CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-“isononyl” phthalate; [2] [DINP]

EC Number: 271-090-9 and 249-079-5

CAS Number: 68515-48-0 and 28553-12-0

Index Number: -

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ANNEX 1: EU RAR for DINP, section on toxicity to reproduction

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate; [2] [DINP]
EC number:	271-090-9 [1] and 249-079-5 [2]
CAS number:	68515-48-0 [1] and 28553-12-0 [2]
Annex VI Index number:	Substance not listed in Annex VI
Degree of purity:	100%
Impurities:	

The substance is generally known as DINP. Two different types of DINP are available on the market covering two CAS numbers with variable composition of the alkyl chain backbones. The two alkyl chain backbones are either mainly C_8H_{17} to $C_{10}H_{21}$ isomers (1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich) or C_9H_{19} isomers (di-"isononyl" phthalate) (ECPI 2014). 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 report the abbreviation DINP will be used as a common name representing both CAS numbers.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Repr. 1B; H360Df
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B; H360Df

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not assessed in this dossier
2.2.	Flammable gases	None		None	Not assessed in this dossier
2.3.	Flammable aerosols	None		None	Not assessed in this dossier
2.4.	Oxidising gases	None		None	Not assessed in this dossier
2.5.	Gases under pressure	None		None	Not assessed in this dossier
2.6.	Flammable liquids	None		None	Not assessed in this dossier
2.7.	Flammable solids	None		None	Not assessed in this dossier
2.8.	Self-reactive substances and mixtures	None		None	Not assessed in this dossier
2.9.	Pyrophoric liquids	None		None	Not assessed in this dossier
2.10.	Pyrophoric solids	None		None	Not assessed in this dossier
2.11.	Self-heating substances and mixtures	None		None	Not assessed in this dossier
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not assessed in this dossier
2.13.	Oxidising liquids	None		None	Not assessed in this dossier
2.14.	Oxidising solids	None		None	Not assessed in this dossier
2.15.	Organic peroxides	None		None	Not assessed in this dossier
2.16.	Substance and mixtures corrosive to metals	None		None	Not assessed in this dossier
3.1.	Acute toxicity - oral	None		None	Not assessed in this dossier
	Acute toxicity - dermal	None		None	Not assessed in this dossier
	Acute toxicity - inhalation	None		None	Not assessed in this dossier

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.2.	Skin corrosion / irritation	None		None	Not assessed in this dossier
3.3.	Serious eye damage / eye irritation	None		None	Not assessed in this dossier
3.4.	Respiratory sensitisation	None		None	Not assessed in this dossier
3.4.	Skin sensitisation	None		None	Not assessed in this dossier
3.5.	Germ cell mutagenicity	None		None	Not assessed in this dossier
3.6.	Carcinogenicity	None		None	Not assessed in this dossier
3.7.	Reproductive toxicity	**Repr. 1B; H360Df**		None	
3.8.	Specific target organ toxicity –single exposure	None		None	Not assessed in this dossier
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not assessed in this dossier
3.10.	Aspiration hazard			None	Not assessed in this dossier
4.1.	Hazardous to the aquatic environment	None		None	Not assessed in this dossier
5.1.	Hazardous to the ozone layer	None		None	Not assessed in this dossier

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

Labelling: Pictogram with signal word: GHS08 (danger)
Hazard statements: H360Df;
Precautionary statements: Not part of Annex VI entry

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

A harmonised classification of DINP has not previously been discussed under the CLP Regulation. In 2003, it was concluded that classification was not justified according to the criteria of Directive 67/548/EC and based on the information available at that time (European Chemicals Bureau 2003a).

The substance is registered under REACH with the following volumes: CAS 68515-48-0: 100,000-1,000,000 tonnes/year, CAS 28553-12-0: 100,000-1,000,000 tonnes/year. Neither of the two DINP variants has been self-classified by the registrants.

2.2 Short summary of the scientific justification for the CLH proposal

The available data summarised below show that DINP causes developmental toxicity and indicate toxicity to reproductive organs potentially leading to fertility effects. These effects are considered to be specific and not secondary non-specific consequences of other toxic effects.

DINP induces effects on the developing male reproductive system. Key findings in animal studies on reproductive effects of DINP are:

a) Structural abnormalities: skeletal effects (rudimentary ribs) were seen two developmental toxicity studies (Hellwig et al., 1997;Waterman et al., 1999) (1000 mg/kg bw/day),

b) Effect on altered growth: decreased body weight in offspring in a two-generation study (Waterman et al, 2000) (from 159 mg/kg bw/day),

c) Functional deficiency: dose-dependent long-lasting decrease in sperm motility in rat offspring exposed perinatally (Boberg et al., 2011) (from 600 mg/kg bw/day),

d) Structural abnormalities: increased nipple retention and decreased anogenital distance in infant or prepubertal male rats exposed perinatally (Boberg et al., 2011; Gray et al., 2000, Lee et al., 2006; Clewell et al., 2013b) (mostly from 750 mg/kg bw/day),

e) Structural abnormalities: increased incidence of permanent changes (permanent nipples, malformations of testes and epididymis, histological changes in testes and epididymides) in rats exposed perinatally (Gray et al., 2000; Masutomi et al., 2003) (at 750 and 1165 mg/kg bw/day, respectively),

f) A comparable pattern of adverse effects and of mode of action as seen for other phthalates classified as reproductive toxicants in category 1B, e.g. DEHP, DBP, DIBP and BBP (Boberg et al., 2011; Borch et al., 2004; Hannas et al., 2011; Clewell et al., 2013a, Li et al., 2015).

These findings all demonstrate a developmental toxicity effect of DINP, i.e. an effect on offspring of exposed dams. It may be noted that a study on prenatal exposure to DINP showed no effect on AGD at gestation day (GD) 20, and a study on perinatal exposure to DINP, revealed decreased anogenital distance (corrected by body weight) only at PND 14 and not PND 2, and revealed no change in nipple retention and no/few permanent malformations of reproductive organs (Clewell et al., 2013a; Clewell et al., 2013b). Two other studies showed no effect of DINP on anogenital distance, although testicular effects were described in offspring (Masutomi et al., 2003, Li et al., 2015). The statistical power of these two studies was low due to small group sizes which may explain the lack of effect on anogenital distance (Masutomi et al., 2003, Li et al., 2015).

Furthermore, there is evidence of adverse effects on male reproductive organs with possible consequences for fertility. Key findings for effects of DINP on fertility are:

g) 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/day), 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/day), and a 13-week study in mice (Hazleton 1992) (2600 and 5770 mg/kg bw/day),

h) reduced sperm count and effects on sperm motion parameters after 28 days of exposure of juvenile rats (Kwack et al., 2009) (one dose only, 500 mg/kg bw/day),

i) dose-dependent long-lasting reduced sperm motility in rats exposed perinatally (Boberg et al., 2011) (from 600 mg/kg bw/day),

Sperm parameters were not examined in any other repeated dose study or in parental animals in the one- or two-generation studies. In contrast to the reduction in testes weights in other studies at high doses, increases in parental absolute and relative testis and epididymis weights were seen at 1100 mg/kg bw/day in a one-generation study in rats (Waterman et al., 2000). In a subsequent two-generation study using lower doses up to 550 mg/kg bw/day, no change in testis or epididymis weights of parental animals were seen (Waterman et al., 2000).

In addition to the effects observed in male reproductive organs after exposure to DINP, reduced absolute and relative ovary weights at high doses were observed in parental animals of a one-generation study in rats (Waterman et al., 2000) (1186 mg/kg bw/day) and in a prepubertal female assay in rats (1380 mg/kg bw/day) (Sedha et al., 2015).

The structural and functional adverse effects and the mode of action of DINP are comparable to those of other phthalates classified as reproductive toxicants. DEHP, DBP, DIBP and BBP are classified as reproductive toxicants based on the same developmental effects listed above for DINP (ECHA 2008a, b, c, ECHA 2014). DEHP additionally affected fertility of both females and males and induced severe testicular effects including testicular atrophy in numerous repeated dose toxicity studies in rats, mice and ferrets (ECHA 2008a). DEHP is thus classified in category 1B for both fertility and development, whereas DBP, DIBP and BBP are classified in category 1B for development and category 2 for fertility (ECHA 2008a, b, c, ECHA 2014).

A harmonised classification of the phthalate DCHP (Dicyclohexyl phthalate) as Repr. 1B, H360D (RAC 2015) has recently been adopted (cf. the 9th ATP to the CLP Regulation). Some of the effects caused by exposure to this phthalate are also observed for DINP. The opinion adopted by RAC for DCHP thus suggests a classification for developmental toxicity in category 1B based primarily on increased incidence of nipple retention and decreased AGD in male pups in three different studies in the absence of marked maternal toxicity. Further support for this classification was based on observations of prolonged preputial separation and hypospadias and atrophy of the testes in offspring in one supporting study.

The above mentioned arguments further support classification of DINP in category 1B for development and in category 2 for fertility.

The above findings on structural abnormalities (skeletal effects, AGD, nipple retention) and functional deficiency (impaired sperm motility) in rat offspring at dose levels without maternal toxicity provide clear evidence that the classification criteria for developmental toxicity in category 1B are fulfilled for DINP. Furthermore, similar findings have been observed for phthalates such as DEHP, DBP, DIBP and BBP. This indicates that DINP has a similar mode of action and a comparable pattern of adverse effects as phthalates already classified for developmental toxicity.

Based on findings of reduced sperm count and velocity following exposure to DINP in a 28-day study in rats, impact on sperm motility in rats exposed perinatally, and adverse effects on reproductive organs in other rat studies, a classification for toxicity to fertility in category 2 is warranted for DINP. This is further supported by the comparable pattern of adverse reproductive effects by phthalates already classified for toxicity to fertility, i.e. DEHP, DBP, DIBP and BBP.

Overall, this justifies a classification of DINP as Repr. 1B (H360Df).

It is, however, noted that the effects observed for DINP are less marked and generally occur at higher doses compared to other other phthalates which have harmonised classifications for reproductive toxicity.

2.3 Current harmonised classification and labelling

The substance is not included in CLP Annex VI, Tables 3.1 and 3.2

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

Self-classifications of DINP notified by industry (manufacturers, importers or groups hereof) are available in the C&L Inventory (http://echa.europa.eu/information-on-chemicals/cl-inventory-database).

For CAS 68515-48-0 a total of 378 self-classifications have been notified by industry. Out of these 322 have notified “no classification”, 52 notifiers have self-classified Aquatic acute 1, three notifiers have self-classified Repr. 2 (without differentiation for effects on either fertility or development) and one notifier has self-classified Skin irrit, 2 and Eye irrit. 2 (December 2016).

For CAS 28553-12-0 a total of 726 self-classifications have been notified by industry. Out of these 724 have notified “no classification”, one notifier has self-classified Acute tox 4 and Aquatic acute 1 and one notifier has self-classified Aquatic acute 1 (December 2016).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Harmonised classification and labelling for CMR properties is a community-wide action under article 36 of the CLP regulation. The classification of DINP has not been discussed under CLP. In 2003 the EU Risk Assessment Report concluded that classification was not warranted for DINP under Directive 67/548/EC (European Chemicals Bureau 2003a). New scientific data have, however, been published for DINP in the intervening period. Based on these data and considering the similar mode of action of DINP compared to other phthalates classified as reproductive toxicants, the dossier submitter considers that a harmonised classification for reproductive toxicity is justified. Primary attention is thus given to the newer studies of DINP published after the EU RAR (European Chemicals Bureau 2003a). The studies on reproductive toxicity from the EU RAR are thus only summarised in brief in this CLH report. The section from the EU RAR describing toxicity to reproduction and the subsequent conclusion on classification for this endpoint is included as a separate Annex to this CLH report for consistency.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

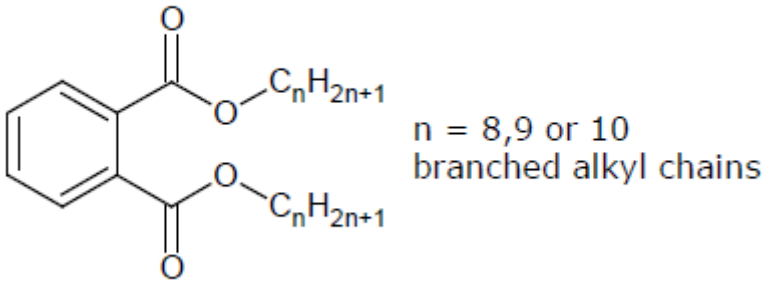
Table 4: Substance identity

EC number:	271-090-9 [1]; 249-079-5 [2]
EC name:	1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate; [2] [DINP]
CAS number (EC inventory):	-
CAS number:	68515-48-0 [1]; 28553-12-0 [2]
CAS name:	-
IUPAC name:	1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich; [1] di-"isononyl" phthalate [2]
CLP Annex VI Index number:	-
Molecular formula:	Average $C_{26}H_{42}O_4$
Molecular weight range:	Average 420.6

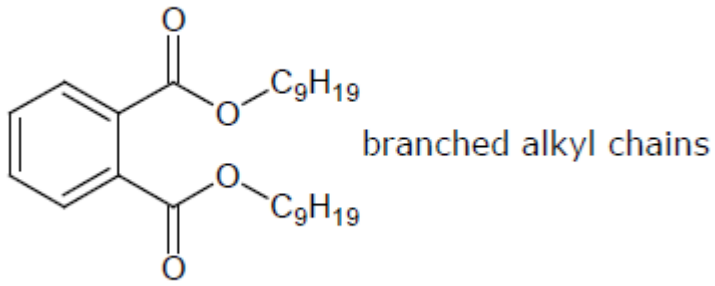
Structural formula:

The substance is generally known as DINP. Two different types of DINP are available on the market covering two CAS numbers with variable composition of the alkyl chain backbones. DINP consists of a multitude of mainly C9-branched isomers with the mean formula $C_{26}H_{42}O_4$ and a mean molecular weight of 420.6 g/mol. Under the EU risk assessment these substances have been considered equivalent from a health and environmental perspective. Representative structures of the substance are shown below:

- CAS 68515-48-0, 1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich corresponds to a UVCB substance including a multitude of constituents showing C8, C9 and C10 alkyl chains branched at various positions:



- CAS 28553-12-0 1,2-benzenedicarboxylic acid, 1,2-diisononyl ester (di-“isononyl” phthalate) corresponds to a UVCB substance including a multitude of constituents showing C9 alkyl chains branched at various positions:



1.2 Composition of the substance

1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich [1] includes the following constituents: di-"isononyl" phthalate (CAS 28553-12-0), isodecyl isononyl phthalate (CAS 85168-75-8), di-"isodecyl" phthalate (CAS 26761-40-0), isononyl isooctyl phthalate (CAS 96532-79-5).

Di-“isononyl” phthalate [2] corresponds to a substance including a multitude of constituents showing C9 alkyl chains branched at various positions. The designation “iso” in the IUPAC name means ‘a mixture of isomers’ (European Chemicals Bureau 2003a). Further, the Platicisers and Flexible PVC Information Centre website also states that di-“isononyl” phthalate contains mainly C9 isomers (ECPI 2014).

Specific information of the content of the above mentioned constituents, content of impurities or additives are not available in the non-confidential part of the REACH registration dossiers. Some information is available from the EU RAR (European Chemicals Bureau 2003a):

Table 5: Constituents, CAS no. 28533-12-0 (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
DINP isononanol isononylbenzoate n-butylisononyl phthalate water	>99.5% (based on ester content) 0.04% 0.03% 0.1% 0.02-0.03%		Data from EU RAR 2003 (European Chemicals Bureau 2003a)

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Not relevant (UVCB)			

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				No information available

Current CLP Annex VI entry: Not applicable

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Clear colorless liquid [1, 2	REACH registration	Measured, Ph. Eur. 2.2.1. and 2.2.2. [1, 2]
Melting/freezing point	-54 °C at 1 atm [1*, 2]	REACH registration	Measured, ASTM D 97-02 [1, 2]
Boiling point	> 400 °C at 1 atm [1, 2]	REACH registration	Calculated [1, 2]
Relative density	0.97 g/cm³ at 20 °C [1*, 2]	REACH registration	Measured, OECD TG 109 [1, 2]
Vapour pressure	0.00006 Pa at 20 °C [1*, 2]	REACH registration	Measured [1, 2]
Surface tension	30.7 mN/m at 20 °C [1, 2*]	REACH registration	Measured, Wilmhelmy plate EC-M-F02 [1, 2]
Water solubility	0.6 µg/L at 21 °C [1, 2*]	REACH registration	Measured, similar to OECD TG 105 [1, 2]
Partition coefficient n-octanol/water	8.8-9.7 at 25 °C [1*, 2]	REACH registration	Measured, similar to OECD TG 117 [1, 2]
Flash point	236 °C [1*, 2]	REACH registration	Measured, ASTM D 93/EU Method A.9 [1, 2]
Flammability	Very low degree of flammability [1, 2*]	REACH registration	Measured, Intertek method [1, 2]
Explosive properties	Not determined [1, 2]	REACH registration	Waived [1, 2]
Self-ignition temperature	400 °C at 1313 hPa[1, 2*]	REACH registration	Measured, ASTM E 659 [1, 2]
Oxidising properties	Not determined [1, 2]	REACH registration	Waived [1, 2]
Granulometry	Not determined [1, 2]	REACH registration	Waived [1, 2]
Stability in organic solvents and identity of relevant degradation products	Not determined [1, 2]	REACH registration	Waived [1, 2]
Dissociation constant	Not determined [1, 2]	REACH registration	Waived [1, 2]
Viscosity	77.6 mm²/s at 20 °C and 27.7 mm²/s at 40 °C [1*, 2]	REACH registration	Measured, OECD TG 114 [1, 2]

*based on read-across/test data for the alternate variant of DINP

2 MANUFACTURE AND USES

2.1 Manufacture

1,2-benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9-rich (CAS 68515-48-0) is manufactured by the "Polygas" process whereas di-"isononyl" phthalate (CAS 28553-12-0) is n-butene based. Isononyl alcohol, used in the synthesis of DINP, is produced via either the

dimerization of butene or the oligomerization of propylene/butene. DINP is produced by esterification of phthalic anhydride with isononyl alcohol in a closed system. Following esterification, excess alcohol is removed under reduced pressure and the product is then typically neutralised, water washed and filtered (ECPI 2014).

2.2 Identified uses

DINP is a high molecular weight general purpose plasticiser added to PVC to impart flexibility. Plasticized PVC with DINP is used in construction, industrial applications and durable goods. DINP is also used in non PVC polymer applications. DINP has been registered under REACH with an annual volume of 100,000-1,000,000 tonnes for both CAS no. 68515-48-0 and CAS no. 28553-12-0, respectively (REACH registration database, ECHA dissemination website, December 2016). According to the Plasticisers and Flexible PVC Information Centre (by the European Council for Plasticisers and Intermediates, ECPI), 95% of DINP is used in PVC applications. The remaining DINP is used in rubbers, adhesives, sealants, paints and lacquers and lubricants (ECPI 2014).

The use of DINP and other high molecular weight phthalates in EU have increased markedly during the last decades due a general shift from the low molecular weight phthalates such as DEHP to high molecular weight phthalates such as DINP, DIDP (di-isodecyl phthalate) and DPHP (di-2-propylheptyl phthalate). The high molecular weight orthophthalates currently represent approximately 70% of the European plasticisers market (ECPI 2014).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The below summaries and citations on toxicokinetics are based on the EU RAR (European Chemicals Bureau 2003a) and the ECHA review of DINP and DIDP from 2013 (ECHA 2013).

4.1.1 Non-human information

Hazleton (1972) administered about 2500 mg/kg/day [^{14}C] DINP orally over 6 days to albino rats (4 treated, 2 controls). The excreted radioactivity in urine ranged from 8-18% within 72 hours. Over 80% of the administered dose was excreted in the feces and most of the radioactivity was excreted within 24-hours after dosing. Between the 60- and 72-hour post dosage periods no animal excreted over 0.2% of the administered dose. Considering the high dose and high level of radioactivity recovered in feces, the absorption process was probably saturated.

Midwest Research Institute (1983), also cited as McKee et al (2002), treated Fischer 344 rats orally with a single radioactive dose of 50 and of 500 mg/kg DINP, with recoveries in urine (~49% at low

dose and 43% at high dose) and feces (~ 51% at low dose and ~ 56% at high dose) within 72 hours after dosing (after normalizing to 100% total recovery, which was 99 and 91% at 50 and 500 mg/kg, respectively). In a repeated dose study over 5 days with 50, 150 and 500 mg/kg, recoveries in urine were 52, 60 and 55 % respectively (after normalizing to 100% total recovery, which was 123, 117 and 115% at 50, 150 and 500 mg/kg, respectively). Almost the entire administered radioactivity was recovered in urine and feces within 72 hours following the last dose; the major portions were eliminated within the first 48 hours. Excretion in urine was higher than in feces at the three dose levels in the repeated dose study.

In general, DINP is rapidly eliminated and less than 0.1% of the radioactivity was recovered in tissues after 72 hours. In tissues, DINP was mainly recovered in GIT, liver and kidney by oral route.

Based on the above studies, the EU RAR concluded that "*these data indicate that a single low dose of [^{14}C]-DINP administered orally to rats was readily absorbed (at least 49%), since metabolites recovered in faeces are the result of bile elimination, and distributed to major tissues, particularly the liver. Absorption was incomplete following a high dose of [^{14}C]-DINP, and after repeated dosing of the compound at all dose levels. DINP metabolites were excreted in urine and to a lesser extent in feces. DINP was de-esterified to the monoester which was further metabolised by side-chain oxidation of the ester group or by hydrolysis to phthalic acid; the formation of oxidation products appeared to increase following the high dose, while hydrolysis to phthalic acid decreased. DINP metabolites reached testes at high dose and were detected in fat. Repeated dosing caused no accumulation of DINP and/or its metabolites in blood and tissue, but resulted in increased formation and elimination of the monoester-oxidation products*" (European Chemicals Bureau 2003a).

4.1.2 Human information

Koch and Angerer (2007) described elimination of major DINP metabolites via urine in a study where one human volunteer was dosed 1.27 mg/kg (n = 1). A recovery of 43.6% of DINP was calculated in urine measurements during 48h of four metabolites. Four metabolite 'groups' of structural isomers were measured. Other possible metabolites (with two or more functional groups or shortened side chains) were not measured.

The recovered percentage is thus likely an underestimation of the actual elimination of DINP via urine (Koch and Angerer, 2007).

Anderson et al. (2011) studied the kinetics of DINP and DEHP in 10 male and 10 female human volunteers (n = 20). Two dose levels were used of the deuterium labelled DINP and DEHP, which were for DINP 0.78 mg (0.010 mg/kg for males and 0.011 mg/kg for females) and 7.3 mg (0.090 mg/kg for males and 0.107 mg/kg for females). A recovery of 32.9 ± 6.4% of the labelled DINP was calculated in urine measurements during 48 h of four metabolites (the same metabolites as in Koch and Angerer)".

4.1.3 Summary and discussion on toxicokinetics

According to the review by ECHA, 2013, the oral absorption in adult rats was estimated to be in the order of 50-55% (based on calculations including excretion in bile). Based on read-across from DEHP, it is assumed that the oral absorption of DINP in humans is 100% (ECHA, 2013).

In the review by ECHA, 2013, the following discussion is made regarding human oral absorption: "*According to the supporting document to the opinion of RAC on the draft version of this report*

(ECHA 2013b), human volunteer studies with DEHP clearly demonstrate that the amount recovered in urine is dependent on the type and amount of metabolites that are measured in those studies. It was considered that measuring all metabolites most likely would result in near to 100% recovery of radioactivity in urine and pointed out that an unknown amount of excretion via bile contributes further to the absorption estimate. It was however acknowledged that the studies in humans have not been designed to determine absorption.

RAC (ECHA 2013a,b) concluded that adult humans orally absorb 100% based on read-across from DEHP. This conclusion differs from the EU Risk Assessments (EC 2003a,b) that assumed a bioavailability factor of 50% for calculating internal oral exposure of adults, derived from toxicokinetic data in rats. For newborns and infants a factor of 100% (i.e. twice as much as for rats) was assumed based on a study from Sjöberg et al. (1985) which seemed to show a greater absorption by oral route of DEHP in young rats compared to older ones."

4.2 Acute toxicity

Not evaluated in this report.

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

Not evaluated in this report.

4.4 Irritation

Not evaluated in this report.

4.5 Corrosivity

Not evaluated in this report.

4.6 Sensitisation

Not evaluated in this report.

4.7 Repeated dose toxicity

Repeated dose toxicity has not been evaluated in this report. The following information on an oral repeated dose toxicity study in rats is included to support the proposal regarding toxicity to reproduction.

