Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Dicyclohexyl phthalate

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

CLH-O-0000001412-86-38/F

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DICYCLOHEXYL PHTHALATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.
All attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website.

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Substance name: Dicyclohexyl phthalate

CAS number: 84-61-7

EC number: 201-545-9

Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	France		MemberState	1
Comment received				
Paragraph 2.2 only explicit the justification for Repro classification. Sensitisation is missing.				
Dossier Submitter's Response				
Thank you for your comment. Yes, this information is missing; unfortunately we forgot to add a scientific justification for why we propose that DCHP should be classified for skin sensitization. The available data (Stimulation index>1.6) from the non-radioactive local lymph node assay (LLNA:BrdU-ELISA OECD TG 442B) clearly indicate that DCHP should be classified as Skin Sens I. There are many epidemiological studies indicating that phthalates may increase the risk for allergic reactions (reviewed e.g. by Jurewicz & Hanke, 2011, and Jaakkola & Knight, 2008). An adjuvant MoA has been discussed (Hansen et al 2007, He et al, 2013). From a regulatory point of view, these effects have been controversial since no phthalate yet is classified as a direct allergen. This is the first example where a phthalate in a regulatory OECD test guideline study is shown to clearly affect the immune system (being a sensitizer), and it is therefore justified to have a harmonized classification for sensitization for this phthalate. Hansen et al., 2007. Adjuvant effects of inhaled mono-2-ethylhexyl phthalate in BALB/cj mice. Toxicology 232: 79-88. He et al., 2013. Effects of airway exposure to di-(2-ethylhexyl)phthalate on allergic rhinitis. Immunopharmacol Immunotoxicol 35(3): 390-5. Jaakkola & Knight, 2008. The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. Environ Health Perspect 116(7): 845-53. Jurewicz & Hanke, 2011. Exposure to phthalates: reproductive outcome and children health. A review of epidemiological studies. Int J Occup Med Environ Health 24(2): 115-41.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.04.2014	Germany		MemberState	2
Comment received				
The DE CA supports the harmonized classification of DCHP as proposed by the SE CA.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted. However, RAC considers that the effects following exposure to DCHP in the 2-generation reproductive toxicity study as well as in the supporting studies are not considered as clear adverse effects on fertility and sexual function. Some effects are reported in the prostate, epididymis and the testes in adult rats but as they occur after *in utero* exposure to DCHP, they can be supportive of developmental effects. Testis tubular atrophy have also been reported when juvenile and adult rats are exposed to DCHP, however, at very high doses. RAC therefore considers that no classification for effects on fertility is required.				

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2014	Norway		MemberState	3
Comment received				
Norway would like to thank Sweden for the proposal for harmonised classification and labeling of dicyclohexyl phthalate, CAS- no. 84-61-7. We support the proposal to classify dicyclohexyl phthalate with repr 1B, H360FD based on the observed findings. This includes the effects on anogenital distance as well as on the occurrence of mammaae/nipple retention in male pups, the effect on male reproductive organs (testicular atrophy, reduced testicular spermatid head count and decreased weight of the prostate and of the levator ani/bulbocavernosus) and the antiandrogenic mode of action. The antroangrogenic mode of action are also seen for other ftalates already classified with repr 1B.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted. See RAC's respons to comments number 2.				

Date	Country	Organisation	Type of Organisation	Comment number
11.04.2014	Finland		MemberState	4
Comment received				
We support the proposed classification according to CLP Regulation: Skin Sens 1 and Repr.1B;H360FD for Dicyclohexyl phthalate.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted. See RAC's respons to comments number 2.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	France		MemberState	5

Comment received

Study from Lake, 1982 (rat, 7d) shows that MCHP (metabolite of DCHP) produces marked testicular atrophy. DCHP displayed tubular atrophy of germ cells at high dose in one animal. However, this study and the one of Grasso are performed at dosage that is too high for the data to be taken into account for classification purpose.

In general, the studies reported in table 13 miss details on maternal and general toxicity to allow clearly picturing overt toxicity. It seems that the high dose (around 500 mg/kg bw/d for the studies of Hoshino and the one of Yamasaki) affects body weight of parents and pups in a way that is not dramatic not demonstrating any overt toxicity. Moreover, the pattern of effects on pups body weight during lactation have been described with other endocrine disruptors such as BPA (Tyl et al., 2008). However, body weight modification can affect AGD for example. Therefore, data should be displayed in order to be able comparing AGD modification with parental and pups body weight modifications.
Comparison of the effects found on prostate across the different studies might be worth performing. This effect appears coherent across studies and might worth digging into it.

