Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9- rich; [1] di-"isononyl" phthalate; [2] [DINP]

EC Number: 271-090-9 [1] 249-079-5 [2]
CAS Number: 68515-48-0 [1] 28553-12-0 [2]

CLH-O-0000001412-86-201/F

Adopted

9 March 2018

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

9 March 2018

CLH-O-0000001412-86-201/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9- rich; [1] di-"isononyl" phthalate; [2] [DINP]**
EC Number:	**271-090-9 [1] 249-079-5 [2]**
CAS Number:	**68515-48-0 [1] 28553-12-0 [2]**

The proposal was submitted by **Denmark** and received by RAC on **29 March 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **4 April 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 May 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC:	**Ralf Stahlmann**
Co-Rapporteur, appointed by RAC:	**Agnes Schulte**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 March 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	607-RST-00-Y	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate; [2] [DINP]	271-090-9 [1] 249-079-5 [2]	68515-48-0 [1] 28553-12-0 [2]	Repr. 1B	H360Df	GHS08 Dgr	H360Df			
RAC opinion	607-RST-00-Y	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate; [2] [DINP]	271-090-9 [1] 249-079-5 [2]	68515-48-0 [1] 28553-12-0 [2]	-	-	-	-			
Resulting Annex VI entry if agreed by COM	607-RST-00-Y	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate; [2] [DINP]	271-090-9 [1] 249-079-5 [2]	68515-48-0 [1] 28553-12-0 [2]	-	-	-	-			

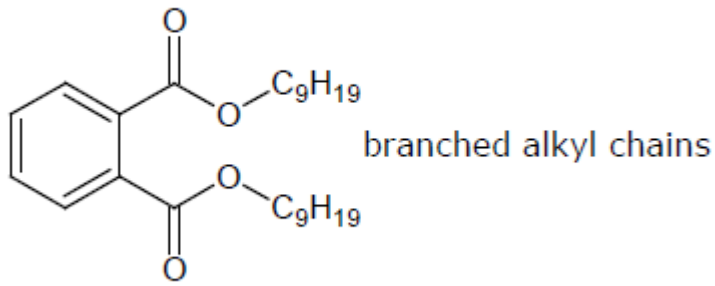
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Substance identity

The abbreviation DINP refers to two substances, described by different EC and CAS numbers, which vary in the compositions of the alkyl side chains.

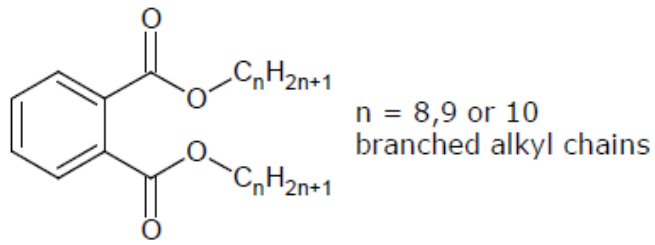
The substance described by EC 249-079-5: di-''isononyl'' phthalate (CAS No. 28553-12-0) contains C9H19 isomers as alkyl side chains (di-"isononyl" phthalate). This substance may be represented by the formula:



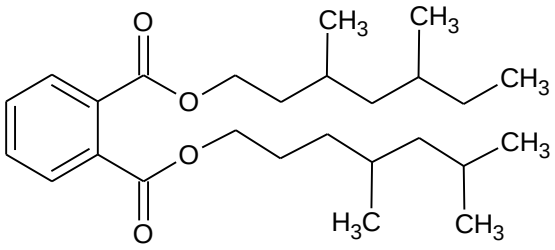
The commercial product, obtained from n-butene, is named DINP2 (cited in some studies).

The alkyl side chains of the substance described by EC 271-090-9: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (CAS-No. 68515-48-0) correspond to a distribution of C8H17 to C10H21 isomers where C9H19 alkyl chains are predominant in the composition.

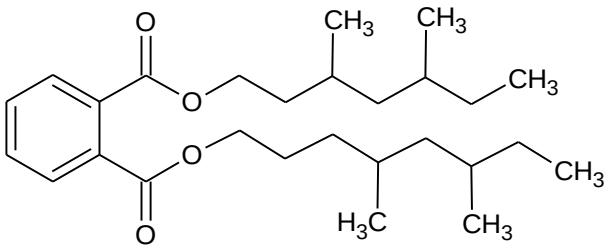
This substance may be represented by the formula:



The constituents present in the composition of this substance may consist of two alkyl chain substituents of the same length e.g.:



or two alkyl chain substituents of different length e.g.:



The commercial product, obtained via the "polygas" process, is named DINP1 (cited in some studies).
Under the EU risk assessment these substances have been considered equivalent from a health and environmental perspective and a single risk assessment has thus been conducted for DINP. Throughout this opinion the abbreviation DINP is used as a common name representing substances identified by the above CAS numbers.

Additional substance identity information was provided by EuPC. Industry also informed that DINP3, another commercial product, was produced from n-butene and iso-butene in the 1980s but is at present not produced on the EU market. The alcohol used for DINP3 consisted of methyl ethyl hexanols (65–75 wt %) and 20-25 wt% dimethylheptanols which result in a very high viscosity plasticiser (approximately double the viscosity of DINP1 and DINP2). The product was decommercialised, since the high viscosity meant that customers could not process it easily to make flexible vinyl articles while leading to poorer performance of the articles. DINP3 is not in the scope of this classification proposal. RAC recognises that studies testing DINP3 are not predictive of the toxicological properties of DINP1 and DINP2.

Industry provided a list of studies with information on the identity of the substance tested (see Addendum to Background Document; BD).

Abbreviations commonly used for describing various phthalates (and metabolites) referred to in the text:

BBP: benzyl butyl phthalate
DBP: dibutyl phthalate
DCHP: dicyclohexyl phthalate
DEHP: di(2-ethylhexyl) phthalate
DIBP: diisobutyl phthalate
DIDP: diisodecyl phthalate
DnHP: di-n-hexyl phthalate
DnOP: di-n-octyl phthalate
DPP: dipentyl phthalate
MEHP: mono-(2-ethylhexyl) phthalate

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's Proposal

Effects on fertility

Non-human information

The dossier submitter (DS) summarised the relevant experimental studies on fertility in a table, see below. RAC comments are listed at the end of the table.

Table: Summary table of relevant experimental studies on fertility (Table 10 of the CLH Report)

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) male/female, 30 rats/sex/group One-generation study Oral: feed 0.5, 1.0 and 1.5 % in diet (nominal conc.) (corresponding to 377-404, 741-796, and 1087-1186 mg/kg bw/d during gestation) CAS 68515-48-0 Vehicle: unchanged (no vehicle) Exposure: Ten weeks before mating and through the mating period, continuing for males until sacrifice following delivery of their last litter sired and females until they were sacrificed following weaning of the F1 animals on postnatal day (PND) 21. (Continuous for parents (males and females)). Equivalent or similar to OECD TG 415 (One-Generation Reproduction Toxicity Study)	No NOAEL could be determined due to effects on liver and kidney weights in parental animals and decreases in offspring body weight at all doses. Parental animals: Lower food consumption and lower body weights were observed before mating primarily in the mid and high-dose parental animals compared with controls. Statistically significant increases in mean absolute and relative right testis weight, left testis and right epididymis weights and mean relative left epididymis and seminal vesicle weights in high dose males compared to controls. In females, there was a statistically significant decrease in the mean absolute and relative right ovarian and mean absolute left ovarian weights of the high dose females compared with controls. No statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices between treated and control animals. Offspring: Dose-related decreases in mean offspring body weight during the postnatal period (PND 0-21). Statistically significant lower mean body weights in the high-dose males (10.2-46.0%) and females (11.3-46.9%), mid-dose females (7.9-26.9%) at all weighing intervals and in mean offspring body weight of the mid-dose males on PND 0, 1, 7, 14 and 21 (5.7-26.5%) compared with controls. Statistically significant lower mean body weights in the low-dose males on PND 0, 1, 14 and 21 (6.9-11.2%) and low-dose females (7.5-10.1%) at all weighing intervals.	No histological evaluation was performed. No evaluation of sperm parameters. This study terminated at PND 21 and no detailed examination of offspring was performed, as the results of this study were used to design a follow-up two-generation reproductive study of DINP.	Waterman *et al*., 2000 Exxon Biomedical Sciences, 1996

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) male/female, 30 rats/sex/group Two-generation study Oral: feed 0, 0.2, 0.4, 0.8 % DINP (nominal in diet), (corresponding to 0, 133-153, 271-307, and 543-577 mg/kg bw/d during gestation) CAS 68515-48-0 Vehicle: unchanged (no vehicle) Exposure: Constant exposure up to about 10 weeks prior to mating, continuing throughout the mating period. Males were sacrificed after mating but treatment of the females continued throughout gestation and lactation until weaning of the offspring on PND 21. Test guideline: EC Dangerous Substances Directive (67/548/EEC), Annex V, Part B; 1987 (identical to OECD TG 416) EPA OTS 798.4700 (Reproduction and Fertility Effects)	No NOAELs for parental or offspring toxicity were derived from this study due to liver changes in parents and reduced mean body weights of offspring at all doses (LOAEL 0.2% corresponding to 114-395 mg/kg bw/d, depending on the exposure period). Parental animals: No maternal toxicity was seen and there were no changes in dam body weight during gestation (P1). No statistically significant differences in male mating, male fertility, female fertility, female fecundity or female gestational indices in the P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility and female fecundity indices was observed in the P2 generation. A statistically significant decrease in the mean left ovary weight of the P1 females at 0.8% (577 mg/kg bw/d) was observed, but in the absence of a clear dose response relationship, similar findings in the right ovary weights, consistent pattern of response between absolute and relative organ weights, or correlating microscopic findings, this decrease was considered incidental and unrelated to treatment in the EU RAR. Offspring: In high-dose males, there was a statistically significant increase of relative right and left epididymis weights in the P2 generation with a concurrent but not statistically significant increase of absolute epididymis weight (by 7.5%). No treatment-related clinical findings and no biologically significant differences in the F1 or F2 offspring survival indices. Statistically significant, dose-related, lower mean offspring bodyweights in all treatment groups compared with controls during the F1 or F2 generations (LOAEL 159 mg/kg bw/d).	No evaluation of sperm parameters.	Waterman *et al*., 2000 Exxon Biomedical Sciences, 1996

The DS noted that these studies together indicate effects of DINP on development (offspring body weight) and fertility (altered reproductive organ weights at the high dose in the one-generation study), but that no clear conclusions from the two-generation study could be drawn as doses were lower and sperm parameters and reproductive organ histology was not examined.

