SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Dicyclohexyl phthalate

EC No 201-545-9

CAS RN 84-61-7

Evaluating Member State(s): Sweden

Dated: 10 August 2023

Template Version 2.1
March 2015

Evaluating Member State Competent Authority

Swedish Chemicals Agency,
Box 2,
SE-172 13 Sundbyberg
Tel: +468-519 41 100
Fax: +468-735 76 98
Email: kemi@kemi.se
Website: http://www.kemi.se/en

Year of evaluation in CoRAP: 2017

Before concluding the substance evaluation a Decision to request further information was issued on: 19 December 2018.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Dicyclohexyl phthalate (called hereafter 'DCHP') was originally selected for substance evaluation in order to clarify concerns about:

- Endocrine disrupting properties – environment

No additional concerns were identified during the evaluation.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

DCHP has been identified as a substance of very high concern (Toxic for reproduction - article 57c and Endocrine disrupting properties - Article 57f human health) and was added to the candidate list for eventual inclusion in Annex XIV of REACH on 27 June 2018. DCHP was recommended for inclusion in Annex XIV in ECHAs 10th recommendation on 14 April 2021. A comprehensive compliance check was concluded without decision in October 2018. A targeted compliance check is ongoing (ECHA web page, latest update 18 August 2022).

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in Table 1 below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	X
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

DCHP has a harmonised classification as repro 1B and has been identified therefore as SVHC. It has also been identified as SVHC based on Reach article 57f due to its endocrine disrupting properties for human health. This means that DCHP in practice meets the criteria for ED cat.1 according to the amended CLP Regulation (Commission Delegated Regulation (EU) 2023/707 of 19 December 2022). The conclusion of this substance evaluation is that DCHP also meets the criteria for classification as endocrine disrupter for the environment cat.1. A harmonised classification for this endpoint is therefore warranted. France is preparing a group entry for C4-C6 phthalates including DCHP. The ED env endpoint will be assessed in this framework and France plans to submit the CLH report in 2024.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

DCHP was listed on the candidate list in 2018 and was recommended by ECHA for inclusion in Annex XIV in 2021. If DCHP is identified as an endocrine disruptor (ED) for the environment and consequently listed on the candidate list also for this property, this may have an impact on the risk management of DCHP. Assessment of risk for the environment would be added to the scope for authorisation. This would have the potential to significantly reduce the emissions to the environment as the applicants have to demonstrate that also the risk for the environment from the use of the substance can be adequately controlled. Furthermore, the authorisation requirement would be extended to use of the substance in cosmetics and food contact materials.

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable, see section 4.

5.2. Other actions

Not applicable, see section 4.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Harmonised classification proposal	2024	France*
SVHC dossier	TBD	TBD

* France is preparing a group entry for C4-C6 phthalates including DCHP. The ED env endpoint will be assessed in this framework and France plan to submit the CLH report in 2024.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

DCHP was originally selected for substance evaluation in order to clarify concerns about:

- Endocrine disrupting properties – environment.

No additional concerns were identified during the evaluation.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disrupting properties for the environment	Concern confirmed: DCHP is concluded to have endocrine disrupting properties for the environment

7.2. Procedure

Sweden and Denmark prepared an Annex XV dossier suggesting that DCHP should be included on the Candidate List of substances of very high concern for Authorisation due to its toxicity to reproduction (art.57 c) and ED-properties for both HH and ENV (art.57 f). The Member State Committee failed to reach an unanimous decision and the case was therefore referred to the commission. In the Commission's decision, DCHP was identified as an SVHC based on toxicity to reproduction and endocrine disruption to human health. The proposal for endocrine disruption for the environment was withdrawn in order to further elaborate on the justification provided in the documentation and to consider requesting further ecotoxicological data during the substance evaluation process. DCHP was put on CoRAP for evaluation 2017.

This substance evaluation is targeted to the environmental ED properties of DCHP. The registration dossier did not contain any relevant information regarding ED effects on aquatic organisms. The evaluation is therefore based on the information available in the Annex XV-dossier for DCHP submitted by Sweden in cooperation with Denmark (February 2016). In addition, relevant information found in open sources has been evaluated and used in the assessment. Two references, LV (2019) and Ahbab *et* al (2017) were retrieved in the literature search performed by the US EPA (EPA-740-R-20-019; 2020).

A fish sexual development test according to OECD guideline 234 has been provided following a substance evaluation decision in 2018.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Dicyclohexyl phthalate
EC number:	201-545-9
CAS number:	84-61-7
Index number in Annex VI of the CLP Regulation:	607-719-00-4
Molecular formula:	C20H26O4
Molecular weight range:	330.418
Synonyms:	*1,2-Benzenedicarboxylic acid, dicyclohexylester* *DCHP*

Type of substance Mono-constituent

Structural formula:

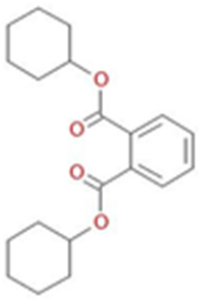


Table 5

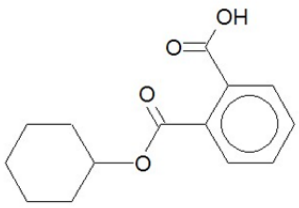
Constituent			
Constituents	**Typical concentration**	**Concentration range**	**Remarks**
Dicyclohexyl phthalate	no other relevant constituent substances reported in the registration dossier		

It is known from studies on other phthalates that the metabolic pathway follows at least two steps: a phase I hydrolysis followed by phase II conjugation. In the first step, the diester phthalate is hydrolysed into the primary metabolite monoester phthalate. Data indicates that the metabolism of DCHP to the monoester (MCHP) would take place primarily in the intestine (Lake et al 1977).

Table 6

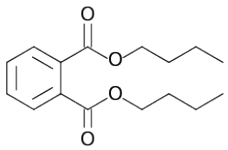
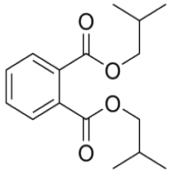
Degradation (transformation) product or metabolite			
metabolite	**Typical concentration**	**Concentration range**	**Remarks**
Monocyclohexyl phthalate	-	-	

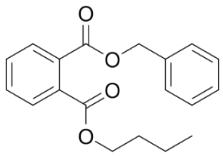
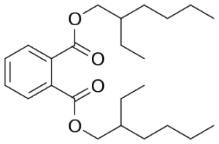
Structural formula:



DCHP belongs to the group *ortho*-phthalates of which DIBP, DBP, BBP, DEHP (Table 7) as well as DCHP have been identified as substances of very high concern (SVHC) due to their endocrine disrupting properties for human health. DEHP has also been identified as SVHC also due to its endocrine disrupting properties for the environment.

Table 7

SUBSTANCE name	EC number	CAS number	Structural formula	Molecular formula	Molecular weight *(g x mol^{-1})*
Dibutyl phthalate (DBP)	201-557-4	84-74-2		$C_{16}H_{22}O_4$	278.348
Diisobutyl phthalate (DIBP)	201-553-2	84-69-5		$C_{16}H_{22}O_4$	278.348

Bensyl butyl phthalate (BBP)	201-622-7	85-86-7		$C_{19}H_{20}O_4$	312.365
Bis(2-ethylhexyl) phthalate (DEHP)	204-211-0	117-81-7		$C_{24}H_{38}O_4$	390.564

7.4. Physico-chemical properties

Table 8

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	White crystalline powder with slightly aromatic odour
Vapour pressure	1.16 $\times 10^{-4}$ Pa at 25 °C OECD Guideline 105
Water solubility	1,015 mg/L (20°C, pH 7), according to the registration dossier[2] (Q)SAR-value (EpiSuite; 25°C, LogKow 4.82): 621 µg/L (Q)SAR-value (EpiSuite; 25°C, LogKow 6.2): 41 µg/L
Partition coefficient n-octanol/water (Log Kow)	4.82 (25°C) OECD Guideline 117 6.2 KowWin (v1.68)
Flammability	-
Explosive properties	-
Oxidising properties	-
Granulometry	D10 127.66 µm D50 442.144 µm D90 889.317 µm
Stability in organic solvents and identity of relevant degradation products	-
Dissociation constant	-
Boiling point	ca. 322.03 °C at 101,3 kPa ASTM E537-07
Melting/freezing point	ca. 65.6 °C at 101,3 kPa ASTM E537-07
Density	0.787 g/ml

[2] The results of the FSDT study (ECHA Decision 19 December 2018) indicates that the true solubility of DCHP is closer to 30 µg/l than 1000 µg/l.

7.5. Manufacture and uses

7.5.1. Quantities

Table 9

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ **100 – 1000 t**	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 10

USES	
	Use(s)
Uses as intermediate	-
Formulation	This substance is used in the following activities or processes at workplace: transfer of chemicals between vessels/large containers, batch processing in synthesis or formulation with opportunity for exposure, mixing in open batch processes, closed, continuous processes with occasional controlled exposure, transfer of substance into small containers, roller or brushing applications, laboratory work, the low energy manipulation of substances bound in materials or articles and high energy work-up of substances bound in materials or articles (e.g. hot rolling/forming, grinding, mechanical cutting, drilling or sanding).
Uses at industrial sites	This substance is used in the following products: polymers and adhesives.
Uses by professional workers	This substance is used in the following activities or processes at workplace: transfer of chemicals between vessels/large containers, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, batch processing in synthesis or formulation with opportunity for exposure, mixing in open batch processes, roller or brushing applications and production of mixtures or articles by tabletting, compression, extrusion or pelletisation
Consumer Uses	This substance is used in the following products: adhesives and sealants, coating products, fillers, putties, plasters, modelling clay, finger paints, non-metal-surface treatment products, inks and toners, polishes and waxes, polymers and textile treatment products and dyes.
Article service life	This substance can be found in products with material based on: plastic (e.g. food packaging and storage, toys, mobile phones).

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 11Table 11 lists the harmonised classification for DCHP according to Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation).

Table 11

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
607-719-00-4	Dicyclohexyl phthalate	201-545-9	84-61-7	Repr. 1B Skin Sens. 1	H360D H317		

7.6.2. Self-classification

- In the registration(s)*:*

  Skin Sens. 1 H 317
  Repr. 1B H 360D
  Aquatic Chronic 2 H 411

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

  Skin Irrit. 2 H 315
  Eye Irrit. 2 H 319
  STOT SE 3 H 335
  Repr. 2 H 361
  Aquatic Chronic 3 H 412

7.7. Environmental fate properties

The text in chapter 7.7 is retrieved from the Annex XV dossier for DCHP (2016) and included for completeness, environmental fate was not evaluated during substance evaluation.

7.7.1. Degradation

The contribution of hydrolysis to the overall environmental degradation of phthalate esters, including DCHP, is expected to be low. Photo-oxidation by OH radicals contributes to the elimination of DCHP from the atmosphere. An atmospheric half-life of about 0.4 days has been estimated (AOPWIN, v1.92).

In a ready biodegradation test 68.5 % degradation by BOD and 91 % degradation by test material analysis was recorded (ECHA 2015). Only very limited details from this study are available and there is no recording of whether or not the 10-day window was met. Hence, it cannot be finally concluded that the substance is readily biodegradable. Simulation degradation studies are not available for DCHP.

7.7.2. Environmental distribution

The distribution of DCHP has been modeled with the EPIWIN 4.1. McKay Level III model. If equal and continuous release of the substance to soil, water and air is assumed the model predicts distribution to soil (77 %) water (13 %) and sediment (10 %) with limited distribution to air (0.2 %).