The testicular toxicity described in different studies is coherent with findings described with other phthalates.
Effects on AGD and areola mammae in males in the Hoshino study are coherent with those described with DnHP which was classified by RAC as Repro 1B FD at equivalent doses (Saillenfait, 2009). Effects on AGD described by Saillenfait with DCHP are also in line with those the team described with DnHP. They are also coherent with DEHP, that decreases relative AGD of 18% at 500mg/kg bw/d (Saillenfait, 2009).
Regarding developmental toxicity no other findings than those above were reported with DCHP. This should be stated and discussed.

A thorough comparison of the effects observed with maternal tox and with existing classified phthalate (dose at which the findings are described) should be provided. In conclusion, the information reported in the dossier cannot allow supporting 1B instead of category 2. Additional data should be provided to ascertain the outcome of the discussions in the Committee.

Saillenfait,A.M., Gallissot,F., and Sabaté,J.P. 2009. Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. Journal of Applied Toxicology: JAT 29(6):510-521.
Saillenfait,A.M., Sabaté,J.P., and Gallissot,F. 2009. Effects of in utero exposure to di-n-hexyl phthalate on the reproductive development of the male rat. Reproductive Toxicology (Elmsford, N.Y.) 28(4):468-476.
Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM (2008) Two-generation reproductive toxicity study of dietary bisphenol a in CD-1 (Swiss) mice. Toxicological Sciences 104, 362-384.

Dossier Submitter's Response

Thank you for your comments and for supporting our view that the observed testis toxicity and the effects on anogenital distance and on areola mammae in males are consistent with what has been observed for other phthalates.

- We do agree that the dose level (2500 mg/kg/day) where atrophy of the seminiferous tubuli (1 out of 5 animals) was observed in young male rats that had been dosed for 7 days (Lake et al., 1982) is too high to warrant classification. The information was included in the CLH report because it adds value to the interpretation of the result in the study by Hoshino et al. In this study, atrophy of the semniferous tubuli was only observed in the F_1 generation at the high dose level (6000 ppm). This can be interpreted as adult animals (F_0) are less sensitive as compared to animals exposed during their entire life cycle (F_1). The results from the study by Lake as well as the reference to the study by Grasso (1979) where testis atrophy was observed in adult animals at a very high dose (4200 mg/kg) were included since these results show that atrophy can indeed be induced in rats that have not been exposed during their entire lifecycle. So although the limited information suggest that the potency of DCHP to induce testicular toxicity is low (as compared to other phthalates), the overall observed age-dependent sensitivity towards tetstis toxicity resembles what has been described for phthalates as a group (see p 46 in the NAS publication "Phthalates and cumulative risk assessment. The task ahead [2008, available at http://www.nap.edu/catalog.php?record_id=12528] and the EU RAR on DEHP [2008, available at http://echa.europa.eu/documents/10162/e614617d-58e7-42d9-b7fb-d7bab8f26feb]).

- We agree with your statement that the anogenital distance is directly correlated to the weight of the pups and that is the reason why both the absolute distance as well as the relative anogenital distance (that has been normalized relative to the cube root of the body weight-in accordance with recommendation in reference literature [Gallavan, R.H. et al., Reproductive Toxicology 13, 383-390, 1999]) is presented in Table 13. Thus by focusing on the relative AGD value effects that are due to effects on fetal weight (that might be secondary to effects on maternal body weight gain) has been taken into account. However we realize that this could have been better specified in Table 15 and in the discussion in section 4.12.4.

- When just looking at the magnitude of the reported values for the relative anogenital distance – see end of this paragraph - the observed effect might at first glance not seem to be convincing. As a reference, Holson and collegues state on page 400 in their chapter "Significance, Reliability, and Interpretation of Developmental and Reproductive Toxicity Study Findings" (Developmental and Reproductive Toxicology – A Practical Approach ,ed R. D. Hood, 2nd edition, Taylor & Francis 2006): *"The most frequently observed adverse effect on the AGD is a reduced distance in males in response to antiandrogenic agents or 5α-reductase inhibitors. When mean litter anogenital distances of approximately 20 litters are evaluated, differences of 5% or greater are generally indicators of reproductive toxicity"*. Thus we conclude that the observed reduction in the relative AGD that was observed in three different studies should be regarded as a clear adverse effect.