4.7.1

Non-human information

4.7.1.1 Repeated dose toxicity: oral

To provide an overview of the general toxicity of the substance, the most relevant repeated dose toxicity studies and chronic toxicity studies discussed in the EU RAR (European Chemicals Bureau

2003a; and cited in ECHA 2013) as well as more recent studies included in the registration dossier for DINP are included in Table 9. A few additional studies are listed in the registration dossier but not repeated here due to lack of data availability or lack of relevance for reproductive toxicity (e.g. studies on effects on the immune system). Regarding repeated dose toxicity and specifically toxicity to the liver, the studies by Aristech 1994 and Exxon 1986 are considered key studies by EU RAR and ECHA 2013, and the study by Bio/dynamics 1986 is considered a supporting study by ECHA 2013. Among the studies published after the ECHA review from 2013, a study by Ma et al., 2014, investigating effects of DINP on liver and kidney may be considered relevant, but due to poor data reporting, this is considered a supporting study only. In addition to the critical effects on liver and kidney, effects on reproductive organs are also noted in Table 9.

Details on key studies with regard to toxicity to reproductive organs are presented after the tables, and discussion of these studies is given in section 4.7.2.

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

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/day N=8	At 1700 mg/kg bw/day: 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/day) (CAS 68515-48-0) N= 15	NOAEL: 0.1% (77 mg/kg bw/day) LOAEL: 0.3% (227 mg/kg bw/day): 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/day; females: 251-880 mg/kg bw/day) (CAS 68515-48-0) N=15	LOAEL: 0,3% (201 – 251 mg/kg bw/day): increased kidney, liver weights, decreased triglycerides and urine chemistry changes. Increased relative testis weight at 1% associated with a slight (NS) increase of absolute testis weight.	No histological data on testes.	Bio/dynamics 1982c
Chronic toxicity 2-year dietary. Sprague Dawley CD rats. Guideline: not indicated in EU RAR 2003. Santicizer 900 (CAS 71549-78-5 according to CHAP 2001) Dietary concentrations of 0, 500, 5	NOAEL: 27 mg/kg bw/day. Spongiosis hepatis was significantly elevated at the mid and high dose in males. In males, the incidence of focal necrosis was significantly		Bio/Dynamics 1986

Method	Results	Remarks	Reference
000, 10 000 ppm Males: ca. 0, 27, 271 and 553 mg/kg bw/day Females: ca. 0, 33, 331, 672 mg/kg bw/day. Dose groups n = 70/sex/dose level	elevated at the low and high doses, while the mid dose was non-significantly elevated.		

Method	Results	Remarks	Reference
Chronic toxicity. 2-year study, rat (CAS 68515-48-0) Dietary concentrations of 0, 0.03, 0.3 and 0.6% (w/) Males: ca. 0, 15, 152, 307 mg/kg bw/day Females: ca. 0, 18, 184, 375 mg/kg bw/day Dose groups n = 220 (110/sex)	NOAEL: 0.03% (15-18mg/kg bw/day) LOAEL: 0.3% (152-184 mg/kg bw/day) 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 - 3,000 - 10,000 - 30,000 ppm (CAS 28553-12-0, purity greater than 99%) which correspond to 333 - 1,101 - 3,074 mg/kg/day at day 7 and to 152 – 512 - 1,543 mg/kg/day at day 91 for the males and to 379 - 1,214 - 3,224 mg/kg/day at day 7 and to 200 – 666 - 2,049 mg/kg/day at day 91 for the females. OECD guideline 408 and in conformity with GLP	LOAEL 3000 ppm for lowered triglyceride level and liver histology changes. At 10000 ppm increased absolute and relative liver and kidney weights. A trend towards a decreasing body weight from 3,000 ppm was observed in males and confirmed at 30,000 ppm with a statistically decreased of the 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 regarded as the result of the clear body weight decrease.	Histological examination of testes and ovaries showed no adverse changes (but 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, 10000, 20000 ppm (Males: 176, 354, 719, 1545 mg/kg bw/day, females: 218, 438, 823, 1687 mg/kg bw/day) N = 10/sex/group CAS 28553-12-0	LOAEL: 2500 ppm (176 mg/kg bw/day in males) increased weight of liver and kidney From 10000 ppm (719 mg/kg bw/day) increase in relative testes/epidydimides weight At 20000 ppm (1687 mg/kg bw/dat) decrease of absolute and relative uterus weight.	No gross of microscopic observations were associated with the weight changes of the ovaries and testes/epidymides	Hazleton Laboratories 1991a

Method	Results	Remarks	Reference
4-week study, B6C3F1 mice, 3000, 60000, 12500, 25000 ppm (Males: 635, 1377, 2689, 6518 mg/kg bw/day) N=10	LOAEL: 3000 ppm (635 mg/kg bw/day in males) increased absolute and relative liver weight. At 6000 ppm (1377 mg/kg bw/day in males) decreased weight of absolute and relative kidney and testes weight	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, 10000, 20000 ppm (365, 972, 2600, 5770 mg/kg bw/day). 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 10000 ppm (2600 mg/kg bw/d), reduced absolute and relative uterus weight at 20000 ppm (5770 mg/kg bw/d). Immature/abnormal sperm forms in epididymis, hypoplasia in uterus and absence of corpora lutea in ovaries at 20000 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 Guideline 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/day, high dose recovery group 637 mg/kg bw/day Females: ca. 0, 36, 109, 442, 885 mg/kg bw/day, high dose recovery group 774 mg/kg bw/day Dose groups n = 70-85/sex and a recovery high dose group of 55/sex	NOAEL: 0.15% (88-103 mg/kg bw/day) LOAEL: 0.6% (358-442mg/kg bw/day): 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,	NOAEL 500 ppm		Aristech 1995

Method	Results	Remarks	Reference
mice. 0, 500, 1500, 4000, 8000 ppm (Males: 0, 90.3, 275.6, 741.8, 1560.2 mg/kg bw/day, females: 0, 112, 335.6, 910.3, 1887.6 mg/kg bw/day)	LOAEL: 1500 ppm increased liver and kidney weights. Decreased absolute and relative testis weight at 741.8 mg/kg bw/day (4000 ppm, 11.1% reduction) and 1560.2 mg/kg bw/day (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/day N=4 males and 4 females	NOAEL: 500 mg/kg bw/day LOAEL: 2500 mg/kg bw/day minor changes: decreased body weight, decreased body weight gain	Another group received Clofibrate 500 mg/kg bw/day 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/day N=4	NOAEL 500 mg/kg bw/day. 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/day and clofibrate 250 mg/kg bw/day on these 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/day CAS 28553-12-0. Purity not described. Vehicle: corn oil Exposure: 28 days (PND 35 to 77)	NOAEL: Not determined LOAEL: 500 mg/kg bw/day. 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. Test material 28553-12-0. 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/day of DINP. N=10	NOAEL: 2 mg/kg bw/day LOAEL: 20 mg/kg bw/day for histological effects on liver (oedema). Increased reactive oxygen species (ROS) in lever and kidney at 200 mg/kg bw/day; decreased glutathione (GSH) content in liver at 20 and 200 mg/kg bw/day and in kidney at 200 mg/kg bw/day.	Other groups received melatonin, or melatonin in combination with DINP (200 mg/kg bw/day). Poor data reporting: no clear description of the number of affected animals at 20 and 200 mg/kg bw/day.	Ma et al., 2014

Further details on key studies regarding toxicity to reproductive organs are presented below. Key studies on hepatic and renal effects are not described in detail in this section, as conclusions by EU RAR (European Chemicals Bureau 2003a) and ECHA 2013 are presented in the discussion section 4.7.2.

Exxon 1986. Groups of 220 (110/sex) Fischer 344 rats, (6 weeks of age at initiation of dosing) were administered dietary concentration of 0 - 0.03 - 0.3 - 0.6% (w/w) DINP (MRD 83-260, CAS 68515-48-0) for a period up to 2 years. The mean daily intakes of DINP over 2 years (Lington et al., 1997) were 15, 152 and 307 mg/kg/day for male rats corresponding to dose levels of 0.03 - 0.3 and 0.6%, respectively and 18, 184 and 375 mg/kg/day for female rats, respectively. Preselected subgroups of 10 rats/sex/dose level were scheduled for interim sacrifice after 6, 12 and 18 months and remaining rats were sacrificed at 24 months. Regarding food consumption, the overall consistent changes consisted of a significant reduction of food consumption in the high-dose males during the last 12 months of the study and of a slight increase of food consumption in the mid and highdose females during the first 12 months of the study. When compared with controls, the high-dose males exhibited a statistically significant, dose-related decrease in body weight beginning at 12 months of treatment and persisting until termination (4 to 7% reduction in body weight compared to the control group). There were not statistically significant changes of body weight in females. Both males and females from the mid and high-dose groups exhibited a statistically significant, dose-related increase in relative kidney and liver weights throughout most of the treatment period; the absolute liver and kidney weights demonstrated a similar trend. At termination in the high-dose level absolute liver and kidney weights were increased, respectively in males by 20% and 8% (liver and kidney weights were 13.94 and 3.19 g vs. 11.63 and 2.95 g) and in females by 27.3 and 9% (liver and kidney weights were 10.25 and 2.30 g vs. 8.27 and 2.10 g). Those changes in organ weights were correlated to histopathological findings at the 18-month sacrifice. At study termination, the mid- and high-dose males exhibited a statistically significant, but not dose-related, increase in absolute and relative spleen weights and the high-dose females a statistically significant increase in relative spleen weight. Relative, but not absolute adrenal weights were slightly, but significantly increased in both sexes. No treatment-related changes were observed in the absolute or relative organ weights for ovaries, brain, heart, or thyroid/parathyroid. At study termination, a statistically significant increase in relative testis weights was observed at the high dose associated with a slight, not statistically significant, increase of 13% in absolute testis weight (6.22 g vs. 5.48 g in the control group).

BASF 1987 Groups of ten Wistar rats of each sex were fed diets containing 0 - 3,000 - 10,000 - 30,000 ppm (CAS 28553-12-0, purity greater than 99%) which correspond respectively to 333 - 1,101 - 3,074 mg/kg/day at day 7 and to 152 – 512 - 1,543 mg/kg/day at day 91 for the males and to 379 - 1,214 - 3,224 mg/kg/day at day 7 and to 200 – 666 - 2,049 mg/kg/day at day 91 for the females. A trend towards a decreasing body weight from 3,000 ppm was observed in males and confirmed at 30,000 ppm with a statistically significant decrease of the body weight compared with the control groups (males up to 18% and females up to 11%). A statistically significant reduction of the triglyceride concentrations was detected in both sexes from 10,000 ppm and as a trend also at 3,000 ppm in both sexes. statistically significant increase of absolute and relative liver weights in males and females was observed from 10,000 ppm. At 30,000 ppm, the increase was about 30% in males and 69% in females (absolute liver weights) and about 59% in males and 89% in females (relative liver weights). A statistically significant increase of relative kidney weight was observed in both sexes from 10,000 ppm and of the absolute kidney weight at 10,000 ppm in males. An increase of the relative testis weight observed at 30,000 ppm is also not regarded as substance induced but regarded as the result of the clear body weight decrease. The histopathological examination of the

testes and ovaries did not show any adverse effect. However, it is noted that the testes were fixed in 4% formaldehyde and not with Bouin fixative, which is generally preferred for testis, and that the determination of the uterus weight was not carried out.

Hazleton Laboratories, 1991 DINP (CAS 28553-12-0) was administered over a 13-week period in the diet of 4 groups of 10 male and female Fischer 344 rats at dose levels of 2,500 - 5,000 - 10,000 - 20,000 ppm (about 176 – 354 – 719 - 1,545 mg/kg/d in males and 218 – 438 – 823 - 1,687 mg/kg/d in females). Body weight gain of males and females treated with 20,000 ppm was significantly lower than controls. There was an increase of the absolute kidneys weight in males from 5,000 and in females from 2,500 ppm and an increase of the relative kidney weight from 2,500 ppm in males and 5,000 ppm in females. There was an increase of the absolute liver weight in males from 5,000 and in females from 2,500 ppm and an increase of the relative liver weight from 2,500 ppm in males and 5,000 ppm in females. Histopathologic examinations showed hepatocellular enlargement in the 20,000 ppm group, periportal in males and centrolobular in females. There was a decrease of the absolute and relative weight of the uterus at 20,000 ppm and an increase of the relative testes/epidydimides weight at 10,000 ppm and higher. No gross or microscopic observations were associated with these organ weight changes. In this study, the LOAEL was set to 2,500 ppm (176 mg/kg/d) based on the increases of liver and kidney weights in males and females observed at this dose level.

Hazleton Laboratories, 1992, performed a 13-week study in mice, described in EU RAR (European Chemicals Bureau 2003a). DINP (CAS 28553-12-0) was administered to B6C3F1 mice in the diet for 13 weeks. Ten mice/sex/group received test material at dose levels of 1,500 - 4,000 - 10,000 and 20,000 ppm (about 365 - 972 - 2,600 - 5,770 mg/kg/d) (groups 2 to 5, respectively). An additional group of 10 mice/sex/group received the basal diet only and served as negative control group (group 1). To evaluate the hepatocellular proliferation and peroxisomal proliferation, additional corresponding groups of 15 mice/sex/group at each dose level (groups 1 to 5) plus a positive control (WY 14,463) (group 6) of 15 mice/sex/group were evaluated 3, 30 and 90 days after study. Weights of testis/epididymis and uterus were reduced and histological changes in epididymis, uterus and ovaries were observed, but only at doses exceeding 1000 mg/kg bw/d (10,000 and 20,000 ppm). A NOAEL of 1,500 ppm (about 365 mg/kg/d) could be determined regarding effect on the liver at 4,000 ppm (enlarged liver in males and increases of absolute and relative liver weight). A NOAEL of 4,000 ppm could be derived for reproductive organs based on decrease in absolute testis/epididymis weights from 10,000 ppm.

A study by Aristech 1995 was used for risk characterization concerning effects on reproductive organs in the EU RAR (European Chemicals Bureau 2003a). Groups of 70 B6C3F1/Crl BR mice /sex were administered daily for at least 104 weeks 0 – 500 – 1,500 – 4,000 and 8,000 ppm DINP (CAS not specified > 99% purity) in the diet (70/sex/group) (groups 1 to 5, respectively). The doses are corresponding to 0 – 90.3 – 275.6 – 741.8 and 1,560.2 mg/kg in males and 112 – 335.6 – 910.3 and 1,887.6 mg/kg in females. A recovery high-dose group of 55 mice/sex was also given 8,000 ppm DINP in the diet for 78 weeks, followed by a 26-week recovery period. At termination, absolute and relative testis weights were decreased in mid-high, high- and recovery high-dose males, (by 11.1, 20.2, and 11.8%, respectively). No histological changes in testes were observed. Liver and kidney weights were reduced at lower doses; i.e. from 1500 ppm. Male body weights were 90 and 83% of controls in the two highest dose groups, respectively.

Hall et al., 1999. In a marmoset monkey study (16-25 months old), four groups of four marmosets/sex/group were administered DINP (CAS number not specified, purity 99.2%) daily by gavage at dose levels of 0 - 100 - 500 - 2,500 mg/kg/d for a period of 13 weeks after 3-week

acclimatisation period. An additional group was treated with 500 mg/kg/d clofibrate to act as a reference control. Body weight losses or low body weight gains were observed for males and females at 2,500 mg/kg/d DINP. There were no treatment-related changes in biochemical parameters (including triglycerides and cholesterol) or in concentrations of estradiol and testosterone. No histological findings were considered to be treatment-related. For testes, no changes in weights or macroscopic /microscopic findings were observed.

Pugh et al., 2000 To evaluate the human relevance of liver effects observed in rats and mice, high doses of peroxisome proliferators were given by gavage in 0.5% methyl cellulose (10 ml/kg) to groups of four adult male cynomolgus monkeys for 14 days (Pugh et al., 1999; 2000). In comparison to vehicle control, DINP (CAS number not specified, 500 mg/kg/d), DEHP (500 mg/kg/d) and clofibrate (250 mg/kg/d) produced no statistically significant changes in body weight, organ weights, urinalysis, hematology, clinical chemistry, or other signs of toxicity. However, relative weights of testes and epididymides (combined) was 76% of control values, but not significantly different from controls. It is noted in the published paper that no distinctive treatment-related effects were observed in the testes, but no details are presented. It is noted that the testes were fixed in 4% formaldehyde and not with Bouin fixative, which is generally preferred for testes. No histological examination of epididymides was performed.

Kwack et al., 2009, exposed groups of 5-week-old SD rats to different phthalates including DINP. Groups of 6 rats were exposed to 500 mg/kg bw/day of DINP (or other phthalates) daily for 4 weeks. Control rats received corn oil. Body weights were reduced to approximately 88% of controls, and relative liver weights were increased to 145% of controls with DINP (and other phthalates). No changes in other organ weights (thymus, heart, spleen, kidney, adrenal, testis, epididymis) were seen for DINP. A statistically significant reduction in sperm counts was observed in the DINP group. Sperm counts were lowered to 75.2% by DINP, whereas e.g. DEHP decreased sperm counts to 34.3% of controls. Different sperm motion parameters were measured by Computer Assisted Sperm Analysis (CASA) including the total percentage of motile sperm and different measures of velocity. The percentage of motile sperm was not affected significantly by DINP (76% motile in control group and 63% motile sperm in DINP group) but by other phthalates. However, the average sperm curvilinear velocity (VCL) and straight-line velocity (VSL) was decreased significantly by DINP. Overall, DINP appeared to affect sperm motion in a similar manner as e.g. DEHP, DBP and BBP, in this repeated dose study of 4 weeks using young animals but with less marked effects. The effects of DINP on sperm count and sperm velocity were seen at a dose level (500 mg/kg bw/day) that is associated with a lower body weight compared to controls. As the dosing with DINP occurred during a period of rapid growth of the rats and establishment of spermatogenesis (PND 35 to 63) it is not clear whether the observed adverse effects on sperm results from direct testicular toxicity or caused by delayed growth and development. Delayed growth may be a non-specific effect of treatment or may be due to impaired testosterone production. Testosterone levels were not examined in that study.

4.7.2 Summary and discussion of repeated dose toxicity

Several of the listed studies showed effects of DINP on male reproductive organs after juvenile or adult exposure including reduced sperm count and velocity (Kwack et al., 2009), decreased absolute and/or relative testis weight (Hazleton 1992; Hazleton 1991; 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 cynomolgus monkeys relative testis/epididymis weigths were 76% of controls, but this was not

statistically significant, and in marmosets no effects on testes were reported (Pugh et al., 2000; Hall et al., 1999). In a one-generation study increased absolute and relative weights of testes and epididymides were observed in parental animals (Waterman et al., 2000), see section 4.11 on toxicity for reproduction.

When evaluating the conclusions from the EU RAR and ECHA 2013, it can be concluded that (human relevant) liver effects are critical to NOAEL determination. These effects are seen at 152-359 mg/kg bw/day in 2-year studies by Exxon 1986 and Aristech 1994 with NOAELs of 152 and 88 mg/kg bw/day, respectively. Reduced kidney weight was seen at the same doses (Exxon 1986).

Overall, reproductive effects after adult exposure of rats and mice were seen at higher doses than those inducing hepatic toxicity. Effects on sperm count and sperm velocity was seen in young adult male rats after 4 weeks of exposure to a higher dose of 500 mg/kg bw/day (Kwack et al., 2009). Chronic exposure to 742 mg/kg bw/day led to reduced absolute and relative testis weight in mice (Aristech 1995). At even higher doses of 2600 mg/kg bw/day, reduced absolute testis/epididymis weights were seen in a 13-week study in mice by Hazleton Laboratories, 1992, a study also showing abnormal sperm and adverse effects on the female reproductive tract at a very high dose of 5770 mg/kg bw/day.

For clarity, discussions from EU RAR 2003 and ECHA 2013 regarding repeated dose toxicity (focusing on liver effects) are presented here:

In the EU RAR, 2003, the overall evaluation of repeated dose toxicity led to a NOAEL of 88 mg/kg/day based on non-peroxisome proliferation related liver effects in a 2-year dietary chronic/carcinogenicity study in rats (Aristech 1994). ECHA 2013 noted that it is not clearly stated in the EU Risk Assessment why the NOAEL of 88 mg/kg bw/day based on the Aristech study was chosen over the lower (15 mg/kg) from the other relevant long term toxicity study by Exxon (1986). ECHA 2013 reevaluated these studies to determine a NOAEL for repeated dose toxicity. They concluded that "…*the Exxon (1986) study was considered the most appropriate to use. Thus a NOAEL of 15 mg/kg bw/day was selected for repeated dose toxicity of DINP. This conclusion was supported by RAC (ECHA 2013a). RAC however noted that the NAEL could be higher given the large dose spacing in the Exxon study*."(European Chemicals Bureau 2003a)

The following summary of repeated dose toxicity focusing on reproductive organs is presented by ECHA 2013 citing EU RAR 2003:

In mice, a NOAEL of 1,500 ppm (276 mg/kg/d) can be derived from a 104-week study (Aristech, 1995c) based on testicular weight decrease observed from 4,000 ppm (742 mg/kg/d) and is used for the risk characterisation. In addition, in 4-week and a 13-week repeated-dose mouse studies, slight decreases of testis weight were observed accompanied by the presence of abnormal / immature sperm forms in the epididymes at doses of 6,500 mg/kg/d and 5,700 mg/kg/d, respectively (25,000 and 20,000 ppm). In those mouse studies (4-week and 13-week) effects were noted in uterus (hypoplasia and absence of endometrial glands) and in ovaries (absence of corpora lutea suggesting an arrest of ovulation) at doses of 20,000 ppm and 25,000 ppm. It should be noted that in the 13-week study in monkeys (Huntingdon Life Sciences, 1998) no changes were reported in testis weight and testis microscopic examination. In addition, there were no treatment-related changes in estradiol and testosterone concentrations assessed. In conclusion, for effects on the liver and kidneys, a NOAEL of 88 mg/kg/d is determined in rats regarding results found in a chronic / carcinogenic study (Aristech, 1994). For reproductive organs, a NOAEL of 276 mg/kg/d can be derived from a mouse study. These NOAELs will be used for the risk characterisation. The effects seen in the repeated dose toxicity tests do not justify classification Xn R48 according to the EU classification criteria." (European Chemicals Bureau 2003a).

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

Not evaluated in this report.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this report.

4.10 Carcinogenicity

Carcinogenicity has not been evaluated in this report. The information on long-term effects on reproductive organs is presented in section 4.7.1 and Table 9 regarding repeated dose toxicity to support the proposal regarding toxicity to reproduction.

4.10.1 Non-human information

4.10.2 Summary and discussion of carcinogenicity

A discussion of carcinogenicity is not relevant for the current proposal. However, a 2-year study in mice by Aristech 1995 was used for risk characterization concerning effects on reproductive organs in the EU RAR, (European Chemicals Bureau 2003a), as the reduced absolute and relative testis weight indicates toxicity to reproductive organs/fertility. See study description in section 4.5.1 and discussion on effects on toxicity for reproduction in section 4.11.4.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Table 10: Summary table of relevant experimental studies on fertility

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/day during gestation) CAS 68515-48-0 Vehicle: unchanged (no vehicle) Exposure: Ten weeks before mating and through the mating	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	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	Waterman et al. (2000) Exxon Biomedical Sciences (1996)

Method	Results	Remarks	Reference
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 PPD 21. (Continuous for parents (males and females)) equivalent or similar to OECD Guideline 415 (One-Generation Reproduction Toxicity Study)	sacrificed at the end of weaning, 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 effects: 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.	used to design a follow-up two-generation reproductive study of DINP.	
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/day 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	No NOAELs for parental or offspring toxicity were derived from this study for DINP 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/day depending on period considered). 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 P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility and female fecundity indices was observed in P2 generation. A statistically significant decrease in the mean left ovary weight of the P1 females at 0.8% (577 mg/kg bw/day) was	No evaluation of sperm parameters.	Waterman et al. (2000) Exxon Biomedical Sciences (1996)

Method	Results	Remarks	Reference
postnatal day (PND) 21. Test guideline: EC Dangerous Substances Directive (67/548/EEC), Annex V, Part B; 1987 (identical to OECD 416) EPA OTS 798.4700 (Reproduction and Fertility Effects)	observed but in the absence of a clear dose response, 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 by the EU RAR. Offspring: In high-dose males, there was a statistically significant increase of relative right and left epididymis weights in P2 generation with a concurrent but not statistically significant (by 7.5%) increase of absolute epididymis weight. 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/day).		

The above study descriptions of a one- and a two-generation study are based on descriptions in the EU RAR (European Chemicals Bureau 2003a; see also Annex 1) and the published paper by Waterman et al., 2000. These reproductive toxicity studies did not examine hormone-sensitive endpoints such as anogenital distance or nipple retention, which are included in a more recent guideline for reproductive toxicity studies (OECD TG 443: Extended One-Generation Reproductive Toxicity Study, OECD 2011).

