Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Dicyclohexyl phthalate

EC number: 201-545-9
CAS number: 84-61-7

CLH-O-0000001412-86-38/F

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

Adopted

04 December 2014

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CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Dicyclohexyl phthalate

EC Number: **201-545-9**

CAS Number: **84-61-7**

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Dicyclohexyl phthalate***
EC number:	***201-545-9***
CAS number:	***84-61-7***
Annex VI Index number:	***None***
Degree of purity:	***Typically 99%***
Impurities:	***Unknown according to REACH registration***

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; H360FD Skin Sens. 1; H317
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 1B; H360FD Skin Sens. 1; H317

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
3.2.	Skin corrosion / irritation	None		None	Conclusive but not sufficient for classification

3.3.	Serious eye damage / eye irritation	None		None	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Not assessed in this dossier
3.4.	Skin sensitization	Skin Sens 1; H317		None	
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; H360FD**		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, conclusive but not sufficient for classification or not assessed in this dossier

Labelling:

Pictogram with signal word: GHS07, GHS08 (danger)
Hazard statements: H360FD; H317
Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There is no previous harmonized classification and labelling for dicyclohexlyl phthalate (DCHP). DCHP was registered within the 100 - 1000 tonnage band (May 30, 2013). The registrants classified DCHP as Skin Sens. 1 - H317; Repr. 2 - H361; Aquatic Chronic 3 - H412, M-factor=1. In addition, the registrant indicated that the data for the following endpoints were conclusive but not sufficient for classification: Acute toxicity oral, acute toxicity dermal, skin corrosion/irritation, serious eye damage/eye irritation, germ cell mutagenicity, carcinogenicity, STOT SE, STOT RE and aquatic acute. For all endpoints regarding physical hazards as well as for acute toxicity – inhalation, respiratory sensitization, aspiration hazard, effects via lactation and hazardous to the ozone layer – the registrants stated that the reason for no classification was lack of data.

2.2 Short summary of the scientific justification for the CLH proposal

The available data indicate that DCHP causes developmental toxicity and toxicity to reproductive organs. DCHP induced effects on the developing male reproductive system. Most pronounced signs seen were areole mammae/nipple retention and decreased anogenital distance, but also a malformation (hypospadias) was noted. Although no clear effect on fertility as assessed by effects on reproductive outcome was reported in either generation in the available studies, toxicity to the reproductive organs was observed in the form of focal and diffuse seminiferous tubules atrophy and a significantly reduced testicular sperm head count. Other signs were reduced weight of the prostate and reduced relative weight of the levator ani/bulbocavernosus muscle. The toxicity to the reproductive organs seemed to be age-dependent as it was only observed in offspring exposed in utero and via the milk but not noted in the adult animals in the reproductive studies. However DCHP can induce testis atrophy also in juvenile and adult rats but only at dose levels much higher than those used in the studies where effects on reproduction of DCHP were examined. The observed effects partly resemble the effects reported for transitional phthalates (reviewed in Fabjan et al., 2006 and in NAS 2008).

In conclusion, the adverse effect on development and on reproductive organs warrants a classification of DCHP in Repro 1B (H360FD).

2.3 Current harmonised classification and labelling

There is no harmonised classification and labelling and thus no entry in Annex VI, Tables 3.1 and 3.2 in the CLP regulation.

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

Self-classification notifications for DCHP by industry are available in the C&L Inventory (http://echa.europa.eu/information-on-chemicals/cl-inventory-database.

The industry has submitted 53 C&L notifications for DCHP forming five notification groups. One group (a joint entry and also representing the registration) classifies DCHP as Skin Sens. 1(H317), Repr. 2 (H361) and Aquatic Chronic 3 (H412; M-Chronic=1). Two notification groups have proposed the same classification but for different forms of the substance (unspecified and liquid, respectively), i.e. Skin Irrit. 2 (H315), Eye Irrit. 2 (H319) and STOT SE 3 (H335). The fourth group (only one notifier) has classified DCHP as: STOT SE 3(H335) and Repr. 1B, (H360), whereas the fifth notification group (24 notifiers) has not classified DCHP at all.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

DCHP has a CMR property (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation. This MSCA disagree with the existing self-classification of skin sensitisation (ranging from category 1 to no classification) notified to the C&L inventory by the industry and considers that the harmonised classification for this endpoint as proposed in this dossier is justified by the information available on this substance.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

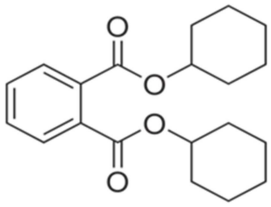
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	201-545-9
EC name:	Dicyclohexyl phthalate
CAS number (EC inventory):	
CAS number:	84-61-7
CAS name:	
IUPAC name:	Dicyclohexyl phthalate
CLP Annex VI Index number:	-
Molecular formula:	$C_{20}H_{26}O_4$
Molecular weight range:	330.418

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
DCHP	99.0 % (w/w)	≥ 99 – 100% (w/w)	Data from REACH registration

Current Annex VI entry: None

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Unknown		>0 - < 1% (w/w)	Data from REACH registration

Current Annex VI entry: Not applicable

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-				No information in REACH registration

Current 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 1013 hPa	White crystalline powder with slightly aromatic odour	REACH registration (2013)	Evidence due to substance observation and handling
Melting/freezing point	ca. 65.6 °C at 101.3 kPa	REACH registration (2013)	Measured, ASTM E537-07
Boiling point	ca. 322.03 °C at 1 atm		Measured, ASTM E537-07
Relative density	Density 0.787 g/ml		Measured, USP 34-NF29 <616>
Vapour pressure	8.7×10^{-7} mm Hg at 25 °C	Werner, 1952	Measured, Dew-Point and Tensimeter method
Surface tension		Data waived in REACH registration (2013)	
Water solubility	1,015 mg/L (20°C and pH 7)	REACH registration (2013)	Measured, OECD 105/1995
Partition coefficient n-octanol/water	Log Pow= 4,82 (25°C)	REACH registration (2013)	Estimated value obtained by extrapolation from the calibration curve, OECD 117
Flash point	180 – 190 °C	Bayern AC, Leverkusen, as cited in IUCLID dataset 2000 for Existing Chemical Substance (European commission 2000a)	Measured, DIN 51376
Flammability	Not determined	Data waived in REACH registration (2013)	
Explosive properties	Not determined	Data waived in REACH registration (2013)	
Self-ignition temperature	Not determined	Data waived in REACH registration (2013)	
Oxidising properties	Not determined	Data waived in REACH registration (2013)	
Granulometry	Average particle size = 442.144 µm	REACH registration (2013)	ISO 13320-1:1999 Particle size analysis - Laser diffraction methods
Stability in organic solvents and identity of relevant degradation products	Not determined	Data waived in REACH registration (2013)	

Dissociation constant	Not determined	Data waived in REACH registration (2013)	
Viscosity	Not determined	Data waived in REACH registration (2013)	

2 MANUFACTURE AND USES

Quantities
The total tonnage band is 100 – 1000 tonnes per annum (ECHA dissimination web site. Information as accessed October 8, 2013).

2.1 Manufacture

Not relevant for this report.

2.2 Identified uses

DCHP is a common plasticizer ingredient in the production of nitrocellulose, ethyl cellulose, chlorinated rubber, polyvinyl acetate, polyvinyl chloride, and other polymers resins and it is also used in paper finishes and makes printing ink water-resistant (HSDB 2013). In Sweden, from 2007-09, DCHP was a component of at-least 18 products (KemI-stat). DCHP is also found in the indoor particulate matter (Rakkestad et al., 2007). In indoor air samples from 27 houses of Tokyo metropolitan area, DCHP was found at a mean concentration of 0.07 $\mu g/m^3$ (Otake et al., 2004). Its metabolite monocyclohexyl phthalate (MCHP) was found in adult urine samples of the US general population (Blount et al., 2000 cited in Saillenfait et al., 2009a).

The Directive 2007/42/EC (European Commission 2007), which relates to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs, limits the use of DCHP as a plasticiser to not more than 4 mg/dm^2 of the coating on the side in contact with foodstuffs (the total quantity of plasticizers may not exceed 6 mg/dm^2).

DCHP was included in EC DG Env Reports "*Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption*" (European Commission, 2000c) and "*Endocrine disrupters: study on gathering information on 435 substances with insufficient data"* (European Commission, 2002). In the 2002 report, DCHP was categorized as high exposure concern since it is used as a softener and plasticizer in commonly used plastics and human exposure is expected for example through food due to leaching from food packages and from plastics in children's toys.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

There is only very limited toxicokinetic data available for DCHP. Lake and coworker (1977) showed that DCHP (similar to dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), di-*n*-octyl phthalate (DOP) and di(2-ethylhexyl) phthalate (DEHP) that also were examined) is hydrolysed in vitro by rat, ferret and primate (baboon) liver and intestinal preparations (as well as by human intestinal preparations) to its corresponding monoester derivatives and to an alcohol moiety (cycklohexanol). For all the compounds examined, the hepatic hydrolase activity generally decreased in the order baboon > rat > ferret (Lake et al., 1977).

Saito and coworkers (2010) showed that eight structurally diverse phthalates (diethyl phthalate (DEP), di-n-propyl phthalate (DPrP), di-*n*-butyl phthalate (DBP), di-*n*-pentyl phthalate (DPeP), di-*n*-hexyl phthalate (DHP), DEHP, *n*-butyl benzyl phthalate (BBP), and dicyclohexyl phthalate (DCHP)) were all hydrolyzed to their corresponding monoesters by both porcine and bovine pancreatic cholesterol esterases. The hydrolysis experiment with bovine pancreatic cholesterol esterases showed complete hydrolysis of every phthalate (5 µmole), except for BBP and DCHP, within 15 min; BBP and DCHP were hydrolyzed within 30 min and 6 h, respectively. The authors concluded that the rates of phthalate hydrolysis could be affected by the bulkiness of alkyl side chains in the phthalate ester

No data were available on absorption or elimination kinetics of DCHP. .

4.1.1 Summary and discussion on toxicokinetics

The data reported suggest that ingestion of DCHP via the oral route results in intestinal absorption of its monoester derivative. The toxicity of DCHP is thus likely related to its rate of hydrolysis to its metabolite monocyclohexyl phthalate (MCHP) as well as to the formation of other not yet identified metabolites and the properties of these metabolites. The rate of hydrolysis for DCHP (which contains a cyclic alkyl chain) is slower as compared to phthalates with straight side chains containing the same number of carbons (or even branched chain containing more carbons).