  Relative anogenital distance

  A. Hoshino et al., 2005, (dietary administration, pups examined on postnatal day 4) at 6000 ppm: F_1: -8% ($p<0.01$); F_2: -9% ($p<0.01$); at 1200 ppm: F_2:-7% ($p<0.01$).
  B. Yamasaki et al., 2009, (oral gavage, pups examined on postnatal day 4) at 500 mg/kg: F_1:-13% ($p<0.05$). No information provided for lower dose levels.
  C. Saillenfait et al., 2009a, (oral gavage, fetuses examined on GD 21): -8 ($p<0.01$), -11 ($p<0.01$), -14% ($p<0.01$) at 250, 500 or 750 mg/kg, respectively.

  - As we see it, the information presented in Table 13 of the CLH report (available at http://echa.europa.eu/documents/10162/09a4185e-cdc4-4978-83b1-cc5d86188ec9) clearly describes the level of toxicity (parental as well as developmental) in the studies by Hoshino et al. (2005); Yamasaki et al. (2009) and Saillenfait et al. (2009a). Unfortunately, the paper by Aydogan Ahbab & Barlas (2013) does not

contain detailed information and thus the information tabulated for this study is not as detailed as for the three other studies. For an overview of reproductive and general toxicity of a number of ortho-phthalates we would like to refer the reader to Table 19 and Annex 1 of the Background document to the RAC Opinion on 1,2-Benzenedicarboxylic acid, Dihexylester, branched and linear, that is available at http://echa.europa.eu/documents/10162/3a1a1bf3-8721-4bc5-a0f3-e367217ad6d6.

- It is correct that there are no other developmental effects than decreased anogenital distance, nipple retention, and hypospadias described in the studies, i.e. no effects on fetal viability or other malformations were reported. This is clearly stated in table 13 and in the text of the CLH report.

We conclude that reduction of the relative anogenital distance and nipple retention were observed in three independent studies and hypospadia was observed in the study by Yamasaki et al. (2009). The effects were recorded in absence of overt toxicity, i.e. the effects are considered to be specific and not secondary unspecific to other toxic effects. Therefore there are clear evidence (and not "some evidence") of specific adverse effect on development. Furthermore there is no indication that the proposed mechanism (antiandrogenic effect) is not relevant for human. Therefore classification in Category 1B is warranted for developmental toxicity.
See response to comment number 6 for discussion on the classification of effects on fertility and response to comment number 8 for further comments on the MoA.

RAC's response

Noted. See RAC's respons to comments number 2.

Date	Country	Organisation	Type of Organisation	Comment number
15.04.2014	Netherlands	RIVM	National Authority	6

Comment received

Effects on sexual function and fertility

Effects on sexual function and fertility by DHCP are mainly observed in the F1 and F2 of multiple generation studies but not in the F0 of these studies. This indicates that these effects are most likely due to the in utero exposure to DCHP. Therefore, these effects should be used for classification for developmental toxicity and not for effects on sexual function and fertility. This is also in line with the classification of a number of other phthalates. The only evidence of a direct effect on sexual function in adult animals is in the repeated dose toxicity study (Lake et al., 1982). At a dose of 2500 mg/kg bw/day one out of 5 exposed showed bilateral tubular atrophy affecting 30-40% of the germinal cells. In the absence of further information, this effect in a single animal would not result in classification for effects on sexual function and fertility. Taken into account the known effects of most phthalates on sexual function and fertility, it could be argued to also classify also DCHP for effects on sexual function and fertility but in category 2 and with an SCL above the GCL (for example 10%) at most seen the low potency.

Developmental effects

We agree that the observed developmental effects including the effects on the sexual function in the F1 and F2 generation warrant classification in category 1B as the effects are consistent with other phthalates. However, the available data indicate that the potency of DCHP is low and could be close to or above the border of 400 mg/kg bw/day between low and medium potency for setting SCLs. Therefore, it is suggested to calculate the ED10 for

the effects warranting classification to determine the need for an SCL.

Dossier Submitter's Response

Thank you for your comments.

Fertility

We agree with the NL CA that the fertility classification is not clear cut and we think that there are two things that need to be clarified:

A. Should the observed testicular toxicity that was observed in the F_1 generation in the study by Hoshino et al. (2005) be regarded as a "developmental toxicity" or as a "fertility" finding?

B. Can the observed effects be viewed as "clear evidence" (i.e. Category 1B) or "some" evidence (i.e. Category 2)?

A. The wordings of the different paragraphs in Annex I of the CLP regulation that defines developmental toxicity and effects on fertility and sexual function are not consistent.