In the one-generation study (Waterman et al., 2000/ Exxon Biomedical Sciences, 1996), four groups of Crl:CDBR, VAF Plus rats (30 rats/sex/group) were administered daily in the diet DINP (MRD 92-455, CAS 68515-48-0) at doses of 0, 0.5%, 1.0% and1.5% (corresponding to 0, 377-404, 741-796, and 1087-1186 mg/kg bw/day during gestation).

Overall, no effects on fertility were detected in the first study (one-generation), but 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 vesicle) in the highest dose group. A reduction in absolute and relative ovary weights of high dose females at termination (end of lactation) was seen concomitantly with a 23% reduction in body weight. However, no microscopic evaluation of reproductive organs and no evaluation of sperm parameters were performed. DINP exposure reduced the percentage of live birth pups, and reduced pup survival in the highest dose group. In offspring, dose-related decreases in mean offspring body weight were seen during the postnatal period (PND 0-21) and in adulthood. In both males and females, significantly reduced body weights were seen at all dose levels. At the end of weaning, pups in the high dose group weighed 53-54% of controls.

This study was followed up by a two-generation study using lower doses (see description below).

In the two-generation study (Waterman et al., 2000/ Exxon Biomedical Sciences, 1996), male and female SD rats were exposed to DINP from 10 weeks before mating and through mating, gestation and lactation until terminal sacrifice. Doses were 0-0.2%-0.4%-0.8% corresponding to 133-153, 271-307, and 543-577 mg/kg bw/day during gestation. Overall, no changes in fertility or parental male reproductive organ weights were seen. This study was based on guidelines not including evaluation of sperm parameters. An increase in relative epididymis weights were 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 was found. In F1 and F2 generations, lower mean offspring bodyweights were seen in all treatment groups compared with controls. However, when the litter size was taken into account, effects were only significant in high-dose males on PND 0, in males and females of the mid and high-dose levels on PND 7 and 14 and in all treated animals on PND 21. In addition, the weights of all F1 and F2 treated offspring were within the historical control range of the laboratory with the exception of the F2 high-dose males and females on PND 0 and the F2 high-dose males on PND 1 (considering litter size). These findings were considered by the laboratory as the results of maternal stress and/or direct effects of DINP via exposure through lactation. In both generations of offspring, increased liver and kidney weights were seen in males and females. These findings were most marked in F1 offspring showing effects from 0.4%, whereas the second generation showed effects at 0.8% only.

The finding of decreased body weights in offspring at all doses in the two-generation study was applied to set a LOAEL of 159 mg/kg bw/day for developmental toxicity (European Chemicals Bureau 2003a and ECHA 2013).

These two studies together indicate effects of DINP on development (decreased offspring body weight), but no clear conclusions regarding toxicity to fertility can be drawn as sperm parameters and reproductive organ histology were not examined. Furthermore, findings in repeated dose studies (see section 4.7) showed reductions of testis weights at high doses of DINP, whereas the one-generation study showed increases of testis and epididymis weights. Discussion of effects of DINP on fertility is presented in section 4.11.4.

4.11.1.2 Human information

Table 11: Summary table of relevant human data on fertility

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 ratio of testosterone:LH.		Joensen et al., 2012
Cross-sectional study, N=589, Male partners of pregnant women, Greenland, Poland, 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

Method	Results	Remarks	Reference
Cross-sectional study and retrospective interview regarding time to pregnancy, N=938 women and 401 men, Greenland, Poland, 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

A study by Joensen et al., 2012, investigated a group of 881 Danish young men in 2007 to 2009. The association between phthalate metabolites in blood and urine, and individual levels of testosterone, LH and sperm quality measures was examined. Exposures based on urinary metabolite levels were presented as quartiles. For the DINP metabolite MINP, statistically significant reductions in free androgen index (serum levels of total testosterone relative to levels of sex hormone binding globulin) was seen in each of the three highest quartiles compared to the lowest quartile (15% lower free androgen index in highest compared to lowest MINP quartile; p<0,001). In the highest quartile of DINP exposure, FSH levels were 13% lower (p<0.05) and the total testosterone to LH ratio was 9% lower (p<0,05) than in the lowest quartile. The free androgen index relative to LH levels were 19% lower in the highest versus the lowest MINP quartile, whereas there were no significant differences in LH or testosterone levels as such. Overall, the same picture was seen for DEHP metabolites. There was little evidence of associations between urinary phthalate metabolites or sums of phthalates with reproductive hormones or semen quality. The authors suggested that testosterone production and central gonadotropin secretion were affected by phthalate exposure, but emphasized that there may be other causes of the observed associations.

Specht et al., 2014: In a cross-sectional study of 589 male partners of pregnant women from Greenland, Poland and Ukraine, serum levels of both individual secondary metabolites of DINP, and a summed metric for DINP metabolites (summary-MINP), was used to estimate internal exposure. The men gave semen and blood samples and were interviewed. Six phthalate metabolites of DEHP and DINP were measured by liquid chromatography tandem mass spectrometry in serum. The metabolites were summed according to their molar weight and recalculated to an estimate of the internal concentration of the mother compounds; a new variable determined proxy-MINP. Trend analysis indicated a statistically significant negative association between serum levels of individual secondary metabolites and summary-MINP with the serum concentrations of testosterone (p<0.05). These associations were only seen across study sites, but were not statistically significant within each site. Trend analysis also showed negative associations between the DINP metabolite 7cx-MMEHP and sex hormone-binding globulin. There were no associations between individual secondary metabolites or summary-MINP on observed semen parameters. For some DEHP metabolites negative associations were seen with sperm volume and sperm count. The authors

concluded that findings were compatible with a weak anti-androgenic action of DEHP metabolites, but less so for DINP metabolites.

Specht et al., 2015: In 938 pregnant women and from Greenland, Poland and Ukraine, serum levels of DEHP and DINP metabolites (and summarized measures of DEHP and DINP metabolites) were compared with time to pregnancy and other parameters investigated by face-to-face interviews. For a subset of male partners of these women (401 males), blood samples and interview data were also available. Fecundability ratios (FR) were calculated and represented the probability of conceiving during a time period (e.g., one month or one menstrual cycle) within one group compared to the probability in the reference group. Time to pregnancy (TTP) was not consistently associated with phthalates among men or women at the three study sites. The FR was slightly elevated among women with high levels of DEHP (FR=1.14, 95% CI 1.00;1.30) suggesting a shorter TTP in these women. The FR was unrelated to DINP in women, but for first time pregnant women from Greenland separately, a FR of 0.72 indicated that having 2.7 times higher Proxy-MINP serum levels would lead to a 28% lower probability of conceiving in a menstrual cycle. First-time pregnant women from Greenland with high serum DINP levels had a significantly longer TTP. For men the results were inconsistent pointing in opposite directions. According to the authors, this study spanning large contrast in environmental exposure did not indicate adverse effects of phthalates on couple fecundity.

In a cross-sectional study, Mieritz et al., 2012, studied a total of 555 healthy boys (age 6.07–19.83 years) as part of the COPENHAGEN Puberty Study. Anthropometry and pubertal stages (PH1-6 and G1-5) were evaluated, and the presence of gynaecomastia was assessed. Non-fasting blood samples were analysed for serum testosterone and morning urine samples were analysed for the total content of 12 phthalate metabolites (MEP, MnBP, MIBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MINP, MHiNP, MiONP and MCiOP) by LC-MS/MS. Of the 555 boys and men, 38 had gynaecomastia at the time of examination. Urinary levels of phthalate metabolites were not associated with pubertal timing, serum testosterone or with the presence of pubertal gynaecomastia.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 12: Summary table of relevant experimental studies on developmental toxicity (chronol. order). 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 Guideline 414	NOAEL of 200 mg/kg bw/day for maternal and developmental effects (skeletal effects (rudimentary ribs)) at 1000 mg/kg bw/day.		Hellwig et al. (1997)

Method	Results	Remarks	Reference
(Prenatal Developmental Toxicity Study) CAS 68515-48-0 and 28553-12-0			
rat (Sprague-Dawley), n=25 oral: gavage doses: 0 (Control), 100, 500 and 1000 mg/kg/day nominal conc.) CAS: 68515-48-0 Vehicle: corn oil Exposure: Gestation Day 6 through Day 15 (Daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	Maternal NOAEL of 500 mg/kg bw/day due to reduced body weight gain and mean food consumption at 1000 mg/kg bw/day. Developmental NOAEL of 100 mg/kg bw/day 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/day.		Waterman et al, 1999 ExxonMobil (1994c)
Rat (SD), n=19 in control group, n=14 in DINP group. Oral: gavage Dose: 0, 750 mg/kg/d CAS: 68515-48-0. Vehicle: corn oil Exposure: GD 14 to PND 3	NOAEL: Not determined LOAEL: 750 mg/kg bw/day 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 to PND 10	NOAEL: ≈230 mg/kg bw/day LOAEL:≈1165 mg/kg bw/day Reduced testes weights in offspring in prepuberty. Slight histological changes in testes in adulthood.		Masutomi et al, 2003
Rat (Wistar), n= 8 dams per group	NOAEL: Not determined	One dose only.	Borch et al., 2004

Method	Results	Remarks	Reference
Oral: gavage Dose: 0, 750 mg/kg bw/day CAS no. 28553-12-0, purity >99% Vehicle: peanut oil Exposure: GD7 to GD 21	LOAEL: 750 mg/kg bw/day Statistically significant decreased testicular testosterone content GD 21($p \leq 0.01$)		
rat (Wistar-Imamichi), n=4 litters oral: diet Doses: 0, 40, 400, 4000, 20000 pm. Food consumption or dose in mg/kg-d were not reported. Exposure GD 15 to PND 21	NOAEL: Not determined LOAEL: 40 ppm (est. 2 mg/kg bw/day) Reduced AGD in males PND 1 in all doses. Increased AGD in females at 20000 ppm (est. 1000 mg/kg bw/day). 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. CAS no. 28553-12-0, purity >98%)	Lee et al., 2006
rat (SD), n=7-8 oral: gavage 0, 250, 750 mg/kg/day Vehicle: corn oil Exposure: GD 13 to GD 17	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 (GD19)	Adamsson et al., 2009
rat (Wistar), n=9-10 litters oral: gavage 0, 300, 600, 750, 900 mg/kg/d CAS 28553-12-0, purity 99% Vehicle: corn oil Exposure: GD 7 to PND 17	NOAEL: 300 mg/kg/d LOAEL: 600 mg/kg/d At 600 mg/kg bw/day: Reduced percentage of motile sperm and histological changes in foetal testis. At 750 mg/kg bw/day: reduced pup body weights, increased male nipple retention, and altered female behaviour. At 900 mg/kg bw/day: reduced male AGD. Increased sperm count per g cauda epididymis.	Satellite study examined foetal testes (n=3-4 litters)	Boberg et al., 2011; Corrigendum Boberg et al., 2016
rat (Harlan SD), n= 3-6	NOAEL: Not determined	Similar effects of the two different CAS	Hannas et al.,

Method	Results	Remarks	Reference
litters oral: gavage Doses: 0, 500, 750, 1000, 1500 mg/kg/d Two types of DINP: CAS 28553-12-0 and CAS 68033-90-2 Vehicle: corn oil Exposure: GD 14 to 18	LOAEL: 500 mg/kg/d Reduced testis testosterone production GD 18 at all tested doses (reduced to 31% of control level at top dose; see table below)	numbers of DINP	2011
rat (SD), n=8-9 oral: gavage Doses: 0, 50, 250, 750 mg/kg/day CAS 68515-48-0 Vehicle: corn oil Exposure: GD 12 to 19	NOAEL: 50 mg/kg/d LOAEL: 250 mg/kg/d Statistically significant decrease in testis testosterone content and increased presence of multinuclear gonocytes at GD 19 in animals exposed to 250 mg/kg/d and 750 mg/kg bw/d.	Effects on Leydig cell clustering at 750 mg/kg bw/day	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 to PND 14	NOAEL: 56 mg/kg/d (760 ppm) LOAEL: 288 mg/kg/d (3800 ppm) At 3800 ppm: Increased number of animals with multinuclear gonocytes at PND 2, 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, increased incidence of Leydig cell aggregates and multinuclear gonocytes at PND 2, reduction of absolute weight of LABC at PND 49/50.	DBP used as positive control. Measurements of blood metabolites.	Clewell et al., 2013b
Rat (SD), n=3-4 per group Oral: gavage Dose: 0, 750 mg/kg/day CAS no. 28553-12-0, 98,8%	Inhibition of testosterone synthesis, testosterone production significantly reduced at 750 mg/kg/day	Short-term in vivo One dose only. Similar effects of two different CAS numbers of DINP	Furr et al. 2014

Method	Results	Remarks	Reference
and 68515-48-0, 99% Exposure: GD 14 to GD 18		DINP is weak positive.	
Rat (SD), n=6 per group Oral: gavage Doses: 10, 100, 500, 1000 mg/kg bw/day CAS 28553-12-0 purity >99% Exposure: GD 12 to 21	No effect on AGD in neonatal pups. Statistically significant reduction of testicular testosterone content at 1000 mg/kg bw/day (dose-dependent decrease at all doses, NS). Histological changes in testes (clustering of Leydig cells from 10 mg/kg; dysgenesis of seminiferous chords and presence of multinucleated gonocytes from 100 mg/kg). Reduced mRNA levels of Insl3 from 10 mg/kg; reduced mRNA levels of several genes involved in steroidogenesis from 100 mg/kg.	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

The developmental toxicity studies by Hellwig et al., 1997, and Waterman et al., 1999, were performed according to OECD Guideline 414 and did not examine testicular histopathology or hormone-sensitive endpoints such as anogenital distance or testicular testosterone production (Hellwig et al., 1997, Waterman et al., 1999, see also Annex I). A number of studies with DINP have been published examining hormone-sensitive endpoints in offspring before or after birth. Most of these studies were published after the EU RAR from 2003. These studies are described below (based on descriptions in EU RAR or publicly available scientific papers) and discussed in relation to reproductive toxicity in section 4.11.4.

Hellwig 1997. Either CAS 68515-48-0 or 28553-12-0 (two preparations) was applied in three sub-studies. In each screening study, eight to ten pregnant Wistar rats (Chbb/THOM) per group were administered by gavage daily doses of 0 - 40 - 200 - 1,000 mg/kg/d (in olive oil DAB 9/10) on days 6-15 post-coitum. One half of the foetuses were examined for soft tissue abnormalities and the remaining foetuses for skeletal abnormalities.

With DINP CAS 68515-48-0 dams at 1,000 mg/kg/d dams consumed less food without concurrent statistically significant decrease of the body weight (decrease around 3-4% compared to the control group). At autopsy a statistically significant increase in relative kidney weights was recorded at 1,000 mg/kg/d; the relative liver weights were slightly, but not statistically significantly, increased. Absolute liver and kidney weights data were not reported. An increased occurrence of foetal skeletal variations was seen at 1,000 mg/kg/d consisting mainly of rudimentary cervical and/or accessory 14th ribs. The NOAEL for the conceptuses was considered to be 200 mg/kg/d and the NOAEL for the dams is considered 200 mg/kg/d, based on slight decrease of food consumption and a slight increase of relative kidney weights.

With DINP CAS 28553-12-0 no significant decrease of food consumption, of the body weight, and of the corrected body weight gain was observed. Absolute and relative liver and kidney weights were not affected by the test substance. The only substance-related foetal effect was a statistically significantly increased incidence of a skeletal variation namely accessory 14th rib(s): 5/10 vs. 0/10 in controls on a per litter basis at 1,000 mg/kg/d. The respective values were distinctly above the

historical control values. It was assumed that the NOAEL for the conceptuses was 200 mg/kg/d and the NOAEL for the dams was 200 mg/kg/d.

In a third sub-study with another preparation of DINP CAS 28553-12-0, developmental toxicity was present in the form of increased rates, of certain skeletal retardation (unossified or incompletely ossified sternebrae) and skeletal (rudimentary cervical and/or accessory 14th ribs) variations. The statistically significant increased occurrence of 1,000 mg/kg/d foetuses with rudimentary cervical (78% vs. 0 in controls on a per litter basis) and/or accessory 14th ribs (89% vs. 0 in controls on a per litter basis) was considered to be related to the test substance administration to the dams. The respective values were far above the actual and historical control values.

Waterman et al., 1999. In a developmental toxicity study, four groups of 25 Sprague Dawley /Crl:CDBR rats were administered daily by gavage DINP (MRD 92-455, CAS 68515-48-0) at doses of 0 - 100 - 500 - 1000 mg/kg (in corn oil) on days 6-15 of gestation. One half of the foetuses were examined for visceral abnormalities and the remaining foetuses for skeletal abnormalities. Statistically significant decreases of the dam body weight gain (42.6 g vs. 50 g in control group) and of the mean food consumption (178.6 g vs. 195.9 g in control group) were observed at 1,000 mg/kg/day during the treatment period (6-15 days of gestation). However, mean body weight gain of all treated group females was essentially equivalent for the overall gestation period and after correction for gravid uterine weight when compared with controls. No statistically significant differences in mean foetal body weight and no statistically significant increases in total or individual external, visceral or skeletal malformations between treated and controls were observed. However, statistically significant increases in foetuses with skeletal lumbar rudimentary ribs and with visceral (dilated renal pelves) variations were seen at 1,000 mg/kg/d on a per litter basis. Specifically, dose-related increase was seen in the total number of foetuses with visceral (mainly dilated renal pelves) variations on a per foetus basis (7/190, 8/198, 9/178 at doses of 100- 500 - 1,000 mg/kg 1/194) and on a per litter basis (3/25, 4/24, 6/23 at doses of 0 - 100 - 500 - 1,000 mg/kg vs. 0/23 in controls). However, variations were only significantly increased at the high-dose level on a per litter basis (6/23 vs. 0/24 in controls. Skeletal variations, mainly rudimentary lumbar and cervical ribs showed a dose-response trend on a per litter as on per foetus basis (32/191, 28/186, 55/194, 76/174 on a per foetus basis and 15/24, 16/25, 22/24, 20/23 on a per litter basis at doses of 0 - 100 - 500 -1000 mg/kg, respectively). A statistically significant difference from controls on a per litter basis was observed only at 1,000 mg/hg/d (22/24 vs. 15/24 in controls). When considered individually, only rudimentary lumbar ribs were statistically significantly different from controls on a per litter basis at the high dose of 1,000 mg/kg (18/23 vs. 6/24 in controls). Based on the clear dose-response profile, together with the fact that incidence of dilated pelves was zero in controls, a NOAEL of 500 mg/kg/d was assumed for developmental toxicity. The NOAEL for the dams was 500 mg/kg/d.

Gray et al., 2000 performed a study on several phthalates. Pregnant rats were gavaged daily with DINP, DEHP, BBP, DEP, DMP and DOTP at single dose of 750 mg/kg/d in corn oil as vehicle from gravid day 14 through postnatal day 3. n=19 control litters and 14 DINP exposed litters. Male offspring with areolas were observed in the DEHP, BBP and DINP dose groups at day 13. Males in the DINP (7.7%, $p<0.04$), DEHP, (91%, $p< 0.0001$), and BBP (84%, $p< 0.0001$) treatment groups had malformations of testis, epididymis, accessory reproductive organs and external genitalia. DINP treatment (750 mg/kg bw/day) modestly reduced maternal weight gain, but maternal body weights were not significantly reduced at any time, and by the end of dosing on PND 3, dams showed no weight change from the start of dosing. DINP did not affect pup weight, nipple retention or AGD in contrast to DEHP and BBP. DINP did not change weights of male reproductive organs in contrast to BBP and DEHP, which reduced several organ weights at PND (testes, levator ani

bulbocavernosus muscle, seminal vesicle, ventral prostate, glans penis, epididymis) of the chemicals altered serum testosterone or weights of kidney, liver, pituitary, or adrenals.

Masutomi et al., 2003, examined the influence of perinatal exposure (GD 15 to PND 10) to DINP on anogenital distance, prepubertal organ weights, puberty onset, adult organ weights and histology of endocrine organs in SD rats (in parallel with studies on methoxychlor and genistein). Dietary doses of 400, 4000 or 20000 ppm corresponded to 30.7, 306.7, and 1164.5 mg/kg bw/day in the gestational period and to 66.2, 656.7, and 2657 mg/kg bw/day in the lactational period (n=5-6 litters per dose group). Reduction in maternal food consumption and body weight gain in the high dose group was seen during gestation and lactation. A slight decrease in mean number of live offspring was seen at the highest dose level of DINP (82% of controls, NS), and pup body weight gain was significantly decreased at PND 2-10. Anogenital distance was reduced to 90% of controls at the two highest doses, but this was not statistically significant, and as body weight was also reduced, it is not clear whether anogenital index (body weight-corrected anogenital distance) would be different from controls in this study with a small number of litters. Prepubertal offspring exposed to DINP at 4000 and 20000 ppm had reduced body weight, whereas reduced absolute brain weight and increased relative brain weight was seen at 20000 ppm. Absolute and relative testes weights were reduced at the highest dose level (prepuberty). Absolute ovary and uterus weights were reduced at 20000 ppm with no change in relative weights (prepuberty). Numerical values for these endpoints are listed in table 13 below.

Table 13: Data from birth to prepubertal (PND21) necropsy

Group (ppm DINP)	**0**	**400**	**4000**	**20000**
No. of litters	5	5	5	5
Maternal body weight gain (g per day)				
Gestational period (GD 15–GD 20)	12.3 ± 2.1	13.3 ± 2.6	14.9 ± 2.4	5.5 ± 1.5**
Lactational period (PND 2–PND 10)	4.8 ± 1.6	5.2 ± 2.8	5.4 ± 1.5	0.7 ± 2.9*
Maternal food consumption (g per day)				
Gestational period (GD 15–GD 20)	26.6 ± 2.1	26.8 ± 1.7	26.9 ± 2.1	19.2 ± 2.7**
Lactational period (PND 2–PND 10)	51.4 ± 4.2	54.2 ± 4.9	54.9 ± 3.8	40.2 ± 5.5**
Calculated maternal intake (mg/kg per day)				
Gestational period (GD 15–GD 20)	0	30.7 ± 16.3	306.7 ± 12.8	1164.5 ± 152.9
Lactational period (PND 2–PND 10)	0	66.2 ± 35.2	656.7 ± 35.8	2656.7 ± 462.6
No. of live offspring	11.2 ± 2.9	12.6 ± 1.5	13.0 ± 2.0	9.2 ± 3.3
Body weight, PND 2 (g)				
Males	7.7 ± 0.7	7.8 ± 0.7	7.0 ± 0.9	6.5 ± 1.3
Females	7.1 ± 0.3	7.5 ± 0.6	6.6 ± 0.7	6.3 ± 1.6
AGD, PND 2 (mm)				
Males	3.3 ± 0.4	3.2 ± 0.3	3.0 ± 0.1	3.0 ± 0.5
Females	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	0.9 ± 0.2
Body weight gain (g per day)				
Males				
PND 2–PND 10	1.8 ± 0.3	1.6 ± 0.3	1.4 ± 0.2	0.8 ± 0.2**
PND 10–PND 21	2.6 ± 0.4	2.8 ± 0.3	2.7 ± 0.1	2.4 ± 0.2

Group (ppm DINP)	0	400	4000	20000
Females				
PND 2–PND 10	1.8 ± 0.4	1.6 ± 0.3	1.4 ± 0.2	0.8 ± 0.1**
PND 10–PND 21	2.6 ± 0.4	2.8 ± 0.3	2.7 ± 0.2	2.4 ± 0.4
Organ weights, prepubertal necropsy				
Males (no. of rats)	5	5	5	5
Body weight (g)	88.5 ± 6.4	84.4 ± 3.8	72.6 ± 7.9**	50.6 ± 7.9**
Brain				
Absolute (g)	1.63 ± 0.11	1.59 ± 0.01	1.56 ± 0.08	1.41 ± 0.06**
Relative (g/100 g BW)	1.85 ± 0.13	1.89 ± 0.06	2.16 ± 0.22	2.84 ± 0.39*
Adrenals				
Absolute (mg)	20.4 ± 0.5	21.0 ± 2.5	18.0 ± 3.1	16.0 ± 4.7
Relative (mg/100 g BW)	23.1 ± 1.3	25.0 ± 3.1	24.7 ± 3.0	31.5 ± 6.9
Testes				
Absolute (g)	0.56 ± 0.02	0.58 ± 0.05	0.44 ± 0.08	0.26 ± 0.06**
Relative (g/100 g BW)	0.63 ± 0.03	0.67 ± 0.04	0.61 ± 0.05	0.51 ± 0.05**
Females (no. of rats)	5	5	5	5
Body weight (g)	76.7 ± 4.7	79.6 ± 5.0	75.0 ± 4.3	46.6 ± 2.4**
Brain				
Absolute (g)	1.53 ± 0.08	1.54 ± 0.08	1.54 ± 0.04	1.36 ± 0.02**
Relative (g/100 g BW)	2.00 ± 0.06	1.93 ± 0.09	2.06 ± 0.11	2.92 ± 0.12**
Adrenals				
Absolute (mg)	18.4 ± 2.3	20.0 ± 3.5	20.2 ± 2.2	16.0 ± 1.1
Relative (mg/100 g BW)	24.1 ± 3.4	25.0 ± 4.7	27.0 ± 2.9	34.4 ± 4.4**
Ovaries				
Absolute (mg)	24.2 ± 2.4	21.0 ± 1.9	21.8 ± 2.3	17.0 ± 2.4**
Relative (mg/100 g BW)	34.3 ± 5.5	26.4 ± 2.3	29.1 ± 2.9	30.7 ± 4.9
Uterus				
Absolute (g)	48.4 ± 12.3	52.0 ± 3.1	48.0 ± 5.7	25.4 ± 4.2**
Relative (g/100 g BW)	63.3 ± 16.5	65.2 ± 7.5	64.1 ± 8.2	54.8 ± 11.4

Values are shown as mean + SD. *p<0.05, **p<0.01, and ***p<0.001.

