study found decreased foetal testosterone concentration in rats exposed to DBP at doses from 50 mg/kg bw/d with a NOAEL of 10 mg/kg bw/d. This corresponded to a reduction in the expression of genes involved in steroid synthesis (SR-B1, StAR, P450scc, 3βHSD) from 50 mg/kg bw/d. At lower doses some of these genes were also sporadically reduced, but effects were not statistically significant at 10 mg/kg bw/d and the most consistent effects were seen above 50 mg/kg bw/d. The power of this study was relatively low with 3 to 5 foetuses examined for each endpoint, and a larger study might have revealed effects on e.g. gene expression at lower doses.

Derivation of point of departure for DNEL setting

Overall, therefore, the critical N(L)OAEL for derivation of the DNEL for DBP is the **LOAEL of 2 mg/kg bw/d** from Lee et al. (2004). This LOAEL is based on delayed germ cell development and persistent male mammary gland changes.

DIBP (from ECHA, 2016)

Fewer reproductive toxicity studies have been published on DIBP compared to the number of studies published for DEHP and DBP. No two-generation studies are available. In addition, DIBP has only been studied at doses >100 mg/kg bw/d (Saillenfait et al. 2006, 2008; Borch et al. 2006; Boberg et al. 2008; Howdeshell et al. 2008; Hannas et al. 2011, 2012).

Description of key studies

Saillenfait et al., 2008
In Saillenfait et al. (2008), pregnant Sprague-Dawley rats were exposed from GD 12 to 21 by gavage to 0, 125, 250, 500, or 625 mg/kg bw/d of DIBP (n=11-14 dams per group). Reduced male neonatal anogenital distance and an increased number of nipples in males were observed from 250 mg/kg bw/d exhibiting clear dose-response relationships over the tested dose range. A subtle and not statistically significant reduction in anogenital distance was seen in the lowest dose group. Prostate weight at postnatal week 16-17 was significantly reduced at all doses except at 250 mg/kg bw/d, but these data did not show a clear dose-response. At postnatal week 11-12, reductions in prostate weight were statistically significant from 250 mg/kg bw/d with data showing a clear dose-response pattern and a non-significant reduction also at the lowest dose group. Reductions in other reproductive organ weights were seen from 500 mg/kg bw/d. At the highest doses, 500 and 625 mg/kg bw/d,

delays in preputial separation and incidence of malformations (hypospadias, cleft prepuce and undescended testes) were observed in young adulthood and histological changes of testes were observed in adulthood.

The observation of histological changes of testes was most marked at 500 and 625 mg/kg bw/d, but mild/infrequent effects were also seen at the two lowest doses. Two of 24 control males had tubular degeneration grade 1 (of 5 grades), whereas 2 of 20 males exposed to 125 mg/kg bw/d DIBP had tubular degeneration at grade 2 and grade 5, respectively, and 7 of 28 males exposed to 250 mg/kg bw/d DIBP had tubular degeneration at grade 1 to grade 5. No statistical analysis is presented for histological data. The results seen for the positive control DBP at a dose of 500 mg/kg bw/d showed comparable effects to those seen with 500 and 625 mg/kg bw/d DIBP. Specifically the effects on anogenital distance, nipple retention, reproductive organ weights and reproductive tract malformations (hypospadias, exposed os penis, cleft prepuce and cryptorchidism) and puberty onset seen with 500 mg/kg bw/d DIBP were comparable or *less* marked than the effects seen with 500 mg/kg bw/d DBP, whereas the effects seen with 625 mg/kg bw/d DIBP were comparable or *more* marked than the effects seen with 500 mg/kg bw/d DBP.

Howdeshell et al., 2008

Howdeshell et al. (2008) described that DIBP decreased foetal testosterone production in rats at doses from 300 mg/kg bw/d (NOAEL 100 mg/kg bw/d). In this study, pregnant Charles River Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/d DIBP from GD 8 to 18 by gavage in corn oil (n=5 to 8 dams per group). Maternal body weight at GD 18 was reduced at 600 and 900 mg/kg bw/d, whereas maternal body weight gain, the number of live foetuses and total resorptions were decreased at 900 mg/kg bw/day. Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old foetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for DIBP and the other tested phthalates (BBP, DBP, and DEHP) at 300 mg/kg bw/d and above, and for DPP (dipentyl phthalate) from 100 mg/kg bw/d.

Hannas et al., 2011

In Hannas et al. (2011), pregnant Harlan Sprague-Dawley rats were exposed to 0, 100, 300, 600, or 900 mg/kg bw/d DIBP from GD 14 to 18 by gavage (n=3 dams per group). Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old foetuses for 3 hours and testosterone measurement in the media. Dose-related decreases in testosterone production was seen for DIBP and the other tested phthalates (DEHP and DIHP (diisohexyl phthalate)) from 300 mg/kg bw/d and above (NOAEL 100 mg/kg bw/d), and for DINP from 500 mg/kg bw/d.

Furr et al., 2014

Furr et al. (2014) studied foetal testosterone production in several experiments with the aim to develop and validate the 'Foetal Phthalate Screen' assay.

In the single dose experiment, 27 substances were tested. Pregnant rats were dosed daily by oral gavage at 0 and 750 mg/kg bw/d from GD 14 to 18 (n=3-4 dams per group). At GD 18, foetal testes were removed and 3 testes were per litter were used to measure ex vivo testosterone production. The testosterone production was reduced to a level of about 20% of the control in the DIBP dosed animals and at about 12% for DEHP, DBP and BBP.

In the dose-response experiment, 11 substances were tested. Pregnant Harlan Sprague Dawley rats were dosed daily by oral gavage at 0, 100, 200, 300, 500, 600, 750 and 900 mg/kg bw/d using the same protocol as for the single dose experiment. The ED_{50} was about 290 mg/kg bw/d with DIBP in Harlan SD rats. The ED_{50} was about 160 and 320 mg/kg bw/d with DBP (Harlan and Charles River SD rats respectively); 120 and 340 mg/kg bw/d with DEHP (Harlan SD and Charles River SD rats respectively); 170 mg/kg bw/d with BBP in Harlan SD rats; and 750 mg/kg bw/dy with DINP in Harlan SD rats. Harlan SD rats appeared to be more sensitive than Charles River SD rats to reduction of foetal testosterone production from phthalate exposure.