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 Skin corrosion/irritation

The information relevant for this endpoint was assessed and the conclusion was that no classification was appropriate for this endpoint.

4.5 Serious eye damage/eye irritation

The information relevant for this endpoint was assessed and the conclusion was that no classification was appropriate for this endpoint.

4.6 Respiratory sensitisation

Not evaluated in this report. No data was available in the REACH registration.

4.7 Skin sensitisation

4.7.1 Non-human information

Table 11: Summary table of relevant skin sensitisation studies

Method	Remarks	Results	Reference
Mouse local lymph node assay (LLNA OECD Guideline 442B Mouse (CBA/JN) female **Test material:** Dicyclohexyl-phthalate **Positive control** hexyl cinnamic aldehyde (CAS No 101-86-0) 25% w/w in acetone: olive oil, 4:1 (v/v) **Vehicle:** acetone/olive oil (4:1 v/v)	Key study	**Preliminary phase**: Test conc: 25, 10, 5, 2.5, 1% w/w. No toxicity signs (clinical signs or toxicologically relevant body weight losses) were observed at any concentration tested. According to the results of the irritation screening, the concentration judged as minimally irritant was 10% w/w. **Main study:** Test conc; 10, 5 and 2.5% w/w, in acetone:olive oil 4:1 (v/v).In a first experiment the calculated stimulation indices were 1.80, 1.91 and 1.24 respectively at low, mid and high dose groups. Since these results were considered borderline, a second experiment was repeated to confirm them. In the second experiment, increases in cell proliferation of draining lymph nodes were observed in all test item treated groups, with the calculated stimulation index equal to 2.22, 2.82 and 1.94 respectively at low, mid and high dose level. In this experiment, the observed increases were statistically significant at the low and mid- dose level (Groups 2 and 3) but not in the high dose level (Group 4). No dose response relationship was observed.	Research Toxicology Centre S.p.A. (2012e), as cited in REACH registration (2013)

The CPSC review for dicyclohexyl phthalate (2011) briefly and poorly describes the results from two studies (data not available toDS) as follows:

1. "*Eastman Kodak Co. (1965) reported that DCHP was not a skin sensitizer in guinea pigs. No further information was available.*"
2. "*Male guinea pigs were repeatedly exposed to 500 mg Nuoplaz 6938 on intact skin for 24 hours (under occluded conditions) for 10 applications and re-challenged at a different site after a 2-week rest period. Four of 10 animals showed erythema and slight edema 24 and 48 hours after the challenge application (Nuodex, 1979d).*"

   Nuoplaz 6938 is a mixture consisting of DBP (21.9%), n-butyl cyclohexyl phthalate (near

61.2%), DCHP (15.2%) and 1.7 % DMP (European Commission, 2000b). Thus the information provided regarding the skin sensitising effects caused by Nuoplaz 6938 cannot be used to draw a conclusion regarding skin sensitising effects of DCHP.

4.7.2 Human information

No information provided in the REACH registration.

4.7.3 Summary and discussion of skin sensitisation

The potential of DCHP to cause skin sensitisation reactions following topical application to the skin of CBA/JN (CBA/J) mice, was assessed using the LLNA:BrdU-ELISA method (OECD TG 442b). In the first experiment, the stimulation index (SI) values of the low and intermediate test concentration (but not the high test concentration) were above the threshold for a positive result (SI= 1.6) but within the range (1.6 – 1.9) that the test guideline defines as a borderline positive result. Therefore the study was repeated. In the repeat study the SI values for all 3 test concentrations were above the threshold for a positive result as well as above the range for a borderline positive result. Therefore, the results obtained in this study indicate that the test item elicits a sensitisation response in mice following dermal exposure.

4.7.4 Comparison with criteria

Current CLP legislation does not specify how data from OECD TG 442B, which is a non-radioactive modification to the local lymph node assay (LLNA, OECD TG 429) that was adopted 2010, should be used for classification. However, the Guidance on the Application of the CLP criteria (section 3.4.2.2.3.2) acknowledges that this test method has been validated for identifying skin sensitising compounds. The data can only be used to identify a compound with a significant sensitising effect (category 1, if Stimulation Index $\geq$ 1.6) but cannot be used for sub categorisation into 1A or 1B. According to CLP Annex I, section 3.4.2.2.1.1, skin sensitisers shall be classified in Category 1 when data are not sufficient for sub-categorisation.

4.7.5 Conclusions on classification and labelling

DCHP meets the criteria in the CLP regulation for classification as Skin Sens. 1 (without sub-categorisation).

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal The proposal for classification of dicyclohexyl phthalate (DCHP) for skin sensitisation (Skin Sens. 1) was based on a single local lymph node assay (LLNA). The study was consistent with OECD Technical Guideline (TG) 442B, and included positive controls which were not however reported in the CLH report. In the LLNA assay using CBA/JN female mice and the BrdU ELISA method, a 10% solution was determined as the minimal irritant concentration, and therefore 10%, 5% and 2.5% (w/w) solutions (in acetone:olive oil 4:1 (v/v)) were used in the main study. In an initial experiment, the stimulation index (SI) values calculated from the mice exposed to the low and intermediate test material concentrations (but not the high concentration) were above the threshold for a positive result (SI= 1.6) but within the range (1.6 – 1.9) which was defined as a borderline positive result in OECD TG 442B. The study was repeated, and the new SI values calculated were 2.22, 2.82 and 1.94 at the low, mid-

and high-dose, respectively. Since for all 3 test concentrations the SI in this repeat study were above the range for a borderline positive result (albeit barely in one case), the DS concluded that based on the LLNA assay, dicyclohexyl phthalate is a skin sensitiser in mice. Sub-categorisation for skin sensitisation was not possible based on the data and therefore the DS proposed classification as Skin Sens. 1.

Comments received during public consultation

Comments were received during public consultation from 2 member states (MS) on this hazard class. One MS supported the proposed classification. Another MS did not agree that the data met the criteria for classification for skin sensitisation and noted that the scientific justification for the proposal for skin sensitisation classification was missing from the CLH report.

In their response the DS noted that the responses in the repeat experiment were above the threshold for a positive result. According to the DS, the response in the high dose group (with a lower SI than in the middle and low dose groups) may have been due to an overload effect, in which the balance between effector and suppressor cells which constitutes the sensitisation response may have been affected by the high dose (Andersen *et al*., 1985).

Assessment and comparison with the classification criteria

One key study, a mouse local lymph node assay (LLNA) with DCHP was included by the DS in the CLH proposal. According to the CLP Guidance (November, 2013), section 3.4.2.2.3.2, the definition of a significant skin sensitising effects is described as an SI ≥ 1.6. RAC therefore concludes in agreement with the DS that DCHP should be classified as a skin sensitiser in Category 1.

Regarding a potency evaluation, the key study summarised in the CLH report did not include sufficient information for sub-categorisation since no EC3 value was derived, and DCHP should therefore be classified in **Category 1 (Skin Sens. 1) without sub-categorisation.**

4.8 Repeated dose toxicity

Table 12: Summary table of relevant repeated dose toxicity studies

Method	Test substance & Dose	Results	Reference
SD rats, males (30 day old) Oral (gavage) Group size not clearly specified Necropsy on day 8: kidneys, liver and testes preserved for histopathology /biochemical analysis. In case of DCHP, histopathological examination of liver, kidney and testes was only done for animals dosed with 0, 1500 or 2500 mg/kg bw/day	0, 500, 1000, 1500, 2000 or 2500 DCHP (≥ 99% purity) mg/kg bw/day for 7 days MCHP: 1130 mg/kg bw/day Cyclohexanol: 455 mg/kg bw/day for 7 days Vehicle: corn oil Dose volume: 5ml/kg	No information on clinical signs, body weights or food consumption. Dose-related increase in relative liver weigh. At 1500 mg/kg bw/day the increase was 42.4% (no data for other dose groups). Slight hypertrophy of centrilobular cells were observed at 1500, effects were more marked at 2500. Ultrastructural examination revealed marked proliferation of smooth endoplasmic reticulum of centrilobular cells but no effects on other organelles at the intermediate dose level (no data given for high dose and low dose animals). No evidence of perixsome proliferation. No adverse effect at 1500 mg/kg on testes or kidney weights. Histopathology of one of five treated animals showed bilateral tubular atrophy affecting 30-40% of the germinal cells at 2500 mg/kg/day. Of the DCHP metabolites, monocyclohexyl phthalate (MCHP) and cyclohexanol, MCHP produced marked testicular atrophy.	Lake et al., 1982

4.8.1 Non-human information

4.8.1.1 Repeated dose toxicity: oral

The information on repeated toxicity is only provided as supportive information to the reprodata.

The Lake study (Lake et al., 1982, see Table 11) has a low reliability but might indicate that the liver and testis are target organs for DCHP. Additional information on effects on these and other organs is also obtained from the reproductive toxicity studies. Thus, there is some information on repeated dose toxicity in the 2-generation reproductive toxicity study (data are presented in Table 13 in this dossier) where Hoshino and co-worker (2005) reported an increased relative liver weight (F_0 and F_1, LOEL = 6000 ppm ~401 – 534 mg/kg bw/day). An increased incidence of diffuse hypertrophy (severity score slight) of hepatocytes (both genders of F_0 and F_1 generation) was also observed at the 6000 ppm dose level and, at a lower incidence, in F_0 males and females at 1200 ppm (~80 – 105 mg/kg bw/day) in that study. Effects on liver weights were also reported by Yamasaki (2009) (F_0 females, males not exposed; +7 and + 24% in the 100 and 500 mg/kg bw/day, respectively) and Saillenfait (2009a) (only females exposed: +17 and +28% in the 500 and 750 mg/kg bw/day, respectively). Effects on thyroid weight (+ 15-24% relative weight, F_0 females at 6000 ppm) and an increased incidence of thyroid follicular cell hypertrophy (severity slight) at the 6000 ppm dose levels (both genders in F_0 and F_1) and in F_0 males at the 1200 ppm dose level were

also recorded in the study by Hoshino (2005). In that study, an increase of hyaline droplets in the renal proximal tubular epithelium was observed in both F_0 and F_1 males including controls without a dose response for the slight severity grade. However, for the moderate severity grade a high incidence (F_0, 15 as compared to 1 in controls; F_1, 8 as compared to 1 in controls) was recorded in males at the 6000 ppm dose level. In addition, the study by Hoshino identified the F_1 generation as being more sensitive as compared to the F_0 generation regarding effects on the weight of the prostate (LOAEL was 6000 ppm [-21%] for effects on the relative weight and no NOAEL was identified for effects on the absolute weight of the prostate in the F_1 generation; no effects in the F_0 generation), as well as regarding atrophy of the seminiferous tubules (LOAEL = 6000 ppm for severity grading severe and 1200 ppm for severity grading slight in the F_1 males; no effects in the F_0 generation), and in the number of testicular homogenization resistant spermatids (LOAEL= 1200 ppm [15% less] in the F_1 generation; no effect observed in the F_0 generation). A decreased relative weight of the prostate was also recorded in offspring exposed in utero and up until weaning and then necropsied at 10 weeks (Yamasaki, via oral gavage). No NOAEL for this effect was recorded in this study (see section 4.12 for further information).

