Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide

EC Number: 278-355-8
CAS Number: 75980-60-8

CLH-O-0000007023-85-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007023-85-01/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: **Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide**

EC Number: **278-355-8**

CAS Number: **75980-60-8**

The proposal was submitted by **Sweden** and received by RAC on **30 June 2020.**

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

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 **24 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 October 2020**.

ADOPTION OF THE OPINION OF RAC

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

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

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 **16 September 2021** by **consensus.**

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

	Index No	Chemical name	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	015-203-00-X	diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide	278-355-8	75980-60-8	Repr. 2	H361f	GHS08 Wng	H361f			
Dossier submitters proposal	015-203-00-X	Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide	278-355-8	75980-60-8	**Add** Skin Sens. 1B **Modify** Repr. 1B	**Add** H317 **Modify** H360Fd	**Retain** GHS08 **Add** GHS07 **Modify** Dgr	**Add** H317 **Modify** H360Fd			
RAC opinion	015-203-00-X	diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide	278-355-8	75980-60-8	**Add** Skin Sens. 1B **Modify** Repr. 1B	**Add** H317 **Modify** H360Fd	**Retain** GHS08 **Add** GHS07 **Modify** Dgr	**Add** H317 **Modify** H360Fd			
Resulting Annex VI entry if agreed by COM	015-203-00-X	diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide	278-355-8	75980-60-8	Repr. 1B Skin Sens. 1B	H360Fd H317	GHS08 GHS07 Dgr	H360Fd H317			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) (index number 015-203-00-X) was placed on Annex VI of CLP with a classification as Repr.2 H361f in ATP03. Limited information on reproductive toxicity was available and the assessment was based upon three studies: an oral 28-day repeated dose toxicity study, an oral 90-day repeated dose toxicity study, and a second (non-GLP compliant) oral 28-day and 90-day repeated dose toxicity study. Since then, one OECD TG 421 study two OECD TG 414, as well as an OECD TG 429 study have been performed and are included in the current assessment.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS included one Local Lymph Node Assay (LLNA) performed according to OECD TG 429 and GLP in mice. Stimulation Indices (S.I.) of 2.22, 2.96, and 3.46 were determined at concentrations of 10, 25, and 50% (w/w) TPO in acetone:olive oil (4+1 v/v), and an EC3 value of 27.0% (w/w) was calculated. The DS concluded that a classification as Skin Sens 1B, H317 was justified.

Comments received during consultation

Three Member States (MS) commented on and supported the proposed classification as Skin Sens. 1B, H317.

Assessment and comparison with the classification criteria

One LLNA according to OECD TG 429 and GLP was included by the DS for the assessment of skin sensitising properties of TPO. The DS considered the study to be reliable without restriction. In the study, 5 female CBA mice per dose group were exposed once daily for three days to TPO in acetone:olive oil (4+1 v/v). Hexyl cinnamic aldehyde (CAS No 101-86-0) was used as positive control. No significant increase in ear weights, as well as no signs of local or systemic toxicity were observed. All treated animals survived the scheduled study period (Study report, 2012). Stimulation Indices (S.I.) of 2.22, 2.96, and 3.46 were determined at concentrations of 10, 25, and 50% (w/w) TPO in acetone:olive oil (4+1 v/v), respectively. Based on the S.I.'s obtained with TPO at a concentration of 25 and 50%, an EC3 value of 27.0% (w/w) was calculated.

There is no information available on skin sensitisation in humans.

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1, which comprises of two sub-categories, 1A and 1B. The classification can be based on data from LLNA studies, where a category 1A is justified if the EC3 value is ≤ 2 while a category 1B is justified if the EC3 value is ≥2.

Overall, RAC is of the opinion that a classification of TPO as **Skin Sens. 1B, H317** is justified based on the EC3 value of 27%.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS summarised that the classification of TPO as Repr. 2, H361f adopted by RAC in 2010 was based on three studies: an oral 28-day repeated dose toxicity study, an oral 90-day repeated dose toxicity study, and a second (non-GLP compliant) oral 28-day and 90-day repeated dose toxicity study. In 2010 RAC concluded that the testes are a target organ in rat (and could potentially lead to reduced male fertility) and that the adverse effects occur in the absence of significant general toxicity. However, due to the limitations of the studies, the evidence was not sufficiently convincing to classify the substance as Category 1B.