Hannas et al., 2012

In Hannas et al. (2012), steroidogenesis-related gene expression was investigated in foetal testes at GD 18. A number of steroidogenesis-related genes were downregulated at 300 mg/kg bw DIBP and above and also by other phthalates examined (dihexyl-, diheptyl-, dipentyl-, and diisononyl phthalate), but not by diisodecyl phthalate. DIBP downregulated the expression of: StAR, Cyp11a1, HSD3b, Cyp17a1, Scarb1, Insl3, Cyp11b1 and Rxrg, and upregulated the expression of Amhr2 and Sox9. These data were applied for potency ranking of these phthalates and it was concluded that several phthalates including DIBP affected the same pathways.

Other studies

Other studies on DIBP were considered for determination of the starting point for DNEL setting but were not considered critical. The described effects on male anogenital distance and foetal testosterone production are consistent with the findings by Borch et al. (2006). Decreased anogenital distance and decreased testicular testosterone production and content were seen in this study in foetal male Wistar rats exposed to 600 mg/kg bw/d DIBP from GD 7 to 21.

The available registration dossier for DIBP also includes the following studies, which all applied oral doses at or above the LOAEL of 250 mg/kg bw/d and were therefore not taken into consideration for NOAEL determination: Saillenfait et al. (2006), Boberg et al. (2008) and Zhu et al. (2010). A study by Ray et al. (2012) applied intraperitoneal exposure to DIBP and was not considered relevant for DNEL determination. In addition, the registration dossier for DIBP presents a study on DBP (NTP 1995) and justifies use of read-across for effects on fertility; however, this study is not used for NOAEL/LOAEL determination.

For direct effects on male adult fertility, these were reviewed by CPSC (CPSC 2011). Short-term oral exposure to DIBP causes significant adverse testicular effects in male adolescent rats including decreased testes weights, increased numbers of apoptotic spermatogenic cells, disorganized or reduced vimentin filaments in Sertoli cells, elevated testicular testosterone levels, decreased testicular zinc levels, and marked inhibition of spermatogenesis and desquamation of spermatocytes. Effects were seen at doses as low as 500 mg/kg bw/d (Zhu et al., 2010; Oishi and Hiraga, 1980a).

Derivation of point of departure for DNEL setting

In the Background Document to the Opinion on the Annex XV dossier proposing restrictions on four phthalates (ECHA 2012b), a LOAEL of 125 mg/kg bw/d from Saillenfait et al. (2008) was used as a starting point for DNEL derivation for DIBP. RAC (ECHA 2012b) noted that the LOAEL for histological effects of DIBP on the adult testes and epididymides can be considered “conservative” given the low incidences found at the LOAEL, but that a steep dose-response curve was seen in this study. Also the available registration dossier for DIBP used a LOAEL of 125 mg/kg bw/d for DIBP as a point of departure.

A starting point of 9.8 mg/kg bw/d was applied by US consumer product safety commission (CPSC) in their toxicity review of DIBP (CPSC 2011). This was based on a BMDL10 for effects on foetal testosterone in the study by Howdeshell et al. (2008).

Few reproductive toxicity studies have been published on DIBP compared to the number of studies available on DBP. No two-generation studies are available. The dose-response curve in Saillenfait et al. (2008) is steep with high incidences (up to 100%) of histological changes in testes and nipple retention at 500 and 625 mg/kg bw/d. Subtle effects are also seen at 125 mg/kg bw/d on anogenital distance, tubular degeneration, oligospermia/azoospermia, and prostate weight. The experimental data leave a high degree of uncertainty when the selected point of departure is a LOAEL (125 mg/kg bw/d) as DIBP has not been tested below 100 mg/kg bw/d. Therefore it was considered important to evaluate new mechanistic evidence relevant to the reproductive potency of DIBP and to explore the potential to derive a new point of departure using all of the available evidence.

DIBP is structurally very similar to DBP. Indeed, DIBP is a branched isomer of DBP having the same molecular weight and physicochemical properties. Health Canada (2015) grouped DIBP and DBP in the same subcategory of medium chain phthalate esters, with the longest carbon backbone length of 3-7. Biomonitoring studies often assume that the molar urinary excretion fraction (FUE value) of DIBP is equal to that of DBP (e.g., UBA 2011; Kasper-Sonnenberg et al. 2014).

Structure activity relationship analysis found DBP to be more potent than DIBP with regard to reducing expression of 5 genes in the steroidogenic pathway (SR-B1, StAR, Cyp11a, 3bHSD, and Cyp17a1). DIBP was found to

be slightly more potent than DBP in reducing foetal testosterone levels, and DIBP and DBP being equipotent in reducing AGD (all reviewed in Health Canada 2015). Overall, DIBP and DBP affect similar mechanistic targets leading to similar adverse developmental effects as other phthalates within the medium chain phthalate esters group, and the mono ester of DBP is the closest structural analogue of the mono ester of DIBP.

The observed effects of DIBP at 500 mg/kg bw/d and 625 mg/kg bw/d in Saillenfait et al. (2008) on anogenital distance, nipple retention, reproductive organ weights, and puberty onset were comparable to the effects seen with 500 mg/kg bw/d of DBP. The potency difference between DIBP and DBP for these reproductive developmental endpoints thus appears to be minor.

Overall, the current data suggest that DIBP has similar potency to DBP, and thus the LOAEL of 125 mg/kg bw/d used previously as the starting point for DNEL derivation does not seem to appropriately reflect the similarity in potency. This is mainly due to the fact that for DIBP, effects on delayed germ cell development seen with DBP have not been investigated in the available studies.

