Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

diisooctyl phthalate

EC Number: 248-523-5
CAS Number: 27554-26-3

CLH-O-0000001412-86-193/F

Adopted

9 March 2018

9 March 2018

CLH-O-0000001412-86-193/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:

Chemical name: **diisooctyl phthalate**

EC Number: **248-523-5**

CAS Number: **27554-26-3**

The proposal was submitted by **France** and received by RAC on **28 February 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France 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 **14 March 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 April 2017**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Stine Husa**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 March 2018** by **consensus**.

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 and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	diisooctyl phthalate	248-523-5	27554-26-3	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
RAC opinion	607-RST-VW-Y	diisooctyl phthalate	248-523-5	27554-26-3	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
Resulting Annex VI entry if agreed by COM	607-RST-VW-Y	diisooctyl phthalate	248-523-5	27554-26-3	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Technical diisooctyl phthalate (DIOP) is a UVCB substance that includes a number of constituents having alkyl chains showing different type of branching but with an overall carbon number corresponding to eight. Commonly it includes in its composition 70-75% isomers with a C4-C6 ester backbone (with additional carbons in the branching to make C8 in total) and less than 25% of isomers with C7 backbone. In the publication by Saillenfait *et al.*, (2013a), it is described that the DIOP used was >99% pure as assessed by GC/MS, although this is difficult to interpret in the case of an isomeric mixture.

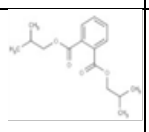
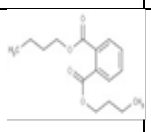
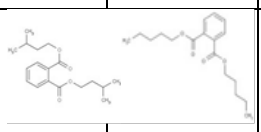
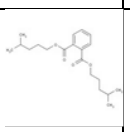
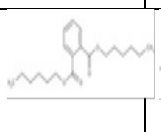
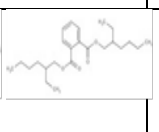
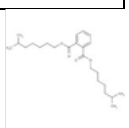
HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

One publication with three different studies assessing reproductive toxicity following exposure to DIOP were included by the dossier submitter (DS) in the CLH dossier (Saillenfait *et al.*, 2013a). In addition, a further study of low quality was found in the literature. Due to the limited data, the DS proposed to use a category approach with *ortho*-phthalates having an alkyl side-chain length between C3 and C7. This group already includes DIBP, DBP, DIPP, DnPP, DHP, DnHP, and DEHP. DIOP is an UVCB that normally includes 70-75% of isomers with C4-C6 ester backbone and less than 25% of isomers with C7 backbone. The *ortho*-phthlates included in the category with alkyl side-chain length between C3 and C7, and which already have a harmonised classification for reproductive toxicity, are shown in the table below:

Table: *Comparison of structure and classification as proposed by the dossier submitter*

Name*	**DIBP**	**DBP**	**DIPP****	**DnPP**	**DHP*****	**DnHP**	**DEHP******	**DIOP**
CAS No.	84-69-5	84-74-2	605-50-5	131-18-0	68515-50-4	84-75-3	117-81-7	27554-26-3
Chemical formula	$C_{16}H_{22}O_4$	$C_{16}H_{22}O_4$	$C_{18}H_{26}O_4$	$C_{18}H_{26}O_4$	$C_{20}H_{30}O_4$	$C_{20}H_{30}O_4$	$C_{24}H_{38}O_4$	$C_{24}H_{38}O_4$
Side chain length**	3C (4C)	4C	4C (5C)	5C	5C (6C)	6C	6C (8C)	7C (8C)
Structure								
Harmonised classification	Repr.1B; H360Df	Repr. 1B; H360Df	Repr.1B; H360FD	Repr.1B; H360FD	Repr.1B; H360FD	Repr.1B; H360 FD	Repr.1B; H360FD	none

*DIBP: diisobutyl phthalate, DBP: dibutyl phthalate, DIPP: diisopentyl phthalate, DnPP: di-n-pentyl phthalate, DHP: 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear, DnHP: di-n-hexyl phthalate, DEHP: di-(2-ethylhexyl) phthalate, DIOP: diisooctyl phthalate.
**In parentheses, the total number of carbon atoms in the side chain
*** Harmonised classification based on read across
**** According to the ECHA website, a synonym to DEHP is DOP (dioctyl phthalate)

Effects on sexual function and fertility

No human data is available to assess sexual function and fertility for DIOP. Furthermore, there are no one- or two generation reproductive toxicity studies available for this substance.

In a prenatal toxicity study (comparable to OECD TG 414) with female rats, exposure to DIOP by oral gavage at doses of 0, 100, 500 and 1000 mg/kg bw/d on gestation days (GD) 6-20, showed no maternal toxicity. However, mal-positioned testes were observed from 500 mg/kg bw/d (Saillenfait et al., 2013a).

In a study on *ex-vivo* testosterone production by the foetal testes (non guideline), pregnant rats were exposed to DIOP by oral gavage (doses of 0, 10, 100, 500 and 1000 mg/kg bw/d) during GD 12-19. The study showed a dose dependent decrease in *ex-vivo* testosterone production in the foetal testes on GD 19 from 100 mg/kg bw/d (Saillenfait *et al.*, 2013a).