RAC noted that doses in the two fertility studies by Waterman *et al.* (2000) were adequately selected. In the two-generation study several organs from parental animals (control and 0.8%

groups) were examined histologically (pituitary, testes, epididymides, prostate, seminal vesicles, vagina, uterus, ovaries, mammary gland).

No effects on male and female reproductive performance were seen in rats at doses up to 1.5% DINP (1087–1186 mg/kg bw/d), as parameters such as fertility indexes, number of pups at birth, or litter size were not adversely affected in any of the two studies.

Human information

The DS summarised the relevant human data on fertility in a table, see below.

RAC comments are listed at the end of the table.

Table: Summary table of relevant human data on fertility (Table 11 of the CLH Report)

Method	Results	Remarks	Reference
Cross-sectional study n=881 Danish young men Study period 2007-2009 Assessment of urinary DEHP and DINP metabolites (spot urine), serum hormone levels and sperm parameters	Differences between highest and lowest quartile of exposure to DINP metabolites with regards to levels of FSH and the testosterone:LH ratio.		Joensen *et al.*, 2012
Cross-sectional study n=589 Male partners of pregnant women (Greenland, Poland and Ukraine) Study period 2002-2004 Assessment of serum DEHP and DINP metabolites, serum hormone levels and sperm parameters	No clear associations between DINP or DEHP exposures and measures of sperm quality and serum hormone levels.		Specht *et al.*, 2014a
Cross-sectional study and retrospective interview regarding time to pregnancy n=938 women and 401 men (Greenland, Poland and Ukraine) Assessment of serum DEHP and DINP metabolites, serum hormone levels and sperm parameters	Some negative associations between DINP metabolites and serum testosterone levels at some study sites. Negative associations between some metabolites and measured sperm and hormone parameters, but overall no clear indications of effects of DINP or DEHP on male reproductive function.		Specht *et al.*, 2014b
Cross-sectional study n=555 boys and young men, Denmark Assessment of urinary phthalate metabolites (morning urine), serum hormone levels, puberty timing and presence of gynaecomastia	No clear associations of phthalate exposure with any of the examined reproductive parameters.		Mieritz *et al.*, 2012

The DS noted that human cross-sectional studies did not show any clear associations between adult exposure to DINP and fertility measures such as sperm parameters, hormone levels or time to pregnancy.

RAC supports the view of the DS on the cross-sectional studies, that the presented studies did not show any clear associations between adult exposure to DINP and fertility measures such as sperm parameters, hormone levels or time to pregnancy. With respect to the data published by Joensen *et al*. (2012) it should be noted that among men in the highest vs. lowest quartiles of % MiNP the associations with lower hormone levels were not accompanied by reductions in sperm quality.

Developmental toxicity

Non-human information

The DS summarised the relevant experimental studies on developmental toxicity in a table, see below. RAC comments are listed at the end of the table.

Table: Summary table of relevant experimental studies on developmental toxicity (chronological order) (Table 12 of the CLH Report). Supporting studies on effects on foetal testosterone production are included here.

Method	Results	Remarks	Reference
Rat (Wistar) Oral: gavage Doses: 0, 40, 200, 1000 mg/kg/d Vehicle: olive oil Exposure: day 6 through 15 post coitum (p.c.) (daily) OECD TG 414 (Prenatal Developmental Toxicity Study) CAS 68515-48-0 and 28553-12-0	NOAEL of 200 mg/kg bw/d for maternal and developmental effects (skeletal effects (rudimentary ribs)) at 1000 mg/kg bw/d.		Hellwig *et al*., 1997
Rat (Sprague-Dawley) n=25 Oral: gavage Doses: 0, 100, 500 and 1000 mg/kg bw/d (nominal conc.) CAS: 68515-48-0 Vehicle: corn oil Exposure: gestation day (GD) 6-GD 15 (daily) Equivalent or similar to OECD TG 414 (Prenatal Developmental Toxicity Study)	Maternal NOAEL of 500 mg/kg bw/d due to reduced body weight gain and mean food consumption at 1000 mg/kg bw/d. Developmental NOAEL of 100 mg/kg bw/d due to findings of visceral (dilated renal pelvis and hydroureter) and skeletal (rudimentary cervical and accessory 14th ribs) variations at 500 and 1000 mg/kg bw/d.		Waterman *et al*., 1999 Exxon Mobil, 1994c

Method	Results	Remarks	Reference
Rat (SD) n=19 in control group, n=14 in DINP group Oral: gavage Dose: 0, 750 mg/kg bw/d CAS: 68515-48-0. Vehicle: corn oil Exposure: GD 14-PND 3	NOAEL: Not determined LOAEL: 750 mg/kg bw/d Increased number of areolas in males, increased incidence of malformations of male reproductive organs.	One dose only.	Gray *et al*., 2000
Rat (SD) n=5-6 dams per group Oral: diet Doses: 0, 400, 4000, or 20,000 ppm CAS: 28553-12-0, purity: >98% Exposure: GD 15-PND 10	NOAEL: ≈230 mg/kg bw/d LOAEL:≈1165 mg/kg bw/d Reduced testes weights in offspring in prepuberty. Slight histological changes in the testes in adulthood.		Masutomi *et al*., 2003
Rat (Wistar) n=8 dams per group Oral: gavage Dose: 0, 750 mg/kg bw/d CAS no. 28553-12-0, purity >99% Vehicle: peanut oil Exposure: GD 7-21	NOAEL: Not determined LOAEL: 750 mg/kg bw/d Statistically significantly decreased testicular testosterone content at GD 21.	One dose only.	Borch *et al*., 2004
Rat (Wistar-Imamichi) n=4 litters Oral: diet Doses: 0, 40, 400, 4000, 20.000 pm. Food consumption or dose in mg/kg bw/d were not reported. Exposure GD 15-PND 21 CAS no. 28553-12-0, purity >98%)	NOAEL: Not determined LOAEL: 40 ppm (estimated to be equivalent to 2 mg/kg bw/d) Reduced anogenital distance (AGD) in males on PND 1 in all doses. Increased AGD in females at 20.000 ppm (estimated to be equivalent to 1000 mg/kg bw/d). Reduced copulatory behaviour in females at all doses. Changes in hypothalamic gene expression.	All groups affected. This study was not considered sufficient by ECHA (2013) to change the developmental NOAEL. No details on corrections for litter effects.	Lee *et al*., 2006
Rat (SD), n=7-8 Oral: gavage 0, 250, 750 mg/kg bw/d Vehicle: corn oil	No effect on testosterone production on GD 19.	No examination of testosterone production at end of dosing (GD 17) but after 2 days recovery period (GD 19)	Adamsson *et al*., 2009

Method	Results	Remarks	Reference
Exposure: GD 13-17			
Rat (Wistar), n=9-10 litters Oral: gavage 0, 300, 600, 750, 900 mg/kg bw/d CAS 28553-12-0, purity 99% Vehicle: corn oil Exposure: GD 7-PND 17	NOAEL: 300 mg/kg bw/d LOAEL: 600 mg/kg bw/d At 600 mg/kg bw/d: Reduced percentage of motile sperm and histological changes in foetal testis. At 750 mg/kg bw/d: reduced pup body weights, increased male nipple retention, and altered female behaviour. At 900 mg/kg bw/d: reduced male AGD. Increased sperm count per g cauda epididymis.	Satellite study examined foetal testes (n=3-4 litters)	Boberg *et al*., 2011
Rat (Harlan SD), n=3-6 litters Oral: gavage Doses: 0, 500, 750, 1000, 1500 mg/kg/d Two types of DINP: CAS 28553-12-0 and CAS 68515-48-0 (corrected) Vehicle: corn oil Exposure: GD 14-18	NOAEL: Not determined LOAEL: 500 mg/kg/d Reduced testis testosterone production GD 18.	Similar effects of the two different CAS numbers of DINP.	Hannas *et al*., 2011
Rat (SD), n=8-9 Oral: gavage Doses: 0, 50, 250, 750 mg/kg bw/d CAS 68515-48-0 Vehicle: corn oil Exposure: GD 12-19	NOAEL: 50 mg/kg bw/d LOAEL: 250 mg/kg bw/d Decreased testis testosterone content GD 19 and presence of multinuclear gonocytes.	Effects on Leydig cell clustering at 750 mg/kg bw/d	Clewell *et al*., 2013a
Rat (SD), n=24 (controls), n=20 (DINP groups). Oral: diet Doses: 0, 760, 3800, 11400 ppm CAS 68515-48-0, 99.9% diester phthalates primarily with alkyl chains of isononyl alcohols (C9H19) with different branching structures Exposure: GD 12-PND 14	NOAEL: 56 mg/kg bw/d (760 ppm) LOAEL: 288 mg/kg bw/d (3800 ppm) At 3800 ppm: Increased number of animals with multinuclear gonocytes, and reduction of male pup weight PND 14. At 11400 ppm: reduction of maternal weight and male pup weight at PND 2, reduction of AGD and anogenital index at PND 14, presence of Leydig cell aggregates at PND 2, reduction of absolute weight of levator ani / bulbocavernosus muscles (LABC) at PND 49/50.	DBP used as positive control. Measurements of blood metabolites.	Clewell *et al*., 2013b

Method	Results	Remarks	Reference
Rat (SD), n=3-4 per group Oral: gavage Dose: 0, 750 mg/kg bw/d CAS no. 28553-12-0, 98,8% and 68515-48-0, 99% Exposure: GD 14-18	Inhibition of testosterone synthesis, testosterone production significantly reduced at 750 mg/kg bw/d	Short-term *in vivo* One dose only. Similar effects of two different CAS numbers of DINP DINP is weakly positive.	Furr *et al*., 2014
Rat (SD), n=6 per group Oral: gavage Doses: 10, 100, 500, 1000 mg/kg bw/d CAS 28553-12-0 purity >99% Exposure: GD 12-21	No effect on AGD in neonatal pups. Statistically significant reduction of testicular testosterone content at 1000 mg/kg bw/d (dose-dependent decrease at all doses, n.s.). Histological changes in testes (clustering of Leydig cells from 10 mg/kg bw/d; dysgenesis of seminiferous chords and presence of multinucleated gonocytes from 100 mg/kg bw/d). Reduced mRNA levels of Insl3 from 10 mg/kg bw/d; reduced mRNA levels of several genes involved in steroidogenesis from 100 mg/kg bw/d.	In the paper, findings are referred to as "in foetal testes", but actually all examinations were performed in neonatal pups (day of birth).	Li *et al*., 2015

n.s = not statistically significant

Summarising the available data, the DS concluded that DINP causes developmental toxicity, and the data indicate toxicity to reproductive organs potentially leading to impaired fertility. These effects were considered to be specifically substance-induced and not secondary, non-specific consequences of other toxic effects.