In paragraph 3.7.1.1 it is stated "*Reproductive toxicity includes adverse effects on sexual function and fertility **in adult males and females**, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC […]."*

Paragraph 3.7.1.3 states " *Adverse effects on sexual function and fertility*

*Any effect of substances that has the potential to interfere with **sexual function and fertility.** This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."*

In paragraph 3.7.1.4 it is stated "*Adverse effects on development of the offspring*

*Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the **conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation**. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means **adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism.**"*

One could thus view the observed testicular toxicity finding in the F_1 generation as developmental toxicity because although the effect is observed at an adult stage, it is a structural abnormality that originates from exposure during pregnancy and, if consistent with other phthalates, early lactational phase.

However, one could also argue that the observed effect should be considered as an effect on fertility because although the criteria partly imply that fertility is an effect observed in adult animals or associated with timing of becoming adult, they do not specify that fertility effects recognized at an adult stage must be associated with exposure during an adult stage in order to fulfill the criteria for classification for effects on fertility. Thus, in these circumstances were the the criteria bring uncertainities on how to assign the effect we would suggest that the observed testicular toxicity in the F_1 generation is not specified with a differentiation - i.e. H360 is used. If RAC does want to include the differentiation we

suggest H360DF.

We note that Annex VI to CLP contains phthalates classified with differentiations both as DF and Df.

B. In the study by Hoshino et al. (2005), diffuse atrophy of the semniferous tubuli (severe grading) was recorded in 3 high dose F_1 males (6000 ppm) and focal atrophy (slight severity) was recorded in 1, 0, 2, 6 F_1 males in the control, low, intermediate and high dose group. In total, 9 out of 22 high dose F_1 males (6000 ppm) displayed signs of testis toxicity. The studies by Yamasaki et al. (2009) and Aydogan Ahbab & Barlas (2013) can only be used as weak supporting studies to the study by Hoshino. The study by Yamasaki just states that "Histologically, decreased testicular germ cells were detected in some rats in the 500 mg/kg/day group". Unfortunately, no further data is provided in the paper on severity grading or on incidence. In the paper by Aydogan Ahbab & Barlas, were histopathology was performed on prepubertal/pubertal/adult rats that had been exposed in utero (0, 20, 100 or 500 mg/kg/day, GD 6-19, oral gavage), incidence data but no severity grading was provided. In this study a dose–dependent increase of the incidence of tubular atrophy (numbers of affected animals: 0/10, 6/10, 5/10, 8/10; 0/10, 3/10, 8/10, 10/10 at the different dose levels of pre-pubertal and pubertal rats, respectively) was recorded. In adult animals, a much lower and not statistically significant incidence of tubular atrophy was recorded (0/10, 2/10, 0/10, 2/10 at the different dose levels): In addition, an increase of the incidence of sertoli cell vacuolization (0/1, 6/10, 4/10,8/10, at the different dose levels) was recorded in the adult animals.
Also other signs of testis toxicity were recorded in the study by Hoshino, such as a dose dependent and statistically significant decrease in the number of testicular homogenization resistant spermatids (spermatid head count in testis) at the high (-24%, $p<0.01$, 6000ppm) and intermediate dose levels (-15%, $p<0.05$, 1200 ppm) in F_1 only. A lack of sperm was observed in the epididymal tubes in the three high dose F_1 males with diffuse atrophic seminiferous tubules; this could partly explain the somewhat lower, but not statistically significant, decrease of the group mean number of epididymal sperm that was recorded in this dose group (599.7 ±219.22 as compared to 728 ± 88 in the control). There were no effects on the group level on the motility or morphology of epididymal sperm in F_0 or F_1. Other relevant effects were reduced relative weight (-19%, $p<0.05$) of the prostate at the high dose level (6000 ppm) in the F_1 males (no adverse finding at histopathological examination). An effect on the prostate weight was also recorded in the the study by Yamasaki (-28% at 500 mg/kg; –16% at 20 mg/kg, no effect at 100 mg/kg) and on the weight of the levator ani/bulbocavernosus muscle (-12% at 500 mg/kg) in adult animals exposed (oral gavage) in utero up to weaning.

We conclude that if one considers the atrophy of the semniferous tubuli (that was observed in the F_1 generation) as being a fertility effect then there is clear evidence of adverse effects on fertility (i.e. Cat 1B). However, if this toxicity should be considered as developmental toxicity, we agree that the remaining effects together with the well known fact that other phthalates do cause testis tox are better described as "some evidence" (i.e. Cat 2).

Developmental toxicity and specific concentration limits for developmental toxicity

We thank the NL CA for supporting our classification of DCHP in 1B for developmental toxicity. Below we provide the calculations of ED_{10} values for effects that warrant classification in 1B.