The Henry's law constant of 0.01 Pa.m3/mol indicates that DCHP will only slowly volatilize from surface waters. A Koc of 4.2 l/kg can be calculated using the Kow method in KOCWIN (v.2.0). The octanol/water partition coefficient (Kow) of DCHP is reported as 4.82 on the dissemination site (ECHA 2015). However, according to QSAR estimates the log Kow is 6.2. This latter value is also more in agreement with experimental values for other phthalates with comparable side chain lengths such as the C6 phthalate Dihexyl phthalate (DHP) which has a recorded log Kow of 6.82. The log Kow value plays a critical role in calculation of many other properties such as fugacity distribution, Koc and bioaccumulation. Hence, the uncertainty relating to the log Kow for DCHP also introduces some uncertainty in the calculation of these other properties.

7.7.3. Bioaccumulation

No experimental information is available for bioaccumulation of DCHP. The high log Kow of DCHP indicates that the substance has a potential for bioaccumulation. However, the actual degree of bioaccumulation *in vivo* is also dependent on the metabolism and the elimination rate of the substance.

QSAR estimates for DCHP from different models are not consistent. The BCFBAF regression based model predicts a BCF of 5700 whereas the Arnot-Gobas model predicts BCF values of 135 (upper trophic, including biotransformation) and 20,000 (upper trophic, assuming zero biotransformation). These values will be lower if the experimental log Kow value of 4.82 is included in the calculations instead of the calculated log Kow of 6.2 which is used as default. However, without experimental information it is not possible to conclude if the BCF for DCHP is above or below the identification criteria for bioaccumulation (threshold of 2000) and for very bioaccumulative (threshold of 5000) (Annex XIII REACH).

7.8. Environmental hazard assessment

The text in chapter 7.8 is retrieved from the Annex XV dossier for DCHP (2016) and included for completeness. The environmental hazard assessment (except ED properties) was not updated during substance evaluation.

7.8.1. Aquatic compartment (including sediment)

Two acute toxicity tests on fish and daphnia, an algal growth study and a 21 day reproduction test with Daphnia are available in the registration dossier. Long term toxicity studies on fish have been waived by the registrant.

- *Oryzias latipes* Fish Acute Toxicity Test (TG 203), LC50 (96h) > 2 mg/L
- *Daphnia magna* Acute Immobilization Test (TG 202), NOEC (24/48h) >2 mg/L
- *Pseudokirchinerella subcapitata* Alga Growth Inhibition Test (TG 201), NOEC (24/72h) >2 mg/L
- *Daphnia magna* Reproduction test (TG 211) (performed 1999-2000 protocol not including sex ratio), NOEC (21d) 0.181 mg/L.

None of these studies include endpoints diagnostic of endocrine disrupting properties.

However two relevant studies have been performed by the Japanese ministry of the environment, MoE (Japanese ministry of the environment, 2003). In addition, a Fish sexual development test according to OECD guideline 234 has been provided following a substance evaluation (SEV) decision request (ECHA Decision 19 December 2018). These studies are summarised in section 7.10.1.

7.8.2. Terrestrial compartment

Not in the scope of this substance evaluation.

7.8.3. Microbiological activity in sewage treatment systems

Not in the scope of this substance evaluation.

7.8.4. PNEC derivation and other hazard conclusions

Not in the scope of this substance evaluation.

7.8.5. Conclusions for classification and labelling

The conclusion of this substance evaluation is that DCHP meets the CLP criteria for classification as endocrine disrupter for the environment cat.1 (see section 7.10.3).

7.9. Human Health hazard assessment

Not in the scope of this substance evaluation. The text in chapter 7.9 is retrieved from the Annex XV dossier for DCHP (2016) and included for completeness, a human health hazard assessment was not conducted during substance evaluation.

7.9.1. Toxicokinetics

There were limited toxicokinetic data available for DCHP. Lake et al. (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 (cyclohexanol). 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.

7.9.2. Conclusions of the human health hazard assessment and related classification and labelling

DCHP has a harmonised classification as repro 1B and has been identified therefore as SVHC. It has also been identified as SVHC based on Reach article 57f due to its endocrine disrupting properties for human health. This means that DCHP in practice meets the criteria for ED human health cat.1 according to the amended CLP Regulation (Commission Delegated Regulation (EU) 2023/707 of 19 December 2022).

7.10. Assessment of endocrine disrupting (ED) properties

The scope of this substance evaluation was ED concern for the environment. *In vitro* data is presented in paragraph 7.10.2.

7.10.1. Endocrine disruption – Environment

Limited information is available on ED relevant effects in aquatic species. One *in vitro* study on ER-receptor binding has been found in the open literature. In this study by Urushitani *et al* (2003) on ER receptor binding using a ERα clone derived from mummichog (*Fundulus heteroclitus*) DCHP and DEHP both show weak but similar receptor binding affinities. The IC_{50} was 3.8 x 10^{-5} M for DCHP and 3.2 x 10^{-5} M for DEHP which was around 10 000 times weaker than 17b-Estradiol (IC_{50} 5.5 x 10^{-9} M).

Two relevant *in vivo* studies, a 21-day fish screening assay (similarities with OECD TG 230) and a partial life cycle test (similarities with OECD TG 234) were identified in which endocrine relevant parameters are included, both of which were conducted by the Japanese Ministry of the Environment (Japanese Ministry of the Environment, 2003)). In addition, a Fish sexual development test according to OECD TG 234 has been provided following a SEV decision request (ECHA Decision 19 December 2018).

The results from the Japanese studies have been published by the Japanese ministry of the environment (2003). A brief overview of the results has also been published by Dang et al. (2011). The original unpublished Japanese study reports were consulted for the Annex XV dossier (2016) in order to evaluate the parameters and to establish validity of the two studies.

In the 21-day fish screening assay on Japanese Medaka (*Oryzias latipes*) no vitellogenin (VTG) induction in male fish was identified. Secondary sex characteristics or VTG in females were not studied. The test was performed with solvent control, control and five exposure groups of 17.9, 38.2, 87.2, 188 and 388 µg/L (average measured concentrations).

The partial life cycle test with similarities to the OECD TG 234 FSDT test was conducted with Japanese Medaka (*Oryzias latipes*). The test used 60 fish (four replicates with 15 fish) in each exposure group consisting of control, solvent control (DMSO) and 5 different concentrations of DCHP. The nominal test concentrations were 0.477, 1.53, 4.88, 15.6, 50.0 µg DCHP/L. The average measured concentrations were 0.429, 1.41, 4.39, 13.3 and 35.8 µg DCHP/L. The test was performed under flow through conditions at a temperature of 24 ± 1°C and pH of 7.5 ± 0.2. The photo period was 16 hours light, 8 hours darkness. The eggs were exposed from shortly after fertilization until 60 days post-hatch. Time to hatch and number of hatchlings were recorded. Secondary sex characteristics, weight and length were measured for all animals. Vitellogenin, testis/ova, hepatosomatic index and gonadosomatic index were measured/determined for 20 randomly selected individuals from each exposure/control group (5 from each replicate). The results are summarised in Table 12Table 12, Table 13Table 13 and Table 14Table 14.

Hatchability was ≥ 90% in all test groups, and no statistically significant differences compared to the control groups were observed. The mean time to hatching was approximately 9 days in all exposure groups and in the solvent control, but slightly longer in the control group (9.7 ± 0.2 days). A statistically significant increase in length compared to the control group was observed at the concentration levels of 0.429, 4.39, 13.3, and 35.8 µg/L, however, not at the concentration of 1.41 µg/L. No statistically significant effects on mortality was observed.

Table 12 Partial Life Cycle Test - Results

Test conc (µg/L) (measured average)	**Hatchability** (%)	**Time to hatch** (day)	**Mortality** (%)	**Total length** (mm)	**Body weight** (mg)
Control	98±3.3	9.7±0.2 0	0	28.0±1.4	220±36
Solvent control	92±13	9.2±0.3	3.3±6.7	27.3±2.8	250±50

(DMSO)					
0.429	100	9.1±0.1	1.8±3.6	28.8±1.5**	225±41***
1.41	93±9.4	9.1±0.1	7.6±11	28.4±2.3	241±44
4.39	92±8.4	9.1±0.1	5.6±7.3	30.0±1.6**	250±47
13.3	100	9.3±0.4	0±0	29.0±1.7**	237±45
35.8	90±8.6	9.1±0.1	13±10	29.8±1.8**	265±48

Data shows mean ± standard deviation.
Statistically significant differences from control and solvent control group (**p<0.01, *p<0.05).
Statistically significant differences from solvent control group (***p<0.05), but not statistically significant differences from control group.

A statistically significant increase in the gonadosomatic index in male fish was observed for the highest exposure group (35.8 µg/l). No statistically significant effects were observed for the hepatosomatic index.

The sex ratio was statistically significant skewed towards more males at the test concentration of 1.41 µg/L but not at other test concentrations (this information was mentioned in the Annex XV dossier report. However, it is not mentioned in the the available study report in English (Japanese Ministry of the Environment, 2003) or in the publication by Dang et al 2011). In addition, one of ten male fish in the highest exposure group developed testis-ova, an intersex condition characterized by both testicular and ovarian tissue in the gonad. The medaka is completely dioecious in nature and emergence of testicular eggs in medaka is only known to be caused by exposure to estrogen agonists or anti-androgenic substances. However, the finding of testis-ova in one fish is not considered sufficiently robust to make a firm conclusion. Finally, a statistically significant effect on vitellogenin induction in male fish was observed at the concentration of 4.39 µg/L but not at higher or lower concentrations. The lack of dose response for the observed effects makes it difficult to interpret the results of this study.

Table 13 Partial Life Cycle Test – Results continued

Test conc (µg/L) (measured average)	**Gonadosomatic Index - Male** (%)	**Gonadosomatic Index - Female** (%)	**No of fishes**	**Testis-ova**
Control	0.75±0.2	4.3±3.3	20	0/13
Solvent control (DMSO)	0.74±0.2	5.2±3.3	20	0/12
0.429	0.83±0.2	5.5±3.1	20	0/13
1.41	0.69±0.2	2.9±2.6	20	0/13
4.39	0.85±0.3	5.8±3.7	20	0/14
13.3	0.76±0.2	3.9±2.8	20	0/11
35.8	1.1±0.3**	5.9±3.1	20	1/10

Table 14 Partial Life Cycle Test – Results continued

Test conc (µg/L) (measured average)	**Hepatosomatic Index - Male** (%)	**Hepatosomatic Index - Female** (%)	**VTG Male** (ng/mg liver)	**VTG Female** (ng/mg liver)
Control	2.7±0.7	3.6±1.0	1.8±2.4	1,600±1,500
Solvent control (DMSO)	2.5±0.4	4.0±0.7	2.2±2.4	1,800±1,300
0.429	2.4±0.4	3.6±0.9	3.8±3.4	2,100±1,100
1.41	2.4±0.6	3.0±0.5	4.7±4.7	1,600±1,400
4.39	2.2±0.6	3.6±0.5	12±16**	1,800±660
13.3	2.1±0.5	3.2±0.7	1.3±2.0	2,400±1,900
35.8	2.2±0.9	3.7±1.0	2.7±2.1	2,900±3,300

It is worth noticing that the study was performed far below the claimed water solubility of DCHP (ca. 1 mg/l according to the information in the registration dossier) and that some of the effects (changed gonadosomatic index and testis-ova) were observed only at the highest test concentration. Given that the LC_{50} of DCHP to Japanese Medaka is > 2mg/l, higher test concentrations could possibly have been used. This was addressed in the SEV-request for the OECD TG 234 study summarised below.