In a peri-postnatal toxicity study (non guideline) pregnant rats were exposed to DIOP by oral gavage at doses of 0, 100, 500 and 1000 mg/kg bw/d during GD 12-21. The study showed permanent postnatal alterations in androgen-dependent structures in male offspring from 500 mg/kg bw/d, but with some maternal toxicity at the highest dose (Saillenfait *et al.*, 2013a).

An additional lower quality 2-generation study with male/female Swiss mice was included in the CLH dossier. The animals were exposed in the diet to 0, 14, 140 or 420 mg/kg bw/d of DIOP beginning 7 days pre-mating, throughout a cohabitation period of approximately 14 weeks. Details on this study were limited and the study could not be adequately assessed.

The DS concluded that classification with Repr. 1B for effects on sexual function and fertility is appropriate, based on the experimental results showing lesions of the male reproductive tract (in particular hypospadias, undescended testes and hypospermatogenesis) in the absence of significant maternal toxicity after *in utero* exposure to DIOP. These findings are further supported by a category approach with C3-C7 (see below) *ortho*-phthalates showing similar toxicity on fertility with the same hypothesized mechanisms of action.

Effects on development

No human data are available for DIOP. The DS considered that the overall evidence from human data for exposure to other phthalates was insufficient to conclude on an effect on development.

The DS presented the same studies for effects on development as for effects on sexual function and fertility. Overall, the DS concluded that these studies after *in utero* exposure to DIOP induced embryotoxicity from 500 mg/kg bw/d. Furthermore, embryolethality and malformations of the male reproductive tract were seen in a few (3) cases from 500 mg/kg bw/day and reaching a high incidence (up to 74% of males with undescended testes) 1000 mg/kg bw/d. The effects seen for DIOP were similar to those seen after exposure to other C3-C7 *ortho*-phthalates and the DS considered that a category approach to fill data gaps is justified.

The DS concluded that based on the embryolethality, embryotoxicity and the permanent postnatal alterations of the male reproductive system, supported by the category approach for C3-C7 *ortho*-phthalates, classification with Repr. 1B for effects on development is justified.

Setting of an SCL

Based on the available data, the DS considered DIOP to be less potent than other C3-C7 *ortho*-pthtalates in the category, and the DS evaluated setting a specific concentration limit (SCL) for DIOP. The lowest calculated ED_{10} value for DIOP is 520 mg/kg bw/d for the percentage of litters with malpositioned testes in the study by Saillenfait *et al*. (2013a). Based on this, DIOP is regarded to be of low potency. However, the DS was of the opinion that the low number of litters in the study (8-12) may underestimate the ED_{10} values. In addition, as the dataset is limited, and other parameters such as effects on germ cells and mammary gland development, which

could be sensitive to phthalate toxicity, were not reported in the study by Saillenfait *et al*. (2013a). Thus, the DS concluded that a SCL should not be assigned for DIOP, but the GCL should be applied.

Comments received during public consultation

Comments were received from two member state competent authorities (MSCAs) and one Industry/trade association.

One commenting MSCA supported classification with Repr. 1B for effects on sexual function and fertility. Another commenting MSCA questioned this classification as they were of the opinion that it could be possible to view the effects seen on the male reproductive tract as a developmental effect instead. In addition, the justification for the read across was questioned based on the side chain length of the included phthalates. DIOP has the longest side chain length of the phthalates in the group, and the read across could therefore rather be regarded as an extrapolation.

One Industry/trade organisation regarded a classification with Repr. 2 for effects on sexual function and fertility to be more appropriate than Repr. 1B, based on limited evidence from structure activity relationships.

All comments were in support of a classification with Repr. 1B for effects on development.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

Three oral gavage studies in rats were included by the DS for the assessment of effects on sexual function and fertility (Saillenfait *et al*., 2013a). In addition, an oral diet reproductive toxicity study in mice, from which only a summary was available, was included. However, there are uncertainties regarding the substance ID for the test substance used in this study, and hence this study has not been taken into account in the weight of evidence assessment for classification. No one- or two-generation studies according to acceptable test guidelines were available following exposure to DIOP.

The first study was a prenatal toxicity study comparable to OECD TG 414 in rats with oral gavage exposure to 0, 100, 500 and 1000 mg/kg bw/d of DIOP on GD 6-20 (Saillenfait *et al*., 2013a). However, less animals were used (8-10 per dose group compared to 20 animals in the OECD TG 414). Maternal toxicity was limited to a decrease in maternal body weight gain and body weight in the high dose group. However, there were no changes in corrected body weight or corrected body weight gain and no effects were reported on food consumption (see table below).