Table 1 Daily chemical intake, unit: mg/kg/day (Table 1, Hoshino et al., 2005)

DCHP (ppm)		Control	240	1200	6000

		(0)			
F_0 Males		-	15.88 ± 1.07	79.57 ± 3.3	401.8 ± 15.6
F_0 Females	Total study period	-	20.80	104.19	**510.7**
	Pre-mating	-	17.70 ± 0.95	88.83 ± 6.39	434.6 ± 29.6
	Gestation	-	14.30 ± 0.88	69.77 ± 4.04	349.0 ± 15.8
	Lactation	-	37.92 ± 2.50	191.6 ± 12.3	932.8 ± 58.2
F_1 males		-	17.84 ± 0.86	89.89 ± 5.01	**457.4 ± 17.3**
F_1 females	Total study period	-	20.95	**107.15**	534.2
	Pre-mating	-	19.27 ± 1.36	98.88 ± 8.34	483.0 ± 25.7
	Gestation	-	14.11 ± 0.98	72.41 ± 4.18	350.9 ± 19.4
	Lactation	-	33.70 ± 2.27	170.6 ± 14.0	896.7 ± 63.2

Effects that warrants classification for developmental toxicity were:
Study by Hoshino et al. (2005):

- Reduced AGD and nipple retention was observed in the F_1 generation at 6000 ppm and in the F_2 generation at 6000 and 1200 ppm. Thus for both endpoints **LOAEL** is 1200 ppm. When calculating the corresponding dose in mg/kg/day we have used the average dose during the total study period for the F_1 female (i.e. **107.15 mg/kg/day**)

- Testisatrophy was observed in F_1 males at the 6000 ppm dose level. Since this endpoint is recorded in the F_1 males after end of study one needs to take the exposure of the F_1 male in utero (total study period dose to F_0 dams) as well as the exposure of the F_1 male ex utero into account i.e ((510.7 + 457.4)/2) = **484.05 mg/kg/day**

Study by Yamasaki et al. (2009):

- Hypospadias: LOAEL 500 mg/kg.
- Nipple retention and reduced **AGD** recorded at 500 mg/kg. However no information provided for lower dose levels.

Study by Saillenfait et al. (2009a):

- Reduced anogenital distance: No LOAEL specified (lowest dose were effects was observed was 250 mg/kg.

Overall the lowest LOAEL for an effect that warrents classification (reduced anogenital distance and nipple retention in F_2 male pups in the study by Hoshino) is **107.15 mg/kg/day.**

ED_{10} calculations

Table 2. Anogenital distance in F_2 pups – data from Table 8 in Hoshino et al., 2005.

Dose (mg/kg/day)	0	20.95	107.15	534.2
F_2 male AGD/$BW^{1/3}$ (mm)	2.072 ± 0.152	2.020 ± 0.125	**1.932** ± 0.158**	1.882 ± 0.129**
		NOAEL	LOAEL	
F_2 female AGD/$BW^{1/3}$ (mm)	0.943 ± 0.072			

** = p<0.01.

From the data in the table above it is obvious that the dose response curve for anogenital distance is very shallow. However, it should be noted that the AGD can never become zero, as the minimum value is probably the AGD expressed in females (having maximum estrogenic influence). If ignoring this fact the ED_{10} value in this case would be the dose that corresponds to an AGD value of 1.8648 mm (i.e. a 10% reduction of the F_2 male control AGD) and that dose would be > 534.2 mg/kg/day. In this situation when the LOAEL (107.15) will be clearly below the the ED_{10} (>534.2) value and falling into a higher potency group compared to the potency group based on ED_{10}, paragraph 3.7.2.5.5.3 of the Guidance on the application of the CLP criteria states that the higher potency group should be used Thus, DCHP is a reproductive toxicant of medium potency (>4 mg/kg bw/day and <400 mg/kg bw/day).

Another way to approach this would be to acknowledge the fact that for males the AGD can at its minimum just be the "female" AGD and take that into account in the calculations. Using this approach, the ED_{10} value would be the dose that corresponds to an AGD of 1.9591 mm (2.072-0.1 x (2.072-0.943)). With this approach one could thus conclude that the ED_{10} value would be somewhere between 20.95 and 107.15 mg/kg.

In conclusion

The lowest ED_{10} value is 107.15 mg/kg/day or somewhere between 20.95 and 107.15. Both these ED_{10} values falls within the limits for a medium potency SCL (ED_{10} > 4 mg/kg bw/day, and < 400 mg/kg bw/day), i.e. a SCL of 0.3% should be applied for developmental toxicity.