The requested OECD TG 234 study was performed using zebrafish (*Danio rerio*) (ECHA Decision 19 December 2018). The decision required that the study should be performed at test concentrations up to the water solubility which was assumed to be 1 mg/l according to the information in the registration dossier. Newly fertilized zebrafish eggs (120/test conc) were distributed between 7 treatment groups each containing 4 replicates of 30 eggs. The fish eggs in five of the 7 groups were treated with DCHP at nominal concentrations of 10, 32, 100, 320, or 1000 µg/L achieved by dilution from stock solutions of DCHP prepared with dimethylsulfoxide (DMSO) as solvent. Two groups served as controls: one as negative control and the other as solvent control (DMSO 0.1 ml/L). The mean measured test concentrations based on measurements in all replicates approx. 3 times a week during the whole test period were: 10.4, 28.2, 66.6, 229.4 and 588.2 µg/l. It was possible to achieve a stable test concentration close to the nominal value only for the two lowest test concentrations. The authors of the study notes a precipitate in the mixing chamber at 320 µg/l and in the aquaria at 1000 µg/l. Thus, it is obvious that the solubility of DCHP in the test system was far below 1000 µg/l All results are therefore based on arithmetic means of measured concentrations i.e. 10.4, 28.2, 66.6, 229.4 and 588.2 µg/L. However, it is possible that the measured concentrations at the three highest exposures to some extent also includes colloidal dispersions and therefore may not represent the concentration of truly dissolved DCHP.

The exposure started as soon as possible after fertilization and before cleavage of the blastodisc commences and no later than 12 h post fertilization.

The test was performed under flow through conditions at 27°C ± 2 in thermostatic bath with a light/dark cycle of 14/10 hours and a light intensity ranged from 540 to 1080 lux (actual value : 620-648 lux) without aeration.

28 days post fertilization the number of fish per replicate were redistributed, so that each replicate contained as equal a number of fish as possible. When mortality occurred, the number of replicates was reduced appropriately so that fish density between treatment levels was kept as equal as possible. The test duration of the study was until 60 days post-hatch.

The endpoints examined were: hatching rate (at 5 dpf), survival, length, weight, sex distribution (based on gonad histology), vitellogenin (VTG) and liver and kidney histology (at 60 dph).

No effect on the hatchability of the eggs were noted up to 66 µg DCHP/l (see Table 15Table 15).
The NOEC for fry survival was also 66 µg/l whereas a marked mortality was noted at 229 µg/l with a survival rate of only 18%. At the highest tested concentration all fry died within 4 days after hatch.

Table 15 Hatching rate (5 dpf) and fry survival (60 dph)

Test conc (µg/L)	**Number of eggs hatched**	**Hatching rate (%)**	**Survival at 60 dph(%)**
Control	111	92.5	96.4
Solvent control (DMSO 0.1ml/l)	116	96.7	94.8
10.4	112	93.3	96.5
28.2	115	95.8	95.6
66.6	115	95.8 **NOEC**	90.6 **NOEC**
229.4	108	90.0 **LOEC**	18.4 **LOEC**
588.2	78	65.0	0.0

The mean length and weight of the fry at 60 dph is shown in Table 16Table 16. Both length and weight was significantly lower at 28.2 µg DCHP/l compared to the controls but not at 66.6 µg/l. The highest test concentration with surviving frys (229.4 µg DCHP/l) was excluded from statistical comparison with the controls due to the low number of surviving fry.

Table 16 Mean length and weight at the end of the study (60dph)

Test conc (µg/L)	**Mean length (cm)**	**Mean weight (mg)**
Control	2.164	157.2
Solvent control (DMSO 0.1ml/l))	2.165	161.2
10.4	2.131 **NOEC**	154.3 **NOEC**
28.2	2.127 **LOEC**	149.2 **LOEC**
66.6	2.180	164.5

*229.4**	*2.017*	*152.4*

**(N=20) Excluded from the statistical analysis due to the low number of fry

According to OECD TG 234 the acceptance criteria related to proportions of sex at termination of the test in the control groups (pooled solvent and water control unless they are significantly different, then solvent only) is 30-70 % (% male or female). It is notable that the sex ratio in the control was statistically significantly different from the solvent control. Only the solvent control was therefore used in the statistical analysis. The 229.4 µg/l test concentration was excluded from the statistical analysis due to the low number of fish (all fry died except in one of the four replicates). The proportion of female at the two lowest test concentrations were higher than the control (78.9 % for 10.4 µg/l and 76.4 % for 28.2 µg/l). However, neither the proportion of females nor the proportion of males in the DCHP exposed groups were significantly different from the solvent control (see Table 17Table 17).
There were no differences in staging of the ovaries in the three lowest exposure groups compared to the control.

Table 17 Sex ratio at 60 dph

Test conc (µg/L)	**Total number**	**Female** (%)	**Male** (%)	**Intersex** (%)	**Undifferentiated** (%)
Control	107	59.8	38.3	1.9	0
Solvent control (DMSO 0.1ml/l))	110	70	30	0	0
10.4	108	78.9	21.1	0	0
28.2	110	76.4	23.6	0	0
66.6	104	72.3 **NOEC**	25.9	0.9	0.9
*229.4**	*20*	*90*	*10*	*0*	*0*

* Excluded from the statistical analysis due to the low number of fry

The results of the VTG measurements are shown in Table 18Table 18. All fish were used for the measurements of VTG. Head and tail of each fish were pooled and the VTG measurements were performed using a zebrafish Vitellogenin ELISA kit. There was no statistical difference between the two controls with respect to female vitellogenin. The female VTG was statistically significantly lower at 10.4 and 28.2 but not at 66.6 µg DCHP/l compared to the pooled controls. Thus the NOEC for female VTG was < 10.4 µg/l.

Also for the male VTG there was no statistical difference between the two controls and they were pooled. The numbers appear to show a decrease in VTG in the exposed males compared to the pooled controls. However, due to the larger variation in the controls, (a few individuals had VTG levels >> 100 ng/g), the statistical analysis did not reveal any significant differences. Thus the NOEC for male VTG was 66.6 µg/l.

Table 18 Vitellogenin concentration

Test conc (µg/L)	**Replicate**	**VTG** (ng/g) **Male**	**Standard deviation**	**VTG** (ng/g) **Female**	**Standard deviation**
Control	1	57.3	87.8	1282.1	1719.5
	2	6.5	3.39	1069.8	1291.3

	3	15.6	30.4	674.8	833.4
	4	66.3	137.5	995.3	1540.7
	average	**36.4**		**1005.5**	
Solvent control (DMSO 0.1ml/l))	1	338.7	716.1	889.2	1178.6
	2	23.4	27.9	811.0	1347.8
	3	12.2	7.3	1145.7	1963.0
	4	30.2	54.5	962.4	1285.7
	average	**101.1**		**952.1**	
10.4	1	11.5	6.0	547.3	1010.8
	2	10.3	5.2	369.0	635.2
	3	7.2	4.2	465.6	790.2
	4	6.9	4.3	728.2	954.2
	average	**9.0**		**527.5**	
28.2	1	11.7	4.3	522.5	622.9
	2	6.7	4.7	273.7	426.4
	3	46.4	74.8	755.1	1493.5
	4	11.8	8.5	522.3	777.8
	average	**19.2**		**518.4**	
66.6	1	17.9	16.6	795.1	1178.6
	2	5.2	4.1	571.1	853.0
	3	56.2	50.2	1077.5	1130.2
	4	11.9	8.1	1148.8	1075.9
	average	**22.8**		**898.1**	
*229.4****	*4*	*89.2*	*119.8*	*680.1*	*1075.3*

* N=20 Excluded from statistical analysis due to the low number of fry

No test item-related findings were noted in kidneys and livers.

To summarise: The results of the study indicates that the true solubility of DCHP is closer to 30 µg/l than 1000 µg/l. The high fry mortality at the two highest test concentrations 82% and 100%, respectively made these test concentrations useless for detecting ED effects. The most significant sign of ED-effects of DCHP was the statistically significant decrease in VTG production in females at the two lowest test concentrations (10.4 and 28.2 µg/l) whereas there was no significant difference at 66 µg/l. The authors of the study report speculates that the reason for this could be explained by problems of solubility of

DCHP in concentrations higher than 30 µg/l with possible formation of colloids making DCHP less bioavailable at higher concentrations.

The significant decrease in female VTG is an indication of an ED-effect. Also the higher proportion of females, not statistically significant compared to the solvent control (statistically significant if compared to the control) at the two lowest test concentrations may be signs of an ED-effect.

7.10.1.1. Studies on mammals - population relevant effects

DCHP has been concluded as an ED for human health, due to its antiandrogenic mode of action. In rat, ED effects were manifested by genital malformations associated with small testis, signs of reduced sperm quality, atrophic tubules in prostate, prostatic intraepithelial neoplasia and testicular changes including tubular atrophy. Further, in male rats, a reduced anogenital distance (AGD) and retained nipples was evident across studies in the absence of marked maternal toxicity. DCHP was classified as Repr. 1B in 2014 for developmental toxicity, based on the adverse effects listed above, and was listed on the candidate list of substances of very high concern for authorisation in June 2018 (Article 57(c) and (f)). DCHP was proposed as an SVHC also due to its ED properties for the environment mainly on the basis of experimental data on mode of action and adverse effects in rodents that were considered relevant for mammals in general (Annex XV report for DCHP, 2016). The proposal was however withdrawn after discussions at MSC 48.

According to the ECHA/EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, adverse effects on growth, development, and reproduction in single species are generally regarded relevant for the maintenance of the wild population.

In a study by Saillenfait (2009) pregnant Sprague–Dawley rats were dosed with 250, 500 or 750 mg/kg bw/day DCHP, by gavage, on gestational days (GD) 6–20. No maternal mortalities or adverse clinical effects were observed. A significantly reduced body weight gain GD 6-9 and GD18-21 (and overall GD 6-21) was observed for the dams dosed with 750 mg DCHP/kg bw/d. The reduced weight gain was associated with significantly lower food consumption at GD 6-9 and GD18-21. The liver weights of the dams were slightly increased in the two highest doses, while serum ASAT and ALAT concentrations were affected in the highest dose only. On GD 21 the dams were killed, and the uterus removed and examined to determine the number of implantation sites, resorptions and dead and live fetuses. Fetuses were also examined for *e.g.*, malformations. There were no effects on post-implantation loss or on sex ratio. No embryolethality or teratogenic effects were observed, but a significantly reduced fetal weight (ca 9 % in both male and female pups) was seen at 750 mg/kg bw/day. Decreased anogenital distance (absolute and relative to the cubic root of bodyweight) were observed 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).

In the study by Yamasaki (2009) pregnant CD (SD) IGS rats (10/dose group) were gavaged with 0, 20, 100, and 500 mg/kg bw/day of DCHP from gestational day (GD) 6 to postnatal day (PND) 20. No body weight changes were detected during the exposure. An increase in dam liver weight was observed being statistically significantly ($p<0.05$) higher at the intermediate and high dose level (+7 and +24 % as compared to controls).

The viability index on PND 4 (%, number of live pups on PND4/number of live pups on PND 0×100) in the 500 mg/kg DCHP group, was lower than in the control group (97.8% compared to 100%). Although small, the difference was statistically significant ($p < 0.05$). No abnormalities were detected in other reproductive parameters. The body weights of male and female pups decreased significantly in the 500 mg/kg DCHP group at PND14 and/or 21. There were, however, no differences in body weight between control and treated pups when sacrificed at 10 weeks of age.

A significantly ($p<0.05$) decreased male anogenital distance was observed on PND4 in the highest dose group (absolute,-15%, as well as relative to the cubic root of the bodyweight, -13%). No information on AGD was provided for lower dose levels. Further, on PND 13, a significant increase in the numbers of pups/litter with areola/nipple retention (NR; 2.7 as compared to 0 in the controls; $p<0.05$) was reported, as well as in the litter incidence of areola/nipple retention (67.6% as compared to 0 in controls; $p<0.05$). No NR data was provided for the lower dose groups. A ca. 2 days delayed ($p<0.05$) preputial separation was observed in high dose males. No information provided for lower dose levels.