However, a possible potency difference between DIBP and DBP has been observed. Based on the available data from Saillenfait et al. (2008), an estimate based on the available data indicates that a 25% higher dose of DIBP would be required to elicit the same reproductive adverse effects as with DBP (anogenital distance, nipple retention, reproductive organ weights and reproductive tract malformations and puberty onset).

If this potency difference of 25% between DBP and DIBP is extrapolated from the high dose area to the lower dose area, a new LOAEL for DIBP would be 25% higher than the current LOAEL of 2 mg/kg bw/d for DBP, leading to a LOAEL for DIBP of 2.5 mg/kg bw/d.

A **LOAEL for DIBP of 2.5 mg/kg bw/day** is therefore selected as the starting point for DNEL derivation.

Derivation of oral DNELs for DIBP and DBP (as described in ECHA, 2016)

Route-specific absorption values for DIBP and DBP are as follows (ECHA, 2012b):

Oral 100%
Dermal 10%
Inhalation 100%

The oral DNELs for DIBP and DBP for consumers and the general public are presented in Table 13 below. In accordance with ECHA guidance Chapter R.8, DNEL calculation uses an uncertainty factor of 2.5 for interspecies differences; an allometric scaling factor of 4 for rats; a factor of 10 for

intraspecies differences; and a factor of 3 for extrapolation from LOAEL to NAEL if no NOAEL is available.

No other assessment factors were considered relevant (e.g., for different duration/exposure time).

Table 13: Overview of oral DNEL derivation for DIBP and DBP (as proposed in ECHA, 2016)

Substance	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Endpoint and study reference	AFs	Oral DNEL (mg/kg bw/d)
DIBP	-	2.5	Read-across from DBP	4x2.5x10x3 = 300	**0.008**
DBP	-	2	Reduced spermatocyte development at postnatal day 21, and mammary gland chan-ges (vacuolar degeneration and alveolar atrophy) in adult male offspring in Lee et al. (2004)	4x2.5x10x3 = 300	**0.007**

It should be noted that these oral DNELs for DBP and DIBP have not been agreed yet by RAC, as they have only been recently proposed in a new draft Annex XV restriction for DEHP, BBP, DBP and DIBP (ECHA, 2016). However, the proposed oral DNEL for DBP (0.007 mg/kg bw/d) is the same value that was previously agreed by RAC (ECHA, 2012b) and it is based on the same starting point (LOAEL of 2 mg/kg bw/d) from the same study (Lee et al., 2004). The proposed oral DNEL for DIBP (0.008 mg/kg bw/d) is different from that previously agreed by RAC (ECHA, 2012b) which was 0.42 mg/kg bw/d. This was derived from the LOAEL of 125 mg/kg bw/d identified in the study by Saillenfait et al. (2008) on the basis of degeneration of seminiferous tubules and oligo-/azospermia in epididymes observed in male rats exposed perinatally (from gestation day 12 to 21) by gavage to DIBP.

7. COMPARISON OF DEVELOPMENTAL EFFECTS ON MALE REPRODUCTION AND ORAL DNELs SET FOR DPP, DIBP AND DBP TO INFORM READ-ACROSS TO DIPP

A comparison of the developmental effects on male reproduction (and the dose levels at which they occur) identified for the three possible candidates (DPP, DIBP and DBP) for read-across to DIPP is presented in the Table below.

Table 14: Comparison of developmental effects on male reproduction in rodent studies for DPP, DIBP and DBP

Phthalate	Protocol (species, strain, duration; doses in mg/kg bw/day)	Effect LOAEL/NOAEL (mg/kg bw/day)	Comment	Reference
AGD				
DIBP	Pregnant rats (SD), gavage, GD 12-21; 0, 125, 250, 500, 650	LOAEL 250 NOAEL 125 (some effects on AGD, not statistically significant).	Overall, effects on male AGD appear around **100** mg/kg bw/d of **DBP** (though only examined in one study) and around **125** mg/kg bw/d of **DIBP** (only one study with several doses available; others (Borch et al., 2006) find decreased male AGD at 600 mg/kg, with this dose the only one tested) Health Canada calculated BMDL10 values (10% decrease in AGD from controls) of 204 and 208 mg/kg bw/d for DIBP and DBP, respectively (Health Canada 2015b) For **DPP**, decreases in male AGD occur at around **100** mg/kg bw/d (investigated only in one study). Overall, based on effects on male AGD, DBP, DIBP and DPP appear of similar potency.	Saillenfait et al., 2008 Saillenfait et al. 2008 included DBP as a positive control, see comparisons from this study below
	Pregnant rats (Wistar), gavage GD 7-21; 0, 600	LOAEL 600		Borch et al., 2006
DBP	Pregnant rats (?), gavage; GD 13-21; 0, 100, 500	LOAEL 100		Martino-Andrade et al.,2009
	Pregant rats (SD), dietary GD 15 – PND 21; 0, 2, 20, 200, 1000	LOAEL 1000 NOAEL 200		Lee et al., 2004
	Pregant rats (SD), gavage; GD12-21: 100, 250, 500 Pregnant rats, gavage; GD 1-PND 21; 0, 50, 250, 500	LOAEL 250 NOAEL 100 LOAEL 250 NOAEL 50		Mylchreest et al., 1999; Zhang et al., 2004
	Pregnant rats (SD), gavage; GD 12-21; 0, 0.5, 5, 50, 100, 500	LOAEL 500 NOAEL 100		Mylchreest et al., 2000
	Pregnant rats (?); gavage; GD12/13-20/21: 100, 500	LOAEL 500 NOAEL 100		Barlow et al. 2004; Johnson et al., 2011
DPP	Pregnant rats (SD); gavage; GD 8-18; 0, 11, 33, 100, 300	LOAEL 100 NOAEL 33		Hannas et al., 2011
↓ fetal testosterone				
DIBP	Pregnant rats (SD), gavage; GD 8-18: 0, 11, 33, 100, 300, 600, 900	LOAEL 300 NOAEL 100	When comparing effects on fetal testosterone production, DIBP and DBP appear to be equally potent, but DPP appears three times more potent. Howdeshell et al. (2008) calculated derived ED_{50} values for DIBP and DBP of 466 and 399 mg/kg/d, respectively (for DEHP 383 mg/kg bw/d) and for DPP a value of130 mg/kg bw/d. Comparing with the potency from Hannas et al. (2011, 2012) the derived ED_{50} value for DIBP was 305	Hannas et al., 2011, 2012; Howdeshell et al., 2008
	Pregnant Harlan (SD) rats, gavage; GD 14-18; 0, 100, 200, 300, 500, 600, 750, 900	ED_{50} 288 (95% CI 248-335)		Furr et al., 2014
DBP	Pregnant rats (SD), gavage GD 8-18: 0, 100, 300, 600, 900	LOAEL 300 NOAEL 100		Howdeshell et al., 2008
	Pregnant rats (SD), gavage; GD 14-18; 0, 100, 200, 300, 500, 600, 750, 900	ED_{50} (Harlan SD rats) 158 (95% CI 101-248) ED_{50} (CR SD) 337 (95% CI 250-454)		Furr et al., 2014
DPP	Pregnant rats (SD), gavage GD 8-18: 0, 100, 300, 600, 900	LOAEL 100 NOAEL 50		Howdeshell et al., 2008