RAC's response

Respons to classification for fertility. See RAC's response to comments number 2.

Respons to the setting of SCL for development: The DS suggests to take into account the female AGD in control animals in the calculation of ED_{10} for effect on AGD in males. However, for the calculation of the ED_{10} for effects on male AGD it is considered that the control value for male AGD should be used.

Date	Country	Organisation	Type of Organisation	Comment number
16.04.2014	Italy	C.O.I.M. SpA	Company-Manufacturer	7

Comment received

In order to support Classification as Reprotox cat. 2 there is a study on laboratory rats about Chronic exposure or carcinogenicity [Lefaux, R. Practical Toxicology of Plastics. Cleveland: CRC Press Inc., 1968, p. 350 **Peer Reviewed**] that evaluated reprotoxicity over four generations: this study give negative results. Particularly, it stated that "Investigations into the reproduction of rats thus treated and various biological and histological exams showed nothing abnormal". This study can support Classification as Reprotox cat 2 because rats did not show any anomalies compared with control rats of same generation and normal reproduction and no anomalies were found in parturition or nursing.

These conclusion are been submitted also in IUCLID 4 dossier created by European Commission – European Chemicals Bureau in 19/02/2000.

Moreover, the results of the studies in toxicological part of Registration Dossier are in line with the Australian National Industrial Chemicals Notification and Assessment (NICNAS) report on phthalate esters, in which 24 phthalates esters (carbon side chain backbone lengths ranging from C1 to C13) were reviewed (NICNAS, 2008). In this assessment reproductive and developmental adverse effects were predominantly associated with phthalate esters with side chain backbone lengths ranging from C4 to C6 (DCHP is C4).

Dossier Submitter's Response
The book that contained the information regarding this study (Lefaux, R., *Practical Toxicology of Plastics.* Cleveland: CRC Press Inc., 1968, p 349- 350.) was not available to the DS. However it is noted that the information in the IUCLID dossier of 19 february 2000 (available at http://esis.jrc.ec.europa.eu/doc/IUCLID/datasheet/84617.pdf) is very minimal, i.e. "***Exposure period:*** *4 generation;* ***Doses:*** *100 mg/kg diet;* ***Result:*** *Normal parturition, no anomalies in parturition and nursing;* ***Remark****: No further information available from the review*". Thus there is no information available on the use of control group or reference to protocol describing the experimental details including examinations done on pups and on the parental generation. The study was performed more than forty years ago and thus it is very unlikely that examination of anogenital distance and nipple retention was performed in that study. Since no information is provided in the IUCLID dossier from 2000 and the comment provided by C.O.I.M. SpA does not contain any additional information regarding details on experimental design of the study (i.e. we do not know what kind of examinations were performed on the pups in this study), the study cannot be taken into consideration in the classification of DCHP for effects on development or on fertility. As indicated in the CLH report (section 4.12.4, page 32) the same agency (NICNAS) has also published a hazard assessment of DCHP where they concluded *"Although data for DCHP are limited, the fertility and developmental effects observed are similar to those phthalates with sidechain backbone of 4-6 carbon atoms in length (C4-C6) (NICNAS 2008a). These C4-6 phthalates previously referred to as ´transitional´ phthalates (Phthalate Esters Panel HPV Testing Group, 2001) have also been associated with male reproductive (seminiferous tubule atrophy) and development (decreased anogenital distance and retention of nipples) effects. Overall DCHP has a similar reproductive profile to the 'transitional' (C4-6) phthalates for which reproductive and developmental effects are recognised".*
RAC's response
Noted, see RAC's responses to comments number 2.

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	Belgium		MemberState	8
Comment received				
Concerning the toxicicty for reproduction endpoint, we have some doubts. We request the DS to better substantiate its proposal for classification, specially the reasons why they are not GLP compliant. The only GLP compliant study available in the dossier indicates no effects on epididymis sperm parameters and no effects on reproductive endpoints. • Fertilty : o In the two-generation (Hoshino et al.) (OECD 416), there are diffuse atrophy of the seminiferous tubules (severe grade) observed in 3 males F1/24 at 457mg/kg bw/d, a decreased weight of the prostate and a dose dependent decrease in the number of testicular homogenization resistant spermatids. However, there is no effects on epididymis sperm parameters (motility, sperm count, morphology) and no effects on reproductive endpoints (mating, gestation index, ..). o In the Yamasaki's study (2009) (non GLP compliant), there is a decreased weight of the ventral prostate ($p<0.05$) at the low and high dose levels. o The Aydogan and Barlas 's study (2013) (non GLP compliant) reveals effects on the morphology of the testis, epididymis and prostate together with an increase in the				