On developmental toxicity, RAC noted that in several adequately designed studies with DINP that no significantly increased incidence of malformations or other signs of severe developmental toxicity were observed, in particular:

1. No signs of pronounced developmental toxicity such as embryolethality or malformations were observed at doses up to 1000 mg/kg bw/d given by gavage; skeletal and visceral variations were significantly increased in the high dose group (Waterman *et al*., 1999)

2. Malformations of male reproductive organs were observed in 2 out of 52 animals (not statistically significant) (Gray *et al*., 2000).

3. Although slight changes in the AGD and the number of nipples in male offspring were observed at PND 1 and 13, these alterations disappeared during postnatal development and were not present at PND 90 (Boberg *et al*., 2011).

4. The highest dose of 11400 ppm DINP in the diet (approx. 720 mg/kg bw/d during gestation and 1513 mg/kg bw/d during lactation) caused a reduced absolute LABC weight, but not in the relative weight (Clewell *et al*., 2013)

Human information

The DS summarised the relevant information from human epidemiological studies in a table, see below.

Table: Summary table of relevant information from human epidemiological studies (Table 24 of the CLH Report).

Method	Results	Remarks	Reference
Human epidemiological study Biological samples from a prospective Danish–Finnish cohort study (1997 to 2001). Analysis of individual breast milk samples collected as additive aliquots 1–3 months postnatally (n=130; 62 cryptorchid/68 healthy boys) for phthalate monoesters e.g. mono-isononyl phthalate (MINP) (DINP). Analysis of serum samples for gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B.	All phthalate monoesters were found in breast milk with large variations [medians (minimum–maximum)]: MINP 95 (27–469 µg/L). 3 months old boys exposed to higher concentrations of phthalate monoesters in breast milk, showed slight, but significant, decreases in levels of reproductive hormones. A positive correlation was found between MiNP and LH.		Main *et al*., 2006
Human epidemiological study Anogenital distance was measured in 196 boys at 21 months of age and analysis of phthalate metabolites was performed on maternal urine from first trimester of pregnancy.	Associations were found between AGD measurements and levels of certain phthalate metabolites. The most significant association was found for AGDas (anoscrotal distance) and DINP metabolites, however the AGDas reduction was small (4%) in relation to more than an interquartile range increase in DINP exposure.		Bornehag *et al*., 2015
Human epidemiological study Case-control study on levels of phthalates in amniotic fluid from the second trimester and occurrence of cryptorchidism and hypospadias. 300 controls, 270 cryptorchidism cases, 75 cases of hypospadias. Metabolites of DEHP and DINP in amniotic fluid were examined for associations with testosterone levels, insl3-levels and cryptorchidism and hypospadias.	The investigated DiNP metabolite showed elevated odds ratio point estimates for having cryptorchidism and hypospadias but was not consistently associated with the steroid hormones or insulin-like factor 3.		Jensen *et al*., 2015
Human epidemiological study Maternal serum levels of DEHP and DINP metabolites were compared with testicular size, semen quality and reproductive hormones in 112 adolescent sons.	Men in the highest exposure tertile of a DINP metabolite had lower total testicular volume, higher levels of FSH and lower semen volume than men in the lowest tertile. Comparable findings were seen for DEHP metabolites. It is concluded that prenatal levels of DINP seemed negatively associated with reproductive function of adolescent men.		Axelsson *et al*., 2015

RAC noted that no clear cut conclusions can be drawn from the epidemiological studies presented in the CLH report. Among a large number of possible associations between exposure levels and reproductive endpoints some positive associations were found which were possibly due to random error. In particular, DINP exposure was not associated with malformations of the male reproductive organs, such as hypospadias or cryptorchidism.

Other relevant information

Repeated dose toxicity studies and chronic toxicity studies

To provide an overview of the general toxicity of the substance, the DS summarised the most relevant repeated dose toxicity studies and chronic toxicity studies discussed in the EU RAR (European Chemical Bureau (ECB), 2003a and cited in ECHA, 2013) as well as more recent studies included in the registration dossier for DINP.

Table: Summary table of selected relevant repeated dose toxicity studies including chronic toxicity studies (chronological order) (Table 9 of the CLH Report)

Method	Results	Remarks	Reference
One-week prechronic oral feeding study, rat (CAS 68515-48-0) Dietary concentration of 2% corresponding to 1700 mg/kg bw/d n=8	At 1700 mg/kg bw/d: Increased absolute and relative weights of liver and kidney. Increased relative testis weight	Another group exposed to 2% DEHP. Formalin fixed testes showed no histological effects of DEHP or DINP.	Bio/Dynamics 1982a
13-week study, Fisher 344 rat 0, 0.1, 0.3, 0.6, 1.0, 2.0% (77, 227, 460, 767, 1554 mg/kg bw/d) (CAS 68515-48-0) n=15	NOAEL: 0.1% (77 mg/kg bw/d) LOAEL: 0.3% (227 mg/kg bw/d): increased kidney, liver weights decreased cholesterol levels from 0.3%. Increased relative testis weight at 2%, but associated with slight decreased absolute testis weight and decreased body weight.	No histological data on testes.	Bio/Dynamics 1982b
13-week study, rat 0, 0.3, 1.0% (Males: 201, 690 mg/kg bw/d; females: 251-880 mg/kg bw/d) (CAS 68515-48-0) n=15 cryptorchidism and hypospadias.	LOAEL: 0,3% (201 – 251 mg/kg bw/d): increased kidney, liver weights, decreased triglycerides and urine chemistry changes. Increased relative testis weight at 1% associated with a slight (n.s.) increase of absolute testis weight.	No histological data on testes.	Bio/Dynamics, 1982c
Chronic toxicity study, 2-year dietary Sprague Dawley CD rats Guideline: not indicated in EU RAR 2003 Santicizer 900 (CAS 71549-78-5 according to CHAP	NOAEL: 27 mg/kg bw/d. Spongiosis hepatis was significantly elevated at the mid and high dose in males. In males, the incidence of focal necrosis was significantly elevated at the low and high doses, while the mid dose was		Bio/Dynamics, 1986

Method	Results	Remarks	Reference
2001) Dietary concentrations of 0, 500, 5000, 10 000 ppm Males: ca. 0, 27, 271 and 553 mg/kg bw/d Females: ca. 0, 33, 331, 672 mg/kg bw/d. Dose groups: n=70/sex/dose level	non-significantly elevated.		
Chronic toxicity, 2-year study, rat (CAS 68515-48-0) Dietary concentrations of 0, 0.03, 0.3 and 0.6% (w/w) Males: ca. 0, 15, 152, 307 mg/kg bw/d Females: ca. 0, 18, 184, 375 mg/kg bw/d Dose groups: n=220 (110/sex)	NOAEL: 0.03% (15-18mg/kg bw/d) LOAEL: 0.3% (152-184 mg/kg bw/d) Males: increased incidence of spongiosis hepatis, increased serum levels of liver transaminases (1.5-2x); increased relative and absolute spleen weights (61%). Male and female: increased liver (11-19%) and kidney weights (5-10%). Other histopathological findings indicating liver toxicity.		Exxon, 1986 Hazleton, 1986a; Lington *et al*., 1987; Lington *et al*., 1997
13-week feeding study Wistar rats, n=10 of each sex Dietary 0, 3000, 10 000 and 30 000 ppm (CAS 28553-12-0, purity greater than 99%) corresponding to 0, 333, 1101 and 3074 mg/kg bw/d at day 7 and to 0, 152, 512 and 1543 mg/kg bw/d at day 91 for the males and to 0, 379, 1214, 3224 mg/kg bw/d at day 7 and to 0, 200, 666, 2049 mg/kg/day at day 91 for the females. OECD TG 408 GLP compliant	LOAEL 3000 ppm for lowered triglyceride level and liver histology changes. At 10 000 ppm: increased absolute and relative liver and kidney weights. A trend towards a decreasing body weight from 3000 ppm was observed in males and this was confirmed at 30 000 ppm with a statistically significantly decreased body weight compared with the control groups (males up to 18% and females up to 11%). An increased relative testis weight observed at 30 000 ppm is not regarded as substance induced but the result of the clear body weight decrease.	Histological examination of testes and ovaries showed no adverse changes (but the method is not considered sensitive due to fixation in formaldehyde and not Bouin's fixative)	BASF, 1987
13-week study, Fischer 344 rats 2500, 5000, 10 000, 20 000 ppm (males: 176, 354, 719, 1545 mg/kg bw/d, females: 218, 438, 823, 1687 mg/kg bw/d)	LOAEL: 2500 ppm (176 mg/kg bw/d in males) increased weight of liver and kidney From 10 000 ppm (719 mg/kg bw/d): increase in relative testes/epidydimides weight	No gross or microscopic observations were associated with the weight changes of the ovaries and testes/epidymides	Hazleton Laboratories, 1991a