4.8.1.2 Repeated dose toxicity: other routes

No information available in the REACH registration.

4.8.2 Human information

No information available in the REACH registration.

4.8.3 Summary and discussion of repeated dose toxicity

The information on repeated dose toxicity is not sufficient to assess this endpoint.

The findings in the liver, thyroid and kidney in the studies by Hoshino (2005) and Yamasaki (2009) were at dose levels and/or of a severity grade outside those where STOT classification is warranted. However the available studies might indicate that the observed effects on the liver and kidney are similar to the ones observed for other phthalates (Fabjan et al., 2006). The effect on testicular histopathology is also similar to what has been observed for transitional phthalates (NAS 2008).

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

Not evaluated in this report.

4.10 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this report.

4.11 Carcinogenicity

Not evaluated in this report.

4.12 Toxicity for reproduction

Table 13: Summary table of relevant reproductive toxicity studies

Reference & Method	Test substance & Dose	Results
Hoshino et al., 2005 **Key study** • Two-generation study (dietary) in accordance with OECD TG 416 of 1983. • 24 animals /sex/dose • Rats (Crj:CD(SD)IGS • F_0: 5 week of age at start of dosing	DCHP (CAS No. 84-61-7, 99.9% purity) 0, 240, 1200, or 6000 ppm (corresponding to for F_0 males : 0, 1, 80 and 402; F_0 female: 0, 21, 105 and 511; F_1 males: 0, 18, 90 and 457; F_1 females: 0, 21, 107 and 534 mg/kg bw/day, respectively, when taking mean daily intake during the entire dosing period into account) F_0 males: dosed at least 10 weeks before mating and during mating F_0 females: dosed at least 10 weeks before start of mating continuing until weaning of F_1 offspring (PND 21). F_1: from PND21 continuing to end of mating for males (mating at ~14 – 15 weeks of age), and females being dosed until lactation day 21.	***Effects on body weights, necropsy and clinical observation*** • F_0 males: no significant effects on body weights. No clinical signs. • F_0 females: slightly decreased body weights ($p<0.01$ from 2 weeks of dosing continuing until end of lactation for high dose group (~ 10-12 % lower body weight, as compared to controls, from premating until PND 21 as judged from the graphical presentation of this data in the paper) and for intermediate group on occasional days (mostly $p<0.05$) up until end of pregnancy and more frequently during the period of lactation ($p<0.05$ /0.01). At end of study the intermediate dose group weighed ~5% less than the controls. No clinical signs. • F_1 males: A very slightly decreased weight from birth and onwards (but statistically significant $p<0.01$) in high dose animals. The effects on body weight got more pronounced as treatment continued over time and after ~10 weeks of dosing decreased body weights ($p<0.01$) was also observed in the intermediate dose group (4% less in the intermediate and 9% less in the high dose group as compared to the controls as judged from the graphical presentation of this data). No clinical signs. • F_1 high dose females showed a somewhat lower weight at birth until weaning ($p<0.01$) and then also during the entire period of gestation and lactation ($p<0.05$/0.0l, being maximum 8-9 % less as compared to controls as judge from the graphical presentation of the data). No clinical signs. <u>Organ weights and histopathology</u> • Increased absolute (+21%) and relative (+24%) liver weight of males and females (+9% and +19%, respectively) in the high dose groups of the F_0 generation. An increased relative liver weight in the F_1 generation (+14 M and +16% F), animals at the high dose level. At the intermediate dose level, an increased relative weight (+6%) in F_0 females and a decreased absolute weight (-12%) in F_1 male were recorded. • At histoptahological examination, an increased incidence of diffuse hypertrophy (severity score slight) of hepatocytes was observed at the high dose

		level (both genders of F_0 and F_1 generation) and at a lower incidence in F_0 males and females at the intermediate dose level. • Increased thyroid weight was seen at the high dose level in the F_0 generation (males: ~+30% both in absolute and relative but only seen in left gland; females: +15-24% in only relative weight of both glands). No effects in F_1 generation. Increased incidence of thyroid follicular cell hypertrophy (severity slight) in high dose animals (F_0 and F_1 animals) and intermediate F_0 males. • Increased hyaline droplets in the renal proximal tubular epithelium were observed in both F_0 and F_1 males including controls without a dose response for the slight severity grade. For the moderate severity grade a high incidence (F_0: 15; F_1: 8), as compared to as compared to the controls (1 in both) was recorded in the high dose males. • Statistically significant decrease in absolute (19%, 16% and 28% less as compared to controls in low, intermediate and high dose groups, respectively) and relative (statistically significant only at the high dose level, -19%) weight of the prostate in F_1 (no effects on prostate weight in the F_0). Diffuse atrophy of the seminiferous tubules (severe grade) was seen in 3 high dose males of the F_1 generation and a lack of sperm in the epididymal tubules was also observed in these animals. Focal atrophy (slight severity) was seen in1, 0, 2, 6 males in the control, low, intermediate and high dose groups, respectively, in the F_1 generation. ***Effects on fertility and hormone levels*** No statistically significant effect on mating or fertility indices or on the number of days between start of mating until day of confirmed copulation, or on gestation length or gestation index for the F_0 and F_1 generations. The values for the mating and fertility indices showed slight tendencies for decrease in the F_1 high dose group (90.5 and 89.5 as compared to 95 and 100%, respectively). The authors considered that this was associated with the testicular changes (soft and/or small size) recognized in three males at necropsy. In the other F_1 high dose males copulation and resultant pregnancies were normal. Dose dependent decrease in number of

		testicular homogenization resistant spermatids in the intermediate and high dose (15 and 24 % less as compared to controls) of the F_1 generation (no effect observed in F_0 and F_2 was not examined.) In the F_1 male parents of the high dose group, soft and small sized testes were observed in one animal, and examination of this rat revealed no sperm. There were no effects on epididymal sperm motility, number or morphology in either F_0 or F_1 generation (endpoint not examined in F_2). Minimal (+5% longer) but statistically significant increase of the estrous cycle length was recorded for the F_0 high dose group (no effect recorded in F_1) but no females displayed abnormal cycles. The effect was thought to be secondary to the suppression of body weight gain by the authors. There were no dose-dependent effects on testosterone/estradiol, FSH and LH levels in F_0 or F_1 animals. ***Developmental effects*** • F_1 and $F_{2:}$ No effects on sex ratio, littersize, viability index or on survival. No effects on physical development as revealed by effects on pinna unfolding or on time point for incisor eruption or eye opening. • Slightly (4-6%, but statistically significant), decreased birth weight in high dose F_1 males and females. The effects on bodyweight were observed throughout lactation and at weaning pups (males and females) weighed 11 - 12% less than the controls. F_2 males and females weighed about the same as the controls at birth and up until post natal day 21 when a slight (8-9%, $p<0.01$) reduced body weight was observed at the high dose level. • Time point for pre-putial separation was delayed (not statistically significant) and coincided with a statistically significantly decreased body weight at day of preputial separation in F_1 high dose males. No effects on day of vaginal opening in F_1 females. • Male pups showed a decreased absolute (F_1: -7%, $p<0.01$; F_2: -9% $p<0.01$) and relative (F_1: -8%, $p<0.01$; F_2: -9%, $p<0.01$) anogenital distance at the high dose level and this effect was also seen at the intermediate dose level in F_2 (-7% and -7% for absolute and relative distance, $p<0.01$). • The percentage of litters with male

		pups that had areole mammae was clearly increased at the high dose level (16.1% in F_1 and 63.2% in F_2, as compared to 0% in controls) The effect was also evident at the intermediate dose level but only in the F_2 generation (18.4% as compared to 0% in the controls). However no nipples were recorded in the male pups of either generation. • NOAEL for effects on the parental animals, including the endocrine system was 240 ppm based on effects on liver and body weights. • NOAEL for reproductive adverse effects on parental animals is 240 ppm for males and 1200 ppm for females. • NOAEL for offspring is 240 ppm for males and 1200 ppm for females.
Yamasaki et al., 2009 **Supporting study** • 40 mated Crl:CD(SD)IGS female rats (F_0) (~12 weeks old) subdivided into 4 equally sized groups (10/group). • Culling at PND 4, to litter size of 8 aiming for 4 pups/sex when possible. • At weaning pups (F_1) in each group was randomly subdivided into 2 sub groups. A. Sacrificed at 10 weeks of age. Examined externally (nipples and effect on external sex organs), vaginal cytology from 8 weeks. Necropsied and examined internally for ectopic or atrophic testes; agenesis of the gubernaculums, epididymides and sex accessory glands; and epididymal granulomas. The following organs were weighed after necropsy: uterus, ovaries, testes, epididymides, ventral prostate, seminal vesicles with coagulation gland, levator ani and bulbocavernosus muscles, brain, liver, adrenals, kidneys, thyroids, and pituitary. B. 2 females and 2 males/dam were mated at 12 weeks to	• 0, 20, 100 or 500 mg/kg bw/day of DCHP (CAS No. 84-61-7, 99.9% purity) via oral gavage between gestation day (GD) 6 and post natal day (PND) 20 • Vehicle: olive oil • Dose volume: 2 ml/kg	**Adult toxicity** • F_0: No effects on body weight. Dose-dependent increased liver weights (absolute and relative), being statistically significantly ($p<0.05$) higher at the intermediate and high dose level (+7 and +24 % as compared to controls). No information on weights of other organs. • F_0: Dyctosia in one high dose female that died on GD 23 before parturition was completed; otherwise no effect on reproductive performance. • F_1 (at necropsy week 10) o Decreased ($p<0.05$) ventral prostate weight at the low and high dose (-16% and -28% as compared to controls), but no dose dependency since the mid dose was less affected (-10%) than the low dose. o Decreased ($p<0.05$) relative weight (-12% as compared to controls) of the levator ani/bulbocavernosus muscle and slight histological changes, including decreased testicular germ cells and degenerated renal proximal tubules (incidence data not shown) in the high dose group. o No statistically significant effects on body weight, relative weights of the brain, pituitary, thyroid, adrenal, kidney, liver, ovary and uterus. • No effect on reproductive performance of F_1-generation at 12 week of age (Sub-group B). **Developmental effects** • F_1:Minimal (-2.2%) but statistically significantly decreased viability index on