Table: *Maternal body weight and body weight gain*

DIOP (mg/kg bw/d)	**0**	**100**	**500**	**1000**
No. (%) pregnant	10 (90.9)	8 (80.0)	12 (100.0)	10 (90.9)
Body weight (g)				
GD 0	224 ± 9[a]	223 ± 12	223 ± 8	222 ± 7
GD 6	258 ± 8	259 ± 12	257 ± 12	254 ± 9
GD 9	270 ± 10	275 ± 13	269 ± 12	262 ± 9
GD 12	292 ± 10	295 ± 12	292 ± 19	279 ± 11
GD 15	315 ± 13	319 ± 15	313 ± 22	299 ± 10
GD 18	358 ± 14	364 ± 21	357 ± 26	335 ± 15*
GD 21	415 ± 23	422 ± 29	410 ± 39	378 ± 18*
Gravid uterine weight (g)	106 ± 12	107 ± 15	100 ± 22	83 ± 21*
Net body weight change (g)[b]	310 ± 16	315 ± 20	311 ± 25	296 ± 14
Body weight change (g)				
GD 0-6	34 ± 4	36 ± 3	34 ± 9	33 ± 6

DIOP (mg/kg bw/d)	0	100	500	1000
GD 6-9	13 ± 4	15 ± 2	12 ± 5	8 ± 7
GD 9-12	22± 6	20 ± 5	22 ± 8	17 ± 6
GD 12-15	22 ± 5	24 ± 7	22 ± 6	20 ± 4
GD 15-18	44 ± 5	45 ± 7	43 ± 7	36 ± 8*
GD 18-21	57 ± 10	58 ± 10	54 ± 15	44 ± 7*
GD 6-21	158 ± 18	163 ± 22	153 ± 34	124 ± 19*
Net weight gain (g)[c]	52 ± 12	56 ± 11	54 ± 16	41 ± 15
Food consumption (g/day)				
GD 0-6	23 ± 1	24 ± 3	23 ± 2	22 ± 1
GD 6-9	22 ± 2	24 ± 3	22 ± 2	21 ± 3
GD 9-12	23 ± 3	24 ± 2	23 ± 3	21 ± 3
GD 12-15	24 ± 2	25 ± 2	23 ± 2	22 ± 2
GD 15-18	26 ± 2	28 ± 2	26 ± 3	24 ± 3
GD 18-21	27 ± 2	28 ± 3	27 ± 3	25 ± 2
GD 6-21	24 ± 2	26 ± 2	24 ± 2	23 ± 2

[a] Values are expressed as means ± SD.
[b] Body weight on GD 21 minus gravid uterine weight.
[c] Body weight gain during GD 6-21 minus gravid uterine weight
*Statistically significant difference from the vehicle control, p<0.05 (Dunnett's test).

The effects reported in the pups related to a potential effect on fertility and sexual function was an increase in the number of male pups with malpositioned testes at 1000 mg/kg bw/d (see the table below):

***Table**: Effects on foetal testes*

Dose (mg/kg bw/d)	0	100	500	1000
Number of foetuses (litters) examined	137 (10)	110 (8)	160 (12)	115 (10)
Testes malpositioned (uni- and/or bilateral)	0	0	1(1)	10[#](5)[*]
Severe	0	0	0	3(3)
Moderate	0	0	1(1)	7(4)

[#]significantly different from the vehicle control, p<0.05 (Mann-Whitney test)
[*] significantly different from the vehicle control, p<0.05 (Chi-2 test)

In the second study the testosterone production was assessed in foetal testes *ex-vivo*. Pregnant rats were exposed by oral gavage from gestation day 12 to 19 to 0, 10, 100, 500 and 1000 mg/kg bw/d of DIOP, and the testes were collected at GD 19 and analysed for testosterone production (Saillenfait *et al.*, 2013a). In this study, an *ex-vivo* dose-dependent decrease in testicular testosterone production was reported compared to control animals (-34%, -72% and -84% in the 100, 500 and 1000 mg/kg bw/d dose groups, respectively). In another study by Saillenfait *et al.*, (2013b) the phthalates DnHP and DEHP were also tested for *ex-vivo* foetal testosterone production. The result showed that these phthalates significantly reduced the foetal testicular testosterone production compared to controls starting at 20 and 50 mg/kg bw/d for DnHP and DEHP, respectively. Note that 50 mg/kg bw/d was the lowest dose tested for DEHP). The foetal testosterone production was also shown to decrease in a dose-related manner for these phthalates.

In the third study (Saillenfait *et al.*, 2013a) pregnant rats were exposed by oral gavage to 0, 100, 500 and 1000 mg/kg bw/d of DIOP between GD 12 and 21, and the animals were examined on postnatal week (PNW) 10 and 12. No test guideline was followed. The maternal effects included a statistically significant decreased body weight at GD 21 in the high dose group (431 g ± 31 g, 417 g ± 33 g, 426 g ± 26 g, 392 g ± 25 g at 0, 100, 500 and 1000 mg/kg bw/d, respectively). The reproductive tract abnormalities reported in the adult male rats at PNW 10 and 12 are included in the table below. This study showed that DIOP induced permanent postnatal alterations of the male reproductive system at PNW 10-12 that may have an effect on fertility as adults.