	Pregnant rats (SD), gavage; GD 8-18: 0, 11, 33, 100, 300, 600, 900	LOAEL 33 NOAEL 11	mg/kg/d, i.e. lower than for DEHP (383 mg/kg/d) and DPP was 8-fold more potent than DEHP. Furr et al.(2014) showed differences in species sensitivity and slightly lower ED_{50} than calculated by Hannas et al.(2011, 2012) and Howdeshell et al.(2008) for DIBP and DBP Overall, in relation to decreased testosterone production, based on the ED_{50} values, DBP and DIBP appear to be roughly of equal potency, but DPP appears to be more potent .	Hannas et al., 2011, 2012; Gray et al., 2016
Gene expression related to stereoid biosynthesis pathway				
DIBP	Pregnant rats (SD), gavage; GD 14-18; 0, 11, 33, 100, 300, 600, 900	LOAEL: 300 (cyp11a, 3bhsd, cyp17a1, sr-b1, star) NOAEL: 100	**DIBP** appears to affect gene expression from around **300** mg/kg bw/d (in the only available study). **DBP** affects gene expression at around **50** mg/kg bw/d. **DPP** appears to affect gene expression at a slightly lower dose level of **33** mg/kg bw/d. Overall, in relation to down-regulation of genes involved in steroidogenesis, DIBP appears less potent that DBP and DPP, which both appear of roughly the same potency.	Hannas et al., 2012
DBP	Pregnant rats (SD), gavage;GD 12-19: 0, 0.1, 1, 10, 50, 100, 500	LOAEL: 50 (sr-b1cyp11a, star, 3bhsd), 500 (cyp17a1) NOAEL 10		Lehmann et al., 2004
DPP	Pregnant rats (SD), gavage; GD 14-18; 0, 11, 33, 100, 300, 600, 900	LOAEL: 33 (Star, cyp11a1, Scarb1, Hsd3b, cyp17a1, Insl3) NOAEL 11		Hannas et al., 2012; Gray et al., 2016
	Pregnant rats (SD), gavage; GD 14-18; 0, 50	LOAEL: 50 (cyp11a1, NrOb1, cyp11b2, Hsd3b, cyp17a1, Scarb1, Insl3, Dhcr7, cyp11b1)		Beverly et al., 2014
Nipple retention in males				
DIBP	Pregnant rats (SD), gavage; GD 12-21; 0, 125, 250, 500, 625	LOAEL 250 NOAEL 125	**DIBP** appears to cause nipple retention from a dose of **250** mg/kg bw/d in the only available study. **DBP** causes nipple retention from a lower dose of around **100** mg/kg bw/d in several studies. **DPP** causes nipple retention from a higher dose of around **300** mg/kg bw/d in the only available study.	Saillenfait et al., 2008
DBP	Pregnant rats (SD), gavage; GD 12-21; 0,5, 5, 50, 100, 500	LOAEL 100 NOAEL 50		Mylchreest et al., 2009

	Pregnant rats (?), gavage ;GD 12-21: 100,500	LOAEL 100	Overall, in relation to nipple retention, DIBP and DPP appear to be of roughly similar potency whilst DBP appears to be more potent.	Barlow et al., 2004
DPP	Pregnant rats (SD), gavage; GD 8-18: 0, 11, 33, 100, 300, 600, 900	LOAEL 300 NOAEL 100		Hannas et al., 2011
Mammary gland development				
DIBP	No studies available investigating mammary gland development after DIBP exposure	-	No studies available investigating mammary gland development after DIBP exposure; For DBP, in the only available study, in the mammary glands, dilatation of alveolar buds and/or ducts was seen in male offspring from 2 mg/kg bw/d with low incidence but not achieving statistical significance in any group. In female offspring, hypoplasia of the alveolar buds of the mammary glands was observed in animals from 2 mg/kg bw/d with a statistically significant increase at 2, 20, 200 and 1000 mg/kg bw/d. Significant increases in vacuolar degeneration in the mammary glands of males was present from 2 mg/kg bw/d but with similar incidence and qualitative gradation of change across the dose groups. No studies available investigating mammary gland development after DPP exposure. Overall, in relation to mammary gland development, data are available only on DBP; therefore a potency comparison with DIBP and DPP is not possible.	-
DBP	Pregnant rats (SD), dietary; GD 15-PND21: 0, 2, 20, 200, 1000	LOAEL 2		Lee et al. 2004;
DPP	No studies available investigating mammary gland development after DPP exposure	-		-
Other reproductive effects				