assess reproductive performance and possible effects on early embryonic development (cesarean sections performed on gestation day 13). Adult males and females necropsied and same organs as in subgroup A was weighed. Non-GLP study		PND 4 in the high dose group. No effect on live birth index, sex ratio at PND 0, number of live pups on PND 4 or PND 21 or on weaning index on PND 21. • F_1: Significantly decreased male and female pup weight at PND 14 and/or PND 21 (detailed data not provided). • F_1 high dose male: o Hypospadias (combined with small testes) in 2 male pups, one sacrificed at 7 weeks due to poor condition. o ~2 days delayed ($p<0.05$) preputial separation in high dose males. No information provided for lower dose levels. o PND 4: Statistically significantly ($p<0.05$) decreased anogenital distance (absolute,-15%, as well as relative to the cubic root of the bodyweight, -13%). No information provided for lower dose levels. o PND 13: An increase in the numbers of pups/litter with areolas/nipple retention (2.7 as compared to 0 in the controls; $p<0.05$) as well as in the litter incidence of areolas/nipples retention (67.6% as compared to 0 in controls; $p<0.05$). No data provided for the lower dose groups • No effects on vaginal opening (examined from day 21 and onwards) or estrous cycling was observed in F_1 females.
Saillenfait et al., 2009a **Supporting study** • Oral (gavage), female SD rats • Main study o 24-25 females/dose level Study protocol resembled that of a Prenatal developmental toxicity study (OECD TG 414). In addition Anogenital distance was measured on GD 21. • Satellite study o 6-9 animals/dose level, dosing interval as main study, for examination of liver effects (Clin Path, enzyme activity and liver weights) on GD 21. Non-GLP study. (No information on how the offspring was randomized into the	• 0, 250, 500 or 750 mg/kg bw/day of DCHP (CAS No. 84-61-7, 99% purity) from GD 6 until GD 20 • Vehicle: olive oil • Dose volume 10 ml/kg	**Main study** ***Maternal body weights & clinical signs*** • There were no mortalities or adverse clinical findings. • Decreased body weight gain during the first 3 days of dosing (30 and 43% in the high and intermediate dose) and in the high dose animals also during late gestation (51% less during GD 18-21) as well as for the entire dosing period (22% less). High dose animals also had a decreased corrected body weight gain for the entire dosing period (50%) indicating clear (but not overt) maternal toxicity at the high dose level. ***Developmental effects*** • No effects on post-implantation loss or on number of dead fetuses or on sex ratio. • Fetal weights (male, females and combined) were decreased (~11%) at the

3 different survival groups)		high dose level • Decreased anogenital distance (absolute and relative to the cubic root of bodyweight) in male fetuses in all DCHP dose groups (absolute distance: -9, -12 and -17% in the low, intermediate and high dose groups, respectively, as compared to the controls; relative distance: -8 , -11, -14% in the low, intermediate and high dose groups, respectively). • Fetal pathology: Diaphragmatic hernia was seen in one control fetus. Three fetuses from three different litters were malformed at the high dose level. One fetus had omphalocele, another had diaphragmatic hernia and a third had a thoracic vertebra malformation. These findings were considered isolated and not related to DCHP treatment by the authors. **Satellite study - liver weights and limited Clinical Pathology** • Significantly increased relative liver weight (+17%; $p<0.01$) in intermediate and high dose (+28%; $p<0.01$) animals. • Dose dependent increased (+75, + 90, +108% as compared to the controls; $p<0.01$) activity of hepatic palmitoyl CoA oxidase (a peroxisomal enzyme marker) at all dose levels. Increase in ASAT, (+49%) and in ALAT (+116%; $p<0.01$) but no statistically significant effects on cholesterol or triglyceride levels, in the high dose group. No adverse finding at the histopathological examination of the liver.
Aydan Ahbab & Barlas 2013 **Supporting study** • Pregnant Wistar rats • After delivery all pups were allowed to grow with their dam for 1 month and then male pups were separated and housed 4/cage until they were killed on PND 20 (pre-pubertal), PND 32 (pubertal) or PND 90 (adult). Group size per age and dose level was 8-10 animals. There is no information on how offspring was randomized into the 3 different survival groups. • At necropsy the F_1 animals were weighed. Testis, epididymis, ventral prostate and seminal vesicle were weighed and processed for histopathological	• DCHP (CAS No. 84-61-7, purity 99%) was administered via gavage at 0, 20, 100 or 500 mg/kg bw/day to separate groups of pregnant dams from GD6 until GD 19. • Vehicle: corn oil Dosing volume 0.25 ml	• No information on maternal clinical signs, food consumption or maternal body weights during gestation or during lactation. No information on effects on littersize at birth or on pupsurvival or on birth weight or weight gain during lactation. No information on clinical signs, food consumption or weights in offspring during the study. Only bodyweight of offspring at termination is reported. No information on effects on anogenital distance. **Body weights (F_1) at termination of study** • ↓ body weight ($p<0.05$) only at the low dose of pre-pubertal stage rats. No effect at any dose levels at the pubertal or adult stages. **Weights of reproductive organ** ↓ absolute testis weight ($p<0.05$) at the low

examination except for left caput epididymis of adult animals which was processed for analysis of sperm head count and sperm morphology. • In connection with sacrifice, blood was collected from the heart samples for analysis of serum concentration of testosterone, estradiol, FSH, LH, inhibin B and MIS/AMH. • Non-GLP study		and high dose group (no dose dependency), and ↑relative testis weight ($p<0.05$) in intermediate dose group at the pre-pubertal stage. ↓ (absolute and relative, $p<0.05$) testis weight at the high dose level, and a ↓ relative weight at the intermediate dose levels (no dose-dependency) at the pubertal stage. No effects on testis weights at the adult stage. • ↓Absolute weight of the epididymis in the low dose group and no effects on the combined seminal and prostate weights were recorded at the pre-pubertal stage. At the pubertal stage no effect was seen on the weight of the epididymis or on the seminal vesicle but a ↑ ($p<0.05$) relative prostate weight was noted at the high dose level. At the adult stage the only effects observed were a ↑ ($p<0.05$) of the absolute weights of the epididymis and of the prostate at the high dose level. **Histopathological examination (no grading of severity was reported)** • Testis: dose dependent ↑ ($p<0.05$) incidence of tubular atrophy (nos. of affected animals: 0/10, 6/10, 5/10, 8/10; 0/10, 3/10, 8/10, 10/10 at the different dose levels of pre-pubertal and pubertal rats respectively) and of germinal cell debris (nos. of affected animals: 0/10, 3/10, 6/10, 9/10; 0/10, 3/10, 10/10/ 10/10 at the different dose levels of pre-pubertal and pubertal animals, respectively). ,In adult animals a much lower and not statistically significant incidence of tubular atrophy was recorded (0/10, 2/10, 0/10, 2/10 at the different dose levels). A dose dependent ↑ ($p<0.05$) incidence of sertoli cell vacuolization (0/10, 6/10, 4/10, 8/10 at the different dose levels) was recorded in adult animals. • Epididymis: dose dependent ↑incidence of presence of spermatogenic cells in lumen at all age stages (incidence in high dose group was 8/10, 10/10 and 8/10 at the pre-pubertal, pubertal and adult stage, respectively as compare to no observations in control animal at any stage of development ,). • Prostate: ↑incidence of atrophic tubules (0/10, 7/10, 9/10, 5/10; 0/10, 5/10, 10/10,10/10; 0/10; 5/10, 8/10, 10/10 at the different dose levels of pre-pubertal, pubertal and adult rats, respectively) and of intraepithelial neoplasia (incidence: 0/10, 7/10,)/10, 5/10; 0/10, 3/10, 10/10, 10/10; 0/10, 5/10, 8/10, 8/10 at the different dose levels of pre-pubertal, pubertal and adult rats, respectively)

		Sperm analysis (manual analysis) • No effects on epidiymal sperm counts. ↑ ($p<0.05$) percentage of abnormal sperms of approximately the same magnitude at all dose levels (10.9, 27.6, 23.0 and 27.4% in the control, low, intermediate and high dose group, respectively).

4.12.1 Effects on fertility

4.12.1.1 Non-human information

Available data are summarized in Table 13.

In the two generation reproductive toxicity study (Hoshino et al., 2005; old study design), diffuse atrophy of the seminiferous tubules (severe grade) was seen in 3 high dose (6 000 ppm, corresponding to 457 mg/kg bw/day) F_1 males, and focal atrophy (slight severity) was seen in 1, 0, 2 and 6 F_1 male in the control, low, intermediate and high dose groups, respectively. A decreased absolute weight (all dose levels; - 19%, $p<0.01$ at the lower dose level) and relative weight (high dose only; -19%, $p<0.05$) of the prostate was recorded in F_1 males only. Dose dependent decrease in the number of testicular homogenization resistant spermatids at the high (-24%) and intermediate dose (-15%; $p<0.05$) (LOAEL= 1200 ppm, corresponding to 90 mg/kg,) was recorded in the F_1 generation. No effects on epididymis sperm parameters (motility, sperm count and morphology) were seen in either F_0 or F_1 generation and no effects on reproductive endpoints such as fertility, mating and gestation and birth index were recorded in this study.

Decreased relative weight of the ventral prostate at the high (-28%, 500 mg/kg, oral gavage) and low dose (-16%, 20 mg/kg) was recorded in F_1 males necropsied at 10 weeks of age (after being exposed in utero and via the milk until weaning) in the study by Yamasaki (2009a). In addition, a decreased (-12%, $p<0.05$) relative weight of the levator ani/bulbocavernosus musle and slight histological changes (including decreased testicular germ cells, incidence data not shown) were also observed at the 500 mg/kg dose level of the F_1 animals.