Method	Results	Remarks	Reference
n=10/sex/group CAS 28553-12-0	At 20 000 ppm (1687 mg/kg bw/d): decrease in absolute and relative uterus weight.		
4-week study, B6C3F1 mice, 3000, 6000, 12 500, 25 000 ppm (males: 635, 1377, 2689, 6518 mg/kg bw/d) n=10	LOAEL: 3000 ppm (635 mg/kg bw/d in males) increased absolute and relative liver weight. At 6000 ppm (1377 mg/kg bw/d in males) decreased absolute and relative kidney and testes weights	Range-finding study for 13-week study (Hazleton, 1992).	Hazleton Laboratories, 1991b
13-week study, B6C3F1 mice, n=10/sex/group. Oral: Diet Doses: 1500, 4000, 10 000, 20 000 ppm (365, 972, 2600, 5770 mg/kg bw/d). CAS 28553-12-0 Exposure: 13 weeks	NOAEL: 1500 ppm (365 mg/kg bw/d) LOAEL: 4000 ppm (972 mg/kg bw/d): Enlarged livers from 4000 ppm in males (from 10 000 ppm in females). Reduced absolute testis/epididymis weights from 10 000 ppm (2600 mg/kg bw/d), reduced absolute and relative uterus weight at 20 000 ppm (5770 mg/kg bw/d). Immature/abnormal sperm forms in epididymis, hypoplasia in uterus and absence of corpora lutea in ovaries at 20 000 ppm (5770 mg/kg bw/d).	Positive control for peroxisomal proliferation WY 14,463	Hazleton Laboratories, 1992
Chronic toxicity. 2-year dietary, rat (Fisher 344). Guideline: equivalent or similar to OECD TG 452. GLP compliant (CAS 68515-48-0). Dietary concentrations of 0, 0.05, 0.15, 0.6 and 1.2% and high dose (1.2%) recovery group. Males: ca. 0, 29, 88, 359, 733 mg/kg bw/d, high dose recovery group 637 mg/kg bw/d Females: ca. 0, 36, 109, 442, 885 mg/kg bw/d, high dose recovery group 774 mg/kg bw/d Dose groups n=70-85/sex and a recovery high dose group of 55/sex	NOAEL: 0.15% (88-103 mg/kg bw/d) LOAEL: 0.6% (358-442mg/kg bw/d): Males: increased incidence of spongiosis hepatis. Males and females: increased absolute and relative liver and kidney weights, increased serum levels of liver transaminases, other histopathological findings indicating liver toxicity		Aristech, 1994 (Aristech, 1995; Covance, 1998; Moore, 1998a; Butala *et al*., 1996)
Chronic toxicity, 2-year dietary study, mice. 0, 500, 1500, 4000, 8000 ppm (Males: 0, 90.3, 275.6, 741.8, 1560.2 mg/kg bw/d, females:	NOAEL: 500 ppm LOAEL: 1500 ppm increased liver and kidney weights. Decreased absolute and relative testis weight at		Aristech, 1995

Method	Results	Remarks	Reference
0, 112, 335.6, 910.3, 1887.6 mg/kg bw/d)	741.8 mg/kg bw/d (4000 ppm, 11.1% reduction) and 1560.2 mg/kg bw/d (8000 ppm, 20.2% reduction) without histological changes.		
13-week study, marmoset (CAS not specified) Gavage in 1% methylcellulose and 0.5% Tween. 100, 500, 2500 mg/kg bw/d n=4 males and 4 females	NOAEL: 500 mg/kg bw/d LOAEL: 2500 mg/kg bw/d minor changes: decreased body weight, decreased body weight gain	Another group received Clofibrate 500 mg/kg bw/d to provide a positive control for liver peroxisome activity	Hall *et al*., 1999 (Huntington Life Science, 1998)
2-week study, adult male cynomolgus monkey, gavage in 0.5% methyl cellulose (10 mL/kg) (CAS 68515-48-0) 500 mg/kg bw/d n=4	NOAEL 500 mg/kg bw/d. No changes in body weight, organ weights, urinalysis, haematology, clinical chemistry, no inflammation or necrosis in the liver, kidney and testes, no change in hepatic peroxisomal β- oxidation or replicative DNA synthesis. No effect on gap junctional intercellular communication *in vitro*.	No effects of additional test compounds DEHP 500 mg/kg bw/d and clofibrate 250 mg/kg bw/d on these endpoints. Clofibrate reduced relative weight of testes/epididymides and thyroid/parathyroid.	Pugh *et al*., 2000
Rat (SD), juvenile male, 5 weeks old, n=6 Oral: gavage Dose: 500 mg/kg bw/d CAS 28553-12-0. Purity not described. Vehicle: corn oil Exposure: 28 days (PND 35 to 77) CAS 28553- 12-0	NOAEL: Not determined LOAEL: 500 mg/kg bw/d. Reduced sperm count (to 75% of control) and sperm velocity. Increased relative liver weight (to 145% of control). Decreased body weights (to 88% of control).	One dose only. Other dose groups exposed to one of eight other phthalate diesters or five phthalate monoesters.	Kwack *et al*., 2009
Male Kunming mice, age 7-8 weeks. 14 days exposure to 0, 0.2, 2, 20, or 200 mg/kg bw/d of DINP n=10	NOAEL: 2 mg/kg bw/d LOAEL: 20 mg/kg bw/d for histological effects on liver (oedema). Increased reactive oxygen species (ROS) in liver and kidney at 200 mg/kg bw/d; decreased glutathione (GSH) content in liver at 20 and 200 mg/kg bw/d and in kidney at 200 mg/kg bw/d.	Other groups received melatonin, or melatonin in combination with DINP (200 mg/kg bw/d). Poor data reporting: no clear description of the number of affected animals at 20 and 200 mg/kg bw/d.	Ma *et al*., 2014

n.s = not statistically significant

RAC noted that for the repeated dose toxicity studies:

1. in the two-year dietary study, testes weights in mice were not decreased when related to body weight, the decrease was only seen relative to brain weight. No histological changes in were found the testes (Aristech *et al*., 1995)

2. in non-human primates (*Callithrix jacchus*) at dose levels up to 2500 mg/kg bw/d given for 13 weeks no significant change in testosterone levels was reported, as well as no changes in absolute or relative testes/epididymis weight. However, hormone levels were highly variable and only four animals were studied in each group. Histopathology of the testes was normal (Hall *et al*., 1999; (Huntington Life Science, 1998; KHK056/983532))

3. reduced sperm counts per g of tissue in rats after treatment with 500 mg/kg bw/d (Kwack et al., 2009), while other authors reported an increase after higher doses (Boberg *et al*., 2011).

Mode of action studies

The DS summarised a Hershberger assay on castrated male rats and a uterotrophic assay in immature and pubertal female rats in the table below in order to clarify the mode of action for adverse effects of DINP.

Table: Summary table of Hershberger assay by Lee and Koo (2007) and uterotrophic study by Sedha et al. (2015). (Table 25 of the CLH Report)

Method	**Results**	**Remarks**	**Reference**
Rat (SD), Hershberger assay in castrated males dosed with testosterone propionate (0.4 mg/kg bw/d) 20, 100 and 500 mg/kg bw/d of DINP Oral: gavage Vehicle: corn oil Exposure: 10 days (PND 35 to 44)	NOAEL: not determined LOAEL: 20 mg/kg bw/d for statistically significant reduction of seminal vesicle weight to 78% of controls. At 500 mg/kg bw/d weights of LABC[1] were significantly reduced to 82% of controls.	Other groups receiving MEHP, DEHP, DBP or DIDP induced comparable changes, but also reduced ventral prostate weight. DNOP and BBP did not affect reproductive organ weights.	Lee and Koo, 2007
Uterotrophic assay in immature Wistar rats and pubertal female assay in Wistar rats, n=6. 3 days exposure (PND 20 to PND 22) or 20 days exposure (PND 21 to 40) Doses: 276 or 1380 mg/kg bw/d of DINP CAS 68515-48-0, 99.5% purity.	No change in uterus weight (both studies) and no change in puberty timing (pubertal study) in DINP exposed groups. High dose: Absolute and relative ovary weights were reduced to 72 or 90% of control values after 20 days of exposure to DINP, but were not affected by DIBP or DES.	Other groups received 250 or 1250 mg/kg bw/d of DIBP. DES (40 µg/kg bw/d or 6 µg/kg bw/d) was used as a positive control.	Sedha *et al*., 2015

The DS concluded that DINP does not act via an estrogenic mode of action, but may possibly interfere with endogenous estradiol production to induce changes in female reproductive

[1] LABC: glans penis, seminal vesicles, ventral prostate, and levator ani/bulbocavernosus muscles

development, although this has not been thoroughly investigated. They further concluded that the same effects as reported in male pups following exposure to DINP were reported following *in utero* exposure to other phthalates with harmonised classifications for development as Repr. 1B, and that an antiandrogenic mode of action was suggested also for these phthalates.

Comparison with other phthalates

The DS listed observations for DINP and other phthalates in a table, see below. The DS concluded that the similarity between the effects of DINP and effects of the listed phthalates further support classification of DINP.

Table: Similarity between effects of DINP and other phthalates classified as toxic to reproduction (Table 26 of the CLH Report).

Substance	**Areola/nipple retention**	**Decreased foetal or neonatal male AGD**	**Hypospadias**	**Harmonized Repr. 1B (H360D) classification**	**Effects on foetal testis testosterone production or -content**	**References**
DIBP	Yes	Yes	Yes	Yes	Yes	Saillenfait *et al*., 2008; Borch *et al*., 2006
DBP	Yes	Yes	Yes	Yes	Yes	Fabjan *et al*., 2006 (review)
BBP	Yes	Yes	Yes	Yes	Yes	Fabjan *et al*., 2006 (review)
DCHP	Yes	Yes	Yes	Yes	Yes	Saillenfait *et al*., 2009; Hoshino *et al*., 2005; Yamasaki *et al*., 2009
DPP				Yes	Yes	Beverly *et al*., 2014
DnHP	Yes	Yes	Yes	Yes	Yes	Saillenfait *et al*., 2009 and 2013
DEHP	Yes	Yes	Yes	Yes	Yes	Fabjan *et al*., 2006 (review)
DINP	**Yes**	**Yes**			**Yes**	Gray *et al*., 2000; Boberg *et al*., 2011; Clewell *et al*., 2013a

RAC noted that the table illustrating the similarity between effects of DINP and other phthalates classified as toxic to reproduction does not reflect the complete data set. For example, reduced AGD was not found in all studies with DINP and was a transient, reversible finding reported in the Boberg *et al*. (2011) publication. A reduction of foetal testosterone has been observed with DINP similar to other phthalates, but this did not cause irreversible, gross structural defects.

Malformations such as hypospadias and cryptorchidism which led to classification of other phthalates were not reported with DINP.

Summary and conclusions of the dossier submitter's classification and labelling proposal

Effects on fertility

Key evidence for effects of DINP on fertility:

- Reduced absolute and relative testes weights at high doses in a 2-year study in mice (Aristech Chemical Corporation, 1995; 742 and 1560 mg/kg bw/d), and at higher doses in studies with shorter durations of exposure, i.e. a 4-week study in mice (Hazleton, 1991; 1377 mg/kg bw/d), and a 13-week study in mice (Hazleton, 1992; 2600 and 5770 mg/kg bw/d).
- Reduced sperm count and effects on sperm motion parameters after 28 days of exposure in juvenile rats (Kwack *et al*., 2009; one dose only, 500 mg/kg bw/d).
- Dose-dependent, long-lasting reduced sperm motility in rats exposed perinatally (Boberg *et al*., 2011; 600, 750 and 900 mg/kg bw/d).