Before weaning (PND 21) the pups from each dose group were randomly assigned to two groups: one group sacrificed at 10 weeks of age, and another group to evaluate the reproductive performance of the F1 generation (caesarean group). Two females and two males per dam in each dose group were assigned to the caesarean group. The treated females in each group were mated with treated males at 12 weeks of age (without brother–sister mating), and the copulation index and fertility index were calculated. Caesarean sections were performed under anesthesia at GD 13, and the implantation index and loss were calculated. No changes in these reproductive parameters were detected.

The effects observed at necropsy (10 weeks of age) were 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.
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.

Hoshino *et* al (2005) performed a two-generation study in accordance with the 1983 OECD TG 416, enhanced with parameters to detect endocrine disrupting activity. Five week old rats (Crj:CD(SD)IGS; 24/sex/dose) were given feed with 0, 240, 1200 or 6000 ppm DCHP. The corresponding average daily doses (mg/kg bw/day) are given in Table 19Table 19. The F0 males were exposed during 10 weeks of pre-mating and mating periods and the F0 females through 10 weeks or more of pre-mating, mating, gestation and lactation, until weaning of F1 (PND 21). The F1 generation was exposed from weaning (PND21) to end of mating (males) and until lactational day 21 (females).

Table 19 DCHP concentration in feed and corresponding daily doses

DCHP concentration in feed (ppm)	**DCHP average daily dose** (mg/kg bw/day)			
	F0 male	F0 female	F1 male	F1 female
0	0	0	0	0
240	16	21	18	21
1200	80	105	90	107
6000	402	511	457	534

No clinical signs of treatment-related changes, or significant differences between parental F0 and F1 animals in the control and the dose groups were observed regarding copulation, mating, gestation, fertility, and birth indices.

Significantly lower F1 body weights were observed in the highest dose group throughout the observation period. In F2, a significantly lower body weight was observed only at PND 21 in the highest dose group. At PND4, male pups showed a significantly decreased absolute (F1: -7%, $p<0.01$; F2: -9% $p<0.01$) as well as relative (F1: -8%, $p<0.01$; F2: -9%, $p<0.01$) anogenital distance at the high dose level, and this effect was also seen at

the intermediate dose level in F2 (-7% and -7% for absolute and relative distance, respectively, $p<0.01$). Retained areola mammae was observed at PND 14 in 16.1 % of F1 males exposed to the highest dose, and at PND 12 in the F2 generation, where areola mammae was observed in the mid (18.4%; ns) and high (63.2%; $p<0.01$) dose groups. Further, seminiferous tubule atrophy was observed in nine out of 22 F1 males in the high dose group, and three males of the 22 showed diffuse atrophy of the seminiferous tubules, with marked testicular atrophy. This is in line with findings in other phthalate ester compound studies.

Aydogan and Barlas (2013) exposed (0, 20, 100 and 500 mg/kg bw/day; n=10/group) Wistar rats (a different strain than used in the other experiments listed above) during gestation up to GD20, with the main effects observed on the male reproductive organs, equal to those in the main studies. The same group (Aydogan and Barlas 2015) exposed (0, 20, 100 and 500 mg/kg bw/day; n=10/group) Wistar rats during gestation (GD6 – GD19), resulting in increased resorptions, a decrease in male AGD index (AGDi) in all dose groups, as well as a decrease in the testosterone/ antimullerian hormone (AMH) and follicle stimulating hormone (FSH)/Inhibin B ratios in the mid- and high dose groups. Further, histopathological alterations of the testis were evident, and observed in a dose-dependent manner showing atrophic and small seminiferous chords, decreased germ cells in chords, Sertoli cell chords only, chords with cells detached from wall and presence of multinucleated germ cells. Results on female rats from the same study were reported in Ahbab *et* al (2017), and AGDi was significantly decreased also in female rats, in all dose groups. No significant differences in maternal body weight gain, food or water intake compared to control were observed. In the two latter publications, a 12% significant increase in maternal relative liver weight was reported in the highest dose group only.

Furr *et al.* (2014) reported that *in utero* exposure to DCHP (Harlan SD rats; 0, 33, 100, 300, 600, or 900 mg/kg bw/day) resulted in a significant decrease ($p<0.01$) of testosterone production in the testis in all dose groups, except the lowest concentration, without a significant effect on maternal weight or fetal viability.

Lv *et al.* (2019) performed a study where adult male SD rats were injected with ethane dimethane sulfone (EDS), in order to eliminate all Leydig cells in the testis. On post-EDS day 7, the animals were treated with DCHP (0, 10, 100 or 1000 mg/kg bw/day), and euthanized at post-EDS days 21 and 28. No general toxicity was observed. On post-EDS day 21, serum testosterone levels in the two lowest dose groups were significantly increased, and the Leydig cell number in the lowest dose group was increased. On post-EDS day 28, serum testosterone and the number of Leydig cells were lowered in the 1000 mg/kg bw/day dose group. Further, DCHP dose-dependently down-regulated mRNA expression of a number of Leydig cell genes (*Lhcgr, Scarb1, Star, Cy*p11*a1, Hsd3b1, Cyp17a1, Hsd17b3, Hsd11b1,* and *Insl3*), and their corresponding protein expression. Thus, exposure to DCHP increased Leydig cell mitosis during the initial phase of Leydig cell regeneration, and later inhibited the differentiation process.

To summarise: DCHP caused adverse effects on the male reproductive system in more than one whole-animal toxicity study with relevant routes of exposure (oral via diet/gavage). The spectrum of effects observed in rats include increased areola mammae retention, decreased anogenital distance, prolonged preputial separation, genital malformations associated with small testis, signs of reduced sperm quality, atrophic tubules in prostate, prostatic intraepithelial neoplasia and testicular changes including tubular atrophy of which almost all can be considered adverse.

7.10.1.2. Thyroid effects

Sugiyama et al. (2005) studied the effect of DCHP and 8 other chemicals in a thyroid hormone (TH) inducible primary screening assay for the identification and assessment of chemicals that interfere with the TH signalling pathway within target cells. The assay developed by Sugiyama *et al.* used a *Xenopus laevis* cell line that was transduced with a

self-inactivating (SIN) lentivirus vector (LV) containing a luciferase gene. The luciferase activation in this cell line was TH-specific: 3,3´,5-L-triiodothyronine (T_3) > 3,3´5-L-triiodothyroacetic acid (Triac) > 3,3´,5-D-triiodothyronine (D-T_3), > L-thyroxine (T_4) > 3,3´,5´-L-triiodothyronine (rT_3). The assay revealed that three phthalates (dicyclohexyl phthalate, n-butylbenzyl phthalate, and di-n-butyl phthalate), two herbicides (ioxynil and pentachlorophenol) and a miticide (dicofol) had T_3-antagonist activity at concentrations ranging from 10^{-6} to 10^{-5} M. DCHP was tested at 0.8, 4 and 20 µM and significantly inhibited the T_3 dependent luciferase activity at 20µM. The IC_{50} was calculated to 11±3 µM. These six chemicals also inhibited the T_3 dependent activation of the *TRβ* gene *in vitro* using the same cell line. The T_3-dependent activation of transcription of TRβ was inhibited to 42±6% by 20 µM DCHP.

The chemicals that were potent in the *in vitro* assays were also tested in an *in vivo* metamorphosis-based assay. The T_3-dependent activation of *TRβ* gene in T_3- induced metamorphosing tadpoles (*Xenopus laevis*) after 5 days exposure was measured. Of the six chemicals, only n-butylbenzyl phthalate and pentachlorophenol exhibited T_3-antagonist activity in this *in vivo* assay. Unfortunately the report is ambiguous as to whether or not DCHP was tested *in vivo*. The results from the in vivo study are presented for n-butylbenzyl phthalate, di-n-butyl phthalate, ioxynil, pentachlorophenol, and dicofol but not for DCHP. Therefore, it remains unclear if DCHP was tested *in vivo* in this study.

An *in vivo* study on Western clawed frog (*Xenopus tropicalis*) was performed by Mathieu-Denoncourt, 2014 as a part of a master of science thesis. (The results are also partly presented in Mathieu-Denoncourt *et* al. 2016.) The study was performed according to the FETAX guideline (ASTM, 1998). Tadpole embryos were exposed to water spiked with monomethyl phthalate (MMP), dimethyl phthalate (DMP) or dicyclohexyl phthalate (DCHP) during 72 hours from NF stage 11-12 to NF stage 46. Only the DCHP results will be presented and discussed in this report.
Each treatment was tested in 6-10 replicates of 30 mL of FETAX solution containing 18-50 Western clawed frog gastrulae each. The nominal (intended) concentrations of DCHP was 0.6, 6, 23, 60 and 600 mg/l, all of which are above the water solubility of DCHP, which is assumed to be below 100µg/l. To achieve the intended concentrations, DCHP was solubilised in 0.82% dimethyl sulfoxide. Rearing media was renewed every 24 h. To verify the DCHP concentrations, rearing media samples (n = 2) were collected before the introduction of embryos (time 0) and 24 h later. No measurements were performed between 24 and 72 h. The measured concentrations were far from the nominal and the results are based on the mean measured concentrations, see Table 20Table 20. Each treatment including a control and a solvent control was tested in 6-10 replicates of 30 mL of FETAX solution containing 18-50 Western clawed frog gastrulae each.

Embryo mortality, malformations (eyes, tails, hearts, guts, gills, head and face) and development were investigated. Body length was used as an indicator of growth inhibition. In addition, the expression of a suite of genes involved in reproduction, TH axis (four diffent genes), cellular stress and transcriptional regulation was assessed For these measurements pools of ten animals (n = 8-9 pools) were preserved at -80 ºC for further gene expression analysis. Each treatment was tested in 4 to 9 replicates of pools of 10 whole embryos.

Table 20 Nominal and measured test concentrations of DCHP in a FETAX test

Nominal conc (mg/l)	**Measured conc at time 0 h** (mg/l ± SD)	**Measured conc at time 24 h** (mg/l ± SD)	**Mean measured conc** (mg/l ± SD)	**% of nominal**
Solvent control (0.82% DMSO)	0.1 ± 0.0	0.0 ± 0.0	**0.1 ± 0.0**	**-**
0.6	0.5 ± 0.1	0.1± 0.0	**0.3 ± 0.2**	**50**
6	2.7± 0.0	0.2 ± 0.0	**1.5± 1.4**	**25**

23	7.0 ± 1.3	1.2 ± 0.2	**4.1± 3.5**	**18**
60	31.8± 4.0	6.3 ± 1.1	**19 ± 14.9**	**32**
600	132.2 ± 34.6	66.3 ± 11.3	**99.3± 43.5**	**16**

The mortality at the two highest test concentrations were 100 and 94.7%, respectively. The 4.1 mg/L treatment lead to 5.5% lethality. Mortality at the two lowest expsore concentration were not different from the solvent control (numbers not given in the report).Most of the mortality occurred between the 24th and 48th hour of the DCHP exposure.

Malformations (Table 21Table 21). The malformation rate in the solvent control was three times higher than, and statistically different from the control malformation rate (36.1% compared to 11.2 %) indicating that the solvent DMSO in itself induced malformations. The malformation rates in the two lowest DCHP concentrations were lower than in the solvent control with the exception of edema and blisterings that had a statistically significantly higher rate at 1.5 mg DCHP/l. At 4.1 mg/l gut malformation and edema & blisterings had a higher rate than the solvent control. The few surviving tadpoles in the 19 mg/l group all had several malformations.

