Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIPHENYL(2,4,6-TRIMETHYLBENZOYL)PHOSPHINE OXIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide
EC number: 278-355-8
CAS number: 75980-60-8
Dossier submitter: Sweden

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	Germany		MemberState	1
Comment received				
The DE CA is of the opinion that the classification of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide as Repr. 1B, H360F is warranted and classification in Category 2 (H360d) could be supported. Fertility The dossier submitter concludes that the substance should be classified as reproductive toxicant Category 1B. We agree with the dossier submitter that adverse effects on sexual func-tion and fertility demonstrated in the recent GLP-compliant reproduction/developmental toxicity screening test with diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (purity 99.32 %) justify this classification proposal (Study report, 2019): • In males, testicular atrophy and reduced number of sperm in the epididymis were observed for all males at 600 mg/kg bw/day (10/10). The histological changes were of "massive" degree, as graded by the pathologist(s) of the study. In addition, reduced testis and epididymis weight, testis size and turgor were observed for males at this dose. Marked testicular degeneration led to substantial reduction of fertility, as none of the females with confirmed mating (6/9) delivered pups. Thus, the fertility index for this dose was 0 %. • In females, irregular cycling during the mating period was reported at 600 mg/kg bw/day, with 3 out of 9 females being in the state of extended diestrus. This				

corresponded to the reduction in the mating index (67 % compared to 100 % in the control). Affected females did not mate in spite of extension of the mating period by additional 7 days and replacement of the mating partner with a male with confirmed mating performance.

The described above effects occurred in the absence of marked systemic effects. The body weight in male rats in the high dose group was reduced by 13 % ($p < 0.05$). Other effects included slight, but not statistically significant reduction in motor activity. In females at this dose, reduction in body weight compared to control was explained by missing pregnancies.

Considering the above, the proposed classification as Repr. 1B, H360F is justified.

Developmental toxicity
The treatment-related effects on limb bones and ribs observed in the GLP-compliant prenatal developmental study in rats (Study report, 2016) would be borderline evidence for the classification.
• In the study, 10 affected foetuses at the top dose (500 mg/kg bw/day) from 5 litters had one or both scapulae bend compared to 1 foetus in the control group. In addition, three of them exhibited bend humerus bones. Based on the classification of the Dev-Tox database (accessed on 30.09.2020) these findings still belong to the "grey zone" findings; thus, a clear definition as malformations would not be possible.
• Additionally, for all of the affected foetuses with bent limb bones (10 foetuses in 5 litters) presence of bent ribs was demonstrated. Overall, a statistically significant increase in incidences of bent ribs was observed for this dose group (69.9 % vs 13.5 % in the controls).
• One more supportive evidence of skeletal effects of in utero exposure to diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide arises from observation of a missing tail in one foetus and filamentous tail structure in another one which has not previously been reported for the historical control animals.
• Reduced ossification of the skull bones (mean litter incidences of 45.9 % vs 12.4 % in control), metatarsals and metacarpals (mean litter incidences of 21 % vs 5.4 % in control) provides another evidence of delayed skeletal development.

Taken together these findings suggest that diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide affects normal skeletal development in utero. High incidences of skeletal findings provide some evidence of an adverse effect on development and consequently suggests that the proposal for classification to Category 2 (H360d) could be supported.

The highest dose (500 mg/kg/bw) in this study indicates the test substance-dependent slight reduction of foetal body weight (statistically significant only in females) in the presence of slight maternal toxicity (absolute corrected weight: - 7 %, corrected weight gain: - 11 %, reduced food consumption (d 6-12), clinical signs).

High incidences of skeletal findings provide some evidence of an adverse effect on development and consequently suggests that the proposal for classification to Caterogy 2 (H360d) could be supported.

Background:

Three studies are cited in the CLP report (De Schaepdrijver *et al.*, 2014; Mitchard & French, 2011; Kimmel *et al.*, 2014) that discuss the relevance of bent limb bones and bent scapulae. The studies' authors concluded that these effects revealed to be reversible post-partum and thus regarded as a transient retardation. Bent limb bones and bent scapulae were considered by the authors as secondary to maternal toxicity and foetotoxicity and should be regarded as variations.
In the experimental Wistar rat study of De Schaepdrijver *et al.* (2014), bent limb bones and bent scapulae were reversible post-partum. Maternal toxicity was marked, including reduced weight gain and lethality of 9/25 dams. In addition, a decreased pup weight was observed. Quantitative information about the reduced bw gain in dams and pups are not given in the study. De Schaepdrijver *et al.* (2014) note, referring to a study from Wilby *et al.* (2007), that bent long bones were also observed spontaneously and at dose levels that produce minimal maternal toxicity.