Effects on the morphology of the testis (tubular atrophy, germinal cell debris, apoptopic cells, sertoli cell vacuolosation) and of the epididymidis (presence of spermatogenic cells in lumen) and prostate (increase in atrophic tubules and of prostatic intraepithelial neoplasia) were also recorded when male offspring were examined at prepubertal, pubertal and adult stages after having been exposed in utero (GD-GD19) in an oral gavage study to dose levels of 20, 100 or 500 mg/kg bw/day (Aydogan Ahbab and Barlas, 2013). This study did not report any effect on epididymal sperm head count but an increase ($p<0.05$) in the the percentage of abnormal epididymal sperms was recorded at all dose levels (10.9, 27.6, 23 and 27.4% in the control, low, intermediate and high dose group, respectively) in the adult animals.

Effects on the testis (bilateral tubular atrophy of 30-40% of the germinal cells) were also observed in 1 out of 5 animals, when juvenile male rats were given 2500 mg/kg bw/day for 7 days via oral gavage (Lake et al., 1982; see section 4.8 for more details). In addition, NICNAS report on DCHP (NICNAS 2008b) refers to a study by Grasso (1979) where rats administered DCHP at 4.2 g/kg via oral gavage for 21 days displayed testicular atrophy (no further information is provided in the NICNAS report). Taken together these findings indicate that DCHP is toxic to the male reproductive organs and that animals exposed in utero/during weaning are more sensitive as compared to adult animals.

4.12.1.2 Human information

No data.

4.12.2 Developmental toxicity

4.12.2.1 Non-human information

Available data are summarized in Table 13.

In a dietary 2-generation reproductive toxicity study (Hoshino et al., 2005) a reduced (~8-9 %) relative (as well as absolute) anogenital distance (LOAEL: F_1 = 6000 ppm, $p<0.05$; F_2 = 1200 ppm, $p<0.01$) was recorded in male pups only. In addition, an increase in the percentage of litters with male pups having areola mammae (which normally only should be present in female pups and in the present study there was no male control pup that displayed an areola mammae) was recorded. The effects were more pronounced in the F_2 generation, where 63% ($p<0.01$) of the F_2 litters as compared to 16.1 % ($p<0.01$) of the F_1 litters at the 6000 ppm dose level were affected, and an increased incidence (18.4%, not statistically significant) was also recorded at 1200 ppm dose level in the F_2 generation. There was no effect on birth index, number of offspring born alive, on the birth sex ratio, on the pup viability index, on the physical development or on sexual maturation recorded in the study. Pup body weight was reduced 4 – 12% (during the entire period of lactation for both male ($p<0.05$ on PND 0 and 4 and $p<0.01$ at the other days of recording) and female pups ($p<0.05$ on PND 0 and $p< 0.01$ on the other days of recording) in the F_1 generation at the 6000 ppm dose level. The pup weight of the F_2 generation was less affected; a decreased pup body weight ($p< 0.01$) was only recorded on PND 21 at the 6000 ppm dose level. The recorded developmental toxicity in the Hoshino et al. study (2005) was observed in absence of marked maternal toxicity. Decreased maternal body weight of approximately the same magnitude (F_0: ~-10%, $p<0.01$, F_1: ~ 8-9%; as judged from the graphical presentation of the data) was observed from premating throughout the period of lactation at the 6000 ppm dose level. Effects on parental body weight (of lower magnitude as compared to the 6000 ppm level) were also observed on occasional days during gestation (GD 7 and 14, $p<0.05$ and 0.01, respectively) and during the lactational period (lactation days 0, 4, 7; $p<0.05$ or 0.01 with no time trend) at the 1200 ppm dose level in the F_0 generation. No other signs of maternal toxicity as mortality, adverse clinical observation or effects on mating index, gestation index, gestational length, were reported in the study.

Signs of developmental toxicity was also observed in the oral gavage study (dose levels: 0, 250, 500 and 750 mg/kg/day) by Saillenfelt et al. (2009a). The study protocol resembled that of an oral prenatal developmental toxicity study (OECD TG414) and anogenital distance was measured on GD 21. There was no effect on fetal viability. A decreased fetal weight (~ -10%, for both female and male) was recorded in the high dose group only. A decreased anogenital distance was observed in males pups at all dose levels (relative distance; $p<0.01$; -8, -11, -14% in the low, intermediate and high dose groups, respectively). No effects were recorded for the anogenotal distance in female pups. No other effect on fetal morphology was recorded at fetal examination. Clear but no marked maternal toxicity was recorded in the study. High dose animals displayed a 50% decreased corrected body weight gain, whereas only a transient decreased body weight gain was recorded at start of dosing in the intermediate dose group. Although an increased liver weight (high and intermediate dose levels) and an increase of ALAT (all dose levels) and hepatic palmiotyl CoA activity (high dose group) was recorded no adverse finding was observed at the histopathological examination of the liver. No mortalities or adverse clinical findings were recorded in the study.

A prolonged preputial separation (~2 days, $p<0.05$) and an effect on the anogenital distance (relative distance: -13%, $p<0.05$) and on areola mammae/nipple retention (2.7 as comp to 0 pups/litter, affecting 68% of the litters; $p<0.05$)) was also reported for male pups at the 500 mg/kg dose level in the study by Yamasaki and coworkers (2009). In this study, mated rats were dosed via gavage (GD 6 – PND 20) at 0, 20, 100 or 500 mg/kg bw/day. Unfortunately the reporting of this study is not optimal since no data is provided regarding these endpoints for the lower dose groups. Hence it is not clear if these findings were only observed at the 500 mg/kg dose level. In the study, no effect on live birth index, sex ratio or on pup survival up to weaning was reported, although a minimal (-2.2) but statistically significant decreased viability index was recorded on PND 4 for the high dose group. The paper states that high dose pups displayed a significant decreased male and female pup weight on PND 14 and PND 21 but no further details were provided in the text. In addition, hypospadias (in association with small testis) was observed in 2 males originating from dams that had been exposed GD 6 – PND 20 via oral gavage at 500 mg/kg. There were no effects on maternal weights (although maternal body weight gain was not reported) and the only sign of possible adverse effects was a dose dependent increase in liver weights (absolute as well as relative). However, histopathological examination was not performed. These findings indicate that DCHP causes developmental toxicity in males in absence of marked maternal toxicity, and based on the result from the Hoshino study (2005) the most sensitive endpoints are presence of areola mammae and decreased relative anogenital distance. In addition, the F_2 generation seems to be more sensitive as compared to the F_1 generation.

4.12.2.2 Human information

No data.

4.12.3 Other relevant information

4.12.3.1 Mode of action/Endocrine disrupting property

Table 14: Summary table of relevant Mode of action studies.

Method & Source	Dose levels	Results	Estrogenic/ androgenic activity
In vivo Crj:CD (SD) rats, females. Uterotrophic assay (intact animals) **Yamasaki et al., 2002**	Subcutaneous injection of 2, 20 or 200 mg/kg bw/day of DCHP (CAS No. 84-61-7, 100% purity) from PND 20 to 22. Vehicle: olive oil Dose volume: 4 ml/kg	No effects on uterine weight whereas an increased weight was recorded in Ethynyl estradiol treated animals (No information why higher dose levels were not tested)	No estrogenic activity
In vivo SD rats, females The estrogenic activity as assessed by effects on the expression of the CABP-9k gene in the uterus from immature rats of butyl benzyl phthalate (BBP), Dicyclo hexyl phthalate (DCHP), diethyl phthalate	Groups of five animals were each given an oral dose of either OP, BPA (98% purity), BBP,DCHP (CAS No. and purity not specified), DEP (99.5%), DEHP (99%) or DBP (99%) at the dose of 600 mg/kg	No significant change in the expression levels of *CaBP-9k* mRNA were recorded for BBP, DCHP, DEP, DEHP, or DBP, i.e. the compounds did not display estrogenic activity in this test system In contrast, 17α-estradiol caused a	No estrogenic activity

(DEP), 2-ethyl hexyl phthalate (DEHP), di-*n*-butyl phthalate (DBP), octylphenol (OP) and bisphenol A (BPA) was determined. 17α-estradiol was used as a positive control and Vehicle (corn oil) treated animals were used as negative controls. Expression of the *Calbindin-D_{9k}*(*CaBP-9k*) gene in the rat uterus is highly regulated by 17α-estradiol and the expression is known to fluctuate during the estrous cycle when the serum 17α-estradiol level is also fluctuating. It was suggested that the expression of CaBP-9k mRNA and protein might be a novel biomarker for estrogenic compounds in immature animals. **Hong et al., 2005**	bw/day on days 14, 15 and 16 after birth and euthanized on day 17. Positive controls received single dose of 17α-estradiol (5 µg/kg BW)	significantly increased expression (both at the mRNA and protein level). The estrogenic compounds OP and BPA also increased the expression of CABP-9k.	
In vitro A series of ring and alkyl-chain isomers of dialkyl phthalates $C_6H_4(COOC_nH_m)_2$ were examined for their ability to displace [3H]17 β -estradiol in the recombinant human estrogen receptor expressed on Sf9 vaculovirus. Exposure time 1 hr (single) **Nakai et al., 1999**	DCHP (CAS No. and purity not specified)	DCHP displaced 17ß-estradiol showing a biphasic binding curve with IC_{50} of 1µM for high binding site and >2,000 µM for low binding site. The binding was three orders of magnitude weaker than 17β-oestradiol.	
In vitro A number of alkyl phthalates were examined for their ability to displace [3H]17β-estradiol from the recombinant human estrogen receptor, which was expressed on Sf9 cells using the vaculovirus expression system. Exposure: 1 hour (single) **Asai et al., 2000** (as cited in the REACH registration, 2013)	**Dicyclohexyl phthalate**	Both dicyclohexyl phthalate and dicyclohexyl 4-hydroxyphthalate showed biphasic binding curves (indicating 2 binding sites of high and low affinity). Hydroxy-derivative had increased binding affinity at high affinity site vs. non-hydroxy form (no difference at low affinity site). Investigators commented that benzene ring mimics the steroid-A ring of 17β-estradiol, but still extremely weak in comparison.	Estrogenic activity
In vitro Yeast two-hybrid assay for estrogenic activity (ER α)	DCHP (no CAS No. and purity not specified)	Dicyclohexyl phthalate was negative in this yeast two-hybrid assay (REC10 > 3 x 10^{-4} M; REC10 is the concentration of the test chemical showing 10% of the	No estrogenic activity