In the current proposal an oral reproduction/developmental toxicity screening test (OECD TG 421) was included in addition to the previously assessed studies. Based on this study, the DS concluded that a Category 1B is justified, as the substance exhibits adverse effects on the testes and epididymides, in the absence of marked general toxicity, which lead to reduction in fertility. Reduced weight of the testes and histopathological effects was also noted in the 28-day (Study report, 1989) and 90-day (Study report, 1991) repeated dose toxicity studies.

Adverse effects on development

For the assessment of adverse effects on development the DS included two OECD TG 414 studies (in rat and rabbit) and one OECD TG 421 study in rat. All these studies were performed after the last assessment by RAC in 2010. The DS considered a classification as Category 2 for developmental toxicity justified based on the high incidence of skeletal malformations and variations seen in the OECD TG 414 study in rat.

Adverse effect on or via lactation

No effects on or via lactation were observed in the OECD TG 421 one-generation reproductive toxicity study, and the DS did not propose any classification.

Comments received during consultation

Three MSCA commented on the proposal, all supported the proposed classification as Repr. 1B, H360Fd. One MSCA asked for consideration of ED10 calculations for potential setting of SCL. The DS responded that the substance can be considered to fall in the medium potency group, and hence no SCL was considered justified.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Since the original classification, **an oral reproduction/developmental toxicity screening test** according to OECD TG 421 has been performed (Study report, 2019), in which, ten Wistar rats per sex and group were exposed to TPO (purity: 99.32%) at dose levels of 0, 60, 200, and 600 mg/kg bw/day by oral gavage. The control group was only administered the vehicle (acetone/olive oil 4:1 v/v). An elongated pre-mating period of 10 weeks was included, to cover at least one complete spermatogenic cycle and at least two complete oestrous cycles.

Two preterm mortalities were reported. In the high dose group, one female was sacrificed *in extremis* during the premating period (day 61) due to animal welfare reasons (moderate lethargy, flat/hunched posture, muscle twitching, piloerection, slight chromodacryorrhoea, slight ptosis, and red snout). 2% body weight loss was noted for this female over Weeks 7-8 of the premating

period, followed by recovery in Week 9. Macroscopic examination at necropsy revealed accentuated lobular pattern of liver and reduced size of the spleen. A relationship to treatment could not be excluded as comparable clinical signs were noted for a surviving high dose female as well. In the control group, one female was found dead on day 43. No definite cause of death could be established; however, macroscopic findings could indicate technical error during gavage. If is further noted that one female in the mid dose group was euthanized on PND4 due to a total litter loss during the lactation period.

Clinical signs were only observed in the high dose group, and in addition to the one sacrificed female, they included transient signs of muscle twitching, hunched posture and piloerection in one female while two other females had piloerection. As similar clinical signs were also noted in the high dose female sacrificed in extremis, these observations were regarded as related to treatment. The piloerection was noted for 2 to 3 consecutive days at the end of Week 13 or 16 of treatment. All males in the high dose group showed transient signs of abnormal calm/lethargic behaviour and one male had breathing rales. The clinical signs were short in duration (lasting for only a few minutes), followed by complete recovery.

No change in absolute or relative food consumption was noted in any group. However, a lower mean value for absolute food consumption was observed in the high dose group females from GD14 onwards, which was considered to be related to their non-pregnancy status.

A dose-response related lower body weight and body weight gain were observed in males in the mid- and high dose group during premating and mating period. In the high dose group, a slightly reduced mean body weight gain from start of treatment and onwards was observed in males (reaching statistical significance on Day 8 and from Day 57 onwards), resulting in a 13% lower mean body weight at the end of treatment when compared with controls. In the mid dose group, the reduced mean body weight observed in males at the end of the treatment period was less than 10 % and considered non-adverse. The lower body weight gain noted in females in the high dose group during the gestation period was considered to be related to the non-pregnancy status of all the females, and not reflecting a systemic toxic effect. In the mid dose group, a slightly reduced mean body weight and body weight gain was observed, however, this was considered related to one dam in this group with resorptions.

Macroscopic observations at necropsy revealed alterations in the reproductive organs of males in the high dose group including flaccid testis (8/10 animals) and testis reduced in size (10/10 animals) and epididymides reduced in size (9/10 animals). Further decreased absolute weight and weight relative to body weight of testes and epididymides were noted. Differences between high dose group and control in organ:body weight ratios were 45% and 34% for testes and epididymis, respectively. Microscopic findings were noted in the testes and epididymides in the mid and high dose groups. Massive tubular atrophy was observed in testes in the high dose group. In addition, in the mid dose group atypical residual bodies were observed in 9/10 examined males and single male had degeneration/depletion of germ cells. In the epididymides a massive reduction in sperm numbers were observed in all 10 examined males in the high dose group (see table below).