The evaluation of published studies by Kimmel *et al.* (2014) showed that bent long bones and bent scapulae in rats often occurred in the presence of maternal toxicity such as reduced maternal bw gain, but the degree of reduction is mostly not mentioned. Some of the cited studies showed bent long bones and bent scapulae but it is unclear whether maternal toxicity did not occur or was not examined.

With regard to Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, maternal toxicity and a reduced foetal bw were only slight and the high incidence of bent limb bones and bent scapulae should not be only secondary to maternal/foetal toxicity. The examined maternal parameters are limited in OECD TG 414 studies. An investigation of the more detailed repeated dose toxicity studies, though longer in duration, could inform whether a reduced bw gain is a sensitive parameter detected at the LOAEL, and which other effects occurred in females that are not examined in OECD TG 414.

Two additional studies discussing bent limb bones and scapulae were found and could be considered:
1. Hofmann *et al.* (2016) Postnatal Fate of Prenatal-induced Fetal Alterations in Laboratory Animals, Reproductive Toxicology, 61, 177–185
2. 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.

Dossier Submitter's Response

Thank you for your support and comments.

We appreciate you bringing to our attention the two additional scientific articles on bent bones and scapulae among other abnormalities (Hofmann *et al.*, 2016 and Mitchard and Stewart, 2014). They are in line with the three studies cited in the CLH report (De Schaepdrijver *et al.*, 2014; Mitchard & French, 2011; Kimmel *et al.*, 2014).

Also, please find a short summary of the observations mentioned in three repeated-dose-toxicity studies in an effort to answer the comments on body weight as a sensitive parameter at the LOAEL and on other effects occurring in pregnant and non-pregnant females in these studies.

In the **OECD Guideline 421** (exposure of at least 12 weeks), clinical signs were only observed in pregnant females at 600 mg/kg bw/day. The clinical signs were transient and

not treatment related and they occurred within the range of background findings. No toxicologically relevant changes in body weight, body weight gain, or food consumption (before or after correction for body weight) were observed in females at 600 mg/kg over the entire treatment period. No effects were seen at the lower dose of 200 mg/kg. See table 11 in the CLH-report for more information.

In the **28-day** oral repeated dose toxicity study (GLP-compliant, Study report 1989), clinical signs (i.e., 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 in non-pregnant females at 750 mg/kg bw/day. Less severe clinical signs were observed at 250 mg/kg. The satellite group recovered immediately following cessation of dosing and appeared normal throughout the treatment-free period.
An increase in body weight gain was observed in week 3 during treatment at 750 mg/kg, while a significant decrease was observed for body weight and body weight gain in week 4. At 250 mg/kg, body weight gain was reduced in week 4. The satellite group was not affected.
No differences in food consumption were observed.
Elevated levels of cholesterol, creatinine and bilirubin as well as increased urinary ketones, increased urine volume and reduced specific gravity were observed at 750 mg/kg. At 250 mg/kg, bilirubin levels were increased. The parameters were reversible as observed during the treatment-free period of the satellite group.
Increased relative liver weights were observed at 250 and 750 mg/kg and periportal hepatocyte vacuolation was observed in two of the high-dose females.
Relative kidney weights were also increased at 750 mg/kg. Basophilia and, in some instances, associated dilatation of distal tubules was observed at 750 mg/kg.
No effects were observed at 50 mg/kg.

In the **90-day** oral repeated dose toxicity study (GLP-compliant, Study report 1991), clinical signs (reduced general state of health) in non-pregnant females were only observed at 1000 mg/kg. Two females at 1000 mg/kg died during treatment. Body weight and body weight gain were reduced by 8% and 16% respectively at 1000 mg/kg. Still, food consumption was increased in this high-dose group.
At 1000 mg/kg, erythrocytes, haemoglobin, haematocrit and thromboplastin time were decreased. Haemoglobin and haematocrit values were also reduced at 300 mg/kg. In contrast, leucocytes, platelets, eosinophilic granulocytes, neutrophilic polymorphonuclears and calcium levels were increased at 300 and 1000 mg/kg.
Kidney and liver weights were increased at 300 and 1000 mg/kg.
Findings considered to be attributed to liver dysfunction, were an increase in serum alkaline phosphatase, gamma-glutamyltransferase activity and cholesterol levels, and a decrease in triglyceride levels and clotting time at 1000mg/kg.
Despite altered blood and urine parameters and increased liver and kidney weight, histopathology revealed no damage on these organs.