Table: *Effects on the reproductive tract in male adult rats assessed PNW10 – PNW12*

DIOP (mg/kg bw/d)	0	100	500	1000
No. males evaluated/litters (%)	84/12	74/12	67/12	39/9[a]
Small penis	0	0	0	8/4 (20.5)[b]
Cleft prepuce	0	0	0	10/5 (25.6)
Hypospadia	0	0	0	14/6 (35.9)
Cleft phallus with exposed os penis	0	0	0	11/4 (28.2)
Testes, undescended (uni or bilateral)	0	0	0	29/9 (74.4)
Testes, undescended (bilateral)	0	0	0	18/6 (46.2)
Testes, enlarged (unilateral)[c]	0	0	1/1 (1.5)	0
Testes, absent or markedly underdeveloped (unilateral)[d]	0	0	0	4/3 (10.3)
Epididymis, absent (unilateral) or markedly underdeveloped (unilateral)[d]	0	0	0	4/4 (10.3)
Epididymis, thin body (unilateral)	0	0	1/1 (1.5)[e]	5/4 (12.8)
Vasa deferentia, crossed	0	0	0	4/3 (10.3)
Vas deferens, absent (uni or bilaterally)	0	0	0	5/4 (12.8)
Seminal vesicles, absent	0	0	0	2/2 (5.1)
Seminal vesicles, markedly underdeveloped[f]	0	0	1/1 (1.5)	15/6 (38.5)
Seminal vesicles, malformed[g]	0	0	0	1/1 (2.6)
Prostate, markedly underdeveloped[f]	0	0	1/1 (1.5)	9/4 (23.1)

[a] One litter had no surviving males when euthanised.
[b] (No. males affected/total males evaluated) x 100.
[c] Approximately 150% of the control weight. Scrotal. Associated with hypospermatogenesis at histological examination.
[d] Less than 10% of the control weight. Undescended testes are not included.
[e] Associated with sperm granuloma at histological examination. Marked hypospermatogenesis was observed in the ipsilateral testes.
[f] Approximately half of the controls or less.
[g] Unilaterally small seminal vesicle.

A two-generation reproductive toxicity study in mice was included in the background document. However, only a summary is available, and in addition there are uncertainties regarding the identity of the substance used. Industry argued that the study was not conducted on DIOP (CAS number 27554-26-3) but on DEHP (CAS number 117-81-7), while the DS stated that this is not correct and that the study was indeed performed on DIOP. However, based on this uncertainty in combination with only limited details from the study being available, RAC considered that the study could not be adequately assessed, and did not consider it further in the evaluation.

Category approach: The background document also includes information regarding the physico-chemical properties of the shorter chain *ortho*-phthalates. The increasing side chain length showed, as expected, a clear trend for decreasing water solubility (from moderate water solubility for 4C (10 mg/L) to very low solubility for 8C (0.09 mg/L)). The log Kow ranged from 4.11 to 8.0, increasing with alkyl chain length and molecular weight indicating, that C3 phthalates would be more extensively absorbed than the C7 phthalates. In particular, physico-chemical properties of DIOP are considered to be close to those of DEHP (C6 backbone).

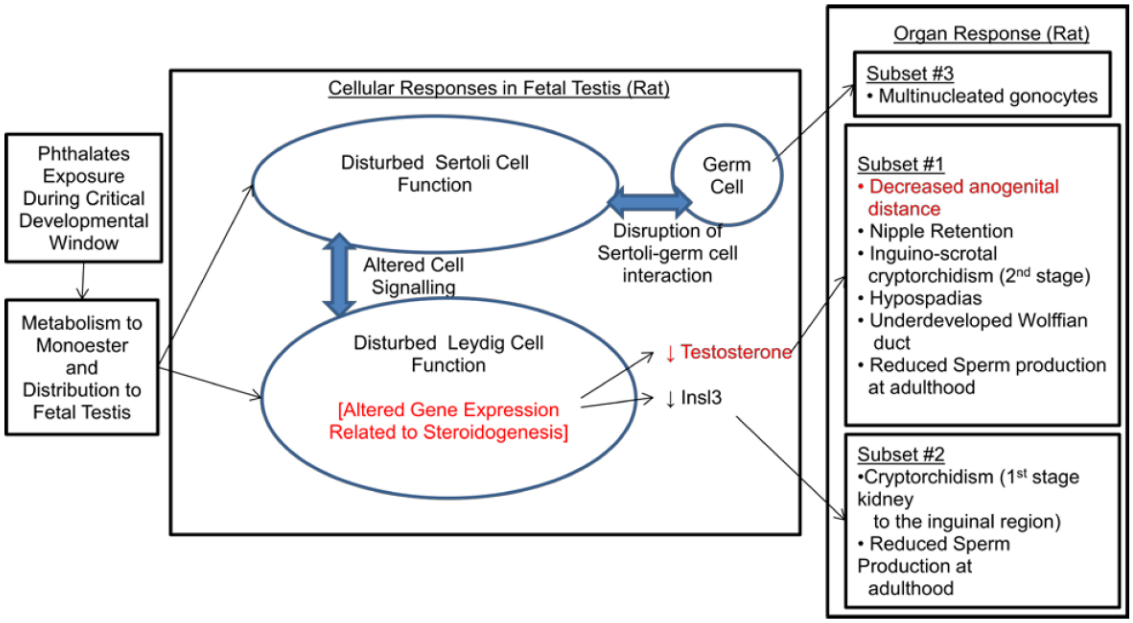
In the CLH proposal, a category approach was chosen, including phthalates with a side chain length of C3 to C7. However, Industry pointed out during the discussion that DIOP should be

regarded as belonging to C3-C6 phthalates as it commonly includes mainly (70-75%) isomers with C4-C6 ester backbone and less than 25% of isomers with C7 backbone.. RAC considered that DIOP would share the toxicological properties of the C3-C6 *ortho*-phthalates rather than the C3-C7 *ortho*-phthalates.