percentage of abnormal epididymal sperms.
The studies do not reveal severe modifications in the fertility parameters. The Aydogan study indicates an increase in the percentage of abnormal epididymal sperms.
• Developmental :
o In the 2-gegeneration (Hoshino et al. 2005) (OECD 416), the results indicate a reduced anogenital distance (LOAEL F1: 6000ppm and F2: 1200ppm), and an increase in the percentage of litters with male pups having areola mammae. The effects are more severe in the F2 generation.
o The Yamasaki study (2009) (non GLP study) reveals a prolonged preputial separation (2days, $p<0.05$), a decrease on the anogenital distance ($p<0.05$) and an areola mammae/nipple retention ($p<0.05$) at 500mg/kg bw/d.
o The saillenfelt et al. study (2009) (non GLP compliant) shows a decrease anogenital distance in male pups at all dose ($p<0.01$). however, in this study, a maternal toxicity is observed (decrease bodyweight, increase liver weight, ALAT, …)
The studies do not reveal severe modifications.

Dossier Submitter's Response

Thank you for your comments. Three of the studies were carried out in University/Institute settings and it is not surprising that they were not GLP studies. However, this does not mean that the data should not be taken into account. According to the phrasing in the paper by Hoshino et al. (2005) the study was "*carried out in accordance"* with OECD TG416 (1983). One can also note that the registrant has not performed any additional reproductive toxicity studies. The registration refers to the results of the studies by Hoshino et al. (2005); Saillenfait et al. (2009a) and Yamasaki et al. (2009) (and in addition to the majority of the mechanistic studies listed in Table 14 in the CLH report). The classification proposed by the registrant is Repr 2 H361 - Suspected of damaging fertility or the unborn child.
Considering the reproductive capacity of rats, decreased fertility as revealed by effect on fertility index is a rather insensitive endpoint. Thus it is not surprising that no decrease in the number of pregnant dams was recorded for the F_0 or F_1 generation. See also response to comment 6 for our response regarding classification for fertility.

Developmental toxicity
See response to comment 5 for our view on how the signal strength should be interpreted.

Mode of Action
As stated in the CLH report, the in vitro mechanistic studies show that DCHP behaves as an antagonist to 5α-DHT at androgen receptors and also inhibits the enzymes involved in the biosynthesis of androgens. Therefore the antiandrogen mode of action can be presumed for the observed adverse effects on the male pups. This presumption is further supported by the fact that the length of the perineum (anogenital distance) and the apoptosis of the nipple anlagen are all under control of dihydrotestosterone. As indicated in Table 15 of the CLH report it is interesting to note that the so called transitional phthalates all have in common that they cause areola mammae/nipple retention, a reduced anogenital distance and hypospadias. In addition many of these compounds have been shown to reduce testosterone production in the fetal testis (Howdeshell et al., 2008, Toxicol Sci 105: 153-165). Evidence that DCHP indeed does affect the synthesis of testosterone at the presumed target organ (fetal testis) as well as a comparison of the potency of a number of phthalates (including DCHP) has been provided recently by the the research group of Leon Gray at the US EPA (Furr et al., 2014, Toxicol Sci (in press, freely available at http://toxsci.oxfordjournals.org/content/early/2014/06/06/toxsci.kfu081.full.pdf+html).
Their aim has been to develop and validate a rapid, medium-throughput in vivo screen that

detects disruption of fetal testosterone synthersis and uses a minimum number of animals to identify phthalate esters with potential to induce the phthalate syndrome. This study examined 27 chemicals including phthalate esters, phthalate ester alternatives and pesticides known to inhibit steroidogeneis using a standardized dose. In addition, dose response studies were conducted on some of these compounds. Pregnant Sprague Dawley rats were treated by oral gavage from gd 14 to 18 (i.e. the critical period for sexual differentiation of the reproductive tract) and necropsied on GD 18. On GD 18 testis production was measured ex vivo from three fetuses/litter from 3 litters (the used sample size was adequate to detect reductions in testosterone production that were greater than 50%). When comparing the relative potency of chemicals that significantly ($p<0.01$) reduced fetal testosterone production,the substance could be ranked as follows DPeP > DHP > **DCHP** > DPeP > DEHP > DBP > BBP > DiBP > DHeP > DINP.