Table 21 Rate of malformation in tadpoles exposed to DCHP

DCHP (mg/l)	**Malformed individuals** % (n)	**Malformations** %						
		Eye	Tail	Heart	Gut	Gills	Head & face	Edema & blistering
Control	11.2 (10)	10.1	2.2	0.0	3.4	0.0	0.0	0.0
Solvent control	36.1 (56)	24.5	20.6	10.3	8.4	2.6	2.6	2.6
0.3	21.8 (32)	6.8	15.0	4.8	4.1	3.4	0.7	3.4
1.5	40.0 (60)	12.0	4.7	7.3	5.3	4.7	0.7	33.3*
4.1	100.0 (216)*	6.5	11.6	7.9	79.6*	5.1	6.0	100.0*
19	100.0 (17)*	23.5	82.4*	94.1*	0.0**	94.1*	5.9	29.4*

*Significantly different from solvent control
** None of the animals from the 19.0 mg/L DCHP group exhibited malformed gut, since 100% of the guts were severely underdeveloped

Development (Table 22Table 22). The solvent control had significant impact on the frequency of developmental delay signs. No developmental delay compared to the solvent control was observed at the two lowest test concentrations. At 4.1 mg/l the regression of the cement gland was significantly delayed compared to the solvent control and the tadpoles were significantly shorter. Treatment seemed to be three developmental stages (NF43) behind water-only control. The few surviving tadpoles from the 19.0 mg DCHP /L treatment were all found to display at least one sign of underdevelopment. They were significantly shorter, bared incompletely coiled guts and had no cement glands resulting in a five developmental stages delay (NF41) when compared to water controls

Table 22 Effects on development of tadpoles exposed to DCHP

DCHP (mg/l)	**Underdeveloped individuals** % (n)	**Signs of developmental delay observed** (%)			**Mean tadpole length** (µm ± SD)
		Eye	Cement gland	Gut	
Control	4.5 (4)	1.1	2.2	2.2	5,668.5 ±398.0
Solvent control	27.7 (43)	12.3	14.8	11.6	5,617.5 ± 389.2
0.3	25.9 (38)	5.4	15.6	16.3	5,499.3 ± 473.2
1.5	34.7 (52)	4.7	23.3	13.3	5,400.6 ± 222.2
4.1	47.2 (102)*	6.9	33.3*	18.1	4,674.5 ± 377.4*
19	100.0 (17)	17.6	0.0	100.0*	3,723.1 ± 442.3*

* Significantly different from solvent control

Also the expression of the different genes investigated suffers from from the solvent control in many cases being lower (in some cases statistically significantly lower) than the culture medium control alone. The only exception was one of the genes involved in thyroid hormone regulation (*dio 1*) wich was upregulated at 1.5 and 4.1 mg DCHP/l.

This study suffers from several shortcomings. DCHP was tested above its water solubility and the nominal test concentrations were far from reached, despite the use of a solvent (DMSO 0.82%). Furthermore, the test concentrations decreased significantly from 0 h to 24 h. The authors of the study claims that significant degradation was observed in the 6 mg/l (nominal) test conc. It is unclear what is behind this observation and the decrease at this test concentration is not different from the decrease at the other concentrations. Another possible explanation to the decrease is adsorption to the test equipment and/or to the embryos. The mortality 100 and 94.7 %, respectively observed at the two highest test concentrations may not be an effect of the intrinsic toxicity of DCHP as it is observed several orders of magnitude above the water solubility. It is also doubtful if the delayed development and malformations observed at 4.1 mg DCHP/l is caused by the intrinsic toxicity of DCHP. The only conclusion considered possible to draw is that DCHP does not seem to cause malformations or developmental delay in Western clawfrog at concentrations at or below its water solubility in this study.

There are also indications of effects on thyroid in mammalian species. In their 2-generation study on rats, Hoshino et al (2005) reports an increased absolute and relative thyroid weight, and hypertrophy of follicular cells. Increased thyroid weight was seen in the high dose group (6000 ppm equivalent to 400 – 500 mg/kg bw/day DCHP) in the F0 generation (males: ~+30% both in absolute and relative but only seen in left gland; females: +15-24% in only relative weight of both glands). An increased incidence of thyroid follicular cell hypertrophy (severity slight) was observed in in both F0 and F1 high dose animals (7 out of 24 examined males and 6 out of 24 examined females in both generations). Thyroid follicular cell hypertrophy was also observed in 3 out of 24 examined F0 males in the intermediate dose group (1200 ppm equivalent to ~100 mg/kg bw/day). No effects on thyroid weight were seen in the F1 generation.

No effects on thyroid weight was seen in the study by Yamasaki *et al.* (2009), however, the dosing period in this study was shorter (GD6–PND20) than in the study by Hoshino *et al*. (where at least three weeks of premating was also included).

Göktekin & Barlas (2017) studied the effects of DCHP on biochemistry and histopathology in the offspring of female rats exposed via gavage during gestation. Pregnant Wistar albino rats were distributed on a random basis into control, vehicle control, and three treatment groups (n=10), and housed individually. The dams were treated by gavage at GDs 6–19 with DCHP in corn oil; 20, 100, and 500 mg/kg bw/day. After delivery, all pups were allowed to grow with dam for one month. Female and male pups were housed at four per cage and allowed to free access for standard rat diet and tap water *ad libitum*. At PD 90 the animals were weighed and sacrificed. Blood was collected, and T3, T4 and TSH measured by using commercially available ELISA kits for rats. For histopathological examination, thyroid (thyroid and parathyroid were removed together) tissues were dissected out and weighed in order to calculate the organ and relative organ weights for each animal. Subsequently, the tissues were processed for histopathology.

There were no significant differences in body weights of male adult rats among groups. However, in female rats final body weights were significantly increased in DCHP–treated rats at doses of 100 and 500 mg/kg bw/day. There were no significant differences in absolute and relative thyroid weights in any of the dose groups. However, in thyroid glands of male and female rats, an elevated incidence of follicular and colloidal degeneration, increase in connective tissue, and adipose tissue between follicular tubules were observed in all treatment groups.

TSH levels were significantly higher compared to the male and female controls in the 20 and 100 mg/kg bw/day DCHP groups. In the 500 mg/kg group, however, the TSH levels were significantly lower than control. Elevated T_3 levels were found in the 100 mg/kg group but not in the 500 mg/kg group of female rats. In male rats T_3 levels were elevated in a dose dependent manner in both the 100 and 500 mg/kg groups. The T_4 levels in female rats in the 20 and 100 mg/kg groups was significantly higher compared to controls, whereas the T_4 levels in the 500 mg/kg group was not. In male rats, serum T_4 levels were significantly higher in the 100 mg/kg group, while it was significantly lower than control in the 500 mg/kg group.

To conclude, across these listed rat studies effects on the thyroid were observed. Alterations of thyroid hormones, effects on histopathology, and in some studies increased thyroid weights, reveal that DCHP may, in addition, be a thyroid disruptor.

7.10.1.3. Other Phthalates

The effects of DCHP are similar to the effecs seen for the phthalates (DiBP, DBP, BBP and DEHP) that so far have been identified as SVHC due to their endocrine disrupting properties for human health. DEHP has in addition been identified as a SVHC due to its endocrine disrupting properties for the environment. Below, as an example, is a summary of the ED-related effects observed for DEHP and DBP.

DEHP

Environmental data

DEHP was identified as a substance having probable serious effects to the environment in accordance with article 57(f) of REACH due to its environmental endocrine disrupting properties and was listed on the candidate list 2014. Overall DEHP acts as a weak estrogen and/or anti-androgen. Several studies on fish reports endocrine mediated effects including changed sex ratio of fish, induction of ovo-testis, decreased reproductive output in combination with Vtg induction, as well as decreasing male reproductive output. Some othe studies are shortly described here. More information is given in the Annex XV dossier for DEHP (Danish EPA, 2014).

Norrgren *et al.* (1999) observed a slight, but yet significant, skewing of sex ratio in Atlantic salmon after feeding 1500 mg/kg dwt DEHP for 4 weeks after yolk sac resorption followed by a 4 month depuration period

Ye *et al.* (2014) observed decreased egg production of female Marine medaka (*O. melastigma*) after 6 month exposure from the larval stage to either DEHP (0.1 and 0.5 mg/L) or MEHP (0.1 and 0.5 mg/L). Moreover, exposure to both DEHP and MEHP resulted in a reduction in the fertilization rate of oocytes spawned by untreated females paired with treated males. Besides, DEHP induced histological changes in the testes and ovaries: the testes displayed a reduced number of spermatozoa, and the ovaries displayed an increased number of atretic follicles.

Uren-Webster *et al.* (2010) investigated the effects of DEHP on the reproductive health of male zebrafish (*Danio rerio*). Males treated with 5000mg DEHP kg^{-1} (body weight) for a period of 10 days via intraperitoneal injection resulted in a reduction in fertilization success of oocytes spawned by untreated females.

Wang *et al.* (2013) demonstrated several effects on steroidogenesis in Chinese rare minnow (*G. rarus)*: ERα was significantly up-regulated in the liver of males and females and the authors argue that DEHP might act directly on ER genes, especially *ERα*, to stimulate Vtg synthesis. Exposure to DEHP caused a significant decrease of E2 and an increased T/E2 ratio in females but a significant increase of E2 and decreased T/E2 ratio in

males. These results could be explained by significant changes in both *CYP17* and *CYP19a* gene transcriptions.

Carnevali *et al.* (2010) exposed female *Danio rerio* to environmentally relevant doses of DEHP (20 ng – 40 µg/L) and a significant decrease in ovulation and embryo production was observed for all doses. The embryo production in the 40 µg/l dose was about 1% of control production.

Corradetti *et al.* 2013 observed a >90% decreased embryo production (P<0.01) after exposure of male zebrafish (Danio rerio) to 0.2 µg/l DEHP for three weeks. The authors hypothesize that the effect is a result of impaired male reproductive behaviour and testes hormone production.

Norman et al. (2007) observed a statistical significant induction of ovo/testis in the highest exposure group of 1500 mg/kg in male *S. salar*.

Mammalian data

Bis(2-ethylhexyl) phthalate (DEHP) is classified as a substance toxic to reproduction Repr. Cat. 1B; H360Df (May cause harm to the unborn child; Suspected of damaging fertility.). DEHP is identified as a substance of very high concern in accordance with Article 57(c), as well as 57(f) of Regulation (EC) 1907/2006 (REACH), as it is a substance with endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health.

DEHP has been shown to adversely affect the endocrine system of mammals primarily through in vivo findings on reduced fetal testosterone. These findings are further substantiated by mechanistic findings, also in vivo, of down-regulation of genes in the steroidogenic biosynthesis pathway. The spectrum of effects observed in male rats include increased incidence of nipple retention and genital malformations, decreased anogenital distance, reduced number of spermatocytes and testicular changes including multinucleated gonocytes, tubular atrophy and Leydig cell hyperplasia of which almost all are considered adverse (OECD 2008).

The overlap of observed effects on the male reproductive system between DCHP, DEHP, and DBP exposure is substantial: Increased incidence of nipple retention (mammae areolae), reduced AGDi, signs of reduced sperm quality, seminiferous tubule atrophy, and decreased testis weight.

Adverse effects caused by exposure to DEHP have also been identified in non-mammalian wildlife where the sex ratio and reproductive output was affected in fish. Furthermore, several studies in fish indicate that DEHP has an estrogenic MoA which may cause the sex reversal of male fish to female fish and/or affect the reproductive output. Hence the current data indicates also in fish that DEHP has endocrine disruptive properties leading to adverse effects related to sexual development and reproduction.

When available information from mammalian and ecotoxicological studies are combined, DEHP can be considered an endocrine disruptor for the environment according to the amended CLP Regulation (Commission Delegated Regulation (EU) 2023/707 of 19 December 2022) and fulfils the WHO/IPCS definition of an endocrine disruptor, as well as the recommendations from the European Commission’s Endocrine Disrupters Expert Advisory Group for the substance to be identified as an endocrine disruptor.