Alteration of the male reproductive system with the same proposed mechanism of action was reported for the C3-C6 *ortho*-phthalates, and RAC therefore considered that due to the similarities between C3-C6 *ortho*-phthalates, the proposed category is supported. Health Canada (2015) proposed that the category "medium chain phthalate esters (longest carbon backbone length 3 to 7)" (including DIOP) showed activity in assays for important events in the mode of action for androgen-dependent effects on the developing male reproductive system.

Mode of action (MoA): The DS included a MoA for reproductive toxicity for phthalates described as the "phthalate syndrome" that is linked to a disturbance of the Sertoli and Leydig cell function. This was based on an assessment included in the Health Canada report from 2015 (see figure below, included in the background document).

Figure: Presentation of the cellular targets for the "phthalate syndrome" with associated changes in gene expression and subsequent hormonal and organ responses (Health Canada, 2015).



This report evaluated the toxicity of various phthalates to the male reproductive system related to an antiandrogen MoA. The DS only analysed the *ortho*-phthalates with a harmonised classification for reproductive toxicity and with a linear or branched alkyl chain from C3 to C7. The analysis found that for all tested phthalates (DIBP, DBP, DnHP and DEHP) reduced steroid biosynthesis gene expression was observed, with DBP being the most potent and DEHP the least potent. Further, the *ex-vivo* foetal testosterone production following *in utero* exposure was analysed for the following phthalates; DIBP, DBP, DnHP, DEHP and DIOP. All phthalates were shown to reduce the foetal testosterone production with DEHP and DnHP being more potent than DIOP.

RAC agreed with the DS that an antiandrogen MoA may explain the adverse effects on the male reproductive organs in male pups observed following *in utero* exposure to DIOP. These effects may impair fertility in adulthood since DIOP induced permanent postnatal alterations of the male reproductive system (seen at PNW 10 - 12). This MoA is also considered to be relevant for humans.

Comparison with the CLP criteria:

A classification as Repr. 1A is not justified based on the lack of adequate human data following exposure to DIOP.

According to the CLP Regulation, classification of a substance in Category 1B is largely based on data from animal studies. There was no fertility study available for DIOP performed according to acceptable test guidelines. The only data available assessing effects on fertility was a non-guideline study in mice. However, this study could not be considered due to uncertainties regarding the test substance used in the study.

No two-generation study according to acceptable test guidelines was available for DIOP. In the developmental toxicity study, DIOP induced permanent postnatal alterations (PNW 10 - 12) of the male reproductive system that may have an effect on fertility as adults. It has been questioned whether the effects on male reproductive organs, induced following *in utero* exposure to DIOP, should be considered as an effect on developmental toxicity or as an effect on sexual function and fertility. In CLP Regulation Annex 1 section 3.7.1.4, the following is included *"… 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 lifespan of the organism…".* However, RAC considers that the effects following exposure to DIOP could be considered an effect on fertility impairment, since in the CLP Regulation no reference to a specific exposure period for the induction of effects on fertility is given (CLP, Annex 1, section 3.7.1.3). However, more importantly, it is concluded that classification in category 1B for sexual function and fertility is supported by a category approach with C3-C6 *ortho*-phthalates where the phthalates with a side chain length closest to DIOP are already classified as Repr. 1B for fertility. The phthalates in the category showed similar toxicity on male reproductive organs as was reported for DIOP. Furthermore, mechanistic studies indicate an anti-androgenic MoA that is considered relevant for humans. RAC therefore considers that read across to the C3-C6 *ortho*-phthalates category is justified, and that DIOP should be classified as Repr. 1B for sexual function and fertility.

In conclusion, RAC considers that classification for DIOP as Repr. 1B, H360F, is justified.

Developmental toxicity

For the assessment of effects on development the DS used the same three studies in rats as was used for the assessment of effects on sexual function and fertility (Saillenfait *et al*., 2013a).

The *first study* was a prenatal toxicity study (comparable to OECD TG 414) in rats with oral gavage exposure to 0, 100, 500 and 1000 mg/kg bw/d of DIOP on GD 6-20 (Saillenfait *et al*., 2013a). However, less animals were used (8-12 per dose group compared to 20 animals in the OECD TG 414). Maternal toxicity was limited to a decrease in maternal body weight gain and body weight in the high dose group. However, there were no changes in corrected body weight or body weight gain (see the table "Maternal body weight and body weight gain" above). In the 500 mg/kg bw/d dose group, decreased foetal body weight was reported but the decrease was not statistically significant when measures on a per-litter basis. In the high dose group (1000 mg/kg bw/d), an increase in post-implantation losses (17.8% vs. 4.7% in the control group) and resorptions (16.4% vs. 4% in the control group). Malpositioned testes were reported in one foetus at 500 mg/kg bw/d and in 10 foetuses from 5 litters at 1000 mg/kg bw/d (statistically significant). Skeletal variations were reported in all dose groups, evident as 14th supernumerary lumbar ribs (5(4), 9(4), 33(10)[1] and 42(10)) in the 0, 100, 500 and 1000 mg/kg bw/d dose