***Table**: Summary microscopic findings in males, testis and epididymides*

			Dose level (mg/kg/bw/d)			
			0	60	200	600
Testes (10 tissues /dose group)	Atrophy tubular	Massive				10
	Atypical residual bodies	Slight			9	
	Atypical residual bodies	Moderate			1	
	Multinucleated giant cells	Moderate			1	
	Degeneration germ cells	Moderate			1	
	Depletion germ cells	Moderate			1	
Epididymides (10 tissues/dose group)	Cell debris	Minimal				2
		Slight				5
		Moderate			1	1
	Reduced sperm	Slight			1	
		Massive				10

Further microscopic findings were noted in the thyroid gland. Hypertrophy of the follicular cell and the colloid alteration of the thyroid glands were observed in the mid- and high dose group up to a slight degree in males and in females in the high dose group (see table below).

***Table:** Summary microscopic findings in males and females, thyroid gland*

			Males				Females			
			Dose level (mg/kg bw/day)							
			0	60	200	600	0	60	200	600
Thyroid glands (10 tissues/dose group)	Hypertrophy follicular cell	Minimal	3	5	2	5	5	3	3	4
		Slight			4	3				2
	Colloid alteration	Minimal			3	2				2
		Slight				4				1

No treatment-related changes were noted in any of the remaining parameters investigated in this study.

In the high dose group, 3 out of 9 females showed no evidence of mating after a prolonged cohabitation period of a total of 21 days with two different males. The mating index in the high dose group was 67% compared with 100% in concurrent control and 99% as mean historical control value. During the mating period, an extended di-oestrus was observed in the 3 high dose females for which mating could not be confirmed. An extended di-oestrus occurred also at low incidence in untreated controls, however, a relation to treatment with the test item could not be excluded.

***Table:** reproductive performance/function*

Dose (mg/kg bw/day)	Female/male (number)	In-life reason	Histopathology
0	-	-	-
60	56/16	Not pregnant	-
200	67/27	Total litter loss	
	68/28	Implantation sites only	Testes: moderate degeneration and depletion germ cells
600	72/32 and 36	No evidence of mating	Testes: massive tubular atrophy Epididymides: massive reduced sperm
	74/34 and 38	No evidence of mating	
	79/39 and 40	No evidence of mating	
	71/31	Not pregnant	
	73/33	Not pregnant	
	76/36	Not pregnant	
	77/37	Not pregnant	
	78/38	Not pregnant	
	80/40	Not pregnant	

The fertility index was 100%, 90%, 100 % and 0% for the control, low-, mid-, and high dose group, respectively. In the high dose group 9/9 couples failed to deliver pups, compared to 2/10 in the mid dose group and 1/10 in the low dose group. All males in the high dose group showed massive tubular atrophy in the testes and reduced luminal sperm with luminal cell debris in the epididymides which could explain the lack of offspring. In the mid dose group, one female had a total litter loss while the other female showed implantation sites only. The lack of offspring for the female which had only implantations could be explained by the moderate depletion and degeneration of sperm cells with multinucleated giant cells in the testes and moderate cell debris and slight reduced sperm in the epididymides in the male. The rest of the males in the mid dose group all showed atypical residual bodies, however with no effect on fertility. In the low dose group, the finding of one female which failed to get pregnant could be considered as not related to treatment. In all dose groups, no morphological findings in the reproductive organs of females related to the treatment were observed. The males in the low dose group did not show any treatment related effect on spermatogenesis.

In the **28-day oral repeated dose toxicity study,** according to a Japanese guideline and GLP (Study report, 1989), TPO (purity 99%) was administered to five Sprague-Dawley rats per sex and group at dose levels of 0, 50, 250, and 750 mg/kg bw/day. The control group was only administered the vehicle (Arachis oil). Two satellite groups, each of five rats per sex were treated with 750 mg/kg bw/day or vehicle alone throughout the 28-day study period and then maintained without treatment for additionally fourteen days.

One female from the satellite high dose group was found dead on Day 4 and one female from the satellite control group died on Day 42 (post-treatment period).

In the high dose group, increased salivation, red/brown staining around the snout and mouth, wet fur, red/brown staining of the fur, hair loss, piloerection, hunched posture, lethargy, ptosis, diuresis, diarrhoea and abdominal distension, and single incidence of vocalisation were observed from Day 3 and onwards. Satellite animals recovered immediately following cessation of dosing and appeared normal throughout the treatment-free period. The mid dose group showed the same clinical signs from day 4 however with less severity and without diarrhoea, abdominal distension, and vocalisation.