At the highest dose of DINP, body weights at puberty onset were reduced in male and females, but no changes was seen in the age of puberty onset (Table 14). In adulthood (week 11) no changes in body or organ weights were detected in DINP exposed groups. Ovaries in some adult females exposed to 20000 ppm showed an increase of secondary follicles/decrease of corpora lutea, although the change was not statistically significant. Testicular histology was slightly affected at 20000 ppm, as degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells were observed in the testes. In these cases, scattered cell debris was found in the epididymal ducts. These changes were evaluated as minimal to slight in severity, but observed in four out of five animals at this dose. In the ECHA review from 2013, these histological findings were discussed and it was noted that "*It may be concluded that perinatal exposure at dose levels causing systemic toxicity in the dams induce minimal or slight but permanent changes in testes and ovaries of the offspring. The NOAEL for the permanent (as well as nonpermanent) changes observed in this study is 307-657 mg/kg bw/day (4000 ppm)*" (p. 140) (ECHA, 2013).

Table 14: Data from puberty to final necropsy

Group (ppm DINP)	**0**	**400**	**4000**	**20000**
No. of rats	8	8	8	8
Body weight gain (g per day)				
Males				
PND 21–PND 42	7.9 ± 0.3	8.3 ± 0.6	7.5 ± 0.6	6.5 ± 0.7**
PND 42–PND 77	6.6 ± 0.5	6.9 ± 0.5	6.7 ± 0.8	6.4 ± 0.5
Females				
PND 21–PND 42	5.9 ± 0.5	6.2 ± 0.3	5.9 ± 0.8	5.2 ± 0.7
PND 42–PND 77	3.0 ± 0.4	3.2 ± 0.4	3.1 ± 0.3	3.0 ± 0.4
Onset of puberty				
Vaginal opening				
Age (days)	36.0 ± 1.3	34.8 ± 0.7	35.5 ± 0.8	35.8 ± 0.7
Body weight (g)	139.5 ± 13.1	133.7 ± 5.9	129.5 ± 11.8	112.7 ± 20.8*
Preputial separation				
Age (days)	41.8 ± 0.5	41.9 ± 0.4	42.1 ± 0.6	42.3 ± 0.5
Body weight (g)	216.0 ± 9.3	225.1 ± 14.5	205.2 ± 15.2	177.3 ± 17.2**
Estrous cyclicity				
Normal	8	8	8	8
Organ weights, final necropsy				
Males (no. of rats)	5	5	5	5
Body weight (g)	504.3 ± 27.2	527.0 ± 24.7	478.6 ± 57.6	456.4 ± 17.9
Brain				
Absolute (g)	2.06 ± 0.09	2.13 ± 0.09	2.11 ± 0.06	2.00 ± 0.05
Relative (g/100 g BW)	0.41 ± 0.03	0.40 ± 0.02	0.45 ± 0.05	0.43 ± 0.01
Pituitary				
Absolute (mg)	15.0 ± 2.0	16.4 ± 2.1	14.6 ± 2.2	14.2 ± 1.8
Relative (mg/100 g BW)	2.99 ± 0.52	3.11 ± 0.40	3.07 ± 0.45	3.11 ± 0.40
Adrenals				
Absolute (mg)	47.8 ± 6.5	52.4 ± 9.6	53.8 ± 8.8	52.2 ± 5.9
Relative (mg/100 g BW)	9.5 ± 1.0	10.0 ± 2.1	11.3 ± 1.7	11.5 ± 1.4
Testes				
Absolute (g)	3.11 ± 0.54	3.43 ± 0.17	3.16 ± 0.14	2.92 ± 0.32
Relative (g/100 g BW)	0.61 ± 0.09	0.65 ± 0.03	0.67 ± 0.07	0.64 ± 0.07
Prostate				
Absolute (g)	0.69 ± 0.17	0.66 ± 0.12	0.63 ± 0.10	0.56 ± 0.25
Relative (g/100 g BW)	0.14 ± 0.03	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.05
Females (no. of rats)	5	5	5	5
Body weight (g)	307.5 ± 23.2	314.2 ± 20.5	304.0 ± 34.2	292.3 ± 23.7
Brain				
Absolute (g)	1.96 ± 0.08	1.93 ± 0.09	1.98 ± 0.08	1.92 ± 0.13
Relative (g/100 g BW)	0.64 ± 0.06	0.62 ± 0.05	0.66 ± 0.07	0.65 ± 0.10

Group (ppm DINP)	0	400	4000	20000
Pituitary				
Absolute (mg)	16.8 ± 2.2	17.2 ± 1.6	16.4 ± 2.7	17.2 ± 2.9
Relative (mg/100 g BW)	5.48 ± 0.70	5.48 ± 0.49	5.38 ± 0.51	5.87 ± 0.72
Adrenals				
Absolute (mg)	59.6 ± 12.5	55.8 ± 9.54	63.8 ± 14.8	56.6 ± 6.8
Relative (mg/100 g BW)	19.5 ± 4.6	17.8 ± 3.3	20.9 ± 3.6	19.6 ± 3.6
Ovaries				
Absolute (mg)	94.6 ± 14.0	92.0 ± 15.0	92.6 ± 5.8	88.6 ± 11.9
Relative (mg/100 g BW)	30.9 ± 5.1	29.4 ± 5.1	30.7 ± 3.5	30.7 ± 6.0
Uterus				
Absolute (g)	0.46 ± 0.10	0.42 ± 0.06	0.47 ± 0.12	0.47 ± 0.12
Relative (g/100 g BW)	0.15 ± 0.02	0.13 ± 0.01	0.16 ± 0.05	0.16 ± 0.05

Values are shown as mean + SD, expect from estrous cyclicity, which is presented as number of animals with corresponding cyclicity. *p<0.05, **p<0.01, and ***p<0.001.

In study by Borch et al., 2004, pregnant Wistar rats were exposed from GD 7 to GD 21 with DINP (750 mg/kg bw/day), DEHP (300 mg/kg bw/day) or a combination of DINP (750 mg/kg bw/day) plus DEHP (300 mg/kg bw/day). Testicular testosterone production and testicular testosterone content was statistically significantly ($p \leq 0.01$) reduced (to around one third of control levels) in DINP exposed male foetuses at GD 21. Plasma testosterone levels were reduced by 25%, but the reduction was not statistically significant. Similar effects were seen with DEHP, and the effect was more marked in the group receiving a combination of DEHP and DINP. Slight reductions in plasma testosterone and slight increases in plasma LH were not statistically significant.

Lee et al (2006) examined the effects of perinatal exposure to DINP (CAS No 28553-12-0, purity >98%) on hypothalamic gene expression, hormonal levels and sexual behaviour. In addition to DINP, effects of DBP and di-(ethylhexyl)adipate (DEHA) were examined. Pregnant female Wistar-Imamichi rats were exposed to DINP in dietary concentrations of 0, 40, 400, 4000 and 20,000 ppm from GD 15 to the weaning on PND 21. The adjusted (normalised) AGD (AGD per cube root of body weight ratio) was decreased on PND 1 in males at all tested dose levels (40, 400, 4000 and 20,000 ppm) showing some dose dependency. The adjusted AGD in females was increased at the dose level of 20,000 ppm. Exact numerical values could not be obtained from the graphical illustrations presented in published paper.

Female offspring showed a significant decrease in the lordosis quotient (number of lordosis reflexes/10 mounts by males x 100%), a measure of sexual responsiveness, at all tested dose levels. The lordosis quotient of female offspring was approximately 75, 50, 45 and 25% at 0, 40, 400 and 4000 ppm, respectively, which indicates a clear dose-depend effect. Reduced copulatory behaviour was seen in the low dose group male offspring, without dose dependence.

In the ECHA 2013 review, the limitations of this study are discussed with the conclusion that the drastically reduced female sexual behaviour observed in this study need to be followed up before any firm conclusions can be drawn.

Adamsson et al., 2009, examined the influence of DINP (and pp'-DDE) on testicular testosterone production, testicular mRNA and protein levels for steroidogenesis and testicular histology in SD rats exposed during gestation. Three groups of 7-8 pregnant dams were exposed by gavage from

GD 13 to GD 17, and male foetuses were examined after caesarean on GD 19. No change in testosterone production of foetal testes at GD 19 after exposure to 250 and 750 mg/kg bw per day of DINP were found. DINP did not alter the histology of steroidogenic cells in the foetal testes or adrenals. Adamsson et al. (2009) found increased mRNA levels of P450scc and Insl3, genes that are known to be reduced by other phthalates and that are likely involved in the anti-androgenic effects of these compounds. This discrepancy between previous studies on phthalate effects on steroidogenic factors and the results reported by Adamsson et al., 2009 may be due to the fact that their study included a recovery period of two days between the last dosing and the time of examination. The authors describe in their discussion that the detected increase in P450scc and Insl3 may be a "rebound effect" due to low testosterone production at the time of dosing a few days earlier (Adamsson et al., 2009). This is also commented by ECHA 2013 (citation in italics): "*It is considered plausible that testicular T content, if reduced due to exposure, has recovered in two days between the last dosing and the point in time when T levels were measured, and that the gene expressions associated with steroidogenesis were increased for several days after cessation the exposure and after the exposure-induced decrease"*. The study did not include any examination of testosterone production or levels at the end of dosing at GD 17.

In the study by Boberg et al., 2011, pregnant Wistar rats were gavaged from gestation day 7 to postnatal day (PND) 17 with vehicle, 300, 600, 750 or 900 mg DINP/kg bw/day. Offspring (subgroup 1, n=3-4) was examined on GD 21 or in adulthood (subgroup 2, n=9-10). A corrigendum from Boberg et al., 2016a provided further description of the statistical methods than in the 2011 paper. Furthermore, minor errors in descriptive statistics (but not in statistical results) were corrected in a letter from Boberg et al. (submitted to Reproductive Toxicology November 2016).

DINP treatment did not alter maternal body weight and weight gain during pregnancy at any dose.

In foetal testes histopathological effects known for certain other phthalates were observed (statistically significant from 600 mg/kg bw/day). As seen in Table 15 multinucleated gonocytes (MNGs) were seen in some animals in the lowest dose group with dose dependent increase at higher doses. In addition, most testes at the two highest dose levels had an increased number of gonocytes with a central location in seminiferous chords, and chord diameters were significantly increased.

Table 15: Testis histopathology GD 21 in rat fetuses exposed to 0, 300, 600, 750 or 900 mg/kg bw/day of DINP from GD 7 to 21. One testis section evaluated from 1 to 4 males per litter. Table lists: % affected, affected animals/total number of animals (affected litters/total number of litters). Results in **bold** are significantly different from controls in a one-sided Fisher's exact test ($p \leq 0.05$).

Testis histopathology GD 21	**Control**	**DINP 300**	**DINP 600**	**DINP 750**	**DINP 900**[#]
Total number of animals with histological changes	0%, 0/7 (0/3)	25%, 2/8 (2/4)	**60%, 3/5** (2/3)	**100%, 7/7 (3/3)**	**100%, 6/6 (3/3)**
Multinucleated gonocytes	0%, 0/7 (0/3)	25%, 2/8 (2/4)	**60%, 3/5 (3/3)**	**86%, 6/7 (3/3)**	**100%, 6/6 (3/3)**
Many gonocytes with central location in chords	0%, 0/7 (0/3)	0%, 0/8 (0/4)	40%, 2/5 (2/3)	**86%, 6**/7 **(3/3)**	**80%, 4/5** (2/2)
Enlarged diameter of seminiferous chords	0%, 0/7 (0/3)	0%, 0/8 (0/4)	40%, 2/5 (2/3)	**57%, 4/7** (1/3)	**60%, 3/5** (2/2)

[#] In one of the six animals evaluated in this group, the testis was damaged and testis histology could not be fully evaluated; however the presence of multinucleated gonocytes was noted.

Slight effects on testicular testosterone content and –production were observed, though this was only statistically significant at 600 mg/kg bw/day for testicular testosterone content (approximately 50% of control values). The low number of litters available for these analyses likely causes a low sensitivity for detecting possible changes in hormone levels.

In male offspring, DINP caused dose-dependently increased nipple retention (statistically significant from 750 mg/kg bw/day), reduced anogenital distance at PND 1 (statistically significant at 900 mg/kg bw/day when corrected for body weight changes), and reduced body weights at PND 13 (no change in birth weight) (Table 16). The reduction in AGD was seen in the absence of (significantly) reduced male pup birth weight.

Table 16: Body weights, anogenital distance at birth (AGD) and nipple retention on PND 13 for pups exposed to DINP from GD 7 to PND 17 (mean ± SD). Values for DINP 900 group were corrected in letter by Boberg et al. (submitted).

Males	**Control**	**DINP 300**	**DINP 600**	**DINP 750**	**DINP 900**
Birth weight[a](g)	6.39±0.39	6.30±0.12	6.12±0.32	6.14±0.49	6.00±0.56
Weight PND 13[a](g)	28.64±5.21	28.60±2.99	27.44±2.25	26.25±3.58	**25.57±2.96****
AGD[a] (units)	21.50±1.82	21.09±1.58	20.65±2.11	20.67±1.55	**19.82±1.41***
AGDi[b] (units)	11.60±1.04	11.43±0.82	11.31±0.20	11.29±0.75	**10.94±0.86***
Nipples	1.98±0.83	2.00±0.64	2.91±0.69[c]	**3.14±1.21***	**3.22±0.89***
Females					
Birth weight[a](g)	5.97±0.31	5.90±0.19	6.01±0.32	5.88±0.41	5.76±0.29
Weight PND 13[a](g)	29.49±4.69	27.93±2.53	26.47±2.49	**24.40±4.62***	24.67±3.19 [d]
AGD[a] (units)	11.05±0.90	11.32±0.85	11.38±1.30	11.27±0.86	10.70±0.66
AGDi[b] (units)	6.10±0.50	6.27±0.48	6.26±.0.67	6.25±0.38	5.98±0.41
Nipples	12.30±0.26	12.25±0.19	12.3±0.24	12.24±0.14	12.34±0.25

[a] Analyzed with body weight as a covariate
[b] AGDi is defined as AGD divided by the cube root of the body weight
*ANOVA followed by Dunnett's test $p < 0.05$. **ANOVA followed by Dunnett's test $p<0.01$
[c] ANOVA followed by Dunnett's test $p=0.058$, [d] ANOVA followed by Dunnett's test $p=0.052$

In adulthood (PND 90), a dose-dependent reduction in sperm motility was seen from 600 mg/kg bw/day (14-19% reduction; Table 17). When data were analyzed without a low outlier in the group exposed to 750 mg/kg bw/day the reduction in sperm motility was statistically significant ($p<0.05$) from 600 mg/kg bw/day when (using Dunnett's test). Data for sperm count per g cauda epididymis was increased at 900 mg/kg bw/day, whereas there was no change in the total number of sperm in cauda epididymis. The percentage of progressive sperm was decreased (23% reduction) at 750 mg/kg bw/day where one animal had very low sperm motility (also small testes and epididymides). When data were analyzed without a low outlier in the group exposed to 750 mg/kg bw/day, the decrease in progressive sperm was only borderline significant ($p=0.08$).

Table 17: Semen quality analysis in male 90-day old rats exposed to 0, 300, 600, 750 or 900 mg/kg bw per day of DINP from GD7 to PND 17 (n = 6–10). Mean± SD.

Group mean values	% motile sperm	% progressive sperm	sperm/gram cauda	cauda	sperm count
Control	59.25±7.09	32.19±6.94	428.44±93.80	0.23±0.02	97.84±20.73
DINP 300	57.19±7.34	33.56±5.02	474.78±61.51	0.21±0.02	101.14±17.08
DINP 600	51.25±8.25 *	27.81±7.82	455.85±77.20	0.23±0.02	102.10±17.87
DINP 750	47.92±12.74 **, ##	24.69±8.01 *	423.44±96.36	0.21±0.03	91.68±25.14
DINP 900	49.43±8.16 **, #	27.07±5.86	499.34±68.71 *, #	0.21±0.01	107.04±17.44

*p < 0.05, **p < 0.01 in test for differences of LSmeans; # p<0.05, ## p<0.01 using Dunnett's test correcting for multiple comparisons.

Mean testicular T content was 63% of control levels at the highest dose level on PND 90, but this was not statistically significant. There were no changes in adult reproductive organ weights, testis testosterone or serum inhibin B levels or in the histopathology of adult male reproductive organs. There was no statistically significant difference between groups in the number of nipples or AGD on PND 90, however four adult DINP exposed animals had permanent malformations such as epididymal and testicular dysgenesis and permanent nipples. One animal from each of the groups exposed to 600 and 750 mg/kg bw per day of DINP had very small testes and epididymides. Two other males exposed to 600 and 750 mg/kg/day of DINP, had 4 and 6 thoracic nipples, respectively. These findings corroborate the findings of very low incidences of malformations of male reproductive organs in the study by Gray et al., 2000.

DINP affected spatial learning as female offspring in the highest dose group performed better than controls and similarly to control males in the Morris Water Maze, indicating masculinization of behaviour in DINP exposed females. A dose-related reduction in swim length and latency to reach the platform was seen in females with the highest dose group being significantly different from control females but comparable to control males (Figure 1).

Effects of DINP on female behaviour were not observed on the other test days in the Morris water maze, nor in the other behavioural tests performed (motor activity and habituation capability, spatial learning and memory in the radial arm maze, and sweet preference).

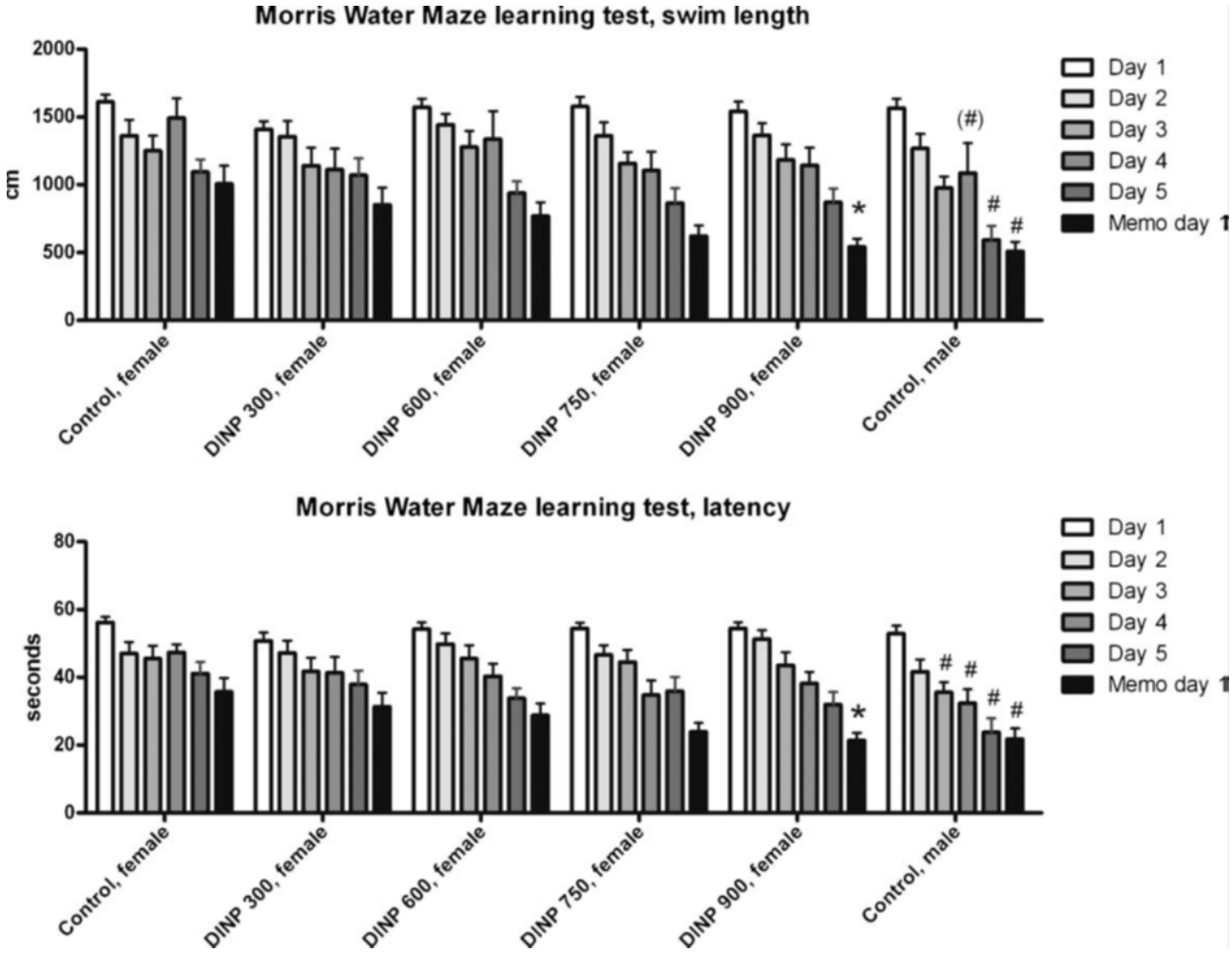


Figure 1: Results of a learning test in female rats exposed to 0, 300, 600, 750 or 900 mg/kg bw per day of DINP from GD 7 to PND 17 (n = 7–10). Mean+ Standard Error Mean (SEM), *p < 0.05 compared to control females, #p < 0.05 control males compared to control females. A dose-related reduction in swim length (A) and latency (B) to reach the platform was seen in females with the highest dose group being significantly different from control females but comparable to control males.

Hannas et al., 2011, describes a study in which pregnant Sprague-Dawley rats were exposed to 0, 500, 750, 1000, or 1500 mg/kg bw/day of DINP from GD 14 to 18 by gavage (in parallel with other phthalates). The study included three blocks of animals exposed to two different CAS numbers for DINP: CAS# 28553-12-0 from BASF was administered to 3-6 dams per dose group across two separate blocks, and CAS# 68033-90-2 was administered to 3 dams per dose group in a single block. DINP did not affect mortality, maternal body weight or litter size at any dose. Testicular testosterone production ex vivo was assessed by incubation of testes of 18 day old foetuses for 3 hours and testosterone measurement in the media. Dose-related statistically significant decreases in testosterone production were seen for DINP from 500 mg/kg bw/day (Table 18) and for the other tested phthalates (DEHP, DiBP and DiHP (diisohexyl phthalate)) from 300 mg/kg bw/day and above. No NOAEL could be obtained for DINP, as effects were seen at all dose levels, whereas the other phthalates were tested in lower doses and showed a NOAEL of 100 mg/kg bw/day. DINP was 2.3 fold less potent than DIBP, DIHP and DEHP in reducing foetal testicular T production (studied

with a similar test set up) and 18-fold less potent that DPeP. The mean expression of mRNA for the steroidogenic factors StAR and CYP11a was reduced at all doses of DINP, though this was only statistically significant at 1000 and 1500 mg/kg bw/day. Overall, no differences were seen for the two different DINP formulations.

Table 18: Effects of DINP exposure from GD 14 to 18 (two different formulations) on testicular testosterone production ex vivo and testicular mRNA expression of StAR and Cyp11a on GD 18.