Nishihara et al., 2000		agonist activity of 10^{-7} M E2, which is the optimum concentration for E2. When the activity of the test substance was higher than REC10 within the concentration tested the chemical was judged as positive).	
In vitro Estrogenic activities of phthalate di and monoesters were studied by using the MCF-7 cell proliferation assay. Anti-estrogenic activities were also examined by estimating the suppression of cell proliferation in the presence of 10^{-11}M 17β – estradiol. **Okubo et al., 2003**	DCHP (CAS No and purity not specified): 10^{-6} – 10^{-3} M MCHP 10^{-5} – 10^{-3} M.	Maximum cell proliferation (80% of that of $3x10^{-11}$ M 17ß-estradiol) by DCHP at $5x10^{-5}$ M, i.e. DCHP was $17x10^{5}$ times less potent as compared to 17ß-estradiol. DEHP and BBP stimulated cell proliferation only slightly at conc> 10^{-3} M**.** MCHP had no proliferative effect Mono-n-pentyl phthalate (MPP), monocyclohexyl phthalate **(MCHP),** monobenzyl phthalate (MBZP), Monoisopropyl phthalate (MIPrP) and BBP were suggested to have anti-estrogenic activities at conc higher than 10^{-4}M.	DCHP but not MCHP: estrogenic activity, and MCHP possibly anti-estrogenic activity
In vitro MCF-7 cell culture and cell proliferation assay in vitro (E-screen). To determine whether phthalates mimic an estrogenic effect in cell proliferation, the potential ability of phthalates to promote anchorage-dependent growth of MCF-7 cells was determined. Treatment (10^{-9} M) with 17β estradiol (9-fold) and 17α estradiol (9-fold increase of proliferation) was used as positive controls. Exposure time: 6 days **Hong et al., 2005**	DCHP (Sigma Aldrich, but CAS No. and purity not specified) BBP (98%), DEP (99.5%), DEHP (99%) or DBP (99%) 10^{-6}, 10^{-5}, and 10^{-4} M	DCHP caused an increased cell proliferation at 10^{-5} M (5-fold increase) and 10^{-4} M (8-fold) as compared to vehicle control. In comparison at 10^{-4} M, butyl benzyl phthalate, 2-ethyl hexyl phthalate and di-*n*-butyl phthalte caused a 6-fold, 6-fold and 7-fold increase in proliferation). In comparision,17β-estradiol caused a 9-fold increase in cell proliferation at 10^{-9}M. In this assay DCHP displayed oestrogenic activity	Estrogenic activity

In vitro Human and rat testis microsomes were used to investigate the inhibitory potencies on 3β-hydroxysteriod dehydrogenase (3β-HSD) and 17β- hydroxysteroid dehydrogenase type 3 (17β- HSD3) activities of 14 different phthalates with various carbon numbers in the ethanol moiety. The two enzymes are involved in the biosynthesis of androgens in Leydig cells. Exposure time: 90 minutes **Yuan et al., 2012**	Up to 1 mM of the test substance was added (but no confirmation of concentration and stability of compound was reported, neither were CAS No. and purity specified).	• Phthalates with 1-2 or 7-8 carbon atoms in the ethanol moieties had no effects on both enzymes activities even at 1mM. • The results demonstrated that the half-maximal inhibitory concentrations (IC(50)s) of dipropyl (DPrP), dibutyl (DBP), dipentyl (DPP), bis(2-butoxyethyl) (BBOP) and dicyclohexyl (DCHP) phthalate were 123.0, 24.1, 25.5, 50.3 and 25.5μM for human 3β-HSD activity, and 62.7, 30.3, 33.8, 82.6 and 24.7μM for rat 3β-HSD activity, respectively. However, only BBOP and DCHP potently inhibited human (IC(50)s, 23.3 and 8.2μM) and rat (IC(50)s, 30.24 and 9.1μM) 17β-HSD3 activity • The mode of action of DCHP on 3β-HSD and 17β-HSD3 activity was competitive with the substrate pregnenolone and androstenodione, respectively.	Effect on synthesis of androgens in vitro at μM concen-trations.
In vitro The affinity of 22 ortho-phthalates to human estrogen and androgen receptors was examined in reporter gene assays. Chinese Hamster ovary cell line (CHO-K1) transfected with expression vectors for human ERα, ERß, and AR. **Takeuchi et al., 2005**	DCHP (purity >99% but no CAS No. provided): 10^{-7} – 10^{-5} M	• REC_{20} (relative effective con.c showing 20% of the agonistic activity of 10^{-9} M 17ß-estradiol) via ERα was $2.8x10^{-6}$ M for DCHP. o The relative potencies of their estrogenic activities descended in the order BBeP > DCHP > DiHP > DiBP, DBP, DPeP,DHP > DEHP, DiHepP. • RIC_{20} (relative inhibitory conc. showing 20% of the antagonistic activity of 10^{-10} M 17ß-estradiol) via ERß was $2.5x10^{-6}$ M for DCHP, and DCHP exhibited the most potent inhibitory effects on ERβ among the studied phthalates. • None of the examined phthalates showed androgenic activity. • RIC_{20} (relative inhibitory conc showing 20% of the antagonistic activity of 10^{-10} M 5α-dihydrotestosterone) via AR was $3.8x10^{-6}$ M for DCHP. Eight other phthalates (DAP, DiBP, DBP, BBeP, DpeP, DiHP, DHP and DiHepP) also	Estrogenic, antiestrogenic and antiandrognic activity

		possessed antiandrogenic activity	
In vitro A reporter gene assay for rat ERα – mediated transcriptional activation. EC50 values were calculated. In addition the PC50 and PC10 values defined as the test chemical concentrations estimated to show 50 and 10%, respectively, of the transcriptional activity of positive control wells (1 nM of 17β--estradiol)) were also calculated Vehicle: DMSO Exposure: 24 hours (single) **Yamasaki et al., 2002**	DCHP (CAS No. 84-61-7, 100% purity) 10 pM to 10µM	No EC50, PC0 or PC10 value could be calculated for DCHP. DCHP was negative in the reporter assay	No estrogenic activiy

DCHP gave negative estrogenic results in a couple of *in vivo* studies where it had no effect on *CaBP-9k* mRNA and protein levels in the uterus (Hong et al., 2005) and was negative (did not increase uterine weight) in a uterotrophic assay (Yamasaki et al., 2002). DCHP gave mixed results in estrogenic *in vitro* assays. It induced MCF7 cell proliferation (Hong et al., 2005 and Okubo et al., 2003) whereas its metabolite inhibited the 17ß-estradiol induced MCF7 cell proliferation (Okubo et al., 2003). In a study by Nakai et al. (1999) it showed a characteristic biphasic binding curve with different affinities for the high and low binding sites on the estrogen receptor. Nishihara et al. (2000) found DCHP to be negative in a yeast two-hybrid assay with ERα, whereas in another assay it was agonistic to ERα and antagonistic to ERß (Takeuchi et al., 2005). In vitro mechanistic studies show that DCHP is not an androgen receptor agonist but behaves as an antagonist to 5α-DHT at the androgen receptor (Takeuchi et al., 2005). It also inhibits the enzymes involved in biosynthesis of androgen in testes (Yuan et al., 2012).

4.12.4 Summary and discussion of reproductive toxicity

Effects on fertility

No clear effect on fertility as assessed by effect on reproductive outcome on a group level was reported in the dietary two-generation reproductive toxicity study (Hoshino et al 2005) or in the study by Yamasaki and coworkers (2009a) where effects on fertility and overall development were examined in offspring that had been exposed in utero throughout the gestation and via the milk until weaning.

However, in both studies toxicity to the reproductive organs was consistently reported. Hoshino et al. reported the occurrence of focal (LOAEL 1200 ppm 90 mg/kg bw/day) and diffuse (LOAEL 6000 ppm 457 mg/kg bw/day) atrophy of the seminiferous tubules and a significantly reduced testicular spermatid head count (LOAEL 1200 ppm 90 mg/kg bw/day) in the F_1 males only. Necropsy data revealed soft and/or small size testis in 3 F_1male pups at 6000 ppm. No effects on the motility, morphology or number of sperm in epididymis were recorded in either generation. Although not so well reported, the studies by Yamasaki (2009) and Aydogan Ahbab and Barlas (2013) support the testicular histopathological findings reported by Hoshino (2005).

Taken together these studies demonstrate that DCHP has adverse effects on male reproductive organs and that animals exposed in utero/during weaning are more sensitive as compared to adult animals. Based on poor studies, it is known that DCHP can induce testis toxicity also in adult and juvenile animals but only at dose levels much higher than those used in the above mentioned studies. Effect on the testis (bilateral tubular atrophy of 30-40% of the germinal cells) was observed in 1 out of 5 animals, when juvenile male rats were given 2500 mg/kg bw/day for 7 days via oral gavage (Lake et al., 1982), and a NICNAS report on DCHP (NICNAS 2008b) refers to a study by Grasso (1979) where rats administered DCHP at 4.2 g/kg bw/day via oral gavage for 21 days displayed testicular atrophy (no further information is provided in the NICNAS report). This age-dependent sensitivity for testis toxicity is similar to what has reported for transitional phthalates (reviewed in NAS 2008). Other relevant effects were reduced relative weight of two androgen-dependent accessory sex tissues – the ventral prostate (effects observed in F_1 in both studies) and the levator ani/bulbocavernosus muscle (F_1, only examined in the study by Yamasaki).

Developmental toxicity

DCHP causes developmental toxicity. The toxicity was revealed as decreased anogenital distance (absolute as well as relative to the cubic root of the fetal weight) and an increase in the incidence of areola mammae or areola mammae/nipple retention. The effects were observed in multiple studies (Hoshino et al., 2005; Yamasaki et al., 2009, Saillenfait et al., 2009a) and in absence of marked maternal toxicity. In addition, hypospadias (in association with small testis) was observed in the study by Yamasaki (only study where this endpoint was examined) and effects on pup weights were also recorded although these could partly be explained by effects on maternal body weights. No effects on pup or fetal viability were recorded and the fetal examination in the study by Saillenfait did not reveal any other effects than the effects on anogenital distance in the male pups. In line with this The US Consumer Product Safety Comission's toxicity review of dicyclohexyl phthalate (CPSC, 2011, page 25) also concluded that "*there was 'sufficient animal evidence' for the designation of DCHP as a 'developmental toxicant'*.

The in vitro mechanistic studies presented in the current report show that DCHP behaves as an antagonist to 5α-DHT at androgen receptors and also inhibits the enzymes involved in the biosynthesis of androgen. Therefore, an antiandrogenic mode of action can be presumed for the adverse effects on the development of the male pups. This presumption is further supported by the fact that the length of the perineum (anogenital distance) and the apoptosis of the nipple anlagen are all under control of dihydrotestosterone (reviewed in NAS 2008). The observed effects on male anogenital distance, areola mammae/nipple retention and hypospadias are also observed after in utero exposure to members of the transitional phthalate group (see Table 15). All these transitional phthalates have been harmonized classified as developmental toxicants in Repro 1B (in addition they all also have been classified in category 1 regarding effects on fertility as well) and mechanistic wise they have all been shown to inhibit the production of testosterone in the fetal testis.