In the high dose group body weight and body weight gain was significantly reduced in week four for both males and females. At the end of treatment, body weight was reduced by 5% (mean) in the mid dose group and 14% (mean) in the high dose group compared to controls. Females of the satellite group were not affected, and satellite high dose males quickly recovered during the treatment-free period. A marked reduction in food efficiency was observed during the last week of treatment in the mid and high dose groups. Food efficiency turned back to normal in the 750 mg/kg satellite group following cessation of treatment.

Decreased testes weight (mean absolute weight 3.09 grams and mean relative weight 0.91) compared to controls (mean absolute weight 3.39 grams and mean relative weight 1.04) and size, microscopically identified as testicular atrophy, was observed in all high dose males. Grading showed increased severity of testicular atrophy at the high dose. Although one animal from each of the remaining treatment group also had a minimal degree of testicular atrophy, the study author considered it to be spontaneous in origin and unrelated to treatment at these dose levels. Testicular atrophy was also present amongst males in the satellite group (750 mg/kg), although the incidence was reduced (3/5).

In the **90-day oral repeated dose toxicity study** similar to OECD TG 408, GLP (Study report, 1991), the test substance (purity 94.8%) was administered by gavage once daily (5 days/week)

to ten Wistar rats per sex and group at dose levels of 0, 100, 300, or 1000 mg/kg bw/day. The control group received the vehicle (CMC (carboxymethyl cellulose), 0.5% in water) alone.

Females in the high dose group showed a reduced general state of health, and two females in this group died on day 44 and 48. There were no mortalities or severe clinical signs reported in males in any dose group. The body weight and body weight gain were reduced in females (8 % and 16 %, respectively) and in males (23 % and 38 %, respectively) in the high dose group compared to control animals, while in the mid dose group reduction was only observed in males (10 % and 16 %, respectively) compared to the control group.

In males in the mid- and high dose group, the testes were reduced in size from week 6 and onwards. The absolute and relative testes weights were decreased, on average by about 50% in all males in these dose groups compared to control animals.

All males in the mid and high dose group had moderate to marked degree of diffuse atrophy of the testicular parenchyma and a slight to moderate degree of interstitial oedema. In the low dose group one male exhibited moderately reduced spermiogenesis. All males of this dose group showed a minimal to moderate vacuole degeneration of spermatogonia in some seminiferous tubules. These lesions and the focal atrophy findings were also seen in the control group up to the same grading and were not considered to be substance related by the study author.

In the **second 28-day oral repeated dose toxicity study** similar to OECD TG 407, not GLP (Study report 2001), three male Wistar rats were exposed to 0 or 1000 mg TPO/kg bw/day (purity: 99.3%) by gavage. The controls received the vehicle (CMC,) 0.5% in water) alone. Age at study initiation was between 41-43 days. No testicular effects, mortalities or clinical signs were reported.

In the **second 90-day oral repeated dose toxicity study** similar to OECD TG 408, not GLP (Study report, 2001), ten Wistar rats were exposed to 0 or 1000 mg TPO/kg bw/day (purity: 99.3%) by oral gavage. The controls received the vehicle (CMC, 0.5% in water) alone. Age at study initiation was 34 days. No mortalities or adverse clinical signs were reported. Body weight in the exposed animals was reduced by 10% compared to the controls. In 8/10 males, testes were reduced in size and loss of turgor was observed. Mean testes weights were significantly lower in the exposed rats (mean absolute weight 2.1 grams and mean relative weight 0.718 %) compared to the control group (mean absolute weight 3.286 grams and mean relative weight 0.996 %). All testes showed slight to severe degree of diffuse atrophy of seminiferous tubules. In 8/10 males, epididymides was reduced in size and histopathology revealed oligo- to azoospermia (*i.e.* reduction or absence of mature sperms). Four males had oedema and Leydig cell hyperplasia of minimal to slight degree.

In summary, the current harmonised classification of TPO as Repr. 2 (H361f), adopted by RAC in 2010, is based on an oral 28-day repeated dose toxicity study, an oral 90-day repeated dose toxicity study and the non-GLP compliant oral 28-day and 90-day repeated dose toxicity study showing reduced weight of the testes and histopathological effects. If was concluded that the testes are a target organ in rat and that the adverse effects occur in the absence of marked general toxicity.

In the more recent oral reproduction/developmental toxicity screening test according to OECD TG 421 (Study report, 2019) with doses up to 600 mg/kg bw/day adverse effects on testis and epidiymides was observed in the absence of marked general toxicity. The fertility index in the high dose group of 600 mg/kg bw/day was 0% and all the males in this dose group showed massive tubular atrophy in the testes and reduced luminal sperm with luminal cell debris in the epididymides.