DIBP with DBP as positive control	Pregnant rats (SD), gavage ; GD 12-21; 0, 125, 250, 500, 650 ; DBP dose: 500	LOAEL 125	The effects (reproductive tract malformations, AGD, nipple retention, reproductive organ weights, delayed puberty onset) seen with 500 mg/kg bw/d DIBP were comparable or slightly less marked than the effects seen with 500 mg/kg bw/d DBP, whereas the effects seen with 625 mg/kg bw/d were comparable or more marked than the effects seen with 500 mg/kg bw/d DBP. The potency difference between DIBP and DBP thus appears to be minor. Prepubertal spermatogenesis was not investigated in this study, but reduced spermatocyte development for DIBP in adult rats was associated with tubular degeneration, occuring in all DIBP treated groups. Its severity increased with the dose. These effects are not reported for DBP. With DPP, mild testis atrophy was seen from 33 mg/kg bw/d (in post-pubertal animals); abnormalities of a number of male reproductive organs (at 6-7 months of age) were seen from 100 mg/kg bw/d; delayed descent of testes and reduced testis weight were seen in foetuses at 300 mg/kg bw/d; delayed preputial separation and hypospadias were seen at puberty at 300 mg/kg bw/d. Overall, in relation to other reproductive effects, DIBP and DBP appear to be of similar potency causing effects at around 500 mg/kg bw/d whilst DPP seems to be more potent than DIBP and DBP with effects seen from a dose of aroubd 100 mg/kg bw/d.	Saillenfait et al., 2008
DPP	Pregnant rats (SD), gavage ; GD 14-18; 0, 11, 33, 100, 300	LOAEL 33 NOAEL 11		Grey et al., 2016

As it can be seen from the table above, DBP, DIBP and DPP appear to be of similar potency in relation to effects on AGD; however, DPP appears to be more potent than DBP and DIBP in relation to decreases in foetal testosterone production and other reproductive effects on male offspring (testis atrophy, delayed descent of testes, hypospadias, delayed preputial separation and other abnormalities of the male reproductive tract). DPP is also of similar potency to DBP in relation to down-regulation of testicular genes involved in steroidogenesis, but more potent than DIBP. Only in

relation to effects on male nipple retention, DPP and DIBP appear to be less potent that DBP. In relation to effects on mammary gland development, which appears to be a very sensitive parameter of the anti-androgenicity of these phthalates, a potency comparison is not possible as data are available only on DBP. Overall, based on these comparative data, it can be concluded that DPP is either of similar potency to DBP and DIBP or even more potent than both. This is confirmed by a number of publications which have investigated the developmental toxicity of DPP together with other medium-chain phthalates (see chapter 4). On the basis of these publications, DPP appeared to be more potent in causing reproductive toxicity than other medium-chain (4-10 carbons) phthalates, including DEHP, DBP, DINP, DIBP and BBP. Depending on the dose descriptor, the strain of rat, the endpoint and the window of prenatal exposure selected for the comparison, DPP appeared to be 1.26 up to 8-fold more potent than DEHP.

Nevertheless, a comparison of the critical endpoints and the resultant DNELs for DPP, DBP and DIBP (see table 15 below) shows that the lowest oral DNEL (0.007 mg/kg bw/d) has been derived for DBP. This is very similar to the oral DNEL (0.008 mg/kg bw/d) derived for DIBP. The oral DNEL for DPP is much higher (0.1 mg/kg bw/d). This would suggest that DBP and DIBP are approximately 1.3-1.4 orders of magnitude more potent than DPP. However, it is still possible that DPP is actually of similar potency to DIBP and DBP, if not even more potent than DBP and DIBP (as shown by the comparison of key developmental effects on male reproduction and the publications described in chapter 4), and that the observed difference could be due to the fact that the critical endpoints on which the DNELs for DIBP and DBP are based upon (delayed germ cell development and persistent male mammary gland histopathological changes) were not investigated with DPP, but might also occur with DPP at similarly lower dose levels. These considerations illustrate the issue that relative potency measurements are significantly influenced by the individual substance's size of the database, quality and design of the available studies and sensitivity of the parameters investigated.

Overall, based on these comparative data, it can be concluded that DPP, DBP and DIBP appear to be of similar potency and to belong to a family of medium-chain phthalates of relatively high potency. On this basis, the most appropriate candidate among these three structurally similar phthalates for read-across to DIPP is DBP, as its extensive and robust database results in the lowest DNEL. Although uncertainties remain in the proposed read-across because there are no relevant reprotoxicity data on DIPP, selecting DBP for read-across to DIPP represents a more conservative choice, which errs on the side of caution. In addition, despite this uncertainty, the level of confidence in the proposed read-across from DBP is rather high, as DIPP has high structural similarity to DPP, DBP and DIBP; hence DIPP is expected to belong to the same family of medium-chain phthalates of relatively high reprotoxicity potency.

Table 15: Comparison of critical endpoints and oral DNELs for DPP, DIBP and DBP

Substance	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Endpoint and study reference	AFs	Oral DNEL (mg/kg bw/d)

DPP	11	33	Reductions in foetal testosterone levels, decreases in foetal testis gene expression and mild foetal testis atrophy in the absence of overt maternal toxicity observed in a rat oral developmental toxicity study (GD 8-18) with post-natal assessment (Hannas et al., 2011; 2012; Gray et al., 2016)	4x2.5x10	**0.1**
DIBP	-	2.5	Read-across from DBP	4x2.5x10x3 = 300	**0.008**
DBP	-	2	Delayed germ cell development at postnatal day 21, and mammary gland changes (vacuolar degeneration and alveolar atrophy) in adult male offspring in Lee et al. (2004)	4x2.5x10x3 = 300	**0.007**

8. DERIVATION OF INDICATIVE DNELs FOR DBP BY ALL RELEVANT ROUTES OF EXPOSURE

Route-specific absorption values for DBP are as follows (ECHA, 2012b):

Oral 100%
Dermal 10%
Inhalation 100%

DNELs will be derived for developmental toxicity (from the oral LOAEL of 2 mg/kg bw/d – Lee et al., 2004) for workers and the general public in accordance with the ECHA guidance on chemical safety assessment , chapter R8 (ECHA, 2010).