Moreover, effects of DEHP on thyroid histology indicating hyperactivity of the gland have been described in rat studies (Poon et al., 1997; Hinton et al., 1986; Howarth et al., 2001).

DBP

Environment

Also for other phthalates e.g. DBP studies showing endocrine mediated effects on fish and amphibians are available. DK proposed DBP for the candidate list for both its HH- and ENV-ED properties and provided an Annex XV report in 2014. The evidence for Env-ED was not considered sufficient at that time and the proposal for ED-Env was withdrawn. However, several studies indicating ED mediated effects of DBP in fish and amphibians are summarised in the the Annex XV report (Danish EPA, 2014) and shortly described here. More detailed information is available in the annex XV report.

Bhatia et al. (2013) reported that the circulating levels of plasma vitellogenin were significantly lower in the female Murray rainbowfish (*Melanotaenia fluviatilis*) exposed to 500 µg/L and 1000 µg/L DBP ($p<0.05$) .

Aoki et al. (2011) observed a significant decline in the concentration of androgen dependent spiggin protein in adult male three-spined sticklebacks (G. aculetaus) after exposure to 35 µg DBP/L for 22 d.

In a study by Tollefsen et al. (2002) steroidogenesis was affected by DBP binding competitively to the Atlantic salmon (S. salar) Sex steroid Binding Protein (SBP).

Jarmołowicz et al. (2013) observed that DBP seriously disturbed sex differentiation process of pikeperch (*Sander lucioperca*) after exposure during the sex differentiation period (age 61–96 days post hatch). Histopathological analyses revealed that the administration of 1 and 2 g DBP/kg feed significantly affected the sex ratio. The feminization process (intersex gonads) at concentrations of 1 g and 2 g di-n-butyl phthalate/kg feed were observed. All analyzed concentrations (0.125 – 2 g/kg feed) delayed testicular development.

The Annex XV dossier also contains a couple of studies on amphibians indicating estrogen agonist/androgen antagonist activity of DBP.

Lee et al. (2005a) reported structural changes of testes as hypoplasia, denudation of germ cells, vacuolization of Sertoli cell cytoplasm, thickening of lamina propria of seminiferous tubules, and focal lymphocytic infiltration in developing male *Xenopus laevis*.

Othani et al. (2000) investigated the effect of DBP on genetically male tadpoles of *Rana rugosa* and found that gonads of the control tadpoles all showed the typical structure of testes. In contrast, after 0.1, 1, and 10 µM DBP (27.8, 278 and 2780 µg/l) treatment, 0, 7, and 17% of tadpoles, respectively, develop gonads of complete or partial ovarian structure.

In addition, studies on amphibians indicates antithyroidal activity of DBP.

Two *in vitro* studies revealed antithyroidal (T3-antagonist) activity of DBP in amphibians (Shimada & Yamauchi. (2004); Sugiyama et al. (2005)). The publication of Sugiyama (2005) also included an *in vivo* study which could not confirm DBP T3-antagonism. Shen et al. (2011) observed decelerated spontaneous metamorphosis in *X. laevis* at concentrations of 10 and 15 mg/L. Moreover, Lee et al. (2005b) investigated developmental effects of DBP on *Xenopus* embryos using the 96-h frog embryo teratogenesis assay–Xenopus (FETAX). At 96 h the incidence of developmental malformations in the surviving tadpoles was 7, 9, 15, 37, 51, 53, 90, and 100% at 0.1, 0.5, 1, 5, 10, and 15 ppm DBP, respectively.

Mammalian

Dibutyl phthalate (DBP) is classified as a substance toxic to reproduction Repr. Cat. 1B; H360Df (May cause harm to the unborn child; Suspected of damaging fertility.). Further, DPB is identified as a substance of very high concern in accordance with Article 57(c), as well as 57(f) of Regulation (EC) 1907/2006 (REACH), as it is a substance with endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health.

DBP has been shown to adversely affect the endocrine system of mammals primarily through *in vivo* findings on reduced fetal testosterone. These findings are further substantiated by mechanistic findings, also *in vivo*, of down-regulation of genes in the steroidogenic biosynthesis pathway. The spectrum of adverse effects observed in rats include increased nipple retention, decreased anogenital distance, genital malformations, reduced number of spermatocytes and testicular changes including multinucleated gonocytes, tubular atrophy and Leydig cell hyperplasia.

In conclusion, the overlap of observed effects on the male reproductive system between DCHP, DEHP, and DBP exposure is substantial: Increased incidence of nipple retention (mammae areolae), reduced AGDi, signs of reduced sperm quality, seminiferous tubule atrophy, and decreased testis weight.

7.10.2. Endocrine disruption - Human health

The following text is extracted from the Annex XV dossier for DCHP (2016) and included for completeness, an assessment for endocrine disruption of human health was not conducted during substance evaluation. DCHP is already identified as an ED for Human health.

Estrogenic/Anti-estrogenic activity

DCHP gave mixed results in estrogenic in vitro assays. It induced MCF7 cell proliferation (Hong et al. 2005, Okubo et al. 2003) whereas its metabolite mono-cyclohexyl phthalate (MCHP) inhibited the 17β-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 ERβ (Takeuchi et al. 2005). 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), thus no estrogenic effects were detected. In summary, studies with DCHP have shown different results for estrogenic/anti-estrogenic activity in vitro. The available in vivo tests in rats do not show estrogenic/anti-estrogenic activity for DCHP.

Androgenic/anti-androgenic

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).

Steroidogenesis

DCHP inhibits the enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), both of which are involved in biosynthesis of androgens in testes (Yuan et al. 2012). Furthermore, in a screening assay assessing fetal testosterone production after in utero exposure DCHP significantly reduced testosterone production in the fetal testis (Furr et al. 2014).

Other hormones

Other *in vitro* assays indicate potential activity relating to effects on adipogenesis, thyroid receptor transcription and membrane signalling via the nicotinic acetylcholine receptor (nAChR) (Sargis et al 2010, Sugiyama et al 2005, Liu and Lin 2002, Lu et al 2004). Data showing adverse effects related to these hormonal effects are, however, not available, even though a change of thyroid weight and histopathological hyperthrophy of thyroid follicular cells were reported in the 2-generation study (Hoshino et al. 2005).

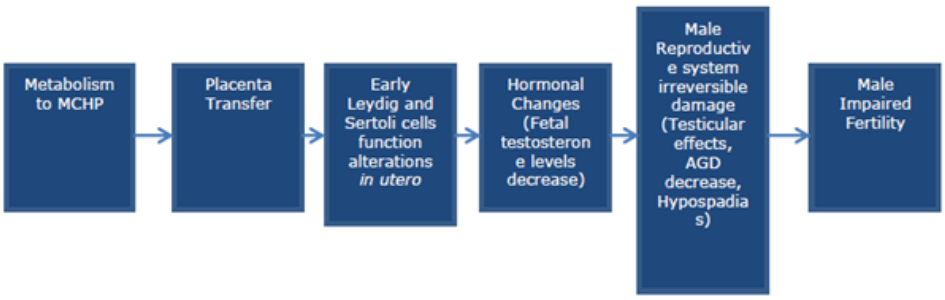
For the purpose of identification of DCHP as an endocrine disrupter, the WHO/IPCS Mode of Action/ Human Relevance Framework (MoA/HRF)7 has been used.

The assessment of the reliability of the available in vivo DCHP studies is presented in Annex III. All available information has been used in a weight of evidence approach using the Bradford Hill considerations.

The MoA/HRF framework focused on:

• the weight of evidence analysis
• the establishment of the MoA endocrine mediated irreversible effects observed with DCHP in experimental species
• the establishment of human relevance

The MoA/HRF analysis is based on the following hypothesised mode of action:



The detailed MoA analysis is presented in Annex II of the Annex XV dossier for DCHP including the elements of male reproductive system adverse effects, endocrine mediated mode of action, establishment of a plausible causal relationship between adverse effects and the endocrine mode of action, and human relevance. The summary of the species concordance analysis (human relevance) as developed with the use of the WHO/IPCS MoA/HRF are presented below.

Qualitative Concordance				Quantitative Concordance	
Key Event (name)	*(Evidence in Experimental Species)*	*(Evidence in Humans)*	*Confidence*	*(Evidence in Experimental Species)*	*Confidence*
Metabolism of DCHP to MCHP	Evidence based	Plausible and some evidence based	High		
Placenta Transfer	Likely, sufficient evidence available	Plausible	Medium		
Early Leydig and Sertoli cells function alterations *in utero*	Evidence based	Plausible	Medium	Evidence based (see section 5a in Annex II)	Medium
Hormonal changes (Fetal testosterone levels decrease)	Evidence based	Plausible	High	Evidence based (see section 5a in Annex II)	High
Male Reproduction system irreversible damage Testicular effects (Leydig cells, Sertoli cells, Epididymis)	Evidence based	Plausible	High	Evidence based (see section 5a in Annex II)	High
Male Impaired Fertility	Likely, not chemical specific (DCHP) based	Plausible	Medium		

Adverse effects:

DCHP caused adverse effects on the male reproductive system in more than one whole-animal toxicity study (Hoshino et al. 2005, Yamasaki et al. 2009, Saillenfait et al. 2009, Aydogan and Barlas 2013 and 2015) of acceptable quality (one OECD test guideline compliant standard 2-generation study and four non-standard studies) with relevant routes of exposure (oral via diet/gavage). The spectrum of effects observed in rats include increased areola mammae retention, decreased anogenital distance, prolonged preputial separation, genital malformations associated with small testis, signs of reduced sperm quality, atrophic tubules in prostate, prostatic intraepithelial neoplasia and testicular changes including tubular atrophy of which almost all can be considered adverse (OECD, 2008).

Endocrine mode of action:

The overall weight of evidence analysis shows that the male reproductive effects observed following in utero exposure to DCHP are mediated via an endocrine (antiandrogenic) mode of action that involves irreversible effects induced by interference with steroidogenesis during fetal development.

Plausible link between adverse effects and endocrine mode of action: The MoA framework analysis establishes a plausible relationship between the distinct key events identified for the hypothesised endocrine mediated mode of action. There is a plausible mechanistic link between the toxic effects of concern and endocrine disruption (an anti-androgenic mode of action) as the cause of the adverse effects in the male reproductive system, observed in the studies described above. This takes into account dose-response relationships and temporal association.

Human relevance:

The effects observed in experimental animals are judged to be relevant to human health on the basis of biological plausibility, taking into account existing knowledge on established pathways for male reproductive system development across species, as well as the absence of contradicting data to exclude human relevance.

7.10.3. Conclusion on endocrine disrupting properties and related classification and labelling

Environment

DCHP is identified as an endocrine disrupter for mammals with an antiandrogenic mode of action. The database relevant for assessing the environmental ED properties for DCHP affecting aquatic environmental species is however limited. One *in vitro* study indicating weak ER-receptor binding and two *in vivo* studies have been identified of which one gave some indications of endocrine mediated effects. In addition an FSDT study on zebrafish has been performed following a SEV decision (SEV decision 19 December 2018.)

For other similar phthalates more information is available. DEHP has been identified as an environmental endocrine disrupter and was listed on the candidate list in 2014. Several *in vivo* fish studies report ED-effects (oestrogen agonist/androgen antagonist) including:

-Inducing vitellogenin (e.g. male *P.promelas* & *D. rerio*)
-Inducing ovo-testis (*Salmo salar*)
-Skewing phenotypic sex ratio (*Salmo salar*)
-Effects on steroidogenesis (e.g *P. promelas*)
-Reduced reproductive output (*D. rerio*)

Also for DBP several *in vivo* studies reports oestrogen or anti-androgenic effects:

-Decrease vitellogenin (female *M. fluviatilis*)
-Decrease Spiggin (male *G. aculetaus*)
-Skewing phenotypic sex ratio (*S. lucioperca*)
-Effects on steroidogenesis (*Salmo salar*)
-Phenotypic sex reversal in tadpoles (*R. rugosa*). Genetically male tadpoles developed gonads of complete or partial ovarian structure.