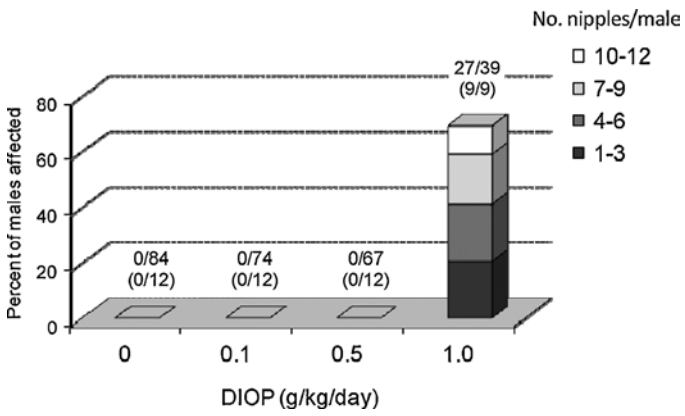
[1] This number has been corrected to 33(10) after communication with the study author. Originally 3(10) was reported in the Saillenfait *et al*., 2013a, however, this was confirmed with the author to be a typographical error.

groups respectively. The effects were statistically significant at ≥ 500 mg/kg bw/d. In addition, an increase in sternebrae ossification bipartite incomplete or absent was reported in the high dose group (1(1), 0(0), 0(0), 12(6)).

In the *second study,* the testosterone production was assessed in foetal testes *ex-vivo*. Pregnant rats were exposed by oral gavage from GD 12 to 19, i.e. during the critical period of male sexual differentiation, to 0, 10, 100, 500 and 1000 mg/kg bw/d of DIOP, and the testes were collected at GD 19 and analysed for testosterone production (Saillenfait *et al*., 2013a). In this study, an *ex-vivo* dose-depended decrease in testicular testosterone production was reported compared to control animals (-34%, -72% and -84% in the 100, 500 and 1000 mg/kg bw/d dose groups, respectively).

In the *third study*, pregnant rats were exposed by oral gavage to 0, 100, 500 and 1000 mg/kg bw/d of DIOP on GD 12-21 and the animals were examined on PNW 10 - 12. No test-guideline was followed (Saillenfait *et al*., 2013a). The maternal effects included a statistically significant decreased body weight at GD 21 in the high dose group (431 ± 31, 417 ± 33, 426 ± 26, 392 ± 25 at 0, 100, 500 and 1000 mg/kg bw/d, respectively). A statistically significant decrease in the number of live pups per litter was reported in the high dose group (13.0 ± 3.1, 11.6 ± 4.5, 11.7 ± 1.5, 8.9 ± 3.6 at 0, 100, 500 and 1000 mg/kg bw/d, respectively). The reproductive tract abnormalities reported in the adult male rats at PNW 10 and 12 are included in the table "Effects on the reproductive tract in male adult rats assessed PNW 10 – PNW 12" above. These effects are considered to be consistent with the high incidence of malpositioned testes in the first study, and with the decrease in *ex-vivo* testosterone reported in the second study. Further, an increase in the incidence of males with thoracic and/or abdominal areolas/nipples as adult stage (PNW 10 or 12) was reported in the high dose group (a total of 69%, and 41% with 6-12 nipples and/or areolas per male) (figure below).

Figure: Incidence of males with thoracic and/or abdominal areolas/nipples as adult stage (PNW 10 or 12) (from Saillenfait *et al*., 2013a)



Mode of Action (MoA): Most of the developmental effects reported following *in utero* exposure to DIOP are considered to be related to a decrease of androgens (see figure presenting the cellular targets for the "phthalate syndrome", above). However, recent publications have questioned the relevance of anti-androgenic effects induced by phthalates in rats for humans. Some experimental animal studies using *in vitro* or xenograft models did not show any decrease of testosterone following exposure to different phthalates including DBP, MEHP and MBP in human foetal testes, although this effect was clearly observed in rat foetal testes (ANSES, 2015). However, RAC agrees with the DS that these data are not sufficiently robust to conclude that the effects reported in rat testes following exposure to phthalates are not relevant for humans. It was also concluded in the RAC restriction dossier on four phthalates (ECHA, 2016) that the available epidemiology studies were associated with such uncertainties that the studies did not allow to conclude on a direct causal relationship between the effects investigated (congenital

malformation of the male genitalia, semen quality, pubertal timing and testicular cancer) and phthalate exposure. Other developmental effects reported following *in utero* exposure to phthalates can be considered independent of testosterone production. For example, delayed prenatal transabdominal migration of the testes is considered to be partially related to an impairment of the production of Insl3 (Insulin-like 3) protein of the foetal Leydig cells. Reduction of Insl3 gene expression and/or alteration of transabdominal migration of the testes have also been reported with various C3-C7 *ortho*-phthalates, showing a similar MoA for these substances (ANSES, 2015).