Workers

For workers, only inhalation and dermal DNELs will be derived.

Inhalation

Modification of the starting point

For development toxicity, the starting point is an oral LOAEL of 2 mg/kg bw/d in rats exposed during gestation and lactation (GD 15 – PND 21).

The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 100% oral absorption and 100% inhalation absorption, the equivalent inhalation LAEL expressed on a body weight basis would be:

2 x 100%/100% = *2 mg/kg bw/d*

Taking into account a rat ventilation rate (at rest) for 8 h of 0.38 m^3/kg bw, the following 8h-inhalation rat LAEC value would be calculated:

2/0.38 = *5.3 mg/m^3/d*

In accordance with the ECHA (2010) guidance, an adjustment for the higher ventilation rate (x 0.67) of a worker under light activity (compared to the experimental rat at rest) is required resulting in the following corrected inhalation 8h-LAEC:

5.3 x 0.67 = *3.5 mg/m^3/d (corrected inhalation 8h-LAEC)*

As the experimental animals were exposed 7 days/week whilst workers are assumed to be exposed 5 days/week, a further adjustment for the difference in the weekly exposure duration is required as follows:

3.5 x 7/5 = *4.9 mg/m^3/d (corrected inhalation 8h-LAEC for 5 days/wk)*

Application of default assessment factors (AFs)

For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 5 for workers will be applied. For extrapolation of the LAEC to the NAEC, RAC has already applied a factor of 3 when deriving the oral DNEL for DBP from the same starting point (ECHA, 2012b). The resulting inhalation (8-hr) DNEL for workers is shown below.

As already indicated by RAC (ECHA, 2012b), there is no need to apply an AF for severity of the effect (effects at the LOAEL were mild) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

$DNEL_{(worker, inhalation, development)}$: $\frac{4.9\ mg/m^3/d}{2.5 \times 5 \times 3}$ = **0.13 mg/m³/d**

Dermal

Modification of the starting point

For development toxicity, the starting point is an oral LOAEL of 2 mg/kg bw/d in rats exposed during gestation and lactation (GD 15 – PND 21).

The first modification step for the derivation of the dermal DNEL is route-to-route extrapolation from oral to dermal by taking into account 100% oral absorption and 10% dermal absorption. This would results in the following equivalent dermal LAEL:

2 x 100%/10% = *20 mg/kg bw/d*

As the experimental animals were exposed 7 days/week whilst workers are assumed to be exposed 5 days/week, an adjustment for the difference in the weekly exposure duration is required as follows:

20 x 7/5 = *28 mg/kg bw/d (corrected dermal LAEL for 5 days/wk)*

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 5 for workers will be applied. For extrapolation of the LAEL to the NAEL, RAC has already applied a factor of 3 when deriving the oral DNEL for DBP from the same starting point (ECHA, 2012b). The resulting dermal DNEL for workers is shown below.

As already indicated by RAC (ECHA, 2012b), there is no need to apply an AF for severity of the effect (effects at the LOAEL were mild) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

$DNEL_{(worker, derm, development)}$: $\frac{28\ mg/kg\ bw/d}{4 \times 2.5 \times 5 \times 3}$ = **0.19 mg/kg bw/d**

The Table below summarises the inhalation and dermal DNELs for workers in relation to DBP-induced developmental toxicity.

Table 16: Inhalation and dermal DNELs for workers for development toxicity for DBP

Endpoint	Inhalation (8-hr)	Dermal
Developmental toxicity	**0.13 mg/m^3/d**	**0.19 mg/kg bw/d**

General public

For the general public, inhalation, dermal and oral DNELs will be derived.

Inhalation

Modification of the starting point

For development toxicity, the starting point is an oral LOAEL of 2 mg/kg bw/d in rats exposed during gestation and lactation (GD 15 – PND 21).

The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 100% oral absorption and 100% inhalation absorption, the equivalent inhalation LAEL expressed on a body weight basis would be:

2 x 100%/100% = *2 mg/kg bw/d*

Taking into account a rat ventilation rate for 24 h of 1.15 m^3/kg bw, the following 24h-inhalation rat LAEC value would be calculated:

2/1.15 = *1.7 mg/m^3/d (corrected inhalation 24-hr LAEC)*

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. For extrapolation of the LAEC to the NAEC, RAC has already applied a factor of 3 when deriving the oral DNEL for DBP from the same starting point (ECHA, 2012b). The resulting inhalation (24-hr) DNEL for the general public is shown below.

As already indicated by RAC (ECHA, 2012b), there is no need to apply an AF for severity of the effect (effects at the LOAEL were mild) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{(public, inhalation, development)}$: $\frac{1.7 \text{ mg/m}^3\text{/d}}{2.5 \times 10 \times 3}$ = **0.02 mg/m^3/d**

Dermal

Modification of the starting point

For development toxicity, the starting point is an oral LOAEL of 2 mg/kg bw/d in rats exposed during gestation and lactation (GD 15 – PND 21).

The first and only modification step for the derivation of the dermal DNEL is route-to-route extrapolation from oral to dermal by taking into account 100% oral absorption and 10% dermal absorption. This would results in the following equivalent dermal LAEL:

2 x 100%/10% = *20 mg/kg bw/d*

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. For extrapolation of the LAEL to the NAEL, RAC has already applied a factor of 3 when deriving the oral DNEL for DBP from the same starting point (ECHA, 2012b). The resulting dermal DNEL for the general public is shown below.

As already indicated by RAC (ECHA, 2012b), there is no need to apply an AF for severity of the effect (effects at the LOAEL were mild) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

DNEL$_{\text{(public, dermal, development)}}$: $\frac{20 \text{ mg/kg bw/d}}{4 \times 2.5 \times 10 \times 3}$ = **0.07 mg/kg bw/d**

Oral

Modification of the starting point

For development toxicity, the starting point is an oral LOAEL of 2 mg/kg bw/d in rats exposed during gestation and lactation (GD 15 – PND 21).