DINP dose, mg/kg bw/day	**Testosterone production, ng/testis**	**N, testosterone production**	**StAR expression, % of control**	**Cyp11a expression, % of control**	**N, gene expression**
	Mean ±SE		Mean ±SE	Mean ±SE	
0	7.00 ± 0.36	9	100.0 ± 19.0	100.0±9.6	7
500	4.90 ± 0.20*	5	72.8±19.0	83.1±9.1	5
750	3.87 ± 0.56**	5	63.8±11.0	72.4±11.3	5
1000	2.98 ± 0.19**	9	41.5±9.2 **	52.4±7.0***	9
1500	2.14 ± 0.27 **	9	30.5±4.0 **	51.1±7.6***	8

*p≤0.05, **p≤0.01 and ***p≤0.001

Clewell et al., 2013a (designated Clewell et al 2011a by ECHA 2013), performed a study on foetal exposure (GD 12 to 19) of rats to DINP. Dams (27 controls and 8 DINP exposed dams) were dosed from GD 12 to 19 and caesarean sections were performed 2 or 24 hours after last dosing (GD 20). Three doses of DINP were administered by gavage: 50, 250 or 750 mg/kg bw/day. Another subset of animals (25 controls and 8 DINP exposed dams) was similarly dosed from GD 12 to 19 and caesarean sections were performed 2 hours after last dosing. The animals sacrificed 2 hours after dosing were applied for measurement of testicular testosterone and metabolite disposition, whereas the animals sacrificed 24 hours after dosing were applied for anogenital distance measurement, testicular testosterone measurement, testicular histopathology, and metabolite disposition. Other animals were sacrificed 0.5, 1, 6, and 12 hours after dosing and applied for metabolite disposition only, and these analyses will not be discussed in detail here.

DINP exposure by gavage did not alter maternal body weight or weight gain during pregnancy and did not alter foetal body weight at sacrifice. No change in fetal AGD at GD 20 was found (only one measure of AGD is listed in the published paper, and it is assumed that this measure is for male fetuses). In the foetuses exposed to 250 and 750 mg/kg bw/day of DINP an increased number of multinuclear gonocytes in testes was seen, and testicular testosterone production 2 hours after dosing was decreased.

It is noted by ECHA 2013 that foetal testicular T levels reduced by 50% at 250 mg/kg bw/day dose level and by ~60% at 750 mg/kg bw/day dose level indicating that at lower dose levels the reduction is larger than would be expected by linear extrapolation from higher dose levels.

At the highest dose of 750 mg/kg bw/day also the incidence of Leydig cell aggregates was increased. Table 19 below on histopathological findings in testes on GD 20 is adopted from ECHA 2013 and Clewell et al., 2013a:

Table 19: Histopathological findings at GD 20 in testes of rats exposed to 0, 50, 250, or 750 mg/kg bw/day of DINP from GD 12 to19.

	Control	**DINP 50 mg/kg bw/day**	**DINP 250 mg/kg bw/day**	**DINP 750 mg/kg bw/day**
Number of animals examined	27	8	8	8
Animals with multinucleated gonocytes	0	0	2	6*
Animals with increased number of gonocytes	0	0	0	2
Animals with large Leydig cell aggregates	2	3	1	7*

* indicates $p<0.001$, one-way ANOVA.

Kinetic studies were performed to calculate internal and external exposure doses and compare potencies with DEHP and DBP. The highest measured intratesticular concentrations of the DINP metabolites MINP and MCiOP related to decreased T levels were 92.9 vs 15.5 µM for MINP at one hour after the final dose of 250 and 50 mg/kg bw/day, respectively, and 70.6 vs 25.0 µM for MCiOP at 6 hours after the final dose of 250 and 50 mg/kg bw/day, respectively. The potency of DINP (internal and external dose) on reducing testicular testosterone content was compared with the potencies of DEHP and DBP (internal and external dose). This is presented in Table 3 of the publication, which is reproduced below (Table 20):

Table 20: Comparison of the potencies of DINP, DEHP, and DBP for reduced testosterone in fetal testes based on external ED50 and internal IC50 values.

	DBP	**DEHP**	**DINP**
External dose (ED50) (mg/kg/day)	39[a]	100[a]	250[c]
Maternal plasma monoester (IC50) (µM)	15[b]	10_b	51[c]
Fetal plasma monoester (IC50) (µM)	10[b]	6[b]	42[c]

[a] ED_{50} calculated from current data and data of other studies using one-site inhibition non-linear regression algorithm in Prism 4.0 (GraphPad Software, Inc., La Jolla, CA).
[b] Fetal or adult rat average monoester plasma concentrations (AUC/h) predicted from ED_{50} values.
[c] Experimentally determined from Clewell et al., 2013a.

For the parent compound it appeared that DINP was 2.5 and 6 times less potent than DEHP and DBP, respectively. For the calculated foetal plasma concentration it was calculated that the DINP metabolite MINP was 7 and 4 times less potent than MEHP and MBP, respectively.

The study by Clewell et al 2013a was evaluated by ECHA 2013 concluding a NO(A)EL for effects on the developing male rat reproductive tract of 760 ppm (50 mg/kg/day) and a LO(A)EL of 3800

ppm (250 mg/kg/day) based on the significant increase in MNGs on GD 21 and reduction in testosterone at GD 19.

Clewell et al., 2013b (designated Clewell et al 2011b by ECHA 2013), performed a dietary study on the developmental effects of DINP on the male reproductive system. Dams were exposed through diet to 0, 760, 3800 or 11400 ppm of DINP from GD 12 to PND 14. DBP was used as a positive control at a dose of 7600 ppm. The target doses for these dietary concentrations were: 0, 50, 250 and 750 mg/kg bw/day of DINP and 500 mg/kg bw/day of DBP. The control group included 24 dams, each DINP group contained 20 dams and the DBP group contained 21 dams.

The most sensitive endpoint appeared to be the presence of multinucleated gonocytes in testes on PND 2, as this was increased significantly from 3800 ppm (250 mg/kg bw/day) with 7 of 20 animals affected, and 18 of 19 animals affected at 11400 ppm (750 mg/kg bw/day). At the same dose (3800 ppm), male pup weight at PND 14 was reduced to 90% of control weight. At the high dose of 750 mg/kg bw/day several endpoints were affected: maternal weight and weight gain, male pup weight at PND 2 (88% of control weight), anogenital distance and anogenital index ("scaled AGD"; AGD divided by cube root of body weight) at PND 14, presence of Leydig cell aggregates in testis on PND 2, and reduction of absolute, but not relative, weight of the levator ani/bulbucavernosus muscle (LABC) on PND 49-50. Numerical values for statistically significant effects at the high dose (11400 ppm DINP) are listed in Table 21.

Table 21: Significant effects on dam and pup weight, male pup AGD, testis histopathology and reproductive organ weights at high dose level of 11400 ppm DINP. Data aggregated from several tables in Clewell et al., 2013b.

	Day	**Control**	**11,400 ppm DINP (750mg/kg bw/day)**
Maternal weight (g)	GD20	377±32	345±24**
Maternal weight (g)	PND2	308±21	270±21***
Maternal weight (g)	PND14	334±24	287±32***
Maternal weight gain (g)	GD10-20	100±16	70±23***
Male pup weight (g)	PND2	8.3±0.2	7.3±0.2**
Male pup weight (g)	PND14	37.7±0.8	27.5±0.7***
Absolute AGD (mm)	PND 14	11.39±0.19	9.57±0.27***
Scaled AGD ($AGD/BW^{1/3}$)	PND14	3.40±0.04	3.17±0.08*
Leydig cell aggregates (# animals)	PND2	4	19**
LABC (g)	PND49-50	0.61±0.01	0.55±0.01**

Mean + SD. * $p<0.05$, **$p<0.01$, ***$p<0.001$

No change in anogenital distance or anogenital index was seen at PND 2. No changes in the number of nipples were seen at PND 14 or 49-50, and no significant changes in genital tract malformations or reproductive organ weights were observed at PND 49-50. However, at PND 14 control pups had a relatively high number of nipples/areolae (1.8±0.4 average number/pup), and subtle effects on single animals may be difficult to detect without analysis of original data.

A few cases of incomplete or flaccid epididymides (6% of pups), interstitial edema in epididymides, undescended testis, and slight hypospadias were seen in the DINP exposed groups. It is not clear whether these effects were dose related as the incidence was low and a few controls also had flaccid epididymides (2% of pups) and slight hypospadias. The reduction in maternal body weight was related to a significantly reduced food intake, which may be related to food palatability according to the authors. . The reduction of maternal body weight and body weight gain in gestation in the highest dose group was related to a reduced food intake and thus not considered a sign of toxicity.

The positive control DBP showed more marked effects: reduced anogenital distance and anogenital index on PND 2 and 14, reduced testis weight on PND 2, increased number of nipples on PND 14 and 49-50, increased number of multinucleated gonocytes and Leydig cell aggregates on PND 2, reduced weights of seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle, and kidneys, increased incidence of incomplete or flaccid epididymis, and increased incidence of enlarged testis.

The study by Clewell et al 2013b was evaluated by ECHA 2013 concluding a NO(A)EL of 760 ppm (50 mg/kg/day) and a LO(A)EL of 3800 ppm (250 mg/kg/day) based on the significant increase in MNGs PND 2, and decreased pup body weight on PND 14.

A study by Furr et al 2014 was designed to develop and validate a short-term in vivo protocol to detect phthalate esters (PEs) and other chemicals that disrupt foetal testosterone synthesis and testis gene expression in rats. Pregnant rats were dosed from gestational day (GD) 14 to 18 at one dose level (750 mg/kg) with one of 27 chemicals including PEs, PE alternatives, pesticides known to inhibit steroidogenesis, an estrogen and a potent PPARα agonist. Ex vivo testis testosterone production (T Prod) was measured on GD 18. Dose-response studies were conducted with 11 of the chemicals to determine their relative potencies. DINP was tested for two different CAS numbers (CAS no. 28553-12-0 and 68515-48-0). DINP inhibited the testosterone synthesis and rats exposed to 750 mg/kg/day had an average testosterone production of 3.23±0.34 ng/testis which is a statistical significant reduction compared to controls with a production of 5.25±0.45 ng/testis ($p<0.01$). Fetal viability was not affected by DINP, but no other toxicity data were presented in the paper.

Li et al., 2015, investigated the effects of DINP in rats exposed by gavage from GD 12 to 21. DINP was administered in doses of 10, 100, 500 and 1000 mg/kg bw/day to pregnant SD rats, n=6 for each dose group. The published paper describes that dams gave birth at GD 21.5, and that body weight and anogenital distance was measured in all pups. Pups were then sacrificed, and testes were removed. The paper states that "fetal" testes were collected for examination of testosterone content, gene expression and histology, but most likely these examinations concern the testes of neonate pups. One dam from the high dose group died at GD 21.5, but the live foetuses were retrieved and included in the studies.

DINP reduced body weight of male pups in all dose groups, but did not affect male AGD or AGD/cube root of body weight (Table 22).

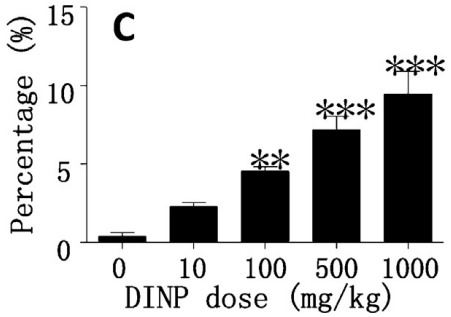
Table 22: Body weight at birth of male pups exposed to 0, 10, 100, 500 or 1000 mg/kg bw/day of DINP.

Male pups	**Control**	**10 mg/kg/day DINP**	**100 mg/kg/day DINP**	**500 mg/kg/day DINP**	**1000 mg/kg/day DINP**
Bodyweight (g)	7.3±0.6	6.5±0.5*	6.1±0.6*	6.7±0.7*	6.6±0.8*

Values are mean + SEM. N=6 n. * P<0.001 (compared to control)

Testicular testosterone level was dose-dependently reduced at all exposure levels, but this reduction was only statistically significant at 1000 mg/kg bw/day (reduction to 43% of controls). The number of testes with focal dysgenesis were significantly increased with 0 out of 6 testes affected in the control group, 2 out of 6 testes affected at 100 mg/kg bw/day, 4 out of 6 testes affected at 500 mg/kg bw/day, and 5 out of 6 testes affected at 1000 mg/kg bw/day of DINP. The percentage of seminiferous chords with multinucleated gonocytes (MNGs) were increased in a dose-dependently manner at all dose levels, and the increasing were statistically significant at 100, 500 and 1000 mg/kg bw/day DINP (Figure 2)

Figure 2: Percentage of seminiferous chords with multinucleated gonocytes in study by Li et al., 2015.



Mean + Standard Error of Mean (SEM). ** p<0.01 and ***p<0.001. Graphical illustration retrieved from Li et al.,2015.

Increased clustering of Leydig cells were seen at all dose levels (Table 23). Gene expression studies showed a marked reduction of Insl3 expression at all dose levels and a dose-dependent reduction of testicular expression of several genes involved in steroidogenesis (Cyp11a1, HSD3b1, Cyp17a1) from 100 mg/kg bw/day. Exact numerical values could not be obtained from the graphical illustration presented in the published paper.

Table 23: Frequency distribution of cluster sizes of fetal Leydig cells after in utero exposure to DINP

Cell no. per cluster	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
1	26 ± 5	21±6	11 ± 5***	14 ± 4*	16 ± 7*
2–4	48 ± 5	40 ± 1*	38 ± 4***	39 ± 4**	35 ± 7***
5–16	29 ± 5	33 ± 7	41 ± 6*	33 ± 6	33 ± 6
>16	0.16 ± 0.23	6 ± 3***	11 ± 3***	14 ± 3***	14 ± 7***
Average	4 ± 0	6 ± 1***	8 ± 1***	8 ± 1***	8 ± 3***

Values are shown as percentage with mean ± Standard Error of Mean. *p<0.05, **p<0.01 and ***p<0.001.

4.11.2.2 Human information

Table 24: Summary table of relevant information from human epidemiological studies.

Method	Results	Remarks	Reference
Human epidemiological study Biologic samples from a prospective Danish–Finnish cohort study (1997 to 2001). Analyse 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 metabolite). Analyze 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, decrease in levels of LH, free testosterone, or the ratio between LH and free testosterone. For DINP metabolites, the only significant finding was a positive correlation between MINP and the ratio between LH and free testosterone. No associations were seen between phthalate metabolite levels and cryptorchidism.		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. Data obtained in 2009-2010.	Associations were found between AGD measures and certain phthalate metabolites. The most significant association was found for AGDas (anoscrotal distance) and DINP metabolites, however the AGD as 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 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	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

Method	Results	Remarks	Reference
cryptorchidism and hypospadias.			
Human epidemiological study. Urinary concentrations of 12 phthalate metabolites in maternal blood (week 28 of gestation) were compared with anogenital distance and penile width of their sons 3 months after expected date of birth. N=245.	No consistent associations were seen between any prenatal phthalate exposure and AGD or penile width.	Lower phthalate levels were registered in this cohort than in a recent Swedish study on phthalate exposure in first trimester.	Jensen et al., 2016
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

A study by Main et al. 2006 was carried out as a case-control study on biologic samples from a prospective Danish–Finnish cohort study (1997 to 2001). Individual breast milk samples were collected as additive aliquots 1–3 months postnatally (n = 130; 62 cryptorchid/68 healthy boys): The study did not find any association between phthalate monoester levels and cryptorchidism. However, there was a positive correlation between MINP in breast milk and serum LH:testosterone ratio in boys 3 months of age without cryptorchidism (Spearman correlation r=0.323, p=0.034). The same was seen for the LH:free testosterone ratio (Spearman correlation r=0.319, p=0.038). P-values were not adjusted for multiple testing. For LH, the positive correlation was not statistically significant (0.273, p=0.078). For comparison, MEP and MBP correlated positively with sex-hormone binding globulin (SHBG), monomethyl phthalate (MMP), monoethyl phthalate (MEP) and monobutyl phthalate (MBP) with LH:free testosterone ratio and MBP correlated negatively with free T. The positive correlation between MINP and LH:free testosterone ratio was stronger for the other mentioned phthalate monoester, as correlation factors were: r=0.517 for MEP (p=0.0005), r=0.462 for MBP (p=0.001), r=0.389 for MMP (p=0.010). Other phthalate monoesters (monobenzyl phthalate and monoethyl phthalate) showed similar but nonsignificant tendencies. In the ECHA review from 2013, the following evaluation of that study was made: "*The reproductive hormone levels and information on phthalate exposures in newborn boys suggest that human Leydig cell development and function may be vulnerable to perinatal exposure to some phthalates. Data support also other findings indicating incomplete virilization in infant boys exposed to phthalates perinatally. However, the study groups may have been too small to detect subtle effects and the postnatal exposure assessment during lactation may have missed the critical window.*"

Bornehag et al., 2015, examined the associations between metabolites of different phthalates in maternal urine in first trimester and different measures of anogenital distance in sons at 21 months

of age. The study was carried out in 196 Swedish boys in 2009-2010. Two measures of anogenital distance were performed; AGDap was from anus to the anterior base of the penis, and AGDas was from anus to the posterior base of the scrotum. Associations were found between AGD measures and certain phthalate metabolites. The most significant association was found for AGDas (anoscrotal distance) and DINP metabolites, however the AGDas reduction was small, with association of ΣDINP of $\beta = -1.69$ (range –3.35, –0.02) with a p-value of 0.047 in an adjusted linear regression model. These results provide support to the proposed effects of DINP on reproductive development, but less marked associations were found for metabolites of other reproductive toxic phthalates (present at similar or higher urinary levels), including DEHP.

Jensen et al., 2015, examined the association of DINP and DEHP metabolites with testosterone levels, cryptorchidism and hypospadias in a case-control study (300 controls, 270 cryptorchidism cases, 75 cases of hypospadias). DINP and DEHP metabolites, steroid hormones and the Leydig cell product Insulin-like factor 3 (Insl3) were measured in amniotic fluid samples collected in second trimester of pregnancy in the period 1980-1996. The investigated DINP metabolite (7cx-MMeHP) showed slightly elevated odds ratio point estimates (not statistically significant) for having cryptorchidism and hypospadias but was not consistently associated with the steroid hormones or Insl3. For cryptorchidism the adjusted odds ratio in the highest 7cx-MMeHP exposure tertile was 1.28 (95% CI of 0.80 to 2.01), and a test for trend was not statistically significant (p=0.28). For hypospadias the adjusted odds ratio in the highest 7cx-MMeHP exposure tertilewas 1.69 (95% CI of 0.78 to 3.67), and a test for trend was not statistically significant (p=0.29). The authors conclude that "based on the elevated ORs observed for the DINP metabolite, we cannot exclude (nor statistically confirm) an association with hypospadias and, less strongly, with cryptorchidism". For DEHP metabolites associations with cryptorchidism or hypospadias were even less marked. The authors highlight the advantage of investigating exposures close to the sensitive window important for fetal masculinization, and the study of steroid hormones and insl-3 in amniotic fluid is also considered a relevant matrix. The measured insl-3 is expected to originate from the male fetus, whereas the measured testosterone may also originate from the adrenals, and the authors speculate that the differential associations between metabolite levels reflect differential actions of phthalate load upon the fetal testis and the fetal adrenal gland. However, the findings are not consistent for DINP and DEHP, and no firm conclusions can be made regarding the associations between exposure to DINP or DEHP and possible effect on hypospadias/cryptorchidism in humans.

Jensen et al., 2016, performed a human epidemiological study comparing maternal urinary concentrations of 12 phthalate metabolites including DINP metabolites to anogenital distance and penile width in their sons. Urine samples were collected from 2010 to 2012 from 245 mothers in week 28 of pregnancy (second and third trimester). No consistent associations were seen between any prenatal phthalate exposure and AGD or penile width. Lower phthalate levels were registered than in a recent Swedish study on phthalate exposure in first trimester (Bornehag et al., 2015), and this may limit the ability to detect any possible relationship between phthalate exposure and anogenital distance. In this study the molar sum of DINP metabolites ΣDINPm had a median of 21.4 (25-75 percentile range: 10.3-53.7; fasting spot urine adjusted by osmolality), whereas the mentioned Swedish study had a median of 55.9 (25-75 percentile range: 28.3-124.9; morning urine adjusted by creatinine). Although no statistically significant associations between phthalate metabolites and AGD or penile width were found.

Axelsson et al., 2015, performed a human epidemiological study comparing maternal serum levels of DEHP and DINP metabolites with testicular size, semen quality and reproductive hormones in 112 adolescent sons. Men were recruited in 2008-2010 at military health board examination or through announcements. Corresponding maternal samples were collected from a Swedish biobank

of samples obtained from the 6th to the 35th week of pregnancy (mean 12th week, majority sampled between 8 and 14 weeks). Men in the highest exposure tertile of a DINP metabolite (mono-carboxy-iso-octyl phthalate) had 9.5% lower total testicular volume (4.3 mL reduction; 95%CI: 0.89, 7.6 mL; p=0.001), 30 % higher levels of FSH (95% CI: 3.6, 63%; p=0.02) and 23% lower semen volume (0.87 mL reduction; 95% CI: 0.28, 1.5 mL; p=0.004) than men in the lowest tertile. Two other DINP metabolites were not correlated with these parameters, and for one DEHP metabolite (mono-2-ethyl-5-hydroxylhexyl phthalate) semen volume was 20% lower in the highest exposure tertile compared to the lowest (0.70 mL reduction (95% CI: 0.090, 1.3 mL; p=0.03). Sperm concentration, sperm count, and serum levels of testosterone, free testosterone, LH, estradiol and SHBG were not associated with phthalate levels. It is concluded by the authors that prenatal levels of DINP seemed negatively associated with reproductive function of adolescent men.

4.11.3

4.11.4 Other relevant information

4.11.3.1 Mode of action studies

Several studies described in section 4.11.2 on developmental toxicity have examined the influence of DINP on fetal testosterone production, testicular histopathology and/or anogenital distance (Borch et al., 2004; Adamsson et al., 2009; Boberg et al., 2011; Hannas et al., 2011; Clewell et al., 2013a; Furr et al. 2014; Li et al., 2015). The individual studies were described in section 4.11.2.1. These studies provide clear evidence that DINP exposure dose-dependently leads to reduced fetal testosterone production by testicular Leydig cells. The reduction in testosterone synthesis is associated with structural changes in testes, i.e. Leydig cell aggregation and presence of multinucleated gonocytes, as described above (Borch et al., 2004; Boberg et al., 2011; Clewell et al., 2013a; Li et al., 2015).

Additionally, a Hershberger study on castrated male rats and a recent uterotrophic assay in immature and pubertal female rats are summarized below in order to clarify the mode of action for adverse effects of DINP.

Table 25: Summary table of Hershberger study by Lee and Koo, 2007 and uterotrophic study by Sedha et al., 2015.

Method	Results	Remarks	Reference
Rat (SD), Hershberger assay in castrated males dosed with testosterone propionate (0.4 mg/kg bw/day) , n=6. Oral: gavage Vehicle: corn oil Exposure: 10 days (PND 35 to 44) Doses: 20, 100, 500 mg/kg bw/day CAS number 57-85-2, Purity > 97%	NOAEL: not determined LOAEL: 20 mg/kg bw/day for statistically significant reduction of seminal vesicle weight to 78% of controls. At 500 mg/kg bw/day weights of levator ani/bulbocavernosus muscle 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

Method	Results	Remarks	Reference
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/day 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/day of DIBP. DES (40 ug/kg bw/day or 6 ug/kg bw/day) was used as a positive control.	Sedha et al., 2015

Lee & Koo, 2007, found indications of an anti-androgenic effect of DINP in an *in vivo* study in castrated male SD rats (Hershberger assay), n=6. This study compared the effects of different phthalates administered for 10 consecutive days to 7 weeks old male castrated rats (SD Crl:CD) treated with testosterone propionate (0.4 mg/kg bw/day). Three doses of each phthalate were applied (20, 100 and 500 mg/kg bw/day of DINP). In DINP exposed animals, a decrease in seminal vesicle weight was seen at all doses (78-80% of testosterone exposed control, $p<0.05$), and a decrease in levator ani/bulbocavernosus muscles (LABC) weights was seen at 500 mg/kg bw/day (82% of testosterone exposed control, $p<0.05$). Additionally, serum testosterone levels were decreased and LH levels were increased at 500 mg/kg bw/day (approximately 85 and 130% of testosterone exposed control values, respectively, as estimated from graphical presentation). Reduction in the same organ weights were seen with DEHP, but not DBP, BBP or DnOP. No changes in ventral prostate weights were seen for DINP, and this was in contrast to the effects of DEHP, MEHP, DBP and DIDP. BBP and DnOP did not affect any reproductive organ weights. None of the tested chemicals altered weights of Cowper's gland or glans penis. These findings were considered as indications of anti-androgenic effects by ECHA 2013 (citation in italics):

According to the test guideline, a substance should be considered as positive in the test if at least two of the five organs show an effect. Thus, the dose level of 500 mg/kg bw/day can be considered as anti-androgenic in this test. For comparison, DEHP decreased ventral prostate weights at and above 20 mg/kg bw/day, seminal vesicle weights at >100 mg/kg bw/day, and LABC weights at 500 mg/kg bw/day (ECHA 2013).