Overall, a classification as Repr. 1B for adverse effects on sexual function and fertility is appropriate based on clear evidence of adverse effects on the testes and epididymides, in the absence of marked general toxicity, which lead to reduction in fertility.

In conclusion, RAC is of the opinion that a classification of TPO as Repr. 1B; H360F is justified.

Adverse effects on development

TPO is currently not classified for adverse effects on development. Three studies have been conducted with the substance since the previous assessment by RAC in 2010; one oral prenatal developmental toxicity study (OECD TG 414) in rat (Study report, 2016) and one in rabbit (Study report, 2018), and one oral reproduction/developmental toxicity screening test (OECD TG 421) in rat (Study report, 2019).

In the prenatal developmental toxicity study OECD TG 414 (Study report, 2016), TPO (purity 99.5%) was administered by gavage to 22 mated female Wistar rats per group at dose levels of 0, 50, 150 and 500 mg/kg bw/day (Gestation Day (GD) 6–20). The control group received the vehicle alone (CMC, 1% in water). No mortalities were observed. Clinical signs in the high dose group included salivation (19/22 females), piloerection (7/22 females), hunched posture (4/22 females). Mean body weight and corrected body weight gain were 7% and 11%, respectively, lower than in controls on day 21 in the high dose group, while unaffected in the low- and mid-dose groups. Food consumption was significantly reduced Day 6 to 12 in the high dose group compared to controls, however fully recovered to similar levels as controls from Day 12 onwards. No effect on food consumption was observed in the low and mid dose groups. Four animals were not pregnant, two at 50 mg/kg bw/day, one at 150 mg/kg bw/day and one at 500 mg/kg bw/day. All other females were pregnant and had litters with viable foetuses. Mean litter size (viable foetuses) was 10.6, 10.2, 9.7 and 10.1 in control, low-, mid- and high-dose group respectively. No effects on the number of corpora lutea, implantation sites, and pre- and post-implantation loss was reported up to 500 mg/kg bw/day.

Mean foetal body weight was 6% lower in the high dose group compared to controls, the reduction in foetal weight was significant for females, but not for males. No effect was observed on sex ratio, litter size or litter weight in all dose groups.

External malformations were observed in two foetuses from the same litter in the high dose group. One foetus had no tail, and one foetus has a filamentous tail. Skeletal malformations were observed in the high dose group evident as bent limb bones in 10 foetuses from 5 litters compared to one control foetus. In all cases one or both scapulae were bent and in three foetuses also humeri were involved. Bent limb bones were not observed in the low- and mid-dose group. The finding in the high dose group (10.6% per litter) was far above the upper limit in historical control foetuses (0.0-0.7% per litter based on 5 studies from 2014-2015). The higher incidence of bent limb bones coincided with an increased litter incidence of bent ribs with 13.5%, 23.5%, 22.1% and 69.9% per litter in control, low-, mid-, and high dose group, respectively. The incidence of bent ribs in the high dose group was statistically significantly different from the controls, and all foetuses with bent limb bones in the high dose group also had bent ribs.

Skeletal variations were also observed in the high dose group. The ossification of the scull and unossified metatarsals and metacarpals were statistically significantly reduced compared to the controls. Reduced ossification of the skull had a mean litter incidence of 45.9% ($p < 0.01$) in the high dose group versus 12.4% in controls. The increased incidence of unossified metatarsals and/or metacarpals showed a mean litter incidence of 21.0% ($p < 0.05$) in the high dose group versus 5.4% in controls.

***Table**: Foetal malformations and variations*

% per litter	0 mg/kg bw/day	50 mg/kg bw/day	150 mg/kg bw/day	500 mg/kg bw/day	Historical control data***, % per litter (foetuses/litters)
Malformations					
No tail or filamentous tail[a]	0	0	0	0.7 % (2 foetus/1 litter	0.0% (0/0)
Bent limb bones[b]	0.8 % (1 foetus/1 litter)	0	0	10.6% (10 foetus/5 litters)	0.0-0.7% (1/1)
Variations					
Bent ribs[b]	13.5 %	23.5%	22.1%	69.9% **	0.8-7.7% (34/17)
Reduced ossification of scull bones[b]	12.4 %	12.5 %	21.1 %	45.9 %**	0.0-1.4% (6/4)
Unossified metatarsals and metacarpals[b]	5.4 %	6.6 %	3.2 %	21 % *	0.0-3.7% (18/11)