Modification of the starting point to derive the oral DNEL is not necessary as the starting point is an oral dose.

Application of default assessment factors (AF)

For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

For intraspecies differences, the default factor of 10 for the general public will be applied. For extrapolation of the LAEL to the NAEL, RAC has already applied a factor of 3 when deriving the oral DNEL for DBP from the same starting point (ECHA, 2012b). The resulting oral DNEL for the general public is shown below.

As already indicated by RAC (ECHA, 2012b), there is no need to apply an AF for severity of the effect (effects at the LOAEL were mild) or for the quality of the database (sufficiently large database for reproductive toxicity including reasonably good-quality studies).

$DNEL_{(public, oral, development)}$: $\frac{2 \text{ mg/kg bw/d}}{4 \times 2.5 \times 10 \times 3}$ = **0.007 mg/kg bw/d**

The Table below summarises the inhalation, dermal and oral DNELs for the general public in relation to DBP-induced developmental toxicity toxicity.

Table 17: Inhalation, dermal and oral DNELs for the general public for developmental toxicity for DBP

Endpoint	Inhalation (24-hr)	Dermal	Oral
Developmental toxicity	**0.02mg/m^3/d**	**0.07 mg/kg bw/d**	**0.007 mg/kg bw/d**

9. SUMMARY

DIPP has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The purpose of this review is to evaluate the available information relevant to deriving DNELs for the reproductive toxicity of DIPP.

In the support document for the identification of DIPP as an SVHC, the basis on which DIPP was classified with H360FD (Repr Cat 1B) is unclear. The support document refers to a single developmental toxicity study with DIPP, but mention is made of a possible read-across from DPP and DBP.

The contractor has performed an extensive literature search for DIPP and identified only 1 publication. The contractor has also reviewed registration dossiers for DIPP. Only one 10 tpa registration was identified which did not include any reproductive toxicity data.

The available data on DIPP are insufficient to establish DNELs for fertility and development. Therefore, read-across from the structurally-related low molecular weight phthalates, DPP, DIBP and DBP has been considered by the contractor.

Based on structural similarity and physico-chemical properties, DPP, DIBP and DBP appear all good candidates for read-across to DIPP. In addition, they all share a common anti-androgenic mode of action. However, it is considered that structural similarity and similarity in physico-chemical properties are insufficient to determine which one of these three phthalates is the most suitable for read-across to DIPP. Therefore, dose-response information on developmental effects on male reproduction in rodents, typical of the “phthalate syndrome”, for each of these three candidates has been considered as it provides further insight which can help in determining which one of these three phthalates is the most appropriate for read-across to DIPP.

For DPP, the contractor has been unable to identify recent reviews of its reproductive toxicity; therefore, the primary literature has been examined and DNELs for the most sensitive effect have been derived. For DBP and DIBP, recent reviews of their reproductive toxicity including derivation of DNELs for the most critical effects have already been considered by RAC or published for consultation; therefore information from the most recent draft review on these phthalates (ECHA, 2016) has been used in this document.

DPP, DIBP and DBP all share a common anti-androgenic mode of action. They inhibit foetal testosterone production; reduce male anogenital distance; decrease gene expression related to steroid biosynthesis; increase nipple retention in male offspring; increase the incidence of genital malformations (hypospadias and cryptorchidism); induce delayed puberty onset; reduce semen quality; and induce testicular changes including testicular atrophy in rats. In addition, DBP induces changes in germ cell differentiation (multinucleated germ cells) and histopathological changes in the mammary gland of males, which are considered to be independent of foetal testosterone reduction.

The critical N(L)OAELs for DPP, DIBP and DBP are based on developmental effects on male reproduction.

For **DPP**, an overall **NOAEL of 11 mg/kg bw/d** (Hannas et al., 2011; 2012; Gray et al., 2016) has been identified in relation to the development of the male reproductive tract. This NOAEL is mainly based on reductions in foetal testicular testosterone levels, down-regulation of testis genes involved in steroidogenesis, sexual differentiation and male reproductive development, and testis atrophy in offspring at the next dose level of 33 mg/kg bw/d. This is the most sensitive and robust NOAEL for the derivation of the DNEL for the developmental toxicity of DPP. The resultant oral **DNEL** for the general population for DPP is **0.1 mg/kg bw/d**.

For **DBP**, the key study for selection of the critical N(L)OAEL was Lee et al. (2004). This study found reduced spermatocyte development in prepubertal rats and mammary gland changes in adult male rats perinatally (GD 15 to PND 21) exposed to 2 mg DBP/kg bw/d and above via the diet. No NOAEL was determined. Therefore, an overall **LOAEL of 2 mg/kg bw/d** was established for the derivation of the DNEL for the developmental toxicity of

DBP. The resultant oral **DNEL** for the general population for DBP is **0.007 mg/kg bw/d.**

For **DIBP**, the available data suggest that it has similar potency to DBP, and thus the LOAEL of 125 mg/kg bw/d used previously (ECHA, 2012b) as the starting point for DNEL derivation does not seem to appropriately reflect the similarity in potency. This is mainly due to the fact that for DIBP, effects on delayed germ cell development and histopathological changes in the mammary gland of males seen with DBP have not been investigated in the available studies. However, a possible potency difference between DIBP and DBP has been observed. Comparative data indicate that a 25% higher dose of DIBP would be required to elicit the same reproductive adverse effects as with DBP. If this potency difference of 25% between DBP and DIBP is used, a new LOAEL for DIBP would be 25% higher than the current LOAEL of 2 mg/kg bw/d for DBP, leading to a LOAEL for DIBP of 2.5 mg/kg bw/d. A **LOAEL of 2.5 mg/kg bw/day** has therefore been selected as the starting point for DNEL derivation for the developmental toxicity of DIBP. The resultant oral **DNEL** for the general population for DIBP is **0.008 mg/kg bw/d**.

A comparative analysis of the developmental effects on male reproduction (and the dose levels at which they occur) identified for DPP, DBP and DIBP has been performed to see if this can inform on their relative potency and ultimately on identifying the most appropriate substance among these three phthalates for read-across to DIPP.

