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**RAC/38/2016/08**
**Final**

# APPLICATION FOR AUTHORISATION: ESTABLISHING REFERENCE DNELs FOR DIISOPENTYLPHTHALATE (DIPP)

## Background

At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, the ECHA Secretariat presented a proposal to set DNELs and dose response relationships for substances prior to receiving applications for authorisation (AfAs). This was approved by RAC as a trial exercise. However, in early 2015, ECHA agreed to continue supporting the practice for Annex XIV substances, recognizing its value to the Authorisation process and its efficiency[1].

The DNELs and dose response relationships so derived are intended as non-legally binding 'reference values'. They provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of AfA.

Reference values in the form of DNELs for threshold substances and/or dose response relationships for non-threshold substances (mainly carcinogens) are published in advance of applications, for authorisation, so providing greater consistency and better use of the legally defined periods of opinion-development in the Committee for Risk Assessment (RAC).

Annex 1: Reference DNELs derived for DIPP

[1] At the Conference on "*Lessons learnt on Applications for Authorisation*" co-organised by ECHA and the European Commission that took place on 10-11 February 2015.

# Annex 1 Reference DNELs derived for DIPP

## Relevance of endpoints

DIPP has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The reference DNELs proposed in the present document are only based on the reproductive toxicity of DIPP. It is noted that this is the most sensitive endpoint of the toxicological profile of DIPP.

## Background

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 dipentylphthalate (DPP) and dibutylphthalate (DBP).

An extensive literature search for DIPP has identified only one publication. A review of the registration dossiers for DIPP has revealed only one 10 tpa registration 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, diisobutylphthalate (DIBP) and DBP has been considered.

## Consideration of read-across from DPP, DIBP or DBP

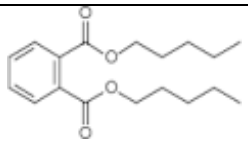
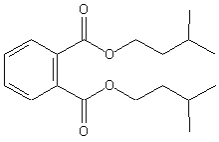
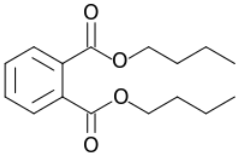
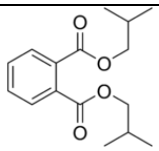
Based on structural similarity and physico-chemical properties, DPP, DIBP and DBP are all suitable candidates for reading across to DIPP to fill data-gaps (see Table 1 below). In addition, they all share a common anti-androgenic mode of action. However, it is considered that structural similarity and 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 is the most appropriate for read-across to DIPP.

For DPP, recent reviews of its reproductive toxicity are not available; therefore, the primary literature was examined for suitable data 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; therefore information from the most recent 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.

**Table 1: 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$^{o}$C | < -25$^{o}$C | -69$^{o}$C | -37$^{o}$C |
| Boiling point | 342$^{o}$C | 339$^{o}$C | 340$^{o}$C | 320$^{o}$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$^{o}$C | 0.025 Pa at 25$^{o}$C | 0.01 Pa at 25°C | 0.01 Pa at 20$^{o}$C |
| Water solubility | 0.8 mg/l at 25$^{o}$C | 1.1 mg/l at 20$^{o}$C | 10 mg/l at 20$^{o}$C | 20 mg/l at 20$^{o}$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) |

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**.

**Comparison of developmental effects on male reproduction for DPP, DIBP and DBP**

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. The results are presented in Table 2 below.

**Table 2: 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<br>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)<br>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)<br><br>For **DPP**, decreases in male AGD occur at around **100** mg/kg bw/d (investigated only in one study).<br><br>Overall, based on effects on male AGD, DBP, DIBP and DPP appear of similar potency. | Saillenfait et al., 2008<br>Saillenfait et al. 2008 included DBP as a positive control, see comparisons from this study below |
| | Pregnant rats (Wistar), gavage<br>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<br>NOAEL 200 | | Lee et al., 2004 |
| | Pregant rats (SD), gavage;<br>GD12-21: 100, 250, 500<br>Pregnant rats, gavage;<br>GD 1-PND 21; 0, 50, 250, 500 | LOAEL 250<br>NOAEL 100<br>LOAEL 250<br>NOAEL 50 | | Mylchreest et al., 1999; Zhang et al., 2004 |
| | Pregnant rats (SD), gavage;<br>GD 12-21; 0, 0.5, 5, 50, 100, 500 | LOAEL 500<br>NOAEL 100 | | Mylchreest et al., 2000 |
| | Pregnant rats (?); gavage;<br>GD12/13-20/21: 100, 500 | LOAEL 500<br>NOAEL 100 | | Barlow et al. 2004; Johnson et al., 2011 |
| **DPP** | Pregnant rats (SD); gavage;<br>GD 8-18; 0, 11, 33, 100, 300 | LOAEL 100<br>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<br>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.<br><br>Comparing with the potency from Hannas et al. (2011, 2012) the derived $ED_{50}$ value for DIBP was 305 mg/kg/d, i.e. lower than for DEHP (383 mg/kg/d) and DPP was 8-fold more potent than DEHP.<br>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<br><br>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; 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<br>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)<br>$ED_{50}$ (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<br>NOAEL 50 | | Howdeshell et al., 2008 |
| | Pregnant rats (SD), gavage; GD 8-18: 0, 11, 33, 100, 300, 600, 900 | LOAEL 33<br>NOAEL 11 | | 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)<br>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.<br><br>Overall, in relation to down- | 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)<br>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)<br>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) | regulation of genes involved in steroidogenesis, DIBP appears less potent that DBP and DPP, which both appear of roughly the same potency. | Beverly et al., 2014 |
| **Nipple retention in males** | | | | |
| **DIBP** | Pregnant rats (SD), gavage; GD 12-21; 0, 125, 250, 500, 625 | LOAEL 250<br>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.<br><br>Overall, in relation to nipple retention, DIBP and DPP appear to be of roughly similar potency whilst DBP appears to be more potent. | Saillenfait et al., 2008 |
| **DBP** | Pregnant rats (SD), gavage; GD 12-21; 0,5, 5, 50, 100, 500 | LOAEL 100<br>NOAEL 50 | | Mylchreest et al., 2009 |
| | Pregnant rats (?), gavage ;GD 12-21: 100,500 | LOAEL 100 | | Barlow et al., 2004 |
| **DPP** | Pregnant rats (SD), gavage; GD 8-18: 0, 11, 33, 100, 300, 600, 900 | LOAEL 300<br>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;<br><br>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 | - |
| **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 | - | | - |