* Significantly different from control at 0.05
** Significantly different from control at 0.01
*** Historical control data from 5 studies, 2014-2015.
[a]: measured in approx. 200 foetuses/20 litters
[b]: measured in approx. 100 foetuses/20 litters

In the publications by De Schaepdrijver *et al*. (2014), Mitchard & French (2011) and Kimmel *et al*. (2014) it has been discussed if bent limb bones should be considered as a temporary variation rather than malformation. All these three publications indicated that the finding of bent limb bones could be transient in nature and should be considered as a variation rather than a malformation. During the general consultation two additional studies by Hofmann *et al.* (2016) and Mitchard and Stewart (2014) were provided. In the study by Mitchard and Stewart (2014) Wistar Hannover rats were exposed from GD6 to GD17, and it was reported that skeletal abnormalities were evident in the foetuses at GD20, however not in pups assessed at PND21 and concluded that these malformations should be regarded as minor rather than major. In the review by Hofmann *et al*. (2016) it was concluded that the data assessed uniformly show that bent scapulae and bent long bones are transient, and not permanent foetal changes, that are completely repaired postnatally and that they should be classified as variations rather than malformations. RAC notes that all these studies have been performed in relation to the regulation of pharmaceuticals and has to be considered in this context.

The bent limb bones observed in the OECD TG 414 study in rats are not considered to be a consequence of maternal toxicity, as there was no marked maternal toxicity reported. In addition, no significant effects on foetal body weights were observed. Furter, there is no follow up study available following the pups postnatally, and it is therefore not possible to assess a possible transient nature of the observed effects on limb bones.

RAC therefore considers that the finding of bent limb bones and the statistically significant increased incidences of skeletal variations, including bent ribs, reduction in ossification of skull bones and unossified metatarsals and/or metacarpals in the high dose group which were outside historical control data, is relevant for a classification for adverse effects on development. These effects were observed in the absence of marked maternal toxicity.

In the second prenatal developmental toxicity study OECD TG 414 (Study report, 2018), TPO (purity 99.32%) was administered by gavage to 22 mated female New Zealand rabbits per dose group of 0, 10, 30 and 100 mg/kg bw/day on gestation day (GD) 6–28. The control group received the vehicle alone (CMC, 1% in water).

The selection of dose levels was based on a dose-range finder study with six females per group at dose levels of 100, 200 and 300 mg/kg bw/day. In this study, females dosed at 200 and 300 mg/kg bw/day, had 5-11% body weight loss with limited to no food consumption, lean appearance and piloerection. Females at 300 mg/kg bw/day also had hunched posture. All females at 300 mg/kg bw/day and 3/6 females at 200 mg/kg bw/day were sacrificed. The remaining females did not show signs of toxicity. Foetal findings at 200 mg/kg bw/day showed reduced litter size (8.7 foetuses/litter) compared to the control group but remained within historical control range.

In the main study two females in the mid-dose groups were euthanized prematurely on GD 26 (female 64) and GD 28 (female 49) respectively. At necropsy female 64 showed intussusception of the caecum with dark red discolouration, which could be a chance finding. Female 49 showed perforation of the left caudal lobe of the lungs which could be related to a dosing related incidence. Further, one female in the mid-dose group and three females in the high dose group were sacrificed prematurely due to early deliveries (GD 27 or 28). Two foetuses (one in the mid-dose group and one in the high dose group) were cannibalised. No external abnormalities were observed for the preterm litters.

No treatment related maternal clinical signs were observed up to the highest dose. Further, no effect was observed on maternal body weight gain. The corrected maternal body weight gain was slightly lower in the high dose group compared to controls, however not statistically significantly different. Further, no effects were observed on food consumption, gross pathology or number of abortions. Number of gravid females were 21 in the control group and 18 in all dosed groups.

No effects on sex ratio, litter size or litter weight. Mean litter size (viable foetuses) was 9.1, 9.1, 9.1 and 9.5 in control, low-, mid- and high-dose group, respectively. However, the foetal body weight was reduced by 5% in the high dose group.

External malformations were observed in the control, low- and mid-dose group in two, three and one foetus(es) respectively. No external malformations were observed in the high dose group. Skeletal variations were observed as a statistically significantly increase in the incidence of malaligned sternebrae in the high dose group (9.2% per litter compared to 3.8% in the controls). However, the finding in the high dose groups is within the historical control data (2.9-10.2% per litter based on 4 datasets, 2013-2017).