Sedha et al., 2015, examined the effects of DINP (20, 100, 500 mg/kg bw/day) on immature and pubertal female rats, n=6. After 3 days of exposure of 20-day old females, no increase in uterus weight was seen as would be expected for estrogenic chemicals and as was observed for the positive control DES. After 20 days of exposure of 21-day old females, no increase in uterus weight was seen, and no change in puberty timing was found. After 20 days of exposure, absolute and relative ovary weights were significantly reduced to 72% and 90% of vehicle controls, respectively. Also slight reductions in uterus weights were discussed by the authors. The authors suggested that DINP may reduce the levels of available estrogens leading to the decreased ovary weight and (non-significant) decrease in the uterus weight. In the same animals, body weight gain was lower, and females weighed 80% of vehicle controls. Unexposed controls weighed significantly more than vehicle controls, and approximately the same as high dose DINP exposed females. It is not clear whether the observed lower absolute and relative ovary weights reflect delayed development, endocrine changes, or a toxic effect of DINP on ovaries.

The lack of estrogenic effect of DINP is in agreement with conclusions in the EU RAR for DINP (2003). In a section on In vivo/In vitro studies on estrogenic activities of DINP it was concluded that DEHP, DINP and DIDP showed no activity in in vitro assays testing the ability of binding to rodent or human estrogen receptors or to induce estrogen receptors-mediated gene expression, and studies on in vivo response on uterine wet weight was considered negative (European Chemicals Bureau 2003a). The ECHA review, 2013, reviewed the literature on estrogenic effects including studies published after the EU RAR and concluded that there were no indications of estrogenic properties of DINP.

4.11.5 Summary and discussion of reproductive toxicity

4.11.4.1 Overview of data.

The available data show that DINP causes developmental toxicity and indicate toxicity to reproductive organs potentially leading to fertility effects. These effects are considered to be specific and not secondary non-specific consequences of other toxic effects.

Developmental toxicity:

DINP induces effects on the developing male reproductive system. Key findings in animal studies on reproductive effects of DINP are:

a) 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) ,

b) 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),

c) Functional deficiency: dose-dependent long-lasting decrease in the percentile of motile sperm in rats exposed perinatally (Boberg et al., 2011) (from 600 mg/kg bw/d),

d) Structural abnormalities: increased nipple retention and decreased anogenital distance in male rats exposed perinatally (Boberg et al., 2011; Gray et al., 2000, Lee et al., 2006) (mostly from 750 mg/kg bw/d),

e) 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),

f) A comparable pattern of adverse effects and of mode of action as seen for the reproductive toxicants (category 1B) DEHP, DBP, DIBP and BBP. In foetal testes, several studies describe 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).

Collectively, these findings show that DINP causes developmental effects in offspring of exposed dams. It may be noted that a study on prenatal exposure to DINP showed no effect on AGD at gestation day (GD) 20, and a study on perinatal exposure to DINP, revealed decreased anogenital distance (corrected by body weight) only at PND 14 and not PND 2, and revealed no change in nipple retention and no/few permanent malformations of reproductive organs (Clewell et al., 2013a; Clewell et al., 2013b). Two other studies showed no effect of DINP on anogenital distance, although testicular effects were described in offspring (Masutomi et al., 2003, Li et al., 2015). The statistical power of these two studies was low due to small group sizes which may explain the lack of effect on anogenital distance (Masutomi et al., 2003, Li et al., 2015).

The skeletal effects and decreased body weight in offspring (Hellwig et al., 1997; Waterman et al., 1999, Waterman et al., 2000) were considered critical to set a LOAEL for developmental toxicity in the EU RAR from 2003 (LOAEL of 159 mg/kg bw/day for decreased body weight). A higher NOAEL of 500 mg/kg bw/day was determined based on visceral and skeletal variations and slight maternal toxicity at 1000 mg/kg bw/day (Waterman et al., 1999). These values were applied for risk characterization, but it was concluded at that time that these findings did not justify classification regarding fertility and development according to directive 67/548/EC (European Chemicals Bureau 2003a).

Other evaluations of these studies have applied even lower NOAELs based on skeletal findings, as reviewed by ECHA in 2013. Reevaluation of the study by Waterman et al, 1999, was performed by the authors in agreement with an evaluation by the US NTP-CERHR panel in 2003. The reanalysis concluded a LOAEL of 500 mg/kg bw/day for skeletal effects in the study by Waterman et al., 1999. A NOAEL of 200 mg/kg bw/day based on the study by Hellwig et al., 1997 was concluded by the NTP-CERHR (2003) and ECHA (2013).

The effects on male pup anogenital distance (Boberg et al., 2011; Gray et al., 2000, Clewell et al., 2013b) are considered a clear adverse effect on development not considered to be secondary to toxic effects on the dam or reductions of offspring body weight, as they are analysed by taking body weight into account, i.e. inclusion of either offspring weight as a covariate in the analysis or using the anogenital index for the analysis. This means that the significant effects described for male anogenital distance cannot be explained by effects on pup body weight, if such an effect is present.

Decreases in anogenital distance and increases in nipple retention in male offspring are clearly related to adverse reproductive effects in offspring such as altered development of reproductive organs, impaired semen quality, smaller penis size and increased incidence of hypospadias and cryptorchidism. The effects are considered robust markers of anti-androgenic effects of chemicals and the strong relationship between anti-androgenic actions in the foetus, reductions in anogenital distance and adverse reproductive effects later in life provides the background for the conclusion in the OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment that statistically significant changes in neonatal AGD that cannot be explained by the size of the animal can be considered adverse (OECD 2008).

The observed permanent structural effects in offspring observed after perinatal DINP exposure (degeneration of meiotic spermatocytes and Sertoli cells, scattered cell debris in ducts in epididymis, decrease in number of corpora lutea (Masutomi et al., 2003); reduced percentile of motile sperm, permanent nipples/areolae in male rats, small testes and epididymides (Boberg et al., 2011; Gray et al., 2000)) are considered to be specific and not secondary non-specific consequences of maternal toxicity or other toxic effects. Furthermore, the finding of reduced testosterone levels in foetal life may be considered adverse: ”*the justification for considering foetal reduced testicular T concentration as adverse is that during the critical developmental window it has shown to induce*

male reproductive developmental effect" *(OECD 2008)*. This is also in line with the conclusions of the ECHA review from 2013, stating that the anti-androgenic effects observed for DINP (reduced testicular testosterone and foetal testicular histopathology) support the findings of permanent changes and lower semen quality in DINP exposed males observed with higher doses of DINP exposure.

The observed dose-dependent reduction of sperm motility (Boberg et al., 2011) is considered key evidence of adverse developmental effects of DINP. Regarding effects on sperm motility, OECD guidance document 43 on mammalian reproductive toxicity testing and assessment states that "*Studies have shown that there is a relationship between sperm motility and fertility, but there is no generally accepted standard of how much of a change in motility should be considered adverse." (…) "A dose-response trend and a statistically significant change in sperm motility would generally be interpreted as indicating a potential effect on fertility in humans*" (OECD 2008, section 183).

Effects of DINP on fetal testosterone production were seen in several studies in rats and clearly indicate an endocrine disrupting mode of action that is 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). A Hershberger study in castrated male rats show indications of anti-androgenic mode of action (Lee et al., 2007), whereas there are no indications that DINP has estrogenic effects (ECHA, 2013; Sedha et al., 2015).

In the ECHA review (2013), a NOAEL of 50 mg/kg bw/day for reproductive toxicity is concluded based on decreased testosterone production/level and histopathological changes in foetal/pup testis at a LOAEL of 250 mg/kg bw/day (based on Clewell et al. 2011a and supported by Clewell et al. 2011b; Hannas et al. 2011a and b and 2012; Boberg et al. 2011). These effects on fetal testosterone production and histopathological changes were considered adverse (ECHA 2013).

Toxicity to fertility:

Key evidence for effects of DINP on fertility:

g) 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/day), 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/day), and a 13-week study in mice (Hazleton 1992) (2600 and 5770 mg/kg bw/day),

h) reduced sperm count and effects on sperm motion parameters after 28 days of exposure of juvenile rats (Kwack et al., 2009) (one dose only, 500 mg/kg bw/day),

i) dose-dependent long-lasting reduced sperm motility in rats exposed perinatally (Boberg et al., 2011) (600, 750 and 900 mg/kg bw/day)

Collectively, these findings point to toxicity of DINP on reproductive organs with subsequent adverse effects for fertility. The reduction in testis weights in the chronic toxicity study in mice (Aristech 1995) was used for risk characterization concerning effects on reproductive organs in the EU RAR (European Chemical Bureau 2003). 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; Exxon 1986).

In contrast to the reduction in testes weights at high doses, increases in parental absolute and relative testis and epididymis weights were seen at 1100 mg/kg bw/day in a one-generation study in rats (Waterman et al., 2000). However, no microscopic evaluation of reproductive organs and no evaluation of sperm parameters was performed (Waterman et al., 2000). This study was followed up by a two-generation study using lower doses showing no significant effects on parental reproductive organ weights (top dose 0.8% corresponding to ~550 mg/kg bw/day). No effects on fertility were seen in parental animals of these one- or two-generation studies (Waterman et al., 2000). In cynomolgus monkeys relative testis/epididymis weights were 76% of controls, but this was not statistically significant, and in marmosets no effects on testes were reported (Pugh et al., 2000; Hall et al., 1999). It may be noted that these monkey studies were carried out with n=4 and that limited details were presented in the published papers. Any clear conclusions regarding the absence or presence of testicular effects of DINP in monkeys would require evaluation of study reports, and therefore the conclusions regarding effects of DINP on reproductive organs are based on rodent studies only. Further discussion on primate studies on other phthalates can be found in section 4.11.4.4.

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 (Joensen et al., 2012, Mieritz et al., 2012, Specht et al., 2014a and 2014b).

It was concluded in the EU RAR from 2003 that the effects in mice did not justify classification for fertility. However, as these studies did not examine sperm count or –quality, direct effects of DINP on fertility were not fully elucidated at that time. The study by Kwack et al., 2009, provides evidence for effects of DINP on sperm count and quality. As fertility assessment by breeding may not be considered a sensitive parameter in rats, the findings by Kwack et al., 2009, are not in conflict with the lack of effect on fertility in the Waterman et al., 2000, studies. As stated in OECD guidance document 43 on mammalian reproductive toxicity testing and assessment: "*Effects on fertility in rodents seem to be a good indicator for effects in humans, and most work on contraceptive agents in humans stems from original studies in rodents (Barlow and Sullivan 1982). However, it should be emphasized that sperm count in rodents must be drastically reduced before an effect on fertility is seen*" (OECD 2008, section 23).

Importantly, "*a statistically significant change in sperm count in a rodent study is considered to be indicative of a potential effect on fertility in humans"* (OECD 2008) due to greater sperm reserves in rats than in humans. Thus, the findings by Kwack et al., 2009 are considered key evidence for toxicity to fertility of DINP.

Also the reduction of sperm motility in rats after exposure during the sensitive perinatal period (Boberg et al., 2011) is considered key evidence that DINP may affect fertility of humans. This effect was seen at dose levels not causing marked maternal toxicity. No histological findings in testes were reported in that study (epididymides were not examined). However, OECD guidance document 43 on mammalian reproductive toxicity testing and assessment states that also in the absence of histological effect, changes in motility can be considered relevant: "*Since sperm motility is dependent on testicular and epididymal function, damage to the testis, as revealed by the histologic appearance, may lead to changes in sperm motility. However, motility can be altered by direct effects on the epididymides or the sperm themselves. Therefore, the absence of a histological lesion does not necessarily mean that a change in motility should be discounted. A dose-response*

trend and a statistically significant change in sperm motility would generally be interpreted as indicating a potential effect on fertility in humans" (OECD 2008, section 183).

The effects on testes and sperm parameters were seen at the same or higher doses than those inducing hepatic toxicity. Body weights in the repeated dose studies were reduced significantly at the same doses, but body weight reductions may not be considered marked enough to exclude these findings for classification purposes. DINP exposed males weighed 88% of controls in study by Kwack et al., 2009; 90 and 83% of controls in the two highest dose groups in study by Aristech 1995. In the offspring of the study by Boberg et al., 2011, the DINP high dose males weighed 98% of controls. The effects on ovary weights were seen concomitantly with markedly lower body weights (77% of controls) in dams at the end of weaning in the one-generation study (Waterman et al., 2000). In the study on pubertal exposure of female rats, body weight gain was lower, and females weighed 80% of vehicle controls (Sedha et al., 2015). It is not clear whether these lower ovary weights reflect delayed development, endocrine changes, or a toxic effect of DINP on ovaries. The adverse effects on testis and sperm parameters are considered not to be secondary non-specific consequences of other toxic effects. The ECHA Guidance on the application of the CLP criteria "*Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity.*" (ECHA, 2015).

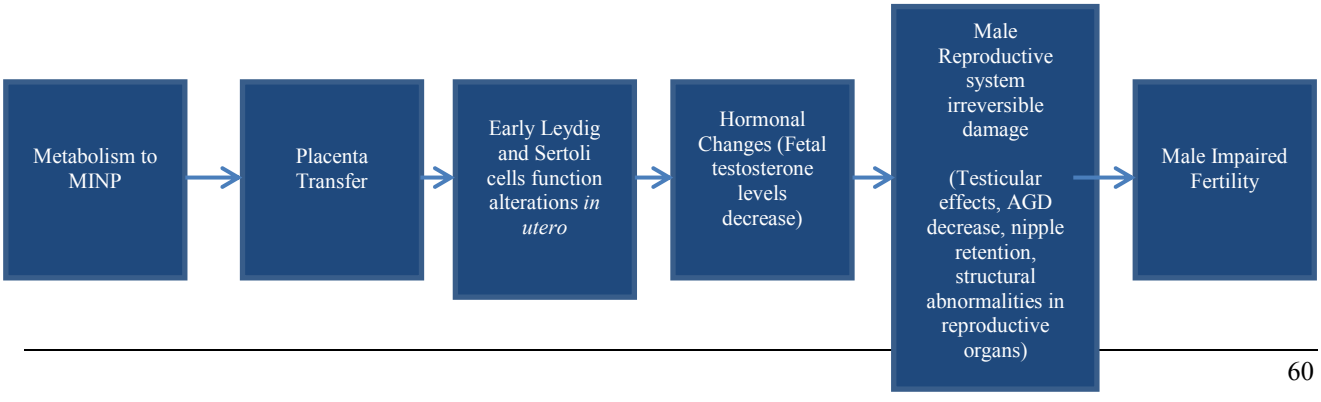
4.11.4.2 Mode of action and similarity with other phthalates

Using the rat as a model, it has been shown that reductions in testosterone concentration in sensitive time windows can cause permanent reproductive toxicity effects.

ECHA (2013) concluded that "the in vivo findings suggest that DINP has anti-androgenic potency but may also exhibit its effects through other modes of action". It was 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".

Overall, DINP acts as an anti-androgen via reduction of testicular testosterone production. This mode of action may explain the adverse effects observed in male offspring. This is supported by the fact that the AGD as well as the normal apoptosis of the nipple anlagen are under the control of dihydrotestosterone.

The following mode of action is hypothesised based on the WHO/IPCS Mode of Action/Human Relevance Framework description which is presented in the SVHC identification proposal for DCHP (Annex XV report DCHP, 2016):



The hypothesised mode of action consists of six main key events. For the key event entitled "male reproductive system irreversible damage", a number of distinct effects described in the studies available, are grouped together under this general title. These effects include increased nipple retention, decreased anogenital distance, and permanent structural changes of male reproductive organs. The key event "male impaired fertility" includes the observation of reduced sperm motility and the finding of nipple retention and AGD decrease in rodents with expected adverse effects in humans regarding infertility (as part of the testicular dysgenesis syndrome). There is strong evidence that these male reproductive system irreversible effects (e.g. sperm quality effects, structural abnormalities in reproductive organs, and decrease in anogenital distance) are linked to fertility adverse effects in mammalian species, including humans. Overall, fetal disturbance of the developing male reproductive system can have multiple effects in mammalian species as described by Skakkebæk et al. (2001) and summarized as the testicular dysgenesis syndrome (TDS).

The same effects as reported in male pups following exposure to DINP were also reported following *in utero* exposure to other phthalates with a harmonized classification for development as Repr. 1B, and an antiandrogenic mode of action was also suggested for these phthalates. DINP does not act via an estrogenic mode of action, but may possibly interfere with endogenous estradiol production to induce changes in female reproductive development, although this has not been thoroughly investigated.

It is well known that phthalate esters with intermediate chain lengths are reproductive toxicants, e.g. DBP and DEHP (European Chemical Bureau 2003b; European Chemical Bureau 2008).

Phthalate esters with chain lengths larger than C6 (high molecular weight phthalate esters) are generally considered less toxic. However, DINP has been found to induce the same adverse effects and to share the same mode of action as the phthalates classified as reproductive toxicants (Boberg et al., 2011; Borch et al., 2004; Hannas et al., 2011; Clewell et al., 2013). For other phthalates than DINP, it has been shown quantitatively that combination effects are seen when rats are exposed to phthalates in utero (Howdeshell et al., 2008). For DINP, one study has similarly indicated combination effects between DINP and DEHP (Borch et al., 2004). This is in line with the conclusions by ECHA that "*DINP causes low incidences of effects, similar to those observed with other phthalates, likely acting via the same modes of action, including androgen deficiency"* and further that "*DINP has anti-androgenic properties and it could be appropriate to include this substance in a combined risk assessment of phthalates with anti-androgenic properties*." (ECHA 2013).

The ECHA review presents potency factors calculated by Benson et al., 2009, comparing the LOAEL for effects of DINP on foetal testosterone concentration with the LOAEL of the most sensitive effect of DEHP (small or absent reproductive organs), i.e. a rather crude estimate of potency. This calculation resulted in relative potencies of 1:0.39 for DEHP:DINP (ECHA, 2013).

The paper by Clewell et al., 2013a, also describes comparisons of the relative potencies of DINP and the classified reproductive toxic phthalates. The potency of DINP (internal and external dose) on reducing fetal testicular testosterone content was compared with the potencies of DEHP and DBP (internal and external dose). For the parent compound it appeared that DINP was 2.5 and 6 times less potent than DEHP and DBP, respectively. For the calculated foetal plasma concentration it was calculated that the DINP metabolite MINP was 7 and 4 times less potent than MEHP and MBP, respectively.

For DINP there is clear evidence of developmental toxicity via the same mode of action as the developmental toxicity known for DEHP, DBP, DIBP and BBP.

Furthermore, a harmonized classification of Dicyclohexylphthalate (DCHP) as toxic to reproduction in category 1B (H360D) has recently been adopted (cf. the 9th ATP to CLP). The classification proposal for DCHP was based on effects that are parallel to those of DINP (RAC 2015). The opinion adopted by RAC for DCHP thus suggests a category 1B classification for developmental toxicity based primarily on increased nipple retention and decreased AGD in male pups in three different studies in the absence of marked maternal toxicity. Further support for this classification was based on observations of prolonged preputial separation and hypospadias and atrophy of the testes in offspring in one supporting study.

The observations for DINP and other phthalates are listed in Table 26. The similarity between the effects of DINP and effects of the listed phthalates further support classification of DINP in category 1B for development.

Table 26: Similarity between effects of DINP and other phthalates classified as toxic to reproduction

Substance	**Areola/nipple retention**	**Decreased fetal or neonatal male AGD**	**Hypospadias**	**Harmonized Repr 1B (H360D) classification**	**Effects on fetal 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		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 2009and 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

In comparison with DEHP, DBP and BBP, the overall evidence for effect of DINP on fertility is limited, as one study in young adult rats showed reduced sperm count and sperm motility (Kwack et al., 2009), whereas no effects on fertility were seen in one- or two-generation studies on DINP (Waterman et al., 2000). Sperm parameters were not assessed in the studies by Waterman et al., 2000.

DEHP affected fertility of both females and males and induced severe testicular effects including testicular atrophy in numerous repeated dose toxicity studies in rats, mice and ferrets (ECHA 2008a). DEHP is thus classified in category 1B for both fertility and development, whereas DBP, DIBP and BBP are classified in category 1B for development and category 2 for fertility (ECHA 2008a, b, c, ECHA 2014). For DEHP, DBP, DIBP and BBP the general pattern of fertility effects on male reproductive organs was decreased testis weight, degeneration of seminiferous tubules and impaired sperm quality after adult or perinatal exposure. DBP, BBP and DEHP furthermore reduced fertility in reproductive toxicity studies (European Chemicals Bureau 2003b, 2007, 2008, ECHA 2014). For DIBP, no fertility studies were available to the evaluation by RAC (ECHA 2009).

It is noted that the effects observed for DINP are less marked and generally occur at higher doses when compared to other classified phtalates.

4.11.4.4 Human relevance

In recent years, several studies have targeted the human relevance of the reproductive toxicity of DEHP and DBP observed in rats, and the issue has been addressed by e.g. the RAC in their opinion on the Annex XV dossier proposing restrictions on four phthalates (RAC/SEAC 2012). As the mode of action of DINP is similar to that of DEHP, DBP, DIBP and BBP, this discussion is equally relevant for DINP.

An evaluation of available studies on interspecies differences lead to the conclusion that the evidence for lack of human relevance of phthalate effects is weak. The conclusion by RAC was that "*that there is too much uncertainty in the data available to allow a conclusion on humans being less, equally or more sensitive than rats"* (RAC/SEAC 2012).

The human relevance of the effects of phthalates is supported by the similarities between the pattern of effects seen in rodent studies of perinatal phthalate exposure and the human "Testicular dysgenesis syndrome", which is a syndrome of reproductive abnormalities suggested to share the same origin, which may be an altered endocrine influence during development (Fisher et al., 2003). Human epidemiological studies are difficult to interpret due to the long period from the time of exposure until possible effects may be observed. In a case-control study, Jensen et al., 2015, found an association between levels of a DINP metabolite in amniotic fluid from second trimester and cryptorchidism or hypospadias in male infants. The same association was not seen for a DEHP metabolite. Bornehag et al., 2015, found associations between AGD measures and exposure to metabolites of certain phthalates (including DINP) in maternal urine from first trimester of pregnancy. In a study on adolescent men, increasing maternal exposure to DINP was associated with decreasing total testicular volume, increasing levels of FSH and decreasing semen volume (Axelsson et al., 2015), Interestingly, a study comparing phthalate exposure in mother's milk and testosterone levels in their infant sons revealed correlations between exposure to certain phthalate monoesters and the ratio of LH to testosterone (Main et al., 2006). This is in good agreement with the marmoset study showing that neonatal phthalate exposure impaired testosterone production and induced testicular effects characteristic for high LH levels (Hallmark et al., 2007). Collectively, this indicates that the neonatal period may be a sensitive window of exposure for humans/primates. As described by Welsh et al., 2008, testosterone levels peak in late gestation in rats, but earlier (week 14-18) in humans, and this coincides with important periods of differentiation of reproductive organs. However, reproductive development continues postnatally and may be sensitive to exposure

to endocrine disrupting compounds during early development (den Hond and Schoeters, 2005, Jacobson-Dickman and Lee, 2009).

For other phthalates than DINP, certain studies are indicative of species similarities and also species differences in the reproductive effects of phthalates, and age at exposure appears to be a central point. In Tomonari et al. (2006), no reproductive effects were seen in male marmosets (n=5-6 per dose group) exposed to DEHP by oral gavage at 100, 500 and 2500 mg/kg bw/day from 3 months of age until sexual maturity (18 months). Similarly, no reproductive effects were seen in Kurata et al., 1998, where male marmosets (n=4 per dose group) were dosed 100, 500 and 2500 mg/kg bw/day during 12-15 months of age. A study with marmosets of 4 days of age (5 co-twins and 4 non-twins, total n=14) treated 14 days with 500 mg/kg bw/day of MBP (monobutylphthalate, a metabolite of DBP), however, revealed increased Leydig cell volume (Hallmark et al., 2007). A second study from the same authors revealed suppressed blood testosterone levels in male marmosets (n = 9) of 2-7 days old at a single dose of 500 mg/kg bw/day of MBP (measurement 5h after dose). These studies are neonatal studies and thus did not expose marmosets at what is considered to be the critical programming window for male development (gestation week 7-15 in marmosets from Mitchell et al. (2008) and McKinnell et al. (2009). In male offspring (n=6) of pregnant marmosets exposed to 500 mg/kg bw/day MBP during gestation (GD 49-105), no effects on testicular morphology, reproductive tract, testosterone levels at birth, germ cell number nor germ cell proliferation were observed (McKinnell et al., 2009). However, unusual clusters of undifferentiated germ cells were found in two of six males examined at birth. The significance of this observation is unclear. In a second study by the same authors, 4 day old co-twin marmosets (5 co-twins, n=10) were exposed to MBP neonatally for 14 days. No effects on germ cell number or differentiation were apparent from this study (McKinnell et al., 2009). Overall, these primate studies underline that the reproductive toxicity of these phthalates may be relevant to primates, and that effects are strongly dependent on timing of exposure in sensitive windows of reproductive development.