The DS concluded that the available data indicate toxicity of DINP to reproductive organs and on sperm count and sperm motility potentially leading to fertility effects. The DS considered these effects to be specific and not secondary non-specific consequences of other toxic effects.

Furthermore, the DS argued that similar findings as for other classified phthalates with the same mode of action supported classification for effects on sexual function and fertility for DINP. Classification as Repr. 2; H361f was proposed, as the evidence was not considered sufficiently convincing to place the substance in category 1.

Developmental toxicity

Key evidence for effects of DINP on development, which are observed in the absence of maternal toxicity, were given as follows:

- Structural abnormalities: skeletal effects (rudimentary ribs) were seen in two developmental toxicity studies (Hellwig *et al*., 1997; Waterman *et al*., 1999; 1000 mg/kg bw/d).
- Effect on altered growth: decreased body weight in offspring was found in a two-generation study (Waterman *et al*., 2000; from 159 mg/kg bw/d).
- Functional deficiency: dose-dependent, long-lasting decrease in the percentage of motile sperm in rats exposed perinatally (Boberg *et al*., 2011; from 600 mg/kg bw/d).
- Structural abnormalities: increased nipple retention and decreased AGD in male rats exposed perinatally (Boberg *et al*., 2011; Gray *et al*., 2000, Lee *et al*., 2006; mostly from 750 mg/kg bw/d).
- Structural abnormalities: increased incidence of permanent structural changes (permanent nipples, malformations of testis and epididymis, histological changes in testis and epididymis) in rats exposed perinatally (Gray *et al*., 2000, Masutomi *et al*., 2003; at 750 and 1165 mg/kg bw/d, respectively).
- A comparable pattern of adverse effects and of mode of action as seen for other phthalates classified in category 1B; i.e. DEHP, DBP, DIBP and BBP. In foetal testes, several studies described the presence of multinucleated gonocytes and reduced testosterone production, as also described for DEHP, DBP, DIBP and BBP (Boberg *et al*., 2011; Borch *et al*., 2004; Hannas *et al*., 2011; Clewell *et al*., 2013, Li *et al*., 2015).

With respect to the developing animal, effects on the AGD as well as on the occurrence of increased nipple retention in male pups were recorded in several studies and those findings were considered by the DS to be specific and not secondary non-specific consequences of other toxic

effects. In addition, dose-dependent permanent decreases in sperm motility in rat offspring following perinatal exposure and increased incidences of permanent malformations of testes, epididymides and external genitalia in rats exposed perinatally were considered to be specific and not secondary non-specific consequences. Mechanistic studies indicated an anti-androgenic mode of action. Based on this, the DS concluded that there is clear evidence of an adverse effect on development and that classification as Repr. 1B; H360D is warranted.

Overall, classification of DINP as Repr. 1B, H360Df was proposed by the DS.

Comments received during public consultation

Fifty-two comments were received during public consultation. Comments were submitted by 27 industry stakeholders, six individuals, two national authorities, six downstream-users, four Member state competent authorities (MSCAs) and seven manufacturers.

In 37 comments, a classification of DINP as Repr. 1B; H360Df was not supported and *no classification* was regarded to be more appropriate (23 by industry stakeholders or trade associations, three by individuals, five by downstream-users, one by an MSCA and five by manufacturers).

This view was based on several points of criticism of the CLH report such as:

- that the CLH proposal did not to follow the CLP criteria and it was stated that the classification proposal was not based on adverse effects, but on small inconsistent changes in some parameters observed in some selected studies with DINP.
- that the study quality and how study limitations affect conclusions with regard to classification had not been assessed in the CLH report, particularly for key studies.
- that most studies cited by the DS had already been assessed extensively by regulators in the past and have been found not to support classification (EU RAR, 2003), and that effects which regulators previously concluded not to support classification were now used to support classification.
- A proposal for *no classification* was argued to be in line with the current REACH registration dossier for DINP (updated in December, 2015), the reason for *no classification* in the registration dossier being that the reproductive data on DINP is "not sufficient for classification".

The commenters also criticised the comparison of DINP with classified, lower molecular weight phthalates in relation to fertility effects. It was argued that "The effects of DINP are not comparable with those seen with classified phthalates (DBP, BBP, DEH and DIBP). This point is also made by the dossier submitter in the dossier in 4.11.4.2 (*In comparison with DEHP, DPB and BBP the overall evidence for effects of DINP on fertility is limited*) and yet the dossier submitter proposes classification for fertility category 2."

It was furher argued that there are no effects shown by DINP similar to those shown by the other classified phthalates. Adverse effects on reproduction are observed with alkyl phthalates exhibiting a carbon chain length of 3 to 6 carbon atoms (with or without methyl or ethyl branching). Past studies have shown that adverse effects on reproduction are not observed in alkyl phthalates when the longest straight carbon chain has 7 to 13 carbon atoms (with or without methyl, ethyl or propyl branching).

Furthermore, the commenters pointed out that the CLH proposal did not discuss the known findings on structure-activity relationships in an adequate way and extrapolated a concern for DINP which is not supported.

Several comments addressed various socioeconomic aspects of a DINP classification as Repr. 1B; H360Df. Those are however not relevant for harmonised classification under CLP.

In 11 comments it was emphasised that the European Commission concluded in 2014 with regard to the regulation of DINP and DIDP, that on the basis of the available information, no unacceptable risk has been characterised for the uses of DINP and DIDP in articles other than toys and childcare articles which can be placed in the mouth, and that in the light of the absence of any further risks from the uses of DINP and DIDP, the evaluation of potential substitutes has been less pertinent.

One MSCA asked the DS to clarify the identity of both substances and to provide concentrations of the constituents.

Two MSCAs commented in support of the proposed classification of DINP as Repr. 1B; H360Df.

In two comments from a national authority, the classification of developmental toxicity in category 1B was supported, but *no classification* for fertility was considered appropriate. It was argued that the evidence for fertility is too inconsistent to derive any firm conclusion, and that instead available data were considered to warrant performing further studies.

One MSCA supported the classification for fertility in category 2 but proposed category 2 also for developmental toxicity as there are some indications of structural/functional effects but no conclusive evidence for DINP to induce irreversible effects in the adult male.

Assessment and comparison with the classification criteria

Effects on fertility

Non-human information

Information from fertility studies

In the one-generation study, rats were exposed via food to 0%, 0.5%, 1.0% or 1.5% DINP (CAS 68515-48-0) in the diet (corresponding to 0, 377-404, 741-796, and 1087-1186 mg/kg bw/d, respectively, during gestation and 0, 490-923, 1034-1731, and 1274-2246 mg/kg bw/d, respectively, during lactation; Waterman *et al*., 2000/Exxon Biomedical Sciences, 1996). In both males and females, significantly reduced food consumption and lower body weights in comparison with control values were seen in males and females at the two highest dose levels before mating (premating day 70: males -10%, females -11% bw at the highest dose) and in females during gestation (GD 21 -12% bw at the highest dose) and the postpartum period (PND 21 -23% bw at the highest dose). No effects on fertility of exposed rats were detected. Absolute ovary weights (ca. 36% (right ovary) and ca. 27% (left ovary)) of high dose females were significantly lower than control values at the end of premating period; this effect was seen concomitantly with 11% lower body weight.
Statistically significant changes were seen in parental male reproductive organ weights (increased absolute and relative weights of testes and epididymides, and increased relative weights of seminal vesicles) in the high dose group at the end of the premating period (day 70). No microscopic evaluation of reproductive organs and no evaluation of sperm parameters were performed.

RAC considers that the observed changes in organ weights of reproductive organs alone are not specific hallmarks for adverse effects on reproductive functions. In the study of Waterman *et al*. (2000) increased absolute and relative weights of testes and epididymides, and increased relative weights of seminal vesicle were observed in the high dose group (1087–1186 mg/kg bw/d). Lower

food consumption and (max. 10%) lower body weights observed before mating in males could be one cause of elevated testes/epididymides weights (max. 10%). Atrophic/degenerative effects on reproductive organs could be indicated by lower weights of reproductive organs, unless these atrophic effects were masked by accompanying inflammatory or other secondary responses which may be associated with normal or increased organ weights. Noting that weight changes may be indicative and are often a sensitive effect, it requires confirmation by accompanying microscopic lesions and/or lower reproductive performance. Rats in the one-generation study of Waterman *et al*. (2000) did not show any changes in the reproductive parameters (e.g. mating and fertility rates, gestational index or length of gestation). Microscopic examinations or evaluation of sperm parameters was not performed.
Relative weights of the ovaries in females at the high dose were reduced, which could be indicative of atrophic or degenerative changes. However, lack of any effect on reproductive parameters raises questions regarding the relevance of the findings. Thus, no indication of a suppressive effect on paternal or maternal fertility were observed up to the high dose (1087-1186 mg/kg bw/d) in the one-generation study of Waterman *et al*. (2000), while some uncertainties remain due to the missing histopathologic examinations.

In the follow-up two-generation study (Waterman *et al*., 2000/Exxon Biomedical Sciences, 1996), rats were exposed to 0%, 0.2%, 0.4% and 0.8% of DINP (CAS 68515-48-0) in the diet. No maternal toxicity was seen, nor were there any changes on body weight during the premating, mating and gestation period. However, dams in the 0.8% group had significantly lower body weights at postpartum days 14 (7%) and 21 (3%) in comparison to control values.
No changes in fertility or parental male reproductive organ weights were seen. A statistically significant decrease was observed in the mean left ovary weight of the P1 females at 0.8% (577 mg/kg bw/d). The DS pointed to the EU RAR (2003) in which it was concluded that in the absence of a clear dose response relationship, similar findings in the right ovary weights, consistent pattern of response between absolute and relative organ weights, or correlating microscopic findings this decrease was considered incidental and unrelated to treatment. This study was based on test guidelines which did not include evaluation of sperm parameters. An increase in relative epididymis weight was detected in adult male offspring. Mean litter size was increased at all doses in both generations, whereas no effects on live births or pup viability were found. In both generations of offspring, increased liver and kidney weights were seen in males and females. These findings were most marked in F1 offspring, which showed effects from the mid dose (0.4% in diet), whereas the second generation showed effects at the top dose (0.8% in diet) only. The pituitary, testes (fixed in Bouin's solution), epididymides, prostate, seminal vesicles, vagina, uterus, mammary gland and gross lesions from all parental animals in the control and 0.8% groups were examined microscopically.