Sporadic visceral malformations were observed in the control and all dose groups, including abnormal lobation of the liver in the high dose group, malpositioned kidneys and testes in control, low- and mid-dose group, transposition of the great vessels in the low dose group and narrow aorta and ventricular septum defect in the control group.

RAC is of the opinion that the malformations observed is incidental and not related to treatment due to the absence of a dose-response relationship, observed frequencies and that the skeletal malformation observed was within the historical control data range.

It is noted that the highest dose of 100 mg/kg was not sufficiently high to cause treatment related general toxicity of the maternal animals, and RAC considers that this study is inconclusive regarding effects of the test item on development in rabbits.

In the **Reproduction/Developmental Toxicity Screening Test** according to OECD TG 421 (Study report, 2019), TPO (purity 99.32%) was administered to ten Wistar rats per sex and group at dose levels of 0, 60, 200, and 600 mg/kg bw/day. The control group received the vehicle alone (acetone/olive oil 4:1 v/v).

In the high dose group, no females were pregnant, and the highest dose for assessment of developmental findings was therefore the mid dose group of 200 mg/kg bw/day where no clinical

signs, no dose-response related changes in absolute or relative food consumption and no dose-response related changes in body weight of females during gestation or lactation were observed. Pregnant females were 9/10 in the control group (1 female sacrificed prior to mating), 9/10 in the low dose group (1 female with 0 implantation sites), and 10/10 in the mid dose group. One female in the mid dose group was euthanized on lactation Day 4, due to litter loss.

As regards foetal findings, one control pup and one pup in the low dose group was missing or found dead at PND 2. In addition, one female in the mid dose group lost her single pup on PND 4. The gestation index was 100% (9/9) in the controls, 100% (9/9) in the low dose group and 90% (9/10) in the mid dose group. The post-implantation survival index was 94%, 90% and 85% for control, low and mid dose group, respectively, and all were within the historical control range.

Dose-response related, but not statistically significant, increase in mean/corrected anogenital distance, in males (2.67/1.42 for controls, 2.78/1.47 at 60 mg/kg, and 2.93/1.54 at 200 mg/kg) and females (1.08/0.59 for controls, 1.15/0.62 at 60 mg/kg, and 1.19/0.64 at 200 mg/kg) was observed.

No other developmental toxicity findings including litter size, live birth index, viability index, lactation index, duration of gestation, parturition and maternal care, clinical signs, body weight, areola/nipple retention, T4 thyroid hormone levels and macroscopic examination were observed by treatment up to 200 mg/kg.

In summary, the developmental toxicity study in rats is relevant for a classification for adverse effects on development based on the finding of skeletal malformations including bent limb bones and the statistically significant increased incidences of skeletal variations, including bent ribs, reduction in ossification of skull bones and unossified metatarsals and/or metacarpals in the high dose group which were outside historical control data, and observed in the absence of marked maternal toxicity.

RAC notes that the developmental toxicity study in rabbits did not show any effects relevant for classification, however, the tested doses were considered not sufficiently high to cause treatment related general toxicity in the maternal animals.

Furthermore, in the OECD TG 421 screening test in rats, where no females were pregnant at 600 mg/kg bw/day, the highest dose for assessment of developmental findings was the mid dose of 200 mg/kg bw/day which limits the assessment for developmental toxicity. The only developmental effect observed was a dose related, however, not statistically significant increase in anogenital distance.

Overall, a classification as Repr. 2 for adverse effects on development is appropriate based on some evidence of adverse effects including increased incidence of skeletal malformations and variations in rats, in the absence of marked general toxicity.

RAC considers that a classification of TPO as Repr. 2; H361d is justified.

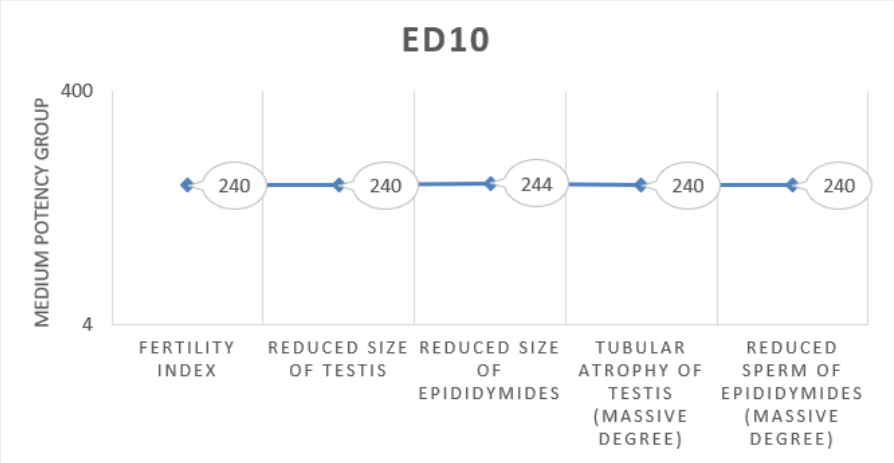
In conclusion, RAC is of the opinion that **TPO warrants classification as Repr. 1B; H360Fd.**