The experimental evidence regarding human relevance or non-relevance of phthalate effects seen in rats can be summarized as follows:

- Correlations have been found between human exposure to certain phthalates and LH:testosterone levels (Main et al., 2006)
- Species similarities are seen between mice, rats and marmosets in the foetal germ cell effects seen with prenatal exposure *in vivo* (McKinnell et al., 2009; Gaido et al., 2007).
- Species similarities are seen regarding germ cell changes in *in vitro* using testes from rats, mice or humans (Chauvigne et al., 2009; Habert et al., 2009; Lambrot et al., 2009; Lehraiki et al., 2009).
- Species similarities and differences have been described in the ability to reduce testosterone production, as DBP exposure reduces testosterone production in neonatal marmosets, but not in marmosets exposed during gestation (McKinnell et al., 2009). This is in contrast to rats, in which DBP exposure during gestation leads to reduced testosterone production (Howdeshell et al., 2008; Shultz et al., 2001). This may be related to different windows of sensitivity for these species.
- Species differences have been described in the sensitivity to the testosterone suppressing effect of phthalates *in vivo*, as a study in foetal mice exposed to DBP revealed changes in several

immediate genes, but no decreases in testosterone levels or in genes related to cholesterol homeostasis or steroidogenesis, as would be expected for rats (Gaido et al., 2007).

- Species differences have been described in sensitivity to the testosterone suppressing effect of phthalates examined *in vitro,* as studies on cultured rat, but not human, foetal testes have shown the ability of phthalates to reduce testosterone production *(Chauvigne* et al*., 2009; Hallmark* et al*., 2007; Lambrot* et al*., 2009)*. In these *in vitro* studies human testis samples were from first or second trimester foetuses, but it is not clear whether these ages correspond to the sensitive window for phthalate exposure in rats (Hallmark et al., 2007; Lambrot et al., 2009).
- An experimental model using transplantation of testicular tissue from foetal rats, mice or humans to a (transgenic) castrated mouse or rat was able to demonstrate a testosterone inhibiting effect of DBP when using rat foetal testis explants, but not when using human foetal testis explants (Mitchell et al., 2012; Heger et al., 2012). However, no conclusions regarding species differences can be drawn from the results of these studies due to several methodological differences in study design between the foetal rat testis graft and the foetal human testis graft studies (Mitchell et al., 2012; Heger et al., 2012).Interestingly, transplant studies using testicular tissue from infant primates showed that DBP inhibited steroid synthesis and spermatogenesis (Rodriguez-Sosa et al., 2014). Also effects on germ cells were comparable across species in several studies (Mitchell et al., 2012; Heger et al., 2012).
- A case-control study found an association between levels of a DINP metabolite in amniotic fluid from second trimester and cryptorchidism or hypospadias in the sons (Jensen et al., 2015).
- An epidemiological study found associations between AGD measures and exposure to metabolites of certain phthalates (including DINP) in maternal urine from first trimester of pregnancy (Bornehag et al., 2015)

Overall, even though some studies cause speculations that reproductive effects of phthalate exposure observed in rats are not of human relevance, there are also several indicators of species similarities showing that the reproductive effects of phthalate exposure may be considered human relevant. There may also be toxicokinetic differences between species.

The current knowledge on species differences is not sufficient to disregard the human relevance of phthalate effects for DINP as well as other reproductive toxic phthalates.

4.11.6 Comparison with criteria

Developmental toxicity:

Key evidence for effects of DINP on development, which are observed in absence of maternal toxicity:

a) Structural abnormalities: skeletal effects (rudimentary ribs) were seen two developmental toxicity studies (Hellwig et al., 1997; Waterman et al., 1999) (1000 mg/kg bw/d),

b) Effect on altered growth: decreased body weight in offspring in a two-generation study (Waterman et al, 2000) (from 159 mg/kg bw/d),

c) Functional deficiency: dose-dependent long-lasting decrease in percentage of motile sperm in rat offspring exposed perinatally (Boberg et al., 2011) (from 600 mg/kg bw/d),

d) Structural abnormalities: increased nipple retention and decreased anogenital distance in infant male rats exposed perinatally (Boberg et al., 2011; Gray et al., 2000; Lee et al., 2006), and decreased anogenital distance in developing male rats exposed perinatally (Clewell et al., 2013b) (mostly from 750 mg/kg bw/d),

e) Structural abnormalities: increased incidence of permanent changes (permanent nipples, malformations of testes 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),

f) A comparable pattern of adverse effects and of mode of action as some of the other phthalates classified as reproductive toxicants in category 1B, e.g. DEHP, DBP, DIBP and BBP (Boberg et al., 2011; Borch et al., 2004; Hannas et al., 2011; Clewell et al., 2013a; Li et al., 2015).

Classification in Repr. 1A is not appropriate as it should be based on human data and no human data specific for DINP is available.

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

Classification in Repr. 2 is not appropriate as there is clear evidence from animal studies. The effects are not considered to be secondary non-specific effects and there is no mechanistic information that raises doubt about the relevance of the effects for humans.

Toxicity to fertility:

Key evidence for effects of DINP on fertility:

g) 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/day), 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/day), and a 13-week study in mice (Hazleton 1992) (2600 and 5770 mg/kg bw/day),

h) reduced sperm count and effects on sperm motion parameters after 28 days of exposure of juvenile rats (Kwack et al., 2009) (one dose only, 500 mg/kg bw/day),

i) dose-dependent long-lasting reduced sperm motility in rats exposed perinatally (Boberg et al., 2011) (from 600 mg/kg bw/day),

These effects are considered to be specific and not secondary non-specific consequences of other toxic effects.

Classification in Repr. 1A is not appropriate as it should be based on human data and no human data specific for DINP is available.

Classification in Repr. 1B is not appropriate, as the evidence on effects of DINP on fertility is not considered clear.

Classification in Repr. 2 is appropriate as there is some evidence from experimental animals of an adverse effect on fertility, but this evidence is not sufficiently convincing to place the substance in Category 1.

4.11.7 Specific concentration limit

Setting of a specific concentration limit (SCL) for DINP has been considered as the reproductive effects observed for DINP occur at relatively high dose levels. However, the classification proposal is based on different types of effects seen in different studies and furthermore, supporting evidence from other classified phthalates also contributes to the total weight of evidence leading to classification. A single ED10 representative for the different findings cannot be derived based on the available data. A potency group and the associated SCL can thus not be assigned for the reproductive effects of DINP.

4.11.8 Conclusions on classification and labelling

The available data show that DINP causes developmental toxicity and indicate toxicity to reproductive organs and on sperm count and sperm motion potentially leading to fertility effects.

With respect to the developing animal, effects on the anogenital distance as well as on the occurrence of increased nipple retention in male pups were recorded in several studies. The findings are considered to be specific and not secondary non-specific consequences of other toxic effects. Dose-dependent permanent decrease in sperm motility in rat offspring following perinatal exposure and increased incidence of permanent malformations of testes, epididymides and external genitalia in rats exposed perinatally are considered to be specific and not secondary non-specific consequences. Mechanistic studies indicate an anti-androgenic mode of action. In conclusion, the available data show clear evidence of an adverse effect on development, and classification as Repr. cat 1B; H360D is warranted.

With respect to effects on fertility, reduced sperm count and velocity of DINP are observed in a 28-day study in rats, and adverse effects on reproductive organs are seen in other rat studies. These effects are considered to be specific and not secondary non-specific consequences of other toxic effects.

Furthermore, similar findings for other classified phthalates with the same mode of action support that a classification for toxicity to fertility is also warranted for DINP. For the effects on fertility a

classification as Repr. cat 2: H361 is proposed, as the evidence is not sufficiently convincing to place the substance in category 1 for effects on fertility.

Overall, the observed findings justifies that DINP is classified in Repr. 1B (H360Df).

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No information available

4.12.1.2 Immunotoxicity

No information available

4.12.1.3 Specific investigations: other studies

No information available

4.12.1.4 Human information

No information available

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

None

7 REFERENCES

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ANNEX 1: EU RAR for DINP, section on toxicity to reproduction (page 225-238)

European Chemicals Bureau

Institute for Health and Consumer Protection

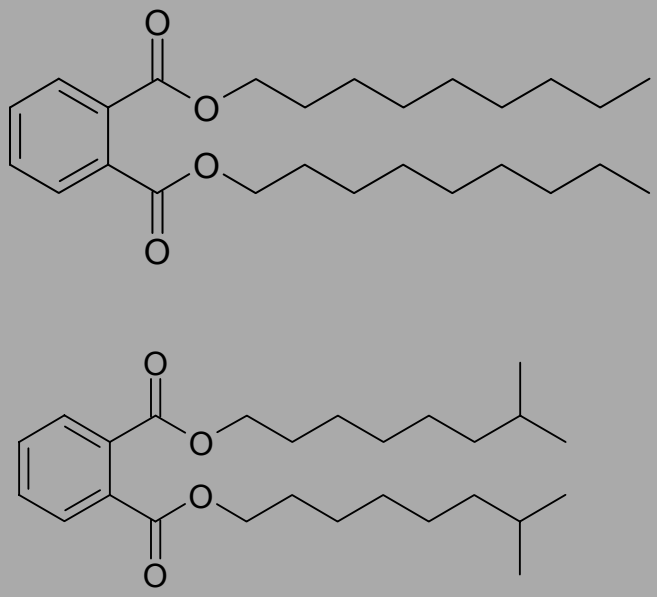
European Chemicals Bureau

Existing Substances

European Union Risk Assessment Report

CAS Nos: 68515-48-0 28553-12-0	EINECS Nos: 271-090-9 249-079-5

1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP)



2nd Priority List

Volume: 35

EUROPEAN COMMISSION
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European Union Risk Assessment Report
DINP
CAS: 68515-48-0, 28553-12-0
EC: 271-090-9, 249-079-5
PL-2
35

European Union Risk Assessment Report

1,2-BENZENEDICARBOXYLIC ACID, DI-C8-10-BRANCHED ALKYL ESTERS, C9-RICH

AND

DI-"ISONONYL" PHTHALATE

(DINP)

CAS Nos: 68515-48-0 and 28553-12-0

EINECS Nos: 271-090-9 and 249-079-5

RISK ASSESSMENT

Cataloguing data can be found at the end of this publication

Luxembourg: Office for Official Publications of the European Communities, 2003

Printed in Italy

1,2-BENZENEDICARBOXYLIC ACID, DI-C8-10-BRANCHED ALKYL ESTERS, C9-RICH

AND

DI-"ISONONYL" PHTHALATE

(DINP)

CAS Nos: 68515-48-0 and 28553-12-0

EINECS Nos: 271-090-9 and 249-079-5

RISK ASSESSMENT

Final Report, 2003

France

The French rapporteur for the risk evaluation of 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate, is the Ministry of the Environment and the Ministry of Employment and Solidarity.

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Date of Last Literature Search:	**2001**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2003**

Regarding MNCL, a clear increase incidence is observed in the two studies conducted with Fisher rats (outside the historical range of spontaneous leukemia), along with shortening of the onset of MNCL. However, MNCL is a common neoplasm in the Fischer 344 rats and the increased incidence after chronic exposure to some substances is likely a strain specific effect with little relevance for humans. Of interest, the IARC categorised MNCL as "an unclassified leukemia with no known human counterpart" and substances which increase MNCL frequency as "not classifiable as to carcinogenicity in humans" (IARC, 1990).

Pertaining to kidney tumours, the species and sex-specific alpha 2u globulin mechanism likely responsible for kidney tumours seen in male rats is not regarded as relevant to humans.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Developmental toxicity and fertility

One-generation study in rats

In this reproduction toxicity study (Exxon Biomedical Sciences, 1996i), test material (undiluted DINP, MRD-92-455 CAS 68515-48-0) diet admixtures were administered ad libitum to 30 rats/sex/group at 3 dosage levels. Group 1 served as a control and received carrier only. Groups 2, 3 and 4 received 0.5%, 1.0% and 1.5% of DINP in feed, respectively. The mean measured dose rat (mg/kg/day) ranges for each group during the premating, gestation and postpartum periods were as indicated in **Table 4.4**7.

P1 males and females received test material daily for at least ten weeks prior to mating and during the mating period. Additionally, P1 female animals received test material during the gestation and postpartum periods, until weaning of the F1 offspring on Postpartum Day (PPD) 21. All animals received test material daily until sacrificed.

Table 4.47 Actual dose related to concentration in diet

Concentration in diet	Actual dose in mg/kg/day	
	Premating: males	Premating: females
0.5%	301 - 591	363 - 624
1.0%	622 – 1,157	734 – 1,169
1.5%	966 – 1,676	1,114 – 1,694
Concentration in diet	**Actual dose in mg/kg/day**	
	Gestation: Females	**Postpartum: Females**
0.5%	377 - 404	490 - 923
1.0%	741 - 796	1,034 – 1,731
1.5%	1,087 – 1,186	1,274 – 2,246

Clinical inlife observations, body weight, and food consumption were recorded for all P1 animals at least weekly during the premating and mating periods (food consumption was not measured during mating due to cohabitation), and for females on Gestation Days (GD) 0, 7, 14

and 21 and on Postpartum Days (PPD) 0, 4, 7, 10 14 and 21 and/or at least weekly until sacrificed. Following birth, the offspring were counted and examined externally daily from Postnatal Day (PND) 0 to 21. Offspring were sexed and weighed on PND 0, 1, 4, 7, 14 and 21. P1 males were sacrificed following the birth of their last litter sired, while females were sacrificed following weaning of their litters on PPD 21. A gross necropsy was performed on all adult animals, selected F1 neonates and on all animals which died during the study. A full macroscopic examination was performed on these animals and selected organs and tissues were collected and weighed.

The results of this study were used to design a follow-up two-generation reproductive study of DINP.

Parental toxicity

There were no clinical signs in the parental animals which were judged to be directly related to treatment with DINP. The majority of animals in all groups had no adverse clinical signs during the premating/mating, postmating, gestation and/or postpartum periods.

Statistically significant lower mean body weights, as well as suppression in body weight gain, were observed primarily in the mid and high-dose parental animals compared with controls. These lower body weights were apparent as early as Week 1 or 2, and were observed during the majority of weighing intervals until scheduled study termination. The greatest decrease from controls (up to 23.3%) was observed during the postpartum period. Similarly, statistically significant lower mean food consumption was observed primarily in the mid and high-dose animals compared with controls. These findings were important in their apparent relationship to findings in the offspring and relative organ weights.

There were statistically significant increases in the mean absolute and/or mean relative liver and kidney weights of both male and female animals at all dose levels tested (0.5, 1.0 and 1.5%) compared with controls. Generally, these increases occurred in a dose-related fashion.

Pertaining to male organ toxicity, there was a statistically significant increase in the mean absolute and relative right testis weight, left testis and right epididymis weights and the mean relative left epididymis and seminal vesicle weights in the high-dose males compared with 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. Histopathological examination was not conducted to confirm if any structural changes occurred.

It was not determined if any structural changes occurred in reproductive organs at any dose level: microscopic evaluation was not performed on any organs in both sexes. Thus significance of organ weight changes could not be assessed because of the limitation of the study. However, there were no statistically significant differences in male mating, male fertility, female fertility, female fecundity, or female gestational indices between treated and control animals. Mean days of gestation of the treated and control groups were essentially equivalent. However, the mean litter size (12.5) and mean live offspring (11.9) of the high-dose animals were lower than controls (14.1 and 13.9, respectively), and the historical control range of this laboratory (16.8-12.6 and 16.1 and 12.2f, respectively). There were no statistically significant differences in the mean sex ratio of the treated offspring compared with controls.

There were no gross post-mortem findings in the parental animals judged to be related to treatment with DINP. The majority of animals in all groups were free of observable abnormalities at post-mortem examination. There were no significant post-mortem findings in the two animals which succumbed prior to scheduled study termination.

Offspring toxicity

The mean live birth index (95.2%), Day 4 survival index (85.6%), Day 14 survival index (92.7%) and lactation index (87.2%) of the high-dose offspring were statistically significantly decreased compared with controls (live birth: 98.2%, Day 4: 93.1%, Day 14: 98.5% and lactation index: 93.9%). The historical control range from this laboratory was the following: live birth: 99.1-95.2%, Day 4: 99.5-89.0%, Day 14: 100-93.7% and lactation index: 100-86.9%.

There were no treatment-related clinical findings observed in the offspring of any group. The majority of offspring in all groups were free of observable abnormalities from PND 0-21.

Dose-related decreases in mean offspring body weight were observed during the postnatal period (PND 0-21). There were 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.

There were also 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.

The majority of animals were free of observable abnormalities at the scheduled terminal sacrifice on PND 21. Similarly, the majority of animals which died prior to scheduled termination (PND 0-20) was free of observable abnormalities, or was too autolyzed to be examined at the necropsy.

For parental systemic toxicity, based on increases in liver and kidney weights from 0.5%, no NOAEL can be determined. Based on decreases in offspring body weight from the lower dose tested, no NOAEL can be determined. No effect was observed on fertility parameters but a decrease of life birth and survival indices occurred at 1.5% which led to a NOAEL of 1%.

Two-generation reproduction studies in rats

In a two-generation study (Exxon Biomedical Sciences, 1996j), four groups of Crl:CDBR, VAF Plus rats (30 rats/sex/group) were administered daily in the diet DINP (MRD 92-455, CAS 68515-48-0) at doses of 0-0.2%-0.4%-0.8%. The actual doses in mg/kg/day were given in **Table 4.48.** These doses were selected based on findings from the previous One-Generation Probe Study.

In this two-generation study (Exxon Biomedical Sciences, 1996j; Waterman et al., 2000**),** performed in accordance with GLP procedures, P1 males and females received test material daily for at least ten weeks prior to mating and during the mating period. Additionally, P1 female animals received test material during the gestation and postpartum periods, until weaning of the F1 offspring on Post Partum Day (PPD) 21. P2(F1) males were dosed from Post Natal Day (PND) 21 for at least 10 weeks prior to mating and through the mating period for F2 litters, until sacrificed following delivery of their last litter sired. P2(F1) females were dosed from PND 21 for at least 10 weeks prior to mating, during mating, gestation, lactation, and until they were sacrificed following weaning of the F2 animals on PPD 21.

Table 4.48 Actual dose related to concentration in diet

Concentration in diet	Actual dose in mg/kg/day	
	Premating P1 generation	**Premating P2 generation**
0.2%	118-215 mg/kg/d	114-264 mg/kg/d
0.4%	236-426 mg/kg/d	235-523 mg/kg/d
0.8%	477-852 mg/kg/d	467-1,090 mg/kg/d
	Gestation P1 and P2 generations	**Post-Partum P1 and P2 generations**
0.2%	133-153 mg/kg/d	159-395 mg/kg/d
0.4%	271-307 mg/kg/d	347-758 mg/kg/d
0.8%	543-577 mg/kg/d	673-1,541 mg/kg/d

Clinical inlife observations, body weight, and food consumption were recorded for all P1 and P2 animals at least weekly during the premating and mating periods (food consumption was not measured during mating due to cohabitation) and for females on gestation days 0, 7, 14, 21 and on PPD 0, 4, 7, 10, 14, and 21 and/or at least weekly until sacrificed. Following birth, the offspring were counted and examined externally daily from PND 0 to 21.

Offspring were sexed and weighed on PND 0, 1, 4, 7, 14, and 21.

Twice daily during the postnatal period, the litters were checked for dead offspring and unusual conditions, and the dams were examined for viability, nesting behaviour, and nursing behaviour. If intact, dead pups were examined externally and internally for anomalies.

P1/P2 males were sacrificed following the birth of their last litter sired, while females were sacrificed following weaning of their litters on PPD 21.

A gross necropsy was performed on all adult animals, selected F1 and F2 neonates (10 offspring/sex/group), and on all animals which died during the study. A full macroscopic examination was performed on these animals and selected organs and tissues were collected and weighed. A range of tissues (vagina, uterus (with cervix), ovaries, coagulating gland, mammary gland (females only), testes (preserved in Bouin's fixation), epididymides, seminal vesicles, prostate, pituitary, liver, kidneys and tissue masses/gross lesions) were examined microscopically for all high-dose and control P1 and P2 (F1) animals used for mating.

Culled pups that appeared normal received only an external examination and tissues were not saved. Culled pups that appeared abnormal were subjected to a visceral examination.

Parental effects

There were no treatment-related deaths and no clinical signs which were judged to be directly related to treatment with DINP in P1 and P2 parental animals.

During the premating period, there were several statistically significantly lower mean body weights in the high-dose males and females in the P2 parental animals compared with controls without an associated decrease of the body weight gain / food consumption. This lower mean body weights in the high-dose animals P2 during the premating period was attributed to lower body weights of the P2 animals at the start of the P2 generation (13% in males and 10% in females), rather than a treatment-related toxicological effect occurring during the P2 premating period.

During gestation, statistically significant lower mean food consumption in the P2 high-dose females compared with controls was recorded without an associated statistically significant decrease of the body weight change during gestation days 0-21.

During the postpartum period, parental toxicity was limited to a lower mean body weight in the high-dose P1 females (< 8%) compared with controls on post partum days 14 and 21 which corresponds to a statistically significant body weight gain suppression (84% decrease) compared with controls during the overall postpartum interval (PPD 0-21) associated with a decreased mean food consumption (9% during the overall postpartum period). Lower mean body weights (8-11%) were observed in the P2 high-dose females with an associated decrease of mean food consumption but without an associated decrease of the body weight gain compared with controls. These lower mean body weights may be due, at least in part, to the lower body weight of the P2 females at the start of the P2 generation (315 g at the high-dose level vs. 331g in the control group).

Biologically significant increases in the mean absolute and/or mean relative kidney weights were observed in the P1 and P2 males from 0.4% but no correlating histologic findings were observed. No gross post-mortem findings judged to be related to treatment were noted in the P1 parental animals. In the P2 generation, there was an increased incidence of dilated renal pelves from 0.2% in males which was mostly unilateral in the high and mid-dose male group.

Additionally, there were statistically significant increases in the mean absolute and mean relative liver weights in P1 and P2 in both sexes at 0.4% and/or 0.8%. Microscopic hepatic changes were noted from 0.2% in P1 and P2 parental animals: minimal to moderate increased cytoplasmic eosinophilia with a rarely enlargement of the affected hepatocytes.

A statistically significant decrease in the mean left ovary weight of the P1 females at 0.8% was observed but in the absence of a clear dose response, 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. Several unconfirmed mated females were noted with red vaginal material approximately 2 weeks after overnight pairing. In high-dose males, there was a statistically significant increase of relative right and left epididymis weights in P2 generation with a concurrent but not statistically significant (by 7.5%) increase of absolute epididymis weight.

Reproductive effects

There were no statistically significant differences in male mating, male fertility, female fertility, female fecundity or female gestational indices in P1 generation. A slight decrease, not statistically significant, of male mating, male fertility, female fertility, female fecundity indices was observed in P2 generation. Mean days of gestation of the P1/P2 treated and control animals were essentially equivalent.

Offspring effects

No treatment-related clinical findings and no biologically significant differences in the F1 or F2 offspring survival indices were observed between the treated and control offspring nor gross post-mortem findings.

There were statistically significant, dose-related, lower mean offspring bodyweights in all treatment groups compared with controls during the F1 or F2 generations. However, when the litter size was taken into account (Waterman et al., 2000), effects were only significant in high-dose males on PND 0, in males and females of the mid and high-dose levels on PND 7 and 14

and in all treated animals on PND 21. In addition, the weights of all F1 and F2 treated offspring were within the historical control range of the laboratory with the exception of the F2 high-dose males and females on PND 0 and the F2 high-dose males on PND 1 (considering litter size). These findings were considered by the laboratory as the results of maternal stress and/or direct effects of DINP via exposure through lactation. Other studies with phthalates concluded that these decreases were apparently due to decreased food consumption by the dams and changes in the quality or quantity of milk (Dostal et al., 1987). Thus the laboratory concluded that the lower body weights in the pups might have resulted from decreased milk consumption.

Based on the microscopic liver changes observed from 0.2%, NOAEL for parental systemic toxicity is considered as lower than 0.2% (114 to 395 mg/kg bw/d seeing that received doses are widely dependent on the period considered). No NOAEL can be derived from this study, but a LOAEL for offspring might be considered as 0.2%, emphasizing a trend observed similarly in males and females, based on the dose dependent reduced mean body weights of the treated offspring. The LOAEL remained approximative since pups switched diet from milk to solid food between PND 14 and 21 but may be estimated 159 mg/kg/d, the lowest dose of the maternal estimated range (159 - 395 mg/kg/d) during post-partum.

No statistically significant differences were observed in reproduction indices.

LOAEL (parents, offspring) =0.2%

4.1.2.9.2 Developmental toxicity studies

(Nikiforov et al., 1994)

A range finding study was conducted to provide information for the selection of dose levels for developmental toxicity study on DINP (CAS 68515-48-0) in the rat. The test substance was administered by oral gavage to 5 groups (7 females/group) of Crl:CDBR mated female rats at doses of 0, 40, 200, 500 and 1,000 mg/kg/d on gestation day 6 through 15. Clinical observations were made daily during gestation, and body weights and food consumption were measured on gestation day 0, 6, 9, 9, 12, 15, 18 and 21. Caesarean sections and gross necropsies were performed, uterine weights with ovaries measured, uterine contents examined and uterine implantation data recorded on gestation day 21. Live foetuses were weighed, sexed, and examined externally for gross malformations.