RAC concluded that no effects on sexual function and fertility were seen in this two-generation study as there were no treatment related effects on reproductive/fertility parameters, organ weights of the reproductive organs nor any microscopic abnormalities in reproductive organs. Some uncertainty remains, as sperm parameters that became standard parameters in the OECD TG 416 in 2001 were not examined in this study which was conducted in 1996 (Waterman et al., 2000).

Both the one- and two-generation studies indicated lower ovary weights in rats. These were markedly lower at 1.5% DINP (1087–1186 mg/kg bw/d) in the one-generation study and most likely incidentally lower in the left ovary only at 0.8% (577 mg/kg bw/d in P1 females of the two-generation study, see above). RAC agreed with the interpretation that no firm indication of atrophic changes on the ovary can be concluded, at least for the doses of 0.8%, at which microscopy did not reveal any abnormalities. No effect on the weight of ovaries was seen at 1%

DINP in the one-generation study, thus pointing to a lack of a clear dose-response relationship up to 1% DINP. Taking the absence of effects on female fertility in both studies into account, the lower ovary weights at 1.5% DINP – based on the data available – were concluded not to contribute to a need for classification. Hence, no effects on male and female reproductive performance were seen at doses up to 1.5% DINP (CAS 68515-48-0; corresponding to 1087-1186 mg/kg bw/d), as parameters such as fertility indexes, number of pups at birth, or litter size were not adversely affected in any of the two studies.

Information on fertility from developmental studies with observation up to PND 90

No indications on maternal toxicity (no effect on body weight gain during pregnancy) or effects on fertility (gestation length, postimplantation loss, litter size, sex ratio or perinatal loss) were observed in the dams of the developmental toxicity study by Boberg *et al*. (2011) at doses up to 900 mg/kg bw/d (GD 7-PND 17).

A reduction in sperm motility was observed in 90 day old rats (subgroup 2a in Boberg *et al*., 2011, Fig. 4 therein) after exposure to 600 mg/kg bw/d DINP and above from GD 7 to PND 17. The corrigendum of the authors (Boberg *et al*., 2016) on the statistical method for pairwise comparisons confirm significant lower percentages of motile sperm at 750 and 900 mg/kg bw/d, but not at 600 mg/kg. Sperm velocity parameters were not affected by DINP treatment. The weights of reproductive organs did not deviate from that of control animals in 90 day old male and female rats up to 900 mg/kg bw/d. One male from the 600 and 750 mg/kg groups, respectively, had small testes and epididymides. No treatment-related histological abnormalities were seen in the testes and epididymides.

Treatment during gestation and the lactation period (GD 7-PND 17) at doses (without maternal toxicity) up to 900 mg/kg bw/d DINP did not affect organ weights or morphology of male reproductive organs, but lowered the % of motile sperms in male rats at doses of ≥750 mg/kg bw/d at 90 days of age.

Reanalysis of the statistical calculations (Boberg *et al*., 2016) and reanalysis of the raw data from this study by Morfeld *et al*. (2017) and Chen *et al*. (2017) showed that the significance of the effect on sperm motility outlined in the original study from 600 mg/kg bw/d could not be reproduced for all dose groups and only effects at 750 and 900 mg/kg bw/d were found to be significantly lower than control values. Morfeld *et al*. (2017) in addition concluded that the increase in sperm counts per g of cauda (at 900 mg/kg bw/d) and the % progressive sperms (at 750 mg/kg bw/d) were not significantly different from controls. These effects, however, were not proposed as justification for classification with category 2 for sexual function and fertility by the DS.

Furthermore, sperm analyses in the study of Boberg *et al*. (2011) was done in 1-3 animals per litter and a total of 6-10 males per dose group, which leaves some uncertainties due to the overall small animal numbers examined. Normally, sperm motility in rats is at least 80%, but sperm motility in control rats described by Boberg *et al*. was only 60%. According to the OECD Guidance Document (OECD, 2008), a minimum of 70% motility is acceptable in controls.

At the Rapporteurs' Open Dialogue the EuPC remarked that none of the values of the control group meet the OECD minimum quality requirements of at least 70% and that all values in the exposed groups were within the historical control variance (of one standard deviation of the mean of historical control data, which they derived from other publications from the same laboratory). RAC took note of this view, but noted that the overall database presented on historical control data from two publications is small (a total of 26 animals from two studies) and noted that the means of the two studies were both lower than 70% (53.8 % in Jarfelt *et al*., 2005 and 65% in Taxvig *et al*., 2007) and this is in the same range as in the study of Boberg *et al*. (2011; 60%).

Whether a significant effect should only be considered as treatment related if the findings were out of the range of one standard deviation, seems questionable. In addition, the animals in the Jarfelt study were examined at PND 190 for sperm parameters, those of the Taxvig study at an age of 7 months, while the rats in the study of Boberg were examined much earlier, on PND 90.

Concerning the findings on sperm motility in the Boberg *et al*. study, RAC took note of this in the overall weight of evidence consideration, and noted that the mean % of motile sperm were significantly reduced at ≥600 mg/kg bw/d following the initial evaluation of Boberg (2011) and were significantly reduced at ≥750 mg/kg bw/d after re-evaluation. The reduction in sperm motility did not show a clear dose-response relationship at the two top doses (750 and 900 mg/kg), and no concomitant microscopic abnormalities were seen in the testes and epididymis

Evidence from additional repeated dose studies in animals

Lower (absolute and relative) testes weights were not seen in any of the available repeated dose studies in rats; the only effects in rats were those on sperm count and velocity of the Kwack *et al*. study (2009). RAC considers lower testes weights at 2600 mg/kg in mice (Hazleton, 1992) as not relevant for classification due to the high dose used. Lower testes weights in two other mice studies were either not accompanied by microscopic abnormalities (741.8 and 1560.2 mg/kg bw/d of the Aristech study) or were not observed in a range-finding study (Hazleton, 1991a) from which microscopic examination were missing (or at least data were not reported).

In the three studies with reduced testes weights, effects on body weights (Hazleton, 1991b; Hazleton, 1992) or lower body weight (and lower testes weight were related to brain weight only) was seen at the same doses (Aristech, 1993). Body weight reductions may not be considered sufficiently marked to exclude these findings for classification purposes. DINP-exposed males weighed 88% of controls in the study by Kwack *et al*. (2009), and 90 % and 83% of controls in the two highest dose groups in the study by Aristech (1995).

In contrast, increased relative testis weights were seen in other studies without changes in absolute testis weight, and were considered to be related to reduced body weights (Bio/dynamics, 1982a, b, c; BASF, 1987). In 2-year old cynomolgus monkeys, relative testis/epididymis weights were 76% of controls following 14 days of gavage treatment with 500 mg/kg bw/d DINP. This was not statistically significant, however the analysis comprised only 4 animals (Pugh *et al*., 2000) and this dose did not induce effects on body weight and relative liver weight. Microscopic examination of the testes revealed no treatment-related effect. The study of Hall *et al*. in marmosets focussed on liver effects and resulted in no change in testosterone levels (assessed in four males/dose group; Huntington Life Science, 1998/Hall *et al*., 1999). No changes were seen in absolute or relative testes/epididymis weight, nor were there any gross abnormalities or histopathology in the testes (epididymis not evaluated).

There was a decrease in absolute and relative weight of the uterus at 719 mg/kg bw/d in the 13-week study, and this was also taken into consideration (Hazleton Laboratories, 1991a). No microscopic lesion has been observed in this study, while no data were available for the ovaries. The hypoplasia in the uterus and absence of corpora lutea in ovaries in combination with lower absolute and relative weights of the uterus observed in mice after 13 weeks of exposure in the diet may indicate that DINP (CAS 28553-12-0) has some adverse effects on female reproductive organs (Hazleton Laboratories, 1992). However, the effects were seen at 5770 mg/kg bw/d and effects at this unusually high dose were not considered relevant for classification purposes.

<u>Human information</u>

Human cross-sectional studies by Joensen *et al*. (2012), Specht *et al*. (2014) and Specht *et al*. (2015) did not show any clear associations between adult exposure to DINP and fertility measures, such as sperm parameters, hormone levels or time to pregnancy. One cross-sectional study

(Mieritz *et al*., 2012) on 555 healthy boys (age 6.07-19.83 years) evaluated anthropometry, pubertal stages and the presence of gynaecomastia. Urinary levels of phthalate metabolites were not associated with pubertal timing, serum testosterone or with the presence of pubertal gynaecomastia.

Summary and conclusion on fertility

Since there is no evidence for effects of DINP on fertility in humans, classification in Category 1A is not appropriate. Also classification in Category 1B for fertility is not appropriate, as there is no clear evidence of effects on fertility.

Classification in Category 2 is based on evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development (and where the information is not sufficiently convincing to place the substance in Category I B).

The available studies provide some suggestions that DINP may affect sexual function and fertility, but do not allow any firm conclusion and the evidence is considered too inconsistent or uncertain to place DINP in Category 2. More specifically:

- The two-generation study by Waterman *et al*. (2000) did not find any significant adverse effects on fertility/reproduction parameters at doses up to 543-577 mg/kg bw/d. It was however noted by RAC that there was no assessment of sperm parameters (not included as standard parameters in the test guideline before 2001) in the study.
- Significant increases in absolute/relative testis and epididymis weights were observed in parental rats at about 1100 mg/kg bw/d in the one-generation study of Waterman *et al*. (2000). The effect is not consistent with lower testes weights seen in other studies at lower and higher doses. Lower body weight in males at this dose in comparison to control values may have contributed to the weight increases, but there was no indication of an atrophic effect (which if present could have been enhanced by lower body weight gain).
- Reduced absolute and relative testes weights at high doses in a 2-year study in mice (Aristech Chemical Corporation, 1995; 742 and 1560 mg/kg bw/d) were not accompanied by any microscopic abnormality. Thus, the biological significance is unclear.
- Lower absolute and relative testes weights at higher doses in studies with shorter durations of exposure, i.e. a 4-week study in mice (Hazleton, 1991b; 1377 mg/kg bw/d), and lower absolute testes weights in a 13-week study in mice (Hazleton, 1992; 2600 and 5770 mg/kg bw/d) were noted, but the missing information on microscopy and the unusually high doses led RAC to conclude that the findings in these studies do not support classification for fertility.
- The study on juvenile rats by Kwack *et al*. (2009) observed relevant adverse effects on sperm parameters, but without concomitant effects on weight or morphology of the testes. The effect on sperm count and velocity is inconsistent with the effects on % motile sperms seen in the Boberg *et al*. study.
- While no effect on sperm counts were observed, Boberg *et al*. (2011) observed decreased sperm motility in the offspring after *in utero*/postpartum exposure to DINP (GD 7-PND 17). Reanalysis of the statistical calculations (Boberg *et al*., 2016) and reanalysis of the raw data from this study (Morfeld *et al*., 2017 and Chen *et al*., 2017) showed that the significance of the effect on sperm motility outlined in the original study, from 600 mg/kg bw/d and higher, could not be reproduced for all dose groups (including 600 mg/kg bw/d). According to the reanalysis only effects at 750 and 900 mg/kg bw/d were significantly lower than control values and were without a clear dose response relationship. Furthermore, sperm analyses in the study of Boberg *et al*. (2011) examined a limited number of animals (1-3 animals per litter and a total of 6-10 males per dose group) and the control values did not meet the minimum OECD quality control standards.