Calculation of ED10-value for assessment of SCL-setting for adverse effects on sexual function and fertility

The DS calculated the ED10-values based on the effects on fertility and reproductive organs observed In the OECD TG 421 study in rats (Study report, 2019). The calculations were performed in accordance with the ECHA Guidance on the Application of the CLP criteria (v.5.0, 2017).

For fertility index an interpolation between the NOAEL (100% at 200 mg/kg bw/day) and LOAEL (0% at 600 mg/kg bw/day) results in a ED10 of 240 mg/kg bw/day based on the following calculation: (600-200) / (100-0) = 4.0 mg/kg per % (steepness). Going from 100% to 90% requires subtraction of 10%. This equals 10% x 4.0 mg/kg per % = 40 plus 200 as the starting point = 240 mg/kg bw/day.

***Figure**: ED10-values calculated from effects observed in the OECD TG 421 study in rats.*



The ED10-values all fall within the medium potency group (4 mg/kg bw/day < ED10-value < 400 mg/kg/bw/day), and hence for a classification in category 1B for adverse effects on sexual function and fertility, the GCL of 0.3% should apply.

Modifying factors are not considered relevant to apply in this case since the calculated ED10-values are not borderline to a higher or lower potency group.

Overall, RAC is of the opinion that no SCL is appropriate based on the ED10 values calculated, and the GCL should apply.

Calculation of ED10-value for assessment of SCL-setting for adverse effects on development

ED-10 value for adverse effects on development was calculated based on the effect on bent limb bones observed in the OECD TG 414 study in rats (Study report, 2016). The calculations were performed in accordance with the ECHA Guidance on the Application of the CLP criteria (v.5.0, 2017).

For bent limb bones the highest tested dose of 500 mg/kg bw/day show an incidence of 10.6% while the incidence in controls is 0.8%. The ED10 is defined as the dose level at which 10% of the test population above the incidence in the concurrent control shows the effect, indicating that in this case the ED10 is approximately 500 mg/kg bw/day (10.6%-0.8%= 9.8%). On this basis the substance could be considered falling into the low potency group for a category 2 classification with an ED10 above 400 mg/kg bw/day. The SCL for the low potency group should be in the range of 3-10%. The limit of 10% may according to ECHA Guidance on the Application of the CLP criteria be considered in certain cases, such as for substances with a ED10 value above 1000 mg/kg bw/day and a NOAEL below 1000 mg/kg bw/day. In this case the ED10 is well below 1000 mg/kg bw/day, and the NOAEL is 150 mg/kg bw/day. A SCL of around 3 % could be considered, however as this is similar to the GCL, no SCL is proposed. This is further supported by assessing the ED10 values for the variations observed in the OECD TG 414 in rats which were calculated to be well below 400 mg/kg bw/day. E.g. calculations based on reduced ossification of scull bones indicate an ED 10 to be approximately 150 mg/kg bw/day or slightly higher (21.1% at 150 mg/kg bw/day-12.4% at 0 mg/kg bw/day =8.7%).

Modifying factors are not considered relevant to apply in this case since the calculated ED10-values are not borderline to a higher or lower potency group.

Overall, RAC is of the opinion the GCL of 3% should be used for adverse effects on development.

Effects on or via lactation

In the OECD TG 421 an oral reproduction/developmental toxicity screening test (Study report, 2019), no adverse effect on or via lactation was observed. No effect was observed on the number of live offspring on Day 20 after littering compared to the number of live offspring on Day 4 (after culling). Further, no pups were found dead/missing between lactation Days 5 and 20.

Overall, RAC is of the opinion that no classification is warranted for effects on or via lactation.

Additional references

Hofmann et al. (2016) Postnatal Fate of Prenatal-induced Fetal Alterations in Laboratory Animals, Reproductive Toxicology, 61, 177–185

Mitchard and Stewart (2014) Reduced post-natal versus pre-natal incidence of bent long bones and scapulae in a preliminary investigation using the Han Wistar rat, Reproductive Toxicology, 45, 39-44.

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).