| | | | | |
|---|---|---|---|---|
| | | | from 2 mg/kg bw/d but with similar incidence and qualitative gradation of change across the dose groups.<br><br>No studies available investigating mammary gland development after DPP exposure.<br><br>Overall, in relation to mammary gland development, data are available only on DBP; therefore a potency comparison with DIBP and DPP is not possible. | |
| **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.<br><br>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 | Saillenfait et al., 2008 |
| **DPP** | Pregnant rats (SD), gavage ;<br>GD 14-18; 0, 11, 33, 100, 300 | LOAEL 33<br>NOAEL 11 | | Grey et al., 2016 |

| | | | | |
|---|---|---|---|---|
| | | | hypospadias were seen at puberty at 300 mg/kg bw/d.<br><br>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. | |

This analysis shows that DBP, DIBP and DPP are 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, do 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. 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 (see Table 3 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 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, in accordance with the ECHA guidance on information requirements and chemical safety assessment, chapter R8 (ECHA, 2012) and using DBP as a critical starting point (LOAEL of 2 mg/kg bw/d), reference DNELs for DBP (and by read-across, reference DNELs for DIPP) have been derived for workers and the general public by relevant routes of exposure. A direct read-across of these DBP DNELs to DIPP has then been performed.

**Table 3: 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** |

## Critical study for DNEL derivation

The key study for selection of the critical N(L)OAEL for DBP (and by read-across, for DIPP) is Lee et al. (2004). In this study, 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) from late gestation (Gestational day 15) to the end of lactation on postnatal day 21 (PND 21). 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.

Overall, this study found reduced spermatocyte development in prepubertal rats and mammary gland changes in adult male rats perinatally exposed to 2 mg DBP/kg bw/d and above via the diet. No NOAEL was determined. A LOAEL of 2 mg/kg bw/d was established.

## Derivation of reference DNELs for DIPP (by read-across from DBP)

For the derivation of DNELs for the oral, inhalation and dermal routes and the application of route-to-route extrapolation, the following route-specific DBP absorption values *specified in ECHA (2012b)* have been used.

**Oral 100%**
**Dermal 10%**
**Inhalation 100%**

DNELs for developmental toxicity (from the oral LOAEL of 2 mg/kg bw/d – Lee et al., 2004) were derived for workers and the general public in accordance with the ECHA guidance on information requirements and chemical safety assessment, chapter R8 (ECHA, 2012).

**Workers**

For workers, only inhalation and dermal DNELs have been 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 the ECHA guidance on information requirements and chemical safety assessment, chapter R8 (ECHA, 2012), 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/m³/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)**: $\frac{4.9 \text{ mg/m}^3\text{/d}}{2.5 \times 5 \times 3}$ = **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)**: $\frac{28 \text{ mg/kg bw/d}}{4 \times 2.5 \times 5 \times 3}$ = **0.19 mg/kg bw/d**

The Table below summarises the inhalation and dermal DNELs for workers in relation to DBP- (and by read-across to DIPP-)induced developmental toxicity.

**Table 4: Inhalation and dermal DNELs for workers for development toxicity for DBP (and by read-across, for DIPP)**

| Endpoint | Inhalation (8-hr) | Dermal |
|---|---|---|
| Developmental toxicity | **0.13 mg/m³/d** | **0.19 mg/kg bw/d** |

## General public

For the general public, inhalation, dermal and oral DNELs have been 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 m³/kg bw, the following 24h-inhalation rat LAEC value would be calculated:

2/1.15 = *1.7 mg/m³/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/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 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(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)**: 2 mg/kg bw/d / 4 x 2.5 x 10 x 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- (and by read-across, to DIPP-)induced developmental toxicity toxicity.

**Table 5: Inhalation, dermal and oral DNELs for the general public for developmental toxicity for DBP (and by read-across, for DIPP)**

| Endpoint | Inhalation (24-hr) | Dermal | Oral |
|---|---|---|---|
| Developmental toxicity | **0.02 mg/m³/d** | **0.07 mg/kg bw/d** | **0.007 mg/kg bw/d** |

Table 6 below gives an overview of all the reference DNELs derived for DIPP (by read-across from DBP).

**Table 6: Overview of derivation of reference 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 reference 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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