RAC agrees with the DS that a decrease in androgens is the most likely MoA for the developmental toxicity reported following *in utero* exposure to DIOP, together with a possible alteration of the Insl3 genexpression, and that this MoA is relevant for humans.

Comparison with the CLP criteria:

Reproductive toxicity category 1A in the CLP Regulation is justified for substances which are known or presumed human reproductive toxicant. For DIOP, RAC consider that classification as Repr. 1A is not justified based on the lack of adequate human data following exposure to DIOP.

According to the CLP Regulation the classification of a substance in Category 1B is largely based on data from animal studies. RAC considers that classification as Repr. 1B is justified based on a statistically significant increase in embryolethality, including post-implantation losses and resorptions, at 1000 mg/kg bw/d in experimental animal studies. In addition, permanent post-natal changes in the male reproductive system were reported in the high dose group and included hypospadias, markedly underdeveloped seminal vesicles, undescended testes, hypospermatogenesis and retained nipples. These effects were reported in the absence of marked maternal toxicity, and can therefore not be considered as a secondary non-specific consequence of other toxic effects. This is supported by a category approach with C3-C6 *ortho*-phthalates showing similar toxicity in male reproductive organs. Further, mechanistic studies indicate an antiandrogenic MoA that is considered relevant for humans.

RAC considers that Repr. 2 is not appropriate since there is clear evidence of developmental toxicity in rats following *in utero* exposure to DIOP that were reported in the absence of marked maternal toxicity.

In conclusion, RAC considers that classification for DIOP as Repr.1B, H360D, is justified.

Specific Concentration Limit (SCL)

The DS included an assessment for setting a lower SCL for DIOP in the CLH dossier, since DIOP was shown to be a less potent reproductive toxicant compared to the other C3-C7 *ortho*-phthalates. The DS calculated various ED_{10} values for effects relevant for classification reported in the Saillenfait *et al*. (2013a) study (see the table below). According to the CLP guidance, the ED_{10} value is the lowest dose which induces reproductive toxic effects fulfilling the criteria for classification for reproductive toxicity with an incidence or magnitude of 10% after correction for the spontaneous incidence.

Table: *Summary of calculated ED_{10} from Saillenfait et al. (2013a)*

Dose (mg/kg bw/d)	0	100	500	1000	ED_{10}
Experiment 1					
% post-implantation loss per litter	4.70	5.00	8.00	17.8	842
% resorption per litter	4.00	5.00	8.00	16.4	858
% foetuses with testes malpositioned	0	0	1.25	17.2	774
% litter with testes malpositioned	0	0	8.33	50.0	520
Experiment 3					
Pup survival to weaning PND21 (%)	89.2	91.2	85.6	68.4	686
Permanent areolas and/or nipple buds (%)	0	0	0	69.0	572
Small penis (% foetus)	0	0	0	20.5	744
Cleft prepuce (% fœtus)	0	0	0	25.6	695
Hypospadia (% fœtus)	0	0	0	35.9	639
Cleft phallus with exposed os penis (% fœtus)	0	0	0	28.2	677
Testes undescended (uni or bilateral) (% fœtus)	0	0	0	74.4	567
Testes. undescended (bilateral) (% fœtus)	0	0	0	46.2	608
Testes absent or markedly underdeveloped (unilateral) (% foetus)	0	0	0	10.3	1000
Epididymis absent (unilateral) or markedly underdeveloped (unilateral) % fœtus)	0	0	0	10.3	1000
Epididymis thin body (unilateral) (% fœtus)	0	0	1.50	12.8	876
Vasa deferentia. crossed (% fœtus)	0	0	0	10.3	1000
Vasa deferens absent (uni or bilaterally) (% fœtus)	0	0	0	12.8	890
Seminal vesicles absent (% fœtus)	0	0	0	5.10	1480
Seminal vesicles markedly underdeveloped (% fœtus)	0	0	1.50	38.5	615
Seminal vesicles malformed (% fœtus)	0	0	0	2.60	2423
Prostate markedly underdeveloped (% fœtus)	0	0	1.50	23.1	697
Hypospermatogenesis (%foetuses)					
Grade 1	0	0	0	8.00	
Grade 2	0	0	0	8.00	
Grade 3	0	0	2.78	8.00	
Grade 4	0	0	0.00	60.0	583
Grade 5	0	0	2.78	4.00	
Hypospermatogenesis (%litter)					
Grade 1	0	0	0	22.2	
Grade 2	0	0	0.00	22.2	
Grade 3	0	0	8.33	22.2	560
Grade 4	0	0	0	88.9	
Grade 5	0	0	8.33	11.1	

As can be seen from the table above all ED10 values calculated by the DS are above the limit for the low potency group (≥ 400 mg/kg bw/d) with the lowest ED_{10} being 520 mg/kg bw/d based on the percentages of litters with malpositioned testes. However, according to the CLP guidance, modifying factors needs to be taken into account such as type of effect/severity, data availability, dose-response relationship, mode or mechanism of action, toxicokinetics and bioaccumulation of substances in order to define the potency group.