- Under the CLP Regulation (Annex I, section 3.7.2.5.7), effects above a limit dose (which for the testing in oral reproductive and repeated dose toxicity studies following OECD test guidelines is specified as 1000 mg/kg bw/d) on testes and reproduction parameters are considered outside the classification criteria unless there are reasons to take them into account for classification (e.g. the anticipated human exposure is sufficiently high or if species differences indicate a higher sensitivity for humans).
- Due to the lack of correlates between weight changes and morphology and the overall heterogeneity of the data on testis weight and sperm parameters, these findings are considered as not sufficiently convincing to outweigh the negative functional studies.

There is no evidence for effects of DINP on fertility in humans. Due to inconsistencies between the different animal studies as well as lack of concommittant microscopic lesions (or information thereon) in the testes, and giving weight to the lack of effects on fertility reproductive parameters in the one- and two-generation studies, RAC concluded that overall there is insufficient evidence for effects on sexual function and fertility in experimental animals. Therefore, RAC considered that **no classification for effects on fertility is warranted**. Studies testing extremely high doses were not considered relevant for classification purposes.

Developmental toxicity

Non-human information

Three developmental toxicity studies consistent with or similar to OECD TG 414 were reported in two publications. Testicular histopathology was not performed and hormone-sensitive endpoints such as AGD or testicular testosterone production were not examined.

In one developmental toxicity study (Hellwig *et al*., 1997**;** cited also as BASF, 1995a in ECHA 2013) either CAS 68515-48-0 or 28553-12-0 (two preparations) was applied in three sub-studies. The differences in the composition are described in ECHA (2013). In each screening study following TG 414 (with 8-10 dams instead of 20 dams/group), eight to ten pregnant Wistar rats per group were administered (by gavage) daily doses of 0, 40, 200 or 1000 mg/kg bw/d on days 6-15 post coitum. Half of the foetuses were examined for soft tissue abnormalities and the remaining foetuses for skeletal abnormalities.

After treatment with DINP (CAS 68515-48-0, 1st sub-study) an increased occurrence of foetal skeletal variations (total no.: 78 (60%) vs. 47 (35%) in controls) was seen at the highest dose (1000 mg/kg bw/d) consisting mainly of rudimentary cervical ribs (incidence (affected litters) at 0, 40, 200, and 1000 mg/kg bw/d: 0, 2(1), 1, and 11(5), respectively), and/or accessory 14th ribs (incidence 0, 0, 2(2), 37(19), respectively). Dams at 1000 mg/kg/d consumed less food (no quantitative information available) and had non-significantly lower body weight (bw at GD 20 around 3-4% lower) compared to the control group. At autopsy, a statistically significant increase in relative kidney weights was recorded at 1000 mg/k bwg/d; the relative liver weights were slightly, but not statistically significantly, increased.

With another preparation of DINP (CAS 28553-12-0, 2nd sub-study) no significant decrease of the food consumption, of the body weight (4% lower on GD 20 at high dose vs. control values), or of the corrected body weight gain was observed. The only substance-related foetal effect was an increased incidence of a skeletal variation, namely accessory 14th rib(s), with incidences of 0, 1, 4(2), 10(5) at 0, 40, 200, and 1000 mg/kg bw/d, respectively (not indicated as significantly increased). The CLH report stated (with unknown source and without further quantitative data) that the respective values were distinctly above the historical control values. The incidences (affected litters) of rudimentary cervical ribs were 0, 0, 1, 4(4).

In a third sub-study with DINP (CAS 28553-12-0, 3rd sub-study) from a different production line which ceased in 1995, mean body weight was significantly lower (8%) at 1000 mg/kg/ bw/d on

GD 15 and non-significantly lower (6%) on GD 20, the food consumption was lower on GD 8-13 (reported in ECB, 2003a). Increased rates of certain skeletal retardation (unossified or incompletely ossified sternebrae) and skeletal (rudimentary cervical (0, 0, 2(1), and 12(7)) and/or accessory 14th ribs (0, 0, 9(5), and 34(8)) variations were seen. The statistically significantly increased occurrence of 1000 mg/kg bw/d foetuses with rudimentary cervical (78% vs. 0 in controls on a per litter basis, 7 of 9 litters affected) and/or accessory 14th ribs (89% vs. 0 in controls on a per litter basis, 8 of 9 litters affected) was considered to be related to the test substance administration to the dams.

No treatment-related effect on the foetal weight was seen in the three sub-studies of Hellwig *et al*. (1997).

In another developmental toxicity study (Waterman *et al*., 1999) with DINP (MRD 92-455, CAS 68515-48-0) no statistically significant differences in mean foetal body weight or external, visceral or skeletal malformations between treated and control animals were observed. However, statistically significant increases in foetuses with skeletal variations, such as lumbar rudimentary ribs and with visceral variations (dilated renal pelves) were seen at the highest dose of 1000 mg/kg bw/d on a per litter basis. Specifically, an increase was seen in the total number of foetuses with visceral variations on a per foetus basis. The total number of variations was significantly increased at the high-dose level on a per litter basis (6/23 vs. 0/24 in controls). The increases in visceral variations were mainly due to increases in dilated renal pelves (0, 3.7*, 4.0*, 4.5* % of foetuses, 0, 12, 16.7, 25.1* % of litters at 0, 100, 500, and 1000 mg/kg bw/d, respectively; *=statistically significant). Skeletal variations, mainly rudimentary lumbar and supernumerical cervical ribs showed a dose-response trend on a per litter as well as on a per foetus basis. The number of foetuses with rudimentary lumbar ribs was significantly higher from 500 mg/kg (% foetuses) and at 1000 mg/kg (% litter). Statistical significance of higher percentages of foetuses and litters with supernumerary cervical ribs was only reached at 1000 mg/kg and in the authors' view this was outside the historical control range (without further details given). In this study, mean food consumption (during GD 9-12) and body weight gain (during GD 6-15) was significantly lower at 1000 mg/kg bw/d (see Table 3 in Watermann *et al*., 1999).

In both developmental toxicity studies skeletal variations were seen (rudimental lumbar or cervical ribs, supernumerary cervical ribs and accessory/or 14^{th} ribs), effects which should normally disappear during postnatal development in rats. These effects are not likely to be of lasting biological significance. In the absence of more profound signs of developmental effects (e.g. malformations, embryolethality) these skeletal variations are considered to be minor effects and potentially reversible. The skeletal effects and decreased body weight in offspring in both studies were considered critical to set a LOAEL for developmental toxicity in the EU RAR (ECB, 2003; LOAEL of 159 mg/kg bw/d for decreased body weight). A higher NOAEL of 500 mg/kg bw/d was determined based on visceral and skeletal variations and slight maternal toxicity at 1000 mg/kg bw/d. These values were applied for risk characterisation, and the EU RAR concluded in 2003 that these findings did not justify classification for developmental effects.

There are a number of additional developmental studies with DINP examining hormone-sensitive endpoints in foetuses and offspring (testicular effects, AGD decrease, nipple retention or structural abnormalities in reproductive organs) that were not standard parameters in the OECD TG 414.

AGD decrease

No statistically significant decrease in AGD was reported in three studies (Boberg *et al*., 2011; Li *et al*., 2015; Masutomi *et al*., 2003). In addition, Gray *et al*. (2000) found no changes in AGD in juvenile male rats (on PND 2). In contrast Clewell *et al*. (2013b) reported that AGD was significantly reduced at 11 400 ppm DINP in diet (~750 mg/kg bw/d, GD 12-PND 14) on PND 14,

but these differences were not observed on PND 2 or PND 49. A shorter treatment period (GD 12-19) at a gavage dose of 750 mg/kg bw/d DINP did not result in effects on the AGD in pups at GD 20 (Clewell *et al*., 2013a).

Boberg *et al*. (2011) reported the AGD as significantly decreased at 900 mg/kg bw/d both without and with correction for body weight at birth. The significance of the decrease was, however, not confirmed in the reanalysis of Chen *et al*. (2017). An effect on AGD was not seen in male rats at PND 90.

Two other studies were reported as showing no effect of DINP on AGD in the CLH report, although testicular effects were described in the offspring (Masutomi *et al*., 2003, Li *et al*., 2015). However, RAC found that the relevance of the testis effects observed at PND 77 in the high dose group of the Masutomi *et al*. study (1165 mg/kg bw/d during gestation and 2657 mg/kg bw during lactation; following an intermittent phase of markedly lower body weight gain, lower body weights and lower weights of several organs) is questionable and may not necessarily be contradictory to the absence of significant effects on AGD on PND 2. Body weight and AGD at PND 2 was slightly (non-significantly) lower (AGD 3.0 at 306.7 and 1164.5 mg/kg bw/d vs. 3.3 in the male control pups) in the Masutomi *et al*. study. Li *et al*. did not see an effect on AGD on PND 1 in their study although the testosterone levels showed a tendency for a dose-dependent decrease from 10 mg/kg bw/d and higher, gaining statistical significance at 1000 mg/kg bw/d DINP. It is noted that the DS found the confidence in their negative outcome on the AGD low due to the low numbers of litters (5-6/dose group) examined.

Nipple retention

Two studies showed increases in nipple retention (Boberg *et al*., 2011; Gray *et al*., 2000).

In the latter study, 2 out of 52 male offspring displayed permanent nipples and 4 out of 52 displayed diverse malformations after oral exposure to 750 mg/kg bw/d DINP on GD 14-PND 3. The results of the study remain unclear regarding the type of malformations and the number of animals affected as no incidence tables were presented. From the information available it is assumed that the two pups with permanent nipples were included in the total number of pups (4 pups; 7.7%) with malformations. The type of malformation(s) in the two other pups is unclear. Nipple retention was not seen in the male control pups of this study. No information was given on the historical background incidence in the laboratory, as permanent nipples may also occur in control animals.

