Committee for Risk Assessment

RAC

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

Adopted

04 December 2014

04 December 2015

CLH-O-0000001412-86-38/F

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

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

Chemicals name: Dicyclohexyl phthalate

EC number: 201-545-9

CAS number: 84-61-7

The proposal was submitted by **Sweden** and received by the RAC on **19 February 2014.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **5 March 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 April 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Christine Bjorge**

Co- rapporteur, appointed by RAC: **Safia Korati**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2013**.

The RAC opinion was adopted by **consensus**.

OPINION OF THE RAC

RAC adopted the opinion that **Dicyclohexyl phthalate** should be classified and labelled as follows:

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	TBD	dicyclohexyl phthalate	201-545-9	84-61-7	Repr. 1B Skin Sens. 1	H360FD H317	GHS07 GHS08 Dgr	H360FD H317			
RAC opinion	TBD	dicyclohexyl phthalate	201-545-9	84-61-7	Repr. 1B Skin Sens. 1	H360D H317	GHS07 GHS08 Dgr	H360D H317			
Resulting Annex VI entry if agreed by COM	TBD	dicyclohexyl phthalate	201-545-9	84-61-7	Repr. 1B Skin Sens. 1	H360D H317	GHS07 GHS08 Dgr	H360D H317			

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The proposal for classification of dicyclohexyl phthalate (DCHP) for skin sensitisation (Skin Sens. 1) was based on a single local lymph node assay (LLNA). The study was consistent with OECD Technical Guideline (TG) 442B, and included positive controls which were not however reported in the CLH report.

In the LLNA assay using CBA/JN female mice and the BrdU ELISA method, a 10% solution was determined as the minimal irritant concentration, and therefore 10%, 5% and 2.5% (w/w) solutions (in acetone:olive oil 4:1 (v/v)) were used in the main study. In an initial experiment, the stimulation index (SI) values calculated from the mice exposed to the low and intermediate test material concentrations (but not the high concentration) were above the threshold for a positive result (SI= 1.6) but within the range (1.6 – 1.9) which was defined as a borderline positive result in OECD TG 442B. The study was repeated, and the new SI values calculated were 2.22, 2.82 and 1.94 at the low, mid- and high-dose, respectively. Since for all 3 test concentrations the SI in this repeat study were above the range for a borderline positive result (albeit barely in one case), the DS concluded that based on the LLNA assay, dicyclohexyl phthalate is a skin sensitiser in mice. Sub-categorisation for skin sensitisation was not possible based on the data and therefore the DS proposed classification as Skin Sens. 1.

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

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

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The DS proposal for classification for reproductive toxicity (for both developmental toxicity and sexual function and fertility) was mainly based on one GLP and OECD TG 416 compliant 2-generation study (Hoshino *et al*., 2005; described as 'old study design') as well as a number of

non-GLP compliant, supporting studies published in the scientific literature. All these studies were conducted in rats which were exposed to the test material (DCHP) via the oral route.

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

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

The DS concluded that taken together these findings indicate that DCHP is toxic to the male reproductive organs and that animals exposed *in utero*/during weaning are more sensitive compared to adult animals. The DS proposed to classify DCHP for its effects on sexual function and fertility (Repr. 1B, H360F).

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

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

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

Comments received during public consultation

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

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

Another MS commented on the quality of the non-GLP studies and noted that the effects seen for both fertility and development were not sufficiently severe for the classification proposed. The DS replied that, considering the reproductive capacity of rats, it was not surprising that there were no reductions in the number of pregnant dams in Hoshino *et al.* (2005). As further information supporting the mode of action, the DS summarised in their response a recent paper (Furr *et al.*, 2014), which showed that testosterone production (measured *ex vivo*) was significantly reduced in foetuses of rats given DCHP (or other phthalates) by oral gavage (doses not stated in the

response) from GD 14 to GD 18 and necropsied on GD 18. The DS argued that considering the overlap of the observed effects with those of other phthalates which affected testosterone production and are currently classified in Category 1B for developmental toxicity, the proposal for classification of DCHP was justified.

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

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

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

Assessment and comparison with the classification criteria

Effects on Development

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

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

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

marked, maternal toxicity was reported in the high dose females with a reduced corrected body weight gain of 50%.

In another supporting developmental toxicity study with exposure from GD 6 to PND 20 (0, 20, 100 and 500 mg DCHP/kg bw/day), effects on AGD, areola/ nipple retention as well as prolonged preputial separation and hypospadias were reported (Yamasaki *et al*., 2009). However, this study was poorly reported. Data were only provided for the high dose group, therefore no information is available on whether these effects were observed in lower dose groups. Effects reported were a statistically significant reduction in relative AGD (13%), an increase in the number of pups/litter with areola/nipple retention (2.7% compared to 0 in controls) affecting 68% of the litters, a prolonged preputial separation by 2 days and hypospadias in 2 offspring in association with small testes (where one of them was sacrified at 7 weeks of age due to poor general condition). These effects were reported in the absence of marked maternal toxicity.

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

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

Effects on sexual function and fertility

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

Regarding effects on mating and fertility following exposure to DCHP, no clear effects were reported in the 2-generation study in the F_0 and F_1 generations exposed to 240 (LD), 1200 (MD) and 6000 (HD) ppm (corresponding to a mean daily intake during the entire dosing period of 18, 90 and 457 mg/kg bw/day, respectively, for males and 21, 107 and 534 mg/kg bw/day, respectively, for females). The absence of an effect on fertility in the study by Hoshino *et al*. (2005) may be explained by the fact that the measurement of reduced fertility is considered as a insensitive endpoint in rats due to the rather high sperm reserve available in rats compared to humans. No effects on fertility were also reported in the F_1 generation rats that were mated at 12 weeks of age, where parental exposure to DCHP was up to 500 mg/kg bw/day from GD 6 to PND 20 (Yamasaki *et al*., 2009).

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

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

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

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

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

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

RAC agrees with the DS that an antiandrogenic mode of action may explain the adverse effects on the development of the male pups. This is supported by the fact that the AGD as well as the normal apoptosis of the nipple anlagen are under the control of dihydrotestosterone (reviewed in NAS, 2008). The same effects as reported in male pups following exposure to DCHP were also reported following *in utero* exposure to transitional phthalates with a harmonised classification for development as Repr. 1B. An antiandrogenic mode of action was also suggested for these phthalates.

Summary

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

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

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

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

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

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

Conclusion

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

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

Additional references

Furr JR, Lambright CS, Wilson VS, Foster PM, Gray LE (Jr) (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. Toxicol. Sci. doi: 10.1093/toxsci/kfu081 (First published online: May 5, 2014)

ANNEXES:

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

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