Overt signs of maternal toxicity were not apparent at any dose level, indicated by the absence of adverse clinical, or post-mortem findings or adverse effects on body weight, food consumption, and/or uterine implantation data.

Similarly, there were no adverse effects for foetal observations or body weight at any dose level. No more information is available (an abstract is only available).

In this range-finding study, the maternal and foetal NOAELs were determined to be 1,000 mg/kg/d.

(Exxon Biomedical Science 1994, Waterman et al., 1999)

In a developmental toxicity study, four groups of 25 Sprague Dawley /Crl:CDBR rats were administered daily by gavage DINP (MRD 92-455, CAS 68515-48-0) at doses of 0 - 100 - 500 - 1000 mg/kg (in corn oil) on days 6-15 of gestation. The female rats were examined for viability and clinical signs throughout the study period. Body weight and food consumption were assessed on gestation day 0, 6, 9, 12, 15, 18 and 21. The dams were sacrificed at day 21 of gestation. Gross necropsies were performed, uterine weights with ovaries attached were measured, uterine contents were examined and uterine implantation data were recorded. All live foetuses were weighed, sexed externally and examined externally for gross malformations. One half of the foetuses were examined for visceral abnormalities and the remaining foetuses for skeletal abnormalities. This study was performed according to GLP procedures except that the analytical chemistry report was not provided as well as the other appendices.

Maternal effects: all dams survived, no treatment clinical signs were observed during gestation and the majority of dams were free of observable abnormalities.

Statistically significant, decreases of the body weight gain (42.6 g vs. 50 g in control group) and of the mean food consumption (178.6 g vs. 195.9 g in control group) were observed at 1,000 mg/kg/day during the treatment period (6-15 days of gestation). However, mean body weight gain of all treated group females was essentially equivalent for the overall gestation period and after correction for gravid uterine weight when compared with controls.

Developmental effects: no statistically significant differences in mean foetal body weight and no statistically significant increases in total or individual external, visceral or skeletal malformations between treated and controls were observed.

External and visceral malformations were observed in both treated and control foetuses during the study and included single or low incidences of anasarca, folded retina, filamentous tail and anal atresia. one mid-dose foetus had *situs inversus viscerum totalis,* unilobular lung and *tetralogy of Fallot*. Skeletal malformations observed throughout the groups included single or low incidences of vertebral agenesis and malformed sternebrae, ribs and/or vertebrae. These findings were considered incidental.

Dose-related increase in total foetuses with visceral (mainly dilated renal pelves) variations on a per foetus (7/190, 8/198, 9/178 at doses of 100- 500 - 1,000 mg/kg 1/194) as on a per litter basis (3/25, 4/24, 6/23 at doses of 0 - 100 - 500 - 1,000 mg/kg vs. 0/23 in controls). However, variations were only significantly increased at the high-dose level on a per litter basis (6/23 vs. 0/24 in controls. Skeletal variations, mainly rudimentary lumbar and cervical ribs showed a dose-response trend on a per litter as on per fœtus basis (32/191, 28/186, 55/194, 76/174 on a per foetus basis and 15/24, 16/25, 22/24, 20/23 on a per litter basis at doses of 0 - 100 - 500 - 1000 mg/kg, respectively). A statistically significant difference from controls on a per litter basis was observed only at 1,000 mg/hg/d (22/24 vs. 15/24 in controls). When considered individually, only rudimentary lumbar ribs were statistically significantly different from controls on a per litter basis at the high dose of 1,000 mg/kg (18/23 vs. 6/24 in controls).

According to the laboratory, the incidences of rudimentary cervical and lumbar ribs at 1,000 mg/kg/d on a per foetus and per litter basis were not within the historical control ranges of this laboratory: percentages of rudimentary cervical and lumbar ribs on a per litter basis were, respectively 30% (historical control ranges: 4.35-16.67%) and 75% (historical control ranges: 29.17-61.90%), but it was considered by the laboratory that rudimentary ribs were probably related to transient maternal toxicity.

Administration of DINP (MRD 92-455, CAS N° 68515-48-0) to the dams resulted in a statistically significant increases in foetuses with skeletal lumbar rudimentary ribs and with visceral (dilated renal pelves) variations at 1,000 mg/kg/d on a per litter basis, which remains the preferred unit of analysis for developmental toxicity studies (Waterman et al., 1999). Rudimentary ribs are common findings in rat foetuses and dilated pelves have been regarded as "normal" delay of renal development unlike hydronephrosis considered as renal malformation (Hayes et al., 1994). However, based on the clear dose-response profile, together with the fact that incidence of dilated pelves was zero in controls, a NOAEL of 500 mg/kg/d can be assumed for developmental toxicity. The NOAEL for the dams is 500 mg/kg/d. This is the most conservative value, since DINP produced transient signs of maternal toxicity at 1,000 mg/kg, as indicated by slight reductions in body weight gain and food consumption. However, normal weight and food consumption patterns were observed during the late gestation period, after exposure ceased, possibly indicating a recovery effect.

Rat prenatal screening toxicity study

In another rat prenatal screening toxicity study, carried out according to GLP procedures, three related forms of DINP (DINP1, DINP2 and DINP3) were investigated:

- DINP1 (CAS 68515-48-0) was of commercial origin. The alcohol moiety mainly consisted of roughly equivalent amounts of 3,4-,4,6-,3,6-,3,5-,4,5- and 5,6-dimethyl heptanol -1;
- DINP2 (CAS 28553-12-0) was produced by BASF with a purity of 99.8%. At least 95% of the main alcohol components was derived from n-butene (alkyl-substituted octanol or heptanol);
- DINP3 (CAS 28553-12-0) was also produced by BASF and synthesised from codimerbutene (n-; isobutene) resulting in at least 60% alkyl-substituted hexanols.

In this study, eight to ten pregnant Wistar rats (Chbb/THOM) were administered daily by gavage at doses of 0 - 40 - 200 - 1,000 mg/kg/d (in olive oil DAB 9/10) on days 6-15 post-coitum. Body weights were recorded on gestation day 0, 6, 10, 15 and 20. The dams were sacrificed at day 20 post-coitum and subjected to gross pathology. The uterus and ovaries were removed and the following data were recorded: weight of uterus, number of corpora lutea, number of implantations, pre/post implantation loss, live foetuses, early/late resorptions. Clinical signs/ symptoms were checked at least daily. Body weights were recorded on days 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20 p.c. and corrected body weight gain was calculated. With the exception of day 0, food consumption was determined on the same days as was body weight. Conception rates, maternal relative liver and kidney weights were recorded. The foetuses were dissected from the uterus, weighed and investigated for external findings. One half of the foetuses were examined for soft tissue abnormalities and the remaining foetuses for skeletal abnormalities.

DINP CAS 68515-48-0 (DINP1) results: during the treatment period the dams at 1,000 mg/kg/d consumed less food (but no quantitative data are available, BASF 1995a; Hellwig et al., 1997b) without concurrent statistically significant decrease of the body weight (decrease around 3-4% compared to the control group). One animal at 1,000 mg/kg/d had vaginal haemorrhage and urine-smeared fur. At autopsy a statistically significant increase in relative kidney weights was recorded at 1,000 mg/kg/d; the relative liver weights were slightly, but not statistically significantly, increased. Absolute liver and kidney weights data were not reported.

The only foetal effects were an increased occurrence of foetal skeletal variations consisting mainly of rudimentary cervical (in 11 foetuses vs. 0 in the control group) and/or accessory 14th ribs (in 37 foetuses vs. 0 in the control group). No treatment-related effects occurred at 40 and 200 mg/kg/d.

Administration of DINP (CAS 68515-48-0, DINP1) resulted in an increased occurrence of foetal skeletal variations at 1,000 mg/kg/d consisting mainly of rudimentary cervical and/or accessory 14th ribs. It can be assumed that the NOAEL for the concept uses is 200 mg/kg/d and the NOAEL for the dams is considered 200 mg/kg/d, based on slight decrease of food consumption and a slight increase of relative kidney weights.

DINP CAS 28553-12-0 (Palatinol N, DINP2) results: the test substance was administered to 9 to 10 pregnant Wistar rats (laboratory report available, GLP study, BASF 1995b; Hellwig et al., 1997b). No significant decrease of food consumption, of the body weight, and of the corrected body weight gain was observed. One dam showed vaginal haemorrhage (gestation days 14 and 15) which might be substance-induced. Absolute and relative liver and kidney weights were not affected by the test substance.

There were no substance-related and /or statistically significant differences of biological relevance between the groups in conception rate, in the mean number of corpora lutea and implantation sites, in pre and post-implantation losses or in the number of resorptions, viable foetuses/dam. The mean foetal weights, placental weights and sex ratio were not influenced by the treatment.

The only substance-related foetal effect was a statistically significantly increased incidence of a skeletal variation namely accessory 14th rib(s): 5/10 vs. 0/10 in controls on a per litter basis at 1,000 mg/kg/d. The respective values are distinctly above the historical control values. Multiple malformations were seen in one foetus among 67 foetuses examined at 1,000 mg/kg/d, namely globular-shaped heart, unilobular lung, hydrocephaly, dilation of the aortic arch and anasarca. At 200 mg/kg/d, one foetus transposition of great vessels was observed in one foetus among 65 foetuses examined.

Administration of DINP (CAS 28553-12-0, DINP2) resulted in a slight increased incidence of a skeletal variation (accessory 14th rib(s)). It can be assumed that the NOAEL for the concept uses is 200 mg/kg/d and the NOAEL for the dams is 200 mg/kg/d.

DINP CAS 28553-12-0 (Palatinol DN, DINP3) results: results for DINP3 are reported here to show the difference among the others, but it is not included in the risk characterisation report, because not any more manufactured since 1995 (cf. Explanatory note).

The test substance was administered to 9 to 10 pregnant Wistar rats (laboratory report available, GLP study, BASF 1995a; Hellwig et al., 1997b). No mortalities were reported. The only clinical findings which occurred were vaginal haemorrhage at 40 mg/kg/d (2 dams), at 200 mg/kg/d (3 dams) and at 1,000 mg/kg/d (1 dam). However, there was no dose-response relationship and gestational parameters (like number of implantations, resorptions, and live foetuses/dam and post-implantation loss values) were not affected. At 1,000 mg/kg/d, food consumption of the dams was significantly reduced at days 8-13 p.c. Mean body weights were statistically significantly lower at days 13-15-17 p.c. at 1,000 mg/kg/d. At the end of the study, the animals weighed about 6% less than the concurrent control group and body weight gain was reduced (about 38% less than control weight). Relative liver weights were statistically significantly increased at this high dose without an associated increase of the absolute weight. Slight increase,

not statistically significant, of the relative kidney weight was observed from 40 mg/kg/d without an associated increase of the absolute weight.

There were no substance-related and/or statistically significant differences between the groups in conception rate, in the mean number of corpora lutea and implantation sites, in pre and post-implantation losses or in the number of resorptions, viable foetuses/dam. The mean foetal weights, placental weights and sex ratio were not influenced by the treatment.

Soft tissue malformations were exclusively found at 1,000 mg/kg/d; in total 5 out of 57 foetuses from 3 out of 9 litters (with a mean percentage of affected foetuses/litter of 10.2) showed malformations of the heart (dilatation of right ventricle, both ventricles: globular shaped heart), the urinary tract (agenesia of kidney(s) and ureter(s); uni- or bilateral) and the testes (abnormal position). While the described dilatation of the heart ventricles appears also sometimes in control foetuses (historical control data: globular shaped heart (0.1% foetuses affected with a range per study of 1.2%) and dilatation of right ventricle (0.02% foetuses affected with a range of 0.8%), the malformations of the urogenital system mentioned above are not within the historical control data base. Even if the rate of malformed foetuses is not increased with statistical significance, a substance-relationship concerning the soft tissue malformations, especially the ones of the urogenital tract cannot be ruled out.

Soft tissue variations (i.e. dilated renal pelvis and hydroureter) were observed most frequently at 1,000 mg/kg/d. Dilated renal pelvis were observed in 20 foetuses out of 57 foetuses from 9 out of 9 litters versus 12 foetuses out of 65 foetuses from 7 out of 9 litters in the control group and incidence of hydroureter was significantly increased at the high-dose level on a per litter basis (89% vs. 33% in controls); foetal and litter incidences are in the range of the historical control and thus not assessed by the laboratory as being associated with the treatment. A substance-relation ship, however, cannot be ruled out for hydroureter. This variation was observed in 12 foetuses out of 57 foetuses from 8 out of 9 litters versus 4 foetuses out of 65 foetuses from 3 out of 9 litters in the control group and appears at a frequency which is clearly outside the historical control range.

Some skeletal malformations (predominantly affecting the long bones) might be assumed to be substance related. No treatment-related effects occurred at 40 or 200 mg/kg/d either in the dams or the foetuses. The malformation rates in these two groups were in the same range as for sham-treated controls.

Developmental toxicity was present in the form of increased rates, of certain skeletal retardation (unossified or incompletely ossified sternebrae) and skeletal (rudimentary cervical and/or accessory 14th ribs) variations. The statistically significant increased occurrence of 1,000 mg/kg/d foetuses with rudimentary cervical (78% vs. 0 in controls on a per litter basis) and/or accessory 14th ribs (89% vs. 0 in controls on a per litter basis) has to be related to the test substance administration to the dams. The respective values are far above the actual and historical control values. Consequently the total number of foetuses with skeletal variations is clearly increased.

Similar studies were conducted on DEHP and reported in DEHP risk assessment. One of them was conducted by the same laboratory (BASF 1995; Hellwig et al., 1997a) and reported at 1,000 mg/kg/d: "a significant lower number of live foetuses/dam as well as a drastically increase rate of malformed fetus/litter". In Tyl et al. (1988), no malformed fetus was reported but the number of live fetus/litter was significantly decreased at 2%. This dose in mg/kg/d is not indicated but probably widely above 1,000 mg/kg/d.

(Hazleton, 1981b)

In a developmental toxicity study, four groups of 25 Sprague Dawley CD albino female rats were administered daily by gavage DINP (CAS not provided) at doses of 0 - 10 - 500 - 1,000 mg/kg (in corn oil) on days 6-15 of gestation. The female rats were examined twice daily for mortality and daily on day 0 and days 6 through 20 of gestation for clinical signs. Body weight was assessed on gestation day 0, 6, 11, 15 and 20. A gross inspection of food consumption for inappetance was made daily. The dams were sacrificed at day 20 of gestation. The dams were examined for visceral gross pathology, the uteri and ovaries were examined. Number of corpora lutea per ovary, uterine implantation data, early and late resorptions, live and dead foetuses and any gross abnormalities were recorded. Foetuses were examined externally, weighed, and crown-rump distances were recorded. One third of the foetuses were examined for visceral abnormalities and the remaining foetuses for skeletal abnormalities. This study was performed according to GLP procedures.

Maternal effects: all dams survived. Inappetance was not observed during the study. No compound-related effects were observed with respect to maternal body weights, clinical signs, pregnancy rates, mean number of corpora lutea, implantation efficiencies, gross pathology or uterine weights changes were observed during gestation and the majority of dams were free of observable abnormalities.

Developmental effects: no treatment-related effects on mean foetal body weight and no visceral or skeletal malformations were observed. A slightly higher incidence of resorptions (10.2% at 1,000 mg/kg/d vs. 4.8% in the control group) and slightly lower foetal viability were noted at 1,000 mg/kg/d when compared to the control group, although no statistical significance was noted.

The incidence of visceral variations (dilated ureters and/or kidneys) was generally higher in the treated groups but this incidence was not statistically significant and did not appear to be compound-related.

In this developmental toxicity study, the maternal and foetal NOAELs were considered to be 1,000 mg/kg/d.

4.1.2.9.3 Summary of developmental studies

Table 4.49 Summary of developmental studies

Species	Protocol/ doses	Results NOAEL/LOAEL	Test substance	References
One-generation studies				
Rat Crl: CDBR	0.5-1-1.5%	LOAEL Parents, offspring 0.5%	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1996i)
Two-generation studies (oral)				
Rat Crl: CDBR	diet 0-0.2-0.4-0.8%	LOAEL parents, offspring 0.2% (159 mg/kg/d)	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1996j) Nikiforov et al. (1995)
Developmental toxicity studies				
Rat Sprague Dawley	gavage 0-100-500-1,000 mg/kg/d	NOAEL (F, dams) 500 mg/kg/d	CAS 68515-48-0 MRD 92-455	Exxon Biomedical Sciences (1994)
Rat Crl: CDBR	range finding study by gavage 0-40-200-500-1,000 mg/kg/d	NOAEL (F, dams) 1,000 mg/kg/d	CAS 68515-48-0	Nikiforov and Koehler (1994)
Rat Wistar	screening study 0-40-200-1,000 mg/kg/d	NOAEL (F, dams) 200 mg/kg/d	DINP1 CAS 68515-48-0	Hellwig et al. (1997b)
Rat Wistar	screening study 0-40-200-1,000 mg/kg/d	NOAEL (F, dams) 200 mg/kg/d	CAS 28553-12-0 DINP 2, Palatinol N (91/26), purity: 99.8%	BASF (1995b) Hellwig et al. (1997b)
Rat Wistar	screening study 0-40-200-1,000 mg/kg/d	NOAEL (F, dams) 200 mg/kg/d	CAS 28553-12-0 DINP 3, Palatinol DN (92/64) purity: >99.9%	BASF (1995a) Hellwig et al. (1997b)
Rat Sprague Dawley	gavage 0-10-500-1,000 mg/kg/d	NOAEL (F, dams) 1,000 mg/kg/d	DINP	Hazleton (1981)

F: fœtus

Prenatal toxicity of C7-9-11 alcohol and of isononanol type 1 and type 2 was assessed in a screening study as described by Hellwig and Jäckh (1997a).

- isononanol type 1 (CAS 68515-81-1) was of commercial origin and mainly consisted of roughly equivalent amounts 3,4-, 4,6-, 3,6-, 3,5-, 4,5- and 5,6-dimethylheptanol-1.
- isononanol type-2 (CAS 68515-81-1) was produced at BASF and mainly consisted of 4, 5-dimethyl-heptanol-1 (about 23%), 4-methyloctanol-1 (29%), 3-ethylheptanol-1 (3%), 6-methyloctanol-1 (15%) and 3-ethyl-4-methylhexanol-1 (1%).
- C7-9-11 alcohol was obtained from BASF, mainly consists of linear alcohols; α-methyl branching is the predominant branching type.

Equimolar dose levels (0, 1, 5 and 10 mmol/kg) were administered by gavage from gestation day 6 to 15 to 10 animals per group. 10 mmol/kg corresponds to 1,440 mg/kg/d for C7-9-11 alcohol and isononanol-2 and to 1,300 mg/kg/d for isononanol-1.

Both isononanols were also investigated in a supplementary experiment at 7.5 mmol/kg/d. Two control groups (distilled water/ distilled water with approximately 0.005% Cremophor EL) were employed.

C7-9-11 alcohol showed no maternal or developmental adverse effects at any dose levels.

Isononanol-1: because of intercurrent death of all dams at 10 mmol/kg, no foetal observations could be obtained.

At 5 mmol/kg maternal symptoms (reduced body weight gain during treatment, apathy, nasal discharge) were visible; foetal weights were numerically (3.60 gr. at 5 mmol/kg vs. 3.90-3.80 gr. in the control groups), but not statistically significantly reduced, and an increased number of skeletal retardations: unossified or incompletely ossified sternebrae (in 34 foetuses among 9 litters vs. 3(3) and 8(4) in the control groups and in 26 foetuses among 7 litters vs. 14(6) and 12(6) in the control groups, respectively) and of soft tissue variations: hydroureter (in 8 foetuses among 5 litters vs. 0 and 3(3) in the control groups) were noted.

At 1 mmol/kg, hydroureters were observed in 8 foetuses among 4 litters 0 and 3(3) in the control groups. The toxicological significance of the increase in hydroureters as an indication of marginal developmental toxicity at 1.0 mmol/kg was considered as debatable by the authors.

At 7.5 mmol/kg, in the supplementary study, severe maternal symptoms and decrease, statistically significant, of the body weights of the dams were noted. An increased number of resorptions (increased postimplantation losses 41.9% vs. 7.2 and 5.8 in the control groups), a decreased foetal weights (3.0 gr. vs. 3.8-3.8 gr. in the control groups) were observed and a statistically significant increase of the incidence of malformations (mainly related to the heart in 5 foetuses among 4 litters vs. 0 in the control groups) and skeletal retardations.

Isononanol-2: isononanol-2 was less toxic to the dams. At 10 mmol/kg, seven of 10 animals survived and in the six litters obtained a low frequency of malformations was found (one *anedeous* foetus and one foetus with meningocele); the number and percentage of foetuses with skeletal retardations also increased (40 foetuses among 6 litters vs. 30 (10) and 29 (7) in the control groups).

At 5 mmol/kg, slight maternal symptoms and slight decrease of the body weight gain were noted associated with a slight increased resorption rates (increased post-implantation losses 12% vs. 3.5 and 5.1 in the control groups) and a slight increased frequency of foetuses with hydroureter (5 foetuses among 3 litters at 10 mmol/kg, 5 (4) at 5mmol/kg, 3 (3) at 1 mmol/kg and, 0 and 3 (3) in the control groups).

At 7.5 mmol/kg, in the supplementary study, clear signs of maternal toxicity were observed associated with higher post-implantation losses (17.2% vs. 7.2% and 5.8% in the control groups) and an elevated number of foetuses with malformations (mainly concerning the thoracic vertebrae).

C7-9-11 alcohol: it showed no adverse effects at any dose levels. Isononanols type 1-2 exhibited a marked degree of maternal and foetal toxicity at 7.5 and 10 mmol/kg and slight foetal effects at 5 mmol/kg. Because of maternal toxicity in the top dose, a statistically significant increase in malformations was obtained only in the dose window of 7.5 mmol/kg, in the supplementary experiment. The authors recommended that the potential for developmental toxicity of primary aliphatic alcohols between 7 and 11 carbon atoms per molecule must be investigated case by case for each individual structure.

4.1.2.9.4 Summary of toxicity for reproduction

Fertility assessment may be inferred from effects on reproductive organs and the two-generation study.

In adult rats, some minor effects were observed not histologically confirmed in any of the studies mentioned: in the one-generation study, a statistically significant increase in the mean absolute and relative right testis, left testis and right epididymis weights and the mean relative left epididymis and seminal vesicle weights in the high-dose males were observed; in a few sub-acute and/or subchronic studies, slight increases (statistically significant) of relative testes weights were also noted at high doses. Taken as a whole, no overt toxicity was observed on reproductive organs in rats.

In mice, very high dose (5,770 mg/kg/d) leads to decrease in testicular weight with abnormal/immature sperm forms and uterus/ovaries atrophy in the 13-week study. In the 104-week chronic study, a NOAEL of 1,500 ppm (276 mg/kg/d) can be assumed for testicular effects, based on decrease in testicular weight (relative and absolute) observed from 742 mg/kg/d. The NOAEL for systemic toxicity in male is 1,500 ppm as well.

In the two-generation study no changes in reproductive indices are observed.

From those assays, no adverse effects on fertility may be anticipated.

In regard with offspring survival in rats, at 1.5% (corresponding to a range of 966-2,246 mg/kg/d), a decrease of life birth and survival indices was observed in the one-generation range-finding study but not observed in the two-generation study, conducted up to 0.8%. For decrease in life birth and survival indices a NOAEL of 622 mg/kg/d (the lowest dose of the estimated range) is determined and is taken into account in the risk characterisation.

In the developmental studies, visceral (dilated renal pelvis and hydroureter) and skeletal (rudimentary cervical and accessory 14^{th} ribs) variations were significantly increased at 1,000 mg/kg/d this lead to a NOAEL of 500 mg/kg/d. Slight (1,000 mg/kg/d) or no (500 mg/kg/d) maternal toxicity was observed in those studies.

A decrease of mean offspring body weight was observed following parental administration of DINP in the one and two-generation studies from the lowest dose tested (0.2% in the two-generation study), leading to a estimated LOAEL of 159 mg/kg/d, the lowest value of the maternal dose range during post-partum. In the two-generation study parental toxicity was limited to lower mean body weight and hepatic changes from 0.2% (eosinophilia and rarely enlargement of the hepatocytes), thus a LOAEL of 114 mg/kg/d (the lowest level of the 0.2% dose) may be derived.

The NOAEL and LOAEL quoted above will be considered in risk characterisation for developmental effects.

Regarding fertility and development, the effects observed in the available studies, do not justify classification according to the EU classification criteria.