DCHP has the same ED effects (antiandrogenic) in mammals as the phthalates listed on the candidate list for ED HH (DEHP, BBP, DBP and DIBP). Given that there are a number of studies on other phthalates including DEHP, DBP and BBP showing endocrine mediated effects (estrogenic or antiandrogenic) in fish and amphibians it is plausible that also DCHP may have endocrine disrupting effects in fish. An ERα receptor binding study by Urushitani *et al.* (2003) where DCHP and DEHP both showed weak but similar receptor binding affinities gives some support to such an assumption.

Of the two available *in vivo* studies with DCHP on fish, one, a 21 day screening test on Japanese medaka, did not indicate ED effects. Vitellogenin in male fish was not induced. The other study was a Medaka partial life cycle test performed by Japanese MoE according to a Japanese guideline. The study was similar to an OECD TG 234 but used fewer eggs. In this study there were some indications of endocrine effects. The sex ratio was significantly skewed towards more males in one out of five test concentrations (the next lowest dose 1.41 µg/l). A statistically significant increase in the gonadosomatic index was observed for males in the highest exposure group (35.8 µg/L). In addition, one of ten fish in the highest exposure group (35.8 µg/L) developed testis-ova. Finally, a statistically significant increase in vitellogenin in the liver of male fish was observed in one of the five test concentrations (the medium high concentration, 4.39 µg/l). The results from this study were not considered strong enough to conclude on the environmental ED-properties of

DCHP due to the lack of dose-response. However, the highest test concentration (35.8 µg/l) was well below the claimed water solubility of DCHP (ca. 1 mg/l).

To investigate if higher test concentrations would give more pronounced ED-effects a FSDT test was requested with a requirement to test up to the limit of water solubility (SEV decision 19 December 2018). The study was performed with Zebra fish and the nominal test concentrations were 10, 32, 100, 320 and 1000 µg/. It turned out that it was not possible to achieve stable test concentrations above 30 µg/l indicating that the true water solubility of DCHP may not be 1 mg/l. The fry mortality at the two highest concentrations, 82 and 100 % made them useless with respect to detection of ED-effects. So, whether or not DCHP may have ED-effects above a concentration above approx. 30 µg/l turned out to be an irrelevant question. The most pronounced indication of ED-effects in this study was significantly decreased female VTG at the lowest test concentrations 10.4 and 28.2 µg/l (measured conc.) There were also indications, although not statistically significant, that DCHP may skew the sex ratio towards females.

The results from the two FSDT studies are somewhat contradictory both within as well as between the studies (see Table 23Table 23). The VTG response in the medaka test, significant increase in male VTG levels at one test concentration, indicates that DCHP may act a weak estrogen agonist or androgen antagonist, while the significant decrease in female VTG at the two lowest test concentrations in the Zebrafish study indicates that DCHP acts as an aromatase inhibitor. The significant shift towards males in one dose in the Medaka study indicates that DCHP may act as an androgen agonist while in the Zebrafish test increased proportion of females in the two lowest test concentrations, although not statistically significant, is indicative of a weak estrogen agonist or androgen antagonist mode of action.

Table 23 Effects of DCHP in the two FSDT-tests and indications of mode of action according to table 1 in OECD TG 234.

Study	**VTG ♂**	**VTG ♀**	**Sex ratio**	**Indication according to table 1 in OECD TG 234**
Medaka	↑ Significantly higher in one of five test concentrations	No significant change	↑♂ Significantly skewed towards males in one test concentration	VTG indicates **weak estrogen agonist** or androgen antagonist. Sex ratio indicates **androgen agonist** However, inconclusive due to lack of dose-response
Zebrafish	No significant change	↓ **Significantly lower** in the two lowest test conc.	↑♀ Skewed towards females in all test concentrations. However, not statistically significant compared to solvent control. (significant compared to control)	VTG response indicate **Aromatase inhibitor** Sex ratio, however not statistically significant, indicates **weak estrogen agonist** or **androgen antagonist.**

To summarise: There are indications of ED effects in both studies and it cannot be ruled out that DCHP has ED effects on fish. However, effects are observed only at single test concentrations. Furthermore, the effects seen are contradictory and points in different

directions in relation to possible mode of action. No firm conclusions can therefore be drawn from the two FSDT studies.

However, a reduced reproductive output was observed when adult male zebrafish were exposed to DEHP for three weeks 0.2 µg/l DEHP before mating (Corradetti et al 2013). The decrease in embryo production was >90% decreased embryo production (P<0.01). A decreased reproductive output was also seen when female zebrafish were exposed to DEHP for three weeks (Carnevali et al. (2010). The embryo production was about 1% of control at an exposure concentration of 40 µg/l. Based on these findings an OECD TG 240 test for DCHP may be warranted as it also covers the reproductive phase of the life cycle. However, the findings in the two FSDT studies on DCHP were weak and contradictory. Furthermore, these studies have also revealed the difficulties in keeping stable test concentrations above 30 µg DCHP/l. It was therefore not considered justified to request an OECD TG 240 test for DCHP.

Thyroid effects.

The effects on thyroid in the 2-generation study (weight changes and hypertrophy) was observed at a dose level equivalent to ca 500 mg/kg bw/day DCHP (Hoshino et al (2005)). In contrast, no effects on thyroid weight was seen in the study by Yamasaki *et al.* (2009), however, the period for dosing in this study was shorter (GD6–PND20) than in the study by Hoshino et al. A study by Göktekin and Barlas (2017) revealed changes in thyroid hormone levels and histopathological changes from 20 mg/kg bw/day DCHP. As DCHP is already identified as an endocrine disrupter having antiandrogen activity, and is listed on the candidate list, it is not considered justified to request further studies on mammals.

The *in vitro* findings in a *Xenopus laevis* cell line suggests that DCHP has thyroid disrupting effects in tadpoles (Sugiyama et al. (2005)). The only available *in vivo* study on tadpoles known to us had a number of shortcomings, but indicates that DCHP exposure did not produce adverse effects on the tadpoles at or below its water solubility (Mathieu-Denoncourt (2014)). The FSDT study on zebrafish as well as the *in vivo* study on tadpoles has revealed that it seems to be impossible to keep stable DCHP concentrations higher than 30µg/l. The effects in the *in vitro* study were seen at a more than 200 times higher concentration than that (20µM, which is equivalent to 6.6 mg/l). No significant effects were seen at lower concentrations (0.8, 2 and 4 µM). Considering also that the FETAX study, although not fully reliable, did not reveal any effects below 4.1 mg DCHP/l it was not considered justified to request further studies on amphibians.

Overall Conclusion

DCHP has an antiandrogenic mode of action giving rise to adverse effects such as genital malformations associated with small testis, signs of reduced sperm quality, reduced AGDi, retained areola mammae, atrophic tubules in prostate, prostatic intraepithelial neoplasia and testicular changes including tubular atrophy. Consequently DCHP is identified as an ED for humans, as well as classified as Repr. 1B for developmental effects. These observations are very similar to the adverse effects observed after exposure to DEHP and DBP.

Considering the information on adverse antiandrogenic adversity from studies on mammals, it is considered plausible that DCHP can cause adverse effects in environmental mammalian species via endocrine activity. The adverse effects concerned such as reduced ability to produce semen or a malformed reproductive system are irreversible / long lasting reproductive changes. No firm conclusions can be drawn in relation to fish.

In the environment, adverse effects concerning development and reproduction are considered endpoints of particular relevance, because such effects are likely to manifest themselves at the population level. Malformed male reproductive organs and impaired spermatogenesis are irreversible developmental effects that manifests at reproductive age. The effects of DCHP observed in rats are of particular concern for wildlife species with a natural low reproductive output, including top predators and other mammals (including

endangered species), as negative effects on reproduction has an even higher potential for causing long-term negative effect at the population level for such taxa.

In addition, it is plausible that DCHP is a thyroid disruptor, as evidenced by observations indicating hyperactivity of the rat thyroid gland. DCHP has also been shown to have T3-antagonist activity *in vitro* in a *Xenopus laevis* cell line and it inhibited the T_3 dependent activation of the *TRβ* gene in the same cell line.

The CLP regulation has been amended with classification criteria for endocrine disruption for human health as well as for the environment (Commission Delegated Regulation (EU) 2023/707 of 19 December 2022).

The criteria for ED environment Cat 1 " *Known or presumed endocrine disruptors for the environment*" are the following:

(a) endocrine activity;

DCHP is identified as an endocrine disrupter for mammals with an antiandrogenic mode of action (see 7.10.2).

(b) an adverse effect in an intact organism or its offspring or future generations;

DCHP gives rise to adverse effects in mammals such as e.g. genital malformations associated with small testis, signs of reduced sperm quality, atrophic tubules in prostate, prostatic intraepithelial neoplasia and testicular changes including tubular atrophy. These effects are considered to be population relevant.

(c) a biologically plausible link between the endocrine activity and the adverse effect.

A biologically plausible link between endocrine activity and the adverse effects has been demonstrated (see 7.10.2).

The eMSCA concludes that, based on the available mammalian data, DCHP fulfills the CLP criteria for classification as endocrine disrupter for the environment category 1.

7.11. PBT and vPvB assessment

Not evaluated.

7.12. Exposure assessment

Not performed.

7.13. Risk characterisation

Not performed.

7.14. References

Ahbab, AM., Güven, C,. Koçkaya, EA. and Barlas, N. (2017): Comparative developmental toxicity evaluation of di-n-hexyl phthalate and dicyclohexyl phthalate in rats. *Toxicology and Industrial Health*, Vol. 33(9) 696–716. https://doi.org/10.1177/0748233717711868

Annex XV report for Dicyclohexyl phthalate (DCHP), Swedish Chemicals Agency in cooperation with Danish Environmental Protection Agency, 17 February 2016: https://echa.europa.eu/documents/10162/0f8a6fd1-835f-4686-8cca-bcbbdc137b73

Annex XV report for Bis(2-ethylhexyl) phthalate (DEHP), Danish Environmental Protection Agency, Denmark, 26 August 2014: https://echa.europa.eu/documents/10162/04233311-4be2-4a41-8c1b-8e6d0c6fe260

Annex XV report for Dibutyl phthalate (DBP), Danish Environmental Protection Agency, Denmark, 26 August 2014: https://echa.europa.eu/documents/10162/d3796777-6d15-4d7a-8ee8-e8eda0aff18f

Aoki KAA, Harris CA, Katsiadaki I, Sumpter JP. (2011): Evidence that DI-n-Butyl Phthalate has antiandrogenic effects in fish. Environmental Toxicology and Chemistry, 30, No. 6, 1338–1345.

Aydogan Ahbab, M. & Barlas, N. (2013): Developmental effects of prenatal di-n-hexyl pthalate and dicyclohexyl phthalate exposure on reproductive tract of male rats: postnatal outcomes. Food and Chemical Toxicology 51:123- 136.

Aydogan Ahbab, M. & Barlas, N. (2015): Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalateon fetal testicular development in rats. Toxicology Letters 233: 125-137.

Bhatia H, Kumar A, Du J, Chapman J, McLaughlin MJ. (2013): Di-n-Butyl Phthalate causes antiestrogenic effects in female Murray Rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry, 32, No. 10, 2335–2344.

Carnevali O, Tosti L, Speciale C, Peng C, Zhu Y, Maradonna F. (2010): DEHP Impairs Zebrafish Reproduction by Affecting Critical Factors in Oogenesis. PLOS One Vol. 5 Issue 4.