To summarise, any significant effect that was observed in the available repeated-dose toxicity studies occurred at high dose levels of 600, 750 and 1000 mg/kg bw/day. At lower dose levels, no marked general toxicity of the females was observed.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2020	France		MemberState	2

Comment received

In the OECD 421 guideline study performed in Wistar rat, histopathological findings were observed in male reproductive organs, mostly at the highest tested dose of 600 mg/kg bw/day. These effects occurred in the presence of general toxicity (decreased body weight and body weight gain, clinical signs). This leads to a lack of fertility at 600 mg/kg bw/day. Moreover, mating index was low at this same dose.

Based on the two 90-day studies performed in Wistar rat, testicular atrophy was found at 300 and 1000 mg/kg bw/day, in the presence of general toxicity (decrease of body weight and body weight gain). When animals were exposed only for 28 days, there was no effect on testis up to 1000 mg/kg bw/day in Wistar rats whereas testicular atrophy was observed at 750 mg/kg bw/day in Sprague-Dawley rats in the presence of general toxicity (decreased body weight and body weight gain, clinical signs).

In conclusion, a consistent reproductive toxicity was found among studies in male rats, leading to infertility in the OECD 421 guideline study. General toxicity is not sufficiently severe to explain these effects. Moreover, it can be noted that the OECD 421 guideline study is a screening assay. As noted in the guideline, this protocol is only "designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition". Statistical analysis is also rather limited since only 10 animals of both sexes are included. Therefore, the fact that significant reproductive effects are observed in this type of study supports that they must be considered as a clear evidence of reproductive toxicity. Based on all the elements, FR supports the proposal: Repro. Cat. 1B for fertility endpoint.

Skeletal findings were reported in the OECD guideline study in rats at the highest tested dose of 500 mg/kg bw/day, in the presence of maternal toxicity (decreased body weight and body weight gain). In contrast, no developmental effect was reported in the prenatal developmental study in rabbit up to highest tested dose of 100 mg/kg bw/day. This dose can be justified based on the higher sensitivity of rabbits as reported in the range-finding study but may not be high enough to detect effect similar as those in the rat study. The absence of skeletal findings in the OECD 421 study may be explained by the fact that it is only a screening study with limited information / statistical powder. Based on all the elements, FR supports the proposal: Repro. Cat. 2 for developmental toxicity.

FR agrees that no classification is required for effects on or via lactation.

Regarding SCL, it would have been appreciated to have the details of the ED10 calculations.

Dossier Submitter's Response

Thank you for your support and comments.

A summary of the effect dose levels at 10% (ED10) are presented in the figure below. The ED10 are calculated for the effects on fertility and reproductive organs observed in the OECD 421 study, and which lay the ground for the classification proposal for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide as Repr 1B. The table below shows the

ED10 calculation for fertility index. We calculated the ED10 according to the ECHA Guidance on the Application of the CLP Criteria, v.5.0, 2017.

Since our proposal for classification is Repr 1B for fertility (generic concentration limit, GCL, of 0,3%, medium potency group of ≥4 – ≤400 mg/kg bw/day) it is not relevant to calculate ED10 for developmental toxicity category 2 (GCL of 3,0%, medium potency group of ≥4 – ≤400 mg/kg bw/day). An ED10 <4 mg/kg bw/day for developmental toxicity would result in a high potency group and a SCL of 0,3%, similar to GCL of 0,3% for Repr 1B.

We do not consider any modifying factor relevant to modify the ED10 since the ED10 is not borderline to a higher potency group. A change in allocation from the medium potency group of ≥4 – ≤400 mg/kg bw/day is therefore not justified.

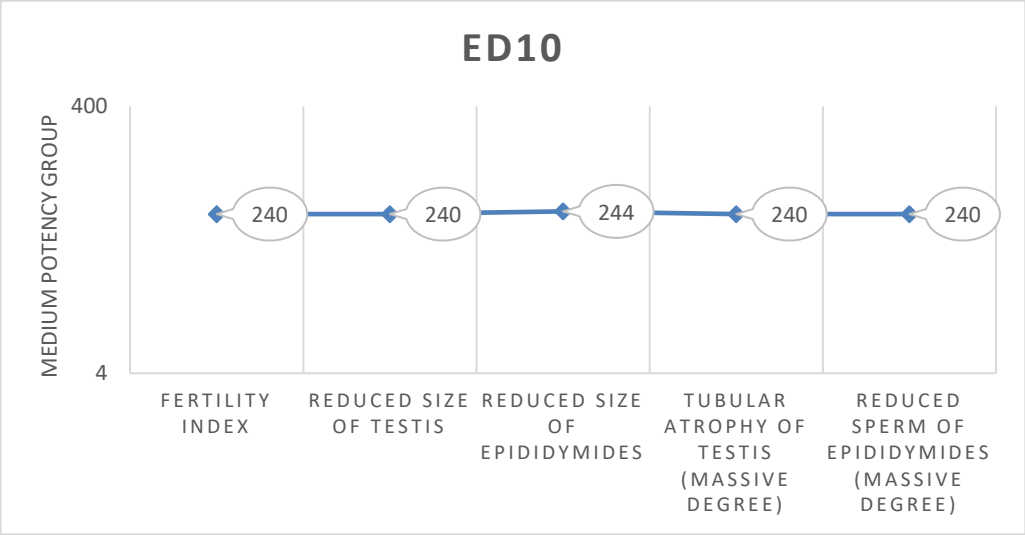

Figure 1. Effect dose levels at 10% calculated from the endpoints in the OECD 421 study.