Boberg *et al.* (2011) found a significant increase in the mean number of nipples/areolas in male pups at 750 and 900 mg/kg DINP (3.14 and 3.17 nipples, respectively, vs. 1.98 in controls, Table 3 in the publication) on PND 13 after exposure from GD 7 (i.e. earlier than in the Gray *et al*. study). Boberg *et al.* reported that no significant difference between incidences in DINP exposed groups and controls remained on PND 90.

Reduction of testosterone level

A dose-related decrease in testis testosterone (50% and 35% of control values; Clewell *et al*., 2013a) has been observed in pups from dams receiving 250 or 750 mg/kg bw/d of DINP on GD 12-19. The effect was only observed when testosterone levels were estimated at 2 hours after the last treatment, while the measurement at 24 hours post treatment was not different from control values. Although a marked drop in testosterone was found, no effect on AGD was seen in this study. The authors hypothesised that this could be due to either a nearly complete inhibition of testosterone being required to affect AGD development, or that AGD is not solely dependent upon testosterone signaling.

Testosterone content was also found to be dose-dependently reduced in pups from dams exposed to 10-1000 mg/kg bw/d DINP on GD 12-21 (reduction to 43% of control values, significant only at 1000 mg/kg bw/d; Li *et al*., 2015). Significant reductions in testosterone production and content to one third of control values were observed at GD 21 in foetuses of dams that received 750 mg/kg bw DINP on GD 7-21, while plasma testosterone levels were non-significantly reduced by 25% (Borch *et al*., 2004). RAC notes that although studies with only one dose group have limitations, as they provide no information on dose-response relationships, the results are valuable in a weight of evidence analysis.

In Hannas *et al*. (2011) doses of 0, 500, 750, 1000 and 1500 mg/kg bw/d DINP was administered to pregnant SD rats on GD 14-18. Testicular testosterone production *ex vivo* was assessed by incubation of testes of 18-day old foetuses for 3 hours. This led to a dose-related reduction in foetal testosterone production of approx. 70% of control values at 500 mg/kg, down to approx. 31% of control values at 1500 mg/kg.

Another *ex vivo* test on testes from GD 18 foetuses led to a reduction in testosterone to 62% of control values when the dams received 750 mg/kg bw DINP on GD 14-18 (Furr *et al*., 2014).
Although lower testicular testosterone concentrations were observed in pups at maternal gavage doses of 250 and 750 mg/kg bw/d DINP from GD 12-19 (Clewell *et al*., 2013a), similar high doses of DINP (11 400 ppm, 750 mg/kg bw/d) when administered in the diet on GD 12 to PND 14 had no effect on testosterone levels in testes of rats on PND 49 in another study of Clewell et al. (2013b). This may be expected as the exposure of the dams stopped at PND 14. Testosterone levels were also reported to be measured in pups on PND 2, but the results varied among pups and no significant differences between DINP-treated rats and controls were seen.

Testicular effects

One study showed reduced testis weights in offspring at prepuberty and slight degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells in 4/5 animals necropsied at PND 77 (after maternal exposure of 1165 mg/kg bw/d in the diet on GD 15 to PND 10; Masutomi *et al*., 2003). The DS cited from the ECHA review (2013) that doses "causing systemic toxicity in the dams induce minimal or slight but permanent changes in testes and ovaries of the offspring". In this study maternal body weight gain was only 45% of control values from GD 15-20 at maternal doses of 1165 mg/kg bw/d and 15% of control values on PND 2-10 at 2657 mg/kg bw/d DINP. At least partly, this can be attributed to the lower food consumption observed. It should be noted that body weight in male and female pups were significantly lower at PND 27 (likely due to low body weight gain on PND 2-10) and final necropsy, but body weights were only slightly (non-significantly) lower at the mid and high dose (-16% in male pups, -11% in female pups) than those in control groups at PND 2, indicating that lower body weight gain in dams during gestation had no significant effect on pup development until birth. Lower weights of testes and other organs may be related to the significantly lower body weight (57% of control values) later on as estimated at PND 27. The body weight gain was significantly lower from PND 21 to 42; the effect on body weight and testes weight disappeared until PND 77.

In summary, the dose given to the high dose dams (causing lower body weight gain) was rather high, and the strongest effect on body weight (gain) in pups was from PND 2-10 and growth normalized from PND 42-77, when slight degenerative lesions in 4/5 male offspring rats were found. Taking into account the uncertainties of the studies, the low number of sacrificed animals per group (5 at PND 27 and 8 at PND 77) and that histopathology of the testes was only conducted on 5 males/group at PND 77, the observation of slight testes degeneration in high dose offspring cannot unequivocally be attributed to DINP-related effects.

Gray *et al*. (2000) reported testes atrophy in 2 of 52 pups at 750 mg/kg (one male with bilateral testes atrophy, one male with unilateral fluid-filled testes without spermatids and epididymal agenesis with hypospermatogenesis).

Multinuclear gonocytes at GD 20 were seen more frequently in testis sections in foetuses 24 hours after last maternal dosing on GD 19 of 250 and 750 mg/kg bw/d (Clewell *et al*., 2013a) in comparison with control foetuses. At 750 mg/kg, a higher number of foetuses showing large Leydig cell aggregates was observed. The same findings reported as clustering of Leydig cells (from 10 mg/kg bw/d) and the presence of multinuclear gonocytes (from 100 mg/kg bw/d) accompanied by dysgenesis of seminiferous chords were observed in GD 21.5 foetuses from dams being exposed on GD 12-21 (Li *et al*., 2015).

Other developmental effects

In the offspring of the one-generation study (Watermann *et al*., 2000), a dose-related significantly lower mean body weight was observed in pups at PND 0 and PND 21 at all doses, and at 1% and 1.5% DINP in the diet during lactation (also from PND 1 to 14). Pup survival was significantly lower at birth and for the lactation period PND 0 to 21. However, maternal weights were significantly lower during premating, gestation and lactation periods at 1.5% (1087–1186 mg/kg bw/d) and during gestation and lactation at 1% (741-796 mg/kg bw/d). Decreased body weight in the offspring of the two-generation study was observed on PND (7-)21 at all doses from 0.2% (133-153 mg/kg bw/d) onwards in the first generation and from 0.4% (271-307 mg/kg bw/d) in the second generation (Waterman *et al*., 2000). Maternal effects were limited to significantly lower body weights of dams during lactation (GD 14-21 in F1, GD 4-21 in F2) at 0.8 % (543-577 mg/kg bw/d).

Human information

Based on the existing epidemiological studies, no clear conclusions on possible effects of DINP exposure on male reproductive organs or other endpoints can be drawn (Main *et al*., 2006; Bornehag *et al*., 2015; Jensen *et al*., 2015; Jensen *et al*., 2016; Axelsson *et al*., 2015).

Other relevant information

Mode of action studies and comparison with other phthalates

Effects of DINP on foetal testosterone production were seen in several studies in rats and might be associated with adverse developmental effects (Boberg *et al*., 2011; Borch *et al*., 2004; Hannas *et al*., 2011; Clewell *et al*., 2013, Li *et al*., 2015, Furr *et al*., 2014). Serum testosterone levels were decreased in rats in a Hershberger study. As the animals are castrated in this assay and do not produce endogenous testosterone, the reduction in testosterone possibly reflects a change in liver metabolism of the exogenously provided testosterone and not a direct anti-androgenic effect of DINP (Lee *et al*., 2007). There are no indications that DINP has estrogenic effects (ECHA, 2013; Sedha *et al*., 2015). ECHA (2013) evaluated new scientific evidence on DINP and concluded that "the *in vivo* findings suggest that DINP has anti-androgenic potency, but may also exhibit its effects through other modes of action". The authors further concluded that the permanent changes seen after exposure to high doses of DINP are "likely to be linked to the reduced perinatal testicular T levels".

Several low molecular weight phthalates cause malformations in multigeneration studies and reduce male reproductive capacity. Phthalate esters with chain lengths longer than C6 are generally considered less toxic.

In contrast to other phthalates, DINP does not induce hypospadias, general reproductive tract malformations or permanent decreases of AGD nor permanent nipple retention.

Summary and conclusion on developmental toxicity

Since there is no evidence for effects of DINP on development in humans, classification in Category 1A is not appropriate.

Classification of DINP in Category 1B is not appropriate because according to the CLP criteria, the data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction should be considered not to be a secondary non-specific consequence of other toxic effects. The evidence of effects on development is not considered clear.

Classification in Category 2 is not considered appropriate, because the evidence from animal experiments for any adverse effect on sexual function, fertility or development is too inconsistent or uncertain.

RAC considers that no classification of DINP is justified for the following reasons:

- In contrast to other phthalates classified as Repr. 1B; H360D, DINP does not induce malformations, such as hypospadias and cryptorchidism in rats, nor permanent decreases of AGD or permanent nipple retention.
- Developmental toxicity studies showed skeletal variations such as rudimentary lumbar or cervical ribs. These changes are considered as minor effects and potentially reversible. More profound signs of developmental toxicity (e.g. malformations, embryolethality) were not observed at doses up to 1000 mg/kg bw/d.
- Shortened AGD on PND 14 was observed (Clewell *et al*. 2013b), but the effect was not seen on PND 2 and had disappeared by PND 49. Other studies did not find significant effects on AGD at comparable doses. No study on DINP reported permanent, irreversible changes in AGD.
- Small increases in nipple retention were reported in two studies, with low incidences or mean incidences above control values in studies with some limitations. No clear dose response relationship was seen on the basis of affected animals/group and no difference could be observed at PND 90. Comparable doses in other studies did not cause increased nipple retention.
- Significantly reduced testosterone content or production in pup testes were observed in several studies at maternal doses from 250 mg/kg bw/d. However, in studies where AGD was examined at the same time, the reduction in testosterone was not linked to AGD effects.
- Histological changes such as multinuclear gonocytes and clustering of Leydig cells were observed in foetal testes after maternal treatment with DINP during the second half of gestation. The toxicological significance of these effects on organ development or developmental toxicity in general cannot be excluded, but remains unclear for DINP.

DINP does not induce irreversible gross-structural malformations such as hypospadias and cryptorchidism in rats, nor permanent decreases of AGD or permanent nipple retention. Reversible histological changes in foetal testes and effects on testosterone production alone are not considered to justify classification. Therefore, RAC concluded that **DINP warrants no classification for developmental toxicity**.

Overall, RAC concluded that **no classification for DINP for either effects on sexual function and fertility, or for developmental toxicity is warranted.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).