Corradetti B, Stronati A, Tosti L, Manicardi G, Carnevali O, Bizzaro D. (2013): Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (Danio rerio). Reprod. Biol. Sept;13(3):195-202.

Dang, Z., Li, K., Yin, H., Hakkert, B., & Vermeire, T. (2011): Endpoint sensitivity in fish endocrine disruption assays: regulatory implications. Toxicology letters, 202(1), 36-46.

ECHA 2015. Dissemination site – Dicyclohexyl phthalate. Available at http://apps.echa.europa.eu/registered/data/dossiers/DISS-dffb4072-e455-47ae-e044-00144f67d031/DISS-dffb4072-e455-47ae-e044-00144f67d031_DISS-dffb4072-e455-47ae-e044-00144f67d031.html (accessed on 23 July 2015).

Fredriksen, H., Skakkebaek, N.E., & Andersson, A.-M. (2007): Metabolism of phthalates in humans. Molecular Nutrition & Food Research, 51:899-911.

Furr, J.R., Lambright C.S., Wilson V.S., Foster P.M. & Gray Jr., L.E. (2014): A short-term In vivo Screen using Fetal testosterone production, a Key Event in the Phthalate Adverse Outcome Pathway, to Predict Disruption of Sexual Differentiation. Toxicological Sciences, Aug 1;140(2):403-24 doi:10.1093/toxsci/kfu081.

Göktekin, E and Barlas, N. (2017): Biochemical and Histopathological Effects of in Utero Di-N-Hexyl Phthalate and Di-Cyclohexyl Phthalate Exposure on the Thyroid Axes and T3, T4, TSH Hormone Levels of Male and Female Rats: at Adulthood. Erciyes Med J 2017; 39(4): 176-82. DOI: 10.5152/etd.2017.17069.

Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P, Bridges JW. (1986): Effects of phthalic acid esters on the liver and thyroid. Environ Health Perspect. Dec;70:195-210.

Hong, E. J., Ji, Y. K., Choi, K. C., Manabe, N. & Jeung, E. B. (2005): Conflict of estrogenic activity by various phthalates between *in vitro* and *in vivo* models related to the expression of Calbindin-D9k. Journal of Reproduction and Development, 51: 253-63.

Hoshino, N., Iwai, M. & Okazaki, Y. (2005): A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. The Journal of Toxicological Sciences, 30: 79-96.

Howarth JA, Price SC, Dobrota M, Kentish PA, Hinton RH. (2001): Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. Toxicol Lett. 2001 Apr 8;121(1):35-43.

Japanese Ministry of Environment, 2003. Available from: http://www.env.go. jp/en/chemi/ed/rt medaka.pdf.

Jarmołowicz S, Demska-Zakęś K, Zakęś Z. (2013): Impact of di-n-butyl phthalate on reproductive system development in European pikeperch (Sander lucioperca). Acta Vet. Brno 82: 197–201.

Lake, B. G., Phillips, J. C., Linnell, J. C. & Gangolli, S. D. (1977): The *in vitro* hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. Toxicology and Applied Pharmacology, 39: 239-248.

Lee SK, Veeramachaneni DNR. (2005a): Subchronic Exposure to Low Concentrations of Di-n- Butyl Phthalate Disrupts Spermatogenesis in Xenopus laevis Frogs. Toxicological Sciences 84, 394–407.

Lee SK, Owens GA, Veeramachaneni DNR. (2005b): Exposure to Low Concentrations of Din-butyl Phthalate during Embryogenesis Reduces survivability and Impairs Development of Xenopus Laevis Frogs. Journal of Toxicology and Environmental Health, Part A, 68:763–772.

Liu, P.-S. & Lin C.-M. (2002): Phthalates Supress the Calcium Signaling of Nicotinic Acetylcholine Receptors in Bovine Adrenal Chromaffin Cells. Toxicology Applied Pharmacolology, 183:92-8 doi:10.1006/taap.2002.9466.

Lu K.-Y., Tseng F.-W., Wu C.-J & Liu P.-S. (2004): Suppression by phthalates of the calcium signalingo f human nicotinic acetylcholine receptors in human neuroblastoma cells SH-SY5Y cells. Toxicology 200:113-21 doi:10.1016/j.tox.2004.03.018.

Lv, Y., Fang, Y., Chen, P., Duan, Y., Huang, T., Ma, L., Xieb. L., Chen, X., Chen, X., Gao, J. and Ge R-S. (2019): Dicyclohexyl phthalate blocks Leydig cell regeneration in adult rat testis. *Toxicology* Volume 411, 60-70. https://doi.org/10.1016/j.tox.2018.10.020

Mathieu-Denoncourt J. (2014): Lethal and sublethal effects of phthalates in western clawed frog. A thesis Submitted to the Division of Graduate studies of the Royal Military College of Canada https://central.bac-lac.gc.ca/.item?id=TC-OKR-101&op=pdf&app=Library&oclc_number=1032927654

Mathieu-Denoncourt J, Martyniuk CJ, Loughery JR, Yargeau V, de Solla SR, Langlois VS. (2016): Lethal and sublethal effects of phthalate diesters in Silurana tropicalis larvae. Environ Toxicol Chem. 2016 Oct;35(10):2511-2522. doi: 10.1002/etc.3413. Epub 2016 Jun 21. PMID: 26924002l

Nakai, M., Tabira, Y., Asai, D., Yakabe, Y., Shimyozu, T., Noguchi, M., Takatsuki, M. & Shimohigashi, Y. (1999): Binding characteristics of dialkyl phthalates for the estrogen receptor. Biochemical and Biophysical Research Communications, 254: 311-314.

Nishihara, T., Nishikawa, J., Kanayama, T., Dakeyama, F., Saito, K., Imagawa, M., Takatori, S., Kitagawa, Y., Hori, S. & Utsumi, H. (2000): Estrogenic Activities of 517 Chemicals by Yeast Two-Hybrid Assay. Journal of Health Science, 46: 282-298.

Norman A, Borjeson H, David F, Tienpont B and Norrgren L. (2007): Studies of uptake, elimination and late effects in Atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Arch Environ Contam Toxicol 52(2):235-42.

Norrgren L, Blom A, Andersson PL, Borjesson H, Larsson DGJ and Olsson P-E. (1999): Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management, Vol 2/3. pp.311-317.

OECD 2008. Guidance document on mammalian reproductive toxicity testing and assessment. Series on testing and assessment No. 43. ENV/JM/MONO(2008)16.

Ohtani H, Miura I, Ichikawa Y. (2000): Effects of Dibutyl Phthalate as an Environmental Endocrine Disruptor on Gonadal SexDifferentiation of Genetic Males of the Frog Rana rugosa. Environmental Health Perspectives, Vol. 108, No. 12. 1189-1193.

Okubo, T., Suzuki, T., Yokoyama, Y., Kano, K. & Kano, I. (2003): Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay *in vitro*. Biological and Pharmceutical Bulletin, 26: 1219-24.

Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. (1997): Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. Food Chem Toxicol. Feb;35(2):225-39.

Saillenfait, A. M., Gallissot, F. & Sabate, J. P. (2009): Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology, 29: 510-21.

Saito, T., Hong, P., Tanabe, R., Nagai, K. & Kato, K. (2010): Enzymatic hydrolysis of structurally diverse pthalic acid esters by porcine and bovine pancreatic cholesterol esterases. Chemosphere 81: 1544-1548.

Sargis, R.M., Johnson, D.N., Choudhury, R.A & Brady, M.J. (2010): Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. Obesity (Silver Spring). Jul;18(7):1283-8. Doi: 10.1038/oby.2009.419.

Shen O, Wu W, Du G, Liu R, Yu L, Sun H, Han X, Jiang Y, Shi W, Hu W, Song L, Xia Y, Wang S, Wang X. 2011. PLoS ONE. April 2011 | Volume 6 | Issue 4 | e19159.

Shimada N & Yamauchi K. 2004. Characteristics of 3,5,3-triiodothyronine (T3)-uptake system of tadpole red blood cells: effect of endocrine-disrupting chemicals on cellular T3 response. Journal of Endocrinology 183, 627–637.

Sugiyama S., Shimada, N., Miyoshi, H. & Yamauchi, K. (2005): Detection of Thyroid System –Disrupting Chemicals using in Vitro and in Vivo Screening Assays in Xenopus laevis. Toxicological Sciences. 88(2):367-74 doi:10.1093/toxsci/kfi330.

Takeuchi, S., Iida, M., Kobayashi, S., Jin, K., Matsuda, T. & Kojima, H. (2005): Differential effects of phthalate esters on transcriptional activities via human estrogen receptors alpha and beta, and androgen receptor. Toxicology, 210: 223-33.

Tollefsen KE, Meysc JFA, Frydenlund J, Stenersen J(2002). Environmental estrogens interact with and modulate the properties of plasma sex steroid-binding proteins in juvenile Atlantic salmon (Salmo salar). Marine Environmental Research 54 697–701.

Uren-Webster TM, Lewis C, Filby AL, Paull GC, Santos EM (2010). Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. Aquatic Toxicology 99 (2010) 360–369.

Urushitani, H., Nakai, M., Inanaga, H., Shimohigashi, Y., Shimizu, A., Katsu, Y., and Iguchi, T. (2003): Cloning and characterization of estrogen receptor a in mummichog, *Fundulus heteroclitus*. *Mol. Cell. Endocrinol.* 203, 41–50. https://doi.org/10.1016/S0303-7207(03)00118-7.

US EPA 2020. Final Scope of the Risk Evaluation for Dicyclohexyl Phthalate; EPA document# EPA-730-R-20-019 https://www.epa.gov/sites/default/files/2020-09/documents/casrn_84-61-7_dicyclohexyl_phthalate_final_scope.pdf

Wang X, Yang Y, Zhang L, Ma Y, Han J. (2013): Endocrine disruption by di-(2-ethylhexyl)-phthalate in Chinese rare minnow Gobiocypris rarus). Environmental Toxicology and Chemistry, Vol. 32, No. 8, pp. 1846–1854.

Yamasaki, K., Takeyoshi, M., Yakabe, Y., Sawaki, M., Imatanaka, N. & Takatsuki, M. (2002): Comparison of reporter gene assay and immature rat uterotrophic assay of twenty-three chemicals. Toxicology, 170: 21-30.

Yamasaki, K., Okuda, H., Takeuchi, T. & Minobe, Y. (2009): Effects of *in utero* through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicology Letters, 189: 14-20.

Yuan, K., Zhao, B., Li, X. W., Hu, G. X., Su, Y., Chu, Y., Akingbemi, B. T., Lian, Q. Q. & Ge, R. S. (2012): Effects of phthalates on 3beta-hydroxysteroid dehydrogenase and 17beta-hydroxysteroid dehydrogenase 3 activities in human and rat testes. Chemico-Biological Interactions, 195: 180-188.

Ye T, Kang M, Huang Q, Fang C, Chen Y, Shen H, Dong S. (2014): Exposure to DEHP and MEHP from hatching to adulthood causesreproductive dysfunction and endocrine disruption in marine medaka(Oryzias melastigma). Aquatic Toxicology 146. 115– 126.

7.15. Abbreviations

AGD Anogenital distance

BBP butylbenzyl phthalate

DBP di-n-butyl phthalate

DCHP dicyclohexyl phthalate

DEHP di(2-ethylhexyl) phthalate

ED endocrine disruptor

FSH Follicle Stimulating Hormone

GD Gestation day

MCHP moncyclohexyl phthalate

MoA/HRF Mode of Action/Human Relevance Framework

MSC Member State Committe

PND Postnatal day

PROC Process category

RAC Risk Assessment Committee

REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning Registration, Evaluation, Authorisation and Restriction of Chemicals

TBD To be determined

WHO/IPCS World Health Organisation/International Programme on Chemical Safety