Table 1. Effect dose level at 10% for fertility index in the OECD 421 study.

Dose (mg/kg)	0	60	200	600	ED10
Fertility index	100%	90%	100%	0%	**240**

Control fertility index 100%. A 10% reduction would be 90%.

Interpolation between NOAEL (classification) (100% at 200 mg/kg) and LOAEL (classification) (0% at 600 mg/kg) leads to an ED10 of 240 mg/kg bw/day.

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.

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	Finland		MemberState	3
Comment received				
Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide currently has a harmonised classification as Repr. 2, H361f. This classification is based on three 28- and/or 90-day repeated dose oral toxicity studies in which the testes were identified as a target organ in the rat. Since then, one re-productive/developmental toxicity screening test (OECD TG 421) in rats and two prenatal devel-opmental toxicity studies (OECD TG 414) in rats and rabbits have been performed in 2016-2019 to further investigate the effects of the substance on the reproductive system. In the reproductive/developmental toxicity screening test, flaccid testes and significantly reduced organ weights (both absolute and relative) of the testes and epididymides were observed in almost all male rats at the highest dose level. Massive testicular tubular atrophy and massively reduced sperm were present in all high-dose males, correlating with the macroscopic findings. Atypical residual bodies in the testes were also observed at slight to moderate degree in all middle-dose males. At the highest dose, the mating index was decreased in females, and the fertility index was 0%. All the findings occurred in absence of marked general toxicity. In the prenatal developmental toxicity study in rats, ten fetuses from five litters were affected with bent limb bones and bent scapulae at the highest dose; three of them also had bent humeri. A significant reduction in ossification of the skull and unossified metatarsals and/or metacarpals were also observed at the highest dose. Bent ribs were observed at all dose levels; the incidence was significantly increased in high-dose rats. No marked maternal toxicity was reported, and there were no significant effects on fetal body weights. Since no follow-up information on pups is available, the possibility that the osseous effects persist into adulthood cannot be excluded. In the prenatal developmental toxicity study in rabbits, no marked toxicity was reported in either dams or pups except for four early deliveries at the middle and highest doses. However, the highest dose was clearly low in comparison to doses used in the two aforementioned rat studies, and therefore the potential for toxic effects cannot be reliably excluded based on the results. There is no information available on reproductive/developmental toxicity in humans. Since there is no evidence on species specificity, the observed adverse effects on sexual function, fertility and development can be considered relevant to humans. FI CA supports the proposed classification of Repr. 1B; H361Fd for diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.				
Dossier Submitter's Response				
Thank you for your support and comments.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
14.10.2020	Germany		MemberState	4
Comment received				
The dossier submitter concludes that the examined substance should be classified as Skin Sens. 1B, H317. The BfR agrees, because the CLP-criteria according to the guidance to Regulation No 1272/2008 are fulfilled.				
Dossier Submitter's Response				
Thank you for your support and comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2020	France		MemberState	5
Comment received				
Skin Sensitization: FR agrees with the proposal: Skin Sens 1B based on the EC3 of 27% in a LLNA.				
Dossier Submitter's Response				
Thank you for your support and comments.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.10.2020	Finland		MemberState	6
Comment received				
The skin sensitisation potential of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide has been investigated in one reliable local lymph node assay (LLNA) in mice. In the study, stimulation indices of 2.22, 2.96 and 3.46 were determined at concentrations of 10, 25 and 50% of the test substance, respectively. A clear dose-response was observed and an EC3 value of 27.0% was calculated. According to the CLP Regulation, a substance may be classified as a skin sensitiser if the stimulation index is ≥3 in the LLNA. This criterion is fulfilled at the test concentration of 50%. In addition, the result allows subcategorisation as the EC3 value is >2%, hence meeting the criterion for sub-category 1B. There is no information available on skin sensitisation in humans. FI CA supports the proposed classification of Skin Sens. 1B; H317 for diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.				
Dossier Submitter's Response				
Thank you for your support and comments.				
RAC's response				
Noted.				