Table 15: Effects on anogenital distance, nipple retention, hypospadias and fetal testis testosterone production after in utero exposure to some transitional phthalates*, and to DIBP or DCHP.

Substance	Areola mammae/Nipple retention	Decreased AGD in male pups	Hypospadias	Harmonized Repr. 1B (H360D) classification	Effects on fetal testis testosterone production (Data from Howdeshell et al., 2008)	Reference
DIBP**	Yes	Yes	Yes	Yes	Yes	Saillenfait et al., 2008
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***		**Not examined**	**Hoshino et al., 2005** ***Yamasaki et al., 2009**
DPP	No info available	No info available	No info available	Yes	Yes	
DnHP	Yes	Yes	Yes	Yes (Propososal supported by RAC)	Yes (2013 paper)	Saillenfait et al., 2009b and 2013
DEHP	Yes	Yes	Yes	Yes (proposal supported by RAC)	Yes	Fabjan et al., 2006 (review)

*Transitional phthalates are defined as those phthalate esters produced from alcohols with straight-chain carbons backbones of C4-6 (ACC Phthalate Ester Panel HPV testing group, 2006, ECHA 2012). DCHP is an ortho-phthalate ester with a side chain ring structure (cyclohexyl). It does not possess simple straight or branched carbon chains as many other phthalates, and strictly DCHP does not belong to the group transitional phthalates although numerically the carbon side chains are within the range C4-6. **DIBP=Diisobutyl phthalate (3C alkyl), DBP=Di-n-butyl phthalate (4C alkyl), BBP= butylbenzyl phthalate, (C4/C5 alkyl) ;DPP=Di-n-pentyl phthalate (5C alkyl), DnHP= Di-n-hexyl phthalate (6C alkyl) DEHP = Diethylhexylphthalate (C6 alkyl).

The similarity between the effects of DCHP and those of transitional phthalates has previously been highlighted. In the hazard assessment of DCHP by the Australian government under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2008b, page 13), it was concluded that *"Although data for DCHP are limited, the fertility and developmental effects observed are similar to those phthalates with sidechain backbone of 4-6 carbon atoms in length (C4-C6) (NICNAS 2008a). These C4-6 phthalates previously referred to as ´transitional´ phthalates (Phthalate Esters Panel HPV Testing Group, 2001) have also been associated with male reproductive (seminiferous tubule atrophy) and development (decreased anogenital distance and retention of nipples) effects. Overall DCHP has a similar reproductive profile to the 'transitional' (C4-6) phthalates for which reproductive and developmental effects are recognised"*

4.12.5 Comparison with criteria

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

The CLP criteria for classification in Repr. 1B are as follows: *"The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."* The existing experimental data on reproduction and development available for DCHP are considered reliable. Effects on the anogenital distance as well as on the occurrence of mammaae/nipple retention in male pups were recorded in multiple studies and the findings were considered to be specific and not secondary non-specific consequences. Effect on male reproductive organs was also recorded (testicular atrophy, reduced testicular spermatid head count and decreased weight of the prostate and of the levator ani/bulbocavernosus) and these findings are considered to be specific and not secondary non-specific consequences. Mechanistic studies indicate an antiandrogenic mode of action. Overall the observed findings justifies that DCHP is classified in Repr. 1B (H360FD).

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.

4.12.6 Conclusions on classification and labelling

The available data justify classification of DCHP in Repro 1B (H360FD).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposal for classification for reproductive toxicity (for both developmental toxicity and sexual function and fertility) was mainly based on one GLP and OECD TG 416 compliant 2-generation study (Hoshino *et al*., 2005; described as 'old study design') as well as a number of non-GLP compliant, supporting studies published in the scientific literature. All these studies were conducted in rats which were exposed to the test material (DCHP) via the oral route.

No clear effects on sexual function and fertility were reported in the F_0 or F_1 generation by Hoshino *et al.* (2005) or in the F_1 generation in a supporting study (Yamasaki *et al*., 2009). However, toxicity to the male reproductive organs was observed in both studies.

Another supporting study (Aydogan *et al*., 2013) revealed, following *in utero* exposure, dose-dependent and significant effects on the morphology of the epididymides and prostate in male offspring at prepubertal, pubertal and adult stages. The DS noted that other potentially relevant information (such as clinical signs, litter size, pup survival, etc.) was not included in the study report.

The DS concluded that taken together these findings indicate that DCHP is toxic to the male reproductive organs and that animals exposed *in utero*/during weaning are more sensitive compared to adult animals. The DS proposed to classify DCHP for its effects on

sexual function and fertility (Repr. 1B, H360F).

The most pronounced developmental effects were decreased absolute and relative (to the cube root of the body weight) anogenital distances (AGD) and increased areolae mammae/nipple retention, but a malformation (hypospadias) was also noted. Although some maternal toxicity was reported in some of the studies, all these findings appeared to be observed in the absence of marked maternal toxicity. In addition, the DS suggested that the F_2 generation may be more sensitive to these effects than the F_1 generation. The DS proposed to classify DCHP for developmental toxicity (Repr. 1B, H360D).

The DS noted that effects on male AGD, areola mammae/ nipple retention and hypospadias were also observed following *in utero* exposure to a number of other phthalates (transitional phthalates; see Table 15 of the CLH report) which have harmonised classifications as Repr. 1B (H360D) and which have been shown to inhibit the production of testosterone in the fetal testis.

Overall, based on the data presented in the CLH report, the DS proposed to classify DCHP as Repr. 1B for both development and sexual function/fertility (H360FD) based on the adverse effects on development and on reproductive organs.

Comments received during public consultation

Comments on this hazard class were received from industry, disagreeing with the proposed classification, and from 6 member states, 3 of which agreed with the proposed classification.

Reservations on the proposed classification were expressed by the other 3 MSs. One MS suggested that the data only supported classification as Repr. 2, on the grounds that the CLH report should have provided a more detailed comparison of the findings (such as AGD) with any concurrent maternal and general toxicity as well as with other phthalates with existing harmonised classifications. The DS responded that the relative AGD (normalised to the cube root of the body weight) took into account effects which were to due to changes in pup body weight (and secondary to effects on maternal weight gain). The DS also noted that since the observed reduction in relative AGD was > 5% in three different studies, this should be regarded as a clear adverse effect. The DS also agreed that marked tubular atrophy observed in a single animal in Lake *et al.* (1982) following exposure to a high dose of DCHP for 7 days did not warrant classification on its own but showed that atrophy can be induced in rats not exposed during their full life cycle.

Another MS commented on the quality of the non-GLP studies and noted that the effects seen for both fertility and development were not sufficiently severe for the classification proposed. The DS replied that, considering the reproductive capacity of rats, it was not surprising that there were no reductions in the number of pregnant dams in Hoshino *et al.* (2005). As further information supporting the mode of action, the DS summarised in their response a recent paper (Furr *et al.*, 2014), which showed that testosterone production (measured *ex vivo*) was significantly reduced in foetuses of rats given DCHP (or other phthalates) by oral gavage (doses not stated in the response) from GD 14 to GD 18 and necropsied on GD 18. The DS argued that considering the overlap of the observed effects with those of other phthalates which affected testosterone production and are currently classified in Category 1B for developmental toxicity, the proposal for classification of DCHP was justified.

Regarding a comment from industry which suggested classification as Repr. 2 based on negative results from a 1968 4-generation study, the DS responded that the information available on that study was too minimal for it to be taken into consideration.

One MS suggested that the effects on the male reproductive system should be used to

classify for developmental toxicity rather than sexual function and fertility, or if so, only in Category 2 with an SCL above the GCL given the low potency based on the repeated dose toxicity study in adult animals. The DS responded that although the findings could be interpreted either way based on the criteria in the CLP Regulation, in this case they could be considered as an effect on fertility, because "although the criteria partly imply that fertility is an effect observed in adult animals or associated with timing of becoming adult, they do not specify that fertility effects recognized at an adult stage must be associated with exposure during an adult stage in order to fulfill the criteria for classification for effects on fertility." The DS also suggested that as an alternative, classification as H360 (without specifying the differentiation) could be considered. The DS also agreed that if the atrophy of the seminiferous tubuli (in the F_1 generation) would be considered as developmental toxicity then the remaining effects together with the well known fact that other phthalates do cause testis toxicity were better described as "some evidence" for effects on sexual function and fertility on this differentiation (i.e. Cat. 2).

In response to another comment from an MS concerning the setting of SCLs, the DS noted that the lowest ED_{10} value (based on reduced AGD and nipple retention in F_2 male pups) was between 20.95 and 107 mg/kg bw/day. Since these values are within the range 4 mg/kg bw/day < ED_{10} < 400 mg/kg bw/day and therefore fall within the limits for a medium potency SCL, an SCL of 0.3% should be applied for developmental toxicity, which is equal to the GCL for a Category 1 reproductive toxicant.

Assessment and comparison with the classification criteria

Effects on Development

A 2-generation reproductive toxicity study in rats by oral exposure performed according to OECD TG 416 and GLP was included in the CLH dossier by the DS (Hoshino *et al*., 2005) together with three non-GLP/OECD TG compliant supporting studies, also in rats and by oral exposure (Yamasaki *et al*., 2009; Saillenfait *et al*., 2009a and Aydogan *et al*., 2013). It was evident from these studies that DCHP induced developmental toxicity, reported as reduced relative AGD, the presence of areola mammae in male pups as well as prolonged preputial separation in the absence of marked maternal toxicity. Furthermore, the study by Aydogan *et al*. (2013) reported adverse effects on the male reproductive organs following *in utero* exposure to DCHP.

In the 2-generation study (Hoshino *et al*., 2005), a reduced relative AGD (8-9%) in the HD (6000 ppm) male offspring was reported. Furthermore, an increase in the percentage of litters with male pups having areola mammae was also reported at the HD. The effect was statistically significant and more pronounced in the F_2 generation with 63% of the F_2 litters having areola mammae compared to 16% in the F_1 litters. An increase (18.4%) was also reported at the MD (1200 ppm) in the F2 generation, however this effect was not statistically significant. Areola mammae are normally only present in female pups, and in the study no areola mammae were reported in the male control pups. However, detailed examination revealed no female-type nipples and only areolae were observed. The effects reported in male pups on AGD as well as areola mammae were present in the absence of marked maternal toxicity. The maternal toxicity reported was a decreased maternal body weight of around 10% in the F_0 and F_1 generations.