Altogether, the study by Furr and collegues, adds further mechanistic support to our conclusion that the effects observed (mammae/nipple retention and reduced relative AGD) are specific effects that are caused by a DCHP induced disturbance of the androgen synthesis. In a weight of evidence analysis and considering the overlap of the observed effects with those of other phthtalates (which similairly affect testosterone production in this functional assay and are classified in Cat 1B for developmental toxicity), classification in category 1B for developmental toxicity is warranted. No clear effect on male fertility as revealed by effects on fertility index, or postimplantation losses were recorded in the studies for DCHP. The reason for this is unclear but might be due to differences in potencies or indicate that DCHP has a more clear antiandrogen effect.

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	France		MemberState	9

Comment received

The data are not dose-related and limited, all the stimulation index are <3. Therefore no EC3 can be defined. We therefore disagree that the data fulfill CLP criteria and warrant a classification as Skin sens cat1.
There is not enough information on the Nuodex, 1979d study to allow interpreting this study thoroughly.

Dossier Submitter's Response

Thank you for your comments.

The potential of DCHP to cause skin sensitisation reactions following topical application to the skin of CBA/JN (CBA/J) mice, was assessed using the LLNA:BrdU-ELISA method (OECD TG 442B), i.e. a modified non-radioactive version of the standard LLNA test (TG429). In contrast to the TG429, the result from the OECD 442B test can not be used for subcategorization (no EC3 value is derived). According to the Guidance on the Application of the CLP criteria (section 3.4.2.2.3.2) the data from TG 442B can only be used to identify a compound with a significant sensitising effect (category 1, if Stimulation Index ≥ 1.6) but cannot be used for sub categorisation into 1A or 1B.

As described in the CLH report (section 4.7.1), in the first experiment the stimulation index (SI) values of the low (1.80) and intermediate (1.91) test concentration (but not the high test concentration, SI=1.24) were above the threshold for a positive result (SI= 1.6) but

within the range (1.6 – 1.9) that the test guideline defines as a borderline positive result. Therefore the study was repeated. In the repeat study the SI values for all 3 test concentrations were above the threshold (2.22, 2.82 and 1.94 in the low, intermediate and high dose group, respectively) for a positive result as well as above the range for a borderline positive result. Therefore, the results obtained in this OECD TG study indicate that the DCHP elicits a sensitisation response in mice following dermal exposure. That is, classification in category 1 is warranted.

The response in the high dose group (lower SI than in the middle and low dose groups) may be due to an overload effect, i.e. the balance between effector and suppressor cells which constitutes the sensitization response may have been affected by the high dose (Andersen et al., 1985). Such non-monotonous dose-response curves have been observed for other sensitisers (Arts et al., 2006).

As indicated on page 14 in the CLH dossier, we agree with the comment that there is not sufficient data to be able to interpret the result from the study performed on Nuoplaz 6938 and therefore the result from this study has not been included in the assessment of skin sensitisation.

Andersen, K. E. et al., 1985. Induction of Formaldehyde Sensitivity. Dose Response Relationship in the Guinea Pig Maximisation Test. Acta Derm Venereol 65: 472-478.

Arts, J. H. E. et al., 2006. Dose-Response Relationships and Threshold levels in Skin and Respiratory Allergy, Critical Reviews in Toxicology 36:219-251.

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	Belgium		MemberState	10
Comment received				
We would you like to thanks Swedish Chemicals Agency for the CLH report on Dicyclohexyl phthalate. We support the classification for skin sensitisation. The mouse local lymph node assay, following the OECD guidance 442B, reveal a stimulation index of 2.22, 2.82 and 1.94 at 2.5, 5 and 10% of the concentration test. These results are above the limit of 1.6 for the classification for this test.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
22.04.2014	France		MemberState	11
Comment received				
4.8.1.1 The data are not well presented and we don't understand why results of the 2gen are discussed here. The reading is rendered difficult. As they are presented, the data needs to have enough information for the RAC to state that no classification is warranted.				

A clear comparison of duration/ dose with criteria should be performed. The relevance of the findings for human should be discussed. We agree that the findings observed in liver, kidney and thyroid are consistent with those of other phthalate as impacting the endoplasmic reticulum. As duration of a 2generation study is coherent with a 90d study, the findings are reported at doses too high for warranting any classification for STOT-RE.
Dossier Submitter's Response
Thank you for your comment. As mentioned in section 4.8.1.1 of the CLH report the repeated dose toxicity data is presented only as supportive information to the reproductive toxicity data.
RAC's response
Noted.