Modifying factors

a) Type of effect/severity

Embryo lethality, malformations of male reproductive tract and nipple retention can be judged as severe effects.

b) Data availability

Only one reliable publication is available to assess developmental toxicity of DIOP (Saillenfait *et al*., 2013a). Data from the C3-C7 *ortho*-phthalate category, as included by the DS, suggest that parameters assessed with DIOP cannot be considered the most sensitive endpoints. This assessment is based on the results from a study performed in rats exposed to DBP from GD 15 to postnatal day (PND) 21 (Lee, 2004). In this study, a LOAEL between 1.5-3.0 mg/kg bw/d was set based on a decreased number of spermatocytes in males adult offspring and on effects on mammary glands in female adult offspring. The occurrence of similar effects has not been studied in experiments performed by Saillenfait *et al*., (2013a) with DIOP. These experiments were conducted during pregnancy and specific assessment on spermatocytes and mammary glands in adulthood was not performed. Therefore, it is unknown if DIOP would induce similar effects as DBP with a similar study design. For other developmental endpoints available for both substances, such as embryo lethality and nipple retention, DIOP seems to be less potent than DBP as described in the background document. However, the design of the studies are not comparable. In contrast, when comparing ED_{50} values for decreased testosterone production, the ED_{50} value for DIOP was 145 mg/kg bw/d while for DBP the ED_{50} value was 400 mg/kg bw/d (Howdeshell *et al*., 2008), which indicates that DIOP could be more potent than DBP.

c) Dose-response relationship

The majority of the effects on the male reproductive organ development were reported in the high dose group (1000 mg/kg bw/d), however, with some incidences in the mid dose group (500 mg/kg bw/d). The only effect that was reported with a clear dose-response was on the decrease in the *ex-vivo* testosterone production.

d) Mode of action

Most of the developmental effects reported with DIOP are characteristics of a decrease of androgens. Recent publications have questioned the relevance of anti-androgenic effects induced by phthalates in rats to humans. Some experimental studies using *in vitro* or xenograft models did not show any decrease of testosterone by different phthalates or phthalate-metabolites (such as DBP, MEHP and MBP) in human foetal testes although this effect was clearly observed in rat foetal testes (ANSES, 2015). However, these data are not considered sufficiently robust to conclude that the effects in testes observed in rats will not also be found in humans. Further, it was also concluded in the RAC restriction dossier on four phthalates (ECHA, 2016) that the available epidemiology studies had such a high degree of uncertainty that it did not allow to conclude on a direct causal relationship between the effects investigated (congenital malformation of the male genitalia, semen quality, pubertal timing and testicular cancer) and phthalate exposure.

Other developmental effects reported with phthalates can be considered independent of testosterone production. For example, delayed prenatal transabdominal migration of the testes

is at least partially related to an impairment of Insl3. In addition, germ cell effects can be due to a direct effect on Sertoli cells (see figure presenting the cellular targets for the "phthalate syndrome", above).

e) Toxicokinetics

No difference in toxicokinetics between animal and humans has been identified.

f) Bio-accumulation

There is no evidence for bioaccumulation of DIOP.

Conclusion on SCL

According to Article 10 of the CLP regulation, a higher SCL may be set in exceptional circumstances where there is adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the GCL.

In the assessment of setting a SCL for DIOP, it was shown that based on the various calculated ED_{10}, DIOP is considered to fall into the low potency group (≥ 400 mg/kg bw/d).

However, according to the data, RAC considers that adequate, reliable and conclusive scientific information is not available to set a higher SCL. This is based on the following;

- The low number of litters in the study by Saillenfait *et al*. (2013a) (8-12 litters with a requirements of 20 litters in the OECD TG 414) may overestimate the ED_{10} values from this study.
- Only a limited data set assessing the possible developmental effects that can be induced by C3-C6 *ortho*-phthalates is available for DIOP. In particular, the category assessment of *ortho*-phthalates show that effects on germ cells and mammary gland development in adult offspring could be sensitive parameters for phthalate toxicity. However, assessment of these parameters was not reported in Saillenfait *et al*., (2013a). Therefore, it cannot be excluded the possibility of reproductive effects at lower dose levels than those reported.

RAC considers that there are uncertainties in the setting of an SCL above the GCL even if all ED_{10} are above the threshold of 400 mg/kg bw/d. This is consistent with CLP Regulation Article 10 which require adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the GCL. Further, in the CLP guidance, Section 3.7.2.6.5.2 on data availability, it is clearly mentioned that limited data availability can be derived from absence of relevant test protocols or relevant parameters for the assessment of reproductive toxicity. It should also be mentioned that for all the other classified phthalates in the C3-C6 *ortho*-phthalate category, the GCL is applied.

In conclusion, RAC consider that the GCL should be applied for DIOP.

Adverse effects on or via lactation

There is no adequate study to assess effect of DIOP on or via lactation included in the CLH dossier. RAC concludes that in the absence of an adequate study, classification for effects on or via lactation cannot be evaluated.

Conclusion on classification

Overall, RAC agrees to classify DIOP as **Repr. 1B; H360FD (May damage fertility or the unborn child)**.

Additional references

Howdeshell, Wilson, Furr, Lambright, Rider, Blystone, Hotchkiss, Gray. 2008. A mixture of five phthalates esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicological Sciences 105(1):153-165.

ANNEXES:

Annex 1 The 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 RAC (excluding confidential information).