An effect on AGD in male pups was also reported in the supporting developmental toxicity study using a study protocol resembling OECD TG 414 (Saillenfelt *et al*., 2009a). In this study, the relative AGD was statistically significantly and dose-dependently reduced in all dose groups by 8%, 11% and 14% at 250, 500 and 750 mg/kg bw/day, respectively. In this study a clear, but not marked, maternal toxicity was reported in the high dose females with a reduced corrected body weight gain of 50%.

In another supporting developmental toxicity study with exposure from GD 6 to PND 20 (0, 20, 100 and 500 mg DCHP/kg bw/day), effects on AGD, areola/ nipple retention as

well as prolonged preputial separation and hypospadias were reported (Yamasaki *et al*., 2009). However, this study was poorly reported. Data were only provided for the high dose group, therefore no information is available on whether these effects were observed in lower dose groups. Effects reported were a statistically significant reduction in relative AGD (13%), an increase in the number of pups/litter with areola/nipple retention (2.7% compared to 0 in controls) affecting 68% of the litters, a prolonged preputial separation by 2 days and hypospadias in 2 offspring in association with small testes (where one of them was sacrified at 7 weeks of age due to poor general condition). These effects were reported in the absence of marked maternal toxicity.

In the supporting study by Aydogan *et al*. (2013), male offspring were examined at prepubertal, pubertal and adult stages after exposure *in utero* during GD 6 to GD 19 to dose levels of 20, 100 or 500 mg/kg bw/day. **In the testis**, a statistically significant dose-depended increase in tubular atrophy and germinal cell debris was reported in prepubertal and pubertal rats. These effects were not observed at the adult stage. However, in adults, a statistically significant increase in Sertoli cell vacuolisation was reported in all dose groups as well as attached seminiferous tubules in all exposed adult rats in the three dose groups. **In the epididymis**, a statistically significant and dose-depended increase in the presence of spermatogenic cells in the lumen was reported at all age stages. Besides, tubules without sperm were observed at the adult stage (statistically significant from 100 mg/kg bw/day but not dose-dependent). Furthermore, a statistically significant and dose-dependent increase in adult animals with a decreased number of sperm in the lumen was reported. **In the prostate**, a dose-depended increase in atrophic tubules and in prostatic intraepithelial neoplasia were also reported at all age stages. No effect on epididymal sperm head count was reported but a statistically significant increase in the percentage of abnormal epididymal sperm was reported in all dose groups in adult rats.

In summary, relative AGD was significantly reduced in male offspring in a GLP-compliant 2-generation study in rats as well as in two supporting studies. Significantly increased incidences of male pups with areola mammae were also seen in all these studies, and the effect was in fact most pronounced in the F2 generation (where only *in utero* exposure is expected). Prolonged preputial separation and hypospadias were also reported in one of the supporting studies. Together with the effects on male reproductive organs following *in utero* exposure to DCHP, which provides clear evidence of a disturbance of the male reproductive tract during development, these findings provide clear evidence of adverse effects on the development of the offspring following parental exposure, at doses which did not result in marked maternal toxicity.

Effects on sexual function and fertility

One 2-generation reproductive toxicity study in rats by oral exposure performed according to OECD TG 416 and GLP (Hoshino *et al*., 2005) was included by the DS together with two non-GLP/OECD TG compliant supporting studies also in rats by oral exposure (Yamasaki *et al*., 2009 and Aydogan *et al*., 2013). It was evident from these studies that DCHP was toxic to the male reproductive organs and that animals exposed *in utero* and/or during weaning, *i.e.* the period of male reproductive organ development, were more sensitive than animals exposed as adults.

Regarding effects on mating and fertility following exposure to DCHP, no clear effects were reported in the 2-generation study in the F_0 and F_1 generations exposed to 240 (LD), 1200 (MD) and 6000 (HD) ppm (corresponding to a mean daily intake during the entire dosing period of 18, 90 and 457 mg/kg bw/day, respectively, for males and 21, 107 and 534 mg/kg bw/day, respectively, for females). The absence of an effect on fertility in the study by Hoshino *et al*. (2005) may be explained by the fact that the measurement of reduced fertility is considered as a insensitive endpoint in rats due to the rather high sperm reserve available in rats compared to humans. No effects on fertility

were also reported in the F_1 generation rats that were mated at 12 weeks of age, where parental exposure to DCHP was up to 500 mg/kg bw/day from GD 6 to PND 20 (Yamasaki *et al*., 2009).

However, adverse effects were reported on the male reproductive organs in the F_1 generation with no effects in the F_0 generation in the 2-generation study as well as in the supporting studies. These included in the 2-generation study a statistically significant decrease in relative **prostate** weight (-19% compared to control animals) in the F_1 generation HD group. Furthermore, diffuse atrophy of the **seminiferous tubules,** graded as severe, was reported in 3 HD males with a lack of sperm in the epididymal tubules. Moreover, focal atrophy with a slight severity was reported in 1, 0, 2 and 6 males in the control, LD, MD and HD groups, respectively and a statistically significant decrease in spermatid head counts were reported in F_1 males in the MD and HD groups.

An effect on **prostate** weight was also reported following *in utero* exposure to DCHP in the supporting study by Yamasaki *et al*. (2009). However, the effect was not dose-related (-16%, -10% and -28%, compared to controls at 20, 100 and 500 mg/kg bw/day, respectively) along with a statistically significant decrease in the relative levator ani/ bulbocavernosus muscle weight at 500 mg/kg bw/day (-12% compared to controls).

In the other supporting study (Aydogan *et al*., 2013) male offspring were examined at prepubertal, pubertal and adult stages after exposure *in utero* during GD 6 to GD 19 to dose levels of 20, 100 or 500 mg/kg bw/day DCHP. In this study, adverse effects were reported in the testis, epididymis and in the prostate in rats examined at the prepubertal, pubertal and adult stage. Since these effects were reported following *in utero* exposure to DCHP they can be considered supportive of developmental effects following exposure to DCHP. A more detailed description of the study is located in the developmental toxicity section.

Testis tubular atrophy was also reported when juvenile and adult rats were exposed to DCHP, but at very high doses, 2500 mg/kg bw/day for 7 days (Lake *et al*., 1982) and 4200 mg/kg bw/day for 21 days (Grasso, 1979). These data indicated that adult animals that were not exposed during the whole lifecycle were also sensitive to the induction of male reproductive organ toxicity, but at very high doses of DCHP.

The systemic toxicity findings reported in the 2-generation reproductive toxicity study were a slight decrease in body weight gain, increased liver and thyroid weight and liver and thyroid hypertrophy. In the supporting study by Yamasaki *et al*. (2009), only an increase in liver weight was reported, and in the supporting study by Aydogan *et al*. (2013), no decrease in final body weight was reported in adult rats up to the highest dose tested (500 mg/kg bw/day).

Mode of action: Several MoA studies were included by the DS. No estrogenic activity was reported in the *in vivo* studies. However, both positive and negative results for estrogenic activity were reported from *in vitro* studies. Several *in vitro* studies indicated that DCHP was not an androgen agonist, but other *in vitro* studies showed antagonist activity towards 5α-dihydrotestosterone (DHT) at androgen receptors and inhibiton of the enzymes involved in the biosynthesis of androgen in the testes. The DS also provided further information from a recent study (Furr *et al*., 2014) on the mode of action of DCHP in a response to comments received during public consultation. This study showed that foetal testosterone production was statistically significantly reduced when measured *ex vivo* in rat fetuses exposed to DCHP or other phthalates from GD14 to GD18 and necropsied on GD18.

RAC agrees with the DS that an antiandrogenic mode of action may explain the adverse effects on the development of the male pups. 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 (reviewed in NAS, 2008). The same effects as reported in male pups following exposure to DCHP were also reported following *in utero* exposure to transitional phthalates with a harmonised classification for development as Repr. 1B. An antiandrogenic mode of action was also suggested for these phthalates.

Summary

According to the CLP criteria classification as Repr. 1A is based on human data. No human data was available for DCHP regarding effects on sexual function and fertility or on development following exposure to DCHP, therefore classification of DCHP as Repr. 1A is not justified.

The experimental animal data for DCHP effects **on development** indicated a reduced AGD and an increased incidence of areola mammae in male pups. These effects were reported in three independent studies in the absence of marked maternal toxicity. In addition, prolonged preputial separation and hypospadias associated with small testis was described in one of the studies. The adverse effects observed in the Aydogan (2013) study in male reproductive organs, including testicular tubular atrophy and atrophic tubules in the prostate, occurred after *in utero* exposure and were considered as supportive evidence for developmental effects. Taken together, all these effects, which were observed following parental exposure in the absence of marked maternal toxicity, provide clear evidence of an adverse effect on development in the absence of other toxic effects. These effects have also been shown to occur following exposure to various transitional phthalates and are consistent with an anti-androgenic action of DCHP, which is considered relevant to humans. Classification as Repr. 1B is therefore warranted.

The experimental animal data available did not show a clear adverse effect of DCHP on **sexual function and fertility**. No effects on fertility parameters were reported in a 2-generation study performed according to OECD TG and GLP. Effects on the male reproductive organs such as testicular atrophy, Sertoli cell vacuolisation, epididymis without sperm and/or abnormal sperm in the tubules and a decreased weight of the prostate as well as atrophic prostate tubules, were observed following *in utero* exposure to DCHP.

Testis tubular atrophy was also reported when juvenile and adult rats were exposed to DCHP, but at very high doses and therefore were not considered relevant for classification for effects on sexual function and fertility.

There was no evidence of severe alteration of the female or male reproductive system, adverse effects on onset of puberty, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes or premature reproductive senescence.

RAC considers that the effects observed are due to *in utero* exposure and are supportive of developmental toxicity and that no classification is required for DCHP for effects on sexual function and fertility.

Conclusion

The adverse effects on development are considered to be specific effects resulting from exposure to DCHP. Mechanistic studies indicate an antiandrogenic mode of action that is considered relevant for humans.

In conclusion, for developmental effects RAC agrees with the DS proposal to classify DCHP for developmental toxicity as **Repr. 1B; H360D.**

4.13 Other effects

4.13.1 Neurotoxicity

No information available in the REACH registration.

4.13.2 Immunotoxicity

No information available in the REACH registration.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

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7 ANNEXES

None