This analysis shows that DBP, DIBP and DPP appear to be of similar potency in relation to effects on AGD; however, DPP appears to be more potent than DBP and DIBP in relation to decreases in foetal testosterone production and other reproductive effects on male offspring (testis atrophy, delayed descent of testes, hypospadias, delayed preputial separation and other abnormalities of the male reproductive tract). DPP is also of similar potency to DBP in relation to down-regulation of testicular genes involved in steroidogenesis, but more potent than DIBP. Only in relation to effects on male nipple retention, DPP and DIBP appear to be less potent that DBP. In relation to effects on mammary gland development, which appears to be a very sensitive parameter of the anti-androgenicity of these phthalates, a potency comparison is not possible as data are available only on DBP. Overall, based on these comparative data, it can be concluded that DPP is either of similar potency to DBP and DIBP or even more potent than both. This is confirmed by a number of publications which have investigated the developmental toxicity of DPP together with other medium-chain phthalates (see chapter 4). On the basis of these publications, DPP appeared to be more potent in causing reproductive toxicity than other medium-chain (4-10 carbons) phthalates, including DEHP, DBP, DINP, DIBP and BBP. Depending on the dose descriptor, the strain of rat, the endpoint and the window of prenatal exposure selected for the comparison, DPP appeared to be 1.26 up to 8-fold more potent than DEHP.

Nevertheless, a comparison of the critical endpoints and the resultant oral DNELs for DPP, DBP and DIBP shows that the lowest oral DNEL (0.007 mg/kg bw/d) has been derived for DBP. This is very similar to the oral DNEL

(0.008 mg/kg bw/d) derived for DIBP. The oral DNEL for DPP is much higher (0.1 mg/kg bw/d). This would suggest that DBP and DIBP are approximately 1.3-1.4 orders of magnitude more potent than DPP. However, it is still possible that DPP is actually of similar potency to DIBP and DBP, if not even more potent than DBP and DIBP (as shown by the comparison of key developmental effects on male reproduction and the publications described in chapter 4), and that the observed difference could be due to the fact that the critical endpoints on which the DNELs for DIBP and DBP are based upon (delayed germ cell development and persistent male mammary gland histopathological changes) were not investigated with DPP, but might also occur with DPP at similarly lower dose levels. These considerations illustrate the issue that relative potency measurements are significantly influenced by the individual substance's size of the database, quality and design of the available studies and sensitivity of the parameters investigated.

Overall, based on these comparative data, it can be concluded that DPP, DBP and DIBP appear to be of similar potency and to belong to a family of medium-chain phthalates of relatively high potency. On this basis, the most appropriate candidate among these three structurally similar phthalates for read-across to DIPP is DBP, as its extensive and robust database results in the lowest DNEL. Although uncertainties remain in the proposed read-across because there are no relevant reprotoxicity data on DIPP, selecting DBP for read-across to DIPP represents a more conservative choice, which errs on the side of caution. In addition, despite this uncertainty, the level of confidence in the proposed read-across from DBP to DIPP is rather high, as DIPP has high structural similarity to DPP, DBP and DIBP; hence DIPP is expected to belong to the same family of medium-chain phthalates of relatively high reprotoxicity potency.

Therefore, using DBP critical starting point (LOAEL of 2 mg/kg bw/d), indicative DNELs for DBP for workers and the general public by relevant routes of exposure have been derived in accordance with the ECHA guidance on chemical safety assessment, chapter R8 (ECHA, 2010). A direct read-across of these DBP DNELs to DIPP has then been performed.

For workers, only inhalation and dermal DNELs have been established. For the general public, inhalation, dermal and oral DNELs have been set. These are summarised in Table 18 below.

Table 18: Overview of derivation of indicative DNELs for workers and general population for developmental toxicity of DIPP (by read-across from DBP) by the inhalation, oral and dermal routes

Point of departure for DNEL derivation by all routes for DIPP (by read-across from DBP) in relation to developmental toxicity (Lee et al., 2004)		
Rat dietary developmental toxicity study (GD 15 – PND 21) (delayed germ cell development on PND 21and persistent histopathological mammary gland changes in adult male offspring)		
LOAEL	**2 mg/kg bw/d**	
Oral absorption	100 %	
Derivation of Indicative DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors[$]		
Interspecies, Allometric scaling	4*	4*
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	1	1
LOAEL to NOAEL	3	3
Hours/day	8	24
INHALATION		
Absorption	100%	100%
Standard respiratory volume in m^3/kg bw/day	0.384	1.15
Breathing rate for workers light activity vs rest	6.7/10	-
5 d/wk exposure for workers vs 7d/wk in animals	7/5	-
LAEC (corrected) in mg/m^3	4.9	1.7
Indicative DNEL INHALATION in mg/m³	**0.13**	**0.02**
DERMAL		
Absorption	10%	10%
5 d/wk exposure for workers vs 7d/wk in animals	7/5	-
LAEL (corrected) in mg/kg bw/d	28	20
Indicative DNEL DERMAL in mg/kg bw/d	**0.19**	**0.07**
ORAL		
LOAEL (mg/kg bw/d)	2	2
Indicative DNEL ORAL in mg/kg bw/dy		**0.007**

*Allometric scaling factor (4 for the rat) not applied only for the derivation of the inhalation DNELs;
[$] Justification for selection of assessment factors is given in the main report;

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11. LITERATURE SEARCH

The following databases, Oshrom, Oshupdate, Web of Science, e-library and Medline were searched for “Diisopentylphthalate” or “Dipentylphthalate” in conjunction with the term “reproductive toxicity”, “developmental toxicity” or “foetal testosterone”. The search covered the period 1980 – 2016.

In addition, PubMed was searched just for the terms “Diisopentylphthalate” or “Dipentylphthalate”.

Furthermore, Google was searched for reviews of “Diisopentylphthalate” or “Dipentylphthalate”